



(51) International Patent Classification:

C12N 1/20 (2006.01)

(21) International Application Number:

PCT/US2018/059595

(22) International Filing Date:

07 November 2018 (07.11.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/582,501 07 November 2017 (07.11.2017) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: PRODUCTION OF MYCOSPORINE-LIKE AMINO ACIDS IN CYANOBACTERIA

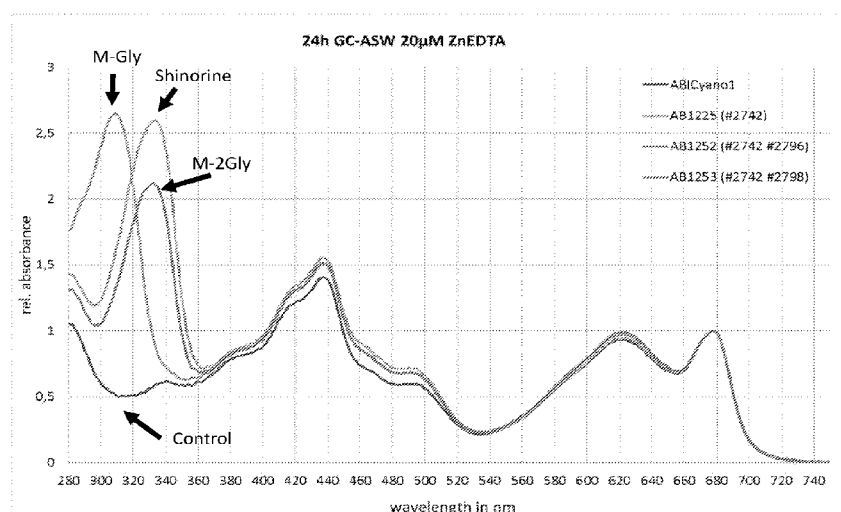


FIG. 12C

(57) Abstract: Modified cyanobacterial cells that are capable of producing various mycosporine-like acids (MAAs), useful as natural UV absorbing compounds, are described.



Published:

- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
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- *with sequence listing part of description (Rule 5.2(a))*

TITLE**PRODUCTION OF MYCOSPORINE-LIKE AMINO ACIDS IN CYANOBACTERIA****STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH**

[0001] Not Applicable.

REFERENCE TO SEQUENCE LISTING

[0002] This application contains a sequence listing submitted by EFS-Web, thereby satisfying the requirements of 37 C.F.R. §§ 1.821-1.825.

FIELD OF THE INVENTION

[0003] The present invention relates generally to the recombinant production of mycosporine-like amino acids (MAAs) in cyanobacterial host cells. MAAs have UV protective ability and can be used as natural skin protectants, such as sunscreens.

BACKGROUND OF THE INVENTION

[0004] Cyanobacteria can be utilized as bio-factories to produce compounds of interest from sunlight, CO₂, and nutrients. The transformation of the cyanobacterial genus *Synechococcus* with genes of interest has been described (U.S. Patent Nos. 6,699,696 and 6,306,639, both to Woods et al.). The transformation of the cyanobacterial genus *Synechocystis* has been described, for example, in PCT/EP2009/000892 and in PCT/EP2009/060526. The transformation of the cyanobacterial genus *Cyanobacterium* sp. has been described (U.S. Patent No. 8,846,369, U.S. Patent No. 9,315,832, and PCT/US2013/077364).

[0005] Penetration of UV radiation through the skin can damage the cellular DNA, induce genetic mutations and can lead to skin cancer. UV-A is regarded as the main cause of skin aging and wrinkling of human skin. Since both UV-A and UV-B are harmful, protection for both kinds of rays are recommended. The FDA has approved a list of active chemical/physical ingredients for use in sunscreens which absorb into the top layers of skin. For some people, however, the ingredients can lead to skin irritation, allergic reactions and even skin damage or aging. Further, the commonly used sunscreen component oxybenzone has recently been found to contribute to

damage to coral reefs by bleaching of corals and disruption of coral reproduction and growth (Downs et al., “Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands”, *Arch Environ Contam Toxicol.*, 70:265-88 (2016). The impacts of other chemical sunscreen substances on aquatic ecosystems are also of concern (Kim et al., “Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review” *Environ Int.*, 70:143-157 (2014). Further, the use of nanoparticles, such as zinc oxide and titanium dioxide, are also controversial. A good alternative sunscreen product or skin care product component would be a natural UV-absorbing substance.

[0006] Mycosporine-like amino acids (MAAs) are natural, water-soluble, carbonaceous, nitrogenous compounds that absorb light in the UV-A/UV-B range between 310 and 362 nm.

[0007] MAAs are naturally produced by a number of organisms, including certain algae species, cyanobacteria, dinoflagellates and corals. MAAs apparently act as natural sunscreens in these organisms. In addition to their ability to act as a sunscreen, MAAs also have antioxidant activity (Wada et al. “Mycosporine-Like Amino Acids and Their Derivatives as Natural Antioxidants” *Antioxidants* 4:603-646 (2015)).

[0008] Several cyanobacteria naturally synthesize MAAs such as mycosporine-glycine, shinorine, porphyra-334, mycosporine-2-glycine, palythine, asterina, mycosporine-glutamic acid-glycine, mycosporine-ornithine, usujirene, and palythene (for a review, see Carreto et al., “Mycosporine-Like Amino Acids: Relevant Secondary Metabolites”, *Chemical and Ecological Aspects Mar Drugs*, 9:387-446 (2011)).

[0009] Some species of cyanobacteria have been found to naturally produce MAAs, and can be cultivated in a sustainable manner for the production of desired ultraviolet screening compounds. Currently, MAA-based bio-sunscreen compounds are exclusively sourced from marine macroalgae, such as *Porphyra umbilicalis*, containing MAAs such as porphyra-334 and shinorine. Purified MAAs are not currently commercially available, but extracts from the red alga *Porphyra umbilicalis* are sold and used in cosmetic products (Helioguard® and Helionori®). The *Porphyra* extracts are reported to contain the MAA porphyra-334 and shinorine with absorption coefficients (ϵ molar) of 42'300 and 44'700 at 334 nm. The concentration of the MAAs in the extract is low: in the range of 1 % of total dry weight (Hartmann et al. “Quantitative analysis of mycosporine-

like amino acids in marine algae by capillary electrophoresis with diode-array detection”, *Jour. Pharm. Biomed. Analysis*, 138:153-157 (2017)).

[0010] However, cyanobacteria naturally produce lower amounts of MAAs (0.03 to 0.98 mg MAA/g dry cell weight) compared to *P. umbilicalis* (10 mg/g dry cell weight), complicating the industrial benefit of cyanobacteria (Garcia-Pichel et al., “The phylogeny of unicellular, extremely halotolerant cyanobacteria”, *Archives of Microbiology*, 169:469–482 (1998)).

[0011] Further, induction of the production of naturally occurring MAAs in cyanobacteria often occurs in response to certain types of stress, such as abiotic stresses and UV radiation, making the commercial production of MAAs with algae or cyanobacteria difficult (Singh et al., “Mycosporine-like amino acids (MAAs): chemical structure, biosynthesis and significance as UV-absorbing/screening compounds”, *Indian J Exp Biol.* 46:7-17 (2008)).

SUMMARY OF THE INVENTION

[0012] Provided herein are modified cyanobacterial host cells, plasmid constructs, and methods to produce high levels of various MAAs from cyanobacterial cultures.

[0013] In an aspect of the invention, a genetically modified cyanobacterial cell having at least one heterologous gene that encodes an enzyme involved in the production of a mycosporine-like amino acid (MAA) is provided. The MAA can be selected, for example, from the group consisting of mycosporine-glycine, shinorine, mycosporine-2-glycine, 4-deoxygadusol, usujirene, porphyra-334, asterina, palythene, palythine, mycosporine-2-(4-deoxygadusolyl-ornithine), mycosporine-ornithine, mycosporine-lysine, mycosporine-alanine, gadusol, 4-deoxygadusol, mycosporine-aurine, palythine-threonine, palythine-serine, mycosporine-methylamine-glycine, mycosporine-methylamine-threonine, mycosporine-methylamine-serine, palythine-threonine-sulfate, palythine-serine sulfate, mycosporine-glycine-valine, mycosporine-glutamic acid glycine, euhalotheca-362, and derivatives thereof. In an embodiment, the enzyme involved in the production of an MAA can be selected from the group consisting of 4-deoxygadusol (DDG) synthase, O-methyltransferase, ATP-grasp family protein, D-Ala-D-Ala-ligase, nonribosomal peptide synthetase (NRPS), clavaminic acid-like synthetase and 3-hydroxyisobutyrate-like dehydrogenase.

[0014] In an embodiment, more than one MAA can be produced by said cyanobacterial cell. The cell can be, for example, a non-filamentous cyanobacterial cell. In an embodiment, the

cyanobacterial cell can be grown photoautotrophically or photomixotrophically. In an embodiment, the heterologous gene can be from an organism such as, for example, cyanobacteria, bacteria, fungi, dinoflagellates, cnidaria, or algae. In an embodiment, the enzyme involved in the production of an MAA has at least 80% identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, and SEQ ID NO: 164 and can be codon optimized for optimal expression in the cyanobacterial cell.

[0015] In a further embodiment of the invention, the genetically modified cyanobacterial cell comprises at least one heterologous gene that encodes an enzyme involved in the production of an MAA having at least 70%, preferably at least 80%, most preferably at least 90% identity to at least one amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 158, SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, and SEQ ID NO: 170. These enzymes are from the mysABCD gene combinations from *Anabaena/Nostoc* and *Anabaena/Aphanathece*, the mysABCD1/D2 gene set from *Trichodesmium*, and the mysABCD1/D2 gene set from *Cyanobacterium* HL-69 for the production of any of the compounds mycosporine-glycine, mycosporine-2-glycine, shinorine, porhyra-344, palythine.

[0016] According to a further embodiment of the invention, the genetically modified cyanobacterial cell for the production of mycosporine-glycine, comprises at least three heterologous genes, wherein the first heterologous gene codes for a first enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 3, SEQ ID NO: 158, or to SEQ ID NO: 164, the second heterologous gene codes for a second enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 6, SEQ ID NO: 160, or SEQ ID NO: 166, the third heterologous gene codes for a third enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 9, SEQ ID NO: 162, or SEQ ID NO: 168. The first enzyme can therefore be selected from the mysA genes from *Anabaena*, *Trichodesmium* and *Cyanobacterium* HL_69, the second enzyme can be selected from the mysB genes from *Anabaena*, *Trichodesmium* and *Cyanobacterium* HL_69 and the third enzyme can be selected from the mysC

genes from *Anabaena*, *Trichodesmium* and *Cyanobacterium* HL_69. These three mysABC genes code for enzymes, which produce mycosporine-glycine (see for example Fig. 1).

[0017] A further embodiment of the invention is directed to a genetically modified cyanobacterial for the production of mycosporine-2-glycine, additionally including a fourth heterologous gene coding for a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 15 or 93. This forth enzyme can therefore be selected from the enzyme for MAA production encoded by the gene Ap3855 or the Enzyme for MAA production encoded by the gene Tery_2970 [*Trichodesmium erythraeum* IMS101] (see also Fig. 1 and 40).

[0018] Another inventive embodiment is directed to a genetically modified cyanobacterial cell for the production of shinorine/porphyra-334 and mycosporine-glycine-alanine, additionally including a fourth heterologous gene coding for a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 12, 15, 85, 93, 95, 170, 172 or 174. The forth enzyme is therefore selected from at least one enzyme out of a group consisting of the enzyme for MAA production encoded by the gene NpF5597, an enzyme for MAA production encoded by the gene Ap3855, an enzyme for MAA production encoded by the gene MysD [*Nostoc commune* var. *flagelliforme* QSY 1], an enzyme for MAA production encoded by the gene Tery_2971 [*Trichodesmium erythraeum* IMS101], an enzyme for MAA production encoded by the gene Tery_2970 [*Trichodesmium erythraeum* IMS101], an enzyme for MAA production encoded by the gene mysD [*Cyanobacterium stanieri* HL-69], an enzyme for MAA production encoded by the gene mysD [*Nostoc verrucosum* KU005], and an enzyme for D-alanine-- ligase [*Actinosynnema mirum* DSM 43827].

[0019] In another embodiment of the invention, the promoter can be a regulatable promoter or a constitutive promoter. In an embodiment, the promoter has at least 80% identity to a promoter selected from the group consisting of PrbcL, PntcA, PnblA, PisiA, PpetJ, PpetE, PcorT, PsmtA, PziaA, PsigB, PlrtA, PhtpG, PhspA, PclpB1, PhliB, PggpS, PpsbA2, PpsaA, PnirA, PnarB, PnrtA, PisiB, PnrsB, PmrgA, PpstS, and PerhC, , PpsbD, PnblA, PrpoA, PrnpA, PrpsL, , PcpcB, PnirA*2, PnirA*3, PnirA*4, PmntC, PrpsL*4, Prbc*, PcpcB, PrpsL*4, PcpcB (SEQ ID NO: 52), PnirA (SEQ ID NO: 22), PlrtA, PmrgA, PnblA, PggpS, PpetJ (SEQ ID NO: 25), PppsA, PrnpA (SEQ ID NO: 46), PpstS, Porf0128 (SEQ ID NO: 31), Porf1486 (SEQ ID NO: 32), Porf3164 (SEQ ID NO: 36), Porf3293 (SEQ ID NO: 33), Porf3621 (SEQ ID NO: 346), Porf3635 (SEQ ID NO: 35), Porf3858, Porf1071 (SEQ ID NO: 27), Porf1072 (SEQ ID NO: 37), Porf1074 (SEQ ID NO: 38),

Porf1075 (SEQ ID NO: 39), Porf1542 (SEQ ID NO: 40), Porf1823 (SEQ ID NO: 41), Porf1824, Porf3126 (PsmA) (SEQ ID NO: 23), Porf3389, Porf0221 (SEQ ID NO: 28), Porf0222 (SEQ ID NO: 42), Porf0223 (SEQ ID NO: 29), Porf0316 (SEQ ID NO: 30), Porf3232 (SEQ ID NO: 43), Porf3461 (PpetJ) (SEQ ID NO: 25), and Porf3749 (SEQ ID NO: 44).

[0020] In an embodiment of the invention, at least one heterologous gene can be located on a modified endogenous or a heterologous extrachromosomal plasmid. In an embodiment, the heterologous genes are located on more than one modified endogenous or heterologous plasmid. In another embodiment, the least one heterologous gene can be integrated into the chromosome.

[0021] In an embodiment, the genus of the cell can be selected from the group consisting of *Arthrospira* sp., *Synechocystis*, *Synechococcus*, *Acaryochloris*, *Anabaena*, *Aphanothece*, *thermosynechococcus*, *Chamaesiphon*, *Chroococcus*, *Cyanobacterium*, *Cyanobium*, *Dactylococcopsis*, *Eubhalothece*, *Gloeobacter*, *Gloeocapsa*, *Gloeothece*, *Microcystis*, *Prochlorococcus*, *Prochloron*, *Chroococcidiopsis*, *Cyanocystis*, *Dermocarpella*, *Myxosarcina*, *Pleurocapsa*, *Stanieria*, *Xenococcus*, *Arthrospira*, *Borzia*, *Crinalium*, *Geitlerinema*, *Halospirulina*, *Leptolyngbya*, *Limnothrix*, *Lyngbya*, *Microcoleus*, *Cyanodictyon*, *Aphanocapsa*, *Oscillatoria*, *Planktothrix*, *Prochlorothrix*, *Pseudanabaena*, *Spirulina*, *Starria*, *Symploca*, *Trichodesmium*, *Tychonema*, *Anabaenopsis*, *Aphanizomenon*, *Calothrix*, *Cyanospira*, *Cylindrospermopsis*, *Cylindrospermum*, *Nodularia*, *Nostoc*, *Chlorogloeopsis*, *Fischerella*, *Geitleria*, *Nostochopsis*, *Iyengariella*, *Stigonema*, *Rivularia*, *Scytonema*, *Tolypothrix*, *Cyanothece*, *Phormidium*, and *Adrianema*.

[0022] In another aspect of the invention, a method of producing an MAA in a cyanobacterial cell is provided, by growing the genetically modified cyanobacterial cell as described herein in a culture medium suitable for production of the MAA in a photobioreactor with adequate light and CO₂ addition; and then isolating the MAA from the cells and/or the culture medium.

[0023] In another aspect of the invention, a pharmaceutical composition or a cosmetic composition is provided, such as a UV sunscreen agent, wherein the UV sunscreen agent can be an MAA that has been produced in a genetically modified cyanobacterial cell as described herein. In an embodiment, the composition comprises at least 2 different MAAs, and further wherein the wavelength range of UV protection can be broader than with a single MAA. In an embodiment, the composition can be a skin care product or a sunscreen. In another embodiment, the UV protection can occur over at least a portion of UV-A and at least a portion of UV-B.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] FIG. 1 is a general biosynthetic pathway for the synthesis of several MAAs in cyanobacteria.

[0025] FIG. 2 is a diagram of the biosynthetic pathway for the production of the MAA “mycosporine-glycine” in cyanobacteria.

[0026] FIG. 3 is a table showing several plasmid constructs for the production of mycosporine-glycine, mycosporine-2-glycine and shinorine, respectively, in cyanobacteria.

[0027] FIG. 4 is a map of the endogenous 6.8 kb plasmid isolated from *Cyanobacterium* sp. ABICyano1 (SEQ ID NO: 16).

[0028] FIG. 5A depicts a map of the 13121 bp circular plasmid construct and sequence annotation of plasmid #2737 (pABICyano1_6.8::Porf0223*-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)). (SEQ ID NO: 54).

[0029] FIG. 5B depicts a map of the 13059 bp circular plasmid construct and sequence annotation of plasmid #2742 (pAB1_6.8::PsmtA-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB) (SEQ ID NO: 17).

[0030] FIG. 6 depicts a map of the 13209 bp circular plasmid construct and sequence annotation of plasmid #2752 (pAB1_6.8::PcpcB*3-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB) (SEQ ID NO: 18)

[0031] FIG. 7 depicts a map of the 10040 bp circular plasmid construct and sequence annotation of plasmid #2796 (pAB1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-NpF5597(ABICyano1opt)) (SEQ ID NO: 19).

[0032] FIG. 8 depicts a map of the 10013 bp circular plasmid construct and sequence annotation of plasmid #2798 (pAB1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-ap3855(ABICyano1opt)) (SEQ ID NO: 20).

[0033] FIG. 9 depicts a map of the 14443 bp circular plasmid construct and sequence annotation of plasmid #2840 (pAB1_6.8::PsmtA-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Porf0221-ap3855(ABICyano1opt)) (SEQ ID NO: 21).

[0034] FIG. 10A is an absorbance spectrum of a culture of strain AB1224 (ABICyano1 strain harboring the plasmid #2737 (FIG. 5A; SEQ ID NO: 54), which allows Cu-inducible expression

of the MAA mycosporine-glycine), after 24 hours of growth in a GC vial. Mycosporine-glycine absorbance maximum is at 310 nm.

[0035] FIG. 10B is an absorbance spectrum of cultures of either strain AB1225 (Zn-inducible) or AB1226 (constitutive) expression of the MAA mycosporine-glycine, after 24 hours of growth in a GC vial in comparison to the wild type control. The mycosporine-glycine absorbance maximum is 310 nm. The graph shows that cultures of several of the clones having the constitutive promoter had a higher absorbance at 310 than a culture of the clone that was Zn-inducible. In comparison, a culture of the wild type did not show a peak at 310 nm.

[0036] FIG. 10C is a line graph showing that the production of mycosporine-glycine (mg/L) made from strain #AB1226 (constitutive expression) was stable over at least a 35 day period, resulting in a final production rate of about 290 mg/L after 40 days.

[0037] FIG. 10D is a line graph of the culture described in FIG. 10C, shown on a dry weight basis (mg MAA/g dry weight.)

[0038] FIG. 11A is a line graph showing the amount of mycosporine-glycine (mg/L) made from strain #AB1225 (ABICyano1 strain transformed with plasmid #2742, which has a Zn-inducible promoter), in 1.2 L indoor photobioreactors with induction by either ZnSO₄ (at 2 μ M per unit OD₇₅₀ increase) or Zn-EDTA (added at 20 μ M, 30 μ M and 30 μ M), compared to the control with no Zn added, over a 27 day period. After 27 days, the Zn-EDTA culture was diluted and re-grown to confirm the stability of production over a 50 day period. XyM in Fig. 11A stands for multiple zinc feeding throughout the cultivation as follows: 20 μ M ZnEDTA on day 1, 13, 22 and 27; 30 μ M ZnEDTA day 2 and 28; 6 μ M ZnSO₄ on day 34 and 36.

[0039] FIG. 11B is a line graph showing the percentage of mycosporine-glycine from FIG. 11A that was found in the culture medium, over the same 50 day period. In general, about 80% of the mycosporine-glycine produced was excreted from the cell.

[0040] Fig. 11C shows the production of mycosporine-glycine by the strain AB1225 with optimized cultivation conditions. The strain was cultivated as described in example 27.

[0041] FIG. 12A is a comparison map of MAA synthesis genes from 3 cyanobacterial species: *Aphanothece halophytica*, *Nostoc punctiforme* ATCC 29133, and *Anabaena variabilis* ATCC 29413.

[0042] FIG. 12B is a diagram showing the chemical structure of shinorine, mycosporine-glycine, and mycosporine-2-glycine, as well the genes that can be utilized to convert mycosporine-glycine into either shinorine or mycosporine-2-glycine.

[0043] FIG. 12C is an absorbance spectrum showing the increased production of shinorine when the additional genes NpF5597 (mysD from *N. punctiforme*) and Ava3855 were transformed to AB1225, using a separate plasmid, pAB1B. Also shown is the increased production of mycosporine-2-glycine when the additional gene Ap3855 (mysD from *A. halophytica*), on a separate plasmid, pAB1B, was transformed to the host cell in addition to the mycosporine-glycine encoding genes.

[0044] FIG. 13A is a line graph showing the production (in mg/L) of several MAAs (mycosporine-glycine, shinorine, and mycosporine-2-glycine) made by the strains AB1225, AB1252 and AB1253 over a 26 day period in 1.2 L indoor photobioreactors.

[0045] FIG. 13B is a line graph showing the production of several MAAs (mycosporine-glycine, shinorine, and mycosporine-2-glycine), as a percentage of dry weight, over a 26 day period in 1.2 L indoor photobioreactors.

[0046] FIG. 13C is a line graph from the same experiment as in FIG. 13A and FIG. 13B, showing the percentage of each MAA that was located in the culture medium over the 26 day period.

[0047] Fig. 13D is a line graph showing the production of shinorine by the strain AB1252 over the course of 3 days under improved cultivation conditions as described in example 27.

[0048] FIG. 14 is a line graph showing the production of the MAA mycosporine-2-glycine (in mg/L) from strain #AB1277 (ABICyano1 transformed with the plasmid #2840, as shown in FIG. 3).

[0049] FIG. 15A is a plasmid map, sequence annotation, and complete sequence of the 11649 bp plasmid construct#2794:pABCyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-Ava_3855 (ABICyano1opt)-TB0050 (SEQ ID NO: 55).

[0050] FIG. 15B is a plasmid map, sequence annotation, and complete sequence of the 11657 bp plasmid construct #2880: pABCyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-mysAB_Pum(ABICyano1opt)-TpsaB (SEQ ID NO: 56).

[0051] FIG. 15C is a plasmid map, sequence annotation, and complete sequence of the 11777 bp plasmid construct #2881: pABCyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-mysCD_Pum(ABICyano1opt)-TpsaB (SEQ ID NO: 57).

[0052] FIG. 15D is a plasmid map, sequence annotation, and complete sequence of the 11732 bp plasmid construct #2882: pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-mysCD_Chc(ABICyano1opt)-TpsaB (SEQ ID NO: 58).

[0053] Fig. 16A shows the dry weight production rate of ABICyano1 and ABCyano4 under different light intensities of 350 $\mu\text{E m}^{-2}\text{s}^{-1}$ and of 1000 $\mu\text{E m}^{-2}\text{s}^{-1}$.

[0054] Fig. 16B shows the total carbon fixation rate of ABICyano1 and ABCyano4 under different illumination conditions.

[0055] Fig. 17A shows the dry weight productivity and turbidostat cultivation for ABICyano1 and ABCyano4.

[0056] Fig. 17B shows the total carbon fixation rate and turbidostat cultivation for ABICyano1 and ABCyano4.

[0057] Fig. 18A shows a microscopic image of ink-negative stained ABCyano4 cells.

[0058] Fig. 18B shows a microscopic image of ink-negative stained ABICyano1 cells.

[0059] Fig. 19A shows a comparison between the growth of two different ABCyano4 strains, AB4092 and AB4094 over a time period of cultivation of nearly 10 days.

[0060] Fig. 19B shows the concentration of MAAs shinorine/porphyra-334 for both ABCyano4 strains AB4092 and AB4094.

[0061] Fig. 19C shows the carbon-partitioning for the cultivation, shown in Fig. 19A and Fig. 19 B.

[0062] Fig. 20A shows a comparison of the growth of an ABICyano1 and ABCyano4 mycosporine-glycine producing cell lines.

[0063] Fig. 20B shows the mycosporine-glycine production of ABICyano1 and ABCyano4 strains.

[0064] Fig. 20C shows the carbon-partitioning of ABCyano4 strains in comparison to ABICyano1 strains, for production of mycosporine-glycine.

[0065] Fig. 21A depicts the growth of mycosporine-2-glycine producing cell lines of ABICyano1 and ABCyano4.

[0066] Fig. 21B shows the production of mycosporine-2-glycine of ABICyano1 and ABCyano4 producing strains.

[0067] Fig. 21C shows the carbon-partitioning for mycosporine-2-glycine producing strains of ABICyano1 and ABCyano4.

[0068] Fig. 22A and 22B show the whole cell absorption spectrum and the HPLC profile of the ABCyano1 strain AB1356. After overexpression of so far undescribed enzymes from *Trichodesmium erythraeum* IMS101 in the mycosporine-glycine (M-Gly) producer strain AB1225, which results in the strain AB1356, a shift from 310 nm (M-Gly) to 320 nm (palythine) can be observed in the whole cell absorption spectrum.

[0069] Fig. 23A and 23B show the whole cell absorption spectrum and the HPLC profile of the ABCyano1 strain AB1358. This strain was produced by transformation of the M-Gly producer strain AB1225 with plasmids carrying genes for so far undescribed enzymes from *Trichodesmium erythraeum* IMS101, resulting in the production of mycosporine-methylamine-glycine and mycosporine-2-glycine (M-2-Gly).

[0070] Fig. 24A and 24B show the whole cell absorption spectrum and the HPLC profile of the ABCyano1 strain AB1360. This strain was produced by transformation of the M-Gly producer strain AB1225 with plasmids carrying genes for so far undescribed enzymes from *Trichodesmium erythraeum* IMS101, resulting in the production of Tery-364, an MAA with yet unknown structure.

[0071] Fig. 25A and 25B show the whole cell absorption spectrum and the HPLC profile of the ABCyano1 strain AB4033. This strain was produced by transformation of the M-Gly producer strain AB1225 with the plasmid carrying the genes *mylCDE* from *Nostoc commune* var. *flagelliforme* QSY 1, resulting in the production of mycosporine-ornithine-4-deoxygadusolyl-ornithine (M-2-DO).

[0072] Fig. 26A, and 26B show the production of 4-deoxygadusol (4-DG) over a time period of 2 days, and the whole cell absorption spectrum of the ABCyano4 strain AB1312. This strain was produced by transformation of ABCyano4 with genes encoding the two enzymes DHQS (Ava_3858) and O-MT (Ava_3857).

[0073] Fig. 27A, 27B and 27C show the production of M-Gly over a time period of 2 days, the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strains AB1322 and AB1361.

[0074] Fig. 28A, 28B and 28C show the production of shinorine/porphyra-334 in a ratio of 60%/40% over a time period of nearly 3 days, the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB1333.

[0075] Fig. 29A, 29B and 29C show the production of mycosporine-2-glycine over a time period of nearly 3 days, the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB1334.

[0076] Fig. 30A, 30B and 30C show the production of shinorine/porphyrin-334 in a ratio of 45%/55% over a time period of 3 days, the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4016.

[0077] Fig. 31A, 31B and 31C show the production of shinorine/porphyrin-334 in a ratio of 10%/90% over a time period of 3 days, the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4046.

[0078] Fig. 32A, and 32B show the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4055, producing palythine. The strain overexpresses thus far undescribed enzymes from *Trichodesmium erythraeum* IMS101 (Tery_2972, a Clavaminic acid synthetase (CAS)-like enzyme and a D-ala-D-ala ligase) in the genetic background of the M-Gly producer strain AB1322.

[0079] Fig. 33A, and 33B show the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4031, producing mycosporine-methylamine:threonine. The strain overexpresses a thus far undescribed enzyme from *Trichodesmium erythraeum* IMS101 (Tery_2969, a homolog of a 6-phosphogluconate dehydrogenase/ 3-hydroxyisobutyrate dehydrogenase) and a D-Ala-D-Ala ligase from *Nostoc punctiforme* in the genetic background of the M-Gly producer strain AB1322.

[0080] Fig. 34A, and 34B show the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4074, producing Tery-322. The strain overexpresses a thus far undescribed enzyme from *Trichodesmium erythraeum* IMS101 in the genetic background of the M-Gly producer strain AB1322.

[0081] Fig. 35A shows a comparison between the production of M-Gly by different ABCyano4 strains transformed with genes operably linked to a constitutive promoter PcpCB (strain AB1365) and the strain AB1322 wherein the heterologous genes for MAA production are operably linked to a zinc-inducible promoter PsmtA.

[0082] Fig. 35B shows a comparison between the production of M-Gly by different ABCyano4 strains transformed with genes operably linked to a constitutive promoter PpsaL (strain AB4049), to a constitutive promoter PpsaA (strain AB4050) and the strain AB1322 wherein the

heterologous genes for MAA production are operably linked to a zinc-inducible promoter PsmtA.

[0083] Fig. 35C shows the copper-dependent production of M-Gly by an ABCyano4 strain transformed with genes operably linked to a copper-inducible promoter PpetE (strain AB4095).

[0084] Fig. 36A, 36B and 36C show a comparison between the production of MAA, the accumulation of biomass and the percentage of carbon partitioning for the different ABCyano4 strains AB1333 and the dual mysA strain AB4028 over the time period of 6 days of cultivation. In comparison to AB1333, the dual mysA strain AB4028 contains a second mysA copy from *Anabaena variabilis* ATCC 29413, operably linked to a zinc-inducible promoter PsmtA.

[0085] Fig. 37 shows the fractionation of the MAAs between the intracellular space of the host cells, cell-associated MAAs with the capsular EPS and secreted MAAs into the culture medium for two different ABCyano4 strains AB1333 and AB1334 over the course of 3 days producing either shinorine/porphyra-334 or mycosporine-2-glycine.

[0086] Fig. 38 shows the fractionation of the MAAs between cell-associated MAAs with the capsular EPS and secreted MAAs into the culture medium for the dual mysA strain AB4028 over the course of 20 days producing a mixture of shinorine and porphyra-334.

[0087] Fig. 39 shows an overview of the MAA biosynthesis gene cluster in *Trichodesmium erythraeum* IMS101.

[0088] Fig. 40 depicts a proposed pathway for the synthesis of palythine, mycosporine-methylamine:threonine, mycosporine-methylamine:glycine, Tery-322 and Tery-364. with the respective heterologous genes encoding the enzymes involved in these pathways

[0089] Fig. 41 depicts the pathway for the synthesis of mycosporine-2-(4-deoxygadusolyl-ornithine) starting with sedoheptulose-7-phosphate.

[0090] Fig. 42A shows a protein sequence alignment of the MysA enzymes from different cyanobacterial species, including the consensus sequence. The first nine MysA variants (*Anabaena* to *Lyngbya*) cluster together to form one monophyletic clade of MysA (type I) while the latter eight MysA variants (*Trichodesmium* to *Hydrocoleum*) form another monophyletic clade of MysA (type II) that differs from the first MysA clade. The conserved amino acid sequences of two fingerprint segments for the classical clade are MLELE_xPNLHE and LDRVIA_xGH. The divergent clade has some conserved motifs (e.g., YxxxEY(G)-xNxxET and QC(D)RPHA(G)YGHTWSP) distinct from the classical clade. Fig. 42B and 42C show the

production of mycosporine-glycine and the C-partitioning (M-Gly produced per dry weight) involving different *mysA* genes. AB1322 contains the *mysABC* operon from *Anabaena variabilis* ATCC 29413 operably linked to a zinc-inducible promoter *PsmtA*. The second strain AB4015 contains the same *PsmtA*-controlled *mysABC* operon from *Anabaena variabilis* ATCC 29413 plus a second *mysA* copy from *Anabaena variabilis* ATCC 29413, operably linked to a zinc-inducible promoter *PsmtA*. The third strain contains the *mysA* gene from *Cyanobacterium* sp. HL-69 and *mysBC* genes from *Anabaena variabilis* ATCC 29413 all operably linked to a zinc-inducible promoter *PsmtA*.

[0091] Fig. 43 shows the plasmid map of one endogenous ABCyano4 plasmid, pABCyano4B, having an approximate copy number of 30-50 copies per cell in ABCyano4. The integration site for the plasmids used for transformation of ABCyano4 via homologous recombination is indicated by an arrow.

[0092] Fig. 44 shows the plasmid map of one endogenous ABCyano4 plasmid, pABCyano4C, having an approximate copy number of 12-20 copies per cell in ABCyano4. The integration site for the plasmids used for transformation of ABCyano4 via homologous recombination is indicated by an arrow.

[0093] Fig 45A depicts a plasmid map of the 8763 bp circular plasmid construct and sequence annotation of plasmid #2870 (pJet1.2::lacI-Ptac-Ava_3858(ABICyano1opt)-IScpBA*1-Ava_3857(ABICyano1opt)-IScpBA*1-Ava_3856(ABICyano1opt)-TB0011). Fig. 45B shows the HPLC profile of *E. coli* BL21 transformed with plasmid # 2870 (Fig 45A) carrying the *mysABC* gene operon from *Anabaena variabilis* ATCC 29413 operably linked to an IPTG-inducible promoter *Ptac* producing mycosporine-glycine.

[0094] Fig. 46 depicts the HPLC profile of *E. coli* BL21 transformed with plasmid # 3119, producing mycosporine-methylamine-threonine.

[0095] Fig. 47 shows the HPLC profile of *E. coli* BL21 transformed with plasmid # 3117, producing palythine.

[0096] Fig. 48 shows the HPLC profile of *E. coli* BL21 transformed with plasmid # 3120, producing Tery-322.

[0097] Fig. 49 depicts the HPLC profile of *E. coli* BL21 transformed with plasmid # 3129, producing Tery-364.

[0098] Fig. 50 shows a sequence alignment of Tery_2966 (O-methyltransferase) homologs from different cyanobacterial species.

[0099] Fig. 51 to 86 show the plasmid maps of various plasmids included in genetically modified host cells of the present invention.

DETAILED DESCRIPTION

[00100] Disclosed herein is a cyanobacterial organism that has been modified so that it is capable of producing large amounts of a mycosporine-like amino acid (MAA). The MAA so produced can be isolated and utilized for consumer products, such as sunscreens, skin treatments, and cosmetics.

[00101] Aspects of the invention utilize techniques and methods common to the fields of molecular biology, microbiology and cell culture. Useful laboratory references for these types of methodologies are readily available to those skilled in the art. See, for example, *Molecular Cloning: A Laboratory Manual* (Third Edition), Sambrook, J., et al. (2001) Cold Spring Harbor Laboratory Press; *Current Protocols in Microbiology* (2007) Edited by Coico, R, et al., John Wiley and Sons, Inc.; *The Molecular Biology of Cyanobacteria* (1994) Donald Bryant (Ed.), Springer Netherlands; *Handbook Of Microalgal Culture Biotechnology And Applied Phycology* (2003) Richmond, A.; (ed.), Blackwell Publishing; and “*The cyanobacteria, molecular Biology, Genomics and Evolution*”, Edited by Antonia Herrero and Enrique Flores, Caister Academic Press, Norfolk, UK, 2008.

[00102] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Definitions

[00103] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[00104] The term “about” is used herein to mean approximately, in the region of, roughly, or around. When the term “about” is used in conjunction with a numerical value/range, it modifies that value/range by extending the boundaries above and below the numerical value(s) set forth. In

general, the term “about” is used herein to modify a numerical value(s) above and below the stated value(s) by a variance of 20%.

[00105] The term “cyanobacterium” refers to a member from the group of photoautotrophic prokaryotic microorganisms which can utilize solar energy and fix carbon dioxide. Cyanobacteria are also referred to as blue-green algae.

[00106] The terms “host cell” and “recombinant host cell” are intended to include a cell suitable for metabolic manipulation, e.g., which can incorporate heterologous polynucleotide sequences, e.g., which can be transformed. The term is intended to include progeny of the cell originally transformed. In particular embodiments, the cell is a prokaryotic cell, e.g., a cyanobacterial cell. The term recombinant host cell is intended to include a cell that has already been selected or engineered to have certain desirable properties and to be suitable for further genetic enhancement.

[00107] “Competent to express” refers to a host cell that provides a sufficient cellular environment for expression of endogenous and/or exogenous polynucleotides.

[00108] As used herein, the term “genetically modified” refers to any change in the endogenous genome of a wild type cell or to the addition of non-endogenous genetic code to a wild type cell, e.g., the introduction of a heterologous gene. More specifically, such changes are made by the hand of man through the use of recombinant DNA technology or mutagenesis. The changes can involve protein coding sequences or non-protein coding sequences, including regulatory sequences such as promoters or enhancers.

[00109] The terms “polynucleotide” and “nucleic acid” also refer to a polymer composed of nucleotide units (ribonucleotides, deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages between them include non-naturally occurring synthetic analogs. It will be understood that, where required by context, when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which “U” replaces “T.”

[00110] The nucleic acids of this present invention may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels,

methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages, charged linkages, alkylators, intercalators, pendent moieties, modified linkages, and chelators. Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions.

[00111] The term “nucleic acid” (also referred to as polynucleotide) is also intended to include nucleic acid molecules having an open reading frame encoding a polypeptide, and can further include non-coding regulatory sequences and introns. In addition, the terms are intended to include one or more genes that map to a functional locus. In addition, the terms are intended to include a specific gene for a selected purpose. The gene can be endogenous to the host cell or can be recombinantly introduced into the host cell.

[00112] In one aspect the invention also provides nucleic acids which are at least 60%, 70%, 80% 90%, 95%, 99%, or 99.5% identical to the nucleic acids disclosed herein.

[00113] The percentage of identity of two nucleic acid sequences or two amino acid sequences can be determined using the algorithm of Thompson et al. (CLUSTALW, 1994, *Nucleic Acids Research* 22: 4673-4680). A nucleotide sequence or an amino acid sequence can also be used as a so-called “query sequence” to perform a search against public nucleic acid or protein sequence databases in order, for example, to identify further unknown homologous promoters, which can also be used in embodiments of this invention. In addition, any nucleic acid sequences or protein sequences disclosed in this patent application can also be used as a “query sequence” in order to identify yet unknown sequences in public databases, which can encode for example new enzymes, which could be useful in this invention. Such searches can be performed using the algorithm of Karlin and Altschul (1990, *Proceedings of the National Academy of Sciences U.S.A.* 87: 2,264 to 2,268), modified as in Karlin and Altschul (1993, *Proceedings of the National Academy of Sciences U.S.A.* 90: 5,873 to 5,877). Such an algorithm is incorporated in the NBLAST and XBLAST programs of Altschul et al. (1990, *Journal of Molecular Biology* 215: 403 to 410). Suitable parameters for these database searches with these programs are, for example, a score of 100 and a word length of 12 for BLAST nucleotide searches as performed with the NBLAST program. BLAST protein searches are performed with the XBLAST program with a score of 50 and a word length of 3. Where gaps exist between two sequences, gapped BLAST is utilized as described in Altschul et al. (1997, *Nucleic Acids Research*, 25: 3,389 to 3,402).

[00114] Database entry numbers given in the following are for the CyanoBase, the genome database for cyanobacteria (<http://bacteria.kazusa.or.jp/cyanobase/index.html>); Nakamura et al. “CyanoBase, the genome database for *Synechocystis* sp. strain PCC6803: status for the year 2000”, *Nucleic Acid Research*, 2000, Vol. 18, page 72.

[00115] The enzyme commission numbers (EC numbers) cited throughout this patent application are numbers which are a numerical classification scheme for enzymes based on the chemical reactions which are catalyzed by the enzymes.

[00116] “Recombinant” refers to polynucleotides synthesized or otherwise manipulated in vitro (“recombinant polynucleotides”) and to methods of using recombinant polynucleotides to produce gene products encoded by those polynucleotides in cells or other biological systems. For example, a cloned polynucleotide may be inserted into a suitable expression vector, such as a bacterial plasmid, and the plasmid can be used to transform a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a “recombinant host cell” or a “recombinant bacterium” or a “recombinant cyanobacterium.” The gene is then expressed in the recombinant host cell to produce, e.g., a “recombinant protein.” A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

[00117] The term “homologous recombination” refers to the process of recombination between two nucleic acid molecules based on nucleic acid sequence similarity. The term embraces both reciprocal and nonreciprocal recombination (also referred to as gene conversion). In addition, the recombination can be the result of equivalent or non-equivalent cross-over events. Equivalent crossing over occurs between two equivalent sequences or chromosome regions, whereas nonequivalent crossing over occurs between identical (or substantially identical) segments of nonequivalent sequences or chromosome regions. Unequal crossing over typically results in gene duplications and deletions. For a description of the enzymes and mechanisms involved in homologous recombination see Court et al., “Genetic engineering using homologous recombination,” *Annual Review of Genetics* 36:361-388; 2002.

[00118] The term “non-homologous or random integration” refers to any process by which DNA is integrated into the genome that does not involve homologous recombination. It appears to be a random process in which incorporation can occur at any of a large number of genomic locations.

[00119] The term “expressed endogenously” refers to polynucleotides that are native to the host cell and are naturally expressed in the host cell.

[00120] The term “operably linked” refers to a functional relationship between two parts in which the activity of one part (e.g., the ability to regulate transcription) results in an action on the other part (e.g., transcription of the sequence). Thus, a polynucleotide is “operably linked to a promoter” when there is a functional linkage between a polynucleotide expression control sequence (such as a promoter or other transcription regulation sequences) and a second polynucleotide sequence (e.g., a native or a heterologous polynucleotide), where the expression control sequence directs transcription of the polynucleotide.

[00121] The term “vector” as used herein is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which generally refers to a circular double stranded DNA molecule into which additional DNA segments may be ligated, but also includes linear double-stranded molecules such as those resulting from amplification by the polymerase chain reaction (PCR) or from treatment of a circular plasmid with a restriction enzyme.

[00122] Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., vectors having an origin of replication which functions in the host cell). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and are thereby replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors” (or simply “expression vectors”).

[00123] A “promoter” is an array of nucleic acid control sequences that direct transcription of an associated polynucleotide, which may be a heterologous or native polynucleotide. A promoter includes nucleic acid sequences near the start site of transcription, such as a polymerase binding site. The promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. The term “promoter” is intended to include a polynucleotide segment that can transcriptionally control a gene of interest, e.g., a pyruvate decarboxylase gene that it does or does not transcriptionally control in nature. In one embodiment, the transcriptional control of a promoter results in an increase in expression of the gene of interest. In an embodiment, a promoter is placed 5' to the gene of interest. A heterologous promoter can be used to replace the natural promoter, or can be

used in addition to the natural promoter. A promoter can be endogenous with regard to the host cell in which it is used or it can be a heterologous polynucleotide sequence introduced into the host cell, e.g., exogenous with regard to the host cell in which it is used. Promoters of the invention may also be inducible, meaning that certain exogenous stimuli (e.g., nutrient starvation, heat shock, mechanical stress, light exposure, etc.) will induce the promoter leading to the transcription of the gene.

[00124] The term “recombinant nucleic acid molecule” includes a nucleic acid molecule (e.g., a DNA molecule) that has been altered, modified or engineered such that it differs in nucleotide sequence from the native or natural nucleic acid molecule from which the recombinant nucleic acid molecule was derived (e.g., by addition, deletion or substitution of one or more nucleotides). The recombinant nucleic acid molecule (e.g., a recombinant DNA molecule) also includes an isolated nucleic acid molecule or gene of the present invention.

[00125] The term “gene” refers to an assembly of nucleotides that encode a polypeptide, and includes cDNA and genomic DNA nucleic acids. “Gene” also refers to a nucleic acid fragment that expresses a specific protein or polypeptide, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

[00126] The term “endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene or “heterologous” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

[00127] The term “fragment” refers to a nucleotide sequence of reduced length relative to the reference nucleic acid and comprising, over the common portion, a nucleotide sequence substantially identical to the reference nucleic acid. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. Such fragments comprise, or alternatively consist of, oligonucleotides ranging in length from at least about 6, 50, 100, 200, 500, 1,000, to about 1,500 or more consecutive nucleotides of a polynucleotide according to the invention.

[00128] The term “open reading frame,” abbreviated as “ORF,” refers to a length of nucleic acid sequence, either DNA, cDNA or RNA, that comprises a translation start signal or initiation

codon, such as an ATG or AUG, and a termination codon and can be potentially translated into a polypeptide sequence.

[00129] The term “upstream” refers to a nucleotide sequence that is located 5' to reference nucleotide sequence. In particular, upstream nucleotide sequences generally relate to sequences that are located on the 5' side of a coding sequence or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

[00130] The term “downstream” refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In particular, downstream nucleotide sequences generally relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[00131] The term “homology” refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known to the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s) and size determination of the digested fragments.

[00132] As used herein, “substantially similar” refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence.

[00133] The term “substantially similar” also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotide bases that do not substantially affect the functional properties of the resulting transcript.

[00134] The terms “restriction endonuclease” and “restriction enzyme” refer to an enzyme that binds and cuts within a specific nucleotide sequence within double stranded DNA.

[00135] The term “expression”, as used herein, refers to the transcription and stable accumulation mRNA derived from a nucleic acid or polynucleotide. Expression may also refer to translation of mRNA into a protein or polypeptide.

[00136] An “expression cassette” or “construct” refers to a series of polynucleotide elements that permit transcription of a gene in a host cell. Typically, the expression cassette

includes a promoter and a heterologous or native polynucleotide sequence that is transcribed. Expression cassettes or constructs may also include, e.g., transcription termination signals, polyadenylation signals, and enhancer elements.

[00137] The term “codon” refers to a triplet of nucleotides coding for a single amino acid.

[00138] The term “codon–anticodon recognition” refers to the interaction between a codon on an mRNA molecule and the corresponding anticodon on a tRNA molecule.

[00139] The term “codon bias” refers to the fact that different organisms use different codon frequencies.

[00140] The term “codon optimization” refers to the modification of at least some of the codons present in a heterologous gene sequence from a triplet code that is not generally used in the host organism to a triplet code that is more common in the particular host organism. This can result in a higher expression level of the gene of interest.

[00141] The term “transformation” is used herein to mean the insertion of heterologous genetic material into the host cell. Typically, the genetic material is DNA on a plasmid vector, but other means can also be employed. General transformation methods and selectable markers for bacteria and cyanobacteria are known in the art (Wirth, *Mol Gen Genet.* 216:175-177 (1989); Koksharova, *Appl Microbiol Biotechnol* 58:123-137 (2002). Additionally, transformation methods and selectable markers for use in bacteria are well known (see, e.g., Sambrook et al, *supra*).

[00142] The term “selectable marker” means an identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene's effect, i.e., resistance to an antibiotic, resistance to a herbicide, colorimetric markers, enzymes, fluorescent markers, and the like, wherein the effect is used to track the inheritance of a nucleic acid of interest and/or to identify a cell or organism that has inherited the nucleic acid of interest. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, spectinomycin, kanamycin, hygromycin, zeocin, chloramphenicol, and the like.

[00143] A “polypeptide” is a polymeric compound comprised of covalently linked amino acid residues. A “protein” is a polypeptide that performs a structural or functional role in a living cell.

[00144] A “heterologous protein” refers to a protein not naturally produced in the cell.

[00145] An “isolated polypeptide” or “isolated protein” is a polypeptide or protein that is substantially free of those compounds that are normally associated therewith in its natural state (e.g., other proteins or polypeptides, nucleic acids, carbohydrates, lipids).

[00146] The term “fragment” of a polypeptide refers to a polypeptide whose amino acid sequence is shorter than that of the reference polypeptide. Such fragments of a polypeptide according to the invention may have a length of at least about 2, 50, 100, 200, or 300 or more amino acids.

[00147] A “variant” of a polypeptide or protein is any analogue, fragment, derivative, or mutant which is derived from a polypeptide or protein and which retains at least one biological property of the polypeptide or protein. Different variants of the polypeptide or protein may exist in nature. These variants may be allelic variations characterized by differences in the nucleotide sequences of the structural gene coding for the protein, or may involve differential splicing or post-translational modification. The skilled artisan can produce variants having single or multiple amino acid substitutions, deletions, additions, or replacements.

[00148] The term “primer” is an oligonucleotide that hybridizes to a target nucleic acid sequence to create a double stranded nucleic acid region that can serve as an initiation point for DNA synthesis under suitable conditions. Such primers may be used in a polymerase chain reaction.

[00149] The term “polymerase chain reaction,” also termed “PCR,” refers to an in vitro method for enzymatically amplifying specific nucleic acid sequences. PCR involves a repetitive series of temperature cycles with each cycle comprising three stages: denaturation of the template nucleic acid to separate the strands of the target molecule, annealing a single stranded PCR oligonucleotide primer to the template nucleic acid, and extension of the annealed primer(s) by DNA polymerase. PCR provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

[00150] The term “mycosporine-like amino acid” (“MAA”) refers to a low molecular weight compound containing a 6 carbon ring structure, substituted with at least one amino acid moiety. Exemplary MAAs include, for example, mycosporine-glycine, shinorine, and mycosporine-2-glycine. These molecules absorb UV light, and exhibit photoprotection functions.

[00151] The term “UV” refers to light between the wavelengths of 290 – 400. This is divided between “UV-A” and “UV-B”.

[00152] The term “UV-A” refers to light between the wavelengths of 320-400 nm.

[00153] The term “UV-B” refers to light between the wavelengths of 290 to 320 nm.

[00154] The term “selection-free” refers to a growth medium that does not include a selection agent, such as an antibiotic, that would allow only cells having a functional selectable marker gene to survive.

[00155] The term “plurality” means more than one.

[00156] In the following, the term “heterologous gene” in the context of genes coding for enzymes involved in the production of MAA and the term “MAA gene” will be used interchangeably. If more than one heterologous gene coding for enzymes involved in the production of MAA is present in a genetically modified cyanobacterial cell, then these genes will be referred to as the first heterologous gene, the second heterologous gene and so on.

[00157] In the following the terms “extracellular polysaccharides” (EPS) and “capsular polysaccharides” (CPS) will be used interchangeably. These terms refer to the extracellular polysaccharide layer of the cyanobacterial host cells of the present invention, in particular the extracellular polysaccharide layers of *Cyanobacterium* sp. host cells, such as *Cyanobacterium* sp. ABICyano1 and *Cyanobacterium* sp. ABCyano4.

Genes and Gene Clusters for the Production of MAAs

[00158] In an embodiment of the invention, cyanobacterial cells can be modified to produce various MAAs. The modified cyanobacterial cells are relatively easy to cultivate, in comparison, for example, to red macroalgae from the genus *Porphyra*. The heterologous genes can be linked to regulatable or constitutive promoters. In an embodiment, a commercially suitable strain of cyanobacteria, already known for its ability to grow well in both indoor and outdoor commercially relevant conditions, and also for the production of ethanol and other compounds, can be utilized to carry the MAA gene clusters. In a further embodiment, endogenous promoters from this strain, as well as their derivatives, can be utilized to produce high amounts of MAAs using sunlight, CO₂, and inorganic nutrients.

[00159] Previously, heterologous systems have been utilized by other researchers to produce larger amounts of MAAs than can typically be found in nature. Cyanobacterial genes

have been transferred to heterologous bacteria, where they can then be grown in fermentor systems without light. For example, heterotrophic production of heterologous MAAs in *E. coli* has been performed utilizing MAA production genes taken from *Anabaena variabilis* ATCC 29413 (Balskus et al., “The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria”, *Science*, 329:1653-1656, (2010)), and *Nostoc punctiforme* ATCC 29133 (Gao, “An ATP-grasp ligase involved in the last biosynthetic step of the iminomycosporine shinorine in *Nostoc punctiforme* ATCC 29133”, *J Bacteriol.*, 193:5923-5928 (2011)).

[00160] The highest productivity obtained with *E. coli* was 637 µg / liter 4-deoxygadusol as a major product, the precursor of mycosporine-glycine and a small amount of shinorine (145 µg / liter) (Balskus et al., supra). Other researchers heterologously expressed the mycosporine-like amino acid biosynthetic genes from the halotolerant cyanobacterium *Aphanothece halophytica* in *E. coli* and identified a new compound mycosporine-2-glycine with productivity of 85.2 µmol per g dry weight (Waditee-Sirisattha et al. “Identification and upregulation of biosynthetic genes required for accumulation of mycosporine-2-glycine under salt stress conditions in the halotolerant cyanobacterium *Aphanothece halophytica*” *Appl Environ Microbiol.* 80:1763-1769 (2014)).

[00161] An MAA cluster from *Cylindrospermum stagnale* sp. PCC 7417 was previously expressed in *E. coli*. The major products were mycosporine-lysine and the novel compound mycosporine-ornithine (Katoch et al. “Heterologous Production of Cyanobacterial Mycosporine-Like Amino Acids Mycosporine-Ornithine and Mycosporine-Lysine in *Escherichia coli*”, *Appl Environ Microbiol.*, 82:6167-6173 (2016)).

[00162] In addition to cyanobacterial MAA gene clusters, heterotrophic bacteria also contain MAA synthesis gene clusters. One example is *Actinosynnema mirum* DSM 43827 whose mysABCD gene cluster was expressed in *Streptomyces avermitilis* SUKA22. The major product was shinorine and minor products were porphyra-334 and mycosporine-glycine-alanine (Miyamoto et al., Discovery of Gene Cluster for Mycosporine-Like Amino Acid Biosynthesis from *Actinomycetales* Microorganisms and Production of a Novel Mycosporine-Like Amino Acid by Heterologous Expression” *Appl Environ Microbiol.* 80:5028-5036 (2014)). The obtained yields were 154 mg/L shinorine and 188 mg/ liter total MAAs (the sum of shinorine, porphyra-334, mycosporine-glycine, and mycosporine-glycine-alanine), which corresponds to 13.9 mg MAAs/ g dry cell weight. Patent application EP20150792252 describes a method for producing a mycosporine-like amino acid by using the genes amir_4256, amir_4257, amir_4258 and

amir_4259 genes from *A. mirum*, expressing them in *Streptomyces* species, *Corynebacterium*, *Aurantiochytrium* sp. *E. coli* JM109 and *Saccharomyces cerevisiae* YPH499XW and recovering the mycosporine-like amino acid from the extracellular culture solution. The extracellular production amount of shinorine in *Streptomyces lividans* 1326 was 510 mg/L and 25 mg/L mycosporine-glycine-alanine after two weeks of cultivation.

[00163] To produce MAAs in a sustainable way, and further to overcome low productivity of MAA levels in wild type cyanobacterial strains, genetically manipulated cyanobacterial cells can be utilized as bio-factories for the production of large amounts of MAAs.

[00164] The genetic basis for the pathway leading to the formation of MAAs was elucidated in *A. variabilis* ATCC 29413. A cluster of four genes (Ava_3858 to Ava_3855) is responsible for synthesis of the MAA shinorine. MAA biosynthesis starts with the conversion of sedoheptulose-7-phosphate (from the Calvin-Benson cycle) to desmethyl-4-deoxygadusol, a cyclohexenone natural product that resembles the mycosporine core, by the DHQS homolog 4-deoxygadusol synthase (Ava_3858). The gene Ava_3857 encodes an O-methyltransferase, converting desmethyl-4-deoxygadusol in 4-deoxygadusol. The product of Ava_3856, an ATP-grasp homolog, adds a glycine to 4-deoxygadusol to synthesize the MAA mycosporine-glycine. A similar cluster of three genes in *N. punctiforme* ATCC 29133 (NpR5600, NpR5599, NpR5598) has also been shown to catalyze the same reactions (Gao, supra).

[00165] Mycosporine-glycine is thought to be the intermediate MAA for attachment of additional amino acids to the core via imine linkages. Ava_3855 encoding a nonribosomal peptide synthetase was described to attach a serine, yielding shinorine (Balskus et al., supra). The genetic basis for the pathway leading to the formation of the shinorine does not apparently share consistency among cyanobacteria. Cyanobacteria that lack homologues of Ava_3855 tend to have a homologue of an ATP-grasp proteins similar to D-Ala-D-Ala ligase involved in peptidoglycan synthesis (Gao, supra). The NpF5597 gene of *N. punctiforme* encodes a D-Ala-D-Ala ligase, attaching a serine to mycosporine-glycine, producing shinorine (Waditee-Sirisattha et al, "Identification and upregulation of biosynthetic genes required for accumulation of mycosporine-2-glycine under salt stress conditions in the halotolerant cyanobacterium *Aphanothece halophytica*", *Appl Environ Microbiol.*, 80:1763-1769 (2013).

[00166] In order to produce high amounts of MAAs with cyanobacteria, heterologous expression of the mycosporine synthesis pathway from *A. variabilis* ATCC 29413, *N. punctiforme*

ATCC 29133 and/or *A. halophytica* was conducted with the highly productive strain *Cyanobacterium* sp. ABICyano1. The derivative AB1225 (ABICyano1 #2742) contains codon-adapted variants of Ava_3858, Ava_3857, Ava_3856 under control of a zinc-inducible promoter and produces 200 mg/L mycosporine-glycine (6 % per dry weight). AB1252 (ABICyano1 #2742 #2796) additionally contains a codon-adapted NpF5597 gene (coding for a D-Ala-D-Ala ligase) under control of a zinc-inducible promoter and yields 110 mg/L shinorine (5 % per dry weight). The third and fourth strains AB1253 (ABICyano1 #2742 #2798) / AB1277 (ABICyano1 #2840) contain instead of NpF5597 the homologous, codon-adapted D-Ala-D-Ala ligase gene from *A. halophytica* (Ap3855) and produce 200 mg/L mycosporine-2-glycine (5 – 6% per dry weight).

[00167] According to another embodiment of the present invention, the genetically modified cyanobacterial cell is for the production of mycosporine-glycine, and comprises at least three heterologous genes, namely a first heterologous gene coding for a first enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 3 or which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO 164, a second heterologous gene coding for a second enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 6, and a third heterologous gene coding for a third enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 9. The first heterologous genes codes for an enzyme, which is highly homologous to an DHQS-like protein, such as the enzyme encoded by the gene Ava_3858, a MysA homologue, converting sedoheptoluse-7-phosphate to desmethyl-4-deoxygadudusol. The second heterologous genes code for an O-methyltransferase, such as the gene Ava-3857, a MysB homologue, which converts desmethyl-4-deoxygadudusol to 4-deoxygadudusol. The third heterologous genes codes for an ATP-grasp protein, such as the gene Ava-3856, a MysC homologue, which converts 4-deoxygadudusol to mycosporine-glycine (see also Fig. 1 for the mode of action of enzymes coded by MysA to MysC). Instead of the *A. variabilis* genes any other MysA to MysC homologues, in particular, the homologues disclosed therein can be employed.

[00168] According to a further variant of the invention, the genetically modified cyanobacterial cell of the previous claim exhibits a low pigment phenotype compared to the wildtype, in particular with respect to the chlorophyll and/or the phycocyanin content of the cell. Such a low pigment type cyanobacterial cell can lead to higher production of MAAs, because cells with a lower amount of pigment do not shade other cells to a great extent, so that the incident light

can penetrate deeper into a cell culture and more light can reach the cells. In particular, the genetically modified cyanobacterial cell can exhibit a low chlorophyll genotype, due to a $\Delta ycf37$ gene inactivation associated with a low chlorophyll phenotype.

[00169] Another embodiment of the invention is directed to a genetically modified cyanobacterial cell for the production of mycosporine-2-glycine, additionally including a fourth heterologous gene coding for a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 15. The fourth heterologous gene codes for an MysD homologous protein, such as the protein encoded by the gene Ap_3855, which converts mycosporine-glycine to mycosporine-2-glycine.

[00170] Another embodiment is directed to a genetically modified cyanobacterial cell for the production of shinorine/porphyra-334, additionally including a fourth heterologous gene coding for a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to the enzyme of SEQ ID NO: 1111. This gene is highly homologous to the gene NpF5597 from *Nostoc punctiforme*, which codes for a D-ala-D-ala ligase protein (MysD homolog), converting mycosporine-glycine to shinorine and porphyra-334, and to some extent also mycosporine-glycine-alanine (see also Fig. 28C and Fig. 40)

[00171] Another embodiment is directed to a genetically modified cyanobacterial cell for the production of shinorine, additionally including a fourth heterologous gene coding for a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 61. This gene is highly homologous to the gene Ava_3855, which codes for an NRPS-like protein, converting mycosporine-glycine to shinorine in the presence of a phosphopantethiene-protein transferase (see also Fig. 1).

[00172] Furthermore, the genetically modified cyanobacterial cell, apart from a first copy of a mysA homologous gene, can further include a second copy of the first heterologous gene which codes for a first enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 3. This additional mysA copy can lead to a higher expression of MysA, higher conversion rate of sedoheptulose-7-phosphate to desmethyl-4-deoxygadusol and finally to higher MAA productivity of e.g. shinorine and porphyra-334 productivity shown in Fig. 42 B and C.

[00173] Furthermore, the genetically modified cyanobacterial cell, apart from a first copy of a classical mysA homologous gene, can further include a second copy of a mysA heterologous

gene from the divergent clade which codes for a first enzyme, which is at least 80%, preferably 90% identical to SEQ ID NO: 164. This divergent clade *mysA* copy can lead to a higher conversion rate of sedoheptulose-7-phosphate to desmethyl-4-deoxygudasol and finally to higher MAA productivity of e.g. shinorine and porphyra-334 productivity shown in Fig. 42 B, C and 19 A, B, C.

[00174] The genetically modified cyanobacterial cells of the present invention can have productivities of 50-100mg/L*d, preferably 120-200mg/L*d over a period of 3-10 days for mycosporine-glycine, mycosporine-2-glycine and shinorine/porphyra-334 under cultivation conditions as described in example 27.

[00175] According to a further embodiment of the present invention, the new MAA compounds Tery-322 and Tery-364 are produced by a genetically modified host cell comprising at least one heterologous gene that encodes an enzyme involved in the production of the compounds Tery-322 or Tery-364. The heterologous genes can be *mysA*, *mysB* and *mysC* from any of the host cells disclosed in this application. The *mysA* gene (for example the genes Ava_3858, Tery_2977, *mysA*_HL-69, NpR5600) encodes a DHQS-like enzyme, which converts sedoheptulose-7-phosphate to desmethyl-4-deoxygudasol. The *mysB* gene (for example the genes Ava_3857, Tery_2976, *mysB*_HL-69, NpR5599), codes for an O-MT enzyme, which further converts the desmethyl-4-deoxygudasol to 4-deoxygudasol. The *mysC* gene (for example the genes Ava_3856, Tery_2975, *mysC*_HL-69, NpR5598), encodes an enzyme that is responsible for the conversion of 4-deoxygudasol to mycosporine-glycine (see also Fig. 1 and Fig. 40). Mycosporine-glycine is then the branching point for a variety of different MAAs dependent on the enzyme involved and the amino acid participating in the conversion (see Fig. 40). For example, *mysD* or *mysD* homologous genes encoding a D-ala-D-ala ligase (for example NpF5597, Ap3855, *mysD*_HL-69, *mysD*_K005, Tery_2971, Tery_2970, Amir_4256) can catalyze the reaction of mycosporine-glycine with glycine to form mycosporine-2-glycine, with mycosporine-glycine and threonine to form porphyra-334, with mycosporine-glycine and alanine to form mycosporine-glycine-alanine or with mycosporine-glycine and serine to result in shinorine (see Fig. 40). Some cyanobacterial species contain an analogous NRPS encoding gene (for example Ava_3855, Tery_2968) instead of or in addition to a *mysD* gene which catalyzes the reaction of mycosporine-glycine with serine to form shinorine.

[00176] Tery-322 in particular can be produced by a genetically modified host cell comprising at least one heterologous gene encoding an enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 103, which is the Tery-2966 homolog of O-methyltransferase from the TERY IMS101 gene cluster from *Trichodesmium erythraeum* IMS101 for production of Tery-322. An exemplary gene encoding the enzyme of SEQ ID NO: 103 is the nucleic acid sequence with the SEQ ID NO: 102. Tery-322 can be produced employing mycosporine-glycine as a starting compound. Thus, either a genetically modified host cell transformed with the above-mentioned enzyme of SEQ ID NO: 103 should be used, which naturally produces mycosporine-glycine, or additional heterologous genes encoding enzymes involved in the production of mycosporine-glycine, for example mysA, mysB and/or mysC have to be present in the host cell. Homologous genes of Tery_2966 with high degree of amino acid sequence similarity can be found in multiple cyanobacterial species. A sequence alignment of Tery_2966 (O-methyltransferase) homologs from different cyanobacterial species is depicted in Fig. 50 which shows the O-methyltransferase-like amino acid sequences from *Synechococcus* sp. PCC7335, *Euhalothece* sp. KZN001, *Croococcidiopsis* sp. TS821, *Trichodesmium erythraeum* IMS101, and *Calothrix* sp. NIES2100. Respective O-methyltransferases from *Synechococcus* sp. PCC7335, *Euhalothece* sp. KZN001, and *Croococcidiopsis* sp. TS821 have been proven to catalyze the same reaction as Tery_2966 from *Trichodesmium erythraeum* IMS101 with mycosporine-glycine as substrate to form Tery-322 as product.

[00177] A further variant of the invention is directed to a genetically modified host cell which further comprises another heterologous gene encoding an enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 99, which is the Tery-2968 gene homolog of NRPS from the TERY IMS101 gene cluster from *Trichodesmium erythraeum* IMS101 for production of Tery-364 employing Tery-322 as starting compound. An exemplary gene encoding the enzyme of SEQ ID NO 99 is the nucleic acid sequence with the SEQ ID NO: 89.

[00178] According to a further embodiment of the present invention, the genetically modified host cell further comprises

- a first enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with either the enzymes of SEQ ID NO: 3 or having at least 80 % sequence identity, preferably at

least 90 % sequence identity with the enzyme of SEQ ID NO: 164 (Ava-3858 mysA variant or distant clade mysA variant),

- a second enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 6 (Ava-3857 mysB variant), and

- a third enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 9 (Ava-3856 mysC variant). These enzymes are able to produce the starting compounds 4-deoxygudasol, desmethyl-4-deoxygudasol or mycosporine-glycine for the production of Tery-322 and Tery-364 (see Fig. 40).

[00179] Another embodiment of the invention is directed to a genetically modified host cell, comprising at least one heterologous gene encoding a protein having at least 80 % sequence identity, preferably at least 90 % sequence identity with the protein of SEQ ID NO: 95 and SEQ ID NO: 93. An exemplary gene encoding the enzyme of SEQ ID NO: 93 Tery_2971 (mysD1_IMS101) is the nucleic acid sequence with the SEQ ID NO: 92. This enzyme is responsible for the formation of mycosporine-2-glycine, employing glycine as the reaction partner and using mycosporine-glycine as the starting compound (see Fig. 40). An exemplary gene encoding the enzyme of SEQ ID NO: 95 Tery_2970 (mysD2_IMS101) is the nucleic acid sequence with the SEQ ID NO: 94. This enzyme is responsible for the formation of shinorine employing serine as reaction partner and using mycosporine-glycine as starting compound.

[00180] The genetically modified host cell of the invention can further comprise at least one further heterologous gene encoding an enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with an enzyme encoded by either one of the nucleic acids of SEQ ID NO: 91 or SEQ ID NO: 97. It is possible that only one, two or all three of the enzymes are present in the host cell. These enzymes are responsible for the formation of mycosporine-methylamine-glycine, mycosporine-methylamine-threonine or palythine, employing either porphyra-334 or mycosporine-2-glycine as starting compounds (see Fig. 40).

[00181] The genetically modified host cell of any of the above described embodiments can be selected from a group consisting of bacteria and eukaryotic cells. Preferably enterobacteria, such as *E. coli*, and cyanobacteria, such as *Cyanobacterium* strains like *Cyanobacterium* sp. ABICyano1 or *Cyanobacterium* sp. ABCyano4, *Anabaena* PCC7120, *Synechocystis* PCC6803, *Synechococcus* PCC7942, or *Synechococcus* PCC7002, can be used as host cells.

[00182] Another embodiment of the invention is directed to the compound Tery-322, which is a mycosporine-like amino acid having an absorption maximum at 322 nm, being producible by culturing a genetically modified host cell including four heterologous genes encoding four enzymes,

- a first enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 164 (Ava-3858 mysA variant or distant clade mysA variant),
- a second enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 6 (Ava-3857 mysB variant),
- a third enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 9 (Ava-3856 mysC variant), and
- a fourth enzyme having at least 80 % sequence identify, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 103 (Tery_2966).

Apart from the unique absorption at 322 nm, Tery-322 also exhibits a retention time, not observed for other MAAs. For example on a HPLC system with a HILIC column (as described in Hartmann et al., 2015) the retention time for Tery-322 is at ~ 6.6 min, for M-Gly is at ~ 6.9 min, for Palythine at ~ 9.0 min, for porphyra-334 at ~ 9,7 min, for Tery-364 at ~ 10,1 min, and for shinorine 10,7 min.

[00183] A further embodiment of the invention is directed to the compound Tery-364, which is a mycosporine-like amino acid having an absorption maximum at 364 nm, being producible by culturing a genetically modified host cell including at least five heterologous genes encoding five enzymes,

- a first enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 164 (Ava-3858 mysA variant or distant clade mysA variant),
- a second enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 6 (Ava-3857 mysB variant),

- a third enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 9 (Ava-3856 mysC variant), and
- a fourth enzyme having at least 80 % sequence identify, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 103 (Tery_2966), and
- a fifth enzyme having at least 80 % sequence identify, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 99 (Tery_2968).

[00184] The genetically modified host cell can further include an enzyme having at least 80 % sequence identify, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 176, which is important for the activity of the enzyme with SEQ ID NO: 99. (Phosphopantethiene-protein transferase).

[00185] Apart from the unique absorption at 364 nm, Tery-364 also exhibits a retention time, not observed for other MAAs. For example on a HPLC system with a HILIC column (as described in Hartmann et al., 2015) the retention time for Tery-322 is at ~ 6.6 min, for M-Gly is at ~ 6.9 min, for Palythine at ~ 9.0 min, for porphyra-334 at ~ 9,7 min, for Tery-364 at ~ 10,1 min, and for shinorine 10,7 min.

Modified Cyanobacterial Strains for MAA “Bio-factories”

[00186] In an embodiment of the invention, genetically modified phototrophic cyanobacteria can produce large amounts of a desired MAA or mixture of MAAs. The MAA productivities of phototrophically grown *Cyanobacterium* sp. ABICyano1 and *Cyanobacterium* sp. ABCyano4, on a dry weight basis, can be in the range of from about 40, 60, 80, 100, 120, 140, 160, 180, 200, or more mg / g dry weight. Further, in comparison to heterotrophic bacteria, the modified cyanobacterial cells do not require the addition of an organic carbon source, as they are able to utilize sunlight and CO₂ to make the MAA compounds.

[00187] In an embodiment of the invention, sustainable production of high amounts (for example, between about 100, 150, 200, and about 250, 300, 350 or more, in particular 400, 600, 800, 1000, 1200, 1400 or more mg/L of the MAAs mycosporine-glycine, shinorine and mycosporine-2-glycine, and others, such as porphyra-334, mycosporine-glycine-alanine and palythine, can be produced, for example, by heterologous expression of the MAA synthesis pathway genes from *A. variabilis* ATCC 29413, *N. punctiforme* ATCC 29133, *Nostoc commune* var. flagelliforme QSY 1, *Nostoc verrucosum* KU005 *P. umbilicalis*, *Cyanobacterium* sp. HL-69,

Trichodesmium erythraeum IMS101, *Euhalothece* sp. KZN 001, *Synechococcus* sp. PCC7335, *Croococcidiopsis* sp. TS-821 and/or *A. halophytica* in the *Cyanobacterium* sp. ABICyano1 and the *Cyanobacterium* sp. ABCyano4.

[00188] In an embodiment of the invention, the cyanobacterial host cell is *Cyanobacterium* sp. ATCC accession number PTA-13311 (“ABICyano1”, also termed “AB1”). This strain has been found to grow well under various indoor and outdoor conditions, and can be genetically modified to produce a compound of interest. The strain, as well as its endogenous plasmid p6.8 (FIG. 4, SEQ ID NO: 16), has been described, for example, in U.S. Patent NO: 8,846,369, U.S. 9,315,832, and U.S. Patent NO: 9,157,101, all of which are hereby incorporated by reference in their entireties.

[00189] In another embodiment of the invention, the cyanobacterial host cell is *Cyanobacterium* sp. ATCC accession number PTA-125253, also called “ABCyano4” or also termed “ABCC1710”. Similar to AB1, this strain has been found to grow well under various indoor and outdoor conditions, and can be genetically modified to produce a compound of interest. As it is shown herein, ABCyano4 can produce more biomass than AB1 in batch cultivation and exhibits a much thicker capsular exopolysaccharide layer (CPS layer) compared to AB1, so that MAAs produced with genetically modified ABCyano4 cells are mainly associated with this CPS layer or are produced intracellularly, enabling an easier purification of the MAAs. The strain, as well as its endogenous plasmids pABCyano4-B and pABCyano4-C (FIG. 43 and 44), has been applied for heterologous MAA production by insertion of MAA biosynthesis genes of interest from different cyanobacterial origin.

[00190] A deposit of the Algenol Biotech LLC’s proprietary strain of *Cyanobacterium* sp., strain ABCyano4, disclosed above and recited in the appended claims has been made with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110. The ATCC Accession Number is PTA-125253.

[00191] The 16S rDNA of *Cyanobacterium* sp. ABCyano4 shows a high sequence identity of around 99% to the 16S rDNA sequences of different cyanobacterial species of the genus *Cyanobacterium*, including *Cyanobacterium* IHB-410, *Cyanobacterium aponinum* ETS-03, and *Cyanobacterium* sp. MBIC10216. The 16S ribosomal RNA (rRNA) gene sequences (16S rDNA) of ABICyano1 was predicted from the genome sequence with the RNAmmer program (Lagesen K, *et al.* (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic*

Acids Research 35(9):3100-3108.), These sequences were then used as a query to search against the NCBI database and 16S rDNA sequences from 4 species belonging to the genus *Cyanobacterium* were retrieved as the top BLAST (Altschul SF, *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl. Acids. Res.* 25(17)3389-3402) hits. ABICyanol and ABCyano4 16S rDNA sequences are 99% identical to the rDNA sequence of *Cyanobacterium* sp. ETS-03.

[00192] In an embodiment, a culture of the cyanobacterial cell is grown in an outdoor photobioreactor system, and the MAA that is produced can be isolated, for example, from the culture medium or from the cells. In another embodiment, a culture of the cyanobacterial cells is grown in an indoor photobioreactor system.

[00193] Cyanobacteria can be modified via addition of MAA biosynthesis genes as shown herein in order to produce various MAAs. The DNA sequences encoding the genes can be amplified by the polymerase chain reaction (PCR) using specific primers. The amplified PCR fragments can then be digested with the appropriate restriction enzymes and cloned into either a self-replicating plasmid or an integrative plasmid. An antibiotic resistance cassette for selection of positive clones can be present on the appropriate plasmid.

[00194] In an embodiment, the nucleic acids of interest can be amplified from nucleic acid samples using amplification techniques. PCR can be used to amplify the sequences of the genes directly from DNA, from mRNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other in vitro amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, and for nucleic acid sequencing.

[00195] To use isolated sequences in the above techniques, recombinant DNA vectors suitable for transformation of cyanobacteria can be prepared. Techniques for transformation are well known and described in the technical and scientific literature. For example, a DNA sequence encoding one or more of the genes described herein can be combined with transcriptional and other regulatory sequences which will direct the transcription of the sequence from the gene in the transformed cyanobacteria.

[00196] In an embodiment, the MAA biosynthesis genes of interest are inserted into the cyanobacterial chromosome. When the cell is polyploid, the gene insertions can be present in all of the copies of the chromosome, or in some of the copies of the chromosome.

[00197] In another embodiment, the inserted MAA biosynthesis genes are present on an extrachromosomal plasmid. The extrachromosomal plasmid can be derived from an outside source, such as, for example, RSF1010-based plasmid vectors, or it can be derived from an endogenous plasmid from the cyanobacterial cell or from another species of cyanobacteria.

[00198] In an embodiment, the inserted MAA genes are present on one or several extrachromosomal plasmids, wherein the plasmids have multiple copies per cell. The plasmids can be present, for example, at about 1, 3, 5, 8, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or more copies per host cyanobacterial cell. In an embodiment, the plasmids are fully segregated.

[00199] In another embodiment, the inserted MAA genes are present on one cassette driven by one promoter. In another embodiment, the inserted genes are present on separate plasmids, or on different cassettes. In a further embodiment of the cyanobacterial cell of the invention, the cassette can be part of an operon.

[00200] In another embodiment of the genetically modified cyanobacterial cell, the cell includes an operon including the first heterologous gene and a second heterologous gene involved in the production of a MAA, the operon including an intergenic sequence between the first heterologous gene and the second heterologous gene. Such an operon allows for a simple overexpression of more than one enzyme involved in the production of MAA, wherein the plurality of heterologous genes of the operon are controlled by one promoter. For example, the operon can include the first heterologous gene, and at least one further gene, the second heterologous gene. In particular, the second heterologous gene can code for an enzyme which further acts on the molecule produced by the enzyme coded by the first heterologous gene. Thus, a consecutive chain of enzymes for MAA production can be overexpressed in one operon, wherein for example a second enzyme for MAA production converts the product of a first enzyme for MAA production into another product, on which then a third enzyme can act. Additional genes, apart from the first and second heterologous genes, such as a third heterologous gene and a fourth heterologous gene coding for a third and a fourth enzyme for producing MAA, respectively, can be present in the operon. For example, shinorine can be produced from sedoheptulose-7-phosphate by overexpressing a 3-dehydroquinate synthase homolog enzyme (DHQS-homolog), an O-methyltransferase (O-MT), an ATP-grasp family protein and a nonribosomal peptide synthetase (NRPS-like protein) or a D-Ala-D-Ala ligase, which subsequently convert sedoheptulose-7-phosphate into shinorine (see also Fig. 1). The

intergenic sequence between the respective heterologous genes contains a ribosomal binding site and further nucleotide sequences, which can influence the overexpression of the enzymes encoded by the heterologous genes.

[00201] In a further variant of the genetically modified cyanobacterial cell of the invention, the intergenic sequence is derived from an intergenic sequence located between two genes coding for a heterodimeric protein. Often, proteins coded by genes arranged in an operon exhibit different levels of expression, wherein proteins whose genes are further downstream in the operon show a lower level of expression compared to the enzymes whose genes are located upstream in the operon. These different levels of expression of different enzymes involved in the production of an MAA can negatively affect the overall yield of the MAA. Therefore, employing the intergenic sequence of an operon coding for two proteins, which are roughly expressed at the same levels can ensure a higher and more equal level of expression of enzymes for MAA production, leading to a higher overall yield of MAAs.

[00202] In particular, the intergenic sequence can be derived from the *cpcB-cpcA* operon coding for phycocyanin. Phycocyanin is a highly expressed, abundant pigment-protein complex from the light-harvesting phycobiliprotein family, which contains two subunits, the alpha and beta subunit. Employing the intergenic sequence of this operon can ensure a higher yield of MAA. According to a further embodiment of the genetically modified cyanobacterial cell of the invention, the intergenic sequence is at least 90 % identical to the sequence with the SEQ ID NO: 177, which is the native IS_{cpcBA} sequence of the *cpcB-cpcA* operon. In particular, a further variant of the genetically modified cyanobacterial cell of the invention includes an intergenic sequence having a ribosomal binding site and wherein the intergenic sequence exhibits nucleotide changes in the ribosomal binding site and in between the ribosomal binding site and a start codon of either the first or second heterologous gene in comparison to the native intergenic sequence. These nucleotide changes can be changes of the nucleotides or even nucleotide additions or deletions in comparison to the native sequence of the intergenic sequence, such as the sequence of SEQ ID NO: 177. These nucleotide changes can further increase in the yield of MAA. One particular example of a modified intergenic sequence is the IS_{cpcBA}*1 sequence of SEQ ID NO: 178.

[00203] In another embodiment of the genetically modified cyanobacterial cell of the invention, the operon comprises a plurality of heterologous genes encoding a plurality of enzymes

for producing MAA, any of the above discussed intergenic sequence being present between all the heterologous genes. Such a design of an operon can ensure roughly the same level of expression for the plurality of the enzymes, thereby enhancing the overall MAA yield.

[00204] In another embodiment, the inserted MAA genes are modified for optimal expression by modifying the nucleic acid sequence to accommodate the cyanobacterial cell's protein translation system. Modifying the nucleic acid sequences in this manner can result in an increased expression of the genes.

[00205] According to another embodiment of the invention, the genetically modified cyanobacterial cell is able to produce high amounts of MAAs over an extended period of time, for example at least $25 \text{ mg L}^{-1}\text{d}^{-1}$, preferably at least $50 \text{ mg L}^{-1}\text{d}^{-1}$ more preferably at least $75 \text{ mg L}^{-1}\text{d}^{-1}$, most preferred at least $100 \text{ mg L}^{-1}\text{d}^{-1}$, or at least $150 \text{ mg L}^{-1}\text{d}^{-1}$ or even $200 \text{ mg L}^{-1}\text{d}^{-1}$ over a period of 1-2 weeks. In particular, the productivity rates can be in the range from $25 \text{ mg L}^{-1} \text{d}^{-1}$ to $250 \text{ mg L}^{-1} \text{d}^{-1}$. The ratio of the MAAs produced in comparison to the dry weight of the cells can be at least 4%, preferably 6%, most preferred at least 8%. Determination of the MAA content in the liquid samples (with or without cells) can be done photometrically via Lambert-Beer's law using the known spectroscopic properties of each MAA (e.g., shinorine: $\lambda_{\text{max}} = 333\text{-}334 \text{ nm}$, $\epsilon = 44.7 \text{ mM}^{-1} \text{ cm}^{-1}$, molar weight 332.3 g/mol).

[00206] Genetically modified cyanobacterial cells able to produce the above mentioned high overall yields comprise at least one or a combination of at least two or three of the following features responsible for the high yield:

- The promoter operably linked to the at least one heterologous gene that encodes an enzyme involved in the production of MAA is a promoter inducible by a change of a metal-ion concentration, in particular the *smtA* promoter or the *petE* promoter. These promoters can be induced by addition of Zn-ions or Cu-ions, respectively, and allow for a tight control of the expression of the enzymes for MAA production. In particular, only traces of MAA production can be observed in the uninduced state of the cells, so that a high cell density can be reached before inducing the cells and then producing a high yield of MAAs. Additionally, the activity of these metal-inducible promoters are easily titratable by a change of a metal ion concentration and allow for high induction.
- Employing an intergenic sequence between different heterologous genes coding for enzymes of MAA production, which is derived from an intergenic sequence located

between two genes coding for a heterodimer protein. As already described herein such an intergenic region can ensure that a plurality of heterologous genes included in an operon operably linked to one promoter can be expressed at roughly the same levels.

- The use of heterologous genes that are codon optimized for optimal expression in cyanobacterial cells. In particular, the following heterologous genes coding for enzymes whose predicted function are included in parentheses can be codon-optimized: *mysA* (dimethyl-4-deoxygadusol (DDG) synthase), *mysB* (O-methyltransferase), *mysC* (ATP-grasp family protein) and *mysD* (D-Ala ligase homolog) or NRPS gene (nonribosomal peptide synthetase).
- Use of heterologous genes or operons containing the heterologous genes located on extrachromosomal high copy plasmids. These high copy plasmids ensure a replication independent of the chromosomes and therefore result in a high copy number of the heterologous genes, resulting in high expression of the enzymes encoded by the heterologous genes. Preferably, the high copy plasmids are present in a cell in at least 10, preferably at least 20, most preferably at least 40 copies per cell. 30 - 50 copies should be present for the pABCyano4B and 12-20 copies of the plasmid pABCyano4C into which heterologous genes for MAA production can be inserted via homologous recombination.
- Use of cyanobacterial strains, which can reach a high amount of biomass during batch cultivation, such as ABICyano1 and ABCyano4. The amount of biomass can be assessed within a specified time period by employing standard lab conditions, such as cultivation in a tubular plastic film or rigid plastic photobioreactor, for example a 1.2L LvPBR (lab-scale vertical photobioreactor); Start OD: 0.5; Cultivation pH: 7.3; CO₂ supply: 15% pH dependent into liquid phase; Aeration: 38 mL min⁻¹; Light: 350 µE fluorescent light from one side, 12h photoperiod; Temperature day 35°C/ night 25°C; Media: ASW BG11 (artificial seawater medium BG11).
- Use of cyanobacterial strains having an extracellular capsular polysaccharide layer (CPS) with a thickness of between 1 and 3 µm, preferably between 1.1 to 2 µm. Such cyanobacterial strains, such as ABCyano4 can greatly ease the purification of the MAA. The MAAs are often associated with the CPS and therefore can more easily be

purified than when they are secreted into the culture medium. Additionally, a purification of the MAAs from the CPS results in higher yields.

In the following Table 1 a description of the genes used to genetically modify the cyanobacterial host cells for MAA production and their respective SEQ ID Nos in the sequence listing is given:

Table 1: Heterologous Genes and enzymes involved in MAA production encoded by the heterologous genes

Description	SEQ ID NO:
Heterologous gene encoding 3-dehydroquinate synthase from <i>Anabaena variabilis</i> (Ava_3858) GenBank: ABA23463.1	1
Ava_3858(ABICyano1opt) codon optimized variant of Ava_3858 for ABICyano1	2
Enzyme for MAA production encoded by the gene Ava_3858(ABICyano1opt)	3
Heterologous gene encoding O-methyltransferase (Ava_3857) from <i>Anabaena variabilis</i> GenBank: ABA23462.1	4
Ava_3857(ABICyano1opt) codon-optimized variant of Ava_3857 for ABICyano1	5
Enzyme for MAA production encoded by the gene Ava_3857(ABICyano1opt)	6
Heterologous gene encoding ATP-grasp enzyme-like protein from <i>Anabaena variabilis</i> (Ava_3856) GenBank: ABA23461.1	7
Ava_3856(ABICYANO1opt) codon-optimized variant of Ava_3856 for ABICyano1	8
Enzyme for MAA production encoded by the gene Ava_3856(ABICyano1opt)	9

Description	SEQ ID NO:
Heterologous gene encoding D-ala-D-ala ligase (NpF5597) from <i>Nostoc punctiforme</i> ATCC 29133 GenBank: ACC83902.1	10
NpF5597(ABICyano1opt) codon-optimized variant of NpF5597 for ABICyano1	11
Enzyme for MAA production encoded by the gene NpF5597(ABICyano1opt)	12
Heterologous gene encoding D-ala-D-ala ligase (Ap3855) from <i>Aphanotece halophytica</i> GenBank: BAO51916.1	13
Ap3855(ABICyano1opt) codon-optimized variant of Ap3855 for ABICyano1	14
Enzyme for MAA production encoded by the gene Ap3855(ABICyano1opt)	15
Heterologous gene encoding Amino acid adenylation nonribosomal peptide synthetase (NRPS) (Ava_3855) GenBank: ABA23460.1	59
Ava_3855(ABICyano1opt) codon-optimized variant of Ava_3855 for ABICyano1	60
Enzyme for MAA production encoded by the gene Ava_3855(ABICyano1opt)	61
Heterologous gene encoding mysAB 3-dehydroquinate synthase O-methyltransferase fusion protein <i>Porphyra umbilicalis</i> GenBank: OSX70340.1	62
mysAB (ABICyano1opt) <i>Porphyra umbilicalis</i> codon-optimized variant of mysAB for ABICyano1	63
Enzyme for MAA production encoded by the gene mysAB (ABICyano1opt) <i>Porphyra umbilicalis</i>	64
Heterologous gene encoding mysCD ATP-grasp enzyme-like protein D-ala-D-ala ligase fusion protein <i>Porphyra umbilicalis</i> GenBank: OSX70339.1	65

Description	SEQ ID NO:
mysCD (ABICyano1opt) <i>Porphyra umbilicalis</i> , codon-optimized variant of mysCD for ABICyano1	66
Enzyme for MAA production encoded by the gene mysCD (ABICyano1opt) <i>Porphyra umbilicalis</i>	67
codon-optimized variant of mysCD ATP-grasp enzyme-like protein D-ala-D-ala ligase fusion protein <i>Chondrus crispus</i> GIDCcT00003179001	68
Enzyme for MAA production encoded by the gene mysCD (ABICyano1opt) <i>Chondrus crispus</i>	69
Heterologous gene encoding dehydroquinase synthase MylA [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54016.1	70
Enzyme for MAA production encoded by the gene dehydroquinase synthase MylA [<i>Cylindrospermum stagnale</i> PCC 7417]	71
Heterologous gene encoding O-methyl transferase MylB [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54017.1	72
Enzyme for MAA production encoded by the gene MylB [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54017.1	73
Heterologous gene encoding D-alanine-D-alanine ligase MylC [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54018.1	74
Enzyme for MAA production encoded by the gene MylC [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54018.1	75

Description	SEQ ID NO:
Heterologous gene encoding ATP-grasp domain protein MylD [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54019.1	76
Enzyme for MAA production encoded by the gene MylD [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54019.1	77
Heterologous gene encoding ATP-grasp domain protein Myle [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54020.1	78
Enzyme for MAA production encoded by the gene Myle [<i>Cylindrospermum stagnale</i> PCC 7417] GenBank: ANS54020.1	79
Heterologous gene encoding desmethyl-4-deoxygadusol synthase MysA [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12742.1	80
Enzyme for MAA production encoded by the gene MysA [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12742.1	81
Heterologous gene encoding O-methyltransferase MysB [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12743.1	82
Enzyme for MAA production encoded by the gene MysB [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12743.1	83
Heterologous gene encoding D-Ala D-Ala ligase MysD [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12744.1	84
Enzyme for MAA production encoded by the gene MysD [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12744.1	85

Description	SEQ ID NO:
Heterologous gene encoding ATP-grasp ligase MysC2 [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12745.1	86
Enzyme for MAA production encoded by the gene MysC2 [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12745.1	87
Heterologous gene encoding ATP-grasp ligase MysC1 [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12746.1	88
Enzyme for MAA production encoded by the gene MysC1 [<i>Nostoc commune</i> var. <i>flagelliforme</i> QSY 1] GenBank: AQU12746.1	89
Heterologous gene encoding Putative Clavaminic acid synthetase-like Tery_2972 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52130.1	90
Enzyme for MAA production encoded by the gene Tery_2972 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52130.1	91
Heterologous gene encoding D-alanine-D-alanine ligase 1 Tery_2971 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52129.1	92
Enzyme for MAA production encoded by the gene Tery_2971 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52129.1	93
Heterologous gene encoding D-alanine-D-alanine ligase 2 Tery_2970 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52128.1	94
Enzyme for MAA production encoded by the gene Tery_2970 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52128.1	95
Heterologous gene encoding 6-phosphogluconate dehydrogenase, NAD-binding Tery_2969 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52127.1	96

Description	SEQ ID NO:
Enzyme for MAA production encoded by the gene Tery_2969 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52127.1	97
Heterologous gene encoding Amino acid adenylation domain Tery_2968 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52126.1	98
Enzyme for MAA production encoded by the gene Tery_2968 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52126.1	99
Heterologous gene encoding conserved hypothetical protein Tery_2967 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52125.1	100
Enzyme for MAA production encoded by the gene Tery_2967 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52125.1	101
Heterologous gene encoding Hydroxyneurosporene-O- methyltransferase Tery_2966 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52124.1	102
Enzyme for MAA production encoded by the gene Tery_2966 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52124.1	103
Heterologous gene encoding 3-dehydroquinate synthase Tery_2977 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52135.1	157
Enzyme for MAA production encoded by the gene Tery_2977 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52135.1	158
Heterologous gene encoding O-methyltransferase Tery_2976 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52134.1	159
Enzyme for MAA production encoded by the gene Tery_2976 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52134.1	160
Heterologous gene encoding ATP-grasp ligase Tery_2975 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52133.1	161

Description	SEQ ID NO:
Enzyme for MAA production encoded by the gene Tery_2975 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG52133.1	162
Heterologous gene encoding 3-dehydroquinate synthase mysA [<i>Cyanobacterium stanieri</i> HL-69] GenBank: AUC61936.1	163
Enzyme for MAA production encoded by the gene mysA [<i>Cyanobacterium stanieri</i> HL-69] GenBank: AUC61936.1	164
Heterologous gene encoding O-methyltransferase mysB [<i>Cyanobacterium stanieri</i> HL-69] GenBank: AUC61935.1	165
Enzyme for MAA production encoded by the gene mysB [<i>Cyanobacterium stanieri</i> HL-69] GenBank: AUC61935.1	166
Heterologous gene encoding ATP-grasp ligase mysC [<i>Cyanobacterium stanieri</i> HL-69] GenBank: AUC61933.1	167
Enzyme for MAA production encoded by the gene mysC [<i>Cyanobacterium stanieri</i> HL-69] GenBank: AUC61933.1	168
Heterologous gene encoding D-alanine-D-alanine ligase mysD [<i>Cyanobacterium stanieri</i> HL-69] GenBank: AUC61934.1	169
Enzyme for MAA production encoded by the gene mysD [<i>Cyanobacterium stanieri</i> HL-69] GenBank: AUC61934.1	170
Heterologous gene encoding D-alanine-D-alanine ligase mysD [<i>Nostoc verrucosum</i> KU005] GenBank: BBC27544.1	171
Enzyme for MAA production encoded by the gene mysD [<i>Nostoc verrucosum</i> KU005] GenBank: BBC27544.1	172
Heterologous gene encoding D-alanine-D-alanine ligase [<i>Actinosynnema mirum</i> DSM 43827] GenBank: ACU38111.1	173
Enzyme for D-alanine--D-alanine ligase [<i>Actinosynnema mirum</i> DSM 43827] GenBank: ACU38111.1	174
Heterologous gene for Phosphopantethiene-protein transferase Tery_4684 [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG53648.1	175

Description	SEQ ID NO:
Enzyme for Phosphopantethiene-protein transferase [<i>Trichodesmium erythraeum</i> IMS101] GenBank: ABG53648.1	176

[00207] Methods to modify cyanobacterial cells with genetic modifications to produce compounds of interest are also described in U.S. Patent No. 9,315,820, U.S. Patent No. 9,965,364, U.S. Patent No. 9,551,014, U.S. Patent No. 9,476,067, U.S. Patent No. 9,493,794, and U.S. Patent No. 9,353,400, all of which are incorporated by reference herein in their entireties.

Codon Improvement of Recombinant Genes

[00208] At least some of the heterologous MAA genes to be expressed in cyanobacterial host cells can be codon improved for optimal expression in the target cyanobacterial strain. The underlying rationale is that the codon usage frequency of highly expressed genes is generally correlated to the host cognate tRNA abundance. (Bulmer, *Nature* 325:728–730; 1987). Codon improvement (sometimes referred to as codon optimization or codon adaptation) can be performed to increase the expression level of foreign genes.

[00209] Codon improvement of the gene of interest can be performed for improved expression in the cyanobacterial host cell. Codon improvement can also be performed by adapting the codon usage of the at least one recombinant gene to the codon usage in *Cyanobacterium* sp., in particular ABICyano1 and ABCyano4. In an embodiment, the G and/or C wobble bases in the codons for the amino acids in the at least one recombinant gene can be replaced by A and/or T because the GC content of the genome of ABICyano1 and ABCyano4 is relatively low at about 36%, respectively.

[00210] In an embodiment, only 2% to 6% or 1% to 10% of the codons of variants of recombinant genes are codon improved. In another embodiment, highly codon improved variants of recombinant genes, at least 25%, to at least 50%, 65% or even at least 70% of the codons have been changed. In another embodiment, recombinant genes are used which are not codon improved.

[00211] Codon improvement of heterologously derived genes (such as genes encoding antibiotic resistance genes, and the recombinant production genes) was conducted using the software Gene Designer (DNA 2.0, Menlo Park, CA), and was guided by the ABICyano1 and ABCyano4 codon usage table derived from ribosomal proteins and highly expressed genes (such

as photosynthesis genes). To improve heterologous gene expression, original sequences of interest were assessed with the online software OPTIMIZER (Puigbò P, Guzmán E, Romeu A, & Garcia-Vallvé S (2007) OPTIMIZER: a web server for optimizing the codon usage of DNA sequences *Nucleic Acids Research* 35(suppl 2):W126-W131) based on the codon-usage table derived from ABICyano1 and ABCyano4 genomes. The pre-optimized sequences were further modified with Gene Designer 2.0 (available at [dna20.com /genedesigner2/](http://dna20.com/genedesigner2/)) to ensure that their codon adaptation index is similar to the ABICyano1 and ABCyano4 codon usage table (Sharp PM & Li W-H (1987).

[00212] The codon adaptation index is a measure of directional synonymous codon usage bias, and its potential applications, (see *Nucleic Acids Research* 15(3):1281-1295). The effective number of codons (see, Wright F (1990) *Gene* 87(1):23-29) are designed match those of highly expressed genes (such as ribosomal proteins) in the ABICyano1 and the ABCyano4 genome. The resulting polynucleotides using improved codons were further modified and optimized to avoid the presence of any known or predicted putative ABICyano1 and ABCyano4 endonuclease restriction sites (*AvaI*, *BsaHI*, *KasI*, *XhoI* *HgiDI*, *HpaII*, *EcoRV* etc.); internal Shine-Dalgarno sequence and RNA destabilizing sequences; an internal terminator sequence; and a repeat sequence of greater than about 10 bp (see, Welch et al., *PLOS One* 4, e7002; 2009; and Welch et al., *Journal of the Royal Society; Interface* 6 (Suppl 4), S467–S476; 2009).

[00213] In an embodiment, the selectable marker genes are also modified so that they will have improved expression in cyanobacteria. For example, the selectable marker gene that confers gentamycin or kanamycin resistance was codon optimized for higher expression in cyanobacteria. Additionally, the selectable marker gene that confers kanamycin resistance was codon optimized for higher expression in cyanobacteria. In an embodiment, as a result of codon improvement, the GC % of the antibiotic resistance genes decreased from 40-53% to 33-40%, which is similar to that of ABICyano1 and to that of ABCyano4 coding genes (about 36% on average). The codon adaptation index of the codon improved antibiotic resistance genes is significantly improved from less than 0.4 to greater than 0.8, which is similar to that of ABICyano1 and to that of ABCyano4 endogenous genes. Table 2, below, depicts the codon usage statistics within the cyanobacterial strain ABICyano1, which is also valid for ABCyano4.

Table 2: Codon Usage – ABICyano1

Amino Acid	Codon	Fraction	Number	Frequency (/1000)
Ala	GCA	0.293	20724	18.356
Ala	GCC	0.214	15144	13.414
Ala	GCG	0.14	9870	8.742
Ala	GCT	0.353	24915	22.068
Arg	AGA	0.347	16040	14.207
Arg	AGG	0.09	4158	3.683
Arg	CGA	0.106	4886	4.328
Arg	CGC	0.131	6043	5.353
Arg	CGG	0.039	1813	1.606
Arg	CGT	0.288	13329	11.806
Asn	AAC	0.22	14609	12.94
Asn	AAT	0.78	51712	45.804
Asp	GAC	0.193	11063	9.799
Asp	GAT	0.807	46399	41.098
Cys	TGC	0.218	2501	2.215
Cys	TGT	0.782	8976	7.95
Gln	CAA	0.806	43747	38.749
Gln	CAG	0.194	10554	9.348
Glu	GAA	0.787	60690	53.756
Glu	GAG	0.213	16451	14.571
Gly	GGA	0.324	22709	20.114
Gly	GGC	0.125	8720	7.724
Gly	GGG	0.151	10542	9.338

Gly	GGT	0.401	28065	24.859
His	CAC	0.251	4859	4.304
His	CAT	0.749	14516	12.858
Ile	ATA	0.195	18334	16.239
Ile	ATC	0.19	17872	15.83
Ile	ATT	0.616	57964	51.342
Leu	CTA	0.088	10776	9.545
Leu	CTC	0.058	7129	6.314
Leu	CTG	0.033	4040	3.578
Leu	CTT	0.116	14162	12.544
Leu	TTA	0.571	69559	61.612
Leu	TTG	0.133	16235	14.38
Lys	AAA	0.836	59396	52.61
Lys	AAG	0.164	11694	10.358
Met	ATG	1	20093	17.797
Phe	TTC	0.172	8420	7.458
Phe	TTT	0.828	40450	35.829
Pro	CCA	0.169	7746	6.861
Pro	CCC	0.275	12613	11.172
Pro	CCG	0.066	3012	2.668
Pro	CCT	0.491	22560	19.982
Ser	AGC	0.088	6435	5.7
Ser	AGT	0.306	22393	19.835
Ser	TCA	0.14	10217	9.05
Ser	TCC	0.102	7465	6.612
Ser	TCG	0.044	3196	2.831

Ser	TCT	0.321	23473	20.791
Thr	ACA	0.26	15649	13.861
Thr	ACC	0.236	14251	12.623
Thr	ACG	0.083	5024	4.45
Thr	ACT	0.42	25340	22.445
Trp	TGG	1	14964	13.254
Tyr	TAC	0.187	7364	6.523
Tyr	TAT	0.813	31912	28.266
Val	GTA	0.28	18541	16.423
Val	GTC	0.117	7778	6.889
Val	GTG	0.184	12184	10.792
Val	GTT	0.419	27713	24.547
End	TAA	0.63	2495	2.23
End	TAG	0.22	848	0.76
End	TGA	0.15	591	0.53

Choice of Promoters

[00214] The inserted genes can be controlled by one promoter, or they can be controlled by different individual promoters. The promoters can be constitutive or regulatable. The promoters can be, for example, inducible. The promoter sequences can be derived, for example, from the host cell, from another organism, or can be synthetically derived.

[00215] Any desired promoter can be used to regulate the expression of the inserted MAA biosynthesis genes. Exemplary promoter types include but are not limited to, for example, constitutive promoters, regulatable promoters such as inducible promoters (e.g., by nutrient source, nutrient starvation, heat shock, mechanical stress, environmental stress, metal concentration, specific metabolites, light exposure, etc.), endogenous promoters, heterologous promoters, and the like. Suitable promoter sequences are also disclosed, for example, in U.S. Patent No. 9,315, 820, U.S. Patent No. 9,551,014, PCT/EP2012/067534, U.S. Patent No. 9,476,067, U.S. Patent No.

9,157,101, PCT/US2013/077364, U.S. Patent No. 9,493,794, and PCT/US2015/000210, all of which are hereby incorporated by reference in their entireties.

[00216] The recombinant MAA biosynthesis gene(s) can be under the transcriptional control of a constitutive promoter. In this way, a sustained level of transcription and, therefore, enzymatic activity of the corresponding protein can be maintained during the whole period of cultivation. For example, the constitutive promoter can be endogenous to the cyanobacterial cell. This has the advantage that no recombinant transcription factor has to be present in the host cell. The endogenous promoter is usually well-recognized by the metabolically enhanced cyanobacterial cell without the need to introduce further genetic modifications.

[00217] Suitable constitutive promoters include, without limitation, the PrpsL promoter (Gene ID: ABICyano1_orf1758), PpsaA promoter (ABICyano1_orf3243), PpsbB (ABICyano1_orf2107), PcpbB promoter (ABICyano1_orf2472), PatpG (ABICyano1_orf1814), PrbcL promoter (ABICyano1_orf1369), and variations thereof. Further suitable endogenous constitutive promoters from genes with unknown function exhibiting appropriate transcriptional activity include, without limitation, the promoters of Gene IDs ABICyano_orf1924, ABICyano_orf1997, ABICyano_orf3446, ABICyano_orf0865, ABICyano_orf1919, ABICyano_orf3278, ABICyano_orf1181, ABICyano_orf1627, ABICyano_orf0265 and ABICyano_orf2536, ABICyano_orf0615, and variants thereof.

[00218] In an embodiment, the promoters can be derived from the cyanobacterial strain *Cyanobacterium* sp. PTA-13311, or they can be derived from another cyanobacterium or from another organism. In an embodiment, the promoters can be about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100 % identical to the promoter sequences described herein.

[00219] The promoters can be regulatable promoters, such as inducible promoters. For example, certain promoters are up-regulated by the presence of a compound, while other promoters can be up-regulated by the absence of a compound (also termed “repressible”).

[00220] Various promoters that can be used include promoters that are regulatable by the presence (or in other promoters, by the absence) of inductors such as different metal ions, different nutrient sources, different metabolites, different external stimuli such as heat, cold, salinity or light. In some embodiments, the regulatable or inducible promoters are induced under conditions such as nutrient starvation, stationary growth phase, heat shock, cold shock, oxidative stress, salt stress, light, darkness, metal ions, organic chemical compounds, and combinations thereof. For example,

a particularly tight control of the expression of gene can be achieved if a gene is under the transcriptional control of a Zn-, Ni-, Cu-, or Co-inducible promoter. Exemplary Zn-regulatable promoters and their variants are described, for example, in International Application No. PCT/EP2013/077496. Exemplary Zn, Ni, and Co-regulatable promoters are described, for example, in International Application No. PCT/2012/076790, both of which are incorporated by reference herein in their entireties.

[00221] In a further embodiment, the regulatable or inducible promoter is inducible by a change of a metal-ion concentration. Such a change of metal-ion concentration includes for instance the addition or depletion of certain metal ions. Suitable inducible promoters include, without limitation, the PziaA promoter, the PsmtA promoter, PaztA promoter, the PcorT promoter, the PnrsB promoter, the PpetJ promoter, the PpetE promoter, the Porf0316, the Porf01460 promoter, the Porf0221 promoter, the Porf0223 promoter, the Porf3126 promoter, the PmntC promoter, and variations thereof. In particular, the promoter being inducible by a change of a metal-ion concentration can be inducible by Zinc-ions and/or Cu-ions, such as the PsmtA promoter or the petE promoter.

[00222] Preferably, the regulatable or inducible promoter is endogenous to the cyanobacterial cell. An endogenous inducible promoter is usually well-recognized by the metabolically enhanced cyanobacterial cell without the need to introduce further genetic modifications.

[00223] In further embodiments, the choice of regulatable or inducible promoters can include, but are not limited to, PntcA, PnblA, PisiA, PpetJ, PpetE, PggpS, PpsbA2, PsigB, PlrtA, PhtpG, PnirA, PnarB, PnrtA, PhspA, PclpB1, PhliB, PcrhC, PziaA, PsmtA, PcorT, PnrsB, PnrsB916, PaztA, PbmtA, Pbxa1, PzntA, PczrB, PnmtA, PpstS, and the like.

[00224] The regulatable or inducible promoter can, for instance, also be a nitrate inducible promoter. Suitable nitrate inducible promoters include, without limitation, the PnirA promoter, the PnrtA promoter, the PnarB promoter, and variations thereof.

[00225] In certain other preferred embodiments, truncated or partially truncated versions of these promoters including only a small portion of the native promoters upstream of the transcription start point, such as the region ranging from -35 to the transcription start can often be used. Furthermore, introducing nucleotide changes into the promoter sequence, e.g. into the TATA box, the operator sequence, 5'-untranslated region and/or the ribosomal binding site (RBS) can be

used to tailor or optimize the promoter strength and/or its induction conditions, e.g. the concentration of inductor required for induction. In some preferred variants, the different inducible promoters are inducible by different metal ions.

[00226] The promoters PhspA, PclpB, and PhliB can be induced by heat shock (raising the growth temperature of the host cell culture from 30°C to 40°C), cold shock (such as, for example, reducing the growth temperature of the cell culture from 30°C to 20°C), oxidative stress (for example by adding oxidants such as hydrogen peroxide to the culture), or osmotic stress (for example by increasing the salinity). The promoter PsigB can be induced by stationary growth, heat shock, and osmotic stress. The promoters PntcA and PnblA can be induced by decreasing the concentration of nitrogen in the growth medium and the promoters PpsaA and PpsbA2 can be induced by low light or high light conditions. The promoter PhtpG can be induced by osmotic stress and heat shock. The promoter PcrhC can be induced by cold shock. An increase in copper concentration can be used in order to induce the promoter PpetE, whereas the promoter PpetJ is induced by decreasing the copper concentration. Additional details of these promoters can be found, for example, in PCT/EP2009/060526, which is incorporated by reference herein in its entirety.

[00227] In an embodiment, the promoters of any of the above embodiments may be selected from the endogenous inducible promoters identified in *Cyanobacterium* sp. with the ATCC accession number PTA-13311 (“ABICyano1”) as listed in table 3A below, and variants thereof.

Table 3A: *Cyanobacterium* sp. ABICyano1 endogenous promoter sequences

Promoter	Sequence	SEQ ID NO:
PnirA	AATTAATAACTTCTTCCTGTACGGGCGAATGGCCATTTGCTCCTAACTAACTCCGTACTGCTTTGCGGAACGAGCGTAGCGAACTCTCCGAATTACTAAGCCTTCATCCCTGATAGATGCAAAAAACGAATTAAAATTATGTGTAAAAAGAAAATGTGTCTTTATTTAGTAGTCAAAGTTACAAAATATTAAGAATCAATTAATAATGTATTGGGCAGTTAAGTATATAAGTCTTTAAATATTTATTTGTATTCAATATATTAACCGAGGACAAATT	22
Porf3126 (PsmA)	CCAATATCTTGTGCATACATACTTATTTGCCTCACTATTAGCCCTATATGTCCTATTGTATTTTTCTTTTCTCCTATTCTAGATCTTGTAATGAATCATTACTCTCTGAAATATAGCTACTAATTTTATGGTTGTTTGTAATAATATATTAACAAATGAACAATAAATCATATTTTGTGTTAATCTAATTATTAGACAACCTGAATTTATATTTCAGATATTACAGATAGGAGAATTTTGATT	23
PnrtA	TATTATTTTTCGTTTATATGCAGATTTAGAATAAACAATAATTCATTTACTGCAATTTTCAAAAAAATGTGACTAAACATACAAAATAAAGAAAAAATAAAGTTTTAAATTATGTACATCAAACTTAAGAAATGTTTAAATTACTTAGA AATTTATAGTTC	24

Porf3461 (petJ)	TTTATATATAAACTCGAATAAAATTATCAATATAAAGTCAAACCTATATCT ATCCTATTTTAACTGCTATTGGTAAGTCCCTTAATTAGTGTTGGGGTGAA TAGATTTTAAAAGGGCAAACCCCCCTTTATCCTCCCTCGAGAGGGGGGA GGGCAAAAGGCAAGGGGCAAGGGAAAAATTAAGAATTAAGAATTAATA ACTCCGAACACCTGTAGGGGCGAATAGCCATTGCTTCCCCTCATCCCC CATCTCCCCAACACCCTAAGCCCCTACTCGTTACTCATTTATTTACATCAT TTATTTACATCATTAAAGAAAAGTAACAAATTTTGACAAGTAGTCTTTTGA CAGGAAAAAGCAAATTCTCGAAGATGAAAACAATAGAAAAAAATTCAA TCTTACAGTAACGATGAAAAAACTTTTAGGCTTAATT	25
PnarB	TGTCTCAAAAAGACAGGTTTTTTTTATGAAAGTAATAAGAAATAAGTAG AAGTGAGGAGTTGGAAGATAGGATTAAGAATTAGGAGTTAACTATTTT CATTCTTTATTCTTCCATTGCCATTGAGAAATCATATCTAAAATCAGCA ACGCCAAATTTAGATGCAAAATAACCATAAATAAAATGCAGAAAAAAG AATACTTTAGATCTTCCGTATCAGAAGATACATTTCTTAACAAAATCTGG TGACAAGATTAAACACACGAAATCCGAGGTTTTATATATTGATTAGTCCT AG	26
Porf1071 (PmntC)	ATTCTGTGAATTGATTAGATTTGAGGTTTTTTAAGAGGTTGATTACCTTG CCTCCAAAAAATCATAACACACTAATGCTCTATATGAAAGGGCTTTAG ACCCATAGGTTTTTGAGAAAAAACTTGCTAACTCTCGGACAATGTCAG CATAACTAAAGTCAATTCTTTTCGTACTTTATAATTGTCTATAATTTAATA TACAACTGTTCTGAACTAGTTTTTCTCTACATTCCTTAGTTTTATCTGAG TAAGGTTGCTTGTAACCTTAACCTCGGTTGGGCCTAAAAATATCCGATTAG GAGCAGGTGTCAGACTTTAATTAATTATTAATTATTAATTGCTTATTGCC AACCCTCGGCGACACCCTTTTTTCATCAGCCCCAGATAAAGATTGATGTT TTAGTTTTGTTTCTTTTTATCCCCTAATTCAACTAATAACAAGTAAACTAA GGTTGTTTATCAAAAATGATGGTTGATGTTTGGGTAAATTTTAAGATATT ATGAAAAGAAAATGAATAAAAAAATGAAAAATCTTT	27
Porf0221	GAATATCTCATCCTTAGCTTCTACTTATACCTTCAGCATAGTTAAAAATC ATCCCTTTATTGATGGTAATAAAAAGAACAGGTTTTATTAGTGAGTAACC TTTTTAATGCTCAATGGTTCTCACTTTACTGCTTCTGAAGTGGAAGTAGT ACATATCATCAAACCTTAGCTAGTGCGAGAATTACCGAGGAAGAATTA CAACAATGGTTCGTAAGGAAAAGTAAGCAGATGAATAATTAAAGCATCA TTTCATCCTCATTTTCATATTCTCCTGTCACCATGGTATGGAAGATTAGGTA AAAATGAGGAAAAAGTTTATT	28
Porf0223	ATACATGGTTGGTTCAGTACTTTTACCCAGTTTTCTCTTTGAACAATTG GCATAACTCTGAAAAAATCAGATCGGGCTTTTGTGTAATTATTTGTTCAA TCAAAGCAAAACCGTGATTGTCTATTTCTTTTTTTTCCCACCACTCATAG ATAAAAATTTATCCCGAACTCAGGTTATATTAAGTTCGGATGATCACTTA AGATAATTGATCAGATTGGTTAAGATAGAGAAAAATCTTTTTTCATAGTG ATTTTCATAATTGATAGTTACAATAACGATTATTATTTAGTAAAAAGATTT TCAAATC	29
Porf0316	TGGTCAAGTTACTATATGTTTAGAAACAACAAAAAAGAAGTCATTATA AAAAATAATTGATACAGGAATTGGCATTAAATAAAGAAGAACAAAAATTAA TTTTTAATCGTTTTTATCGAATCAATAAAGCAAGAAATAGAGAGAAAGG CAGTTGCGGATTAGGTTTAGCTATTGCAAATGCGATCGCGCTTAATCATG GTGGTAGAATAATTTTAGAAAGTCAAGAAAATCAAGGCAGTATTTTAC CGTTTATTTACCGAAAATCATTTTCATCCTAATTTTCATATTCTTTTGACAGA ATCAAAGGTAAAGATAAAAAGAGAGAAACAGTC	30

Porf0128	CCTCAACTACAAGTTCTTTTATATATTACTTTAACCTGAGTTTTGGATAAG CTGAAAGCATTATTTTCTCGTAGTCAGAAAACCTTATAGCTTCTTAGAAA TAACGATAAAATTACCTTAATCCGAACTGACGTTAAATATATTACCCCT ATCACCCCAAAACCCTAAGCCCCTACTCCCCCTTTCCCTTCATCACCTC ATCCCCCATCCCCTAACACTTAACCTTATTCTTTATTCTTAAACCGAACT GAGGTGAAGTTGCAGAATACCCATGGGGGGTTACAGCATTGTAGAAAAA TAAATATTCTTTCATTATTAAGGTTGTTTGGTAAAAATATGTGAAAACCC TAATAATT	31
Porf1486	GGGGACAGACATATTTTTATCATAATGGTAAATTCATAATAATTTTAGAC TTTTTTTTGCAAAAATTAATCTCACTCTCTTCTTCCCTATCTCCCATTGTT TCTTATATCCCAATGCCCAATACCCAAAGCTCAGAAAATAGGTATTAGC GAAGAGGTGTTGATCCCCTCCCCTAGCAAAATATACTCCTATATAGTAAA GTGAGAAAGTGAAGAAATAAGATCAAGTTCGCAATTT	32
Porf3293	TTGACGATTGTATTGACTTACGCCAAATGGCTTACCCTCATAGTGAATAG TTGATAATTAAGAATTA AAAAATCCCGTTCACGACAGAAGGGAGTGTAAG AGCCTTCGGTGCGAACTCTCATCTTCCCTGAAACCTGACACCTGAAACCT GACACCTGAAACCTGACACCTCATCTCCCTAATCCCCTAATTTTAATGAA AAAATACCCTGAGTGGGCATTGAAAAAAAAGAAAAGTTGTTGACTATG AAATAAGAATTCTGCACCTTCGTGAGAAAAAAGGAAATGAAAT	33
Porf3621	CTATTTAACTAGGAAAAGGTAAAGTTAAAGGACAAGGGTAAATAATTA AAAATTAAGAATTAAGA ACTTCTAACTCTCATTACTCATTACTTATTTCC TCCTCTCACCCCTTCTCCTGATCACCTCTTCTCCTCAATACTCGGAACTCA TTTCCCCATGGTGTGACACTCAAATCAAAAGTCTGTTATTGACTTTCAGA TGAAATATTACTATGATAACAATATCCCCCTATGGGTATATAAAAATAT GAGCGATATTAGTTAAAAATCAAATTTGGATTTTTTTTCTGAAAATATTT TAAGATTAAGTAAAGATAAGTAAAGAAATTATAAGCAATTTTGTAAAT CATACC	34
Porf3635	CTCACACTGAAAATATTGCCACAAGAAATAAAGATCAAGCAATAATCCT GACTAAAAAGGAATAAAGTAATTATCCTTTTCCTGATATGTTATCTGACT TGTTGTTTCTTAGTCATGTTCCCTTCATTTTATTTTTGTTTTATCATTTTT ATTACAAAAATTTCTTAATAGGGCTAAAGCATTTAGTTAGTTTTTTAGCT CTCAACAAGTTGACTAATCAATATAATGCCCTAAGTTAATTTGCCCTTGG TTTGACGGAGGATATTGGAAAAAAGAACTTCTCGTTGTATTTACAGG GAAAAGGGGGAAATTTTATTAATACTAAACAATAGAAAAATAATTATTT ATTTATATTATTTTGTGAACAAATGTTCAAGAATTAAAGTGTAATAAGAA AATTTATTTTTTTATATTTATTTAAACTTAGATATAAGCCTAAAGGTCTG AAATTATTATTAGACAATCAATTGATTCAGAGGTAATAGTTTTTTACTTA AAAATATTTTTTCAAATATCCCCTATTTGGGTATTGAAAAATAAATAA ATTCAAGTAATAATATACAGAATAAAGGAAAAATCTAATCTTAAAAATTT TGTGTGTGAGGAATTGAAA	35
Porf3164	CAATCACGAGAATTTATGTAGGGACTATTTTTGGGTTGACGGTGGAGAG TATGTCGCCCTTGAATTATGACCCGAAGATGAAGATGTCGGGGAGGTGG AAGGACGGTCTTTAAGAGGTTTAACATCAAAGTTGGTCATAATCTCTGTC CCTGTTTGATAACTACTATTTAATTTTGAGTTGTTTTAGGTACATCAAAAT ACCCAAATCCTTACTCTCCCCTCAATATACAACAAAAAAAACTTTTTGAT TCACTTTAGTCATAAAAATTAGAATTTATCTACCGAAATATTACATAAAT GTAATGTATATATTTTCTGATTTATTCCGTGTGAGCCATGATTCATAATTT ATAATTCATAATTTCTAAATATGCCCTACAATGGATATAGAATGTCATT TTAATTATAGGTATCATAATCGTGGTAGTTACTCCGAAAAAACTATTGA ATCAAATTCAGTCTCACCTGCTACAGATAGAGTAGCCGTTATTCTT	36

Porf1072	CTACAGGGGCAAGATTTGGCGGAAATCTATATGTGGATTCTCTTTCAAGT GAAGAAGGTGCAGTGCCGACTTATCTGGACTTATTAGAATACGATATTC GCACTATTACTAATGGTTTGTAGCAGGAGTGAACAATTAATAATTTTTT CCTAATTGACGAATAAAAAATCAATGTCAACTAATAGTTAACAATACTC TCTGAAAACCAAAAATTGTCAACCAAAACATAACATAATTTTTACCCAA AAACCTCATTTATAAACTTTAAGGATAAAATCAATG	37
Porf1074	GGGATTAGAGAGTTCAAAGTTAGGAATGAGGTGTCAGGTTTTAGGTTTC AGGTTTAGGGGAGCAATGAGAAAGAGGTTTCAGGTTTCAGGTGTCAGGT TGCAGGTGTCACAGGTGATGAGGGGATGGGGGATGAGGGGGAAACAAG TAAGTAATAAGTGTTTCGGAGTTTTTAATTCTTAATTCTTAATTTTTCTTT GCCTCTTGCTTTTTGCCTTGTCTTAATTACTAATTTCTAATTAATAATGATT GTGTTTTCTAGTTTAGTCTCATGGTTACTTGAACCCTTACAGCATAGTTTT	38
Porf01075	TTACAAACGGCGGGAATTATTATGGTAGTAGCGATGTTAGTAACCCCGG GTGCGATCGCATATTTACTTACAGATCGTTTTGATCAAATGTTAATCTTA TCAATAGTTAGTAGTGTTCTATCTTGTGTTTTAGGCACTTATTTAAGTTAT CATTTTGATGTTTCTACGGGGGGAAGTATTGTCGTTTTAATGACCATAAT TTTTATTTTAGCGATGATTTTTGCTCCTAAATATGGCATCATCAATCAAA ATACCAAAATATATTCTGCTTAACCTGTTTACTGATACTTCAAATAATCA TATAACCTATCTTCCGAGTTAAAAATAATGGATATTATCCAAGTGGGTC GAGAATAGAGTTTCTTTTTTGATAGAATTTTTTTACACCAGTTATTCATTA CTATCATGGGATAAT	39
Porf1542	TAATATAGTGATTATTATAAATGCAATGTGAATCAAACCTATATTTTACC GTACATTGACCATGGAACCTAATTTGAGGTGATTAGTAGAGGGTGCAT CGCCCTATTTGTCAAATAATAAAGATAACATTTGACATTGCTGATTGAAG ACATAAAACACAGAAAAAATCAGGTAAAAATATAAAGCTAAAGTCTAA ATATGGTTTACTTTTGCCTTCGACTTACAACAAAAAATCATAGCTAGAAT CACCAACGCCTAATATTTTATTTAGCTGAAATTTTGGGATGAACTTTTTG TAAAAATCGGGGGTCTAAAAATATAGCAACCACGATATTAAATAACTGA GTGATTATTTTAATCTATTGGGGGCTTATTAACCTAAATACTTGCATTTTA TGGAGGGTTTTAATT	40
Porf1823	AAAGATTATTTTCTACAGAAGCAACCCTTTCATCTTCCGAATTTTCAGGA ATTTCTGCTTTTGTCTGAATATTAGCATAGGCGGCTTTTGCCCACTCT AAAGAAGGTTGAGACTGAATTTCTGAGGTTTCAGAAGGAGCATTAGATT GTTTATCTTCAACAACAGGAGGTTTTTGTTCATATTTTCTTATTCTCTT TTTTACGGCGAAACCAATTAAACATAATGATTGTGCATAAATATTCGTTA ATATATTGTAACCTAGAAAGGAATCGGTTTCAGGTTTATCCCCAGAGA ATGTGAACCTTTACAGAAAGTAAAAAGTCTAAATCGTAGCAACAATAA ATCACAGAAATTGAG	41
Porf0222	GCGATTATCAACCACGAAAACATACAATTATTATCAAACCTGCTGAGAA ATTATCCACAGAAATAGATGTTTCTGCGAAGGGAAAATGGGCTTTTCATT GCCATTTAATGTATCAGATGGATGTGGGAATGTTTCGGACTATTAATGTT ATTTCTTAAAAAATAATAGTATTAAGCCTAAAATTTTTATAAAAAAATT CATGTCTTTTATTAGGGTGAGCATTCTTCCTTTATGTCTCCTTATTTTACC TCTTTAGAGGTAACCTACAACTTAATCAAAAAATTTAGATAATTAATTAT ATCA	42
Porf3232	CATCTTTACTTTTGACTAACATTTTCATAGGTATCATGACGAAAATTTTTTA GTCTGTTATATTTGTTTCATGTAGAGAGATTTTAATTTGTGATTATTTTATT TTCTCTCTATTTTTCTTTTTTGTCTTGTCTTCTCCTCATTTTTCTCTACATTA GTCTAAACTACAGCTCTTTAATCTTCAGTTTCTCTTCTCCTCTCTCTCA TCAAGGTAATCATCCCAATTAATATCTTCTTCTTGTCTAATTTGGGTTGA	43

	GATTGTTGTTTATCAATCATATTTTCATACTCCTAAAACCTTTCTTACTTATT TATCAGTTACTTTTTACCCATTTATGCAATAGTGTAGAAATTTTTTCGAT CGAGTTAATTAATTTTTATTTCAACCATATCTAAATAATTCTTGATGGAC ATTCTAGTTAACTAGAAGGTTTAAGCTAAAAATAATTATTGATATTGCCT TCGGTATAACTAACTATATCCAGAGAAAAAG	
Porf3749	CTCAAGAGATAGTTAAAAAACAAATAGCTTTAGTCTATCAATTAATCGA ATTATTTTTACAAACAAATTTTCATAAACCCATAGAACTAGAGGAGGAA GTTATTTATGTTTAAAAATCTAAAAGAGTTTATATTCCCCTAAAACCCC CTTAGTAAGAGTGACTTTTTTCATCATTTGCCTGTAAATTCTCCTCTTTTA ATAAGAGAGCTAGGGTGTTTAAAAAGAGGATTTTATTGCTTTCCAATTCT AACTACTTCAAAAACCTTATTTTATACTCAATAATTTATTAATCAAGAGGA AATTACC	44
PrbcL	TCGAGCGCTCGTTCCGCAAAGCGGTACGGAGTTAGTTAGGGGCTAATGG GCATTCTCCCGTACAGGAAAGAGTTAGAAGTTATTAATTATCAACAATTC TCCTTTGCCTAGTGCATCGTTACCTTTTTAATTAACATAAGGAAAACT AATAATCGTAATAATTTAACCTCAAAGTGTAAGAAATGTGAAATTCTG ACTTTTATAACGTTAAAGAGGGGAAAAATTAGCAGTTTAAATACCTAGA GAATAGTCTGGGGTAAGCATAGAGAATTAGATTAGTTAAGTTAATCAAA TTCAGAAAAAATAATAATCGTAAATAGTTAATCTGGGTGTATAGAAAT GATCCCCTTCATGATAAGATTTAAACTCGAAAAGCAAAAGCCAAAAAAC TAACTTCCATTAAAAAGAAAGTTGTTACATATAACGCTATAAAGAAAAATTA TATATTTGGAGGATACCAAC	45
PrnpA	AATAGTTGATAATTACTCGTTACTCATTACTCACTTAAACCTGCCACCTG ATACCTGCCACCTCTCCCCCATCACCTCATCCCCTCAACATTCCGAACC CCTTGACACTTTGAACTAAAATTGTATTAAGTGCAAATCTGGACGGGGT TAACCAAGTGTGACTTATAATAGTAAACGCTGTTTTTTATAATAAATAAGC TAAATATTTAAAAACTATGAGTAAATATACACTAAATGGTACTAGACGT AAGCAGAAAAGAACCTCCGGTTTCCGCGCCCGTATGAGAACCAAAAATG GTAGAAAAGTAATTCAAGCTCGTCGTAATAAGGGTAGAAAAAGATTAGC AGTATAAAATTACTGTAAATAAGGAAGCTAAGTTTAGCATTTTAAAGTTT GATATTACTAATCATTAAATTTACTGTGAAATATAGGTGGGACTACCATC AAAGCATCGACTGAAACGGCGTTTAAATTTCCAATCTGTTTATCAACAGG GTATTCGCCGCTCTAGTCGTTATTTTATTGTCCGAGGGTTACGG	46
PrpsL	CTCCGCTTAAAAAATTTTCATTTTTTCGATCAAAAAAGACAAATTATTACTA ATTAGCTCATGGCAATAAATAATCAGTAGTAATCTGTTTTACATTTTAT TGTTAATTTTTATTATTGCTAATATCAACCTTTTCTACTTCTGCTTAATATT TTATTTATGCTCAATGGGAAATCTGAAATAAGATTGAGAACAGTGTTA CCAATAGAAGTATTTAAGGTTTAAAGCATACCTTAAAGATAACATTTTTT TTTGAAAAGAGTCAAATTATTTTTGAAAGGCTGATATTTTTGATATTTAC TAATATTTTATTTATTTCTTTTCCCTTAAATAAGAGCTAAATCTGTTTT TATTATCATTTATCAAGCTCTATTAATACCTCAACTTTTTCAAGAAAAAA TAATAATAATTTTTCCCTCTATTCTCATGACCTTTTAGGAAAATTAATTTT AGAAAACTATTGACAAACCCATAAAAAATGAGATAAGATTATAGATTG TCACTGGTATTTTATACTAGAGGCAAATTATATTTATATATACAAAAATG CTGTATAAAAAACATCT	47
PrpoA	AGTAAAGATTATCACCAACATCTGAAACCTGACTTCATCAACTGAGGAA ATAACCACTGTGGCTGTGTTTAAATCGACTGCGTAGCAAGTAAACTC AAAAAATCAAGGTCAATACGGAAAGTTTGTGCTTGAACCCTTAGAAAA AGGACAAGGCATAACT	48

PpsaA	CTACATCAACTAATCAAAAAGTTAAGAAAAAAGATAGAAACGCCCATGAA TATTAAGATTAATCTGTGTCCTTTAACTTTTTATCCCCTTAAAAGAGCAT AACTAAAACATTGATAGATTTTATAAAGAAAAGTAACAAAATCTTGACT TAAATGAGAAAGGATTA AAAACCAAAGCCTTATCTGAGGGAATGTTAAA CAAATTTTAAATATTGTTAAGCAAGAACCACAATGGTGACAAATAGCCC TTATCATCTTCAGTAATGTAGTAGTTTAAGTATTTGTGCGAGAGAGGAATC CCTC	49
PpsbA2	GATCGAATTTTTGACTATTTAATAATTTCTTTACTATTTCATAATATCTCAA AAGACTTCTATCTTTTTAAGTAACTACCTCCTCTAAGAATAAACACTTA TTGACTATATTCCTTTTTAGTTATAAAATGGCATTATAAGTTACTCAAAA TATTTGCAATCATTCTACAAAACATAGTGTATTTCTTTGTATTAAGCGTA TTGTGTCTGTAGATAATGTAGGAAAGATTGTGAGTTGATAGGTGATAA ATACATAACTCATTAGACAACAAGATAAAGTTGTAGGAGTTCTAAATT	50
PpsbD	AAGAGTTTGGCATTTTTTATTGGTAAGACTATTCTGAGAAAAATGTGACAA TTTGTTAAATATTTGCTAGAAATAGAAAAAGTAATTTGGCAAAGATAC TTAAATCGTATCGAAAAACGGAGTTACATTA ACTCTAACTCATGTATAT TAAGAAAAGTTAATTGCAGATCAGTATTATTGCTGAGTAGCAGTGCCGT CTCCAATAATATAAAGAGAGACAATATAAAAAGTAAACTTGACAAGTTA AAAAAAGAAAGATT	51
PcpcB	AACTTTAGATATTCGTAGTTGGCAATGTCGTAAATGCGGAACAATACAT GGAAAACATATAGATTTGTAATGAGAAAAAGTGTAACAAATATTAAGA AAAAGATCAGAAAAATTTAACAACACGTAATAAAAAAATGCGTCACTAC GGGTTATAAATTTACATGAAAGGTTAAAACACTTTTCTGAGACGATTTTG ATAAAAAAGTTGTCAAAAAATTAAGTTTCTTTACAAATGCTTAACAAAA ACTTGTTTTAAGCACAAAATAAGAGAGACTAATTTGCAGAAGTTTTAC AAGGAAATCTTGAAGAAAAAGATCTAAGTAAAACGACTCTGTTTAACCA AAATTTAACAATTTAACAACAACTAAATCTATTAGGAGATTA ACT ACA	52
PcpcB*3	AACTTTAGATATTCGTAGTTGGCAATGTCGTAAATGCGGAACAATACAT GGAAAACATATAGATTTGTAATGAGAAAAAGTGTAACAAATATTAAGA AAAAGATCAGAAAAATTTAACAACACGTAATAAAAAAATGCGTCACTAC GGGTTATAAATTTACATGAAAGGTTAAAACACTTTTCTGAGACGATTTTG ATAAAAAAGTTGTCAAAAAATTAAGTTTCTTTACAAATGCTTAACAAAA ACTTGTTTTAAGCACAAACGCAAGAGAGACTAATTTGCAGAAGTTTTAC AAGGAAATCTTGAAGAAAAAGATCTAAGTAAAACGACTCTGTTTAACCA AAATTTAACAATTTAACAACAACTAAATCTATTAGGAGATTA ACT ACA	134

[00228] In an embodiment, the promoters of any of the above embodiments may be selected from the endogenous inducible promoters identified in *Cyanobacterium* sp. with the ATCC accession number PTA-125253 (“ABCyano4”) as listed in table 3B below, and variants thereof.

Table 3B: *Cyanobacterium* sp. ABCyano4 endogenous promoter sequences

Promoter	Sequence	SEQ ID NO:
PnirA	CATTCCCTCATCTCTCTCCCCAATAGATGCAAAAAACGAATTAAAATTA TGTGCAAAAAGAAAAATAAGCCTTTATTTAGTAGTCAAAGTTACAAAATG TTAAGAATCAAATTAATAATGTATTGGGCAGTTAAGTATATAAATCTTT AAATATTTATTTGTATTCAATATATTAATCGAGGACACATT	135
PsmA	CTCCTATTCCTAGATCTCGTAATGAATCATTACTCTCTGAAATATACCTC AATTTTATGGTTGTTTGTAATAATATCTTAATAAATGAACAAGCAATCAT ATTTTGTGTAGTCTAATTATTAATAATTACTGAATCTATATTCAGATA TTCACAAATAGGAGAATTTTGATT	136
PpetE	AAATTAAGCAAAAACTCTCTTCACAGAAGACAATGAAAACCTTATTTA CGTATTACTGAGACCCGAATCTTACGCCACCGTAAGCATCCCGTATTG CTGCTACCTTCCGGTCCTGACAAGGTTTGAGCGTCACAGTTGCATAAGT CCGAGTCAGTGACCATTATAACTTAATCTCCTCAACAAACACAAGGTTA ATTGTCCAAAACAATTTTTTAAATTAGATATGACATTAGGTCAAAGTTC ATAGTATGATAGTAGAAAATAAAATTTGACGATCTGTAAAAATAAAAA AAATACA	137
Porf01460	TTAATTTTCGTAACAAATAATACTTAATTTTTTAACCCTTTATTTTATCCCA AACTCAAATCTATTCTTAAATCTTAATTACCCTTTACCCAAACCTAAACA GTAAACCTGTAAACTAGCTTAAAGTAATTGACTCTCTAGCCTACTAGA AGGTTTAACGTATAAATAGAACAAATTTTTTCCTTTAGTGAATTGGAATT GAACTGATT	138
PrbcL	CGATGCTAGAGATGAATTTATATATCAAACAGACTTAGTGAGAAGGGC AAAAAGATATTTTCACCGTGATAATTTACCTTATCCTTTACTTCCCCCT TAATCATAATTAGGGCTGTTTGGGGAAATTTCTTTTTTATCTGAACCTCG AACTCAAATAATTGTCAATTTTCTTCATGATATAAGAAAAACTAATAACT ATAATAATATAATCTCAAAGTGTAAGAAATGTGAAATTTTGACCTTTA TACCGTTAAAAAAGCAAAATGAGCAGATTAAAAATACCCAGAGAATAG TCTAGGGTAAGCATAGAGAATTAGATTAGTTAAGTTAATCAAATTCAGA AAAAATAATAATCGTAAATAGTTGATCTAGGTGTATAGAAAATGATCCC CTTCATGATAAGATTTAAACTCGAAAAGCAAAAGCCAAAAAACTAACT TCCATTAAGAAAGTTGTTACATATAACGCTATAAAGAAAATTTATATA TTTGGAGGATACCAAGC	139
PpsaL	TACGCCTCGCTTTTCGATTTGTAAGAATAATAATCCCATTACGACAGCAG GCATAACTAAGCCAACAGCAGGAATAAAATGGCAGGTAAGAAAGAA GCCGCAAAATCTCCAAGAATCATTTTTTTTATTTTCCTATACTAAAAAAG TTAACCTAGATGGATATTACTGCTAAGTTTAACCAAAAAGAATCAAATA TAAAGATTCTTAACAAAAAAATGTGAATAGACTCCGATCAAGTTAAAGT AAGCTAAAAAATACTTAATTATTTGATAACCAATTTAAGGAGATCGTATA AAAAACA	140
PpsaA	TCGATTGTAAAGGGTTAATTGTCAATTACTAAATGTAAAAGAAAAAATT TATTATTAATATTTGTTTTGTCTATAGTCAGTTTGATAAATTATGATAT CTACTTGAGTCAATCAAAGTTAAGAAAAACAGAGAACTCCCATGA ACATTAAAGATTCATCTCCATCTTTTTATTTTTTATCCCCTTAAAGACC ATAACTAAAAACATTGATAGACTTTATAAAGAAAAGTAACAAAATCTTG ACTTAAATGAGAAAGGATTAAAAACCAAGCCTTATCTGAGGGAATGT TAAATAAATTTTAAATATTGTTAAGCAAGAACCACAATGGTGACAAATA GCCCTTATCATCTTCAGTAATGTAGTAGTTAAGTATTTGTGAGAGAG GAATCCCTC	141
PpsaD	GAGGCGATCGCATCTTATTCTCAAGGATTTAAAGGGGCATACCTATTCC AAAAACCAACACAGACGAGGGAATAGCCTTTATTCTTTGGGAAAAAA TAGAAGATATGGAAGAAAACAAAACGAAGTCCATAACCAAATTATGG	142

	AACAAATGAAGCATTTATTTGCTACCCCTCCTCAAACCAGTTTTTATGAT GTTTTAACAGAATTTTCTCCTTAATAAGAATTTACACTTGAAGGATTAA AGAAATAAACTGTTACAGTTCATAACAAAGATGCACAATAATGGGGAT CTCAGTGGTAGCATTGCCTAGATAGTAAATATAATTTGTACAAAAGCAG TATCTCT	
PpsaF	TTAATCATTTCCAAATGACGACGGGAAGCCAACCTCAGGCACAACCTCCGC CATACTGCTCATGTAGTTTGATTGCGAAGCCACTACATTAGCGATAAC ATTACGATTTGTTACAATGGCGACGCTAGTTTCATCACAACCTACTTTCAA CTGCTAAAATTGTGGGCATTATTTTCTTGATTGTGAAGAATAATAAATT TTCTAACTATTACTTTACTCCATTTACTAATCACAGTAAATCGCATTTG AAGTCATTTTTTCGGCTTCATTTTTGTAAACAAAACTTATTGTTTCGTA GCAAAAGGAAACAATTTT	143
PpcpB	ATGAGAAAAAATGTAAACAAATATTAAGGAAAAAGATCAGGAAAAATTTA ACAACACGTAATAAAAAAAGGTGTCACTACGGGTTATAAATTTACATG AAAGGTTAAAAACACTTTTCTTAGACGATTTTTGTAAAAAAGTTGTCAAA AAATTAAGTTTCTTTACAAATGCTTAACAAAACTTGGTTTTAAGCACA AAATAAGAGAGACTAATTTGCAGAAGTTTACAAGGGAATCTTGAAGA AAAAGATCTAAGTAAACTACTCTGTTTAACCAAAATTTAACAATTTA ACAAAACAACTAAATCTATTAGGAGATTAACTAAAA	144
PpetE*	AAATTAAGCAAAAAACTCTCTTCACAGAAGACAATGAAAACCTT ATTTACGTATTACTGAGACCCGAATCTTACGCCACCGTAAGCAT CCCGTATTGCTGCTACCTTCCGGTCTGACAAGGTTTGAGCGTCA CAGTTGCATAAGTCCGAGTCAGTGACCATTATAACTTAATCTCCT CAACAAACACAAGGTTAATTGTCCAAAACAATTTTTTAAATTAG ATATGACATTAGGTCAAAGTTCATAGTATGATAGTAGAAAAATAA AATTTGACGATCTGTAAAAGGAGAATTTTGATT	145
Porf01460	TTAATTTTCGTAACAAATAATACTTAATTTTTAACCCTTTATTTTATCCCA AACTCAAATCTATTCTTAAATCTTAATTACCTTTACCCAAACCTAAACA GTAAACCTGTAAACTAGCTTAAAGTAATTGACTCTCTAGCCTACTAGA AGGTTTAACGTATAAATAGAACAAATTTTTTCCTTTAGTGAATTGGAATT GAACTGATT	146
Porf01460*2	TTAATTTTCGTAACAAATAATACTTAATTTTTAACCCTTTATTTTATCCCA AACTCAAATCTATTCTTAAATCTTAATTACCTTTACCCAAACCTAAACA GTAAACCTGTAAACTAGCTTAAAGTAATTGACACTCTAGCCTACTAGA AGTGTATACTATAAATAGAACAAATTTTTTCCTTTAGTGAATTGGAATTG AACTGATT	147
PoprB	GCCCTAATAATTTTATAAAACAAGACAAAGTTAAACCAGTCTTAACCTT TAAGGTTTATGATGGACGATGAAAAAACACACCATTATTTTCCAGCTAC TTAATAGTTTTTTTTCTATTACTATATAATAGCTATCTGATAACAAAA AAATAAAAAAGTTGTTATCTTAGTTACA	148
PrpsO	GGATTCGGAGACTACCCGCCATATTTCTACTGTTAGGGGAGTTGGCTAC AAGTTTGATATTGAAAATAGTTAACTAATTGTTGTAATTTATTTGTTTA TGATAGCAGATTTGGAAAGAGAGAATAGTTAATAGTTGATTATTAATAA TATTATTCCGAACCTCAAATAGTTCCTAATTAAAATCAGTTTATGATATAA TCATAGGT	149
PpsaC	GGAGTTTTCTTAATCTTAAGAGACACTGCAAATCTCGCTTTCTTCCAACA ATTATTACCGGAATAGATTTTTACGGCACATTCAAAAGGTAATAGGTAT AGGGTATCAGGTAAACCTAAAATGTATTCAAATTGTATTTTTTGTGCTT ATTAATTTTTAACTCAATCATTGATTTTTATCTATCGTTGTCCATCCTA AAAGAAGAAAAATTGTTACAGAGTTTGAAGTTATATATCAGAAAAGGT GACAGAGATTATATACTGCGTTTA	150
PpcpA	TATCCTCAGCGATTTTCGGCAACAACCTTTGGGATCGATGCCGTAGCCTAA GTTTCCCATTAAGGCTTCACCGCTTAATTTTAATAAAATCCGTTGGTAAG TCATTCTCGGTTGCCTTCATATTCGATAAATTTTCTTCAGTTAAGATAC CAGTTTTTGACTACCATAAATTAGTTTTTTTTTATTAACAACCTACGATAT	151

	GGATAATCAATATCCTCATCCCAATAATTGATAGGTTTTAATTTTCATTG ACAAAACGCAATAAAACTTTACTAAATTCTAGGGAAAACCTGCACGGAG TTTGCACAAAGATTCTGTTTAAATAATCA	
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[00229] In certain other preferred embodiments, truncated or partially truncated versions of these promoters including only a small portion of the native promoters upstream of the transcription start point, such as the region ranging from -35 to the transcription start can often be used. Furthermore, introducing nucleotide changes into the promoter sequence, e.g. into the TATA box, the operator sequence and/or the ribosomal binding site (RBS) can be used to tailor or optimize the promoter strength and/or its induction conditions, e.g. the concentration of inductor required for induction.

[00230] The following Table 3C shows the nucleotide sequences of two intergenic regions IScpBA and IScpBA*1 employed to enhance the expression of the enzymes involved in the production of MAAs as described herein.

Table 3C:

Intergenic sequence	Sequence	SEQ ID NO:
<u>IScpBA</u>	TTTAAACCAAGATTAGAAAATCCATTTTCATTAACGT AAACCAACATAATTAGGAGAAATTAATTACA	177
<u>IScpBA*1</u>	TTTAAACCAAGATTAGAAAATCCATTTTCATTAACGT AAACCAACATAATTAGGAGGATCAGCACA	178

Plasmid Vector Construction

[00231] In an embodiment, the cyanobacterial strain ABICyano1 or ABCyano4 is transformed with heterologous genes in order to produce the MAA. Methods for producing a genetically enhanced, non-naturally occurring *Cyanobacterium* sp. and ABICyano1 as well as ABCyano4 host cells are disclosed herein. In an embodiment, methods include introducing a recombinant nucleic acid sequence into a cyanobacterial host cell. At least one recombinant gene can be introduced into the host cells through the transformation of the host cell by an extrachromosomal plasmid. In an embodiment, the extrachromosomal plasmid can independently replicate in the host cell. In another embodiment, at least one recombinant gene can be introduced into the genome of the host cell. In yet another embodiment, at least one recombinant gene is

introduced into the genome of the host cell by homologous recombination. In another embodiment, at least one recombinant gene can be introduced into the endogenous plasmids of the host cell. In yet another embodiment, at least one recombinant gene is introduced into the endogenous plasmids of the host cell by homologous recombination.

[00232] In an embodiment, a recombinant gene is present on the chromosomal DNA of the cyanobacterial cell. In another embodiment, a recombinant gene is present on an extrachromosomal plasmid that can replicate independently from the chromosomes of the cyanobacterial cell. In an embodiment, the extrachromosomal plasmids described herein are present in high numbers in the host cells so that a compound of interest can be produced in a high yield.

[00233] Genetically enhanced cyanobacterial cells, for example ABICyano1 or ABCyano4 host cells, can include further genetic enhancements such as partial deletions of endogenous genes or other recombinant genes which can increase the overall yield of the compound being produced by the host cells. Examples of such genetic enhancements are described in PCT patent publication number WO 2009/098089 A2, which is hereby incorporated by reference in its entirety.

[00234] In another embodiment, genetic enhancements of the genes encoding enzymes of the carbon fixation and subsequent carbohydrate metabolism can be genetically enhanced to further increase the production of a compound of interest. Genetic enhancement targets include, but are not limited to, components of the photosystems (antennas and pigment modification), and components of the photosynthetic and respiratory electron transport systems. Genetic enhancement targets include local and global regulatory factors including, but not limited to, the two component system, sigma factors, small regulating RNAs and antisense RNAs.

[00235] In an embodiment, a recombinant nucleic acid sequence can be provided as part of an extrachromosomal plasmid containing cyanobacterial nucleic acid sequences in order to increase the likelihood of success for the transformation.

[00236] In another embodiment, the method for producing a genetically enhanced *Cyanobacterium* sp. host cell uses an extrachromosomal plasmid derived from an endogenous plasmid of the host cell to introduce a recombinant nucleic acid sequence into the host cell. This endogenous plasmid can be, for example, an extrachromosomal plasmid derived from the 6.8 kb endogenous plasmid of ABICyano1.

[00237] In an embodiment, the cyanobacterial strain ABICyano1 is transformed with at least one heterologous gene in order to produce the desired MAA. It has been found that the use of modified endogenous plasmids improves the stability of the plasmid in the host cell.

[00238] The cyanobacterial strain ABICyano1 contains three endogenous plasmids. In combination with other genotypic and phenotypic attributes, these endogenous plasmids differentiate ABICyano1 from other cyanobacterial species. One plasmid is 6,826 base pairs (SEQ ID NO: 16), another is 39,702 base pairs, and a third plasmid is 28,554 base pairs. The 6,826 bp endogenous plasmid is alternatively referred to herein as pABICyano1, p6.8 or 6.8. As disclosed herein, plasmid 6.8 has been modified *in vivo* and *in vitro* for use as a plasmid vector containing genes of interest for the production of compounds of interest. In an embodiment, a modified endogenous vector derived from p6.8 from ABICyano1 was developed. The modified endogenous vector from ABICyano1 can be used to transform cyanobacteria from a broad range of genera, including ABICyano1 itself.

[00239] In an embodiment, the cyanobacterial strain ABICyano4 is transformed with at least one heterologous gene in order to produce the desired MAA. It has been found that the integration of heterologous genes into the endogenous plasmids improves expression of the respective enzymes. The cyanobacterial strain ABCyano4 comprises three endogenous plasmids, which, in combination with other genotypic and phenotypic attributes, differentiate ABICyano4 from other *Cyanobacterium* species. One plasmid herein termed “pABCyano4B” is 37990 base pairs. A second, smaller plasmid “pABCyano4C” is 31678 base pairs. In an embodiment, both plasmids can be used for integration of heterologous genes via homologous recombination.

[00240] In certain embodiments, the present invention includes the p6.8 plasmid or the pABICyano1B plasmid, and modified vectors comprising sequences of these plasmids. In an embodiment, the modified endogenous vector contains at least one of the following: a recombinant gene that encodes an enzyme involved in MAA production; and an origin of replication suitable for replication in ABICyano1.

[00241] Other suitable plasmids that can be used to carry genes of interest in several strains of cyanobacteria (such as, for example, *Synechocystis*, *Synechococcus* PCC7002 and *Anabaena*), are the RSF1010-based shuttle plasmids (extrachromosomal broad-host vector) such as pVZ321-6 series (described, for example, in PCT/EP2009/060526 and in PCT/EP2009/000892, both of which are incorporated herein by reference in their entireties). Further, the plasmid vector

described in U.S. Patent NO: 9,476,067 can be utilized to carry heterologous genes of interest in many cyanobacterial species.

[00242] In certain embodiments, a gene coding for a replication initiation factor that binds to the origin of replication can either be present on the modified endogenous vector or can be present in the chromosomes or other extrachromosomal plasmids of ABICyano1. An origin of replication suitable for replication in ABICyano1 and the gene coding for the replication initiation factor binding to that origin of replication ensure that the modified endogenous vector can be replicated in ABICyano1.

[00243] In an embodiment, the nucleotide sequence of an origin of replication of the modified endogenous plasmid vector can have at least 80%, 90%, and 95% identity or can be identical to the nucleotides 3375 to 3408 of the sequence of the endogenous 6.8 kb plasmid (SEQ ID NO: 16).

[00244] In an embodiment, the sequence of the gene coding for the replication initiation factor has at least 80%, 90%, and 95% identity or is identical to nucleotides 594 to 3779 of the sequence of the endogenous 6.8 kb plasmid (SEQ ID NO: 16). In an embodiment, the gene coding for the replication initiation factor codes for a protein having at least 80%, 90%, and 95% sequence identity or is identical to the protein coded by nucleotides 594 to 3779 of the sequence of the endogenous 6.8 kb plasmid (SEQ ID NO: 16) of ABICyano1. This putative initiation replication factor is thought to bind to the putative origin of replication, thereby ensuring the replication of a plasmid containing the initiation factor in ABICyano1.

[00245] In an embodiment, a modified endogenous plasmid vector can contain a sequence having at least 50%, at least 55 %, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% identity to the sequence of the endogenous 6.8 kb plasmid (SEQ ID NO: 16). In another embodiment, the modified endogenous vector contains the entire p6.8 endogenous plasmid from ABICyano1.

[00246] In another embodiment, gene delivery vehicles that are developed using the endogenous 6.8 kb plasmid (or a portion of the plasmid) containing characteristic portions of the endogenous 6.8 kb plasmid may be able to be efficiently transformed into a wide range of cyanobacteria. In an embodiment, characteristic portions of the 6.8 kb endogenous plasmid from ABICyano1 include portions that enable it to replicate in a host cell (origin of replication and replication initiation factor, for example) and can be referred to as the backbone of the endogenous

6.8 kb plasmid. Such vectors may also be able to efficiently produce heterologous proteins and other compounds of interest in cyanobacterial cultures.

[00247] In another embodiment, modifications starting with the backbone of the 6.8 kb endogenous plasmid from ABICyano1 are performed individually or together to increase transformation efficiency, increase the replication rate within the cell, and to increase the production of a desired product from the cyanobacterial cell. Suitable modifications include, for example, insertion of selection markers (such as antibiotic resistance genes), recombinant genes or cassettes for the production of a desired compound, and other modifications to increase the expression or stability of the plasmid in the cyanobacterial cell. In an embodiment, the invention includes cyanobacteria, e.g. ABICyano1, comprising a modified p6.8 plasmid having any of these improved characteristics.

[00248] In an embodiment, the modular design of the p6.8 derived vector allows complex sequence manipulation in cyanobacteria.

[00249] Several exemplary p6.8-derived plasmids and pABICyano1B plasmids, respectively, carrying genes encoding enzymes to produce MAAs are listed below in Table 4A. The plasmid maps of these constructs are shown in the FIG. as indicated below.

Table 4A: ABICyano1 p6.8 and pABICyano1B derived plasmids for production of an MAA

Plasmid Name	Present in Strain #	Figure	SEQ ID NO:
ABICyano1 P6.8 (Native)		14	16
ABICyano1 pABICyano1B (Native)		N/A	53
#2737\pABICyano1_6.8::Porf0223*- Ava_3858(ABICyano1opt)-IScpcBA*1- Ava_3857(ABICyano1opt)-IScpcBA*1- Ava_3856(ABICyano1opt)-TpsaB	AB1224	5A	54
#2742\pABICyano1_6.8::PsmA- Ava_3858(ABICyano1opt)-IScpcBA*1- Ava_3857(ABICyano1opt)-IScpcBA*1- Ava_3856(ABICyano1opt)-TpsaB	AB1225	5B	17
#2752\pABICyano1_6.8::PcpcB*3- Ava_3858(ABICyano1opt)-IScpcBA*1- Ava_3857(ABICyano1opt)-IScpcBA*1- Ava_3856(ABICyano1opt)-TpsaB	AB1226	6	18
#2796\pABICyano1B_Rep::PrpsL-Gm**-TB0014- oriVT-PsmA-NpF5597(ABICyano1opt)	AB1252 (plasmids #2742 + #2796)	7	19
#2798\pABICyano1B_Rep::PrpsL-Gm**-TB0014- oriVT-PsmA-ap3855(ABICyano1opt)	AB1253 (plasmids #2742 + #2798)	8	20

#2840\pABICyano1_6.8::PsmtA-Ava_3858(ABICyano1opt)-IScpBA*1-Ava_3857(ABICyano1opt)-IScpBA*1-Ava_3856(ABICyano1opt)-TpsaB-Porf0221-ap3855(ABICyano1opt)	AB1277	9	21
#2794\pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-Ava_3855(ABICyano1opt)-TB0050	AB1251 (plasmids #2742 + #2794)	15A	55
#2880\pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-mysAB_Pum(ABICyano1opt)-TpsaB	AB1323	15B	56
#2881\pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-mysCD_Pum(ABICyano1opt)-TpsaB	AB1302 (plasmids #2742 + #2881)	15C	57
#2882\pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-mysCD_Chc(ABICyano1opt)-TpsaB	AB1303 (plasmids #2742 + #2882)	15D	58
#2893\pABICyano1_6.8::PsmtA-Ava_3858(ABICyano1opt)-IScpBA*1-Ava_3857(ABICyano1opt)-IScpBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA-ap3855(ABICyano1opt)	AB1324	5151	111
#2966\pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-Tery_2972(ABICyano1opt)-IScpBA*1-mysD1_IMS101(ABICyano1opt)-TpsaB	AB1356 (plasmids #2742 + #2966)	5252	114
#2968\pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-Tery_2972(ABICyano1opt)-mysD1_IMS101(ABICyano1opt)-IScpBA*1-mysD2_IMS101(ABICyano1opt)-IScpBA*1-Tery_2969(ABICyano1opt)-TpsaB	AB1358 (plasmids #2742 + #2968)	5353	115
#2970\pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-Tery_4684-IScpBA*1-Tery_2968(ABICyano1opt)-IScpBA*1-Tery_2966(ABICyano1opt)-TpsaB	AB1360 (plasmids #2742 + #2970)	5454	116
#3049\pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA1710-mylD_QSY1(ABICyano1opt)-IScpBA*1-mylE_QSY1(ABICyano1opt)-IScpBA*1-mylC_QSY1(ABICyano1opt)-TpsaB	AB4033 (plasmids #2742 + #3049)	5555	125

[00250] Several exemplary plasmids for integration of genes into the endogenous plasmids pABCyano4B and pABCyano4C of ABCyano4, respectively, carrying genes encoding enzymes to produce MAAs are listed below in Table 4B. The plasmid maps of these constructs are shown in the Fig. as listed above in Fig. 4A.

Table 4B: Integrative plasmids for production of a MAAs in ABCyano4

Plasmid Name	Generated Strain #	Figure	SEQ ID NO:
pABCyano4B (Native)	ABCyano4		
pABCyano4C (Native)	ABCyano4		
#2906\oriVT-pABCyano4B_HFL1-PsmtA-Ava_3858(ABICyano1opt)-ISpcBA*1-Ava_3857(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-pABCyano4B_HFL2	AB1312 (plasmid #2906)	56	112
#2865\oriVT-pABCyano4B_HFL1-PsmtA-Ava_3858(ABICyano1opt)-ISpcBA*1-Ava_3857(ABICyano1opt)-ISpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-pABCyano4B_HFL2	AB1322 (plasmid #2865)	57	107
#2891\oriVT-pABCyano4C_int1-PsmtA-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCyano4C_int2	AB1333 (plasmids #2865 + #2891)	58	109
#2892\oriVT-pABCyano4C_int1-PsmtA-ap3855(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCyano4C_int2	AB1334 (plasmids #2865 + #2892)	59	110
#2848\oriVT-yef37_ABCyano4_up-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-yef37_ABCyano4-down	AB1361 (plasmids #2865 + #2848)	60	108
#2983\oriVT-pABCyano4B_HFL1-PcpcB-Ava_3858(ABICyano1opt)-ISpcBA*1-Ava_3857(ABICyano1opt)-ISpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Km**-TB0014*-pABCyano4B_HFL2	AB1365 (plasmid #2983)	61	117
#2992\oriVT-pABCyano4C_int1_HFL1-PsmtA-Ava_3858(ABICyano1opt)-PrbcL*-mysD_Pum(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCyano4C_int2	AB4015 (plasmids #2865 + #2992)	62	119
#3004\oriVT-pABCyano4C_int1-PsmtA-mysD_HL-69(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCyano4C_int2	AB4016 (plasmids #2865 + #3004)	63	121
#2991\oriVT-pABCyano4C_int1_HFL1-PsmtA-Ava_3858(ABICyano1opt)-PrbcL*-NpF5597(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCyano4C_int2	AB4028 (plasmids #2865 + #2991)	64	118
#3036\oriVT-pABCyano4C_int1-PsmtA-NpF5597(ABICyano1opt)-Tery_2969(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCyano4C_int2	AB4031 (plasmids #2865 + #3036)	65	122
#2907\oriVT-pABCC1710B_HFL1-PsmtA-Ava_3857(ABICyano1opt)-ISpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-pABCC1710B_HFL2	AB4035 (plasmids #2907 + #3001)	66	113

#3001\oriVT-pABCC1710C_int1-PsmtA-mysA_HL-69(ABICyano lopt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2		67	120
#2907\oriVT-pABCC1710B_HFL1-PsmtA-Ava_3857(ABICyano lopt)-IScpcBA*1-Ava_3856(ABICyano lopt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-pABCC1710B_HFL2	AB4036 (plasmids #2907 + #3002)	77	152
#3002\oriVT-pABCC1710C_int1-PsmtA-mysA_IMS101(ABICyano lopt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2			
#3051\oriVT-pABCyano4C_int1-PsmtA-mysD_K005(ABICyano lopt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCyano4C_int2	AB4046 (plasmids #2865 + #3051)	68	126
#3038\oriVT-pABCC1710B_HFL1-PpsaL-Ava_3858(ABICyano lopt)-IScpcBA*1-Ava_3857(ABICyano lopt)-IScpcBA*1-Ava_3856(ABICyano lopt)-TpsaB-Toop*1-PrbcL-Km**-TB0014*-pABCC1710B_HFL2	AB4049 (plasmid #3038)	69	123
#3039\oriVT-pABCC1710B_HFL1-PpsaA-Ava_3858(ABICyano lopt)-IScpcBA*1-Ava_3857(ABICyano lopt)-IScpcBA*1-Ava_3856(ABICyano lopt)-TpsaB-Toop*1-PrbcL-Km**-TB0014*-pABCC1710B_HFL2	AB4050 (plasmid #3039)	70	124
#3055\oriVT-pABCyano4C_int1-PsmtA-Tery_2972(ABICyano lopt)-IScpcBA*1-mysD1_IMS101(ABICyano lopt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCyano4C_int2	AB4055 (plasmids #2865 + #3055)	71	127
#3094\oriVT-pABCyano4C_int1-PsmtA1710-Tery_2966-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCyano4C_int2	AB4074 (plasmids #2865 + #3094)	72	128
#3126\oriVT-pABCC1710B_HFL1-PsmtA-Ava_3858(ABICyano lopt)-IScpcBA*1-Ava_3857(ABICyano lopt)-IScpcBA*1-Ava_3856(ABICyano lopt)-TpsaB-PsmtA-Amir_4256(ABICyano lopt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710B_HFL2	AB4092 (plasmid #3126)	73	132
#3125\oriVT-pABCC1710C_int1-PsmtA-mysA_HL-69(ABICyano lopt)-TpsaB-PsmtA-mysD_HL-69(ABICyano lopt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4094 (plasmids #2865 + #3125)	74	131
#3115\oriVT-pABCC1710B_HFL1-PpetE*-Ava_3858(ABICyano lopt)-IScpcBA*1-Ava_3857(ABICyano lopt)-IScpcBA*1-Ava_3856(ABICyano lopt)-TpsaB-Toop*1-PrbcL-Km**-TB0014*	AB4095 (plasmid #3126)	75	130
#3146\oriVT-pABCyano4B_HFL1-PsmtA-mysA_IMS101(ABICyano lopt)-IScpcBA*1-mysB_IMS101-IScpcBA*1-mysC_IMS101-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-pABCyano4B_HFL2	AB4097 (plasmid #3146)	76	133

#3077\ pABCC1710C_int1-PsmtA-mysA_HL-69(ABICyano lopt)-PrbcL*-mysD_HL-69(ABICyano lopt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT	AB4106 (plasmid #3077 + #3127)	80	155
#3127\ pABCC1710B_HFL1-PsmtA-mysB_HL-69-ISpcBA*1-mysC_HL-69-TpsaB-Toop*1-PrbcL-ble(ABICyano lopt)-TB0014		81	156
#3034\ pABCC1710C_int1-PsmtA-mysD1_IMS101(ABICyano lopt)-TpsaB-Toop*1-PrbcL-ble(ABICyano lopt)-TB0014	AB4107 (plasmid #3146 + #3034)	78	153
#3035\ pABCC1710C_int1-PsmtA-mysD2_IMS101(ABICyano lopt)-TpsaB-Toop*1-PrbcL-ble(ABICyano lopt)-TB0014	AB4108 (plasmid #3146 + #3035)	79	154

[00251] Several exemplary plasmids for expression of MAA synthesis genes in *Escherichia coli* are listed below in Table 4C. The plasmid maps of these constructs are shown in Fig. as described in table 4C.

Table 4C: Plasmids for production of a MAAs in *Escherichia coli*

Plasmid Name	Generated Strain #	Figure	SEQ ID NO:
#2870\ pJet1.2::lacI-Ptac-Ava_3858(ABICyano lopt)-ISpcBA*1-Ava_3857(ABICyano lopt)-ISpcBA*1-Ava_3856(ABICyano lopt)-TB0011	BL21 #2870	82	179
#3117\ pJet1.2::lacI-Ptac-Ava_3858(ABICyano lopt)-ISpcBA*1-Ava_3857(ABICyano lopt)-ISpcBA*1-Ava_3856(ABICyano lopt)-TB0011-Ptac-Tery_2972(ABICyano lopt)-ISpcBA*1-mysD1_IMS101(ABICyano lopt)-TpsaB	BL21 #3117	83	180
#3119\ pJet1.2::lacI-Ptac-Ava_3858(ABICyano lopt)-ISpcBA*1-Ava_3857(ABICyano lopt)-ISpcBA*1-Ava_3856(ABICyano lopt)-TB0011-Ptac-NpF5597(ABICyano lopt)-Tery_2969(ABICyano lopt)-TpsaB	BL21 #3119	84	181
#3120\ pJet1.2::lacI-Ptac-Ava_3858(ABICyano lopt)-ISpcBA*1-Ava_3857(ABICyano lopt)-ISpcBA*1-Ava_3856(ABICyano lopt)-TB0011-Ptac-Tery_2966(ABICyano lopt)-TpsaB	BL21 #3120	85	182
#3129\ pJet1.2::lacI-Ptac-Ava_3858(ABICyano lopt)-ISpcBA*1-Ava_3857(ABICyano lopt)-ISpcBA*1-Ava_3856(ABICyano lopt)-TB0011-Ptac-Tery_4684-ISpcBA*1-Tery_2968(ABICyano lopt)-TpsaB-Ptac-Tery_2966(ABICyano lopt)-TpsaB	BL21 #3129	86	183

Table 4D: List explaining the abbreviations

ABICyano1opt	Codon usage adapted to ABICyano1 version 1
ISpcBA*1	Modified intergenic sequence of cpcBA operon
TpsaB	Terminator sequence from psaB
pABICyano1B_Rep	Replication region of the endogenous plasmid pABICyano1B
Gm**	Gentamycin resistance codon adapted to ABICyano1
TB0014, TB0014*, TB1002, TT7, Toop*1	Terminator sequence
pABCyano4B_HFL1	Homologous upstream flanking region for pABCyano4B
pABCyano4B_HFL2	Homologous downstream flanking region for pABCyano4B
ble(ABICyano1opt)	Bleomycin resistance codon adapted to ABICyano1
pABCyano4C_int1	Homologous upstream flanking region for pABCyano4C
pABCyano4C_int2	Homologous downstream flanking region for pABCyano4C
Km**	Kanamycin resistance codon adapted to ABICyano1
FRT	Binding site of flippase
flp	Flippase coding gene
ABCyano4opt1	Codon usage adapted to ABCyano4 version 1

[00252] In yet another embodiment, the method for producing a genetically enhanced cyanobacterial cell involves protecting the recombinant nucleic acid sequence, for example a plasmid, against endogenous restriction endonucleases of the host cell by methylating at least a part of the recombinant nucleic acid sequence or modifying and/or eliminating the recognition sequences of the endogenous restriction endonucleases. By changing the nucleic acid sequence of potential recognition sites of restriction endonucleases, a digest of the recombinant nucleic acid sequence can be avoided. It was discovered that endogenous restriction endonucleases of ABICyano1 and ABCyano4, for example, can cut an extrachromosomal plasmid carrying recombinant genes, thereby preventing genetic transformation of this host cell.

[00253] In an embodiment, methyltransferases, for example M. *AvaI*, M. *AcyI* and M. *MspI* can be used to protect recombinant vector extrachromosomal plasmids. The plasmids can either be incubated with the methyltransferases *in vitro* or a helper plasmid can be present in a helper *E. coli* strain in order to methylate the extrachromosomal plasmids *in vivo* before conjugation takes place during the transformation of ABICyano1, ABCyano4 or other cyanobacterium. In another

embodiment, recognition sequences for the restriction enzymes can be modified or deleted. As described by Elhai and Wolk, in “Conjugal Transfer of DNA to Cyanobacteria” *Methods in Enzymology* 167:747-754 (1988), herein incorporated by reference, plasmid pRL528 can be used as a helper plasmid for conjugal transfer. The indicated genes are M. *AvaI* coding for the methyltransferase protecting against the restriction endonuclease *AvaI* and the respective gene coding for M. *AvaII*. The latter is not required for transformation of ABICyano1, as it lacks any endonuclease activity of *AvaII*. M. *MspI* from *Moraxella* sp. ATCC 49670 is required for transformation of ABCyano4.

[00254] In an embodiment, the vector to be transformed into *Cyanobacterium* sp. host cells can be modified to integrate into the cyanobacterial chromosome by adding an appropriate DNA sequence homologous to the target region of the host genome. In another embodiment, the vector to be transformed can be modified to integrate into the cyanobacterial chromosome through *in vivo* transposition by introducing mosaic ends to the vector. Once the plasmid is established in the host cell, it can be present, for example, at a range of from 1 to many copies per cell.

[00255] In an embodiment, an endogenous plasmid derived from ABICyano1 can be modified, either *in vivo* or *in vitro*, to be a plasmid vector capable of introducing exogenous genes encoding enzymes for the production of a compound or compounds of interest into a wide range of host cyanobacterial cells such as ABICyano1, *Cyanobacterium* sp., or other cyanobacterial genera such as *Synechocystis* and *Synechococcus*.

[00256] In an embodiment, the ABICyano1 6.8 kb endogenous plasmid is used as a backbone for a plasmid vector used for transformation of *Cyanobacterium* sp. Since this is the endogenous vector from the species, it is likely to be more stable when transformed into the cell than plasmids derived from completely different organisms. In an embodiment, the entire p6.8 endogenous plasmid is inserted into a vector used for transformation. In another embodiment, a sequence of about 50%, 70%, 75%, 80% 85%, 90%, 95%, 98%, 99%, or 99.5% identity to the entire endogenous plasmid sequence is inserted into the extrachromosomal plasmid vector.

[00257] In an embodiment, a modified p6.8 vector is designed to have several modular units that can be swapped out using specific restriction enzymes. Promoters, genes of interest, selectable markers, and other desired sequences can be moved in and out of the vector as desired. This modular design makes genetic experiments faster and more efficient.

[00258] The modified p6.8 vector, according to certain embodiments of the disclosure, can replicate in both cyanobacteria and in *E. coli*. The vector contains a replication unit that can function in a broad range of cyanobacterial genera. The vector also contains a replicon for propagation in *E. coli* for ease of cloning and genetic manipulation using *E. coli*.

[00259] In an embodiment, a plasmid shuttle vector is provided which is characterized by being replicable in both *E. coli* and cyanobacterial species. The plasmid comprises a promoter capable of functioning in cyanobacteria and *E. coli* and a DNA sequence encoding a sequence capable of functioning as a selective marker for both *E. coli* and cyanobacteria. In another embodiment, the shuttle vector includes two different promoter systems, one functioning in cyanobacteria and the other one functional in *E. coli*. In an embodiment, the plasmid shuttle vector contains at least 50% of the p6.8 plasmid. The plasmid shuttle vector enables the efficient transformation of cyanobacteria and the expression of recombinant genes of interest.

[00260] In another embodiment, the p6.8 derived plasmid vector also contains an origin of transfer (oriT) which is suitable for conjugation. In particular, the plasmid vector can contain a combined origin of replication and an origin of transfer (oriVT), which enables replication in *Enterobacteriaceae*, in particular *E. coli*, and which also enables conjugation with, for example, an *E. coli* donor strain and *Cyanobacterium* sp., in particular ABICyano1 as a recipient strain. Such a plasmid vector can be used for bi- or triparental mating wherein a conjugative plasmid present in one bacterial strain assists the transfer of a mobilizable plasmid, for example a plasmid vector disclosed herein, present in a second bacterial strain into a third recipient bacterial strain, which can be ABICyano1.

[00261] Also disclosed herein is a non-naturally occurring plasmid vector in which a gene encoding an enzyme involved in MAA production is operably linked to a shuttle vector. In an embodiment, cyanobacterial cells are transformed with the recombinant shuttle vector. The recombinant shuttle vector is relatively small in size, relatively stable in a cyanobacterial host cell, and can replicate in a variety of cyanobacterial species. This recombinant vector is useful for expressing a variety of heterologous genes in cyanobacteria.

[00262] In an embodiment for transforming host cells with modified plasmid vectors, a shuttle vector expresses a codon-optimized antibiotic resistance gene (Ab^R), such as codon improved kanamycin or gentamycin resistance genes. In an embodiment, the shuttle vector is constructed based on a modular basis so that all of the key elements (replication origin, Ab^R gene

and reporter gene) are exchangeable via unique restriction sites thus providing versatile cloning options and facilitating the delivery of genes of interest to target organisms. Other antibiotic resistance genes can be used if desired. For example, genes conferring resistance to ampicillin, chloramphenicol, erythromycin, zeocin, spectinomycin or other antibiotics can be inserted into the vector, under the control of a suitable promoter. In some embodiments, the vector contains more than one antibiotic resistance gene.

[00263] In yet another embodiment, the plasmid is modified by several factors so that it is capable of efficient replication in multiple types of cyanobacterial species. The vector has also been organized so that various sequences can be easily replaced with other desired sequences as needed. Thus, a construct having a different gene (or genes) of interest, a different antibiotic, a different promoter, etc. can be made with relative ease. The modified vector allows for rapid testing of various heterologous constructs in a cyanobacterial cell.

[00264] The transfer of exogenous genes into cyanobacteria often involves the construction of vectors having a backbone from a broad-host range bacterial plasmid, such as RSF1010. The RSF1010-based vector has been widely used as a conjugation vector for transforming bacteria, including cyanobacteria (Mermet-Bouvier et al. (1993) "Transfer and Replication of RSF1010-derived Plasmids in Several Cyanobacteria of the Genera *Synechocystis* and *Synechococcus*" *Current Microbiology* 27:323-327). RSF1010 has an *E. coli* origin of replication, but does not have a cyanobacterial origin of replication.

[00265] As an example of additional tools used to transfer exogenous genes into cyanobacteria, several endogenous plasmids from *Synechococcus* sp. PCC 7002 were used as the backbone portions for plasmids to prepare vectors for heterologous gene expression, see Xu et al., *Photosynthesis Research Protocols* 684:273-293 (2011). Other vectors for transformation of cyanobacteria include the pDU1-based vectors. The pDU1 origin of replication is best suited for filamentous cyanobacteria. Attempts to transform ABICyano1, with either RSF1010 or pDU1-based shuttle vectors were unsuccessful using the techniques as described in the art.

[00266] In an embodiment, cyanobacteria disclosed herein can be transformed to add biochemical pathways to produce the MAAs of interest. Recombinant DNA sequences encoding genes can be amplified by PCR using specific primers or produced by gene synthesis. The amplified PCR fragments can then be digested with the appropriate restriction enzymes and cloned

into either a self-replicating plasmid or into an integrative plasmid. An antibiotic resistance cassette for selection of positive clones can be present on the appropriate plasmid.

[00267] In an embodiment, the recombinant nucleic acids of interest can be amplified from nucleic acid samples using known amplification techniques. PCR can be used to amplify the sequences of the genes directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other in vitro amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, and for nucleic acid sequencing.

[00268] In an embodiment, recombinant DNA vectors suitable for transformation of cyanobacteria can be prepared. For example, a DNA sequence encoding one or more of the genes described herein can be combined with transcriptional and other regulatory sequences which will direct the transcription of the sequence from the gene in the transformed cyanobacteria.

[00269] In an embodiment, recombinant genes of interest are inserted into the cyanobacterial chromosome. When the cell is polyploid, the gene insertions can be present in all of the copies of the chromosome, or in some of the copies of the chromosome.

[00270] In another embodiment, recombinant genes are present on an extrachromosomal plasmid. The extrachromosomal plasmid can be derived from an outside source such as RSF1010-based plasmid vectors, for example, or can be derived from an endogenous plasmid from *Cyanobacterium* sp. host cells or from other cyanobacteria.

[00271] In an embodiment, recombinant genes are present on an extrachromosomal plasmid having multiple copies per cell. The plasmid can be present, for example, at about 1, 3, 5, 8, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or more copies per cyanobacterial host cell. In an embodiment, the recombinant plasmids are fully segregated from the non-recombinant plasmids.

[00272] In another embodiment, recombinant genes are present on one cassette driven by one promoter. In another embodiment, the recombinant genes are present on separate plasmids, or on different cassettes.

[00273] In yet another embodiment, recombinant genes are modified for optimal expression by modifying the nucleic acid sequence to accommodate the cyanobacterial cell's protein translation system. Modifying the nucleic acid sequences in this manner can result in an increased expression of the genes.

Transformation of Cyanobacterial Cells

[00274] Some strains of cyanobacteria can be transformed through natural uptake of exogenous DNA. Other cyanobacterial strains can be transformed, for example, by the use of conjugation or electroporation. Some cyanobacterial strains are difficult to transform by any known means. For many of these types of difficult to transform strains, specific methods of preparing the cells for transformation, as well as specific methods of allowing entry of the foreign DNA into the cells, need to be designed *de novo*.

[00275] Exemplary cyanobacteria that can be transformed include, but are not limited to, *Synechocystis*, *Synechococcus*, *Acaryochloris*, *Anabaena*, *Arthrospira*, *Thermosynechococcus*, *Chamaesiphon*, *Chroococcus*, *Cyanobacterium*, *Cyanobium*, *Dactylococcopsis*, *Gloeobacter*, *Gloeocapsa*, *Gloeotheca*, *Microcystis*, *Prochlorococcus*, *Prochloron*, *Chroococcidiopsis*, *Cyanocystis*, *Dermocarpella*, *Myxosarcina*, *Pleurocapsa*, *Stanieria*, *Xenococcus*, *Arthrospira*, *Borzia*, *Crinalium*, *Geitlerinema*, *Halospirulina*, *Leptolyngbya*, *Limnothrix*, *Lyngbya*, *Microcoleus*, *Cyanodictyon*, *Aphanocapsa*, *Oscillatoria*, *Planktothrix*, *Prochlorothrix*, *Pseudanabaena*, *Spirulina*, *Starria*, *Symploca*, *Trichodesmium*, *Tychonema*, *Anabaenopsis*, *Aphanizomenon*, *Calothrix*, *Cyanospira*, *Cylindrospermopsis*, *Cylindrospermum*, *Nodularia*, *Nostoc*, *Chlorogloeopsis*, *Fischerella*, *Geitleria*, *Nostochopsis*, *Iyengariella*, *Stigonema*, *Rivularia*, *Scytonema*, *Tolypothrix*, *Cyanothece*, *Phormidium*, *Adrianema*, and the like.

[00276] Exemplary methods suitable for transformation of cyanobacteria, include, as nonlimiting examples, natural DNA uptake (Chung, et al. (1998) FEMS Microbiol. Lett. 164: 353-361; Frigaard, et al. (2004) Methods Mol. Biol. 274: 325-40; Zang, et al. (2007) J. Microbiol. 45: 241-245), conjugation, transduction, glass bead transformation (Kindle, et al. (1989) J. Cell Biol. 109: 2589-601; Feng, et al. (2009) Mol. Biol. Rep. 36: 1433-9; U.S. Pat. No. 5,661,017), silicon carbide whisker transformation (Dunahay, et al. (1997) Methods Mol. Biol. (1997) 62: 503-9), biolistics (Dawson, et al. (1997) Curr. Microbiol. 35: 356-62; Hallmann, et al. (1997) Proc. Natl. Acad. USA 94: 7469-7474; Jakobiak, et al. (2004) Protist 155:381-93; Tan, et al. (2005) J. Microbiol. 43: 361-365; Steinbrenner, et al. (2006) Appl Environ. Microbiol. 72: 7477-7484; Kroth (2007) Methods Mol. Biol. 390: 257-267; U.S. Pat. No. 5,661,017) electroporation (Kjaerulff, et al. (1994) Photosynth. Res. 41: 277-283; Iwai, et al. (2004) Plant Cell Physiol. 45: 171-5; Ravindran, et al. (2006) J. Microbiol. Methods 66: 174-6; Sun, et al. (2006) Gene 377: 140-149; Wang, et al. (2007) Appl. Microbiol. Biotechnol. 76: 651-657; Chaurasia, et al. (2008) J.

Microbiol. Methods 73: 133-141; Ludwig, et al. (2008) Appl. Microbiol. Biotechnol. 78: 729-35), laser-mediated transformation, or incubation with DNA in the presence of or after pre-treatment with any of poly(amidoamine) dendrimers (Pasupathy, et al. (2008) Biotechnol. J. 3: 1078-82), polyethylene glycol (Ohnuma, et al. (2008) Plant Cell Physiol. 49: 117-120), cationic lipids (Muradawa, et al. (2008) J. Biosci. Bioeng. 105: 77-80), dextran, calcium phosphate, or calcium chloride (Mendez-Alvarez, et al. (1994) J. Bacteriol. 176: 7395-7397), optionally after treatment of the cells with cell wall-degrading enzymes (Perrone, et al. (1998) Mol. Biol. Cell 9: 3351-3365). Biolistic methods (see, for example, Ramesh, et al. (2004) Methods Mol. Biol. 274: 355-307; Doestch, et al. (2001) Curr. Genet. 39: 49-60; all incorporated herein by reference in their entireties).

[00277] In an embodiment, the cyanobacterial strains ABICyano1 and ABCyano4 are used as the host cell for production of the MAA. In addition to the conjugation methods described below in the example section, electroporation can also be used for successful transformation of ABICyano1 and ABCyano4, although at a somewhat lower efficiency. Strain-specific adaptations of standard electroporation protocols may be made to avoid DNA digestion by endogenous restriction enzymes and to allow DNA entry through the extracellular polysaccharide layer (CPS layer) of ABICyano1 host cells. To achieve successful electroporation, DNA may be protected against endogenous restriction enzymes by methylation. Prior to electroporation using techniques well known in the art, ABICyano1 cells may be pretreated with positively charged polyaminoacids such as poly-L-lysine hydrobromide or poly-L-ornithine hydrochloride or combinations thereof (in particular poly-L-lysine hydrobromide) in order to increase the DNA uptake efficiency.

Selecting for successful transformation

[00278] In an embodiment, the presence of a foreign gene encoding antibiotic resistance in a cell is selected by placing putatively transformed cells into a media containing an amount of the corresponding antibiotic and selecting cells that survive. The selected cells are then grown in the appropriate culture medium to allow for further testing.

[00279] In another embodiment, colony PCR methods are used to confirm transformants. In certain embodiments of this procedure, three primer sets are used and are directed against parts of the shuttle vectors to detect specific fragments of the shuttle vector. Transformants exhibiting

the predicted PCR products are analyzed further by plasmid rescue. In one embodiment for plasmid rescue, a 25 mL liquid culture is subjected to DNA isolation.

[00280] In a non-limiting example of plasmid rescue, 500 ng to 1 µg of isolated DNA from transformants containing the transformed plasmids is re-transformed into *E. coli* and usually results in approximately 10-20 transformants per transformation implemented. Plasmid DNA of ten *E. coli* colonies is isolated and analyzed by PCR using specific primers for the transformed plasmids. The plasmid DNA is further analyzed with specific restriction enzymes and then sequenced.

Cultivation of Cyanobacterial Cells or Host Cells to Produce an MAA

[00281] A method of producing an MAA in a cyanobacterial cell or any host cell disclosed herein, comprises:

- a) growing the genetically modified cyanobacterial cell or any host cell disclosed herein in a culture medium suitable for production of the MAA in a photobioreactor with adequate light and CO₂ addition; and
- b) isolating the MAA from the cells and/or the culture medium.

[00282] Another embodiment of the method of the invention is directed to a method, wherein step a) further comprises adding an amino acid to the culture medium. This can be highly advantageous, if amino acids are needed for the production of certain MAAs, such as glycine, serine, threonine, alanine for the production of mycosporine-2-glycine, shinorine, porphyrin-334, and mycosporine-glycine-alanine (See Fig. 40).

[00283] According to another method of the present invention at least 50%, preferably at least 60% more preferred at least 65% of the MAA produced is associated with the cyanobacterial cell and wherein in step b) the MAA is isolated from the cells by separating the cells from the culture medium and isolating the MAA from the cells. Such a method greatly facilitates the purification of the MAAs, because the cells can be concentrated for example by centrifugation avoiding the handling of large culture volumes during the purification of the MAAs.

[00284] Preferably, the cyanobacterial cells employed in the methods have a CPS and the MAA is associated with the CPS of the host cells. As disclosed herein, some cyanobacterial host cells, such as *Cyanobacterium* sp. ABCyano4 or to a lesser extent *Cyanobacterium* sp. ABICyano1

have a thick CPS sheath, wherein a large fraction of the MAA produced stays associated with the host cells and only a minor fraction is secreted into the culture medium.

[00285] According to another embodiment of the method of the invention, in step b) the CPS is removed from the cells, thereby removing the MAA from the cells. This can be done for example by employing methanol-water mixtures.

[00286] In an embodiment, an enzyme involved in MAA production is synthesized in cyanobacterial cultures by preparing host cyanobacterial cells having the gene constructs discussed herein, and growing cultures of the cells.

[00287] Methods of growing cyanobacteria in liquid media and on agarose-containing plates are well known to those skilled in the art (see, e.g., websites associated with ATCC). Any of these methods or media maybe used to culture the cyanobacterial cells. A number of known recipes for cyanobacterial growth medium can be used. In an embodiment, BG11 medium is used, see Stanier, R.Y., et al., *Bacteriol. Rev.* 1971, 35: 171-205, which is hereby incorporated by reference.

[00288] In an embodiment, the cyanobacterial strain is a fresh water strain, and BG11 is used. In another embodiment, the cyanobacteria culture grows best in a marine (salt water) medium, by adding an amount of salt to the BG11 medium. In an embodiment, marine BG11 (mBG11) contains about 35 practical salinity units (psu), see Unesco, *The Practical Salinity Scale* 1978 and the *International Equation of State of Seawater* 1980. *Tech. Pap. Mar. Sci.*, 1981, 36: 25 which is hereby incorporated by reference.

[00289] In an embodiment, the cells are grown autotrophically, and the only carbon source is CO₂ or bicarbonate. In another embodiment, the cells are grown mixotrophically, for example with the addition of another carbon source such as glycerol or glucose.

[00290] The cultures can be grown indoors or outdoors. The light cycle can be set as for continuous light, or for periodic exposure to light, e.g., 16 hours on and 8 hours off, or 14 hours on and 10 hours off, or 12 hours on and 2 hours off, or any alternative variation of on and off hours of light comprising about a day.

[00291] The cultures can be axenic, or the cultures can also contain other contaminating species.

[00292] In an embodiment, the cyanobacteria are grown in enclosed bioreactors in quantities of at least about 1 L, 20L, 50L, 100 L, 500 L, 1000 L, 2000 L, 5000 L, or more. In a

preferred embodiment the bioreactors are about 20 L to about 100 L. In an embodiment, the cyanobacterial cell cultures are grown in disposable, flexible, and tubular photobioreactors made of a clear plastic material.

[00293] In another embodiment, cultures are grown indoors or outdoors with continuous light, in a sterile environment. In another embodiment, the cultures are grown outdoors in an open pond type of photobioreactor.

[00294] The choice of culture medium can depend on the cyanobacterial species. In an embodiment of the invention, the following BG11 medium for growing cyanobacteria can be used. When salt water species are grown, artificial seawater recipe (Table 5) is used to prepare the culture medium to yield artificial seawater medium BG11, denoted “ASW BG11”.

Table 5: Culture Medium

<u>BG11 Medium</u>	
<u>Compound</u>	<u>Amount</u>
NaNO ₃	1.5 g
K ₂ HPO ₄	0.04 g
MgSO ₄ ·7H ₂ O	0.075 g
CaCl ₂ ·2H ₂ O	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
NaCO ₃	0.02 g
Trace metal mix A5	1.0 ml
Distilled water	1.0 L
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<u>Composition of Trace Metal Mix</u>	
<u>Compound</u>	<u>Amount</u>
H ₃ BO ₃	2.86 g
MnCl ₂ ·4H ₂ O	1.81 g
ZnSO ₄ ·7H ₂ O	0.222 g
NaMoO ₄ ·2H ₂ O	0.39 g

CuSO ₄ ·5H ₂ O	0.079 g
Co(NO ₃) ₂ ·6H ₂ O	49.4 mg
Distilled water	1.0 L
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<u>Compsition of artificial seawater</u>	
<u>Chemical Components</u>	<u>g / 1L</u>
Sodium Chloride	25.84
Magnesium Sulfate . 7 H ₂ O	6.36
Magnesium(II) Chloride . 6 H ₂ O	5.06
Potassium Chloride	0.62
Calcium Chloride . 2 H ₂ O	1.36
Salinity Measured (psu)	34 psu

[00295] The present disclosure is further described by the following non-limiting examples. However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present disclosure.

EXAMPLES

Example 1

General Methods

[00296] Restriction endonucleases were purchased from Thermo Fisher Scientific, unless otherwise noted. PCR was performed using a Biometra thermocycler (Biometra, Germany), using Phire Plant Direct PCR Master Mix polymerase or Taq DNA polymerase (Thermo Fisher Scientific) for diagnostic amplifications, and Phusion polymerase for high fidelity amplifications. PCR temperature profiles were set up as recommended by the polymerase manufacturer. Cloning was performed in *E. coli* using NEB10-beta or NEB Turbo competent cells (New England Biolabs) following the manufacturer's protocol. NEBuilder HiFi assembly kits were purchased from New England Biolabs. Gene synthesis was conducted by GeneArt Gene Synthesis (Thermo Fisher Scientific).

[00297] *E. coli* strains HB101 (Promega), NEB10 and NEB Turbo (New England Biolabs), and EC100D (Epicentre) were grown in Luria-Bertani (LB) medium at 37 °C. Carbencillin (100 µg/mL), kanamycin (50 µg/mL), and chloramphenicol (34 µg/mL), zeocin (25 µg/ml), streptomycin (25 µg/ml) were used when appropriate. Cultures were continuously shaken overnight at 200 rpm and at 100 rpm when used for conjugation. ABICyano1 and ABCyano4 were cultured at from 28 °C to 37 °C in liquid BG11 fresh water medium in bubbled bottles under continuous illumination or 12h/12h day/night cycles of approximately 30 – 40 µmol photons*m⁻²*sec⁻¹.

[00298] Plasmid DNA from *E. coli* strains was isolated using a GeneJet Plasmid Miniprep Kit (Fermentas) according to the manufacture's protocol. For plasmid isolation from putative ABICyano1 transformants, total DNA was prepared according to Saha et al. (2005), *World Jour. Microbiol Biotechnol* 21:877-881.

Example 2

Preparation of Cyanobacterial Culture Medium

[00299] For cyanobacterial growth, BG-11 stock solution was purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, MO). Marine BG-11 (MBG-11) was prepared by dissolving 35 g Instant Ocean (United Pet Group, Inc, Cincinnati, OH) in 1 L water and supplementing with BG-11 stock solution. Vitamin B12 (Sigma Aldrich) was supplemented to MBG-11 to achieve a final concentration of 1 µg/L, as needed. Stock solutions of the antibiotics were purchased from Sigma Aldrich or Carl Roth GmbH).

[00300] Unless otherwise noted, the ABICyano1 and ABCyano4 transformants were selected on solid BG11 medium containing 10 - 20 µg/mL of the appropriate antibiotic.

Example 3

Transformation of ABICyano1 with p6.8 kb Based Shuttle Vector

[00301] The endogenous 6.8 kb plasmid of ABICyano1 (p6.8) can be used as a means of shuttling exogenous DNA to cyanobacterial host cells. By inserting an origin of replication that is effective in *E. coli* (such as R6Kori or oriVT), the p6.8 kb plasmid DNA can be manipulated in bacteria, such as *E. coli*, to incorporate genes and sequences of interest into a recombinant p6.8

kb. For example, modifications to decrease the effectiveness of endogenous restriction systems that are present in ABICyano1, such as methylation, can be performed.

[00302] The presence of an origin of replication that is already on ABICyano1 can assist with replication of the recombinant p6.8 kb once it is transferred into a host cell. Multiple cloning sites can be added to allow for several different antibiotic resistance genes to be added, if desired. Multiple cloning sites can also be inserted to allow for ease of insertion of various expression cassettes. In this way, various sequence segments of the plasmid can be replaced with other sequence segments as needed.

Example 4

Competent ABICyano1 Cells for Transformation by Conjugation

[00303] The following method was used to treat cells of the strain *Cyanobacterium* sp. ABICyano1 prior to conjugation. The method involves several steps: treatment of cells with N-acetylcysteine (NAC), washing steps that utilize NaCl, treatment with lysozyme, and subsequent washing followed by a conjugation procedure.

[00304] Two hundred mL of an exponentially growing culture (OD_{750nm} greater than about 0.5 and less than about 1.0) was incubated with NAC for 2 days at 16 °C (end concentration of NAC is about 0.1 mg/mL) without shaking. This pretreatment was followed by several steps to weaken the cell wall. The pretreated culture was pelleted at 4400 rpm and washed with 0.9% NaCl containing 8 mM EDTA.

[00305] For further treatment with lysozyme, the cell pellet was resuspended in 0.5 M sucrose and incubated 60 min at room temperature (RT) with slow shaking (85 rpm). Then, cells were centrifuged and resuspended in 40 mL of a solution containing 50mM Tris (pH 8.0), 10 mM EDTA (pH 8.0), 4% sucrose, and 20-40 µg/mL lysozyme. After incubation at rt for 10-15 min, cells were centrifuged and washed three times using different washing solutions; i) 30 mM Tris containing 4% sucrose and 1 mM EDTA; ii) 100 mM Tris containing 2 % sucrose and iii) with BG11 medium. All centrifugation steps before lysozyme treatment were performed at 4400 rpm for 10 min at 10 °C. All centrifugations after the lysozyme treatment were performed at 2400 rpm for 5 min at 4 °C. Resuspended cells were used for conjugation.

Example 5

Transformation of ABICyano1 by Conjugation

[00306] Gene transfer to ABICyano1 was performed using conjugation. Generated plasmids containing oriVT were used for conjugation. The shuttle vectors were transformed into ABICyano1 following a modified conjugation protocol which includes the pretreatment of ABICyano1 as described in the above Example.

[00307] Triparental mating was performed as follows: *E. coli* strain J53 bearing a conjugative RP4 plasmid and *E. coli* strain HB101 bearing the cargo to be introduced into ABICyano1 and the pRL528 helper plasmid (for in vivo methylation) were used. *E. coli* strains were grown in LB broth supplemented with the appropriate antibiotics overnight at 37 °C with shaking at 100 rpm. An aliquot of 3 – 5 mL of each culture was centrifuged, washed twice with LB medium and resuspended in 200 µL LB medium. Subsequently, the *E. coli* strains were mixed, centrifuged and resuspended in 100 µL BG11 medium. Two hundred mL of exponentially growing cyanobacterial culture (OD_{750nm} of greater than 0.5 and less than 1.0) was centrifuged (3000 rpm, 10 min), pretreated as described in Example 4, and subsequently washed and resuspended in 400 µL BG11 culture medium containing Tris/sucrose buffer (Example 4). A 100 µL aliquot of resuspended cyanobacterial and *E. coli* cultures was mixed and applied onto a membrane filter (Millipore GVWP, 0.22 µm pore size) placed on the surface of solid BG11 medium supplemented with 5% LB. Petri dishes were incubated under dim light (5 µE/m²-sec) for 2 days. Cells were then resuspended in fresh BG11 medium and plated onto selective medium containing 10 and 15 µg/mL kanamycin, respectively. The following selection conditions were used: light intensity of approximately 20 – 40 µE/m²-sec at a temperature of approximately 28 °C. Transformants were visible after approximately 7-10 days. The transformant colonies were then plated on BG11 media containing 15 µg/mL kanamycin and then transferred stepwise to higher kanamycin concentrations (up to kanamycin 60 µg/mL) to aid in the selection process.

Example 6

Transformation of ABICyano1 or ABCyano4 by Electroporation

[00308] Electroporation can also be used for successful transformation of *Cyanobacterium* sp. ABICyano1 and ABCyano4, or other strains such as *Arthrospira*, *Synechococcus*, and *Synechocystis*, using, for example, the same plasmids as for conjugation, but with lower efficiency.

[00309] As with the conjugation transformation protocol (above), strain-specific adaptations of standard electroporation protocols can be made to avoid DNA digestion by endogenous restriction enzymes and to allow DNA entry through the CPS layer. To achieve successful electroporation, DNA is protected against endogenous restriction enzymes by methylation. Prior to electroporation, ABICyano1 and ABCyano4 cells are pretreated with positively charged polyaminoacids such as poly-L-lysine hydrobromide or poly-L-ornithine hydrochloride or combinations thereof (in particular poly-L-lysine hydrobromide) in order to increase the DNA uptake efficiency.

[00310] As an example, 100 mL of exponentially growing ABICyano1 or ABCyano4 cultures (corresponding to a cell density of approximately 2×10^7 cells/mL), were harvested, washed and resuspended in 0.9 % NaCl containing 25 mM Tris-HCl (pH 8.0). Poly-L-lysine hydrobromide was added to the resuspended cells to obtain a final concentration of 50 µg/mL. ABICyano1 or ABCyano4 cells were then incubated for several hours or overnight before electroporation.

[00311] In a typical procedure, 50 mL of poly-L-lysine hydrobromide treated ABICyano1 or ABCyano4 cells were harvested and treated with 30 mL ice-cold BG11 containing 6% DMSO. After incubation on ice for 20 min, cells were harvested and frozen in liquid nitrogen for 15 min. These pre-frozen cells were thawed by adding 15 mL ice-cold buffer containing 1 mM HEPES (pH7.5), 0.2 mM K_2HPO_4 and 0.2 mM $MgCl_2$. The cells were washed sequentially once more with 1 mM HEPES and ETMT buffer containing 0.1 mM HEPES, 0.2 mM K_2HPO_4 and 0.2 mM $MgCl_2$. The cells were harvested by centrifugation at $15,000 \times g$ for 5 min. All of the washes and centrifugations were carried out on ice or in a pre-chilled centrifuge (4 °C). For each electroporation procedure, 3 µg methylated DNA is added to 100 µL of concentrated cells. Cells were electroporated in a cuvette with a 2 mm gap between the electrodes and pulsed once in a Gene Pulse X-cell (Bio-Rad) using an exponential decay protocol (electric field strength of 8 kV/cm, capacitance of 25 µF, resistance of 400 ohms, for a time of approximately 8-9 ms). After electroporation, 1-2 mL BG11 medium was immediately added to the cyanobacterial suspension, which was subsequently transferred to a 50 mL flask containing 15 mL fresh BG11 medium. After incubation for 1- 2 days under normal light (30 – 40 µE/m²-sec) with gentle shaking at 30 °C, recovered cultures were centrifuged, resuspended in 500 µL BG11 medium and placed onto selective media (BG11 containing 20 µg/mL Km or 40-60 µg/mL of spectinomycin).

Example 7

Transformation of *Cyanobacterium* sp. ABCyano4 by Conjugation

[00312] The following method was used for transformation of ABCyano4. An exponentially growing culture ($0.5 < OD_{750nm} < 3$) was pelleted at >4000 RCF and washed twice with 0.9% NaCl containing 8 mM EDTA and once with 0.75 M NaCl. Then, cells were centrifuged and resuspended in BG11 medium containing 10 mM TES pH 7.3.

[00313] Biparental mating was performed as follows. *E. coli* strain EC100D bearing a conjugative RP4 Δ Aph plasmid, the pRL528-based helper plasmid #2709 (pRL528-lacI-Ptac-M.MspI-Toop) for in vivo methylation and the cargo plasmid to be introduced into *Cyanobacterium* sp. ABCyano4. *E. coli* strains were generally grown in LB broth supplemented with the appropriate antibiotics overnight at 37 °C with shaking at 100 rpm. An aliquot of 3–5 ml of each culture was centrifuged, washed twice with LB medium and resuspended in 200 μ l LB medium. Subsequently, the *E. coli* strains were mixed, centrifuged and resuspended in 100 μ l BG11 medium. A 100 μ l aliquot of the resuspended cyanobacterial cells and the *E. coli* cultures was mixed and applied onto a membrane filter (Millipore GVWP, 0.22 μ m pore size) placed on the surface of solid BG11 medium supplemented with 5% LB. Petri dishes were incubated under dim light of 5 μ mol photons $m^{-2} s^{-1}$ for two days. Cells were then resuspended in fresh BG11 medium and plated onto selective medium containing > 10 μ g/ml of the respective antibiotic. The following selection conditions were used: light intensity approximately 20 – 40 μ mol photons $m^{-2} sec^{-1}$ at a temperature of approximately 30 °C. Transformants were visible after approximately 3-4 days. The transformant colonies were then stepwise transferred to higher antibiotic concentrations.

[00314] Integration of heterologous genes into endogenous plasmids of ABCyano4 was conducted via homologous recombination, a method well known to those skilled in the art. The integrative plasmids harbor an oriVT for conjugative transfer and homologous 2-3 kb flanking regions upstream and downstream of an integrative sequence followed by a resistance marker gene. In order to insert MAA synthesis genes into the endogenous plasmids pABCyano4B and pABCyano4C respective integrative regions were applied as shown in Fig. 43 and Fig. 44.

Example 8

Constitutive Production of the MAA “Mycosporine-glycine” in *Cyanobacterium* sp.

ABICyano1

[00315] A plasmid construct was prepared by inserting 3 genes from *Anabaena variabilis* (ava_3858, ava_3857, and ava_3856) to a modified endogenous ABICyano1 plasmid p6.8. The three genes were regulated by a strong constitutive promoter PcpB*3 (plasmid #2752; SEQ ID NO: 18). The ABICyano1 strain containing this plasmid is named ABICyano1 strain # AB1226.

[00316] The plasmid was transformed to ABICyano1 host cells, and the transformation was confirmed. Cultures of the modified cells were grown in a GC vial experiment to confirm that the MAA was being produced (FIG. 10B).

Example 9

Inducible Production of the MAA “Mycosporine-glycine” in *Cyanobacterium* sp.

ABICyano1

[00317] Plasmid constructs #2737 (FIG. 5A; SEQ ID NO: 54) and #2742 (FIG. 5B; SEQ ID NO: 17) were prepared by inserting 3 MAA biosynthesis genes from *Anabaena variabilis* (ava_3858, ava_3857, and ava_3856) into a modified endogenous ABICyano1 plasmid p6.8 (FIG. 4). The three genes were regulated either by a Porf0223 (Cu-inducible) or a PsmtA (Zn-inducible) promoter. The ABICyano1 strain containing plasmid #2737 is named ABICyano1 strain # AB1224 and the strain containing plasmid #2742 is named ABICyano1 strain AB1225.

[00318] The plasmids were transformed to ABICyano1 host cells, and the transformation was confirmed by PCR. As an initial confirmation of MAA production in the transformed cells, the Cu inducible strain AB1224, the Zn inducible strain AB1225 and the constitutive MAA expressing strain AB1226 (previous example) were grown in GC vials for 24 hours, and the absorbance spectra of the cultures were examined (FIG. 10A and 10B). Several AB1226 clones were found to produce high MAA peaks at 310 nm, while the strains AB1224 (Cu inducible) and AB1225 (Zn-inducible) appeared to produce less MAA. To determine whether the ZnSO₄ level needed for MAA induction was somehow deleterious to these cells, the next experiment was run using Zn chelated by EDTA. AB1226 was grown in small (375 mL) indoor vertical photobioreactors under a 12 hour on/12 hour off light cycle, using a light level set at 350 $\mu\text{E}/\text{m}^2\text{-sec}$. AB1226 was shown to produce mycosporine-glycine over a cultivation period of 40 days,

resulting in the production of 250 mg/L mycosporine-glycine after 30 days (Fig. 10C). Over the whole production period up to 300 mg/L mycosporine-glycine was produced.

Example 10

Improvement of Inducible Production of the MAA “Mycosporine-glycine” in *Cyanobacterium* sp. ABICyano1 using Zn chelated by EDTA

[00319] To test the effect of the chelator EDTA, cultures of the modified cells from Example 8 were scaled-up and grown for 50 days in BASW medium (35 PSU) in a 1.2 L indoor photobioreactor, with light set at 350 $\mu\text{E}/\text{m}^2\text{-sec}$ under a 12/12 light/dark cycle (FIG. 11A). Nitrate (at 17 mM) was used as the nitrogen source. The culture was maintained at a pH of 7.3 by supplementation with 15% CO_2 . The medium contained either ZnSO_4 (6 μM at day 8, 10, and 21) or Zn-EDTA (20 μM at day 1, 30 μM day 2).

[00320] The results (FIG. 11A) show that while the promoter was tightly regulated when no Zn was present (about 30 mg/L throughout the run), the addition of Zn (as Zn-EDTA) resulted in a high production of mycosporine-glycine: by 15 days, about 140 mg/L product was produced. At about 20 days, about 160 mg/L of the mycosporine-glycine product was produced. Although the induction with ZnSO_4 appeared to produce less mycosporine-glycine than induction with Zn-EDTA, the production was almost the same by the end of the run. This resulted in a productivity of about 6% MAA per dry weight of algae.

[00321] In contrast, Zn added without the EDTA produced about 83 mg/L at 15 days, and about 130 mg/L by 20 days.

[00322] At about 27 days, the Zn-EDTA culture was allowed to continue growing to better understand the stability of the system. The culture was diluted with fresh medium, and the Zn-EDTA culture continued to produce high amounts of mycosporine-glycine through the 50 day test period. This shows the genetic stability of the modified system: both the modified plasmid and the heterologous genes remain substantially intact in the host cell at least through at least the 50 day test period.

[00323] It was also found that about 80 to 90% of the mycosporine-glycine produced by these cells was excreted into the culture medium (FIG. 11B).

Example 11**Production of the MAA Mycosporine-2-glycine in *Cyanobacterium* sp. ABICyano1 using****MAA Genes on Two Plasmids**

[00324] Another MAA, “mycosporine-2-glycine”, which has 2 amino acid moieties attached to the central ring (see FIG. 12A and FIG. 12B), was prepared by starting with a plasmid that contained the genes for the production of mycosporine-glycine (such as shown above in Examples 6-9), and further inserting an additional gene encoding the mysD gene (ap_3855) from *Aphanothece halophytica*, linked to a zinc-inducible promoter, and localized on a second endogenous plasmid pABCyano1B from the ABICyano1 strain.

[00325] Construct information: AB1253 (ABICyano1:2742:2798) pABICyano1_6.8::PsmA-Ava_3858(ABICyano1opt)-ISpcBA*1-Ava_3857(ABICyano1opt)-ISpcBA*1-Ava_3856(ABICyano1opt)-TpsaB, pABICyano1B_Rep::PrpsL-Gm**-TB0014-orivT-PsmA-ap3855(ABICyano1opt) → mycosporine-2-glycine.

[00326] An absorbance spectrum analysis after 24 hours of growth in a GC vial showed that mycosporine-2-glycine (with an absorbance max of 331 nm) was produced (FIG. 12C).

Example 12**Production of the MAA Mycosporine-2-glycine in *Cyanobacterium* sp. ABICyano1 using****MAA Genes Located on One Plasmid**

[00327] The MAA mycosporine-2-glycine was also produced in the cyanobacterial strains AB1277 and AB1324 by locating the several MAA pathway genes on one extrachromosomal plasmid (plasmid #2840 and #2893, as shown in FIG. 3 and Table 4) instead of divided between two plasmids as shown in the above example.

Example 13**Preparation of Plasmid Construct for Production of the MAA “Shinorine and Porphyrin-334” in *Cyanobacterium* sp. ABICyano1**

[00328] The MAAs “shinorine” and “porphyrin-334” were produced in cyanobacterial host cells using the following method. The modified cyanobacterial host cells that already have the mysABC genes for the production of mycosporine-glycine (such as shown above in Examples 6-

9), were then transformed with a second endogenous plasmid pABICyano1B that contains one additional gene: either *mysD* from *Nostoc punctiforme* (NpF5597) or *Ava_3855*, which encodes a nonribosomal peptide synthetase (NRPS) from *Anabaena variabilis* (see FIG. 12A and FIG. 12B).

[00329] Construct information: AB1252 (ABICyano1:2742:2796) pABICyano1_6.8::PsmA-Ava_3858(ABICyano1opt)-IScpBA*1-Ava_3857(ABICyano1opt)-IScpBA*1-Ava_3856(ABICyano1opt)-TpsaB, pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmA-NpF5597(ABICyano1opt) → shinorine/porphyra-334.

[00330] An absorbance spectrum analysis after 24 hours of growth in a GC vial demonstrated that shinorine/porphyra-334 (with an absorbance max of 334 nm) were produced (FIG. 12C).

Example 14

Scale-up of MAA-Producing *Cyanobacterium* sp. ABICyano1 in 1.2L Photobioreactors

[00331] *Cyanobacterium* sp. ABICyano1 cells harboring the genes for mycosporine, shinorine/porphyra-334, or mycosporine-2-glycine were grown in ASW-BG11 (35 psu) medium in 1.2 liter indoor vertical photobioreactors under a 12 hour on/12 hour off light cycle, using a light level set at 350 $\mu\text{E}/\text{m}^2\text{-sec}$. The starting OD₇₅₀ was 0.2. The culture was aerated, and the pH was set at 7.3, with automated adjustment of pH by the addition of 15% CO₂ as needed. The nitrogen source was 17.3 mM nitrate.

[00332] The graph shows that the mycosporine-glycine producing culture produced the most MAA over the length of the run (FIG. 13A, FIG. 13B), although the mycosporine-2-glycine producing culture was about the same by the end of the run, at about 200 mg/L at 26 days. The mycosporine-glycine producing culture produced about 6% of the cell dry weight at about day 15.

[00333] The shinorine/porphyra-334 producing culture produced about 110 mg/L at 26 days.

[00334] FIG. 13C shows that over time, the MAAs accumulate in the culture medium. By the end of the run at 26 days, about 85% to 90 % of the MAA was present in the culture medium.

[00335] To determine whether several of the MAA pathway genes could be located on one plasmid, and would still be capable of adequate expression and production, the mycosporine-2-glycine producing cells (transformed with a single plasmid, #2840; Strain #AB1277; see Table 4) from Example 11 were also examined for MAA production. The cells were grown in BASW (35

psu) medium in a 1.2 liter indoor vertical photobioreactors under a 12 hour on/12 hour off light cycle, using a light level set at 350 $\mu\text{E}/\text{m}^2\text{-sec}$. The starting OD_{750} was 0.2. The culture was aerated, and the pH was set and maintained at 7.3, with adjustment by the addition of 15% CO_2 as needed. The nitrogen source was 17.3 mM nitrate. The resulting MAA production over time (mg/L) is shown in FIG. 14.

Example 15

Production of Various MAAs in Cyanobacteria

[00336] As an additional example, another MAA is produced in cyanobacteria by the following method. The biosynthetic pathway for the MAA is determined, and suitable enzymes are identified by analysis of the genome sequence from another cyanobacterial species. The genes, linked to constitutive or inducible promoters, are used to transform a suitable cyanobacterial strain, such as ABICyano1. The modified cells are grown and the production of the desired MAA is confirmed. The cells are then scaled-up to a desired culture volume, and grown for a suitable number of days. The MAA is then isolated, purified, and quantified. The MAA so produced is used in skin care and cosmetic products.

Example 16

Scale-up of Modified Cyanobacteria to Produce an MAA

[00337] A culture of modified cyanobacteria is scaled-up to flasks, then 1 liter containers, then 5 liter containers, then to outdoor or indoor photobioreactors, in a suitable culture medium, with the pH set at 7.3 by use of air bubbling with CO_2 addition on demand. After two days, the host cells are induced to produce the MAA and production is allowed to proceed for a specified period of time, usually not more than 30 days of continuous growth under production conditions.

Example 17

Isolation of MAA from Genetically Modified Cyanobacterial Host Cells

[00338] A culture of MAA-producing cyanobacterial cells is grown and the production of the MAA of interest is induced in an indoor or outdoor photobioreactor. After a selected time period of growth, the culture is harvested by centrifugation or other means, and the MAA is collected from the cell-free medium using crossflow ultrafiltration combined with bulk ion exchange chromatography, or alternatively through absorption/de-absorption cycling using an

organic substrate such as activated charcoal as an isolation matrix. The concentrated MAA is quantified and further purified, if desired. If desired, other products of interest are isolated from the culture in addition to the MAA, such as biomass, pigments, proteins, lipids, etc.

[00339] Alternatively, MAAs can be isolated from the concentrated culture biomass. In this instance, the cell biomass is harvested by centrifugation or other means of harvesting, with the result that the cells are separated from the culture medium. The concentrated cell slurry is dried and the cells disrupted by grinding or other means. The cellular material is resuspended in water, mixed for 30 minutes at room temperature, and centrifuged to separate the insoluble solid biomass from the soluble MAA. The MAA-enriched cell extract can then be used without further purification, or it can be further purified, such as, for example, using crossflow ultrafiltration, centrifugation, and ion exchange, as needed. The purity of the MAA is determined using mass spectrometry.

Example 18

Quantitative Analysis of MAAs Using Capillary Electrophoresis and Hydrophilic Interaction Liquid Chromatography (HILIC)

[00340] The amount (and type) of MAA produced is quantified by Capillary Electrophoresis, following the method described in Hartmann et al. “Quantitative analysis of mycosporine-like amino acids in marine algae by capillary electrophoresis with diode-array detection”, *Jour. Pharm. Biomed. Analysis*, 138:153-157 (2017).

[00341] Another suitable method for quantitation and analysis of MAAs is the use of HPLC, as described, for example, in Rastogi et al., *Appl. Microbiol.* 119:753-762 (2015). Hydrophilic interaction liquid chromatography (HILIC) is also utilized (Hartmann et al., *Mar. Drugs* 13:6291-6305 (2015)).

Example 19

Preparation of a Skin Care Agent Using MAAs from Cyanobacteria

[00342] An MAA is produced in an indoor or outdoor photobioreactor containing modified cyanobacterial strain according to the above examples. The MAA is obtained from the culture, and is further purified using either a tangential cross-flow filtration unit or a batch addition of an ion exchange medium. The MAA obtained via ion exchange method has a purity of at least 95%

by HPLC analysis. The MAA is further purified to about 99% using additional chromatography methods. The purified MAA is mixed with one or more different MAAs (of different absorbance maxima) that have also been prepared from cyanobacteria. This mixture of various MAAs increases the breadth of the UV protection over a wider absorbance range. The MAA mixture is then mixed with a naturally obtained, suitable carrier for application to human skin (such as an oil or a lotion) to result in a spreadable material that protects the skin from sun damage.

Example 20

Production of the MAA Porphyrin-334 in *Cyanobacterium* sp. ABICyano1 using MAA Genes on Two Plasmids

[00343] Another MAA, “porphyrin-334”, which has two amino acid moieties attached to the central ring, is prepared by starting with a strain that contains the genes for the production of mycosporine-glycine (such as shown above in Examples 6-9), and further inserting an additional gene encoding the mysCD fusion-gene from *Porphyra umbilicalis* or *Chondrus crispus*, linked to a Zn inducible promoter, and localized on a second endogenous plasmid pAB1B from the ABICyano1 strain.

[00344] Construct information: (ABICyano1:2742:2881) pABICyano1_6.8::PsmtA-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB, pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-mysCD_Pum(ABICyano1opt)-TpsaB → porphyrin-334.

[00345] Construct information: (ABICyano1:2742:2882) pABICyano1_6.8::PsmtA-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB, pABICyano1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-mysCD_Chc(ABICyano1opt)-TpsaB → porphyrin-334.

Example 21

Production of the MAAs Mycosporine-ornithine, Mycosporine-lysine and Mycosporine-2-(4-deoxygadusolyl-ornithine) in *Cyanobacterium* sp. ABICyano1

[00346] As an additional example, other MAAs, like mycosporine-ornithine, mycosporine-lysine and mycosporine-2-(4-deoxygadusolyl-ornithine) were produced by starting with a strain

that contained the genes for the production of mycosporine-glycine (such as shown above in Examples 6-9), and further inserting the additional mylCDE gene cluster from *Cylindrospermum stagnale* PCC 7417 or the mysD-C1-C2 cluster from *Nostoc commune* var. *flagelliforme* QSY, linked to a Zn inducible promoter, and localized on a second endogenous plasmid pABICyano1B from the ABICyano1 strain.

[00347] Fig. 25A and 25B show the whole cell absorption spectrum and the HPLC profile of the ABICyano1 strain AB4033. This strain was produced by transformation of the M-Gly producer strain AB1225 with the plasmid carrying the genes *mylCDE* from *Nostoc commune* var. *flagelliforme* QSY 1, resulting in the production of mycosporine-ornithine-4-deoxygadusolyl-ornithine (M-2-DO). In particular, the heterologous genes *mylD*, *mylE* and *mylC* were introduced in the strain as codon optimized variants under the transcriptional control of the *PsmtA* promoter. Fig. 25A shows the shift in the absorbance spectrum and Fig. 25B shows the large HPLC peak for mycosporine-glycine and smaller peaks for mycosporine-ornithine-4-deoxygadusolyl-ornithine, mycosporine-2-(4-deoxygadusolyl-ornithine) (M-2-DO) and mycosporine-ornithine.

Example 22

Production of the MAAs Palythine, Mycosporine-2-glycine, Mycosporine-methylamine-glycine, and Tery-364 in *Cyanobacterium* sp. ABICyano1

[00348] The MAAs palythine, N-methylamine-glycine and the palythene-like compound Tery-364 were produced by starting with different strains. Fig. 23A and 23B show the whole cell absorption spectrum and the HPLC profile of the ABICyano1 strain AB1358. This strain was produced by transformation of the M-Gly producer strain AB1225 with plasmids carrying genes for thus far undescribed enzymes from *Trichodesmium erythraeum* IMS101, resulting in the production of mycosporine-methylamine-glycine and mycosporine-2-glycine (M-2-Gly). In particular, the heterologous genes *Tery_2972*, *mysD1_IMS101* (*Tery_2971*), *mysD2_IMS101* (*Tery_2970*) and *Tery_2969* were used as codon optimized variants to transform AB1225. Fig. 23A shows the shift in the absorbance spectrum when producing mycosporine-methylamine-glycine and mycosporine-2-glycine (AB1358) instead of mycosporine-glycine (AB1225). Fig. 23B shows the HPLC spectrum identifying the considerably larger peaks for mycosporine-

methyammine-glycine and mycosporine-2-glycine and the much smaller peak for the remaining mycosporine-glycine.

[00349] Fig. 24A and 24B show the whole cell absorption spectrum and the HPLC profile of the ABICyano1 strain AB1360. This strain was produced by transformation of the M-Gly producer strain AB1225 with plasmids carrying genes for so far undescribed enzymes from *Trichodesmium erythraeum* IMS101, resulting in the production of Tery-364, an MAA with yet unknown structure. In particular, the heterologous genes Tery_4684, Tery_2968 and Tery_2966 were introduced under the transcriptional control of the PsmtA promoter. Fig. 24A shows the difference in the absorbance spectrum between the initial AB1225 strain and AB1360. Fig. 24B identifies the HPLC peaks for mycosporine-glycine and Tery-364 with an absorbance maximum at 364 nm.

[00350] In particular, the strains can contain the genes for the production of mycosporine-glycine (such as shown above in Examples 6-9), and further inserting parts of the additional genes from the MAA gene cluster (Tery_2966 – Tery_2972 from *Trichodesmium erythraeum* IMS101), linked to inducible or constitutive promoters, and localized at the same or on a second endogenous plasmid from the ABICyano1 strain.

[00351] The minimum MAA biosynthesis gene cluster consists of the three genes mysABC, which is widely distributed in the five subsections of cyanobacteria. The MAA gene cluster can contain additional genes, which encode for a D-Ala-D-Ala-Ligase (MysD) or an NRPS (non-ribosomal peptide synthase). Some cyanobacterial strains have even more, partially unknown, genes associated within in the MAA gene cluster. One example is the MAA gene cluster in the filamentous cyanobacterial strain *Trichodesmium erythraeum* IMS101. The genome sequence can be found on NCBI (GenBank: CP000393.1). The MMA gene cluster in T. erythraeum IMS101 consists of twelve genes in total. Besides mysABC (Tery_2977 – Tery_2975), two mysD genes mysD1 and mysD2 (Tery_2971, Tery_2970) and a NRPS (Tery_2968) are present. The operon moreover contains two genes which encode for a transporter/permease (Tery_2974, Tery_2973), an additional mysB-like O-methyltransferase (Tery_2966) and three hypothetical genes (Tery_2972, Tery_2969 and Tery_2967) of yet unknown function in conjunction with MAA

biosynthesis. The amino acid sequence of Tery_2972 exhibits homology to a clavaminic acid synthetase, whereas Tery_2969 shows homology to a hydroxyl-isobutyrate dehydrogenase.

[00352] In order to identify a potential function of these proteins in conjunction with MAA synthesis, codon-optimized versions were expressed in ABCyano1 and ABCyano4 along with the core genes for MAA biosynthesis, mysABCD.

Example 23

Production of the MAAs Palythine, Mycosporine-methylamine-threonine, and Tery-322 in *Cyanobacterium* sp. ABCyano4 strains

[00353] Fig. 32A, and 32B show the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4055, producing palythine. The strain overexpressed so far undescribed enzymes from *Trichodesmium erythraeum* IMS101 (Tery_2972, a Clavaminic acid synthetase (CAS)-like enzyme and Tery_2971, a D-ala-D-ala ligase). The strain was created by transformation of the mycosporine-glycine producer strain AB1322 with the heterologous codon optimized genes Tery_2972 and mysD1_IMS101 (Tery_2971), both of which were under the transcriptional control of the PsmtA promoter. Fig. 32A shows the difference in the absorbance spectrum between the initial AB1322 strain and AB4050. Fig. 32B shows the HPLC profile with the peaks for mycosporine-glycine and palythine.

[00354] Fig. 33A, and 33B show the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4031, producing mycosporine-methylamine-threonine. The strain overexpressed a so far undescribed enzyme from *Trichodesmium erythraeum* IMS101 (Tery_2969, a homolog of a 6-phosphogluconate dehydrogenase/ 3-hydroxyisobutyrate dehydrogenase) and a D-Ala-D-Ala ligase from *Nostoc punctiforme* (NpF5597, both under the transcriptional control of the PsmtA promoter. The strain was created by transformation of the M-Gly producer strain AB1322 with plasmid #3036. Fig. 33A shows the huge difference in the absorbance spectrum between the initial AB1322 strain and AB4031. Fig. 33B depicts the HPLC profile with the peaks for mycosporine-methylamine-threonine, porphyra-334 and shinorine.

[00355] Fig. 34A, and 34B show the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4074, producing Tery-322, an MAA with yet unknown structure, with an absorbance maximum at 322 nm. The strain overexpressed a thus far undescribed enzyme from

Trichodesmium erythraeum IMS101, Tery_2966 under the transcriptional control of the PsmtA promoter. The strain was created by transformation of the M-Gly producer strain AB1322 with the plasmid #3094. Fig. 34A shows the difference in the absorbance spectrum between the initial AB1322 strain and AB4074. Fig. 34B shows the HPLC profile with the peak designating Tery-322.

Example 24

Comparison of Dry Weight Production Rate and Total Organic Carbon Fixation Rate Between ABICyano1 and ABCyano4

[00356] Genetically unmodified wild-type strains of ABICyano1 and ABCyano4 are cultured over a period of seven days at different light intensities of $350 \mu\text{E m}^{-2}\text{s}^{-1}$ and of $1000 \mu\text{E m}^{-2}\text{s}^{-1}$. Dry weight and total organic carbon (TOC) measurements were performed over a period of 7 days of a batch cultivation. Cultivations were performed in 1.2 L vPBRs in BG11 artificial seawater (35 psu). The cultures were cultivated at a 12h / 12h dark / light cycle and illuminated from one side of the LvPBRs. During the night the temperature was 25°C with a ramping to 37°C during the day. The strains were cultivated at $\text{pH } 7.3 \pm 0.01$, controlled by CO_2 (15 % CO_2 in air) injection into the liquid phase. Mixing of the culture was ensured by continuous aeration at 38 mL / min. Biomass dry weight was determined as follows. A homogenous biomass sample was filtered through a glass fiber filter to collect all organic particulates. After filtration, the filter was dried for a suitable period in a drying oven at 70°C . Particulate mass was determined by subtracting the initial filter mass from the dry mass of the filter plus the particulates. Measurements were reported in units of dry weight mass per volume water, i.e., g D.W.*L⁻¹. TOC was analyzed with a Shimadzu TOC-VCPH equipped with combustion catalytic oxidation/non-dispersive infrared (NDIR) gas analyzer. Fig. 16A shows that at both light intensities ABCyano4 produced more dry weight in comparison to ABICyano1. Additionally, as evidenced by Fig. 16B, ABCyano4 also had a higher organic carbon-fixation rate in comparison to ABICyano1. Therefore, a skilled person

also would expect genetically modified ABCyano4 cells to produce a higher amount of a compound of interest, for example ethanol or MAAs.

Example 25

Comparison of Dry Weight Productivity and Total Carbon Fixation Rate in Turbidostat Cultivation Conditions for ABICyano1 and ABCyano4

[00357] Wild type ABICyano1 and ABCyano4 cells were cultivated under the following conditions: in 1.2L LvPBRs for continuous cultivation; Operational OD at 750nm: set point sOD_{750nm} at 1pm: 1, 2, 3, 4, 5, and 6; feed medium 35 psu, ASW BG11 with 4mM urea as sole nitrogen source, cultivation pH: 7.3 via pH dependent CO₂ supply (10% in air into the liquid phase; aeration: at a continuous flow of 38 ml/min, mixed via bubbling and illuminated with 350 μ E m⁻² s⁻¹ homogenous light field, from one side with fluorescent light, at a 12h photoperiod over a time period of at least 7 days per operational optical density (sOD set point). Both Fig. 17A and 17B show that the dry weight productivity as well as the total carbon fixation rate of ABCyano4 is higher in comparison to ABICyano1, so that a skilled person would expect a higher productivity for any compounds of interest for genetically modified variants of ABCyano4 in comparison to ABICyano1.

Example 26

Comparison of the Extracellular Polysaccharide Layer of ABICyano1 and ABCyano4

[00358] Wild type strains of ABICyano1 and ABCyano4 were cultured under standard conditions in 1.2 L vPBRs in BG11 artificial seawater (35 psu). The cultures were cultivated at a 12h / 12h dark / light cycle and illuminated from one side of the LvPBRs. Microscopic images of both strains were produced. Fig. 18A, the microscopic image of ABCyano4 cells shows that these cells have thick CPS layer, which is much thicker than the corresponding CPS layer of the ABICyano1 cells (see Fig. 18B). In particular, ABCyano4 cells exhibit in CPS layer having a thickness of around 1 μ m to 3 μ m, preferably 1.1 to 2 μ m, most preferred 1.1 to 1.6 μ m. The thickness of the CPS as determined experimentally via microscopic images employing ink negative stain. The ABCyano4 cells exhibit a CPS layer having a thickness of around 1.28 +/-

0.16 μm at an average cell diameter of $3.0 \pm 0.17 \mu\text{m}$, while ABICyano1 cells do not exhibit a measurable CPS layer at an average cell diameter of $2.3 \pm 0.15 \mu\text{m}$. Owing to the difference in the thickness of the CPS layer, a lot of the compounds of interest, especially mycosporine-like amino acids, produced by genetically modified ABCyano4 cells are maintained either in the cells or are associated with the thick CPS layer, greatly facilitating the purification of the compound of interest (Fig. 37). In contrast to that, a large fraction of a compound of interest produced by genetically modified ABICyano1 cells with thin CPS layer can be found secreted into the culture medium.

Example 27

Comparison of Mycosporine-glycine Production in ABICyano1 and ABCyano4 strains

[00359] Fig. 20A, 20B and 20C show the growth, the mycosporine-glycine production and the carbon-partitioning for an ABICyano1 (AB1225) and ABCyano4 strain (AB1322) over a time period of 9 days. The strain AB1322 is an ABCyano4 strain genetically modified with the heterologous genes Ava_3858, Ava_3857 and Ava_3856 under the transcriptional control of the PsmtA promoter. AB1225 is the analogous ABICyano1 strain transformed with the same heterologous genes under the transcriptional control of the same promoter. Strains were cultivated in 20 mL GC vials filled with 2 mL culture suspension using GC assay medium (ASW BG11 supplemented with 10 mM urea, 10 mM NaHCO_3 , buffered with TES at pH 7.3. The GC vials were placed on a rotary shaker at 450 rpm at 35°C and a $120\text{--}200 \mu\text{E m}^{-2} \text{s}^{-1}$ homogenous light field from the bottom. Initially, 6 mL pure CO_2 was injected through the gas-tight lid. Pre-cultures were grown until an $\text{OD}_{750\text{nm}}$ between 5 and 10 and then diluted to an OD set point of about 5 using fresh medium. For induction of MAA production ZnSO_4 was added and 6 mL pure CO_2 was injected into each GC vial. Induced cultures were placed again on a rotatory shaker with 450 rpm at $120\text{--}200 \mu\text{E m}^{-2} \text{s}^{-1}$ homogenous light field from the bottom. Experiments were performed in biological replicates per strain. From each GC vial culture samples were taken daily and measured for growth (increase in optical density at 750nm) and MAA production. The cultures were supplemented daily with 10 mM fresh urea and 6 mL pure CO_2 . Fig. 20A and Fig. 20B show that after around 3-4 days of cultivation the ABCyano4 strain exhibited a higher growth rate and a higher MAA production. In contrast, the ABICyano1 strain showed higher carbon partitioning to

mycosporine-glycine at the beginning of the cultivation in comparison to the ABCyano4 strain. However, after around 4 days of cultivation the ABCyano4 strain significantly increased the carbon partitioning leading to a higher carbon partitioning value than ABICyano1. The carbon-partitioning was determined by calculation from the OD measured using an experimentally determined DW/OD ratio (1L ABCyano1 culture with an OD of 1 corresponds to 0.2g/L dry biomass). MAA concentration was determined spectrophotometrically using Lambert-Beer's law known absorbance properties of M-Gly as described earlier.

Example 28

Comparison of Growth, Mycosporine-2-glycine Production and Carbon Partitioning for ABICyano1 and ABCyano4

[00360] Fig. 21A, 21B and 21C show the growth, the mycosporine-2-glycine production and the carbon-partitioning for an ABICyano1 (AB1324) and ABCyano4 strain (AB1334) over a time period of 3 days under cultivation conditions described earlier in example 27. The carbon-partitioning was determined by calculation from the OD measured using an experimentally determined DW/OD ratio (1L ABCyano1 culture with an OD of 1 corresponds to 0.2g/L dry biomass). MAA concentration was determined spectrophotometrically using Lambert-Beer's law known absorbance properties of M-2-Gly as described earlier. Fig. 21A and Fig. 21B show that particular after around 1.5 days of cultivation the ABCyano4 strain exhibited a higher growth rate and a higher MAA production. In contrast to that, the ABICyano1 strain had a higher carbon-partitioning to mycosporine-2-glycine at the beginning of the cultivation in comparison to the ABCyano4 strain. However, after around 1.5 days of cultivation the ABCyano4 strain significantly increased the carbon-partitioning, leading to a higher carbon-partitioning value than ABICyano1. Fig. 21B shows a high production of mycosporine-2-glycine over the course of nearly 3 days for the strain AB1324. In particular, for M-2-Gly production with AB1324 (ABICyano1) productivities of ~75-100mg/L*d and for AB1334 (ABCyano4) productivities of ~150-

200mg/L*d over a period of 3-4 days was achieved when employing the cultivation conditions of Example 27.

Example 29

Production of 4-deoxygadusol in ABCyano4

[00361] Fig. 26A shows the production of 4-deoxygadusol (4-DG) by the ABCyano4 strain AB1312 over a time period of 2 days under cultivation conditions described in example 27. This strain was produced by transformation of ABCyano4 with genes encoding the two enzymes DHQS (Ava_3858) and O-MT (Ava_3857) under the transcriptional control of the PsmtA promoter. Fig. 26B depicts the whole cell absorption spectrum showing the differences between the wild-type ABCyano4 and AB1312.

Example 30

Production of Mycosporine-glycine in Low-pigment Strain of ABCyano4

[00362] The ABCyano4 strains AB1322 and AB1361 were created. AB1322 resulted from the transformation of the wild-type ABCyano4 strain with the heterologous genes Ava_3858, Ava_3857 and Ava_3856 (codon optimized variants) under the transcriptional control of the PsmtA promoter. The strain AB1361 was created by transformation of the strain AB1322 with the plasmid #2848 (see table 4B) which leads to a $\Delta ycf37$ gene inactivation associated with a low chlorophyll phenotype (Dühring et al., 2006). The strains were cultivated under conditions described in example 27. Fig. 27A, 27B and 27C show the production of mycosporine-glycine over a time period of 2 days, the whole cell absorption spectrum and the HPLC profile. The Fig. 27A and 27B show that the low pigment strain produced more mycosporine-glycine than AB1322.

Example 31

Production of Shinorine/porphyrin-334 in ABCyano4 with Different Ratios

[00363] Fig. 28A, 28B and 28C show the production of shinorine/porphyrin-334 in a ratio of 60%/40% over a time period of nearly 3 days, the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB1333. This strain was created by transforming the ABCyano4 strain AB1322 with plasmid #2891 (see table 4B). This resulted in a strain being transformed with the heterologous gene NpF5597 (*mysD* from *N. punctiforme*) under the transcriptional control of the *PsmA* promoter and additionally the heterologous genes *Ava_3858*, *Ava_3857* and *Ava_3856* all also under the transcriptional control of the *PsmA* promoter. Fig. 28C shows the HPLC peaks of both porphyrin-334 and shinorine. The quantification of the peak areas from the HPLC profile reveal a relative quantity of 60% for shinorine and 40% for porphyrin-334 of total MAAs produced.

[00364] Fig. 30A, 30B and 30C show the production of shinorine/porphyrin-334 in a ratio of 45%/55% over a time period of 6 days, the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4016. This strain was generated by transforming AB1322 with *mysD_HL-69* under the transcriptional control of the *PsmA* promoter. The quantification of the peak areas from the HPLC profile revealed a relative quantity of 45% for shinorine and 55% for porphyrin-334 of total MAAs produced.

[00365] Fig. 31A, 31B and 31C show the production of shinorine/porphyrin-334 in a ratio of 10%/90% over a time period of 3 days, the whole cell absorption spectrum and the HPLC profile for the ABCyano4 strain AB4046. This strain was generated by the transformation of AB1322 with a heterologous gene *mysD_K005* under the transcriptional control of the *PsmA* promoter. The HPLC spectrum of Fig. 31C clearly shows the peaks for shinorine and porphyrin-334. The quantification of the peak areas from the HPLC profile reveal a relative quantity of about 10% for shinorine and 90% for porphyrin-334 of total MAAs produced.

[00366] The strains were cultivated under conditions described in example 27.

Example 31

Production of Mycosporine-2-glycine in ABCyano4

[00367] The ABCyano4 strain AB1334 was created by transformation of the strain AB1322 with the heterologous gene ap3855 (mysD from *A. halophytica*) under the transcriptional control of a PsmtA promoter. The strains were cultivated under conditions described in example 27. Fig. 29A shows the production of mycosporine-2-glycine over a time period of nearly 3 days. Fig. 29B shows the differences in the whole cell absorption spectrum between AB1322 producing mycosporine-glycine and AB1334 producing mycosporine-2-glycine. Fig. 29C shows the HPLC profile of the produced mycosporine-2-glycine.

Example 32

Constitutive vs. Metal-inducible Production of Mycosporine-glycine in ABCyano4

[00368] Fig. 35A and 35B show a comparison between the production of mycosporine-glycine by different ABCyano4 strains transformed with genes operably linked to a constitutive promoter PcpCB (strain AB1365), PpsaL (strain AB4049), PpsaA (strain AB4050), and the strain AB1322 wherein the heterologous genes for MAA production are operably linked to a zinc-inducible promoter PsmtA. All strains have been transformed with the same set of heterologous genes for MAA production, namely Ava_3858, Ava_3857 and Ava_3856. Fig. 35A and 35B clearly show that the production of mycosporine-glycine was higher for the inducible PsmtA promoter in comparison to the constitutive promoters. The strains were cultivated under conditions described in example 27. Fig. 35C shows a comparison between the production of mycosporine-glycine by different ABCyano4 strains transformed with MAA production genes operably linked to the metal-inducible promoters PsmtA (AB1322) and PpetE (AB4095) inducible with zinc or copper, respectively. As long as no zinc or copper was added to the strains very low amount of mycosporine-glycine was produced, while addition of zinc to AB1322 and copper to AB4095 specifically induced the production of mycosporine-glycine at high level.

Example 33

Production of Shinorine/porphyra-334 with Dual mysA-strain in ABCyano4

[00369] Fig. 36A, 36B and 36C show a comparison between the production of shinorine/porphyra-334, the accumulation of biomass and the percentage of carbon partitioning for the different ABCyano4 strains AB1333 and the dual mysA strain AB4028 over the time period of 6 days of cultivation. In comparison to AB1333, which only contains the gene Ava_3858 as one mysA homolog, the dual mysA strain AB4028 contains a second mysA copy, namely another Ava_3858 gene operably linked to a zinc-inducible promoter PsmtA. The strains were cultivated under conditions described in example 27. Fig. 36A and 36C show that the dual mysA strain had a higher production of shinorine/porphyra-334 and a higher carbon partitioning compared to AB1333 and at the same time comparable accumulation of biomass (Fig. 36B).

[00370] Fig. 19A to Fig. 19C depict the growth, the concentration of shinorine/porphyra-334 and the carbon-partitioning of two different ABCyano4 strains, AB4092 and AB4094 over a time period of cultivation of nearly 10 days under cultivation conditions described in example 27. The strain AB4092 included the mysABC genes from *A. variabilis*, Ava_3858, Ava_3857, Ava_3856 and additionally the mysD gene Amir_4256 from *Actinosynnema mirum*. The strain AB4094 also contained the Ava_3858, Ava_3857, and Ava_3856 genes and additionally a second mysA gene copy from the divergent clade and mysD of *Cyanobacterium* sp. HL-69. While AB4094 exhibited a slower growth compared to AB4092, AB4094 had a larger production of shinorine/porphyra-334 and a higher carbon-partitioning due to the catalytically more active MysA variant from the divergent clade.

Example 34

Production of Palythine, Tery-364, Mycosporine-methylamine-threonine and Tery-322 in *Escherichia coli*

[00371] *E. coli* cells BL21 have been transformed with the plasmids #2870, #3119, #3117, #3120 and #3129 to produce various MAAs under control of an IPTG inducible Ptac promoter. Fig. 45A depicts exemplarily the plasmid map of plasmid construct #2870 for description of general plasmid design used for transformation of *E. coli* to produce MAAs. Strains were

cultivated in 40 ml LB medium over night at 25 or 18 °C after induction with 0.5 mM IPTG during the exponential growth phase. Fig. 45 shows the HPLC profile of *E. coli* BL21 transformed with plasmid #2870 (Ava_3858, Ava_3857 and Ava_3856) producing mycosporine-glycine. Fig. 46 depicts the HPLC profile of *E. coli* BL21 transformed with plasmid # 3119 (Ava_3858, Ava_3857, Ava_3856, Np_mysD and Tery_2969), producing mycosporine-methylamine-threonine. Fig. 47 shows the HPLC profile of *E. coli* BL21 transformed with plasmid # 3117 (Ava_3858, Ava_3857, Ava_3856, Tery_mysD1 and Tery_2972), producing palythine. Fig. 48 shows the HPLC profile of *E. coli* BL21 transformed with plasmid # 3120 (Ava_3858, Ava_3857, Ava_3856, Tery_2966), producing Tery-322. Fig. 49 depicts the HPLC profile of *E. coli* BL21 transformed with plasmid # 3129 (Ava_3858, Ava_3857, Ava_3856, Tery_2966, Tery_4684, Tery_2968, Tery_2966), producing Tery-364. *E. coli* BL21 # 3129 produces more Tery-364 than the corresponding cyanobacterial strain AB1360.

Example 35

Production of MAAs with a Divergent Clade mysA Variant

[00372] Fig. 42A shows a protein sequence alignment of the MysA enzymes from different cyanobacterial species, including the consensus sequence. The first nine MysA variants (*Anabaena* to *Lyngbya*) cluster together to form one monophyletic clade of MysA (type I) while the latter eight MysA variants (*Trichodesmium* to *Hydrocoleum*) form another monophyletic clade of MysA (type II) that differs from the first MysA clade. The conserved amino acid sequences of two fingerprint segments for the classical clade are MLELE_xPNLHE and LDRVIA_xGH. The divergent clade has some conserved motifs (e.g., YxxxEY(G)-xNxxET and QC(D)RPHA(G)YGHTWSP) distinct from the classical clade. The conserved two fingerprint segments are marked in Fig. 42A. The strains were cultivated under conditions described in example 27. Fig. 42B and 42C show the production of mycosporine-glycine and the C-partitioning (M-Gly produced per dry weight) involving different mysA genes. AB1322 contains the mysABC operon from *Anabaena variabilis* ATCC 29413 operably linked to a zinc-inducible promoter PsmtA. The second strain AB4015 (dual mysA strain) contains the same PsmtA-controlled mysABC operon from *Anabaena variabilis* ATCC 29413 plus a second mysA copy from *Anabaena variabilis* ATCC 29413, operably linked to a zinc-inducible promoter PsmtA.

The third strain AB4035 contains the *mysA* gene from *Cyanobacterium* sp. HL-69 (divergent MysA clade type II) and *mysBC* genes from *Anabaena variabilis* ATCC 29413 all operably linked to a zinc-inducible promoter *PsmtA*. Fig. 42B and 42C show that the dual *mysA* strain AB4015 had a higher production of mycosporine-glycine and a higher carbon partitioning compared to AB1322. In comparison to that strain AB4035 with one single *mysA* gene copy from *Cyanobacterium* sp. HL-69 exhibited a higher production of mycosporine-glycine and a higher carbon partitioning compared to AB1322 as well but already at much lower level of induction (at lower zinc concentration). This indicates that application of the *mysA* gene from *Cyanobacterium* sp. HL-69 (divergent MysA clade type II) is advantageous compared to the *mysA* variant from *Anabaena variabilis* ATCC 29413 (common MysA clade type I).

Example 36

Use of *mysABCD* Gene Cluster from *Trichodesmium erythraeum* IMS101 and *Cyanobacterium* sp. HL-69

[00373] As a prophetic example, the integrative plasmids #3077 and #3127 are prepared, having a splitted *mysABCD* cluster from *Cyanobacterium* sp. HL-69 under control of the zinc inducible *PsmtA*, codon optimized for optimal expression in the ABCyano4 host strain. The plasmids are transformed to ABCyano4 cells, following the method described in Example 7, resulting in AB4106. The strain can be cultivated under conditions described in example 27 and tested for production of shinorine/porphyrin-344.

[00374] As a prophetic example, an integrative plasmid #3146 is prepared, having the *mysABC* cluster from *Trichodesmium erythraeum* IMS101, codon optimized for optimal expression in the ABCyano4 host strain. The plasmid is transformed to ABCyano4 cells, following the method described in Example 7, resulting in AB4097. The strain can be cultivated under conditions described in example 27 and tested for production of mycosporine-glycine. The strain AB4097 can moreover be transformed with a second plasmid harboring either the *mysD1* (#3034) or the *mysD2* (#3035) gene from *Trichodesmium erythraeum* IMS101 to produce mycosporine-2-glycine and/or shinorine, resulting in strains AB4107 and AB4108.

Example 37**Constitutive Production of the MAA Mycosporine-glycine in *Synechocystis* PCC 6803**

[00375] As a prophetic example, a plasmid construct is prepared by inserting 3 genes from *Anabaena variabilis* (ava_3858, ava_3857, and ava_3856) to a modified endogenous ABICyano1 plasmid p6.8 or a RSF1010-derived plasmid. The three genes are regulated by a strong constitutive promoter PcpB*3 (plasmid #2752; SEQ ID NO: 18), following the method described in Example 8.

[00376] The plasmid is transformed to *Synechocystis* PCC 6803 host cells, and the transformation is confirmed. Cultures of the modified cells are then grown in a GC vial experiment to confirm that the MAA is being produced. Once production is confirmed, the cultures are scaled up to outdoor large-scale vertical photobioreactors, and commercially useful amounts of mycosporine-glycine are produced.

Example 38**Preparation of of the MAA “Shinorine and Porphyrin-334” in *Synechococcus* PCC 7002**

[00377] As a prophetic example, the MAAs “shinorine” and “porphyrin-334 are produced in *Synechococcus* sp. PCC 7002 host cells using the following method, which generally follows the method of Example 13. Modified *Synechococcus* sp. PCC 7002 host cells that already have the mysABC genes for the production of mycosporine-glycine are then transformed with a second endogenous plasmid pABICyano1B that contains one additional gene: either mysD from *Nostoc punctiforme* (NpF5597) or Ava_3855, which encodes a nonribosomal peptide synthetase (NRPS) from *Anabaena variabilis*.

[00378] Construct information: AB1252 (ABICyano1:2742:2796)
 pABICyano1_6.8::PsmA-Ava_3858(ABICyano1opt)-IScpBA*1-Ava_3857(ABICyano1opt)-
 IScpBA*1-Ava_3856(ABICyano1opt)-TpsaB, pABICyano1B_Rep::PrpsL-Gm**-TB0014-
 oriVT-PsmA-NpF5597(ABICyano1opt) → shinorine/porphyrin-334

[00379] An absorbance spectrum analysis after 24 hours of growth in a GC vial is then performed to determine that shinorine/porphyrin-334 (with an absorbance max of 334 nm) are produced.

[00380] The modified cells are scaled-up to outdoor photobioreactors, and grown for 20 days. The cultures are then harvested and the MAA is isolated and purified.

[00381] Although the present invention has been described in considerable detail with reference to certain embodiments thereof, other embodiments are possible. Therefore, the spirit and scope of the appended claims should not be limited to the description of the embodiments contained therein.

CLAIMS

What is Claimed is:

1. A genetically modified cyanobacterial cell comprising at least one heterologous gene that encodes an enzyme involved in the production of a mycosporine-like amino acid (MAA).
2. The genetically modified cyanobacterial cell of claim 1, wherein the MAA is selected from the group consisting of mycosporine-glycine, shinorine, mycosporine-2-glycine, 4-deoxygadusol, usujirene, porphyra-334, asterina, palythene, palythine, mycosporine-2-(4-deoxygadusolyl-ornithine), mycosporine-ornithine, mycosporine-lysine, mycosporine-alanine, gadusol, mycosporine-taurine, palythine-threonine, palythine-serine, mycosporine-methylamine-threonine, mycosporine-methylamine-serine, palythine-threonine-sulfate, palythine-serine-sulfate, mycosporine-glycine-valine, mycosporine-glycine-alanine, mycosporine-glutamic acid glycine, and euhalothec-362, and derivatives thereof.
3. The genetically modified cyanobacterial cell of any of the above claims, wherein the enzyme involved in the production of an MAA is selected from the group consisting of 4-deoxygadusol (DDG) synthase, O-methyltransferase, ATP-grasp family protein, D-Ala-D-Ala-ligase, nonribosomal peptide synthetase (NRPS), clavaminic acid-like synthetase and 3-hydroxyisobutyrate-like dehydrogenase.
4. The genetically modified cyanobacterial cell of any of the above claims, wherein more than one MAA is produced by said cyanobacterial cell.
5. The genetically modified cyanobacterial cell of any of the above claims, wherein the cyanobacterial cell is grown photoautotrophically.
6. The genetically modified cyanobacterial cell of any of the above claims, wherein in the heterologous gene is from an organism selected from the group consisting of: cyanobacteria, bacteria, fungi, dinoflagellates, cnidaria and algae.
7. The genetically modified cyanobacterial cell of the above claim, wherein the heterologous gene is from a cyanobacterium.

8. The genetically modified cyanobacterial cell of any of the above claims, wherein the at least one heterologous gene that encodes an enzyme involved in the production of an MAA has at least 70%, preferably at least 80%, most preferably at least 90% identity to at least one amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 158, SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, and SEQ ID NO: 170.
9. The genetically modified cyanobacterial cell of any of the above claims, wherein the heterologous gene is codon optimized for optimal expression in the cyanobacterial cell.
10. The genetically modified cyanobacterial cell of any of the above claims, wherein the promoter is a regulatable promoter or a constitutive promoter.
11. The genetically modified cyanobacterial cell of the above claim, wherein the promoter has at least 80% identity to a promoter selected from the group consisting of PrbcL, PntcA, PnblA, PisiA, PpetJ, PpetE, PcorT, PsmtA, PziaA, PsigB, PlrtA, PhtpG, PhspA, PclpB1, PhliB, PggpS, PpsbA2, PpsaA, PpsaL, PnirA, PnarB, PnrtA, PisiB, PnrsB, PmrgA, PpstS, and PcrhC, PpsbD, , PrpoA, PrpsL, PrpoA, PcpcB, PnirA*2, PnirA*3, PnirA*4, PmntC, PrpsL*4, Prbc*, PcpcB (SEQ ID NO: 52), PnirA (SEQ ID NO: 22), PppsA, PrnpA (SEQ ID NO: 46), PpstS, Porf0128 (SEQ ID NO: 31), Porf1486 (SEQ ID NO: 32), Porf3164 (SEQ ID NO: 36), Porf3293 (SEQ ID NO: 33), Porf3621 (SEQ ID NO: 346), Porf3635 (SEQ ID NO: 35), Porf3858, Porf1071 (SEQ ID NO: 27), Porf1072 (SEQ ID NO: 37), Porf1074 (SEQ ID NO: 38), Porf1075 (SEQ ID NO: 39), Porf1542 (SEQ ID NO: 40), Porf1823 (SEQ ID NO: 41), Porf1824, Porf3126 (PsmtA) (SEQ ID NO: 23), Porf3389, Porf0221 (SEQ ID NO: 28), Porf0222 (SEQ ID NO: 42), Porf0223 (SEQ ID NO: 29), Porf0316 (SEQ ID NO: 30), Porf3232 (SEQ ID NO: 43), Porf3461 (PpetJ) (SEQ ID NO: 25), and Porf3749 (SEQ ID NO: 44).
12. The genetically modified cyanobacterial cell of the above claim 11, wherein the promoter is inducible by a change of a metal-ion concentration, in particular the PsmtA promoter or the PpetE promoter.

13. The genetically modified cyanobacterial cell of any of the above claims, wherein at least one heterologous gene is located on a modified endogenous or a heterologous extrachromosomal plasmid.

14. The genetically modified cyanobacterial cell of the above claim, wherein the heterologous genes are located on more than one modified endogenous or heterologous plasmid.

15. The genetically modified cyanobacterial cell of any of the above claims 13 or 14, wherein the heterologous genes are located on an extrachromosomal high copy plasmid being present in a cell in at least 10, preferably at least 20, most preferably at least 40 copies per cell.

16. The genetically modified cyanobacterial cell of the above claim, wherein the high copy plasmid is a genetically modified endogenous high copy plasmid.

17. The genetically modified cyanobacterial cell of any of the above claims, which is able to produce high amounts of MAAs over an extended period of time, for example at least $25 \text{ mg L}^{-1}\text{d}^{-1}$, preferably at least $50 \text{ mg L}^{-1}\text{d}^{-1}$ more preferably at least $100 \text{ mg L}^{-1}\text{d}^{-1}$, most preferred at least $150 \text{ mg L}^{-1}\text{d}^{-1}$, or even $200 \text{ mg L}^{-1}\text{d}^{-1}$ over a period of 1-2 weeks.

18. The genetically modified cyanobacterial cell of any of the previous claims for the production of mycosporine-glycine, comprising at least three heterologous genes, wherein the first heterologous gene codes for a first enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 3, SEQ ID NO: 158, or to SEQ ID NO: 164, the second heterologous gene codes for a second enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 6, SEQ ID NO: 160, or SEQ ID NO: 166, the third heterologous gene codes for a third enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 9, SEQ ID NO: 162, or SEQ ID NO: 168.

19. The genetically modified cyanobacterial cell of the previous claim, exhibiting a low pigment phenotype compared to the wildtype with respect to the chlorophyll and/or the phycocyanin content of the cell.

20. The genetically modified cyanobacterial cell of the previous claim, wherein the cell exhibits a low chlorophyll genotype having a *ycf37* gene inactivation associated with a low chlorophyll phenotype.
21. The genetically modified cyanobacterial cell of the previous claims 18 to 20, wherein the first enzyme is a DHQS-like protein, the second enzyme is an O-methyltransferase, and the third enzyme is an ATP-grasp family protein.
22. The genetically modified cyanobacterial cell of the previous claims 18 to 21 for the production of mycosporine-2-glycine, additionally including a fourth heterologous gene coding for a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 15 or 93.
23. The genetically modified cyanobacterial cell of the previous claims 18 to 21 for the production of shinorine/porphyra-334 and mycosporine-glycine-alanine, additionally including a fourth heterologous gene coding for a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 12, 15, 85, 93, 95, 170, 172 or 174.
24. The genetically modified cyanobacterial cell of the previous claim 23, having productivities of 50-100mg/L*d, preferably 120-200mg/L*d over a period of 3-10 days for the MAAs mycosporine-2-glycine or shinorine/porphyra-334.
25. The genetically modified cyanobacterial cell of the previous claims 23 or 24, further including a second copy of the first heterologous gene which codes for a first enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 3 or which is at least 70%, preferably 80%, most preferred at least 90% identical to SEQ ID NO: 164.
26. The genetically modified cyanobacterial cell of the previous claim 22, having productivities of 50-100mg/L*d, preferably 120-200mg/L*d over a period of 3-4 days for mycosporine-2-glycine.
27. The genetically modified cyanobacterial cell of any of claims 1-12 and 17 to 26, wherein at least one heterologous gene is integrated into the chromosome.

28. The genetically modified cyanobacterial cell of any of the above claims, wherein the genus of the cell is selected from the group consisting of *Arthrospira* sp., *Synechocystis*, *Synechococcus*, *Acartyochloris*, *Anabaena*, *Aphanothece*, *thermosynechococcus*, *Chamaesiphon*, *Chroococcus*, *Cyanobacterium*, *Eubhalothece*, *Cyanobium*, *Dactylococcopsis*, *Gloeobacter*, *Gloeocapsa*, *Gloeothece*, *Microcystis*, *Prochlorococcus*, *Prochloron*, *Chroococcidiopsis*, *Cyanocystis*, *Dermocarpella*, *Myxosarcina*, *Pleurocapsa*, *Stanieria*, *Xenococcus*, *Arthrospira*, *Borzia*, *Crinalium*, *Geitlerinema*, *Halospirulina*, *Leptolyngbya*, *Limnothrix*, *Lyngbya*, *Microcoleus*, *Cyanodictyon*, *Aphanocapsa*, *Oscillatoria*, *Planktothrix*, *Prochlorothrix*, *Pseudanabaena*, *Spirulina*, *Starria*, *Symploca*, *Trichodesmium*, *Tychonema*, *Anabaenopsis*, *Aphanizomenon*, *Calothrix*, *Cyanospira*, *Cylindrospermopsis*, *Cylindrospermum*, *Nodularia*, *Nostoc*, *Chlorogloeopsis*, *Fischerella*, *Geitleria*, *Nostochopsis*, *Iyengariella*, *Stigonema*, *Rivularia*, *Scytonema*, *Tolypothrix*, *Cyanothece*, *Phormidium*, and *Adrianema*, preferably ABICyano1 and ABCyano4.

29. The genetically modified cyanobacterial cell of any of the above claims, wherein the cell is a non-filamentous cyanobacterial cell.

30. The genetically modified cyanobacterial cell of the above claim, wherein the genus of the genetically modified cyanobacterial cell is *Cyanobacterium* sp.

31. The genetically modified cyanobacterial cell of the above claim, wherein the genetically modified cyanobacterial cell is *Cyanobacterium* sp. ABICyano1 or *Cyanobacterium* sp. ABCyano4.

32. The genetically modified cyanobacterial cell of claim 30 or 31, having an extracellular capsular polysaccharide layer (CPS) with a thickness of between 1 and 3 μm , preferably between 1.1 and 2 μm .

33. The genetically modified cyanobacterial cell of claim 32, wherein the genetically modified cyanobacterial cell is *Cyanobacterium* sp. ABCyano4.

34. The genetically modified cyanobacterial cell of any of the above claims, wherein the cyanobacterial cell is grown photomixotrophically.

35. The genetically modified cyanobacterial cell of any of the above claims, including an operon including at least one first and one second heterologous gene encoding a first and a second enzyme involved in the production of a MAA, the operon including an intergenic sequence between the first heterologous gene and the second heterologous gene.

36. The genetically modified cyanobacterial cell of the above claim, wherein the intergenic sequence is derived from an intergenic sequence located between two genes coding for a heterodimer protein.

37. The genetically modified cyanobacterial cell of any the above claims 35 or 36, wherein the intergenic sequence is derived from the cpcB-cpcA operon coding for phycocyanin.

38. The genetically modified cyanobacterial cell of any the above claims 35 to 37, wherein the intergenic sequence is at least 90 % identical to the sequence with the SEQ ID NO: 177 (IScpcBA).

39. The genetically modified cyanobacterial cell of the above claim, wherein the intergenic sequence includes a ribosomal binding side and wherein the intergenic sequence exhibits nucleotide changes in a region between the ribosomal binding site and a start codon of either the first or second heterologous gene in comparison to the sequence of SEQ ID NO: 177, preferably wherein the intergenic sequence is the sequence with the SEQ ID NO: 178.

40. A method of producing an MAA in a cyanobacterial cell, comprising:

a) growing the genetically modified cyanobacterial cell of any of the above claims in a culture medium suitable for production of the MAA in a photobioreactor with adequate light and CO₂ addition; and

b) isolating the MAA from the cells and/or the culture medium.

41. The method of the above claim, wherein step a) further comprises adding an amino acid to the culture medium.

42. The method of the above claims 40 or 41, wherein at least 50%, preferably at least 60% more preferred at least 70% of the MAA produced is associated with the cyanobacterial cell and wherein

in step b) the MAA is isolated from the cells by separating the cells from the culture medium and isolating the MAA from the cells.

43. The method of the previous claim 42, wherein the cyanobacterial cell has a capsular exopolysaccharide layer (CPS) and wherein the MAA associated with the cell is associated with the CPS of the cyanobacterial cells.

44. The method of the previous claim 43, wherein in step b) the CPS is removed from the cells, thereby removing the MAA from the cells.

45. A pharmaceutical composition or a cosmetic composition comprising a UV absorbing compound, wherein the UV absorbing compound is an MAA that has been produced in a genetically modified cyanobacterial cell of any of claims 1-40.

46. The pharmaceutical composition or a cosmetic composition of claim 45, wherein the composition comprises at least 2 MAAs, and further wherein the wavelength range of UV protection is broader than with a single MAA.

47. The pharmaceutical composition or a cosmetic composition of claim 45 or 46, wherein the MAA is isolated from the culture medium.

48. The pharmaceutical composition or a cosmetic composition of claim 45 or 47, wherein the MAA is isolated from the cyanobacterial cells.

49. The pharmaceutical composition or a cosmetic composition of any one of claims 45 – 48, wherein the composition is a sunscreen product ingredient.

50. The pharmaceutical composition or a cosmetic composition of any one of claims 45 – 49, wherein the composition is a skin or hair care product ingredient.

51. The pharmaceutical composition or a cosmetic composition of any one of claims 45-50, wherein UV protection occurs over at least a portion of UV-A and at least a portion of UV-B.

52. A genetically modified host cell producing the compound Tery-322, Tery-322 having an absorption maximum at 322 nm, comprising at least one heterologous gene that encodes an enzyme

involved in the production of the compound Tery-322, having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 103.

53. The genetically modified host cell of the previous claim, producing the compound Tery-364, Tery-364 having an absorption maximum at 364 nm, further comprising another heterologous gene encoding an enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 99.

54. The genetically modified host cell of the previous claim, further including a heterologous gene encoding an enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme phosphopantethiene-protein transferase (SEQ ID NO: 176)..

55. The genetically modified host cell of any of the previous claims 52 to 54 for the production of either Tery-322 or Tery-364, further comprising at least three additional heterologous genes encoding three enzymes,

- a first enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 164 (Ava-3858 mysA variant or distant clade mysA variant),

- a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 (Ava-3857 mysB variant), and

- a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 (Ava-3856 mysC variant).

56. A genetically modified host cell producing shinorine/phorphyra-334 or mycosporine-2-glycine, comprising at least one heterologous gene encoding an enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme encoded by the nucleic acid of SEQ ID NO: 85 or enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either one of the enzymes of SEQ ID NO: 12, 15, 93, 95, 170, 172, or 174.

57. The genetically modified host cell of the previous claim producing palythine, comprising at least one further heterologous gene encoding an enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with an enzyme of SEQ ID NO: 91.

58. The genetically modified host cell of the previous claim 57, producing mycosporine-methylamine-glycine or mycosporine-methylamine-threonine, comprising at least one further heterologous gene encoding an enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with an enzyme of SEQ ID NO: 97.

59. The genetically modified host cell of any of the previous claims 56 to 58, further comprising at least three additional heterologous genes encoding three enzymes,

- a first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 164 (Ava-3858 mysA variant or distant clade mysA variant),

- a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 (Ava-3857 mysB variant), and

- a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 (Ava-3856 mysC variant).

60. The genetically modified host cell of any of the claims 52 to 59, wherein the host cell is selected from a group consisting of bacteria and eukaryotic cells, preferably enterobacteria, such as *E. coli*, and cyanobacteria, such as *Cyanobacterium* sp. like *Cyanobacterium* sp. ABICyano1 or *Cyanobacterium* sp. ABCyano4.

61. A mycosporine-like amino acid having an absorption maximum at 322 nm, being producible by culturing a genetically modified host cell including four heterologous genes encoding four enzymes,

- a first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 80 % sequence identity,

preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 164 (Ava-3858 mysA variant or distant clade mysA variant),

- a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 (Ava-3857 mysB variant),

- a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 (Ava-3856 mysC variant), and

- a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 103 (Tery_2966).

62. A mycosporine-like amino acid having an absorption maximum at 364 nm, being producible by culturing a genetically modified host cell including at least five heterologous genes encoding five enzymes,

- a first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 80 % sequence identity, preferably at least 90 % sequence identity with the enzyme of SEQ ID NO: 164 (Ava-3858 mysA variant or distant clade mysA variant),

- a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 (Ava-3857 mysB variant),

- a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 (Ava-3856 mysC variant),

- a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 103 (Tery_2966), and

- a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 99 (Tery_2968).

63. The mycosporine-like amino acid of the previous claim, wherein the genetically modified host cell further includes an enzyme having at least 70%, preferably at least 80%, most preferably at

least 90% sequence identity with the enzyme phosphopantethiene-protein transferase (SEQ ID NO: 176).

Mycosporine-glycine / Shinorine biosynthesis gene cluster
from *Anabaena variabilis*:

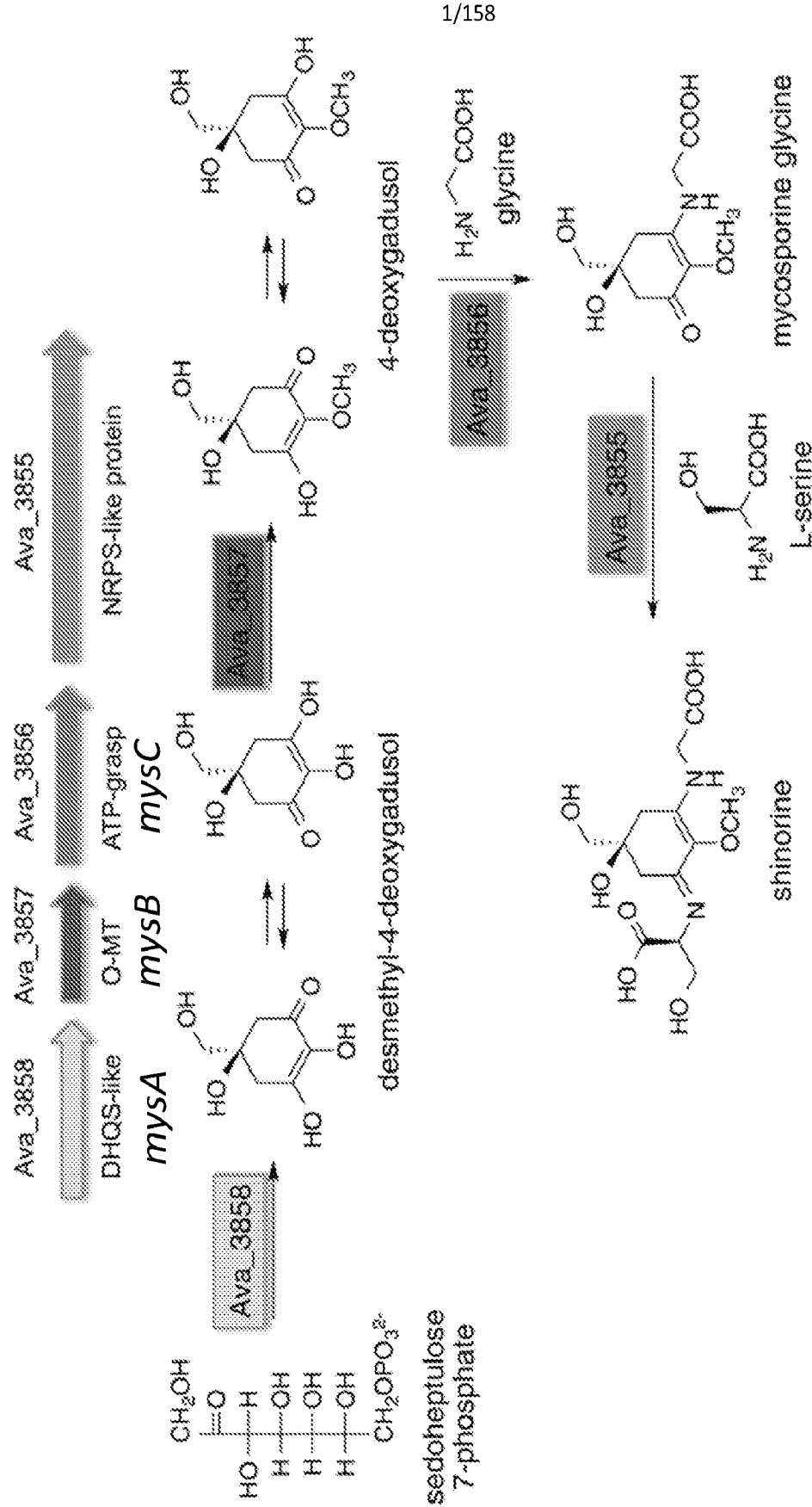


FIG. 1

(from Balskus et al., Science; 329(5999):1653-1656 (2010))

Biosynthetic Pathway for the Production of Mycosporine-glycine

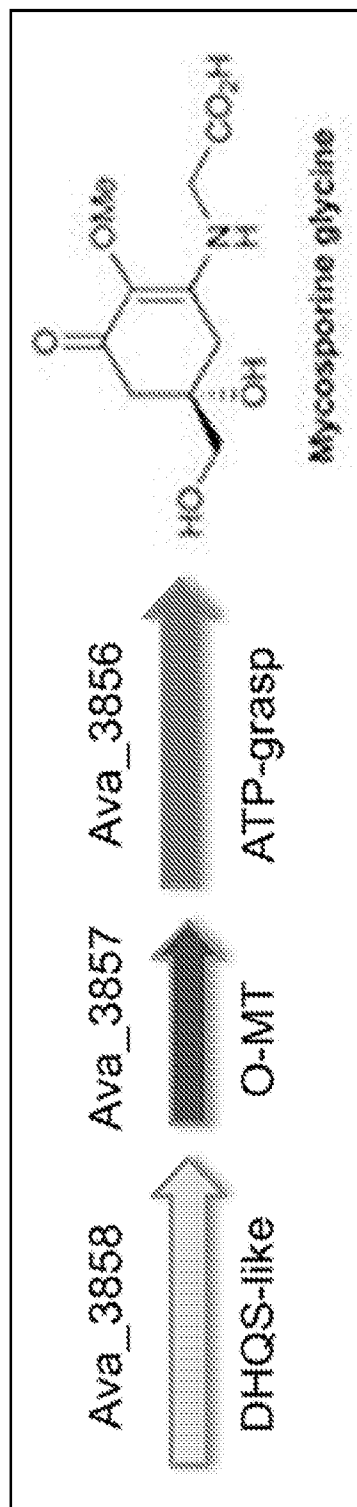


FIG. 2

Plasmid and Algal Strain Details

AB1224	Plasmid #2737	pABICyano1_6.8::Porf0223*-Ava_3858(AB1opt)-IScpBA*1-Ava_3857(AB1opt)-IScpBA*1-Ava_3856(AB1opt)-TpsaB
AB1225	Plasmid #2742	pABICyano1_6.8::PsmA-Ava_3858(AB1Cyano1opt)-Ava_3857(AB1Cyano1opt)-Ava_3856(AB1Cyano1opt)-TpsaB
AB1226	Plasmid #2752	pABICyano1_6.8::PcpB*3-Ava_3858(AB1Cyano1opt)-Ava_3857(AB1Cyano1opt)-Ava_3856(AB1Cyano1opt)-TpsaB
AB1252	Plasmid #2742, #2796	pABICyano1_6.8::PsmA-Ava_3858(AB1Cyano1opt)-IScpBA*1-Ava_3857(AB1Cyano1opt)-IScpBA*1-Ava_3856(AB1Cyano1opt)-TpsaB, ABICyano1B_Rep::PrpsL-Gm*-TB0014-oriVT-PsmtA-NpF5597(AB1Cyano1opt)
AB1253	Plasmid #2742, #2798	pABICyano1_6.8::PsmA-Ava_3858(AB1Cyano1opt)-IScpBA*1-Ava_3857(AB1Cyano1opt)-IScpBA*1-Ava_3856(AB1Cyano1opt)-TpsaB, ABICyano1B_Rep::PrpsL-Gm*-TB0014-oriVT-PsmtA-ap3855(AB1Cyano1opt)
AB1277	Plasmid #2840	pABICyano1_6.8::PsmA-Ava_3858(AB1Cyano1opt)-IScpBA*1-Ava_3857(AB1Cyano1opt)-IScpBA*1-Ava_3856(AB1Cyano1opt)-TpsaB-Porf0221-ap3855(AB1Cyano1opt)
AB1324	Plasmid #2893	pABICyano1_6.8::PsmA-Ava_3858(AB1Cyano1opt)-IScpBA*1-Ava_3857(AB1Cyano1opt)-IScpBA*1-Ava_3856(AB1Cyano1opt)-TpsaB-PsmtA-ap3855(AB1Cyano1opt)

FIG. 3

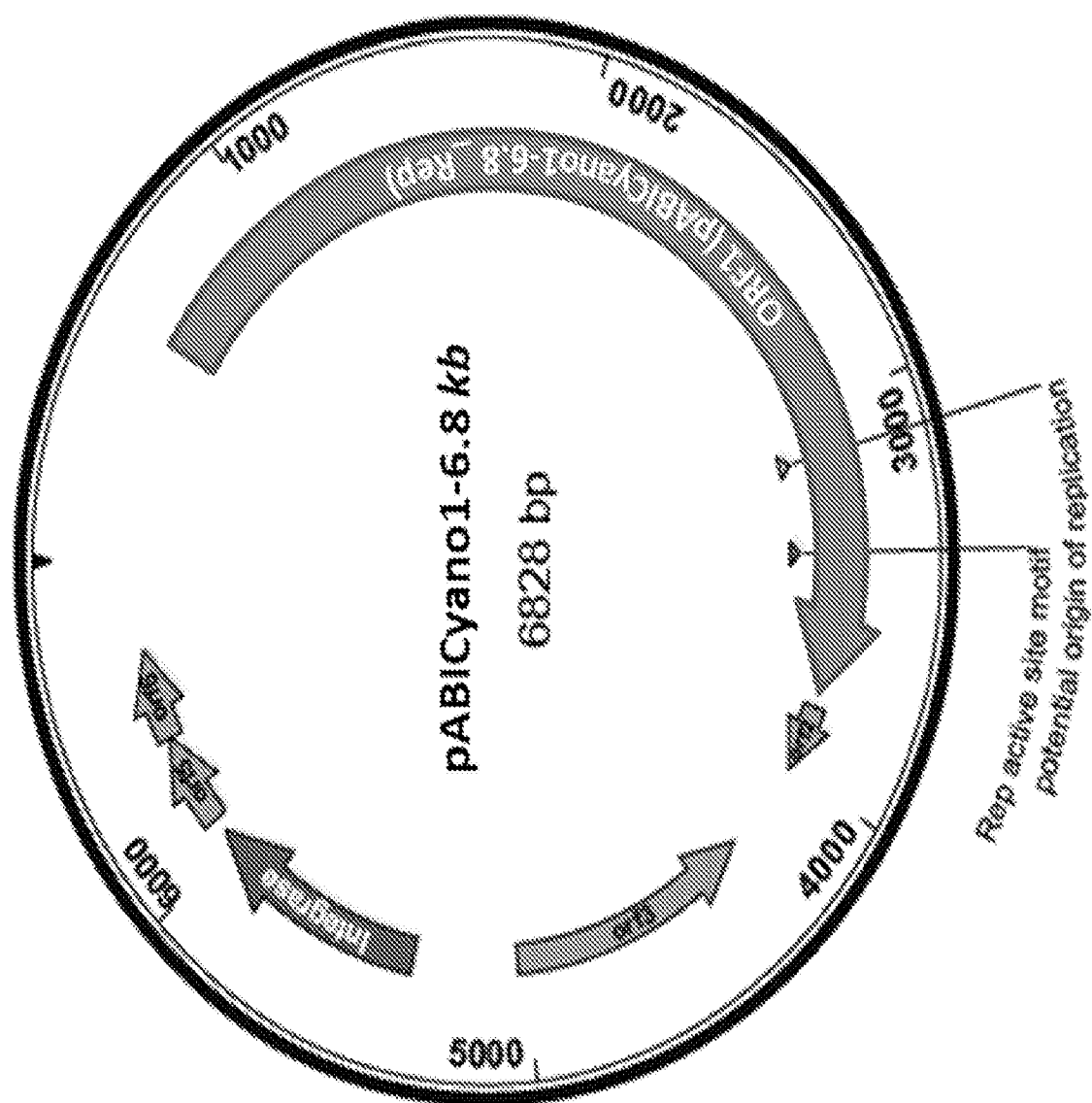


FIG. 4

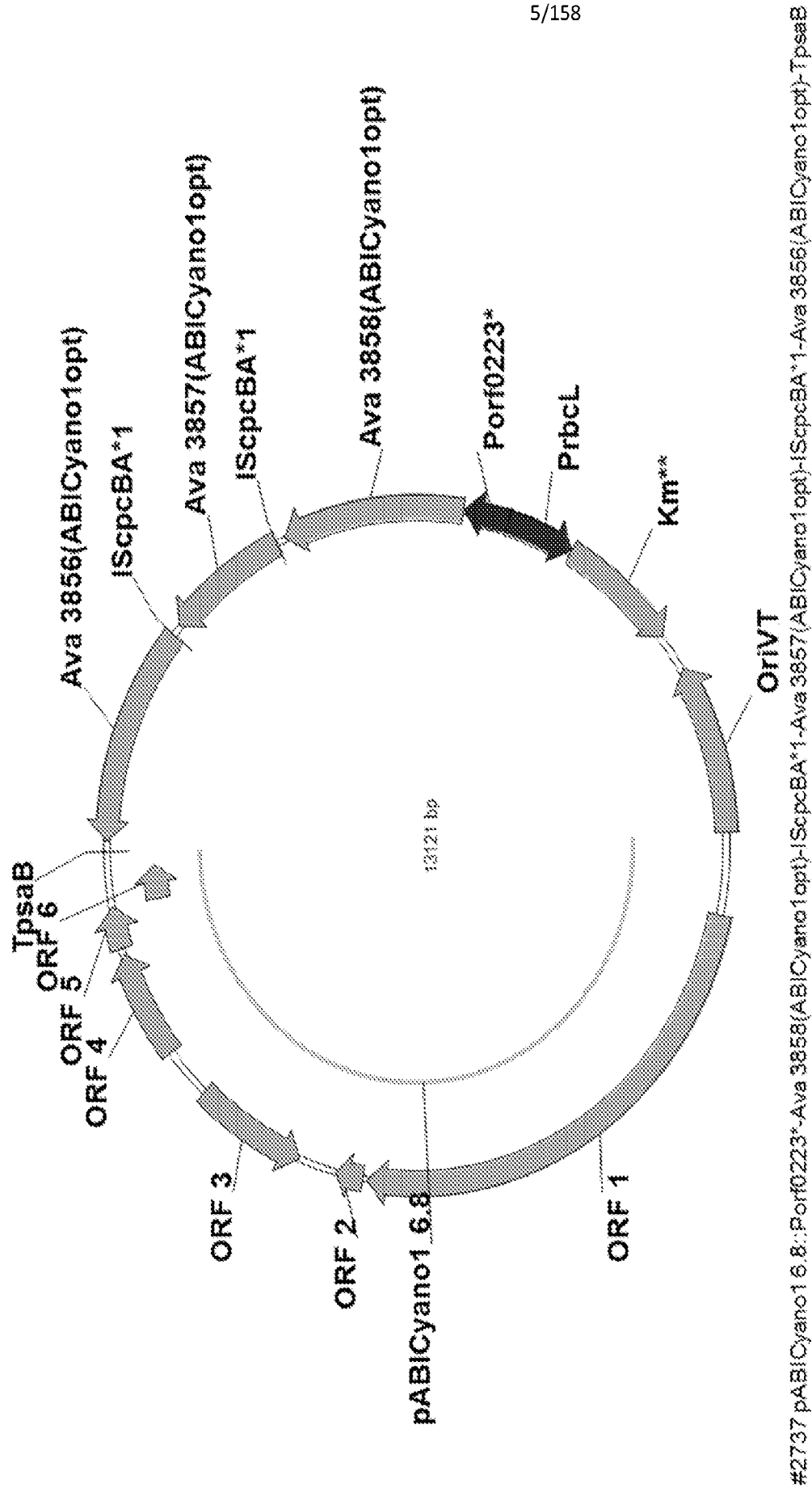


FIG. 5A

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ID      #2737\pABICyano1_6.8::Porf0223*-Ava_3858 (ABICyano1opt)-IScpcBA*1-
Ava_3857 (ABICyano1opt)-IScpcBA*1-Ava_3856 (ABICyano1opt)-TpsaB standard; circular DNA;
13121 BP.
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FT      /type="Custom cloned insert"
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FT      /note="Unknown feature type:insert"
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FT      /vntifkey="33"
FT      /label=oriVT
FT      CDS      6988..10173
FT      /vntifkey="4"
FT      /label=ORF\1
FT      /note="orf1 rep ori binding protein slr7037 homolog Similar to
hypothetical protein slr7037 of plasmid pSYSA (103 kb) of Synechocystis sp. PCC 6803"
FT      CDS      10209..10394
FT      /vntifkey="4"
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FIG. 5A (continued)

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FT      /note="orf2"
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FT      /label=ORF\3
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FT      /label=ORF\4
FT      /note="orf4 Integrase/Recombinase Similar to Site-specific recombinase
of Bacillus thuringiensis serovar israelensis ATCC 35646"
FT      12472..12735
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FT      12732..12980
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FT      /note="orf6"
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FT      /label=PrbcL
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FT      terminator

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FIG. 5A (continued)

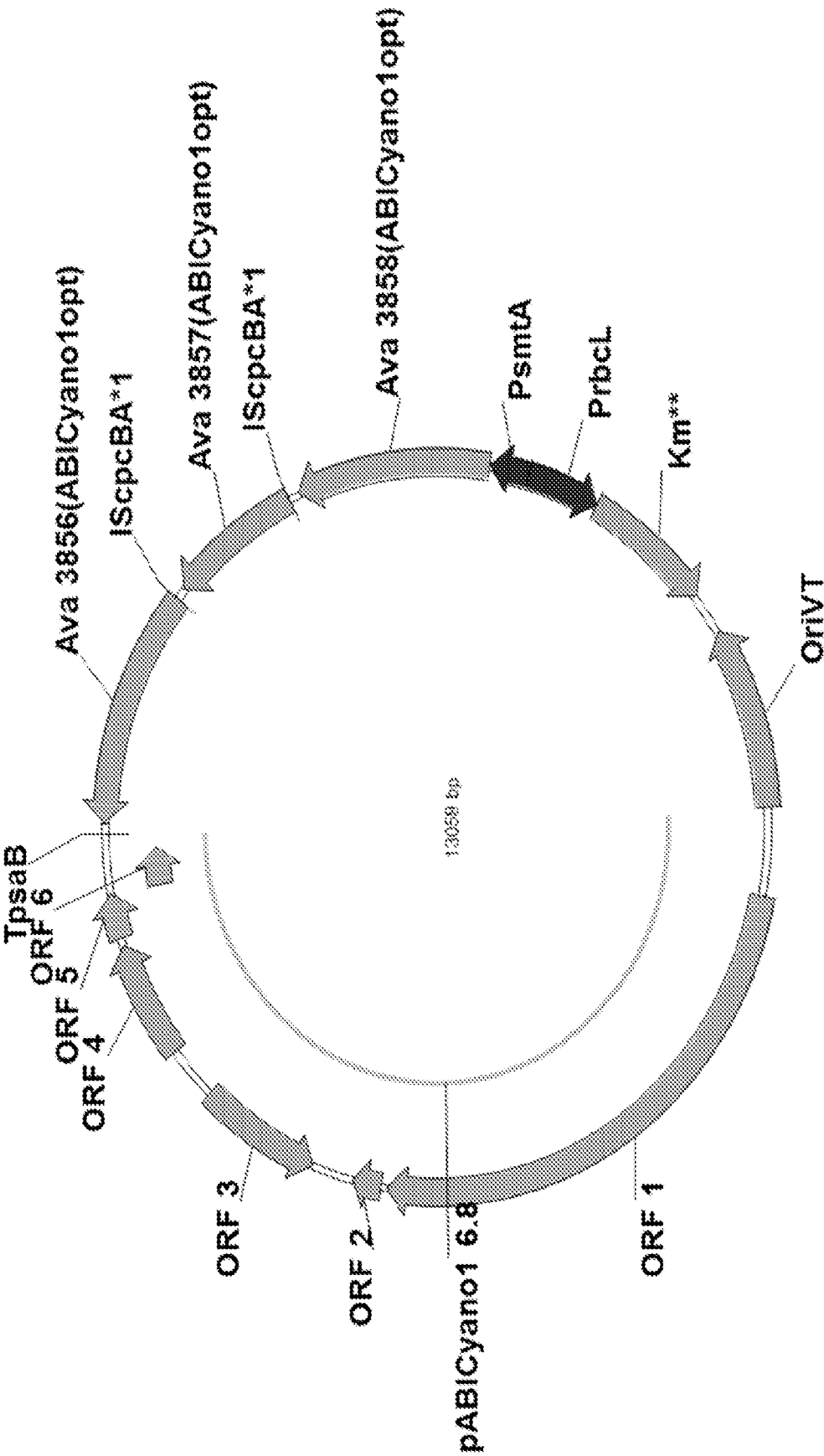
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FT      misc_feature  complement(1378..1442)
FT      /vntifkey="21"
FT      /label=IScpcBA*1
FT      CDS            complement(1443..2282)
FT      /vntifkey="4"
FT      /label=Ava_3857(ABICyano1opt)
FT      CDS            complement(1..1377)
FT      /vntifkey="4"
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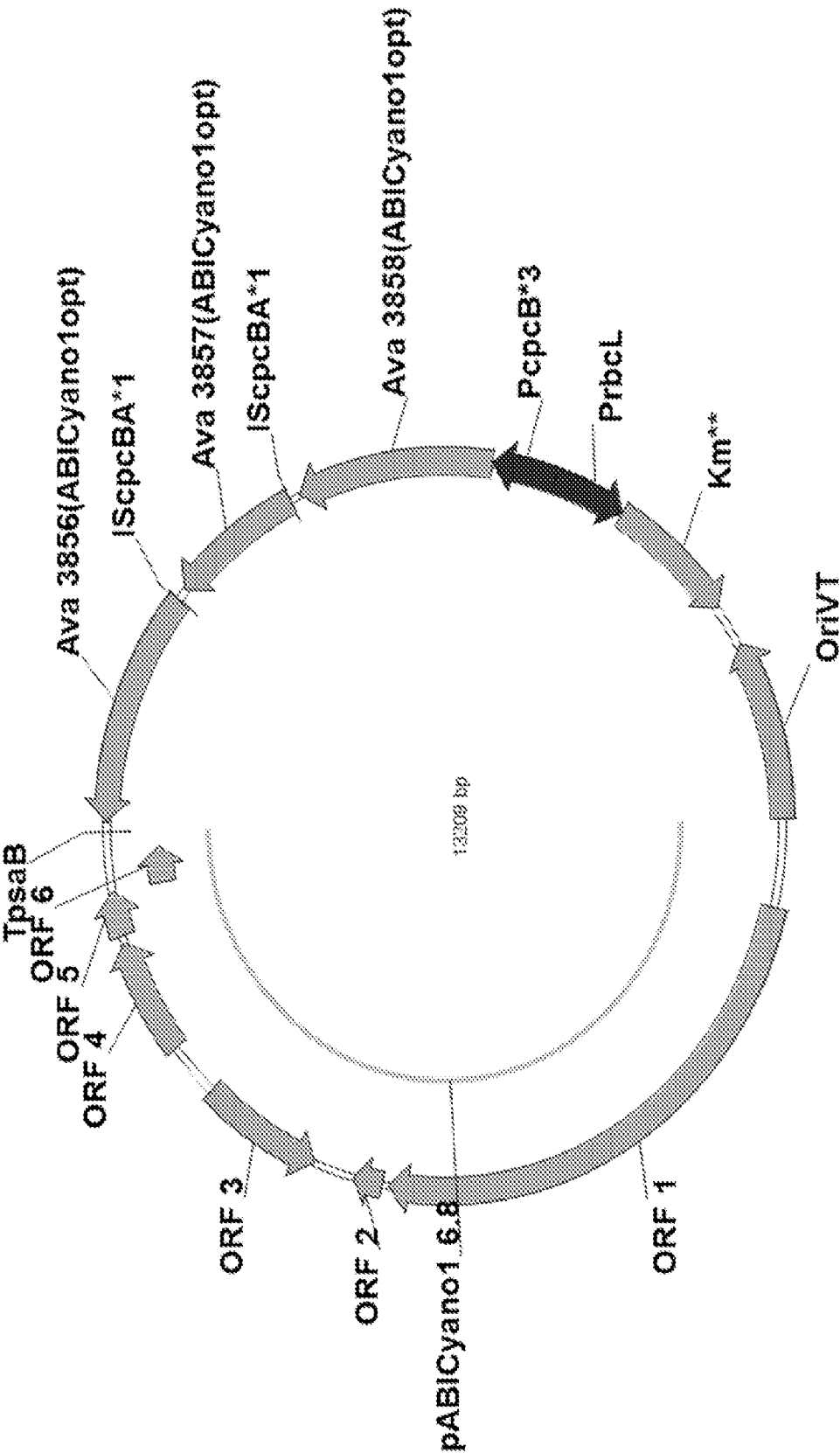
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FIG. 5A (continued)



#2742 pABICyano1 6.8::PsmA-Ava 3858(ABICyano1opt)-IScpcBA*1-Ava 3857(ABICyano1opt)-IScpcBA*1-Ava 3856(ABICyano1opt)-TpsaB

FIG. 5B



#2752 pABICyano1.6.8::PcpCB*3-Ava 3858(ABICyano1opt)-IScpCB*1-Ava 3857(ABICyano1opt)-IScpCB*1-Ava 3856(ABICyano1opt)-TpsaB

FIG. 6

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ID      #2752\pABICyano1_6.8::PcpcB*3-Ava_3858(ABICyano1opt)-IScpcBA*1-
Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB standard; circular DNA;
; 13209 BP.
FH      Key      Location/Qualifiers
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FT      rep_origin      complement(5501..6559)
FT      /vntifkey="33"
FT      /label=oriVT
FT      7076..10261
FT      /vntifkey="4"
FT      /label=ORF\1
FT      /note="orf1 rep ori binding protein slr7037 homolog Similar to
hypothetical protein slr7037 of plasmid pSYSA (103 kb) of Synechocystis sp. PCC 6803"
FT      CDS      10297..10482
FT      /vntifkey="4"
FT      /label=ORF\2
FT      /note="orf2"
FT      complement(10742..11506)
FT      CDS      /vntifkey="4"

```

FIG. 6 (continued)

```

FT      /label=ORF\3
FT      /note="orf3"
FT      11832..12518
FT      /vntifkey="4"
FT      /label=ORF\4
FT      /note="orf4 Integrase/Recombinase Similar to site-specific recombinas
of Bacillus thuringiensis serovar israelensis ATCC 35646"
FT      12560..12823
FT      /vntifkey="4"
FT      /label=ORF\5
FT      /note="orf5"
FT      12820..13068
FT      /vntifkey="4"
FT      /label=ORF\6
FT      /note="orf6"
FT      4443..5258
FT      /vntifkey="4"
FT      /label=Km**
FT      /note="Km**"
FT      3977..4441
FT      /vntifkey="30"
FT      /label=PrbcL
FT      complement(13166..13203)
FT      /vntifkey="43"
FT      /label=TpsaB
t

```

FIG. 6 (continued)

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```

FT      CDS      complement(2348..3577)
FT      /vntifkey="4"
FT      /label=Ava_3858 (ABICyano1opt)
FT      misc_feature complement(1378..1442)
FT      /vntifkey="21"
FT      /label=ISpcpBA*1
FT      CDS      complement(1443..2282)
FT      /vntifkey="4"
FT      /label=Ava_3857 (ABICyano1opt)
FT      CDS      complement(1..1377)
FT      /vntifkey="4"
FT      /label=Ava_3856 (ABICyano1opt)
FT      misc_feature complement(2283..2347)
FT      /vntifkey="21"
FT      /label=ISpcpBA*1
FT      promoter complement(3584..3976)
FT      /vntifkey="30"
FT      /label=PcpCB*3
BASE COUNT      4234 a      2411 c      2470 g      4094 t

```

FIG. 6 (continued)

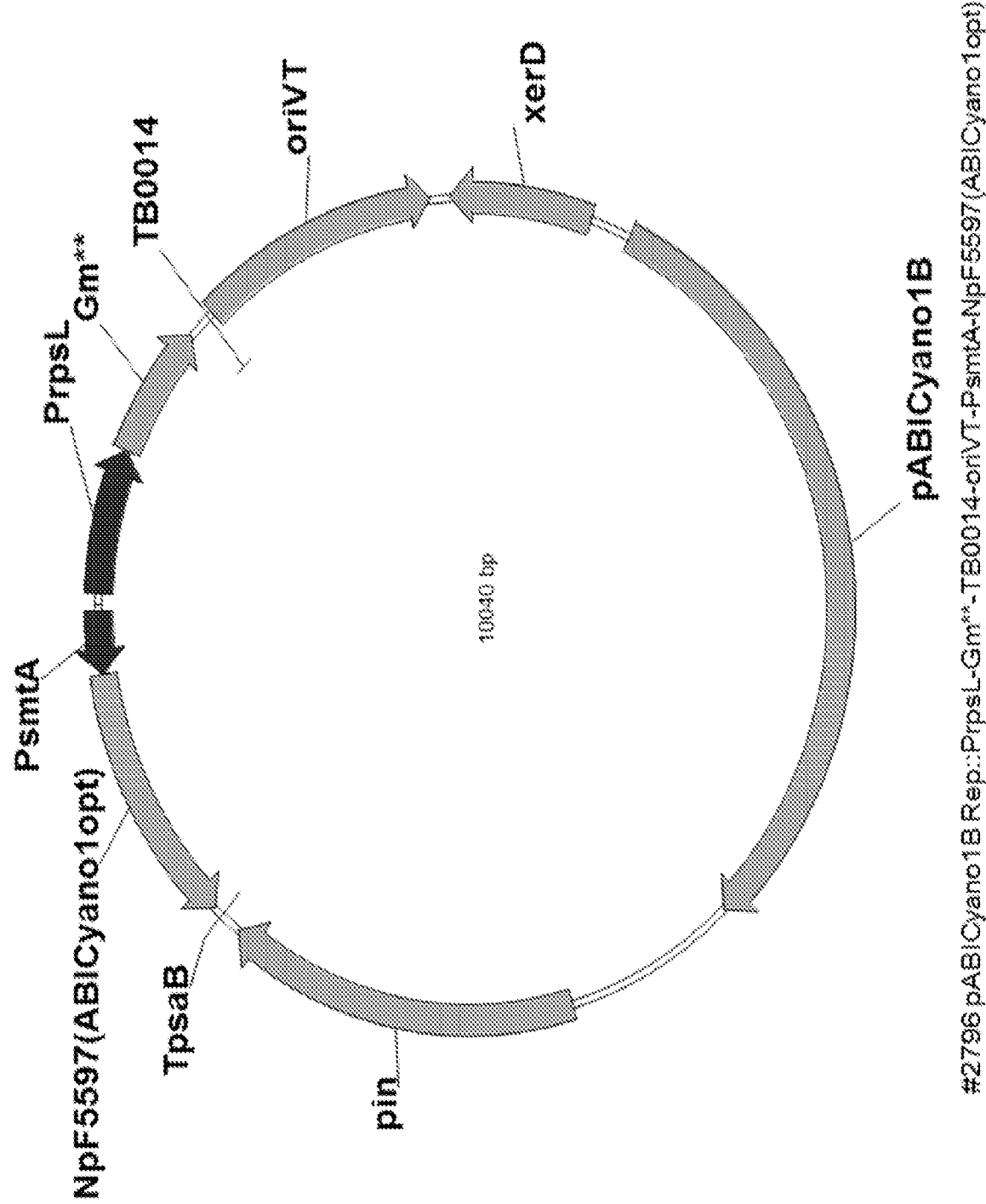


FIG. 7

LOCUS#2796\pABICYANO1B_Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-NpF5597 (ABICYANO1opt)

10040 bpDNAcircular10-MAY-2017

SOURCEORGANISM

FEATURES

rep_originLocation/Qualifiers

1268..2326

/vntifkey="33"

/label=oriVT

rep_origin3206..6328

/vntifkey="33"

/label=pABICYANO1B

CDScomplement (2408..3028)

/vntifkey="4"

/label=xerD

CDS7080..8603

/vntifkey="4"

/label=pin

gene5533..5533

/vntifkey="60"

FIG. 7 (continued)

/note="pABICYANO1B-Rep"
 7080..8603
 /vntifkey="60"
 /note="Pin resolvase [Anabaena variabilis ATCC 29413]"
 complement(2408..3028)
 /vntifkey="60"
 /note="XerD %09Phage integrase (Crocospaera watsonii WH 8501)"
 42..610
 /vntifkey="30"
 /label=PrpsL
 611..1144
 /vntifkey="4"
 /label=Gm**
 1163..1256
 /vntifkey="43"
 /label=TB0014
 complement(9785..10027)
 /vntifkey="30"
 /label=PsmTA
 complement(8698..8735)
 /vntifkey="43"
 /label=TpsaB
 complement(8736..9782)
 /vntifkey="4"
 /label=NpF5597 (ABICYANO1opt)
 3125 a 1856 c 1814 g 3245 t
 BASE COUNT

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FIG. 7 (continued)

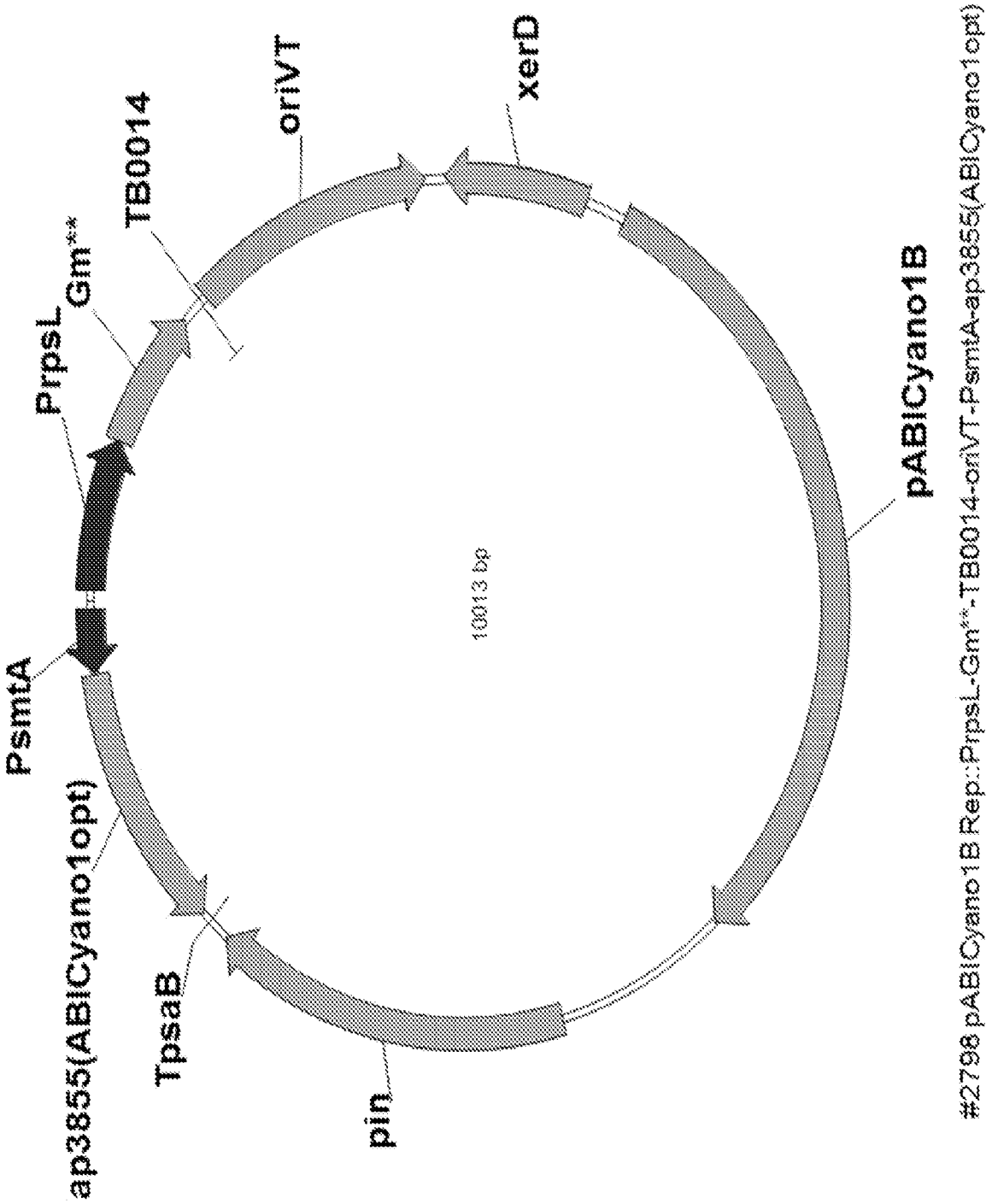


FIG. 8

#2798 pABICyano1B Rep::PrpsL-Gm**-TB0014-oriVT-PsmtA-ap3855(ABICyano1opt)

```

ID      #2798\pABICYANO1B_Rep::PrpsL-Gm*-TB0014-oriVT-PsmtA-ap3855 (ABICYANO1opt)  standard;
circular DNA;      ; 10013 BP.
FH      Key      Location/Qualifiers
FH
FT      rep_origin      1268..2326
FT      /vntifkey="33"
FT      /label=oriVT
FT      rep_origin      3206..6328
FT      /vntifkey="33"
FT      /label=pABICYANO1B
FT      CDS      complement (2408..3028)
FT      /vntifkey="4"
FT      /label=xerD
FT      CDS      7080..8603
FT      /vntifkey="4"
FT      /label=pin
FT      gene      5533..5533
FT      /vntifkey="60"
FT      /note="pABICYANO1B-Rep"

```

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FIG. 8 (continued)

FT 7080..8603
 FT /vntifkey="60"
 FT /note="Pin resolvase [Anabaena variabilis ATCC 29413]"
 FT complement(2408..3028)
 FT /vntifkey="60"
 FT /note="XerD %09Phage integrase (Crocosphaera watsonii WH 8501)"
 FT 42..610
 FT /vntifkey="30"
 FT /label=PrpsL
 FT 611..1144
 FT /vntifkey="4"
 FT /label=Gm**
 FT 1163..1256
 FT /vntifkey="43"
 FT /label=TB0014
 FT complement(9758..10000)
 FT /vntifkey="30"
 FT /label=PsmA
 FT complement(8698..8735)
 FT /vntifkey="43"
 FT /label=TpsaB
 FT complement(8736..9755)
 FT /vntifkey="4"
 FT /label=ap3855(ABICYANO1opt)
 SQ Sequence 10013 BP; 3118 A; 1870 C; 1781 G; 3244 t;

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FIG. 8 (continued)

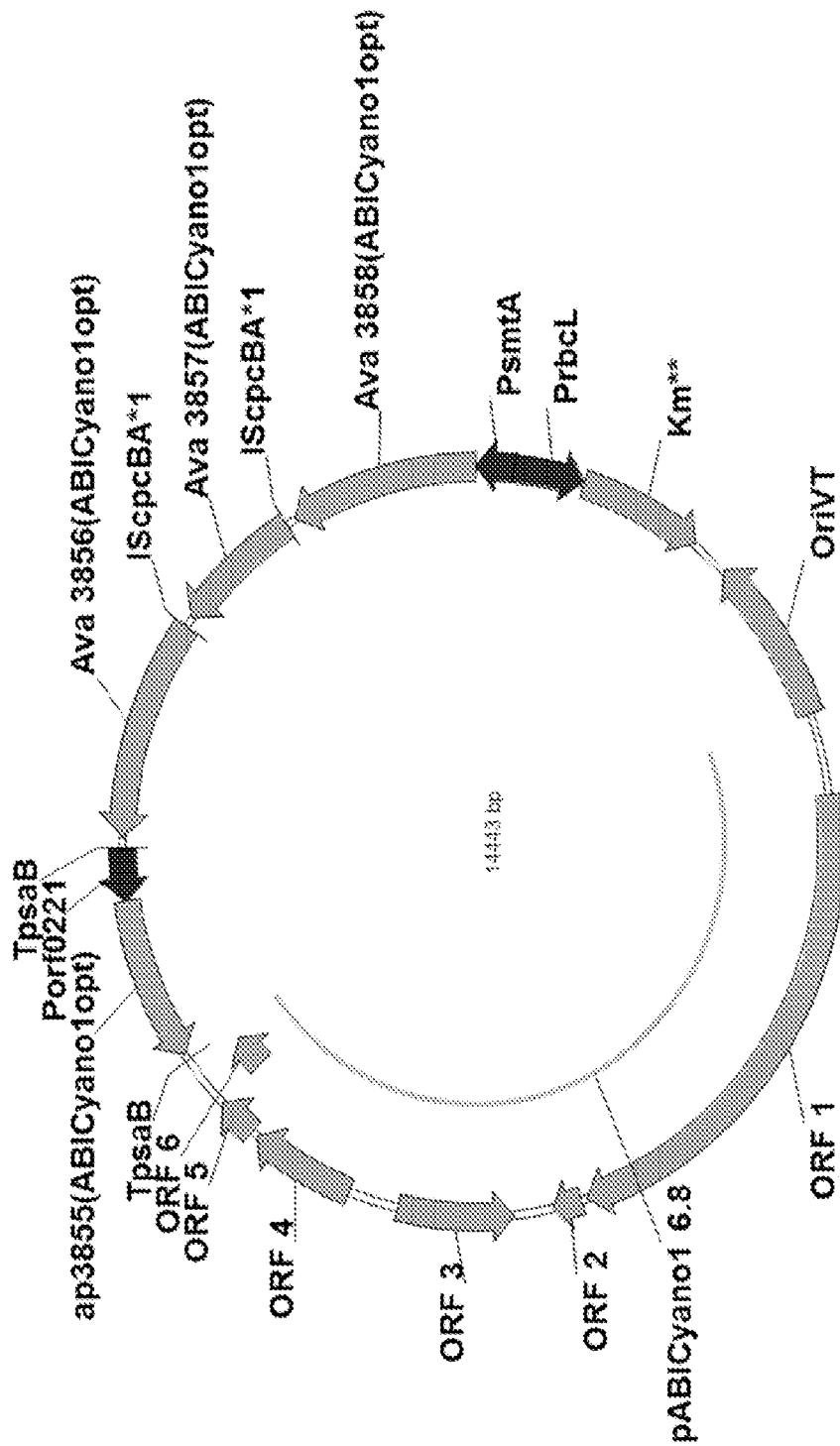


FIG. 9

#2840 pAB|Cyan16.8:Psmt4-Ava 3858|AB|Cyan1opt)|ScpcBA*4-Ava 3857|AB|Cyan1opt)-TpsaB-Port0274-ep3855|AB|Cyan1opt)

```

ID      #2840\pABICYANO1_6.8::PsmA-Ava_3858 (ABICYANO1opt)-ISpcBA*1-Ava_3857 (ABICYANO1opt)-
ISpcBA*1-Ava_3856 (ABICYANO1opt)-TpsaB-Porf0221-ap3855 (ABICYANO1opt) standard; circular
DNA;    ; 14443 BP.
FH      Key      Location/Qualifiers
FH
FT      insertion_seq      6416..13009
FT      /source="pABICYANO1_6.8"
FT      /type="Custom cloned insert"
FT      /vntifkey="14"
FT      /label=pABICYANO1_6.8
FT      /note="Unknown feature type:insert"
FT      rep_origin      complement(5351..6409)
FT      /vntifkey="33"
FT      /label=oriVT
FT      6926..10111
FT      /vntifkey="4"
FT      /label=ORF\1
FT      /note="orf1 rep ori binding protein slr7037 homolog similar to
hypothetical protein slr7037 of plasmid pSYSA (103 kb) of Synechocystis sp. PCC 6803"
FT      CDS      10147..10332
FT      /vntifkey="4"
FT      /label=ORF\2
FT      /note="orf2"
FT      complement(10592..11356)
FT      /vntifkey="4"
FT      /label=ORF\3

```

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FIG. 9 (continued)

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```

FT      /note="orf3"
FT      11682..12368
FT      /vntifkey="4"
FT      /label=ORF\4
FT      /note="orf4 Integrase/Recombinase Similar to Site-specific recombinase
of Bacillus thuringiensis serovar israelensis ATCC 35646"
FT      12410..12673
FT      /vntifkey="4"
FT      /label=ORF\5
FT      /note="orf5"
FT      12670..12918
FT      /vntifkey="4"
FT      /label=ORF\6
FT      /note="orf6"
FT      4293..5108
FT      /vntifkey="4"
FT      /label=Km**
FT      /note="Km**"
FT      3827..4291
FT      /vntifkey="30"
FT      /label=Prbcl
FT      complement(14400..14437)
FT      /vntifkey="43"
FT      /label=TpsaB

```

CDS

CDS

CDS

CDS

CDS

promoter

terminator

FIG. 9 (continued)

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```

FT      CDS      complement(2348..3577)
FT      /vntifkey="4"
FT      /label=Ava_3858(ABICYANO1opt)
FT      misc_feature complement(1378..1442)
FT      /vntifkey="21"
FT      /label=ISpcBA*1
FT      CDS      complement(1443..2282)
FT      /vntifkey="4"
FT      /label=Ava_3857(ABICYANO1opt)
FT      CDS      complement(1..1377)
FT      /vntifkey="4"
FT      /label=Ava_3856(ABICYANO1opt)
FT      misc_feature complement(2283..2347)
FT      /vntifkey="21"
FT      /label=ISpcBA*1
FT      promoter complement(3584..3826)
FT      /vntifkey="30"
FT      /label=PsmTA
FT      CDS      complement(13054..14073)
FT      /vntifkey="4"
FT      /label=ap3855(ABICYANO1opt)
FT      terminator complement(13016..13053)
FT      /vntifkey="43"
FT      /label=TpsaB
FT      promoter complement(14074..14393)
FT      /vntifkey="30"
FT      /label=Porf0221
SQ      Sequence 14443 BP; 4667 A; 2660 C; 2694 G; 4422 t;

```

FIG. 9 (continued)

GC vial assay with AB1224

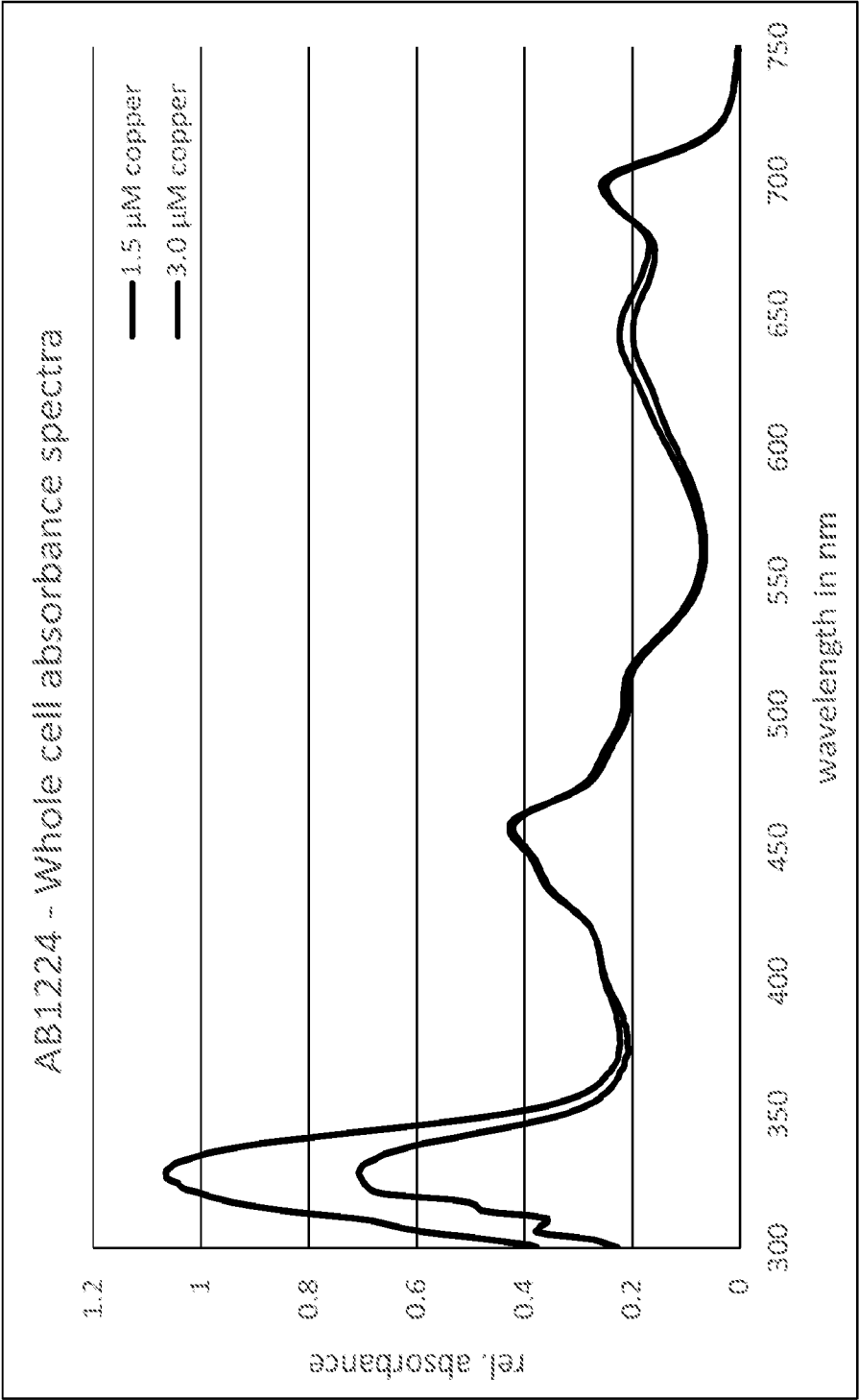


FIG. 10A

GC vial assay comparing AB1225 and AB1226

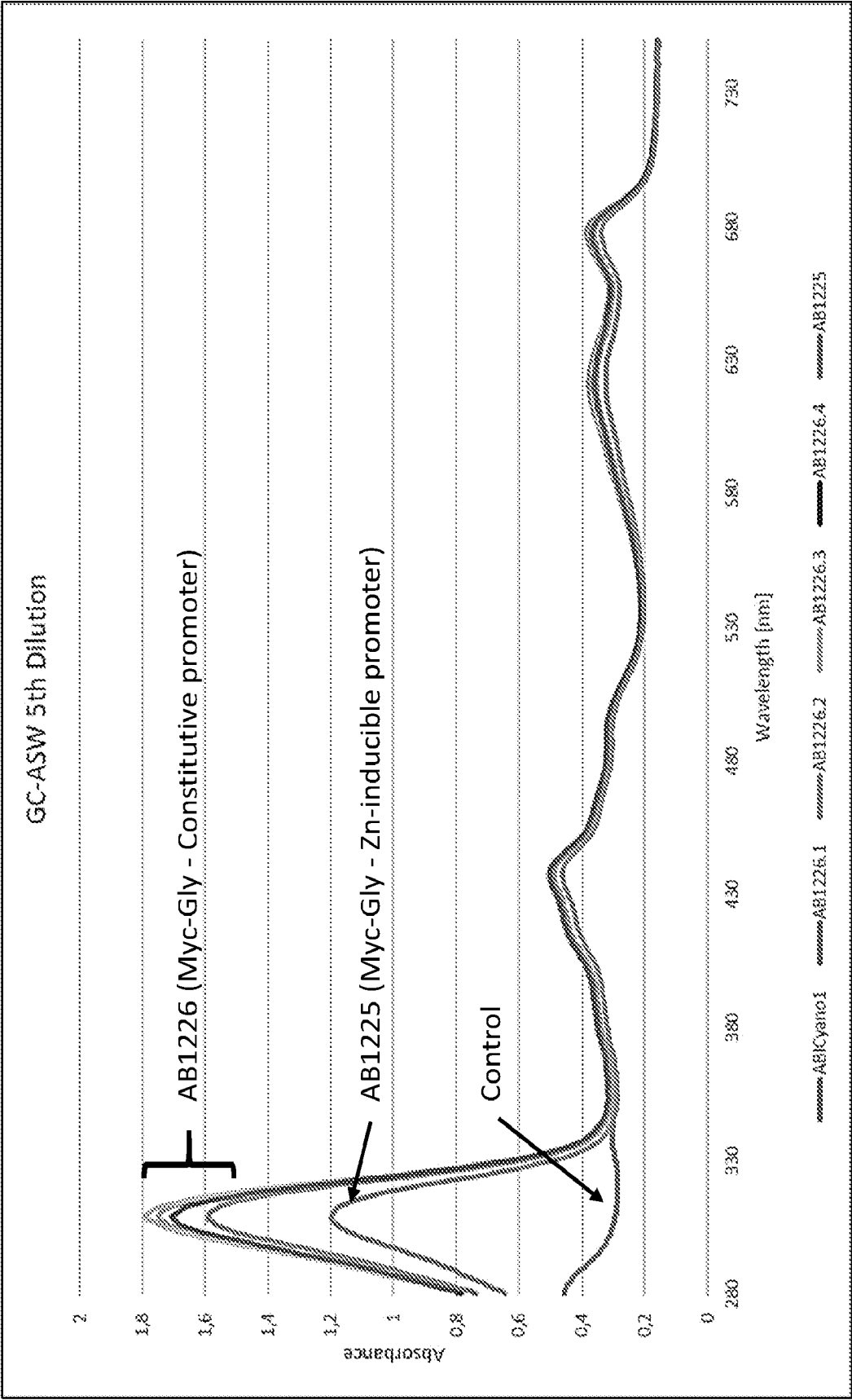


FIG. 10B

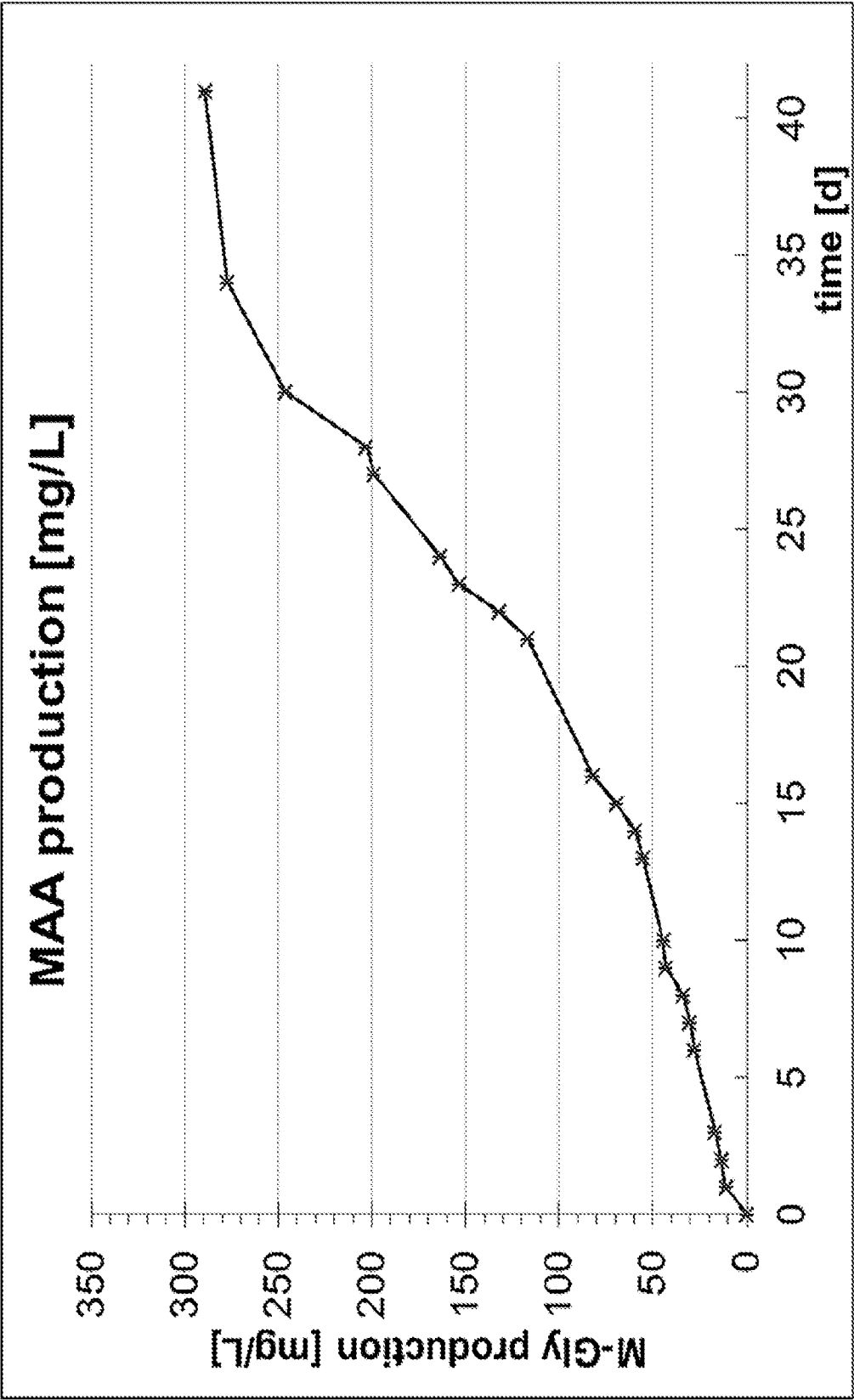


FIG. 10C

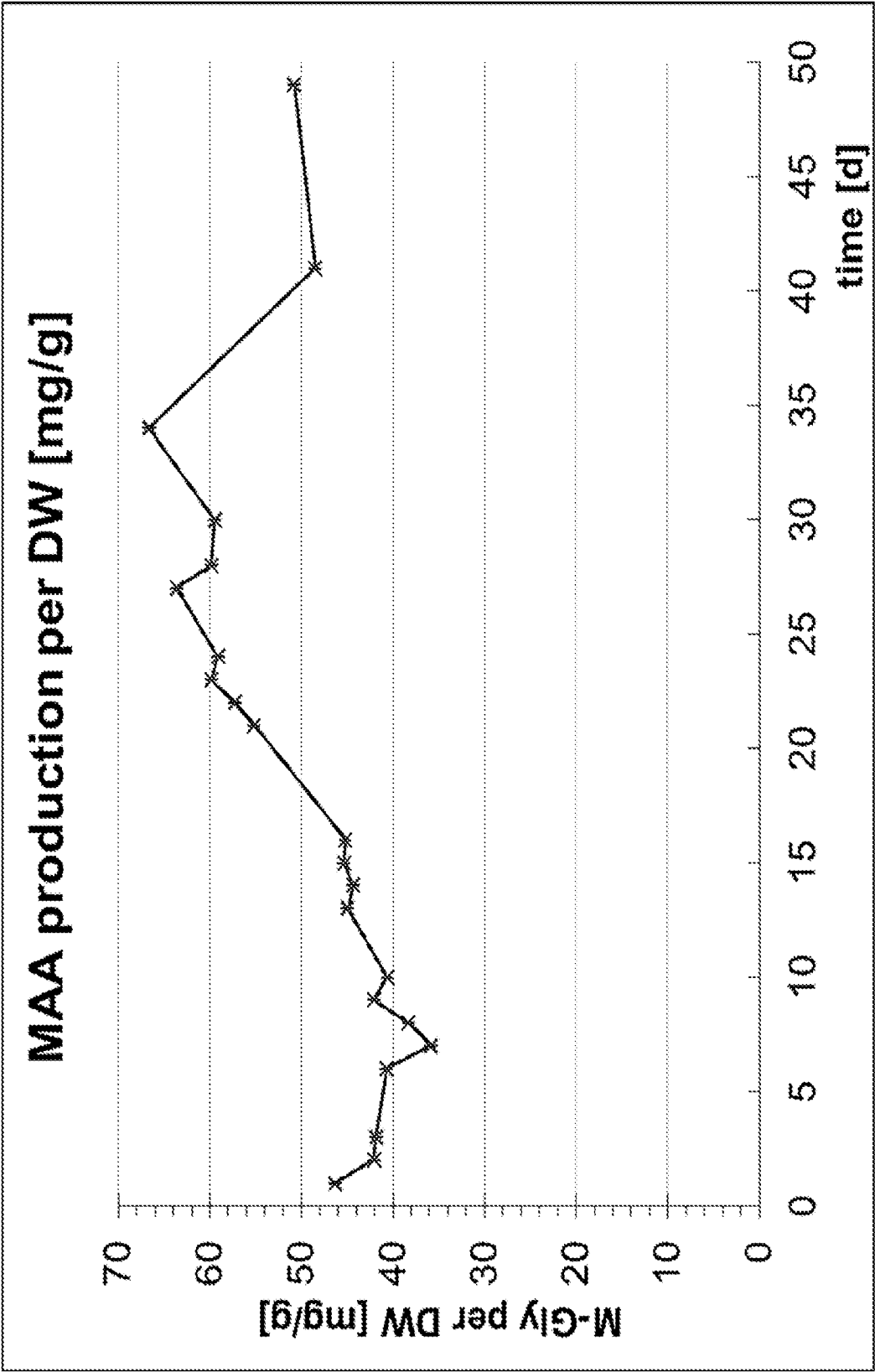


FIG. 10D

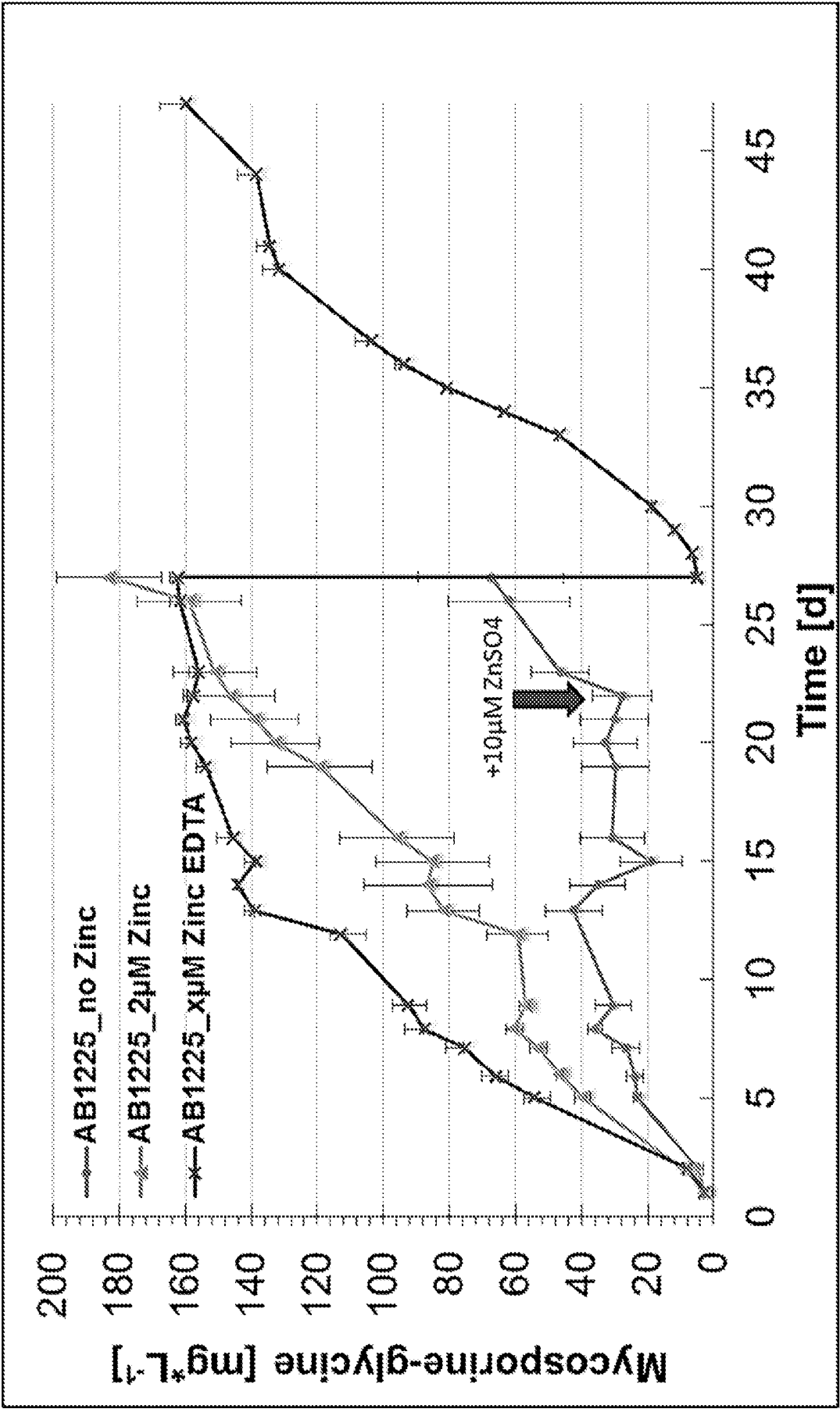


FIG. 11A

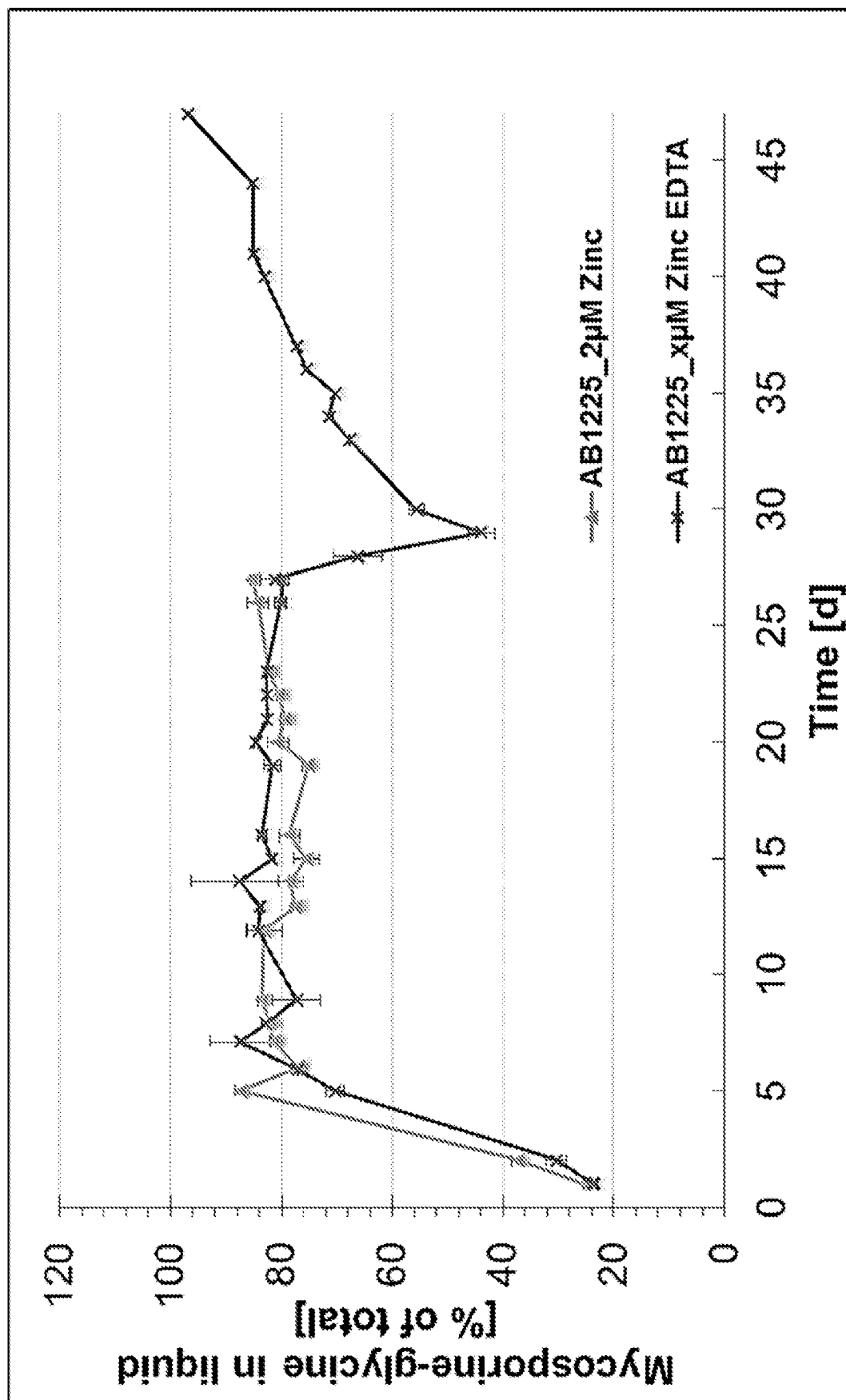


FIG. 11B

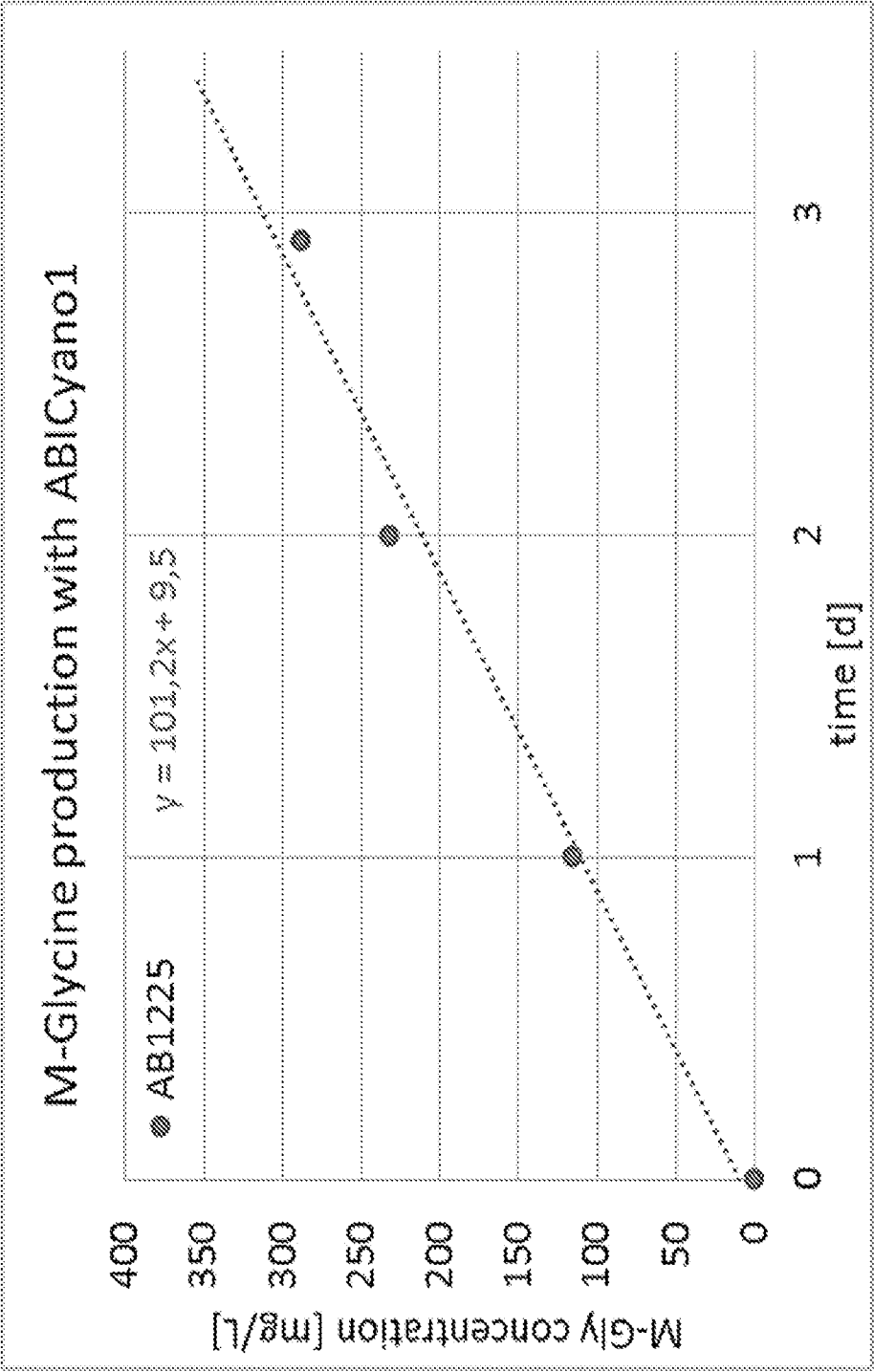


FIG. 11C

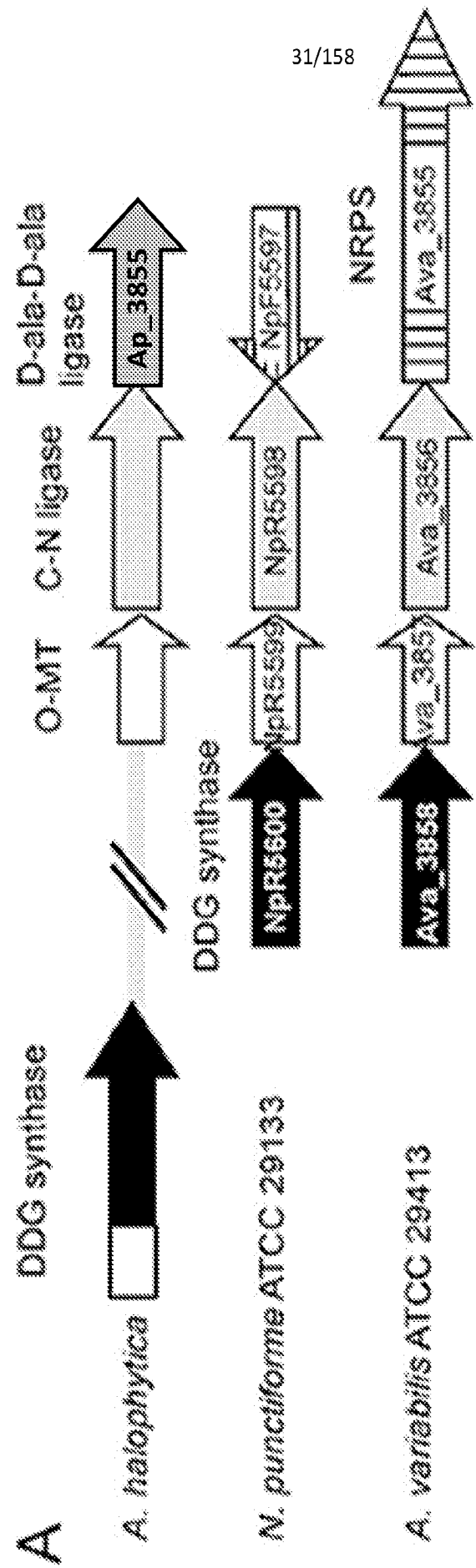


FIG. 12A

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Biosynthetic Pathway from Mycosporine-glycine to Shinorine and Mycosporine-2-glycine

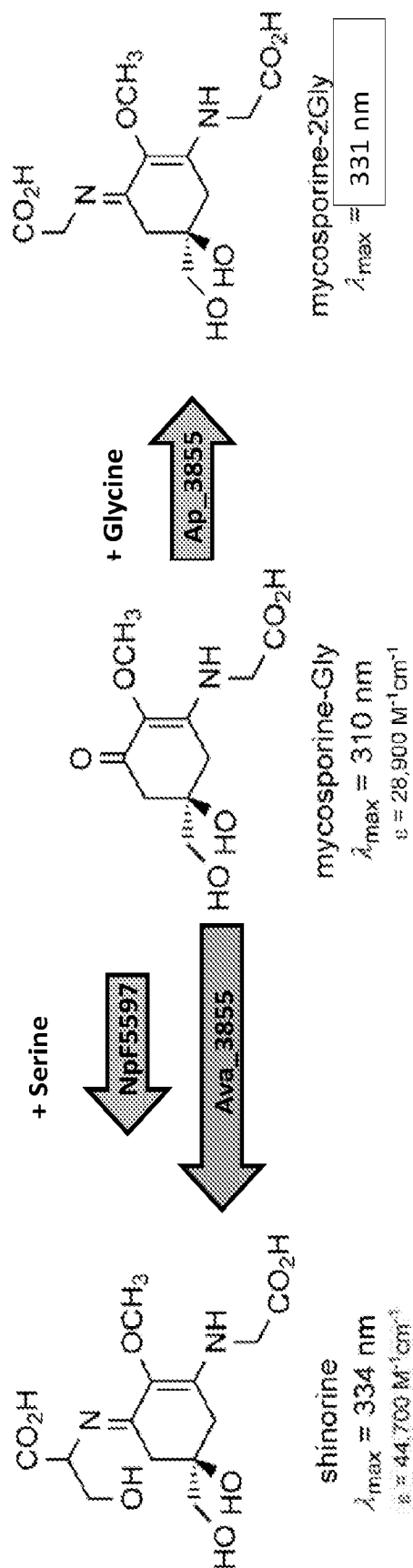


FIG. 12B

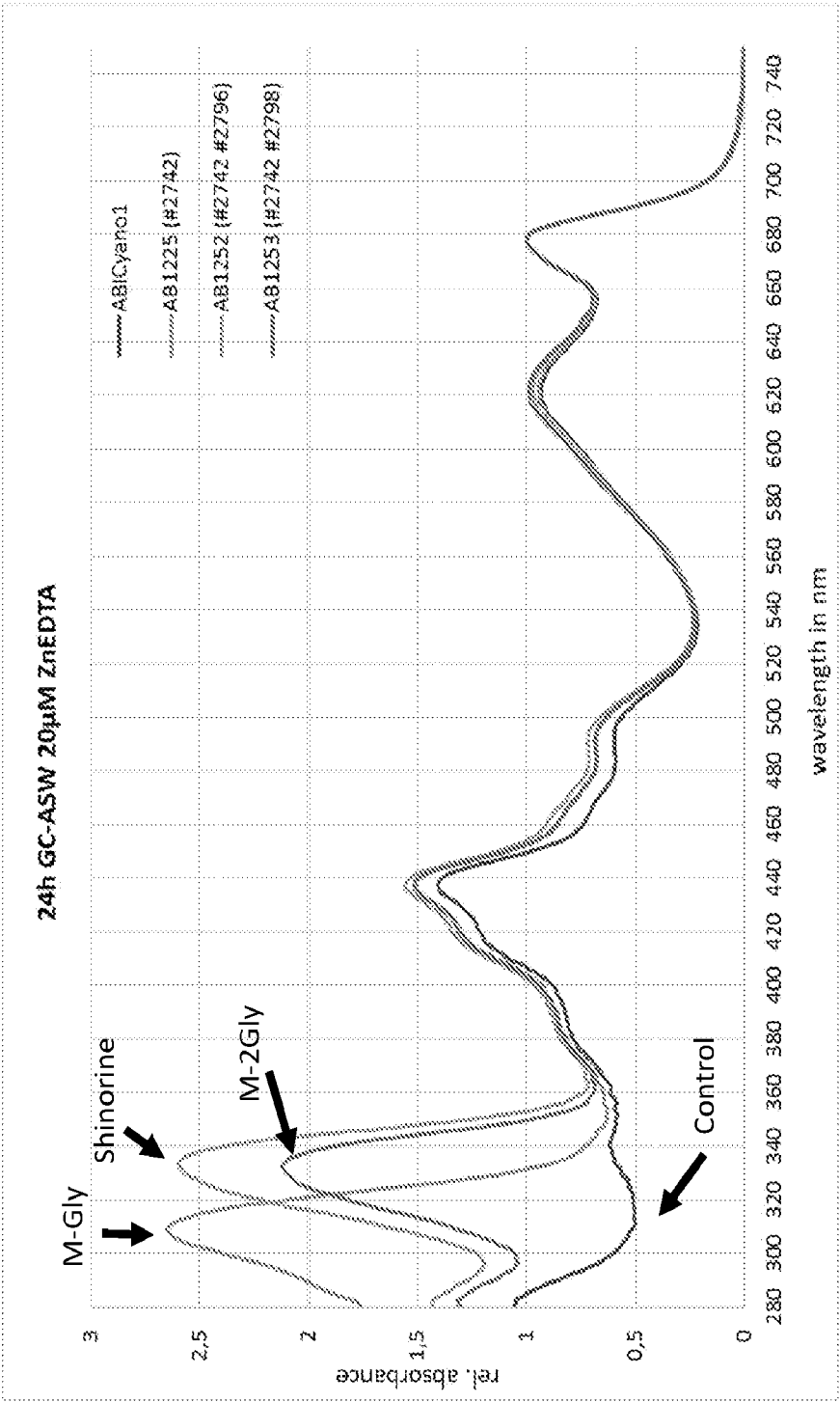


FIG. 12C

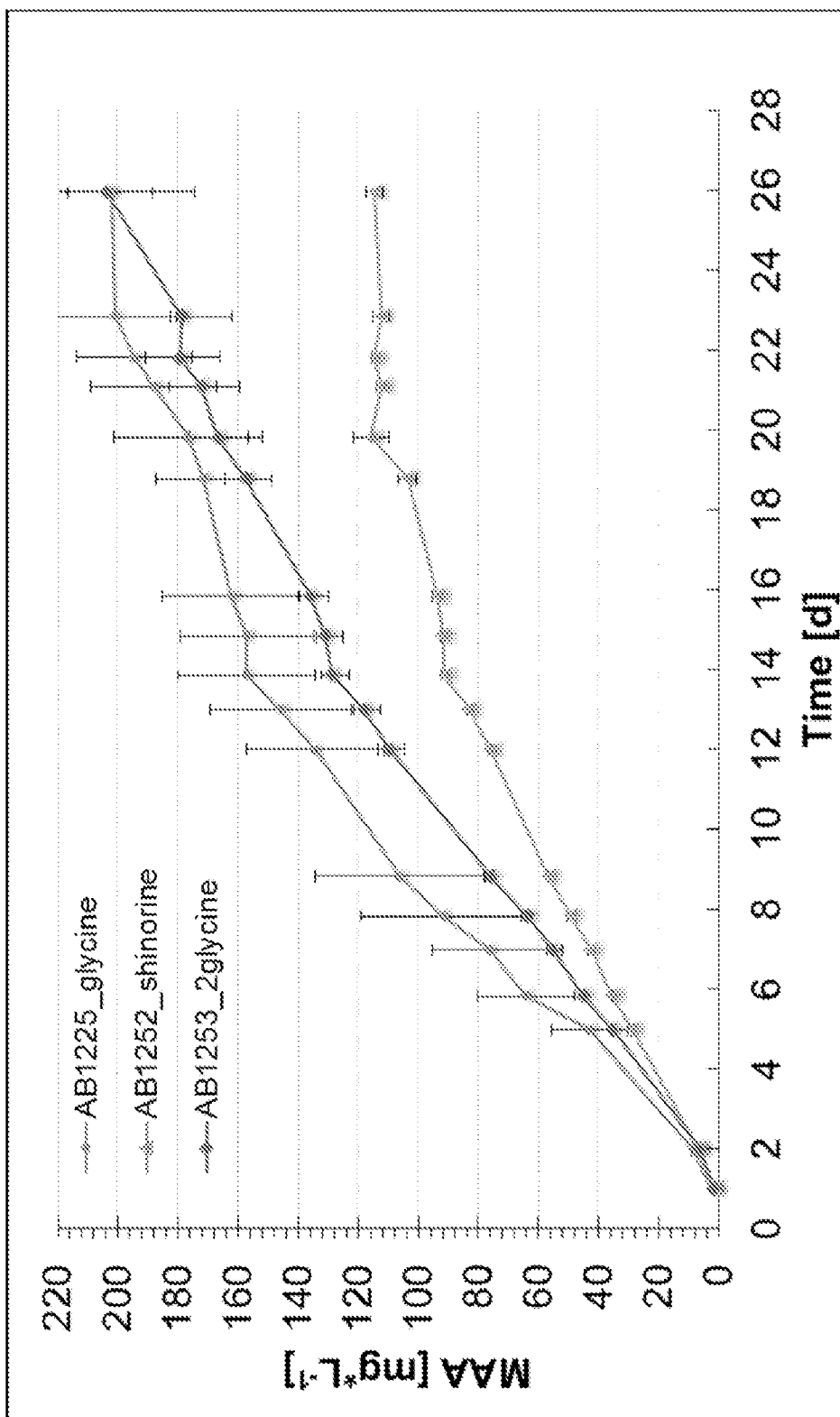


FIG. 13A

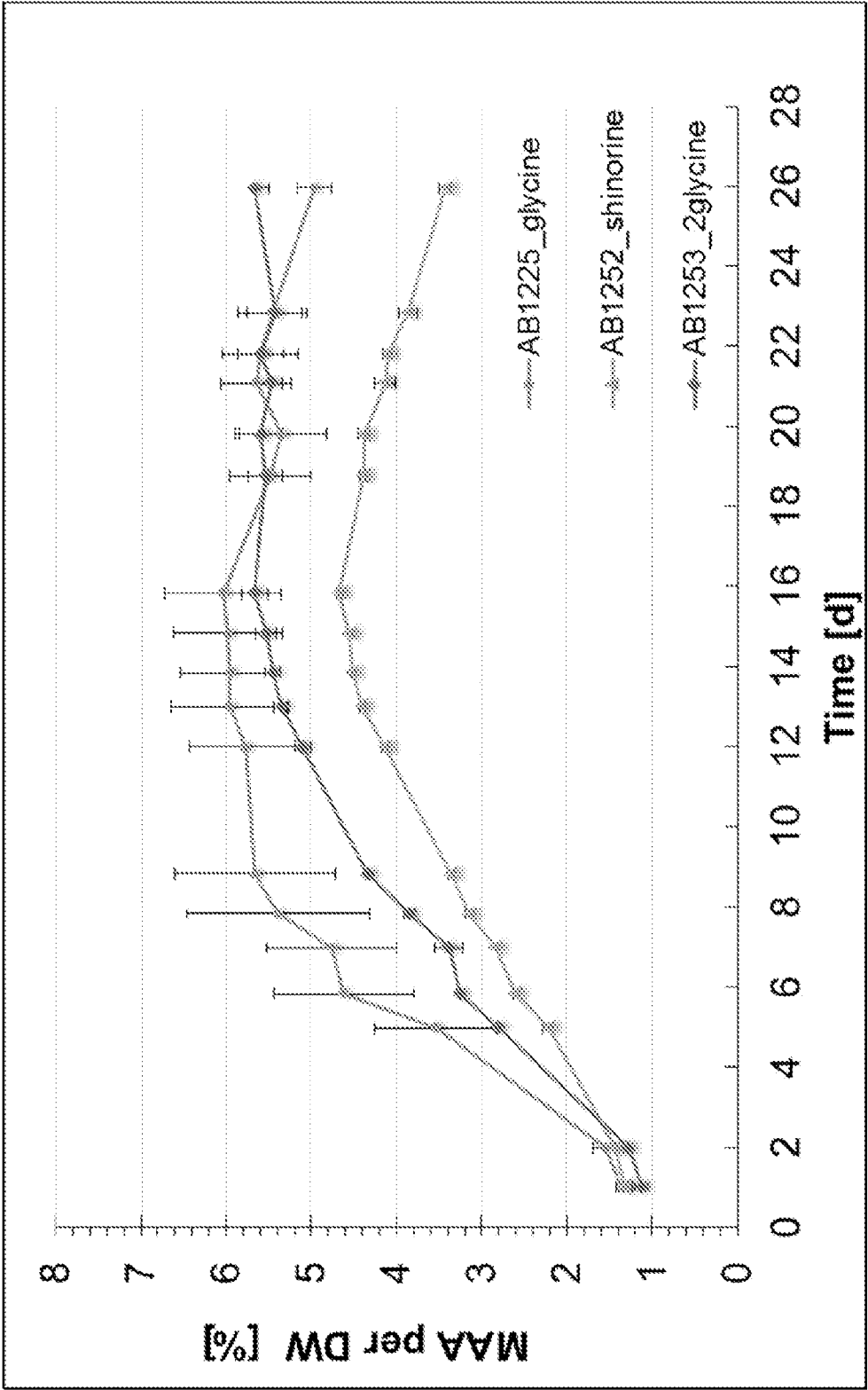


FIG. 13B

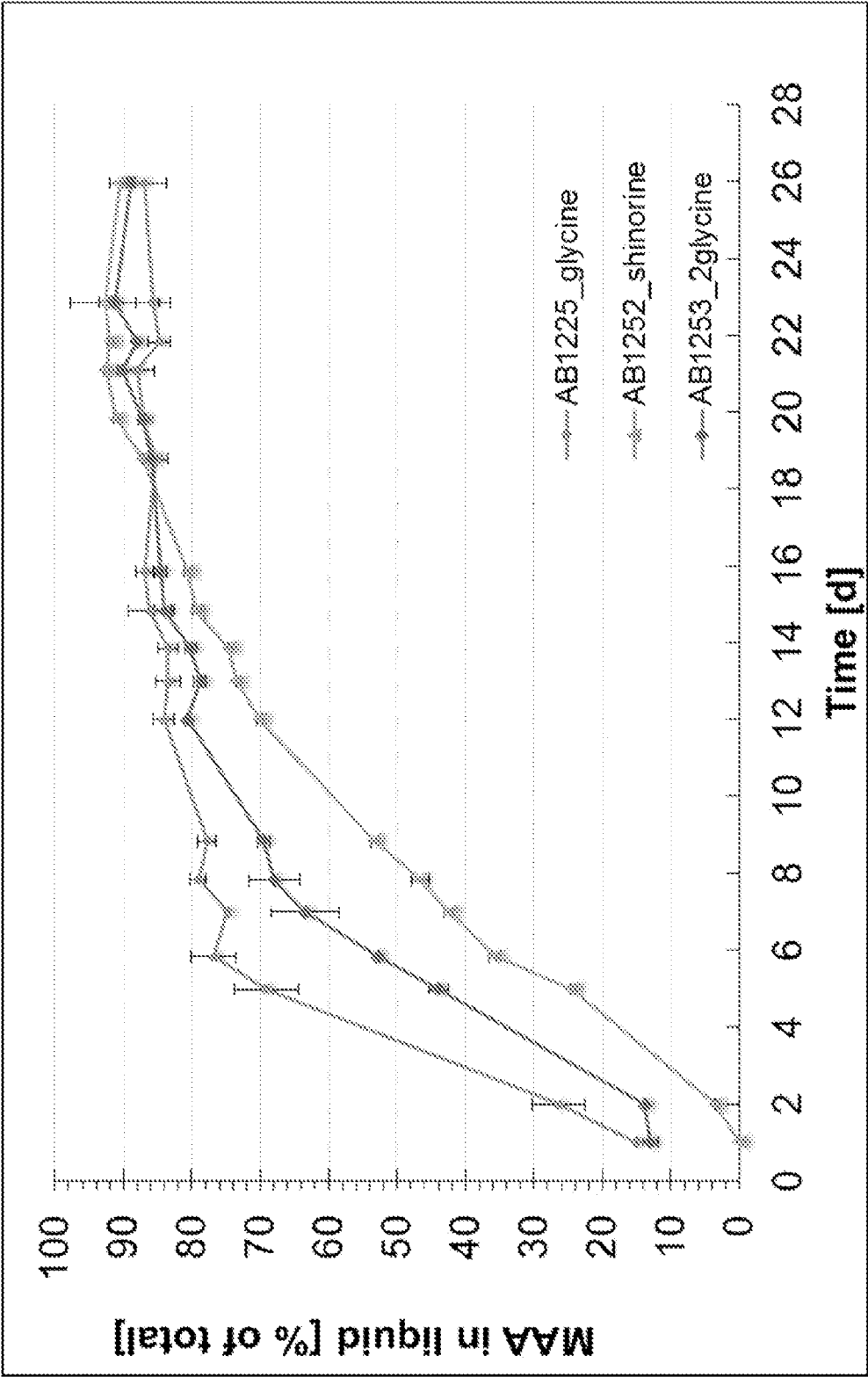


FIG. 13C

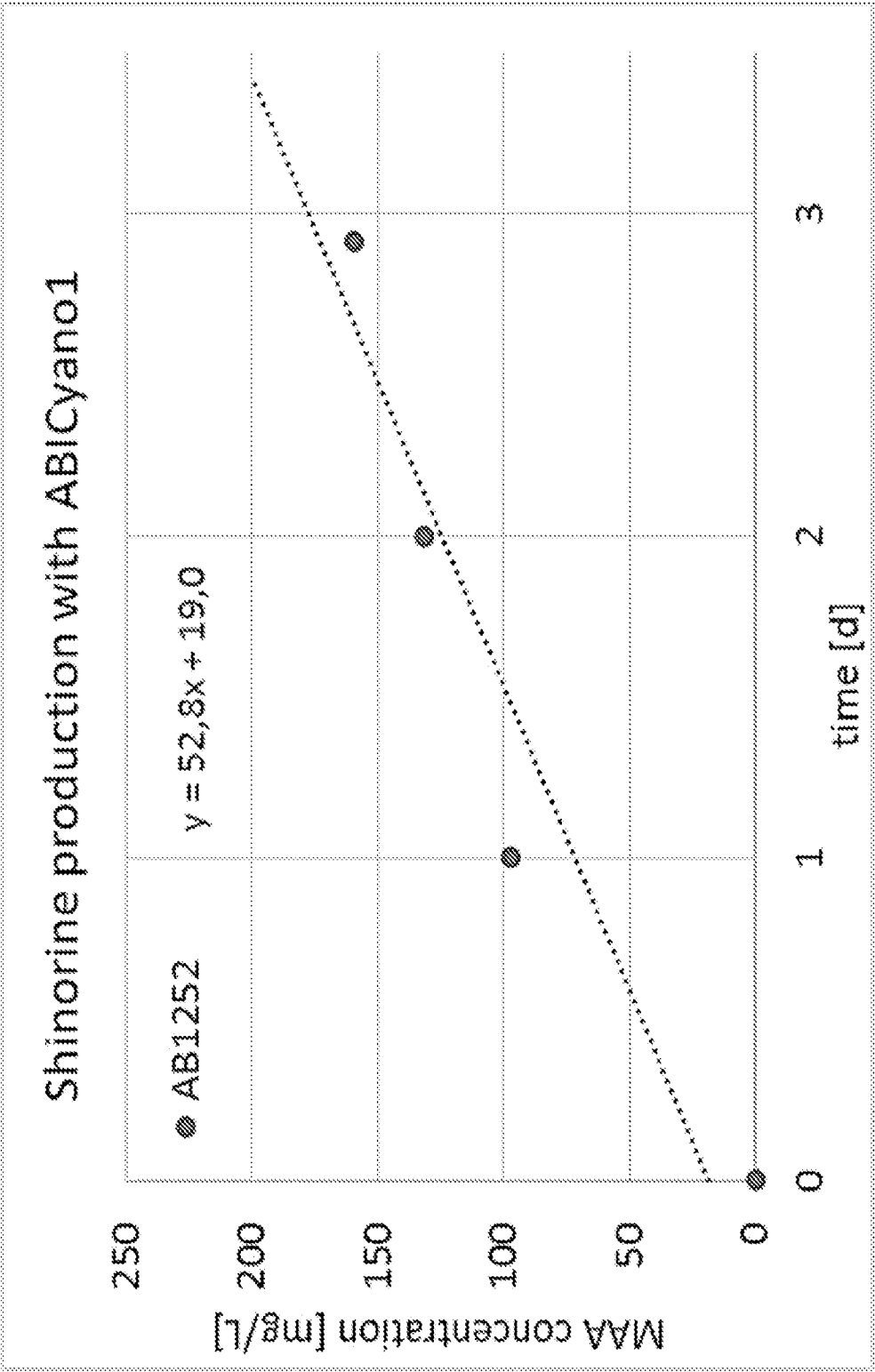


FIG. 13D

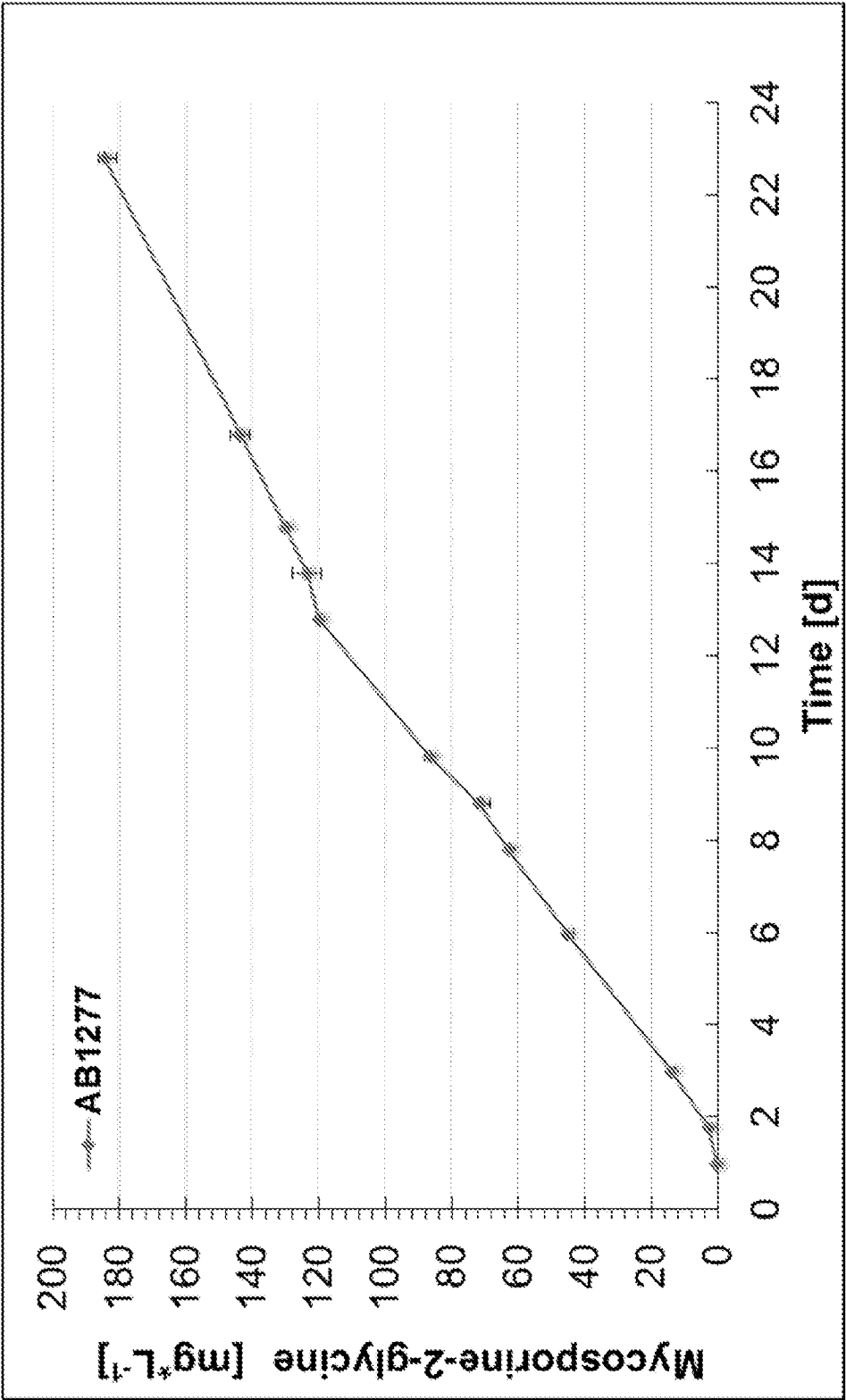


FIG. 14

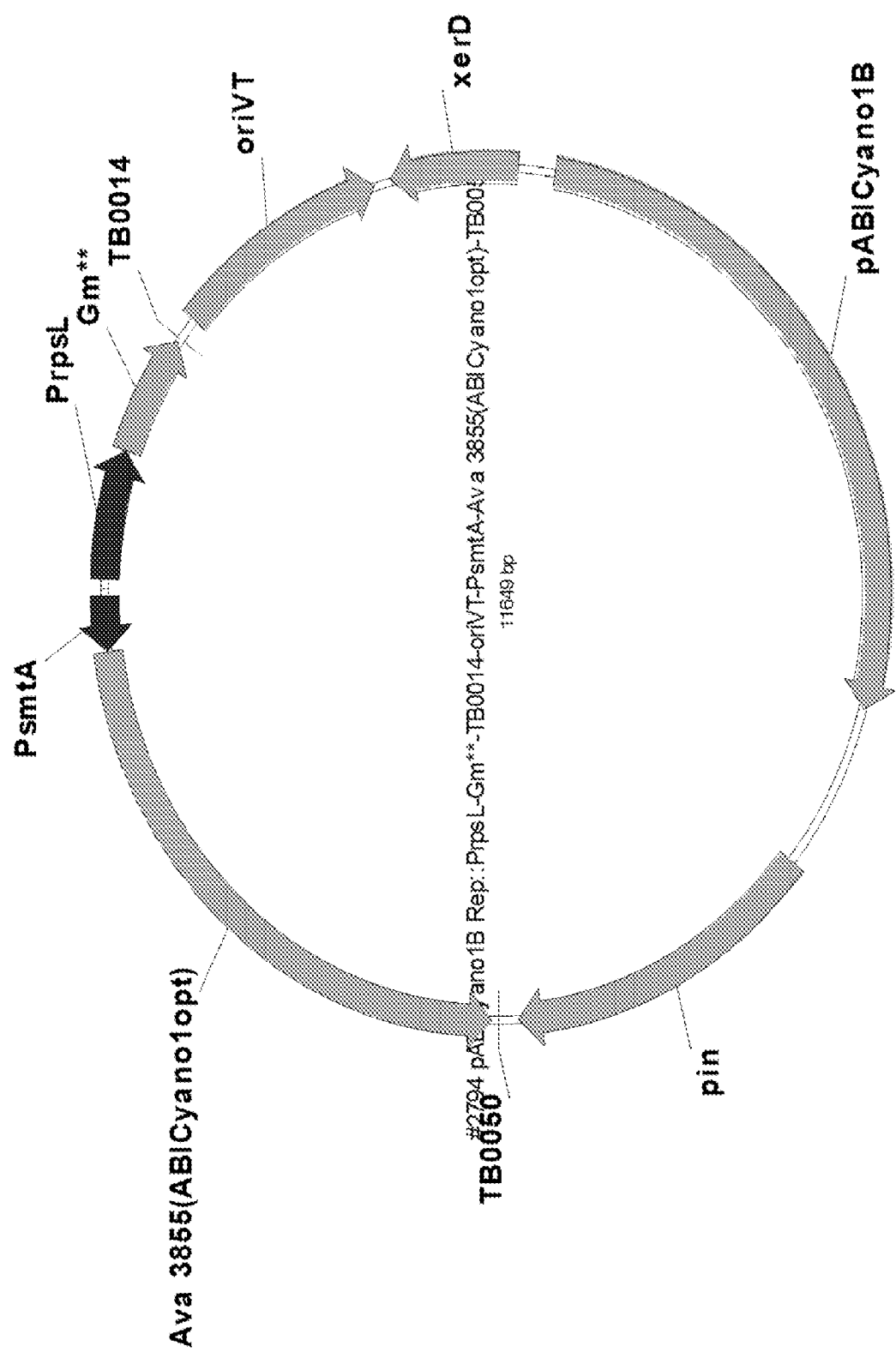


FIG. 15A

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```

ID      #2794\pABCyanolB_Rep::PrpSL-Gm*-TB0014-oriVT-PsmtA-Ava_3855(ABICyanolopt)-TB0050
standard; circular DNA;      ; 11649 BP.
FH      Key      Location/Qualifiers
FH
FT      rep_origin      1268..2326
FT      /vntifkey="33"
FT      /label=oriVT
FT      rep_origin      3206..6328
FT      /vntifkey="33"
FT      /label=pABCyanolB
FT      CDS      complement(2408..3028)
FT      /vntifkey="4"
FT      /label=xerD
FT      CDS      7080..8603
FT      /vntifkey="4"
FT      /label=pin
FT      gene      5533..5533
FT      /vntifkey="60"
FT      /note="pABCyanolB-Rep"
FT      gene      7080..8603
FT      /vntifkey="60"
FT      /note="Pin resolvase [Anabaena variabilis ATCC 29413]"
FT      complement(2408..3028)
FT      /vntifkey="60"
FT      /note="XerD %09Phage integrase (Crocospaera watsonii WH 8501)"

```

FIG. 15A (continued)

```

FT      promoter      42..610
FT      /vntifkey="30"
FT      /label=PrpsL
FT      611..1144
FT      /vntifkey="4"
FT      /label=Gm*+
FT      1163..1256
FT      /vntifkey="43"
FT      /label=TB0014
FT      complement(11394..11636)
FT      /vntifkey="30"
FT      /label=Psmtd
FT      complement(8731..11391)
FT      /vntifkey="4"
FT      /label=Ava_3855(ABICyano1opt)
FT      complement(8698..8730)
FT      /vntifkey="43"
FT      /label=TB0050

```

FIG. 15A (continued)

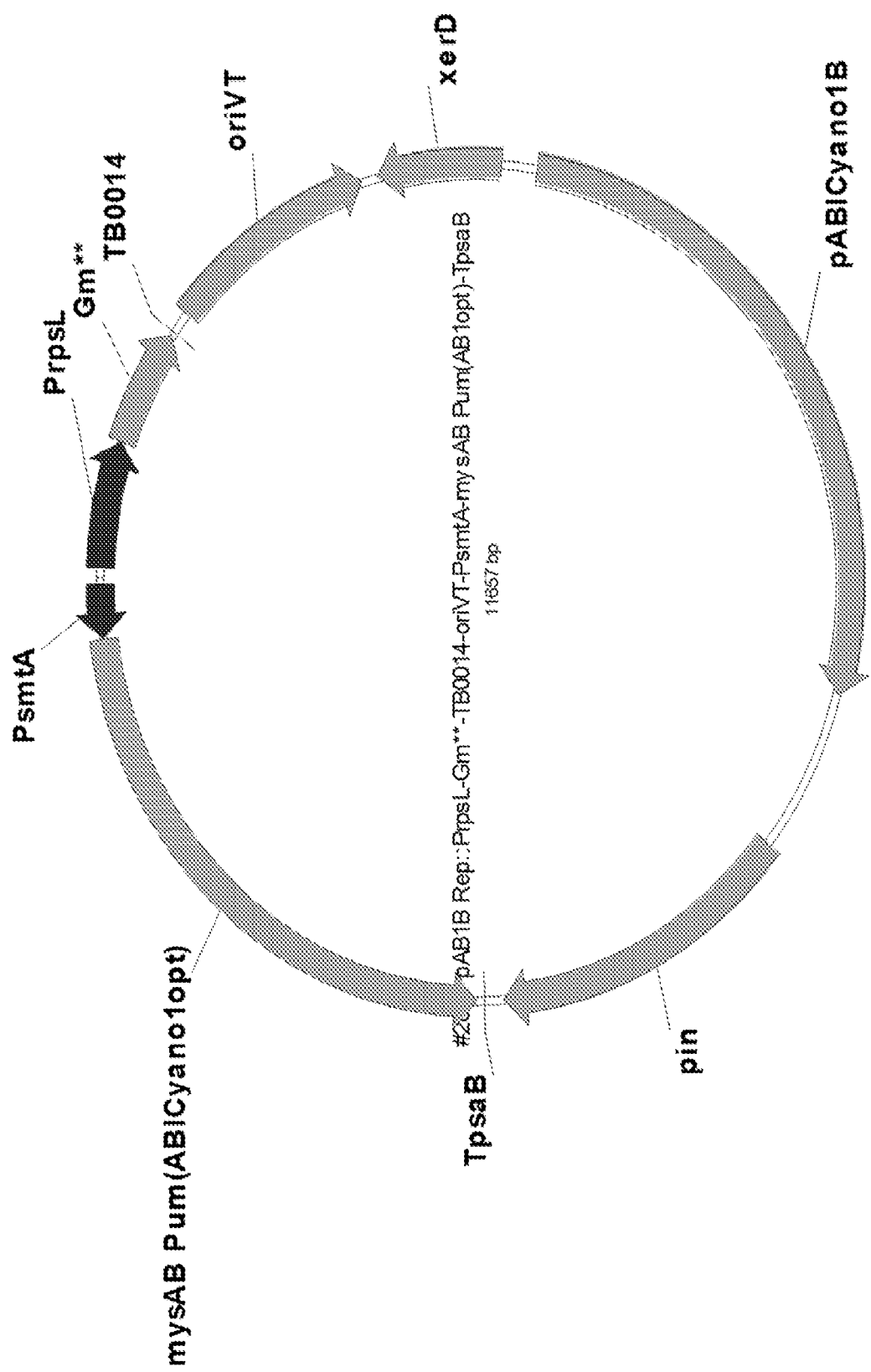


FIG. 15B

ID #2880\pABICyano1B_Rep::PrpsL-Gm*-TB0014-oriVT-PsmtA-mysAB_Pum (ABICyano1opt) -TpsaB
standard; circular DNA; ; 11657 BP.
FH Key Location/Qualifiers
FH rep_origin 1268..2326
FT /vntifkey="33"
FT /label=oriVT
FT rep_origin 3206..6328
FT /vntifkey="33"
FT /label=pABICyano1B
FT CDS complement(2408..3028)
FT /vntifkey="4"
FT /label=xerD
FT CDS 7080..8603
FT /vntifkey="4"
FT /label=pin
FT gene 5533..5533
FT /vntifkey="60"
FT /note="pABICyano1B-Rep"
FT 7080..8603
FT /vntifkey="60"

FIG. 15B (continued)

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```

FT      /note="Pin resolvase [Anabaena variabilis ATCC 29413]"
FT      complement(2408..3028)
FT      /vntifkey="60"
FT      /note="XerD %09Phage integrase (Crocosphaera watsonii WH 8501)"
FT      42..610
FT      /vntifkey="30"
FT      /label=PrpsL
FT      611..1144
FT      /vntifkey="4"
FT      /label=Gm**
FT      1163..1256
FT      /vntifkey="43"
FT      /label=TB0014
FT      complement(11402..11644)
FT      /vntifkey="30"
FT      /label=PsmA
FT      complement(8698..8735)
FT      /vntifkey="43"
FT      /label=TpsaB
FT      complement(8736..11399)
FT      /vntifkey="4"
FT      /label=mysAB_Pum(ABICyanolopt)

```

gene

promoter

CDS

terminator

promoter

terminator

CDS

FIG. 15B (continued)

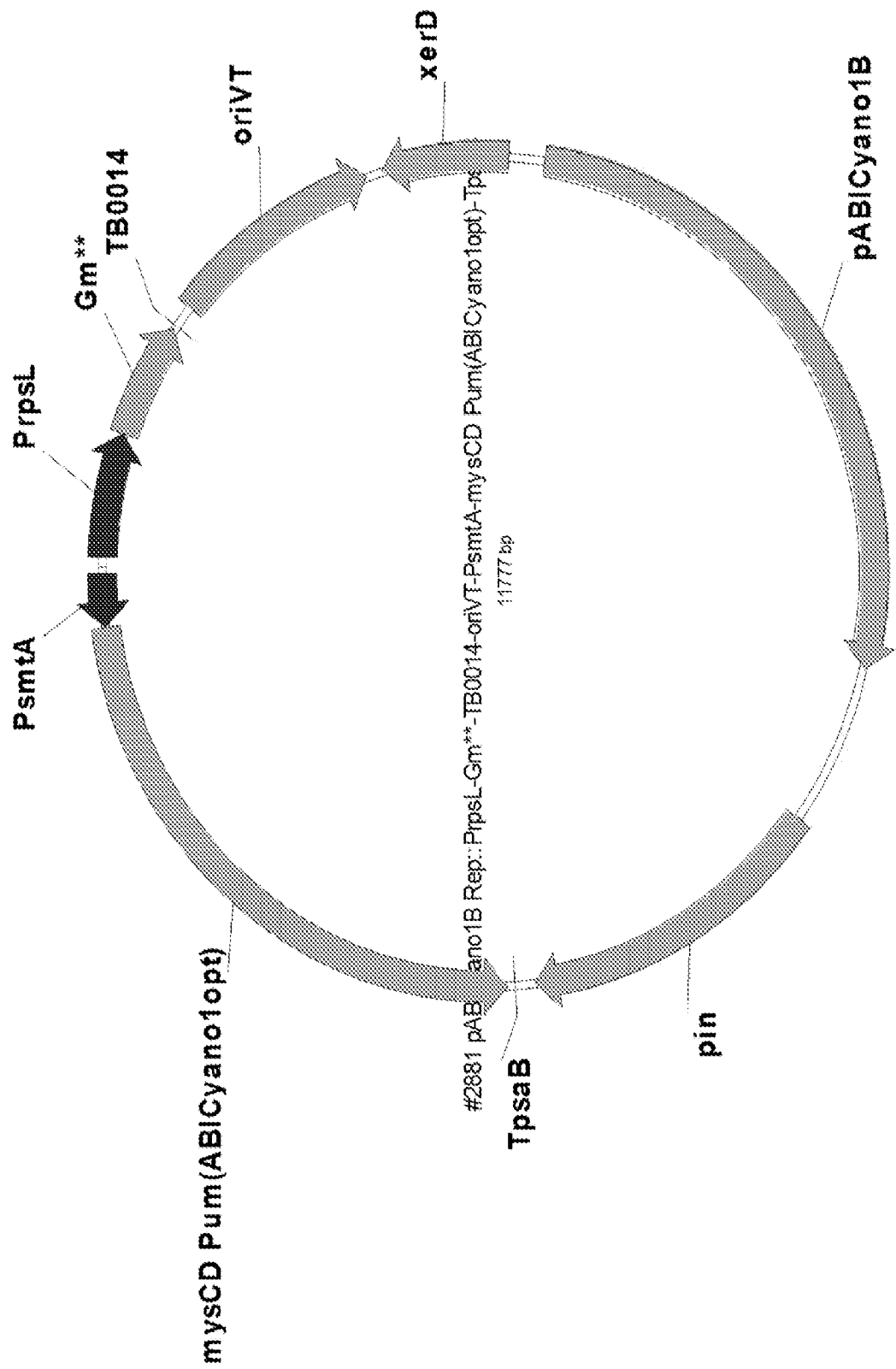


FIG. 15C

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```

ID      #2881\pABCyanolB_Rep::PrpsL-Gm*-TB0014-oriVT-PsmtA-mysCD_Pum (ABICyanolopt) -TpSaB
standard; circular DNA;      ; 11777 BP.
FH      Key      Location/Qualifiers
FH
FT      rep_origin      1268..2326
FT                      /vntifkey="33"
FT                      /label=oriVT
FT      rep_origin      3206..6328
FT                      /vntifkey="33"
FT                      /label=pABCyanolB
FT      CDS              complement(2408..3028)
FT                      /vntifkey="4"
FT                      /label=xerD
FT      CDS              7080..8603
FT                      /vntifkey="4"
FT                      /label=pin
FT      gene             5533..5533
FT                      /vntifkey="60"
FT                      /note="pABCyanolB-Rep"
FT      gene             7080..8603
FT                      /vntifkey="60"
FT                      /note="Pin resolvase [Anabaena variabilis ATCC 29413]"
FT      gene             complement(2408..3028)
FT                      /vntifkey="60"
FT                      /note="XerD %09Phage integrase (Crocosphaera watsonii WH 8501)"
FT      promoter         42..610
FT                      /vntifkey="30"
FT                      /label=PrpsL
FT      CDS              611..1144
FT                      /vntifkey="4"

```

FIG. 15C (continued)

```

FT      /label=Gm**
FT      1163..1256
FT      /vntifkey="43"
FT      /label=TB0014
FT      complement(11522..11764)
FT      /vntifkey="30"
FT      /label=PsmTA
FT      complement(8698..8735)
FT      /vntifkey="43"
FT      /label=TpsaB
FT      complement(8736..11519)
FT      /vntifkey="4"
FT      /label=mysCD_Pum(ABICyano1opt)

```

terminator

promoter

terminator

CDS

FIG. 15C (continued)

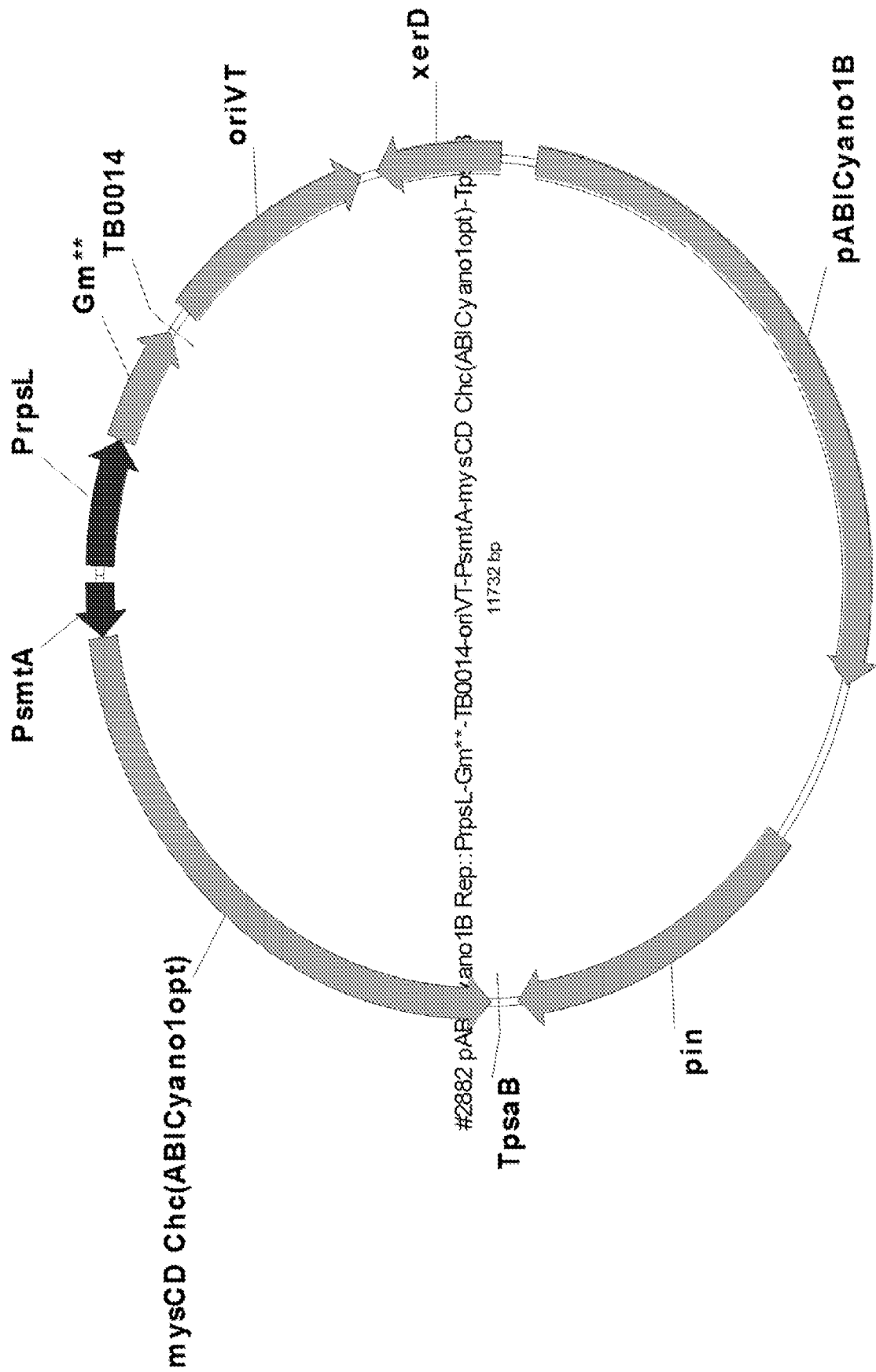


FIG. 15D

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```

ID      #2882\pABICyano1B_Rep::PrpsL-Gm*-TB0014-oriVT-PsmtA-mysCD_Chc(ABICyano1opt)-TpsaB
standard; circular DNA;      ; 11732 BP.
FH      Key      Location/Qualifiers
FH
FT      rep_origin      1268..2326
FT      /vntifkey="33"
FT      /label=oriVT
FT      3206..6328
FT      /vntifkey="33"
FT      /label=pABICyano1B
FT      complement(2408..3028)
FT      /vntifkey="4"
FT      /label=xerD
FT      7080..8603
FT      /vntifkey="4"
FT      /label=pin
FT      5533..5533
FT      /vntifkey="60"
FT      /note="pABICyano1B-Rep"
FT      7080..8603
FT      /vntifkey="60"
FT      /note="Pin resolvase [Anabaena variabilis ATCC 29413]"
FT      complement(2408..3028)
FT      /vntifkey="60"
FT      /note="XerD %09Phage integrase (Crocospaera watsonii WH 8501)"
FT      42..610
FT      /vntifkey="30"
FT      /label=PrpsL
FT      611..1144
FT      /vntifkey="4"

```

FIG. 15D (continued)

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FT	/label=Gm**
FT	1163..1256
FT	/vntifkey="43"
FT	/label=TB0014
FT	complement(11477..11719)
FT	/vntifkey="30"
FT	/label=PsmTA
FT	complement(8698..8735)
FT	/vntifkey="43"
FT	/label=TpsaB
FT	complement(8736..11474)
FT	/vntifkey="4"
FT	/label=mysCD_Chc(ABICyanolopt)

terminator

promoter

terminator

CDS

FIG. 15D (continued)

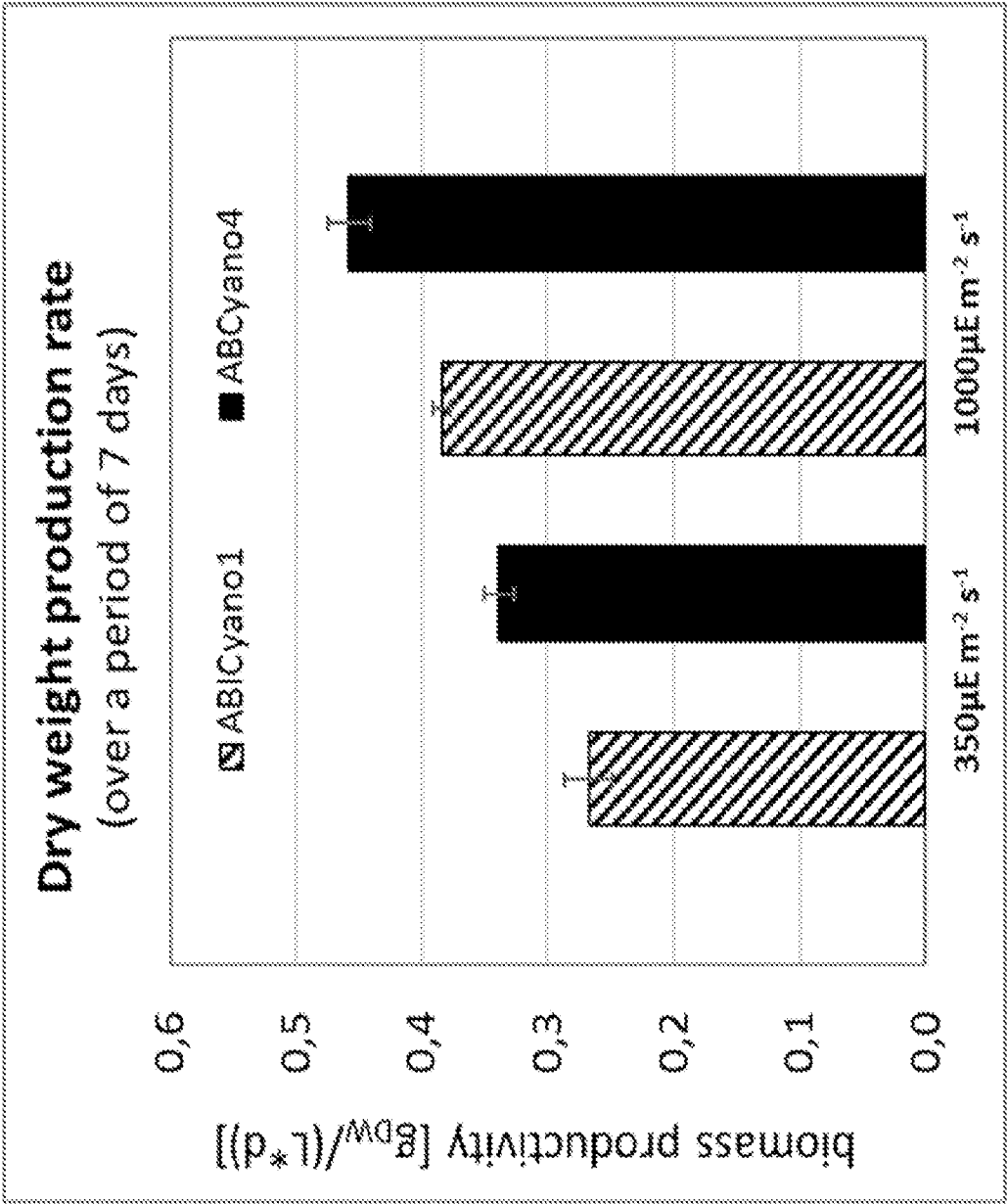


FIG. 16A

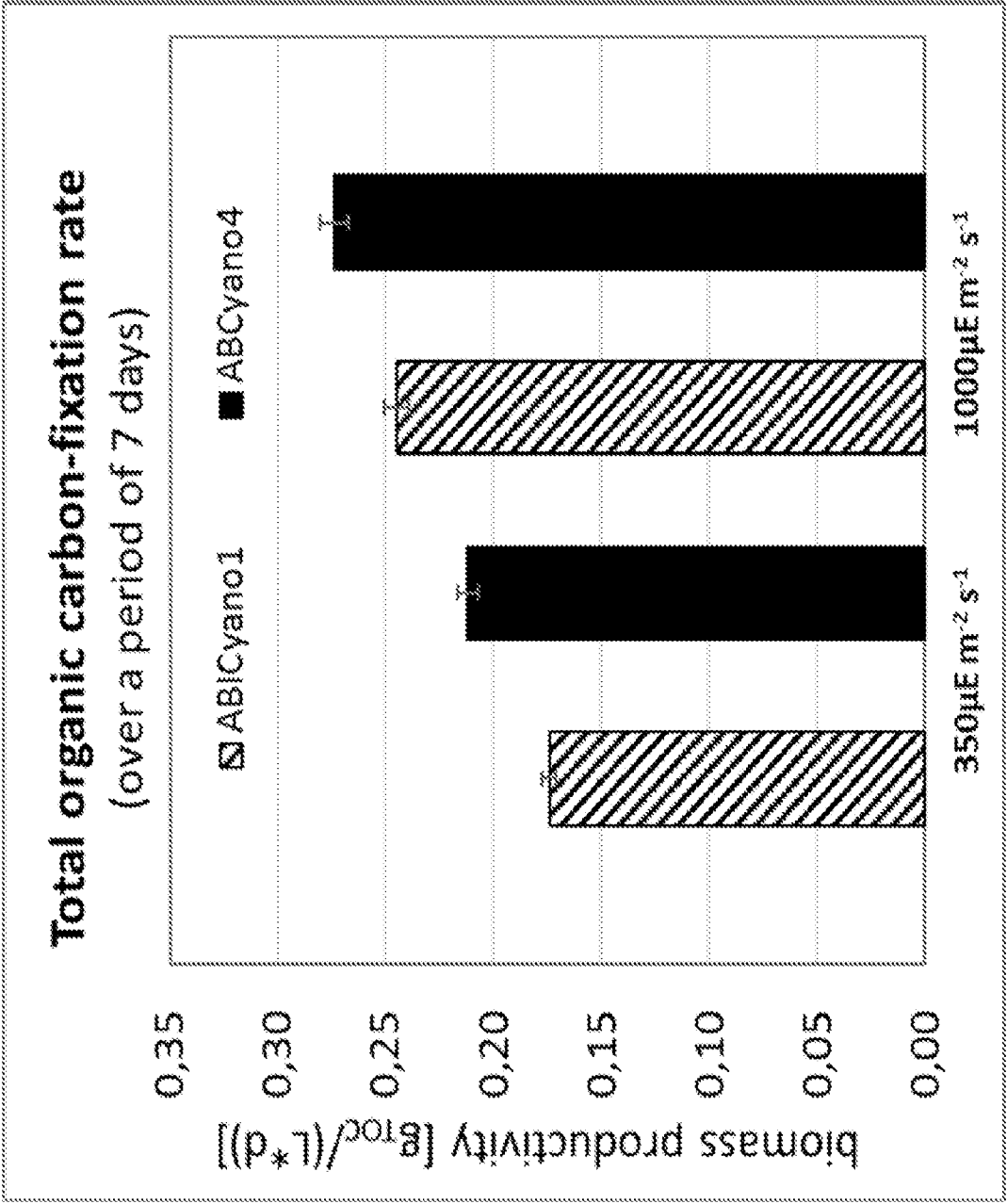


FIG. 16B

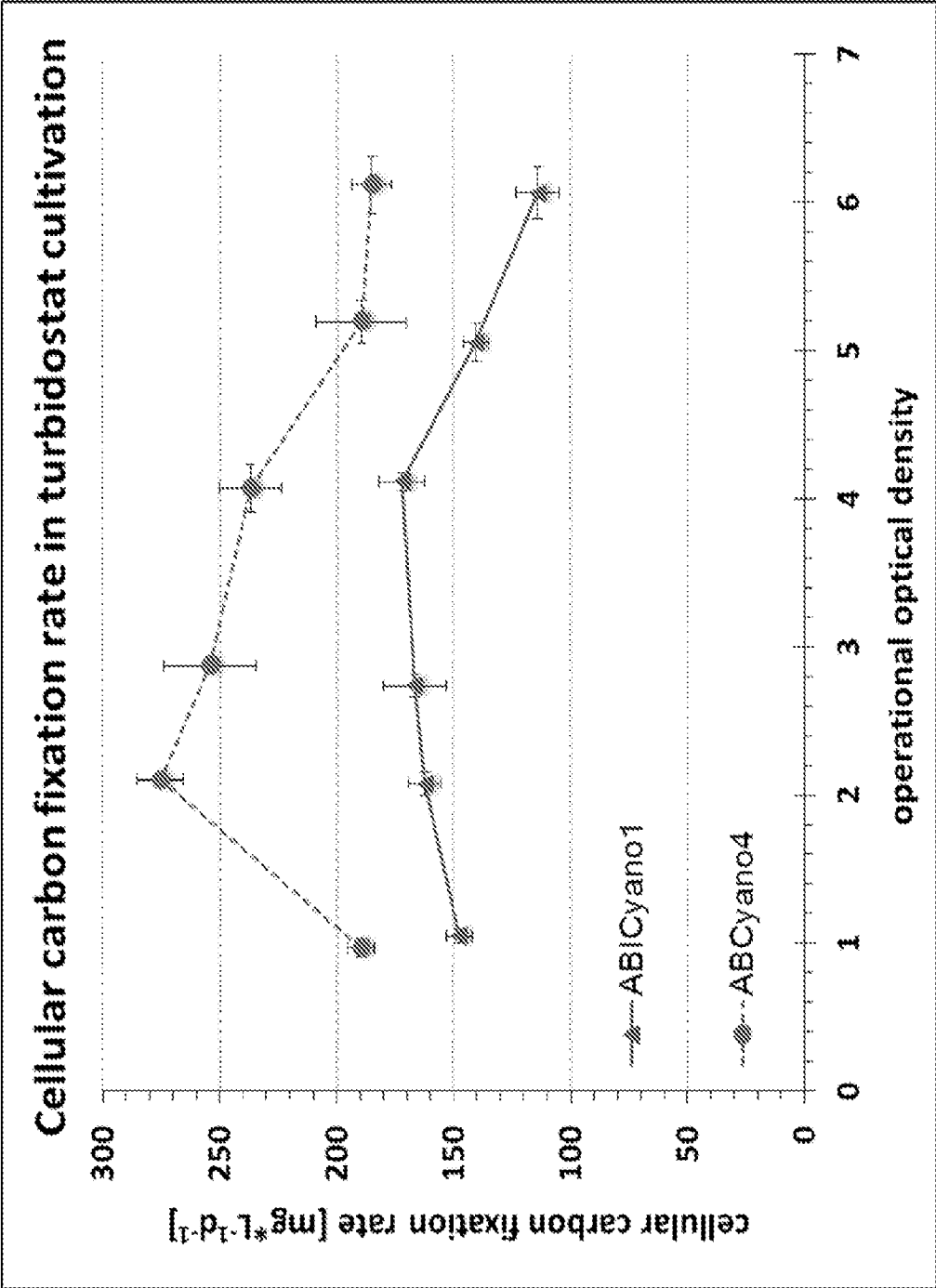


FIG. 17A

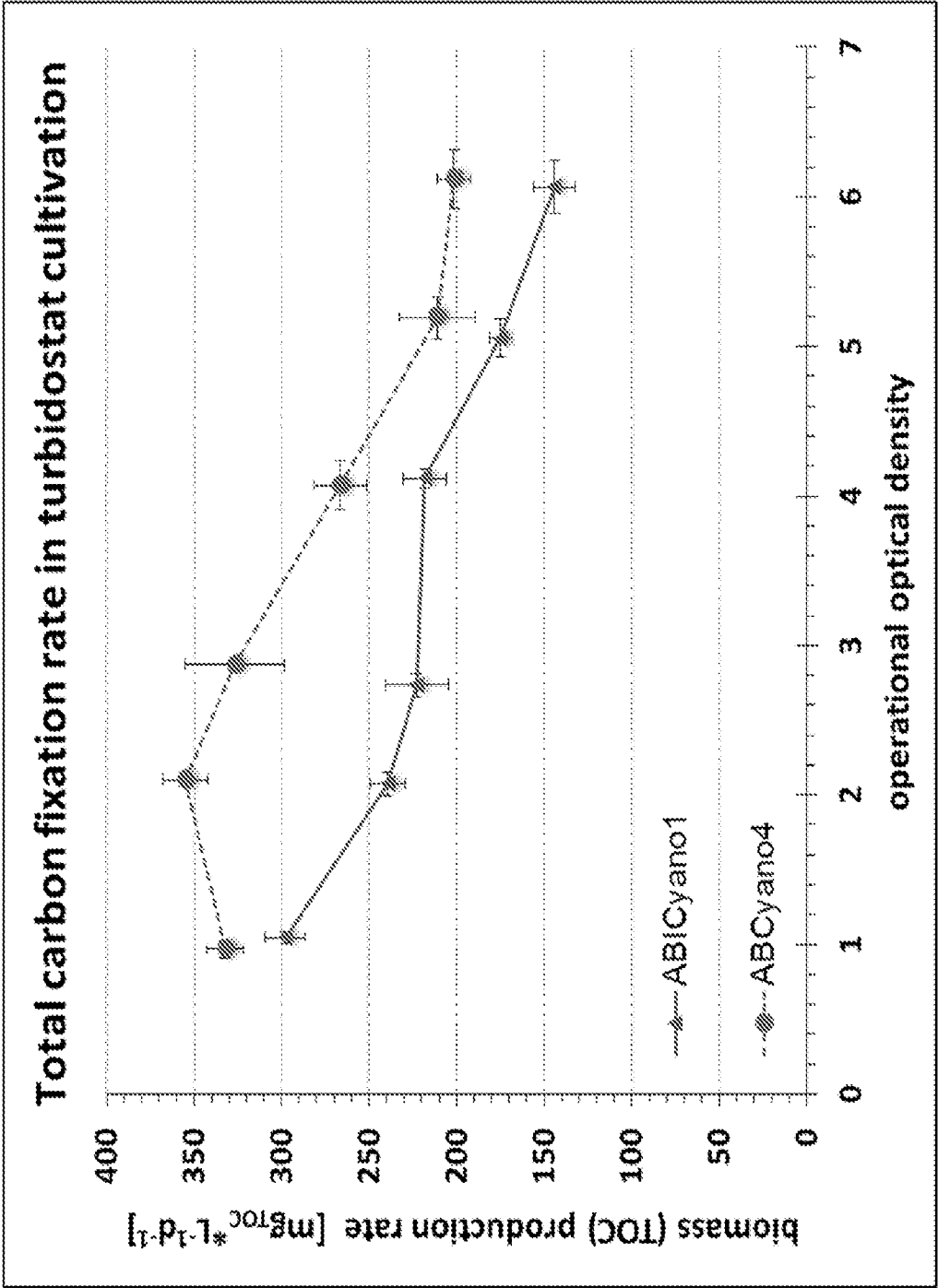


FIG. 17B

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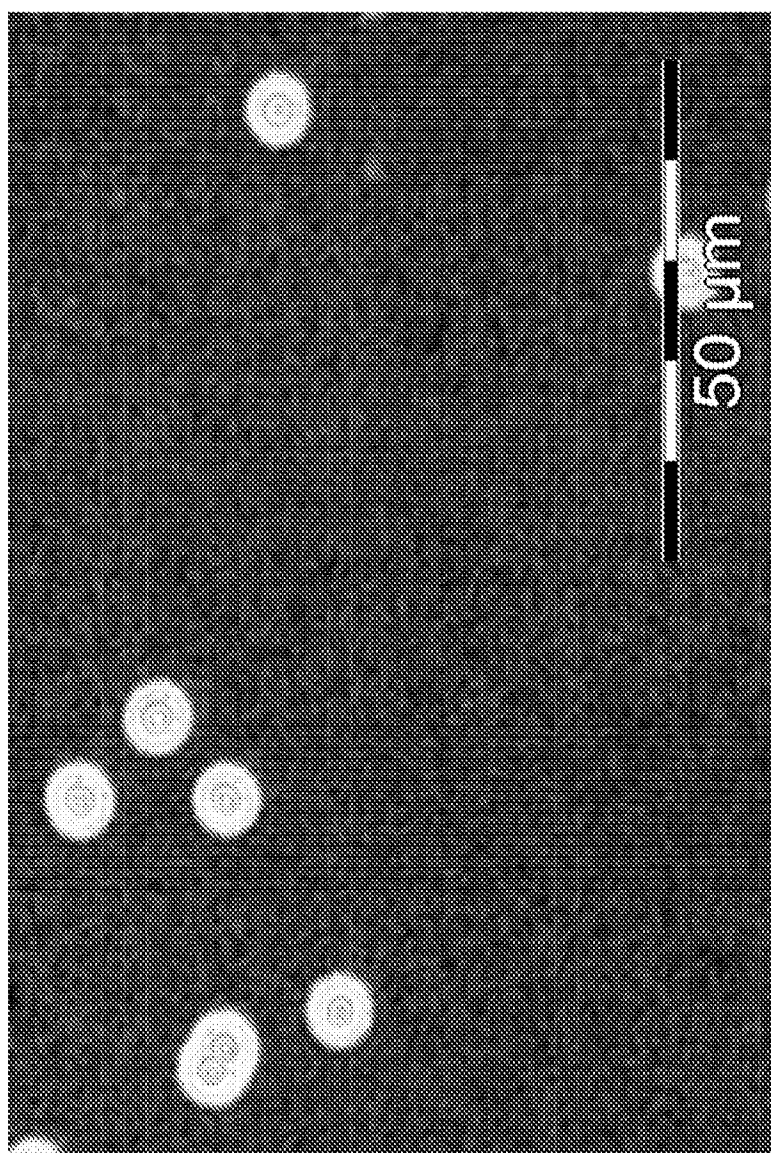


FIG. 18A

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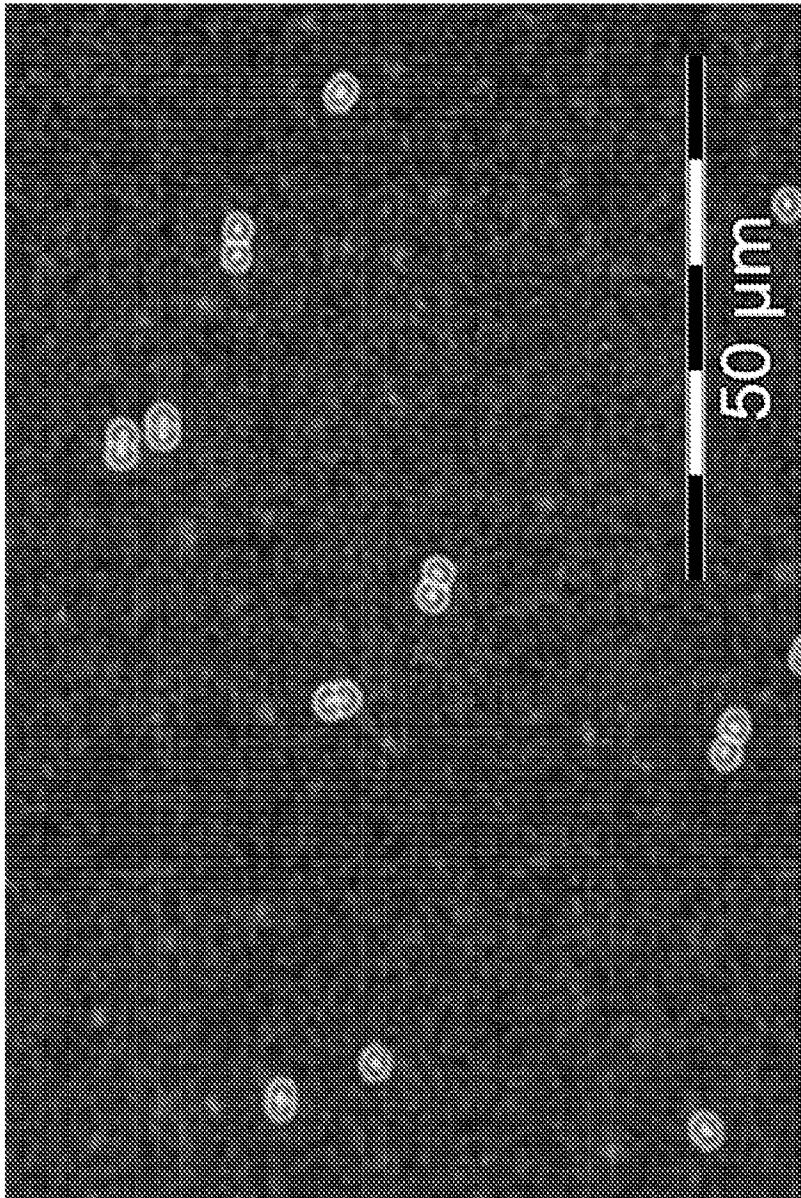


FIG. 18B

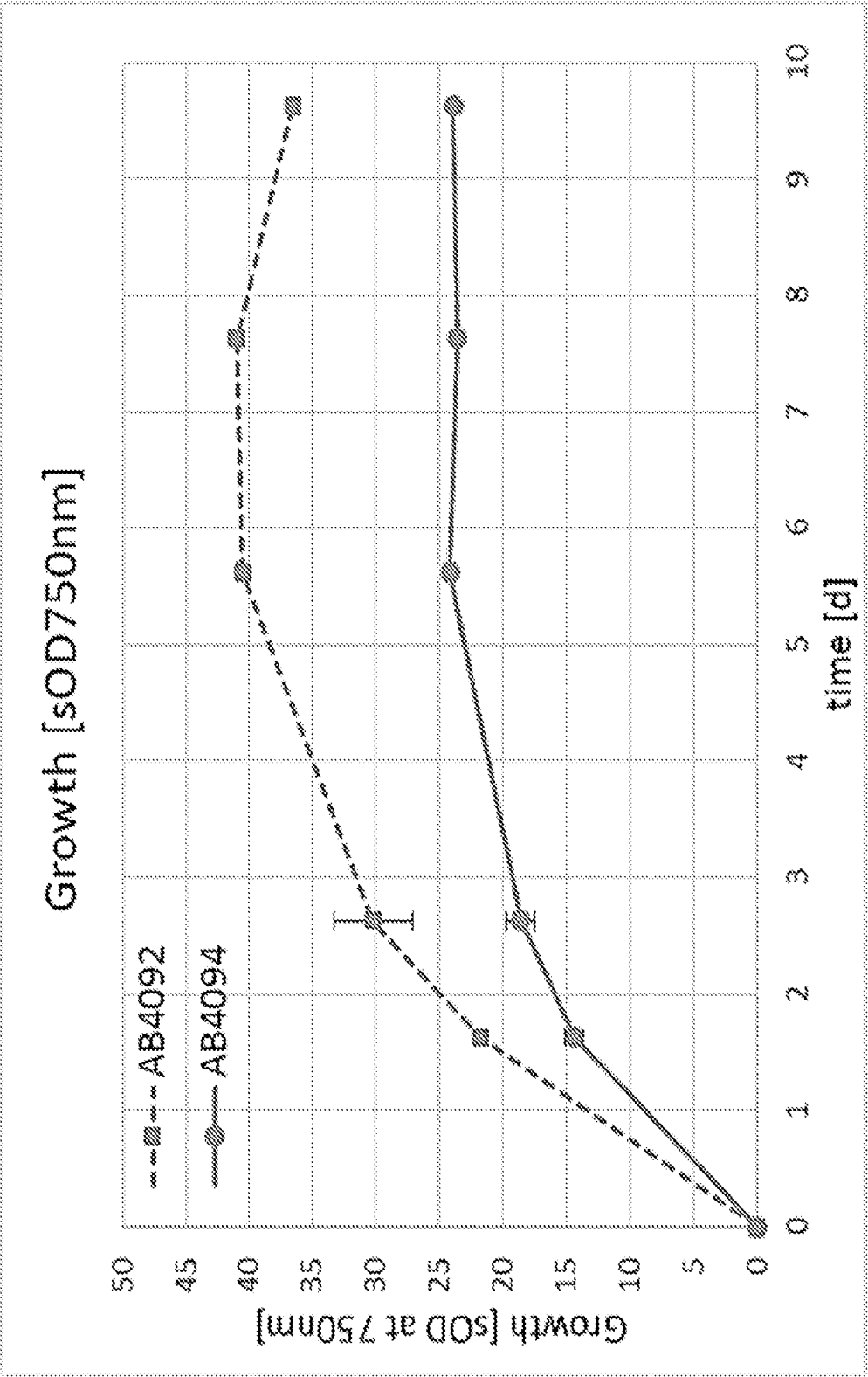


Fig. 19A

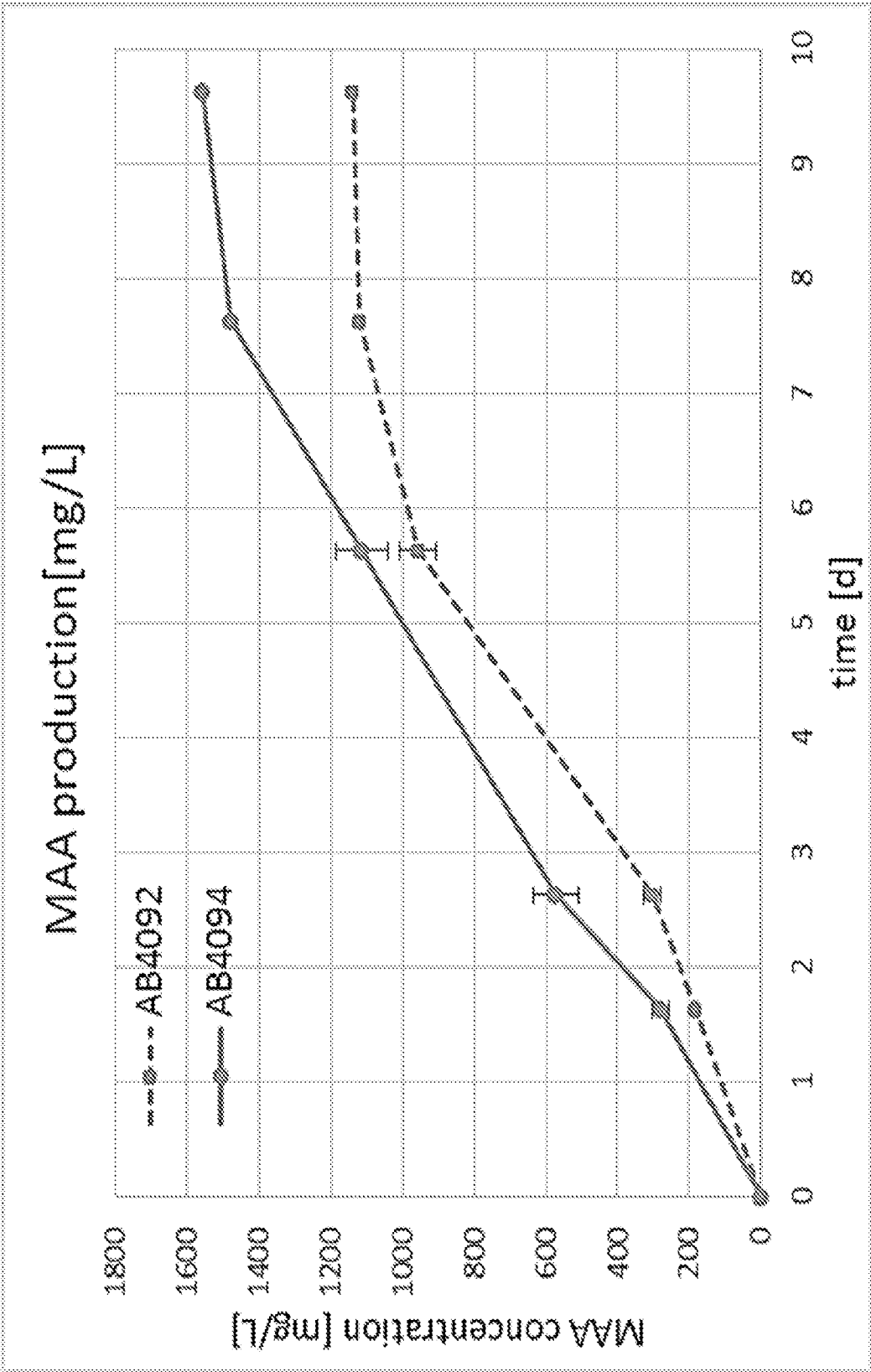


Fig. 19B

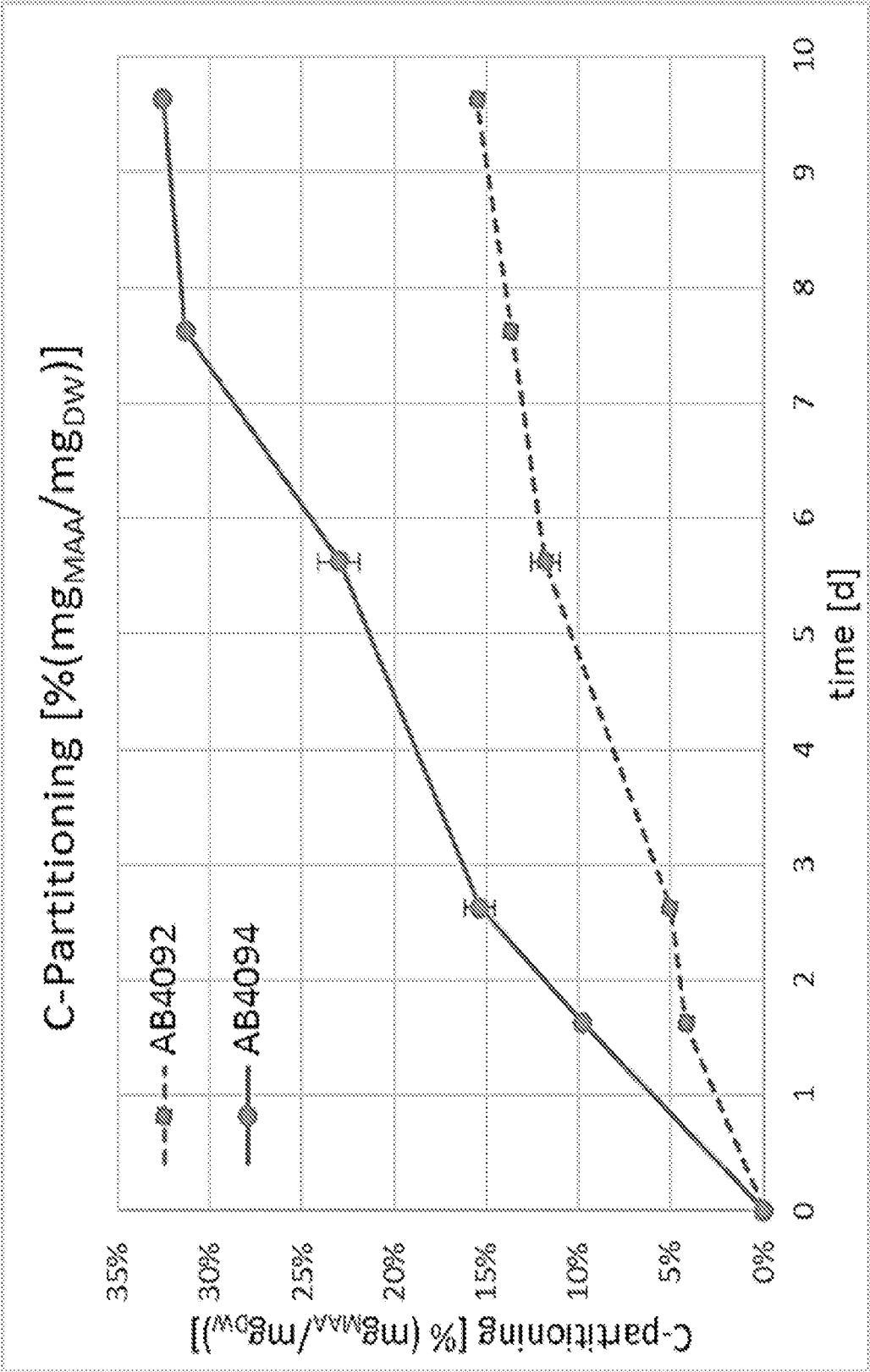


Fig. 19C

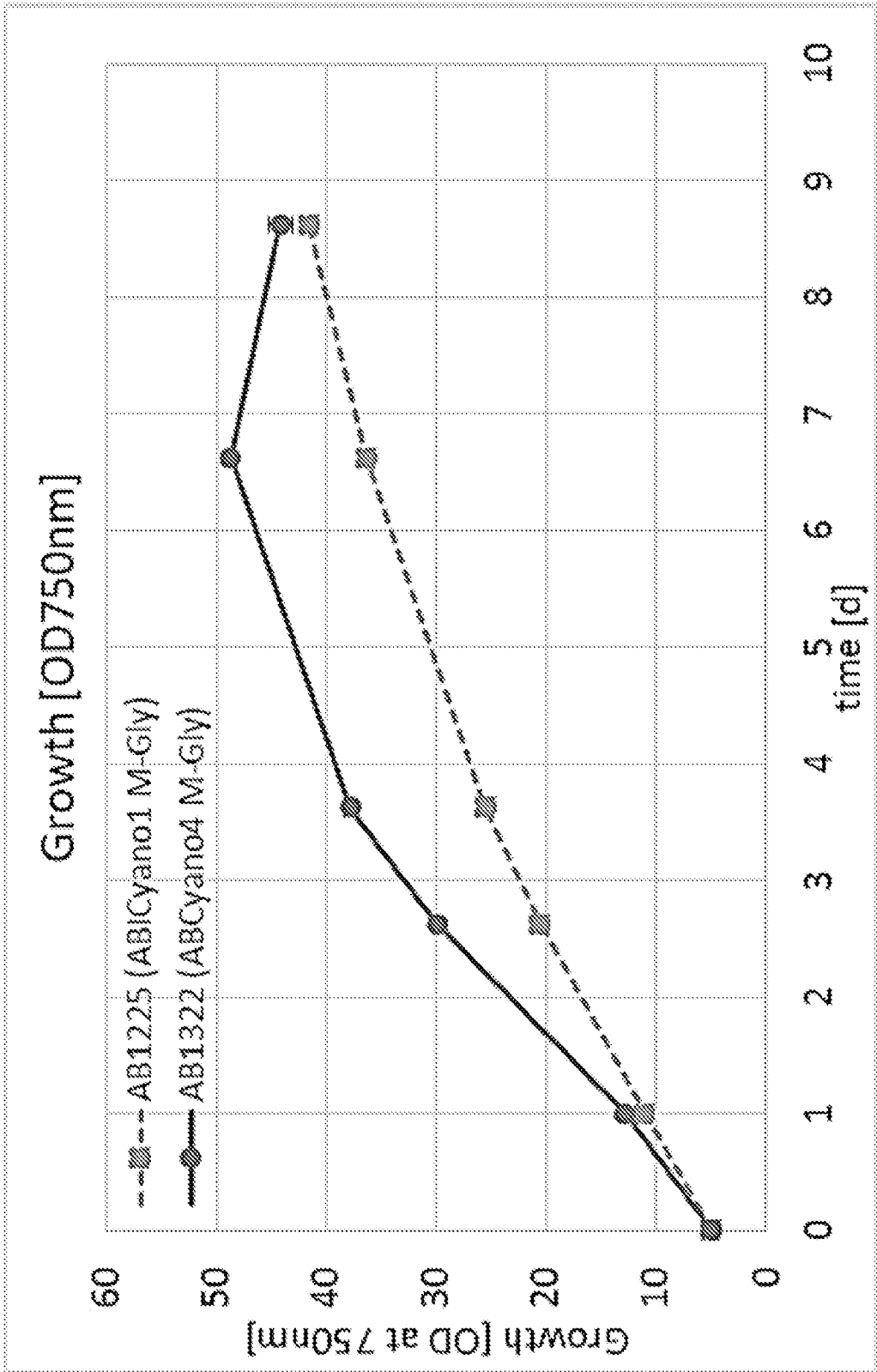


FIG. 20A

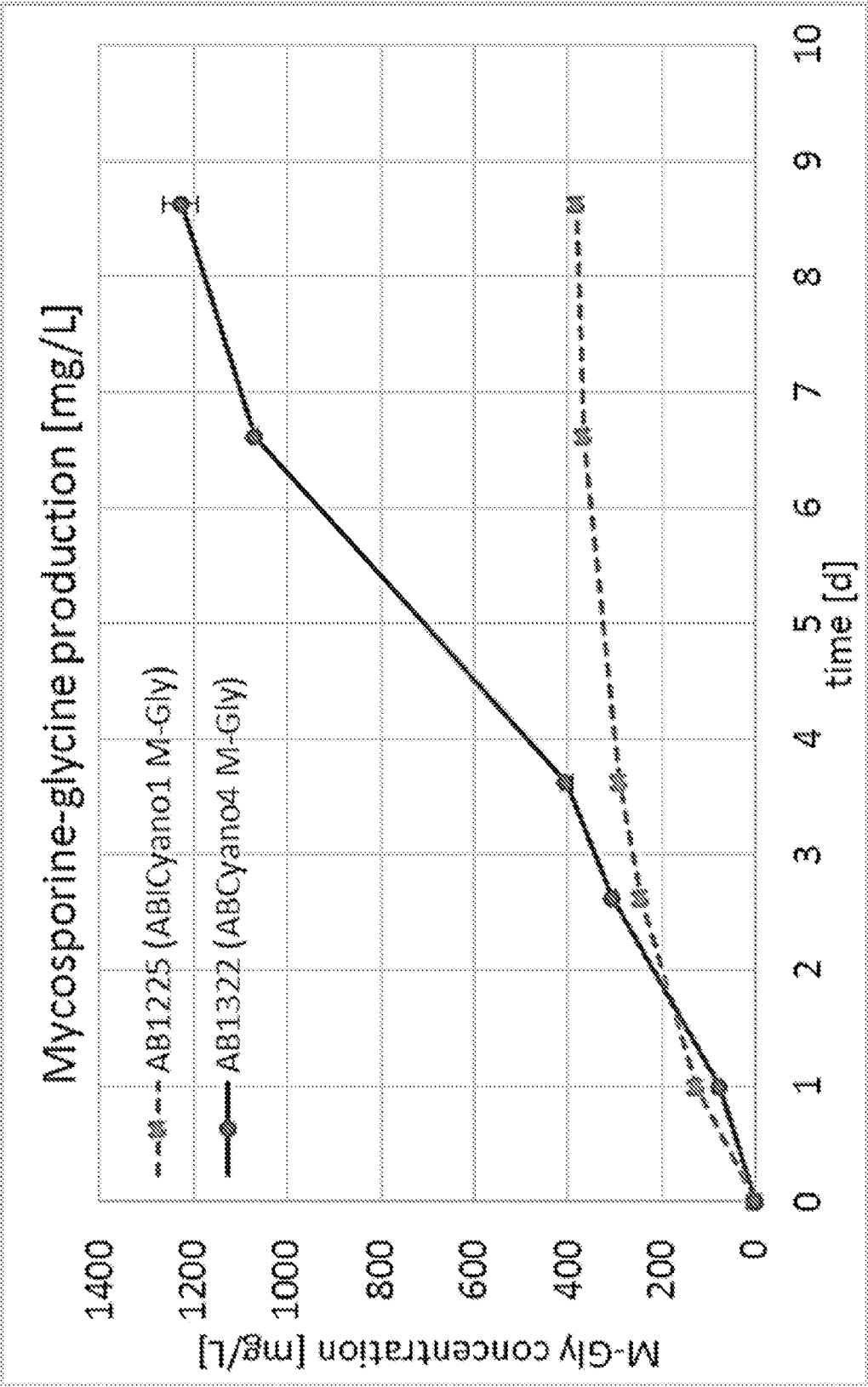


FIG. 20B

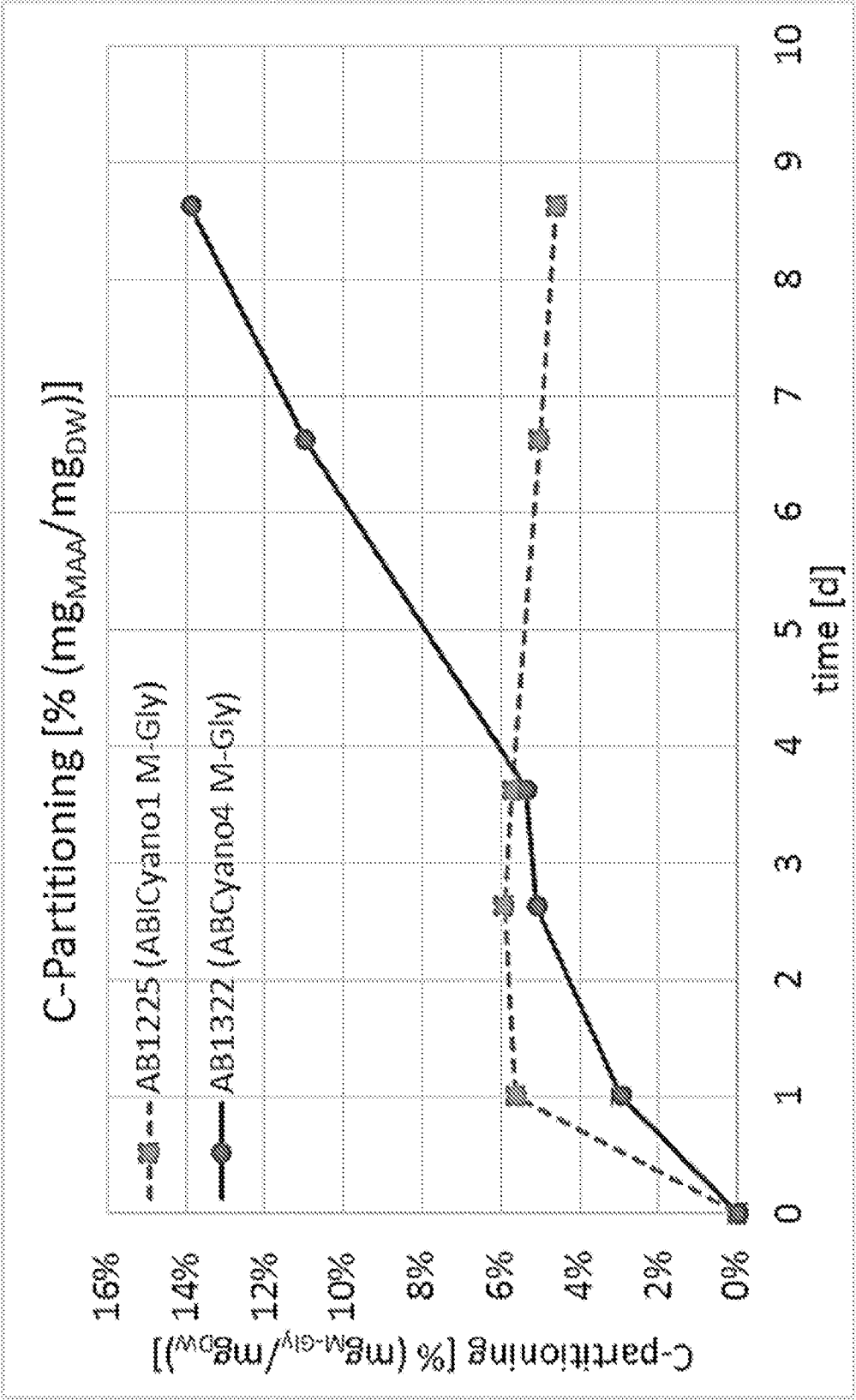


FIG. 20C

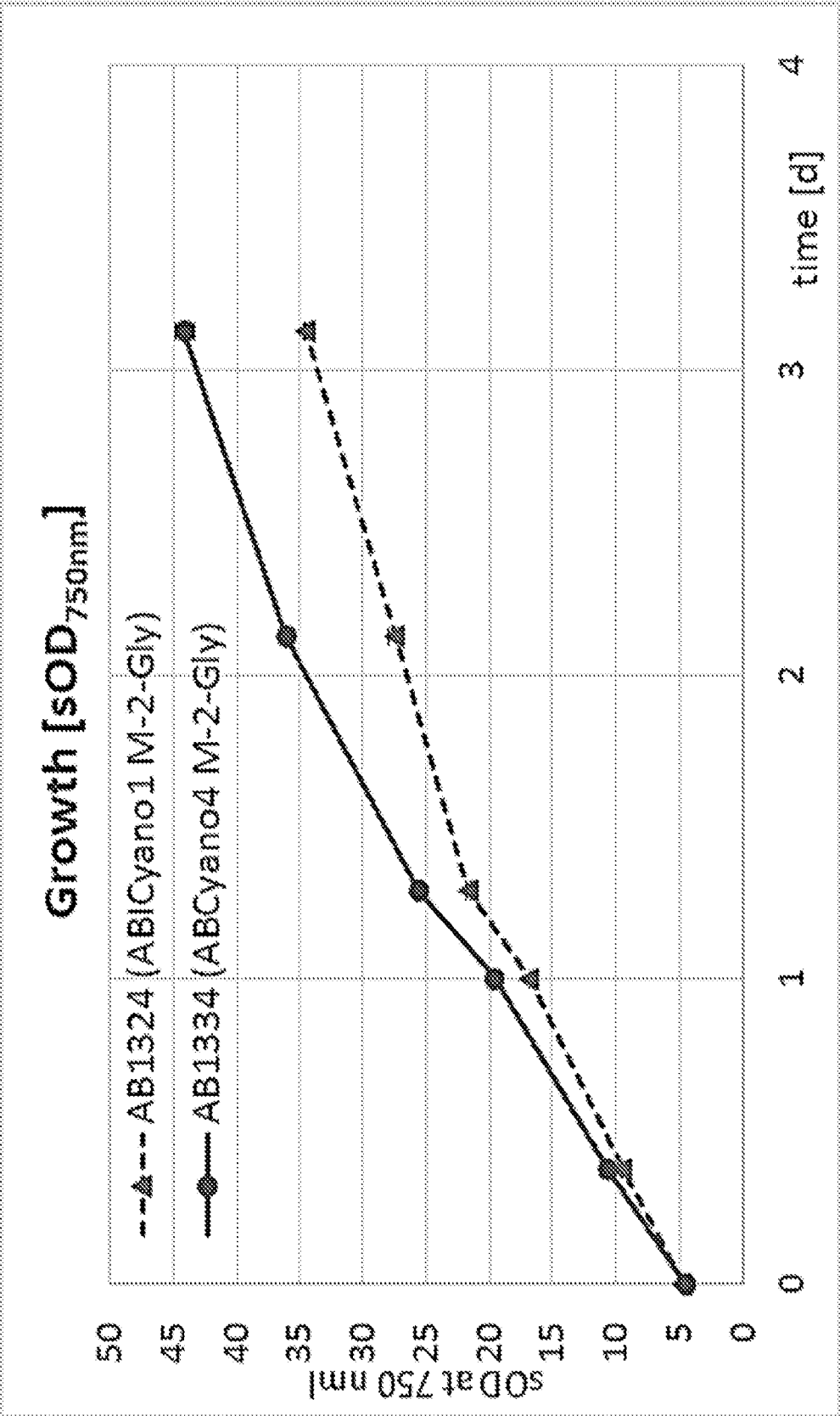


FIG. 21A

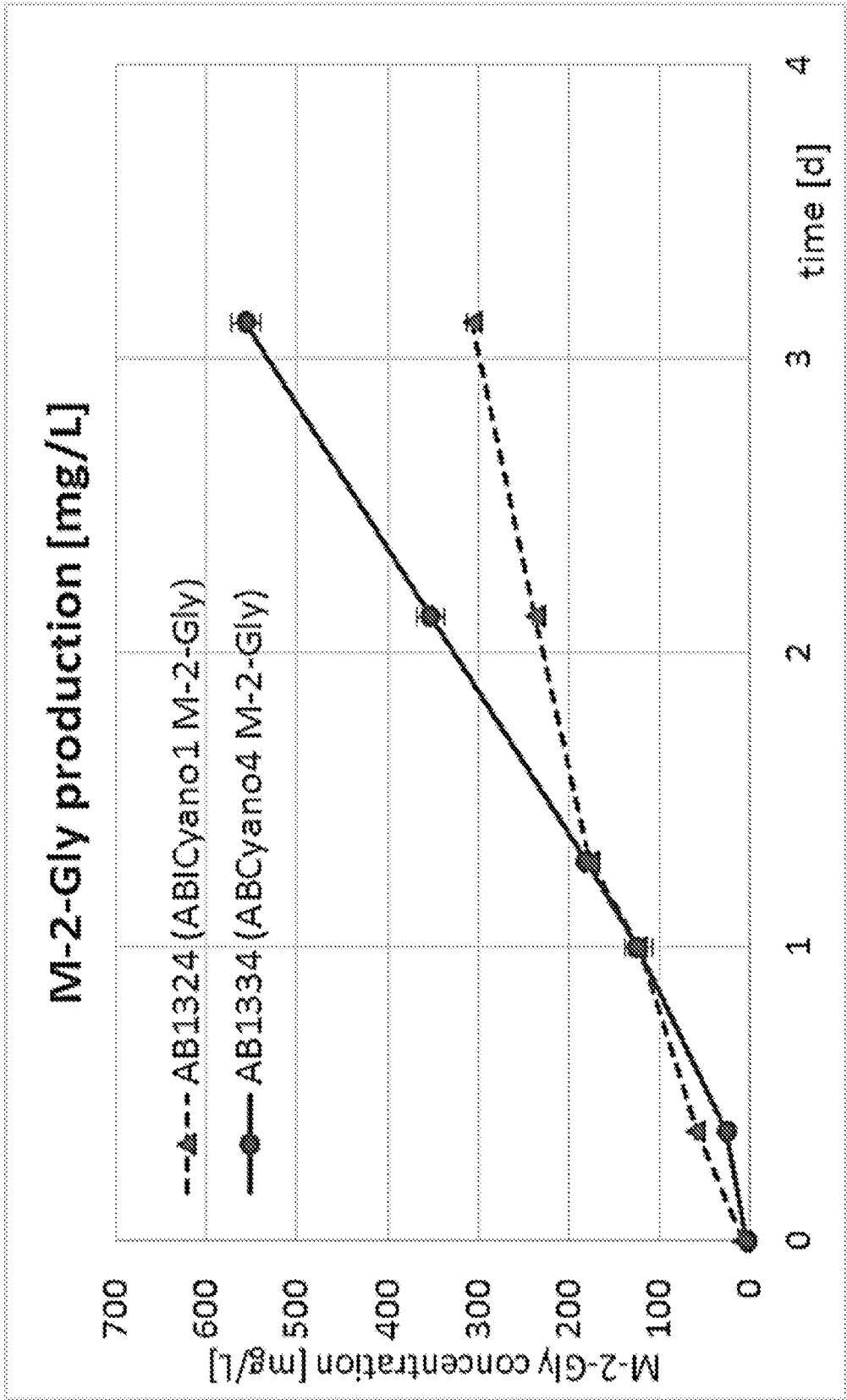


FIG. 21B

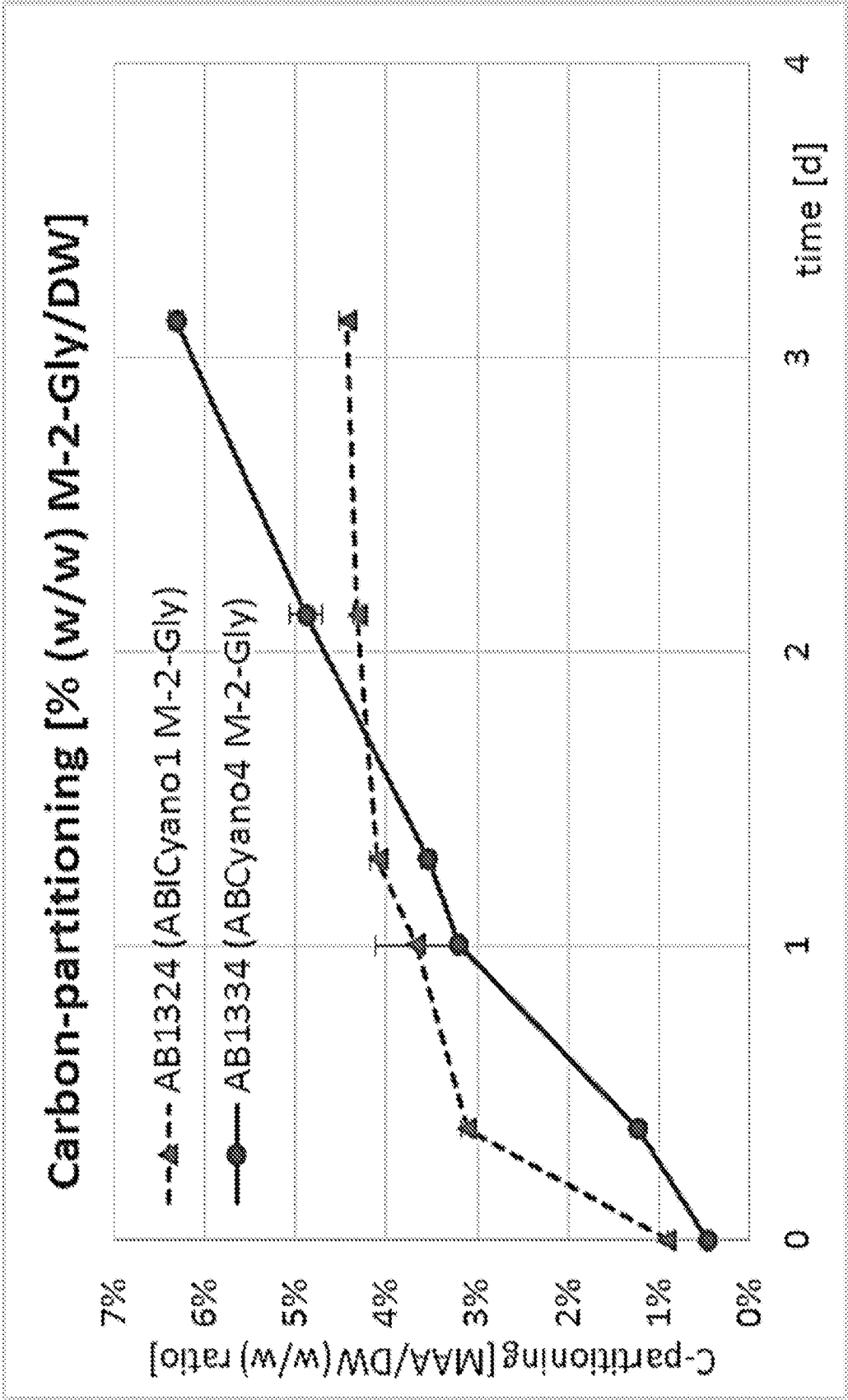


FIG. 21C

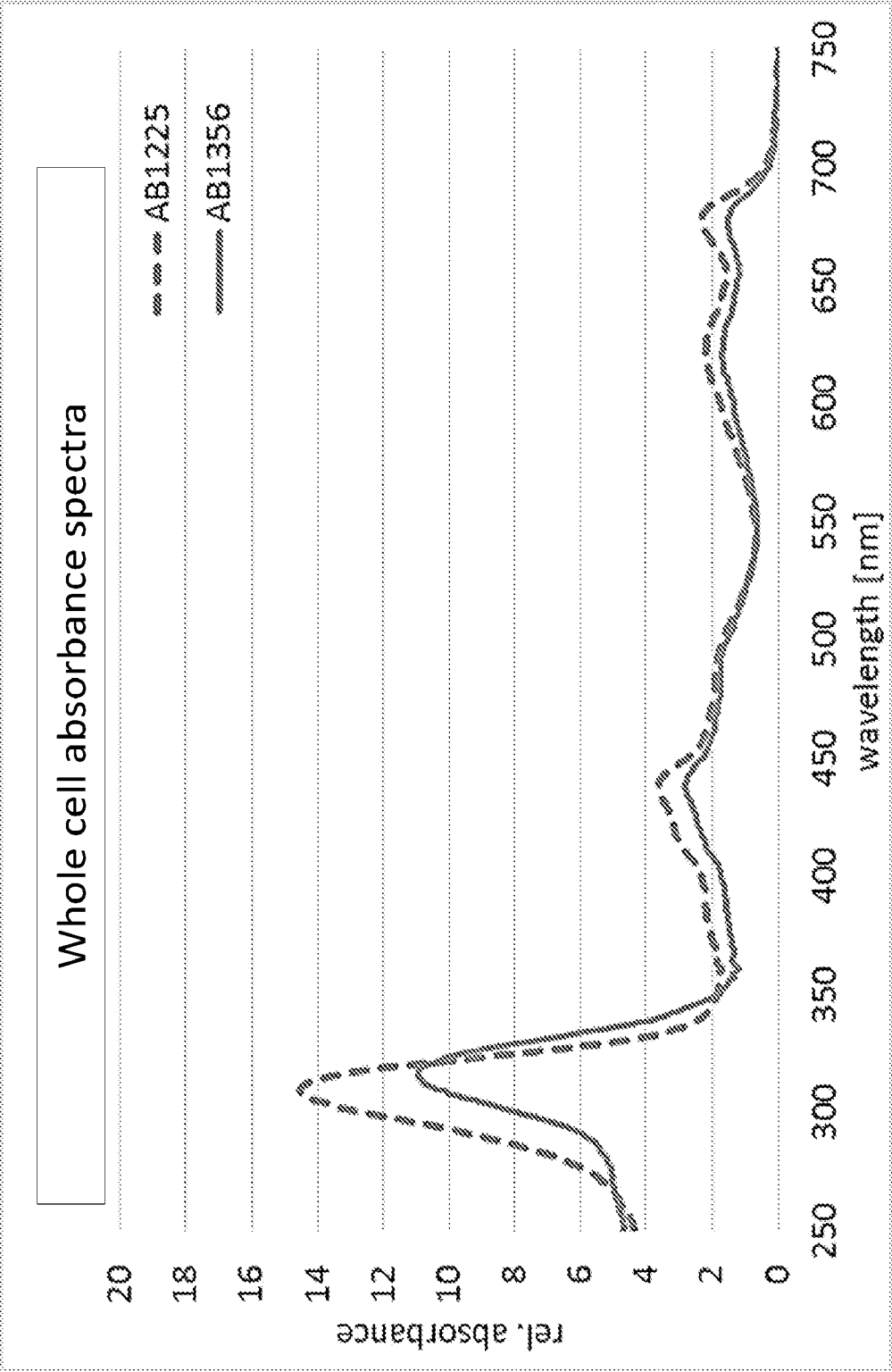


FIG. 22A

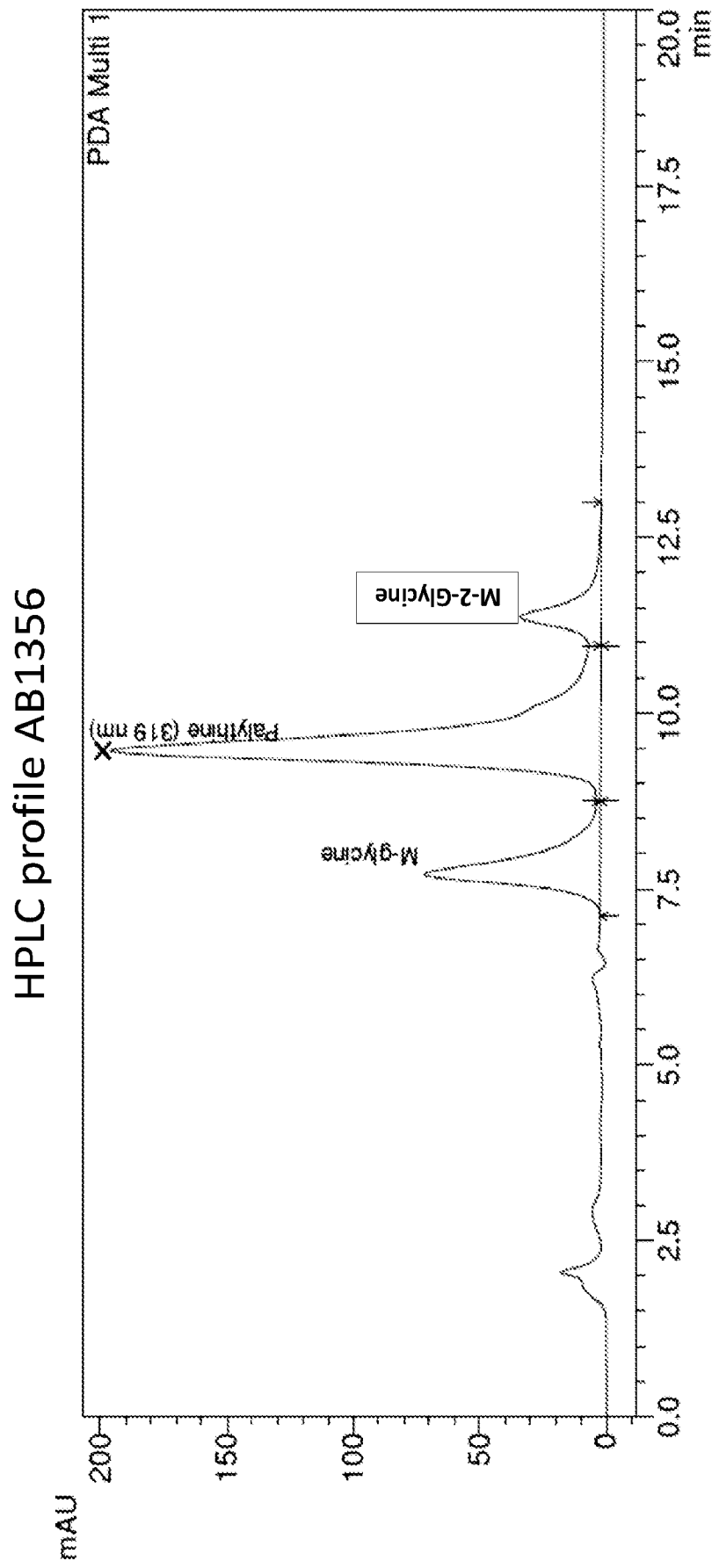


FIG. 22B

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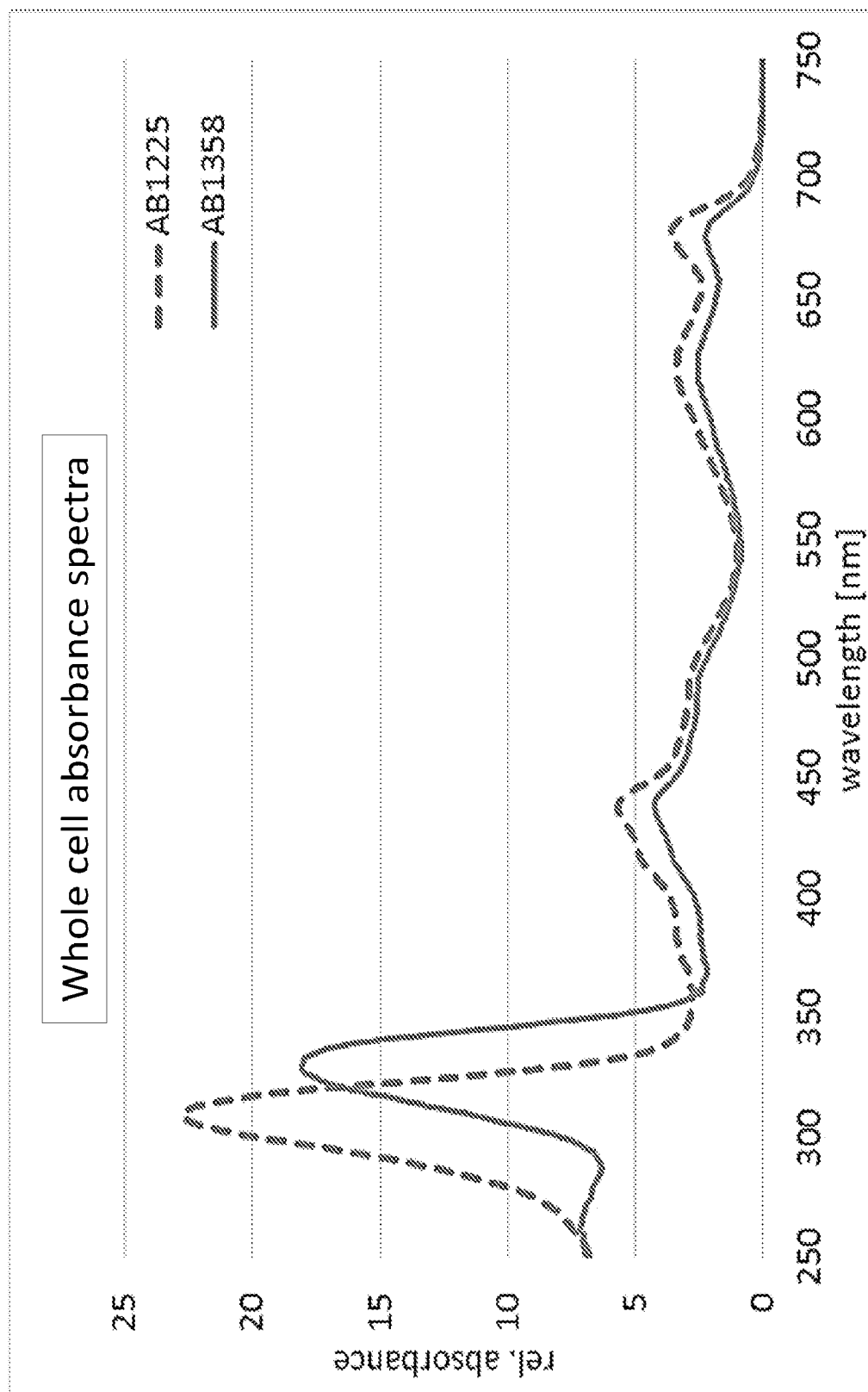


FIG. 23A

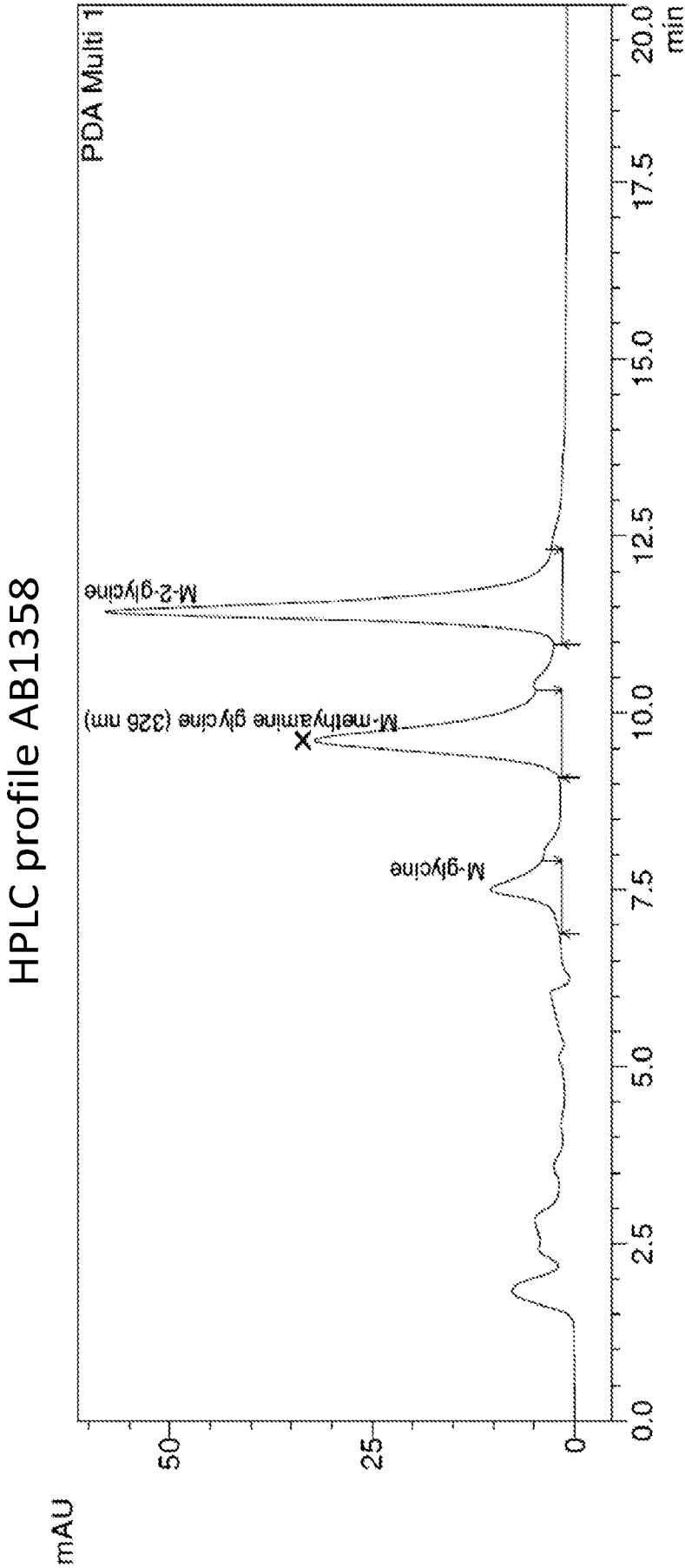


FIG. 23B

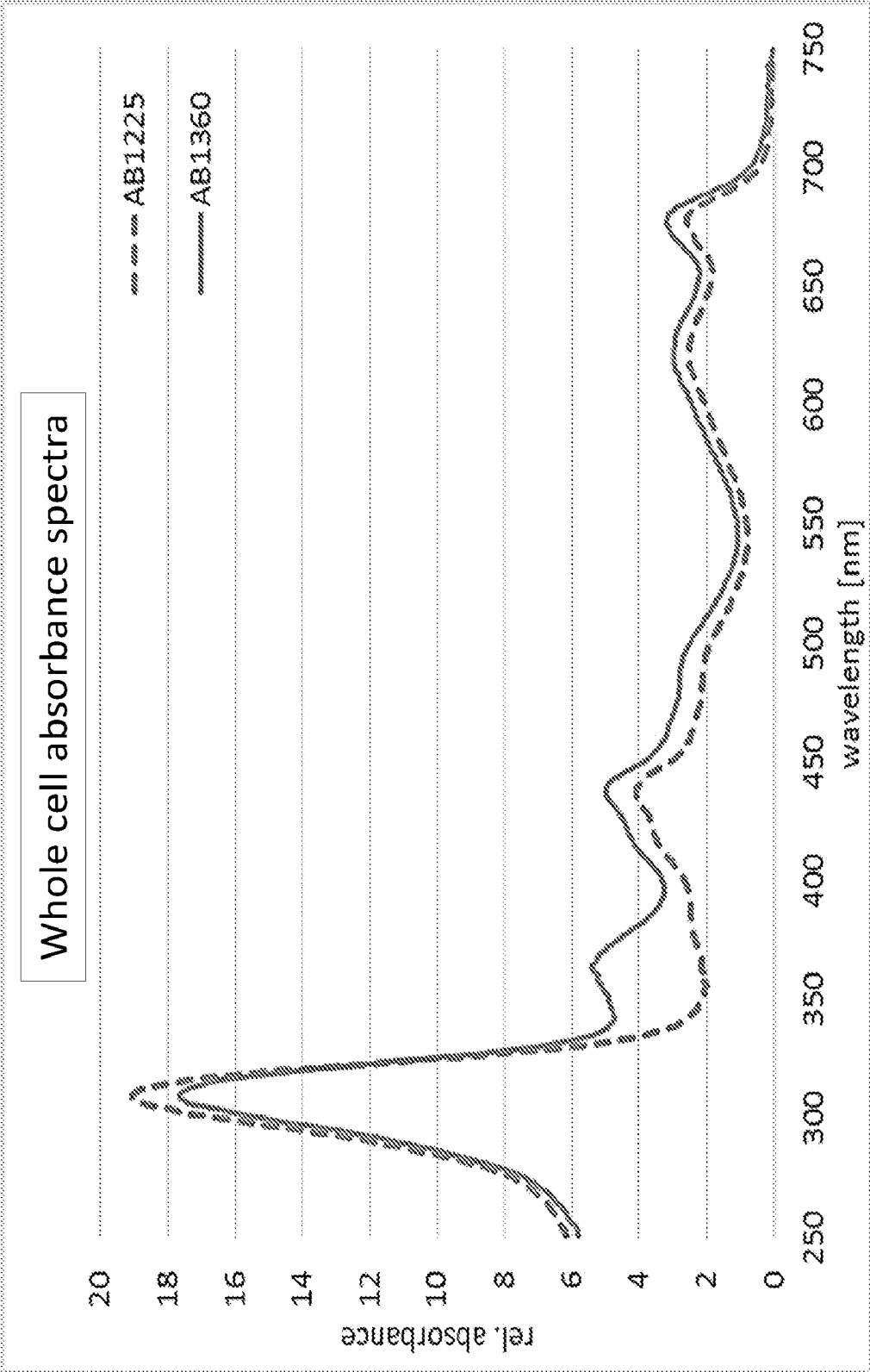


FIG. 24A

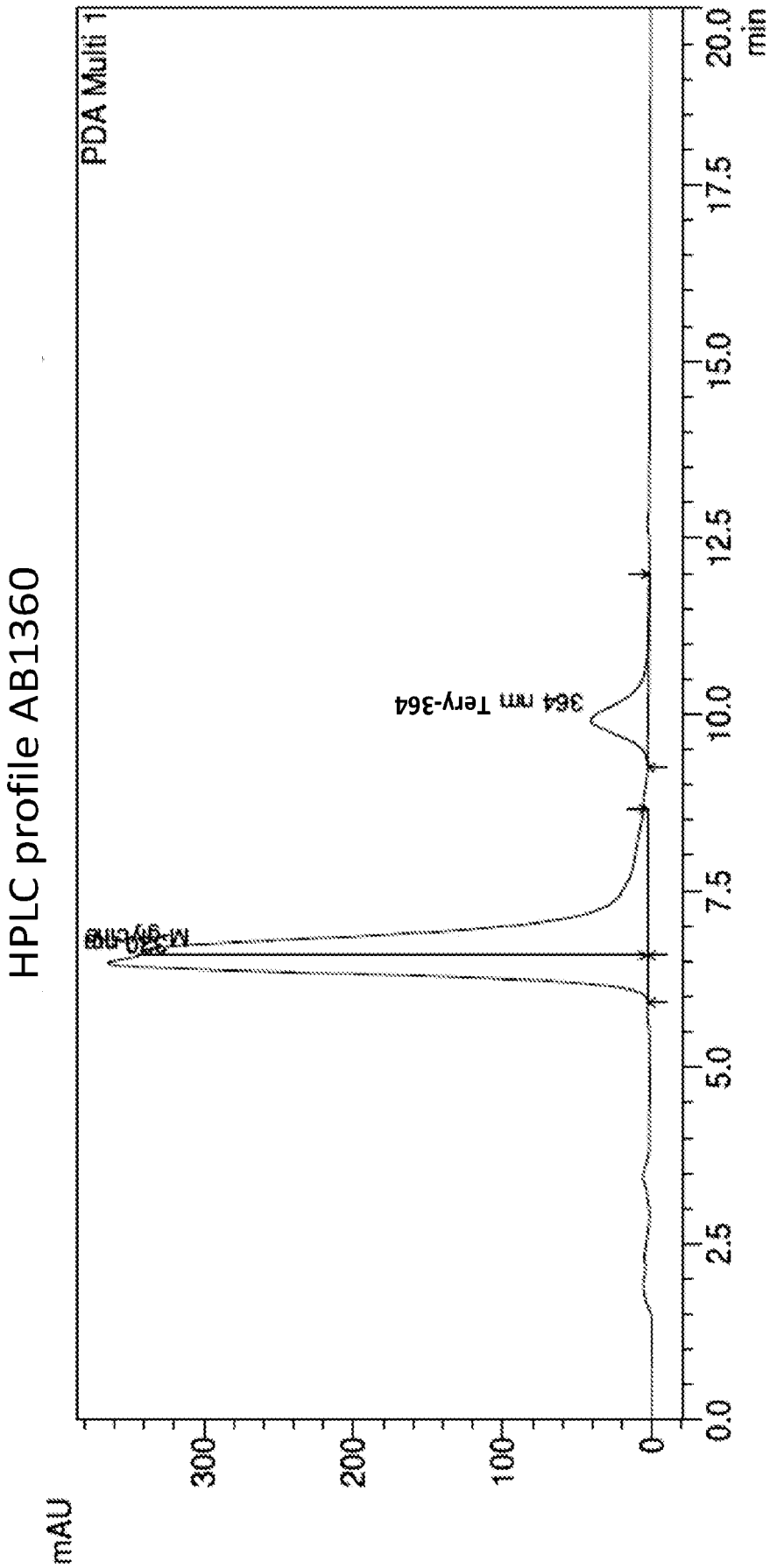


FIG. 24B

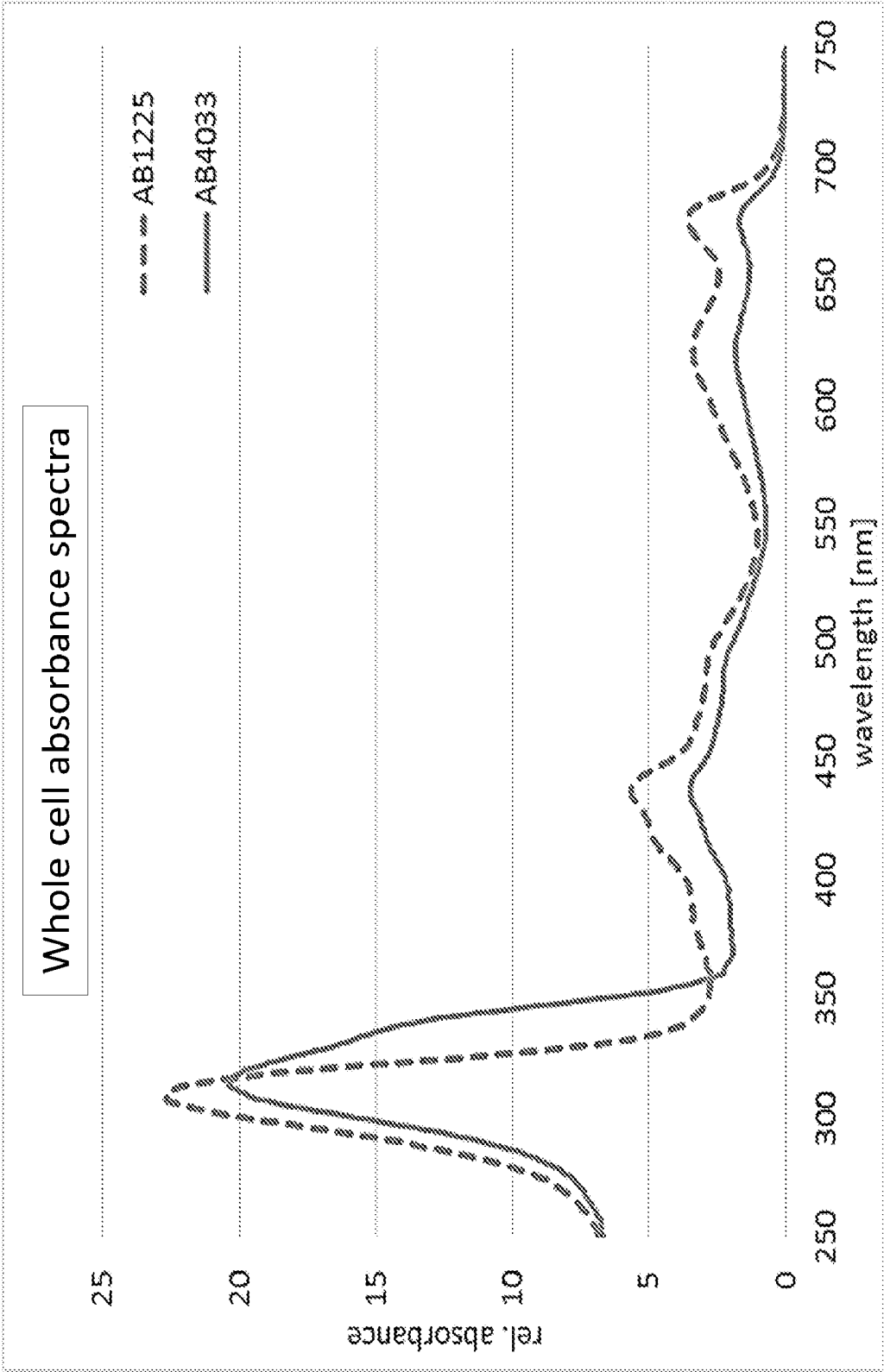


FIG. 25A

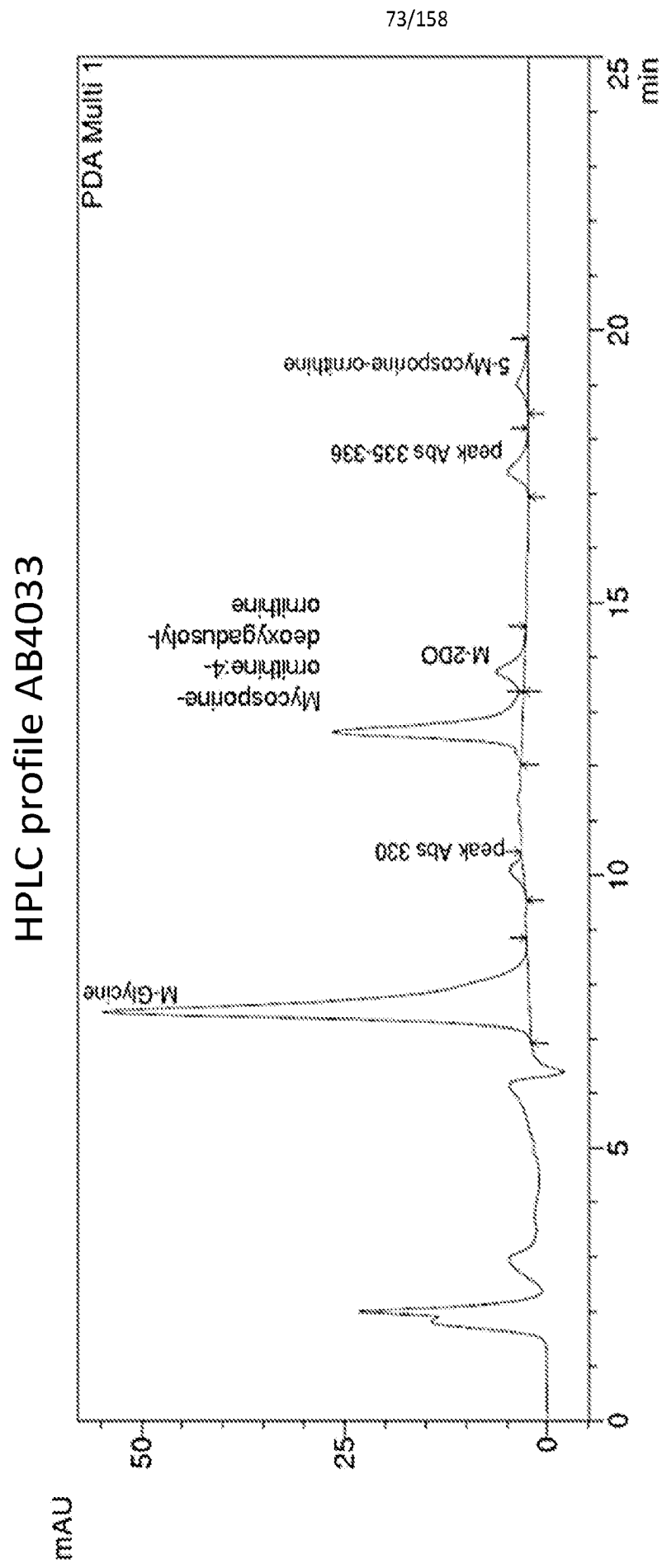


FIG. 25B

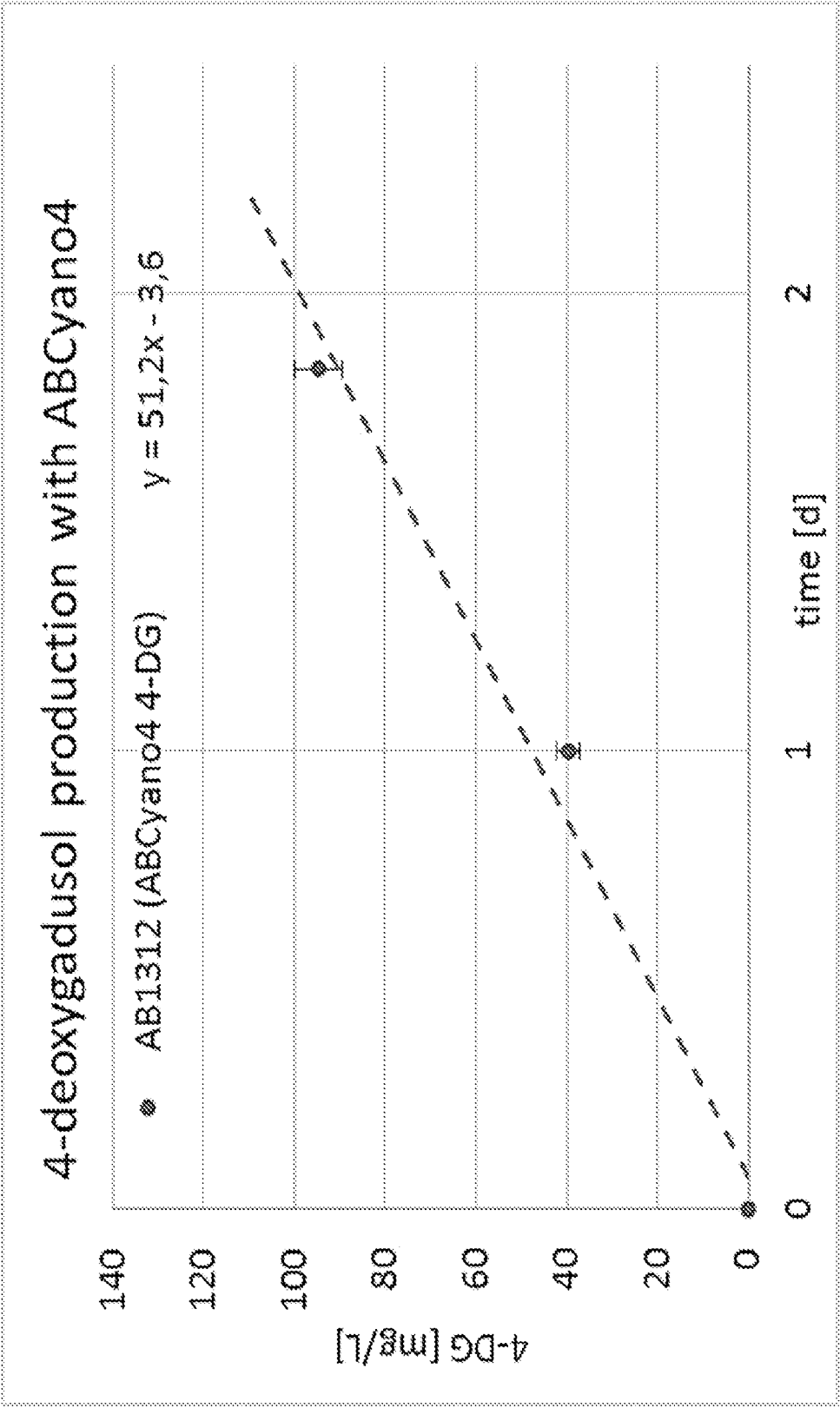


FIG. 26A

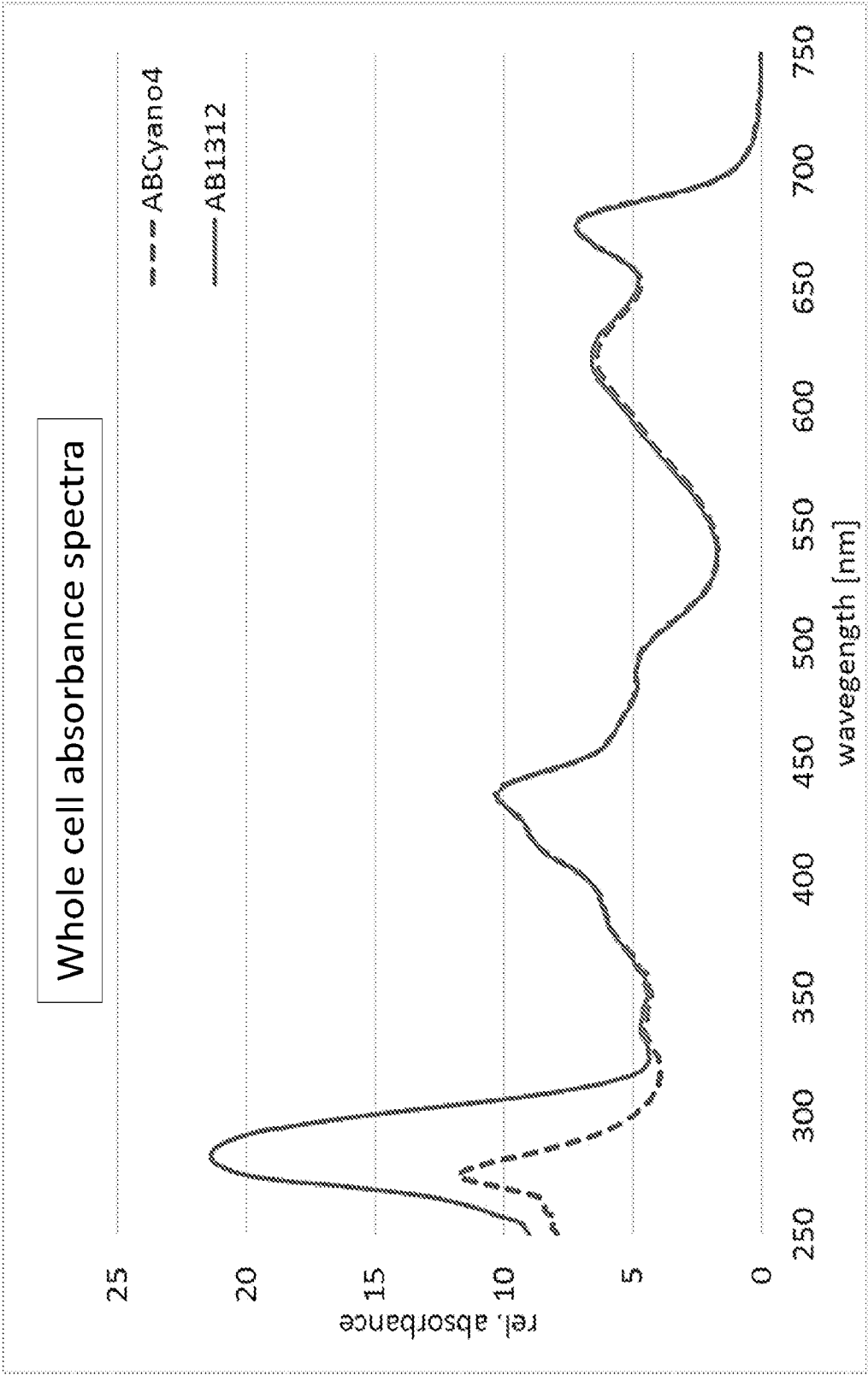


FIG. 26B

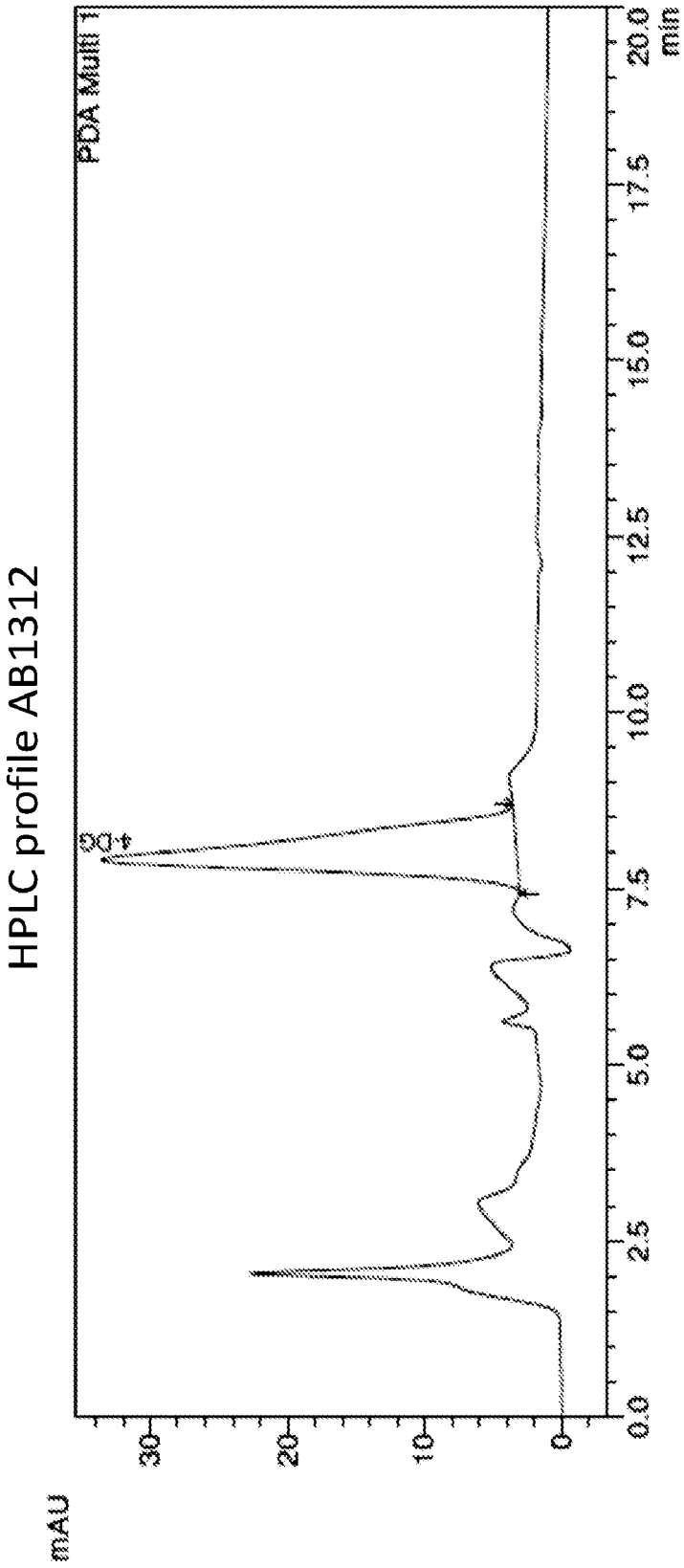


FIG. 26C

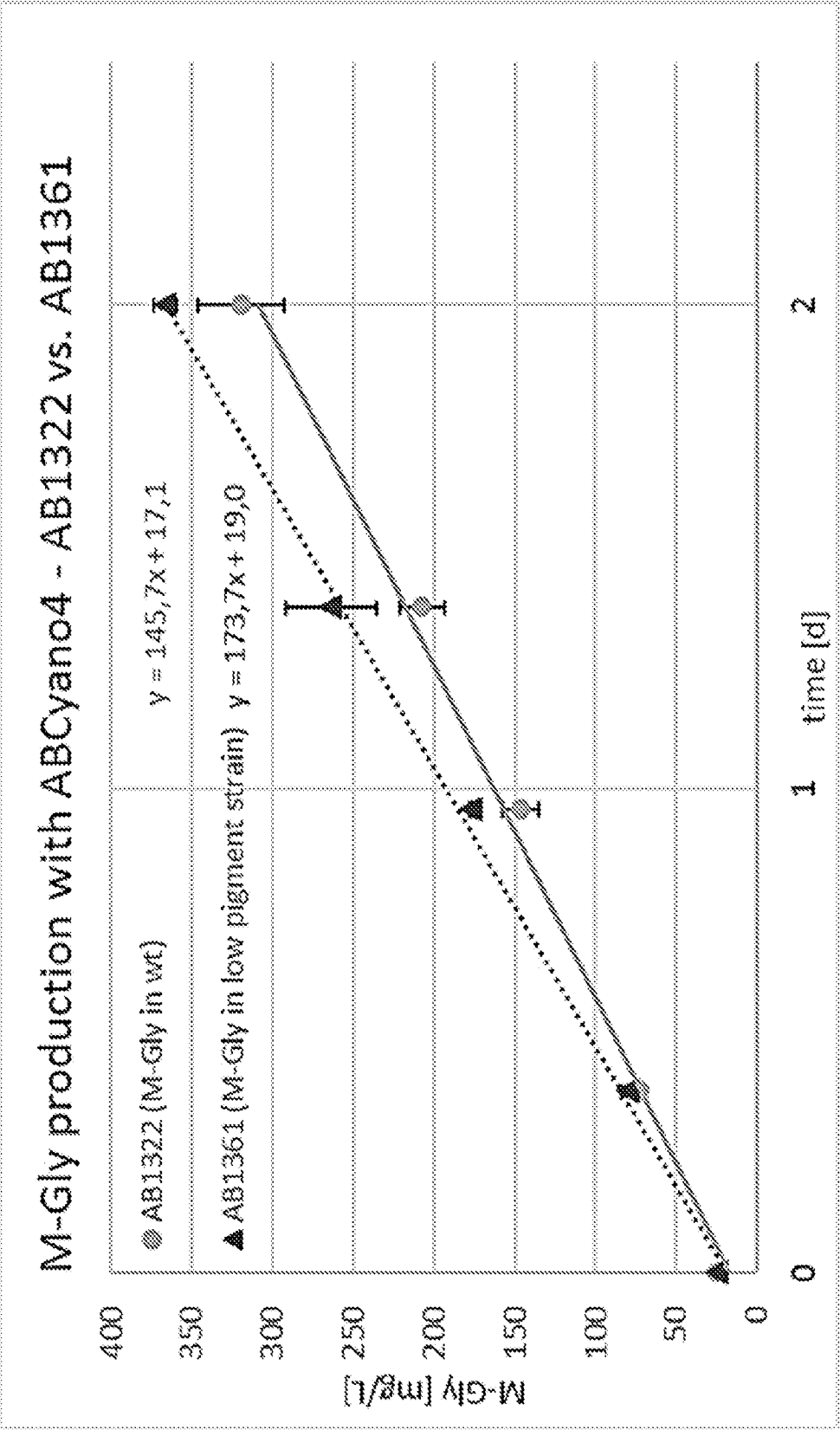


FIG. 27A

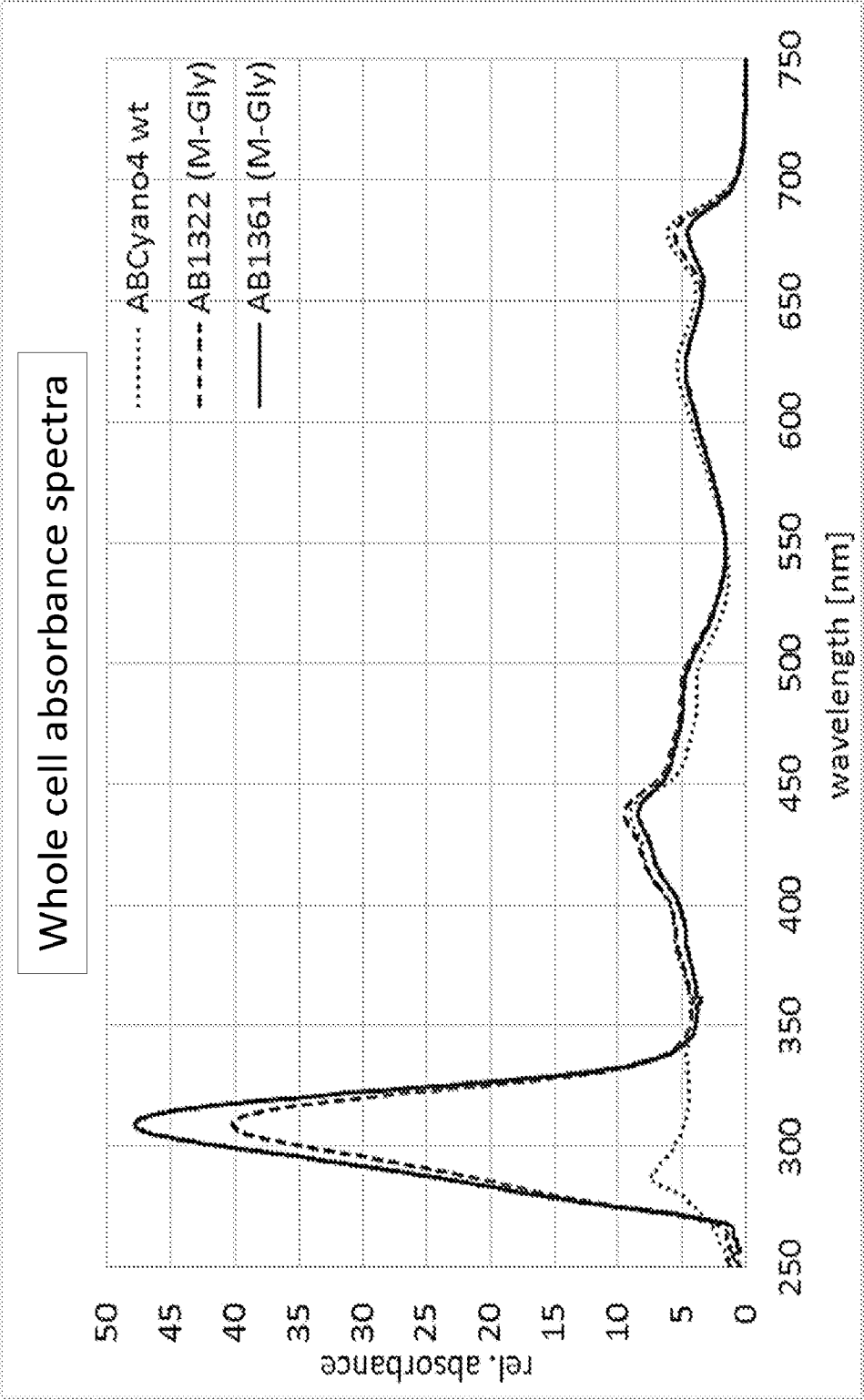


FIG. 27B

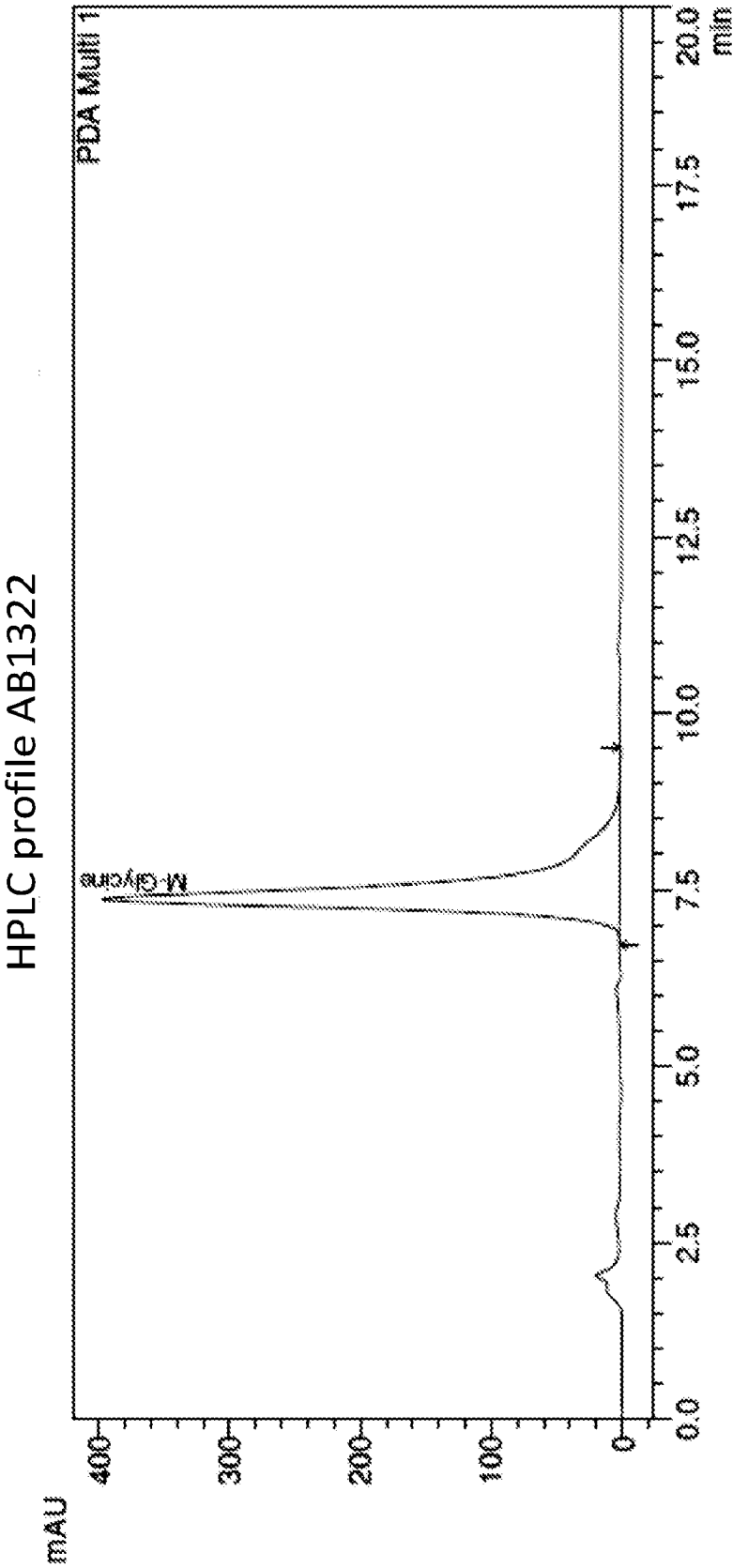


FIG. 27C

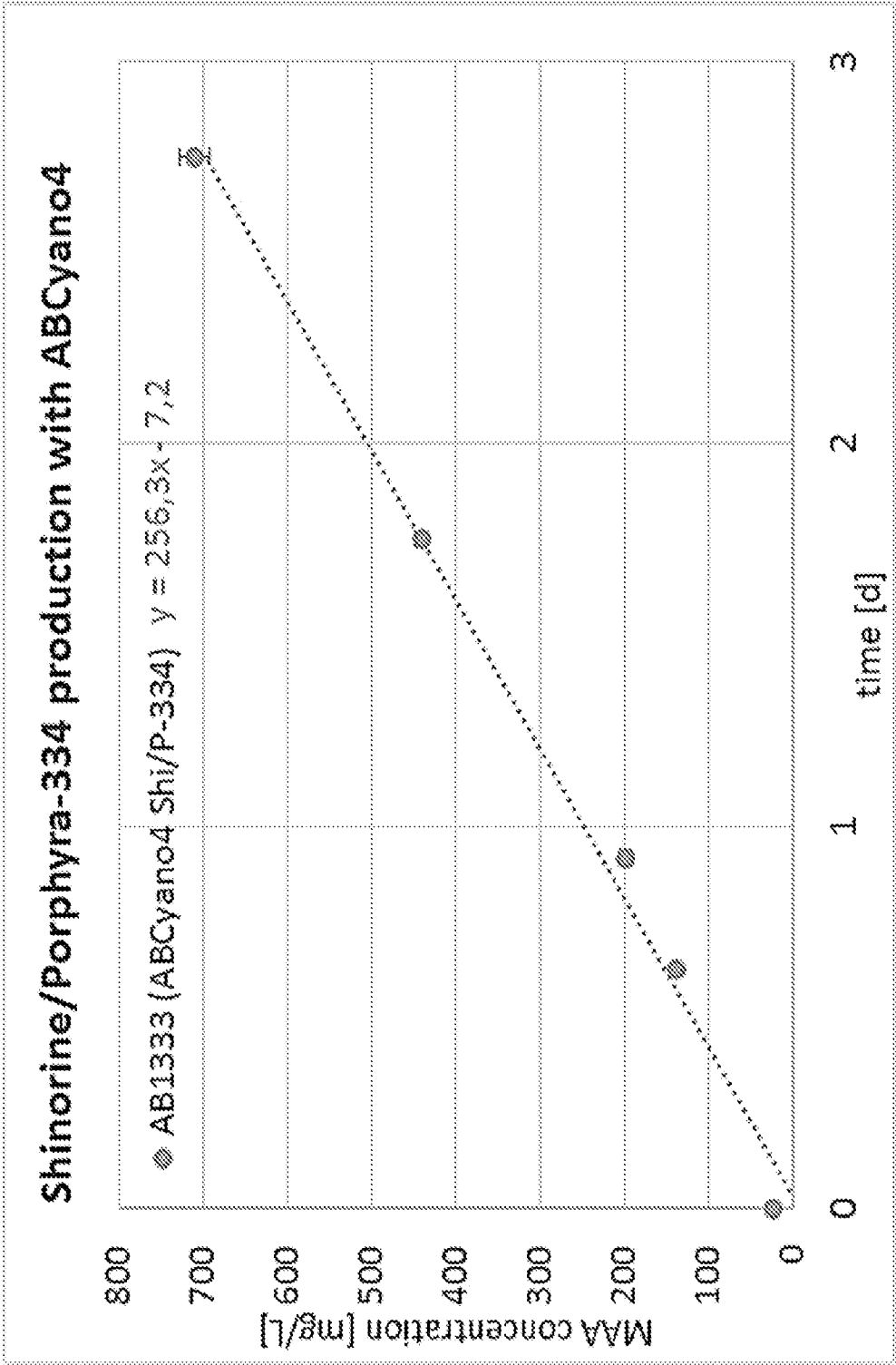


FIG. 28A

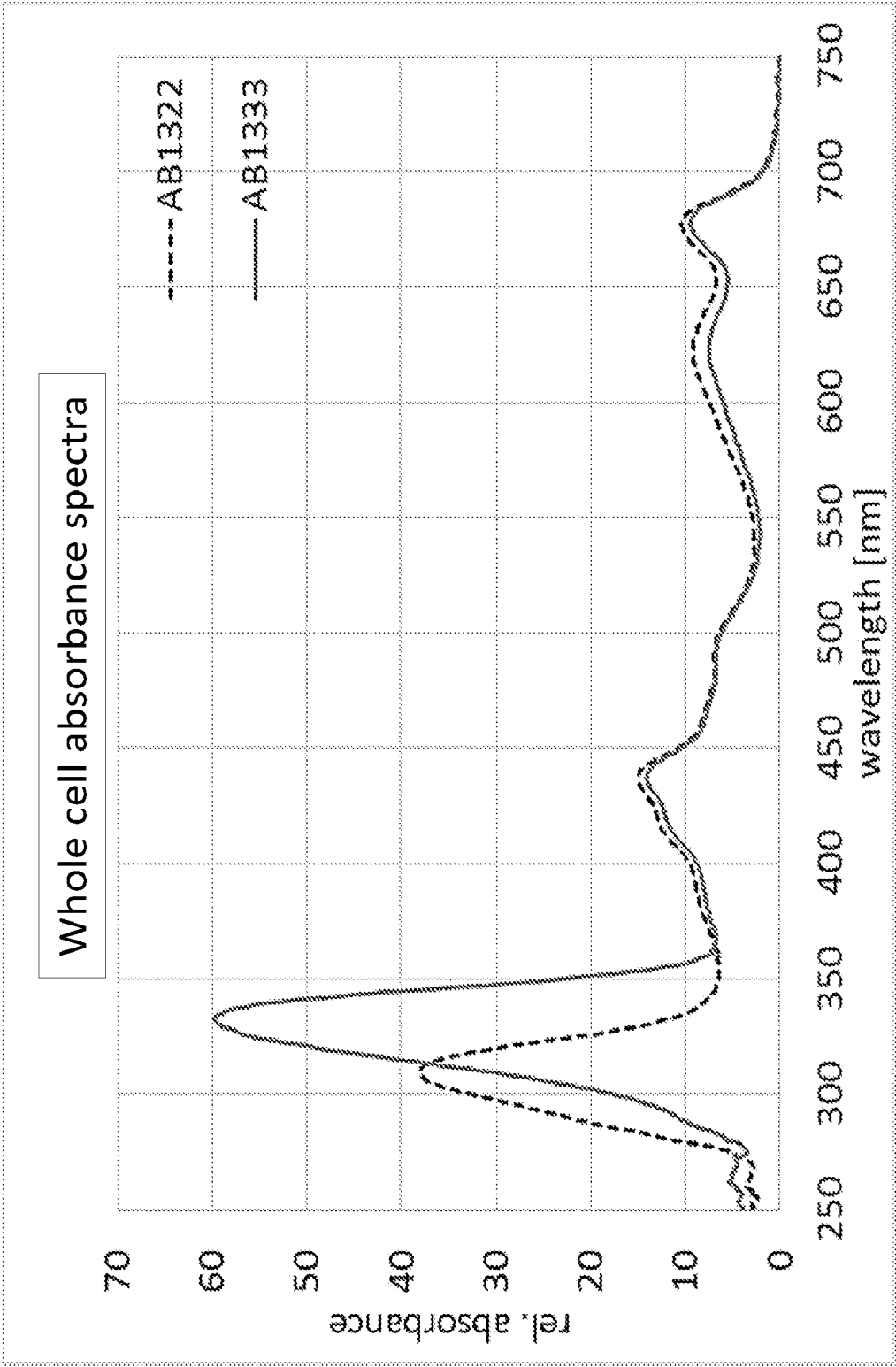


FIG. 28B

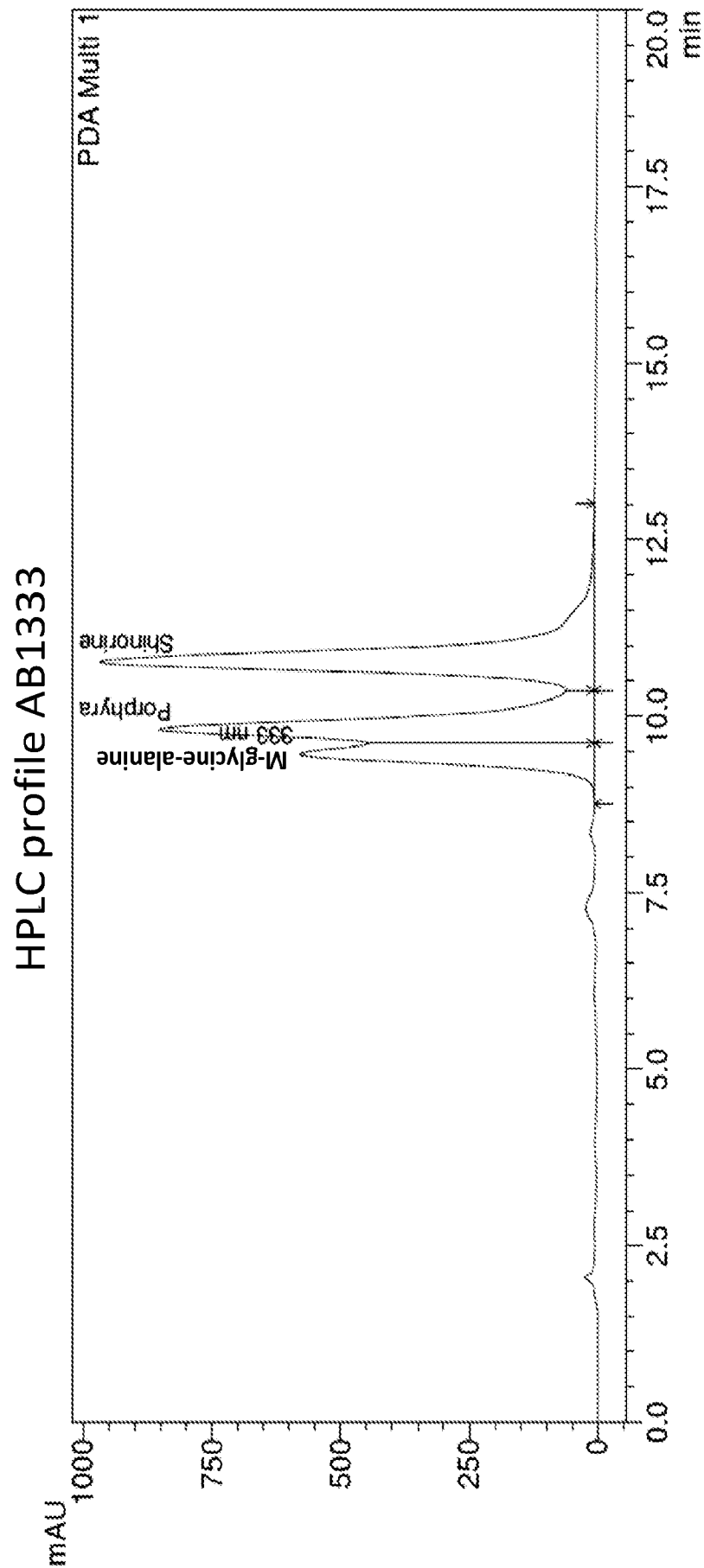


FIG. 28C

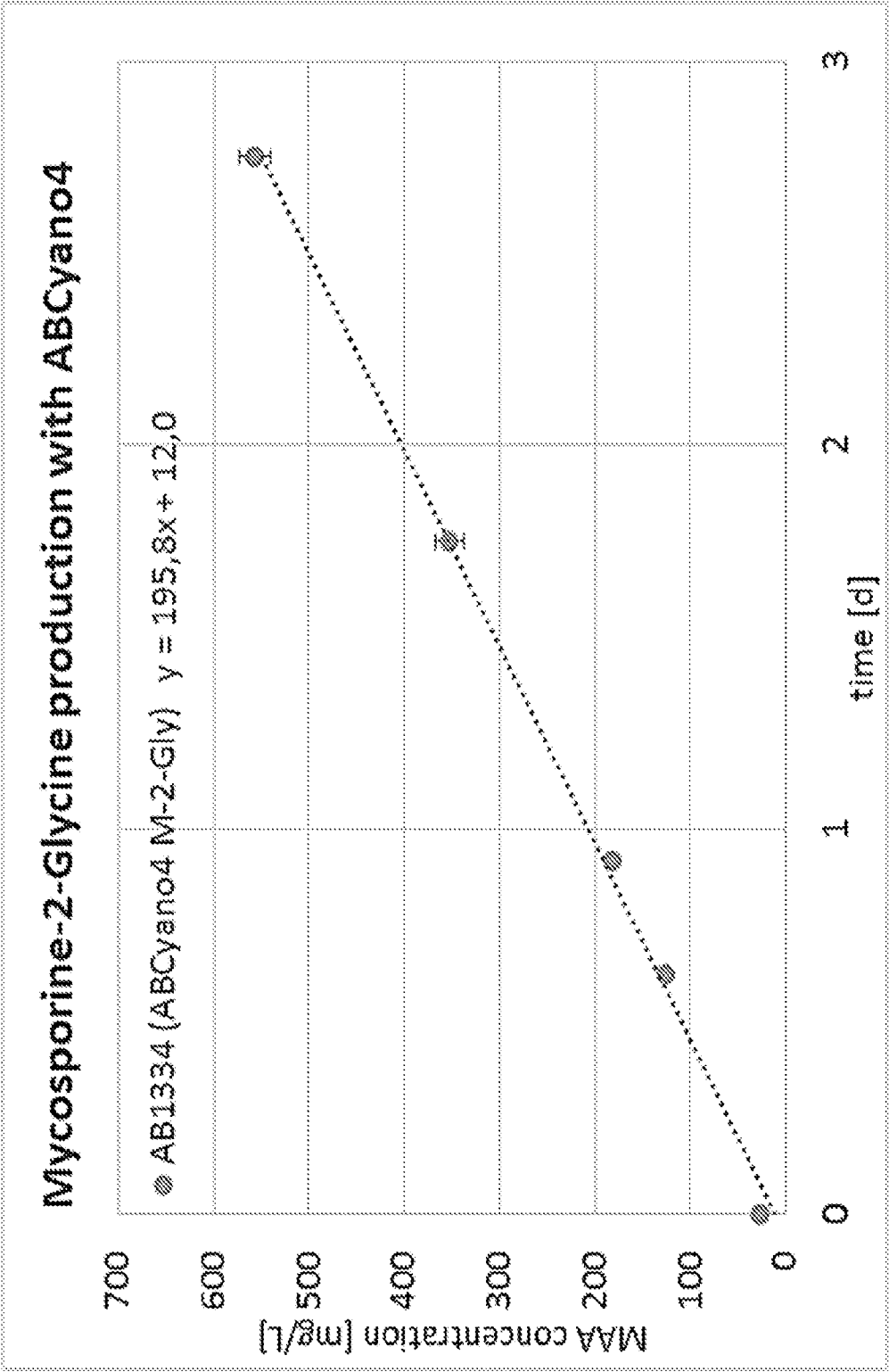


FIG. 29A

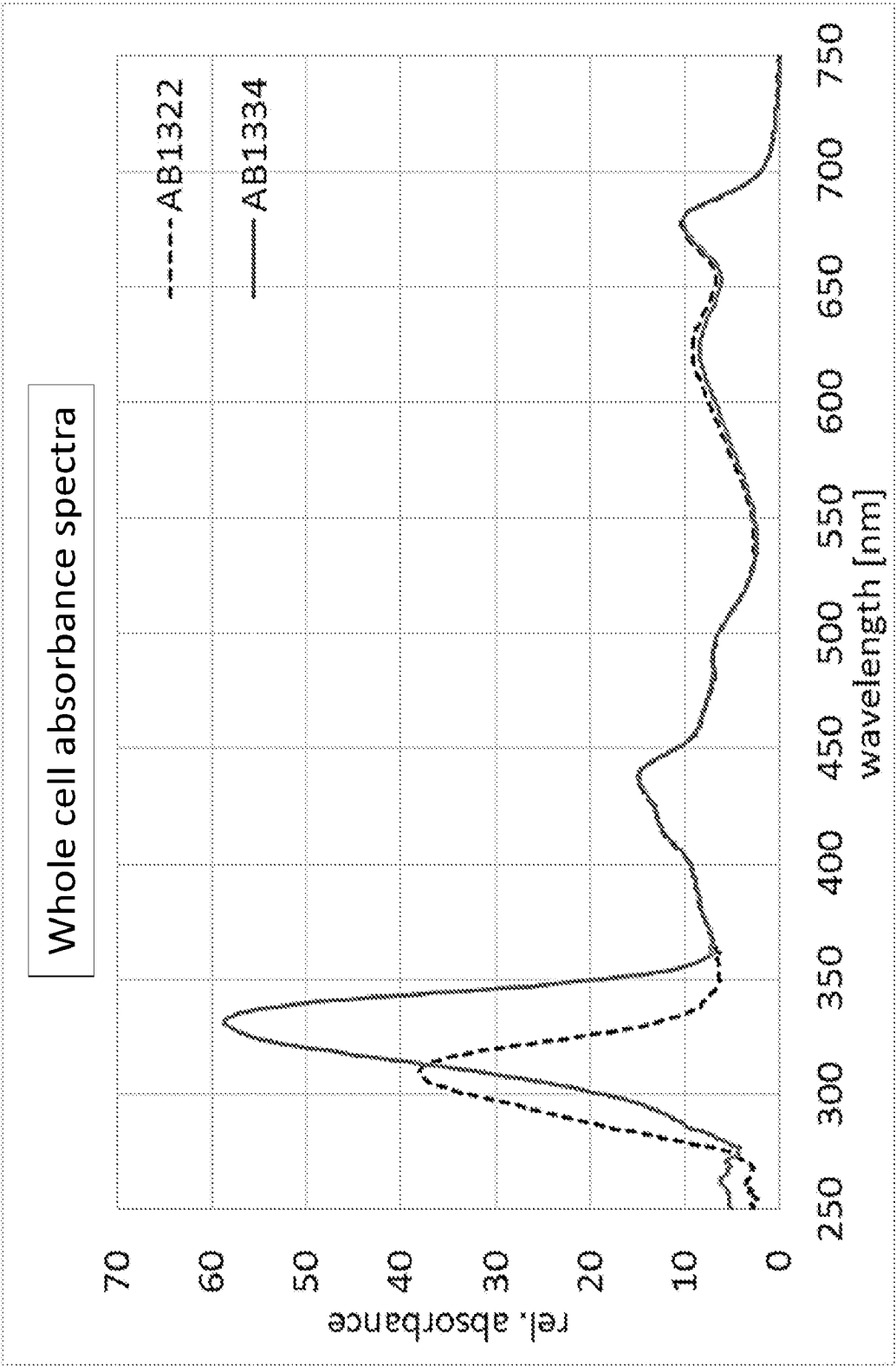


FIG. 29B

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HPLC profile AB1334

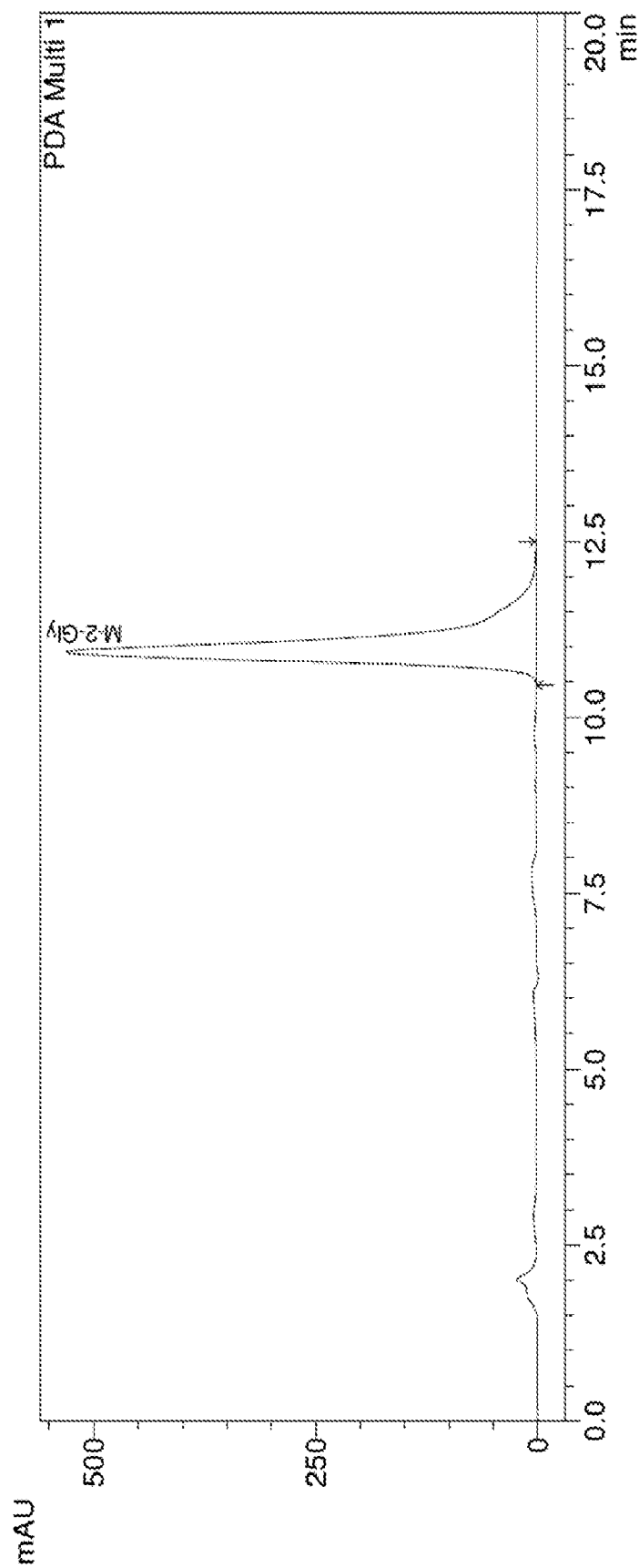


FIG. 29C

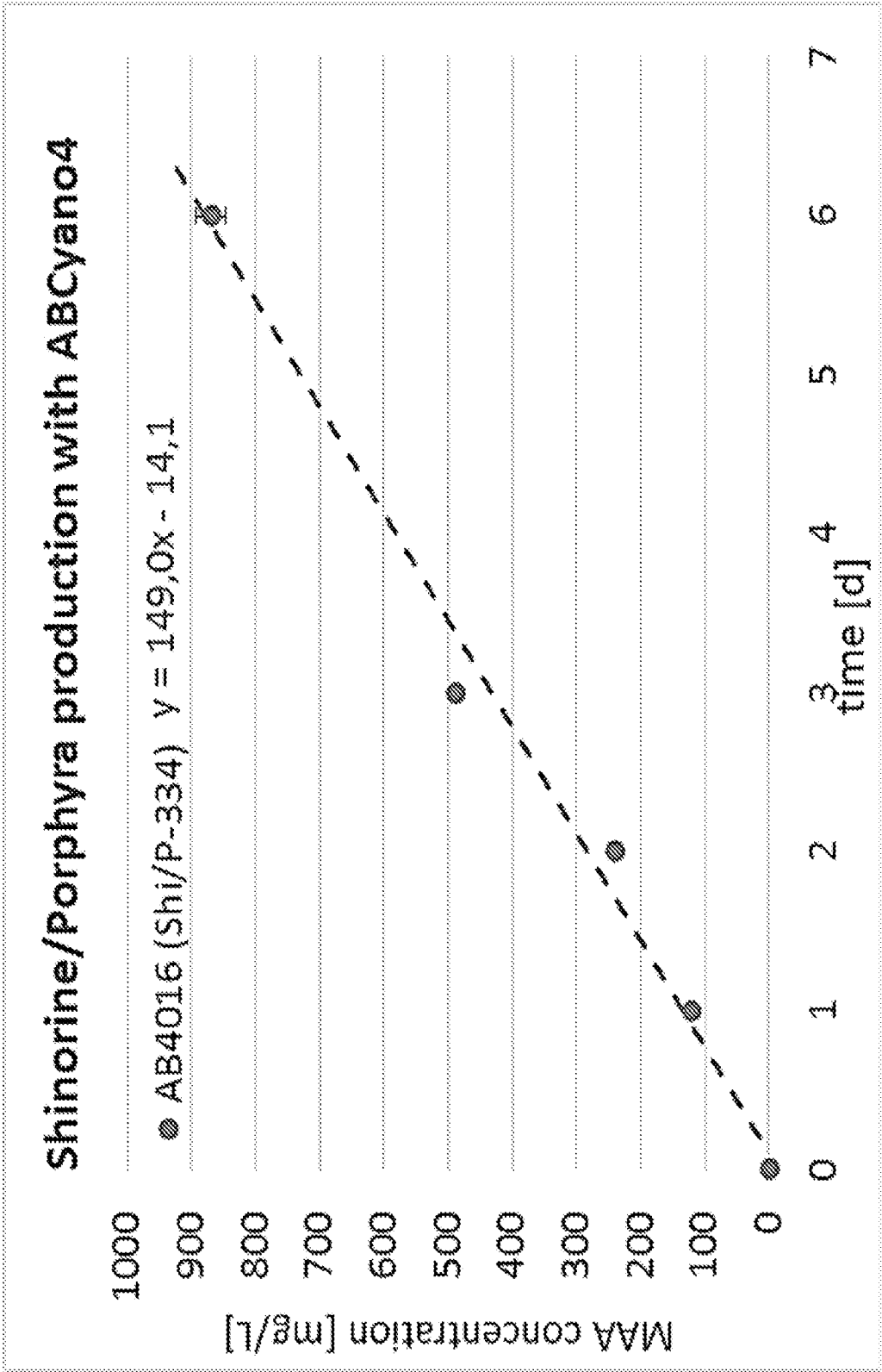


FIG. 30A

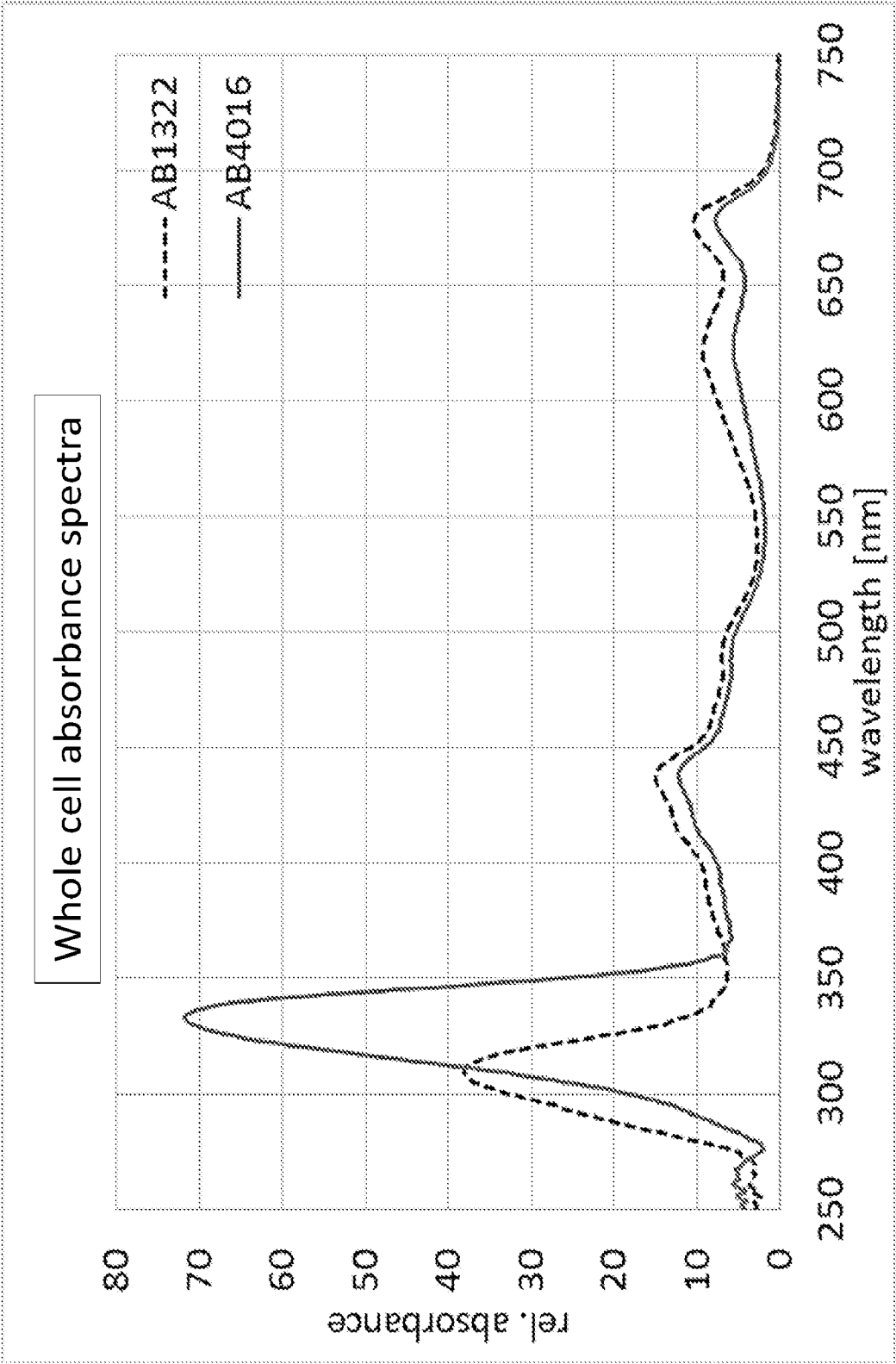


FIG. 30B

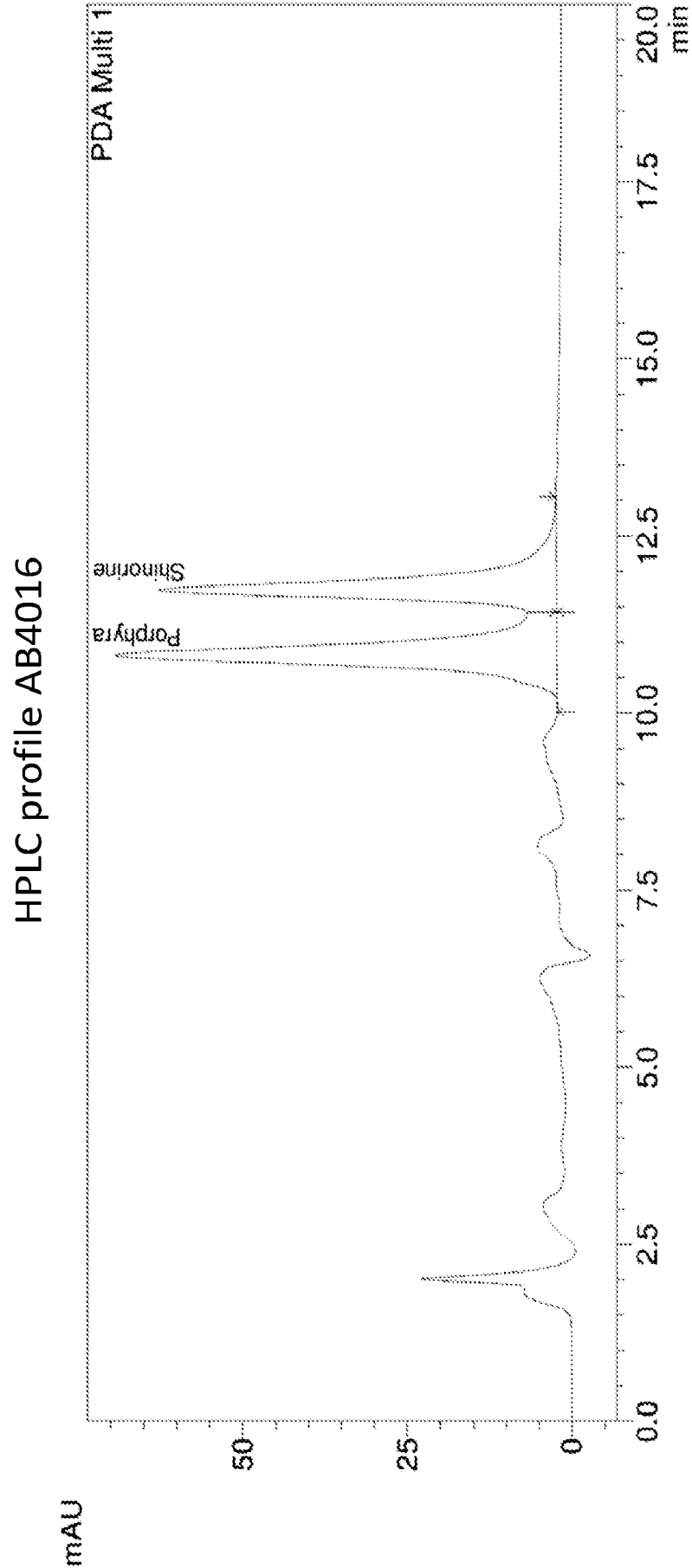


FIG. 30C

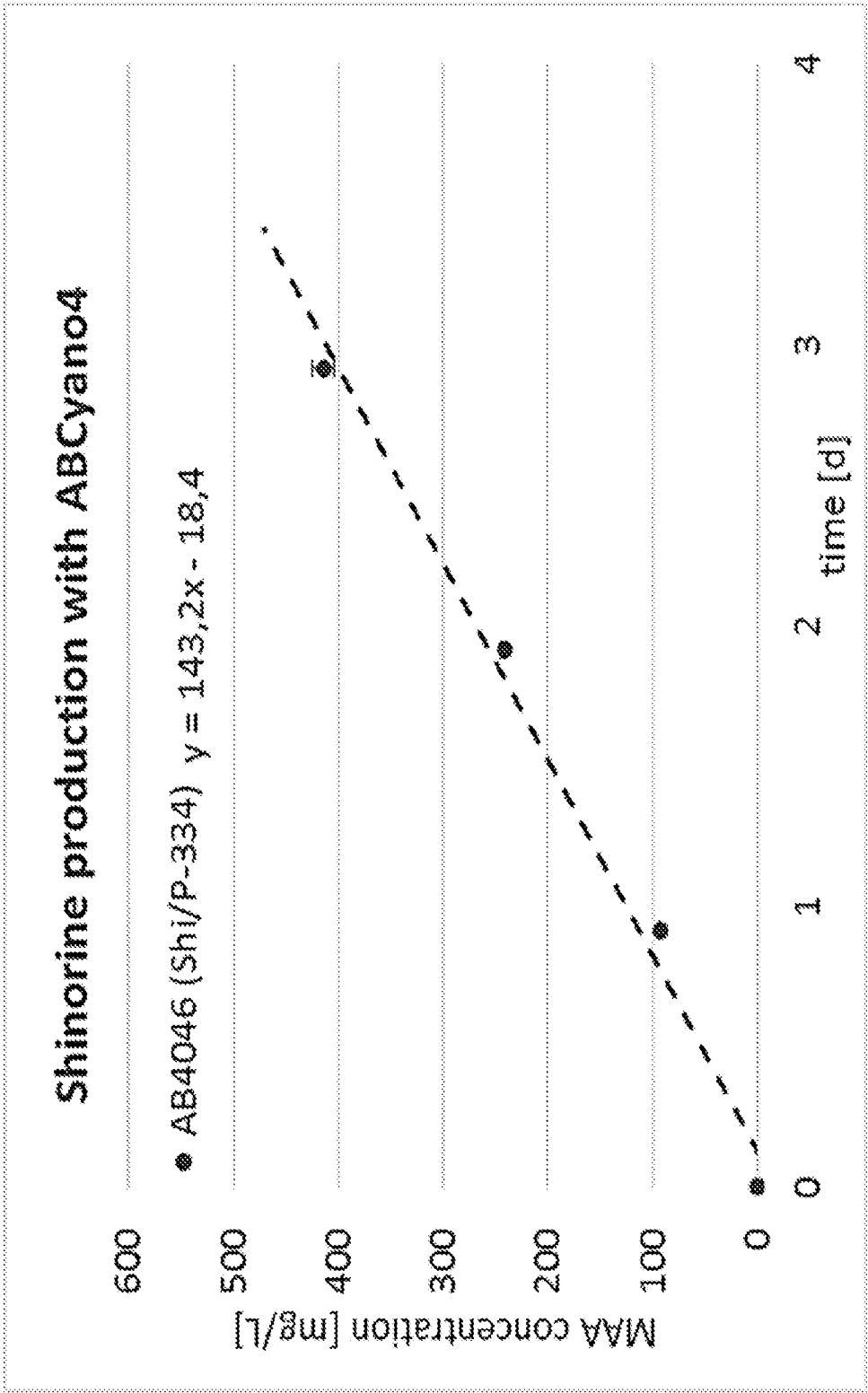


FIG. 31A

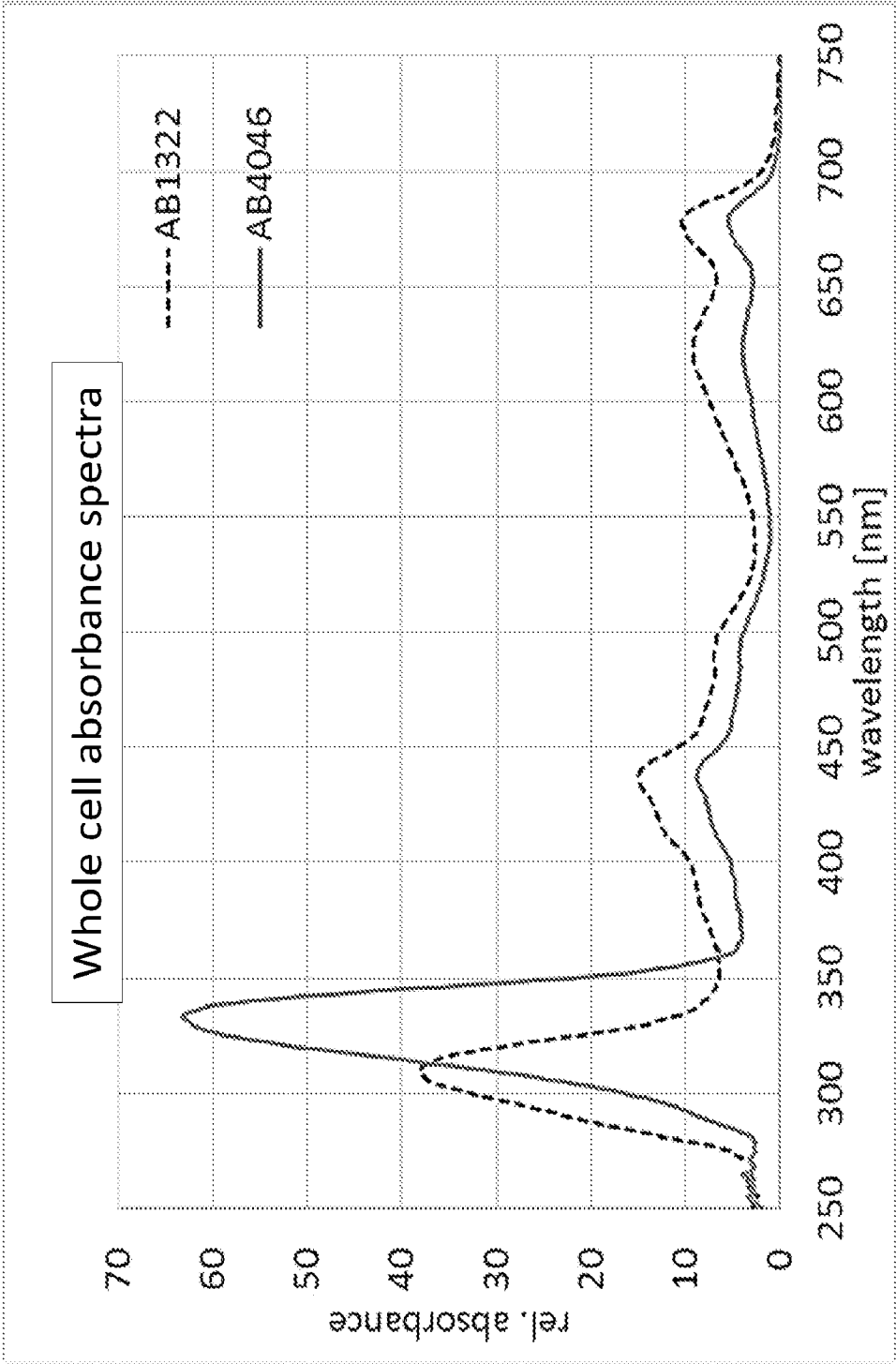


FIG. 31B

HPLC profile AB4046

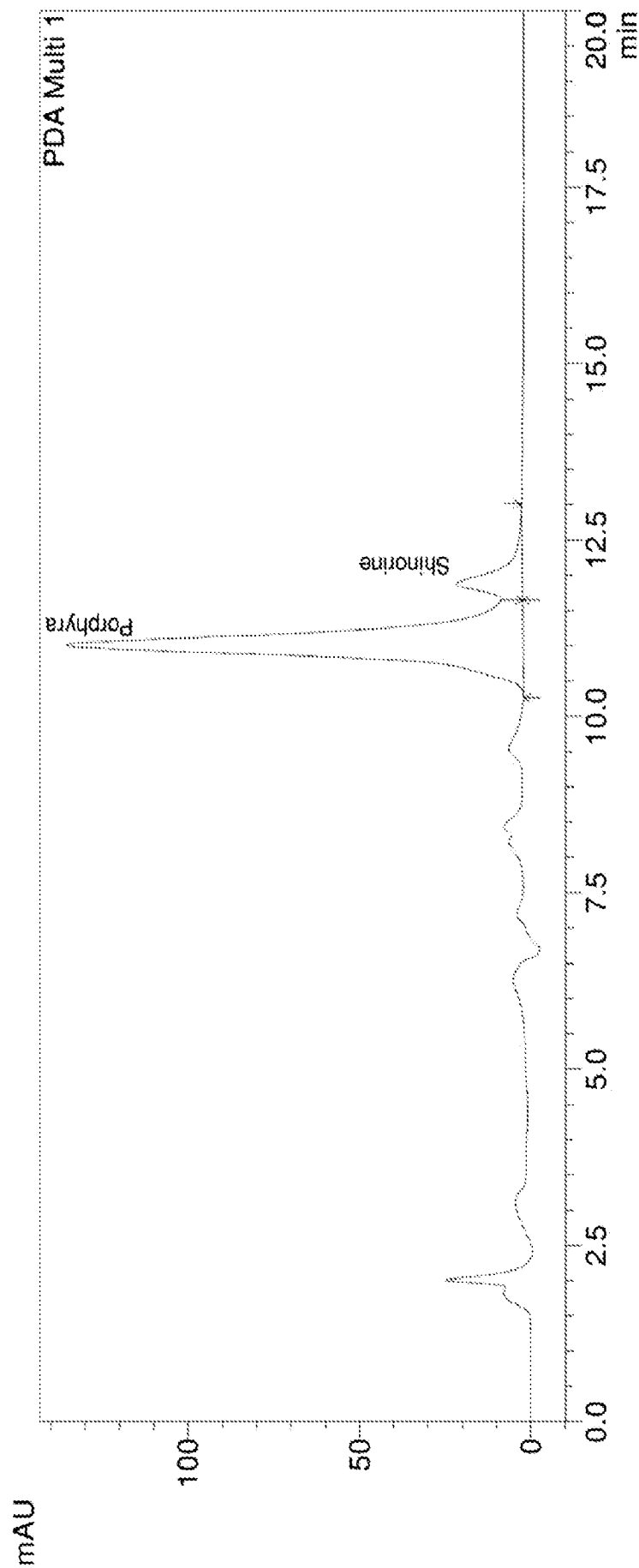


FIG. 31C

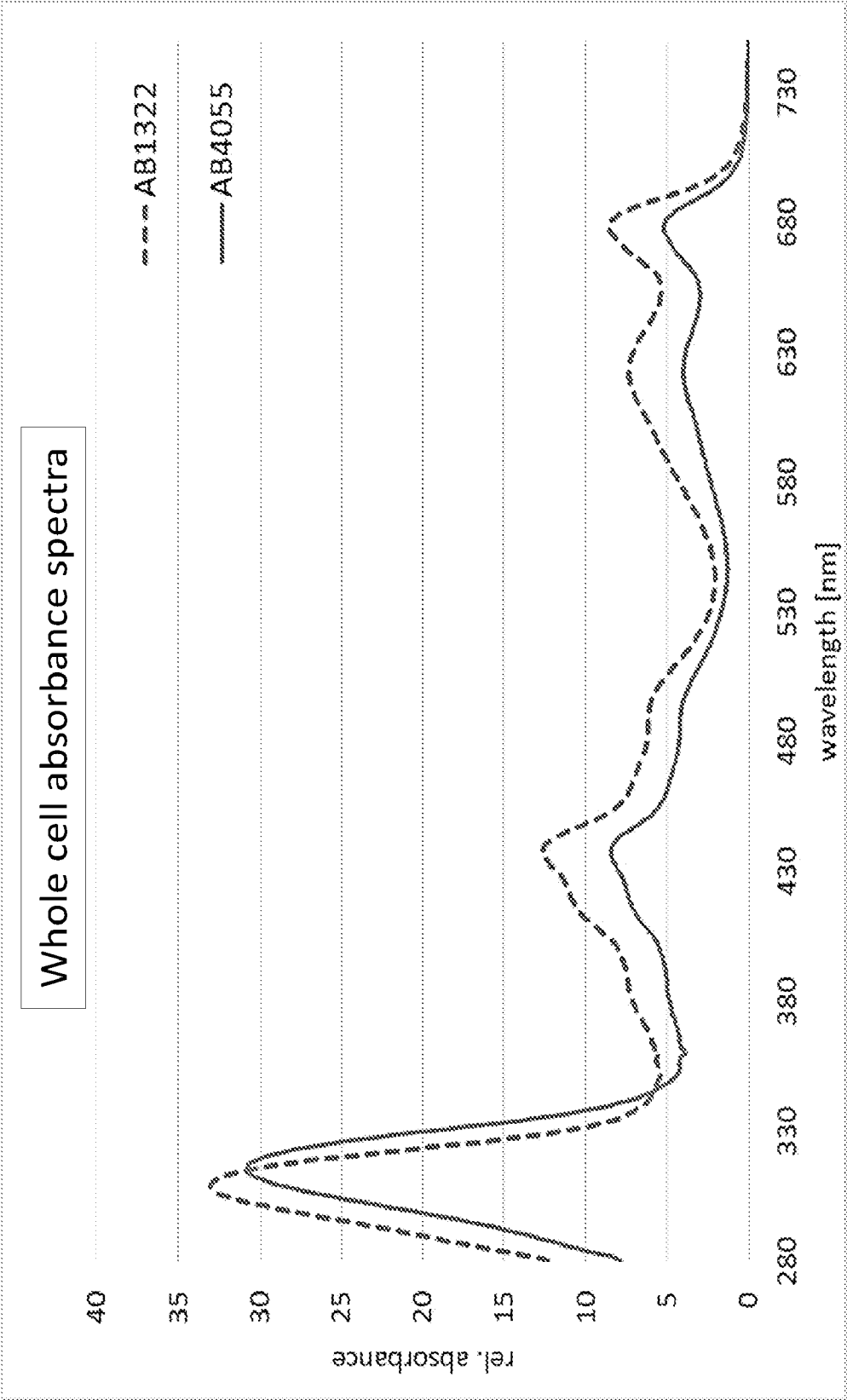


FIG. 32A

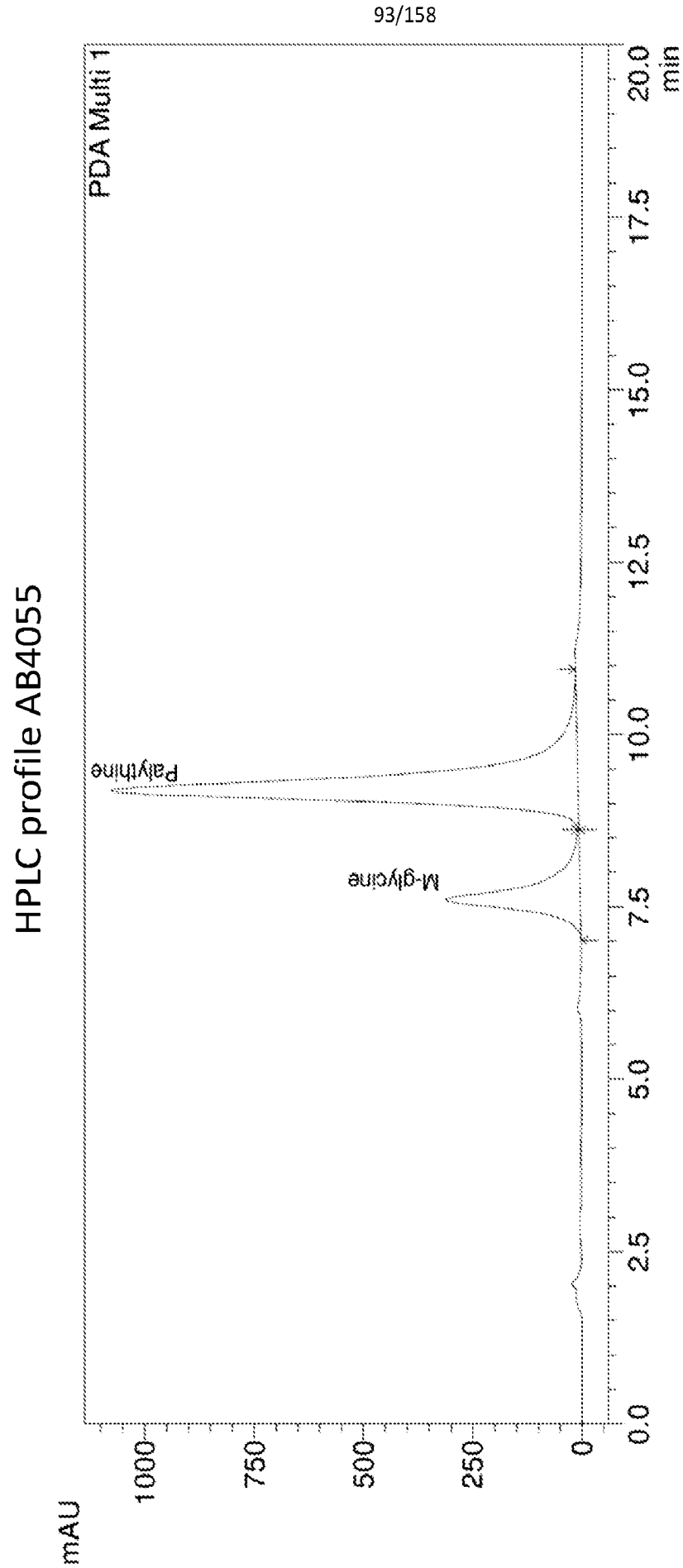


FIG. 32B

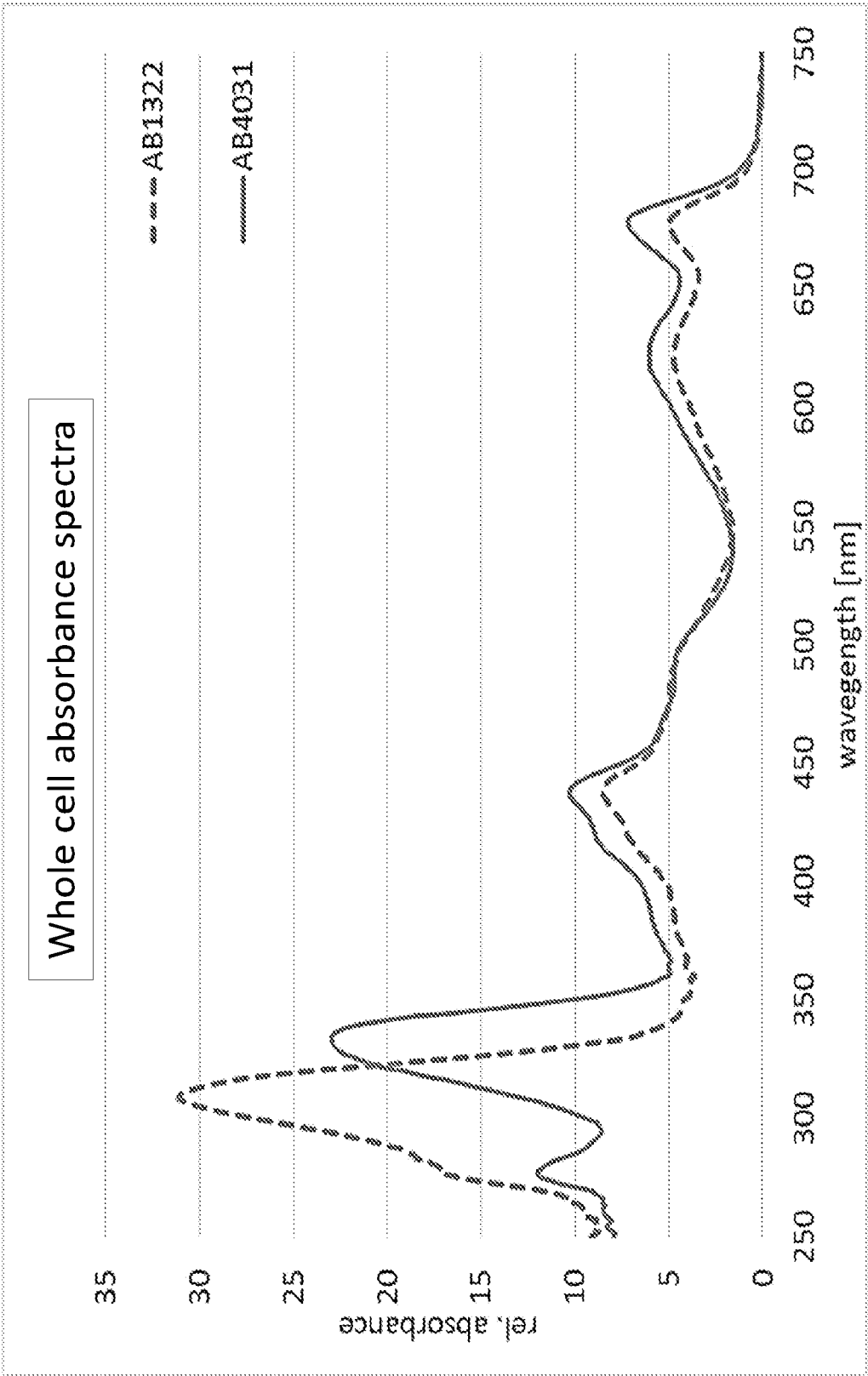


FIG. 33A

HPLC profile AB4031

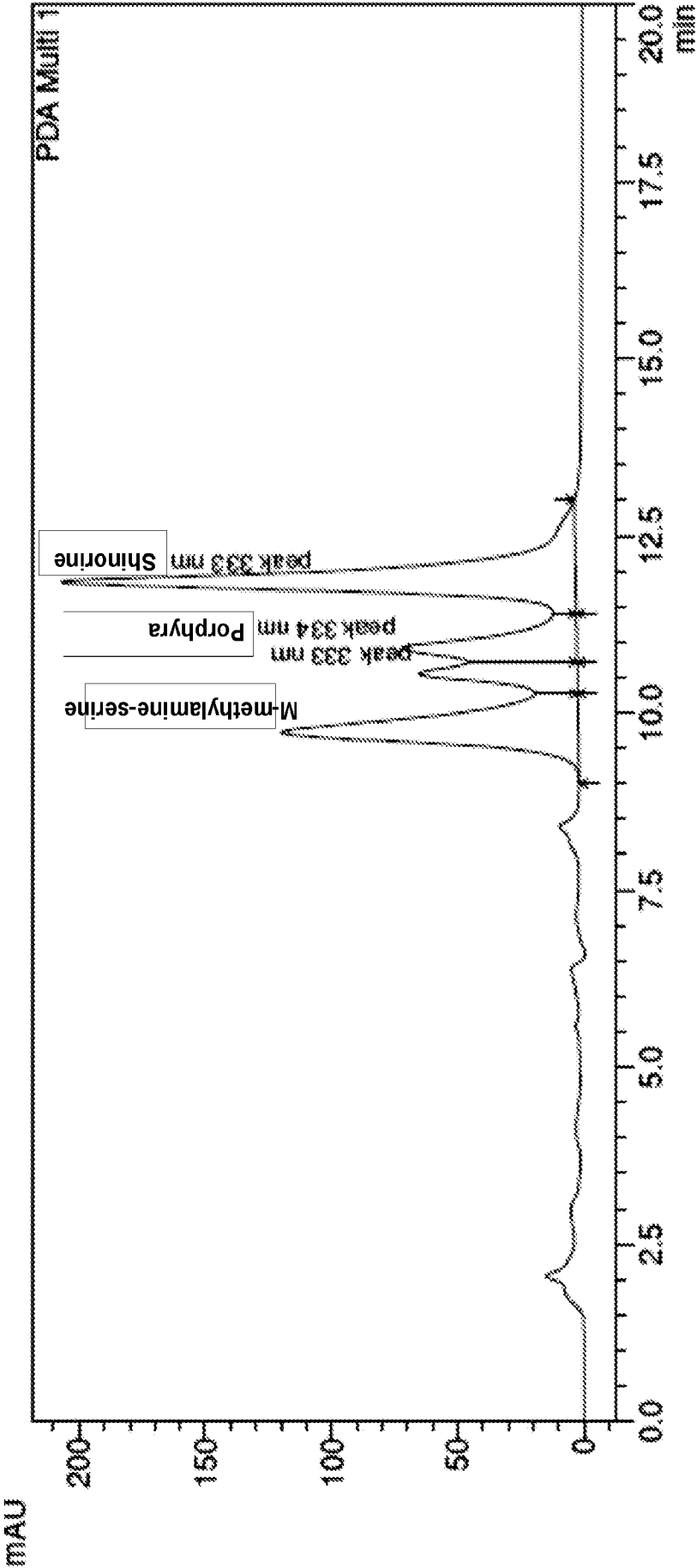


FIG. 33B

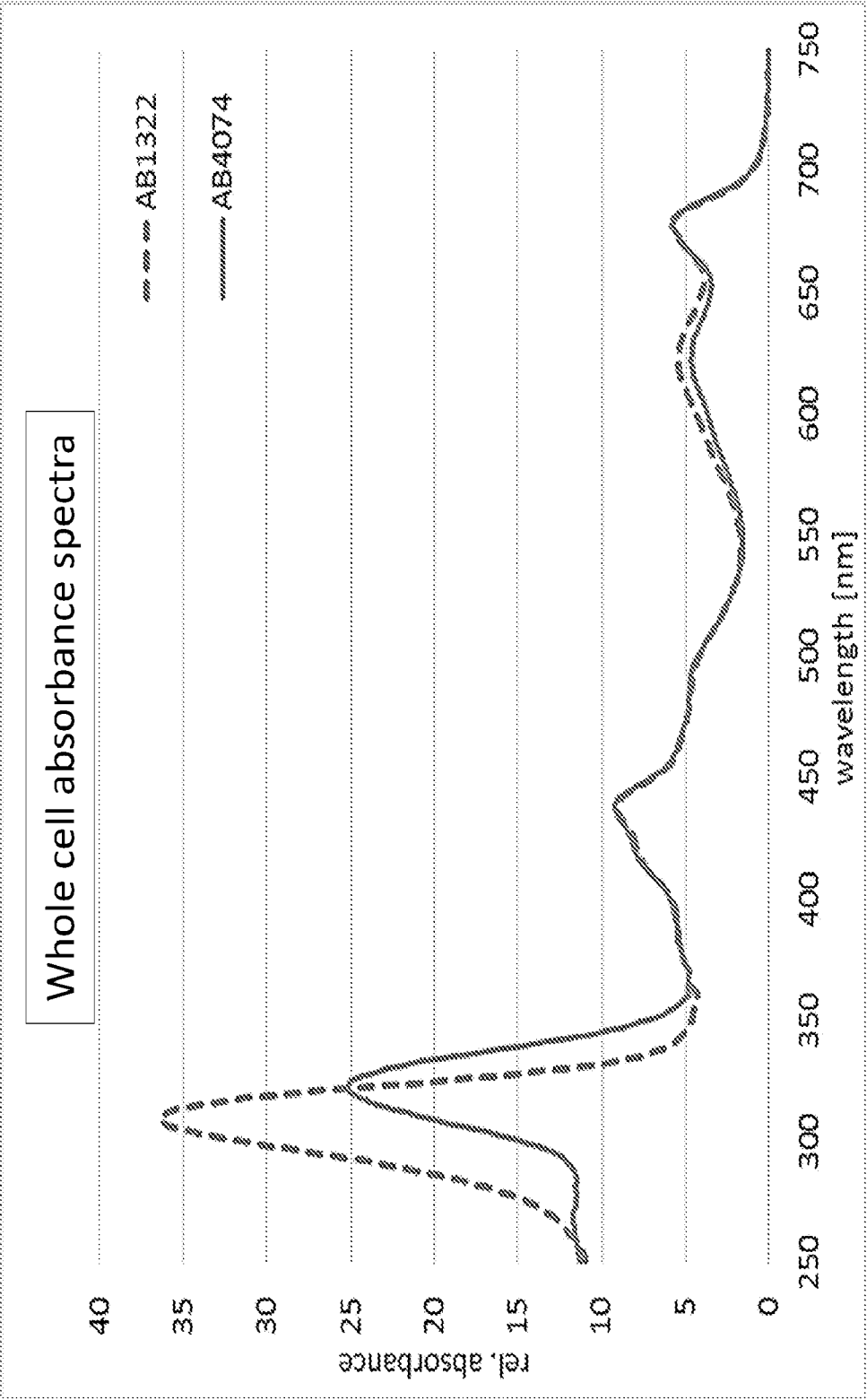


FIG. 34A

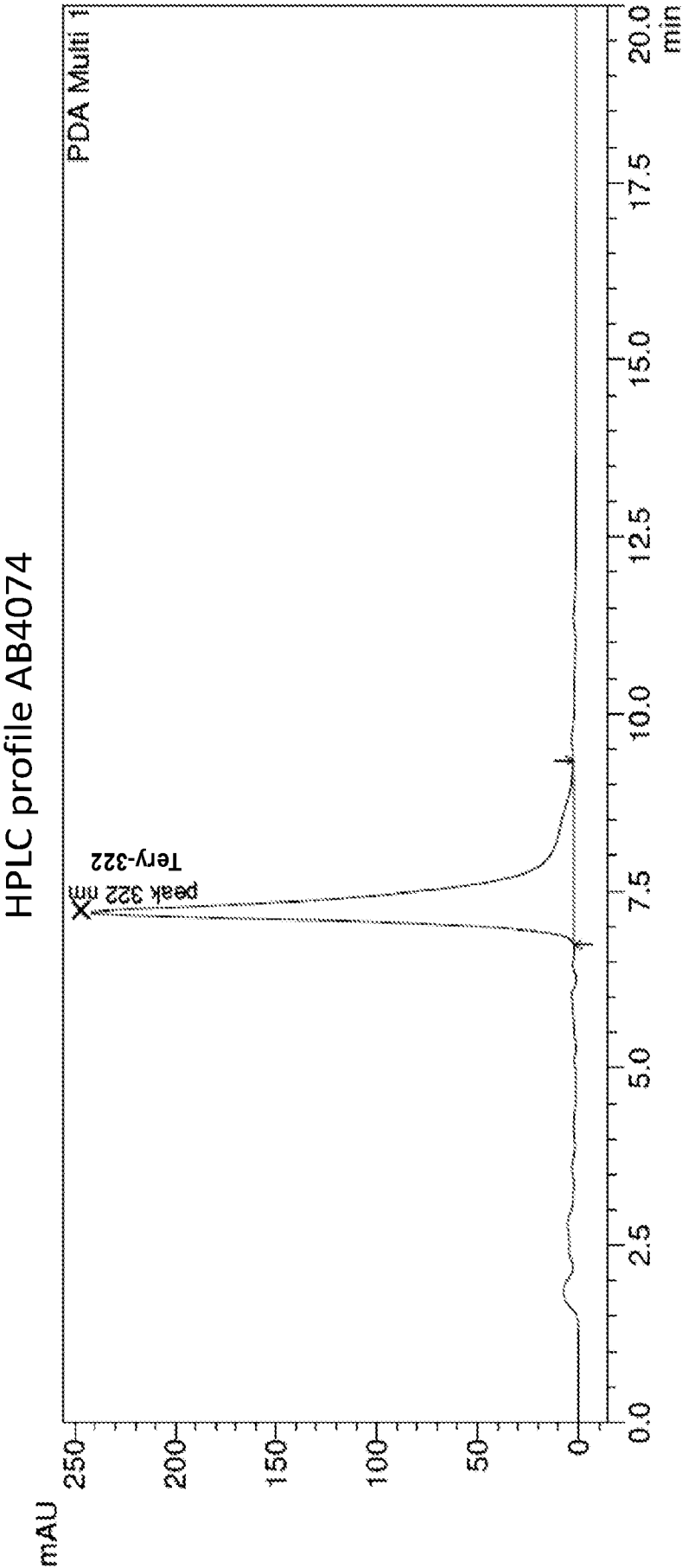


FIG. 34B

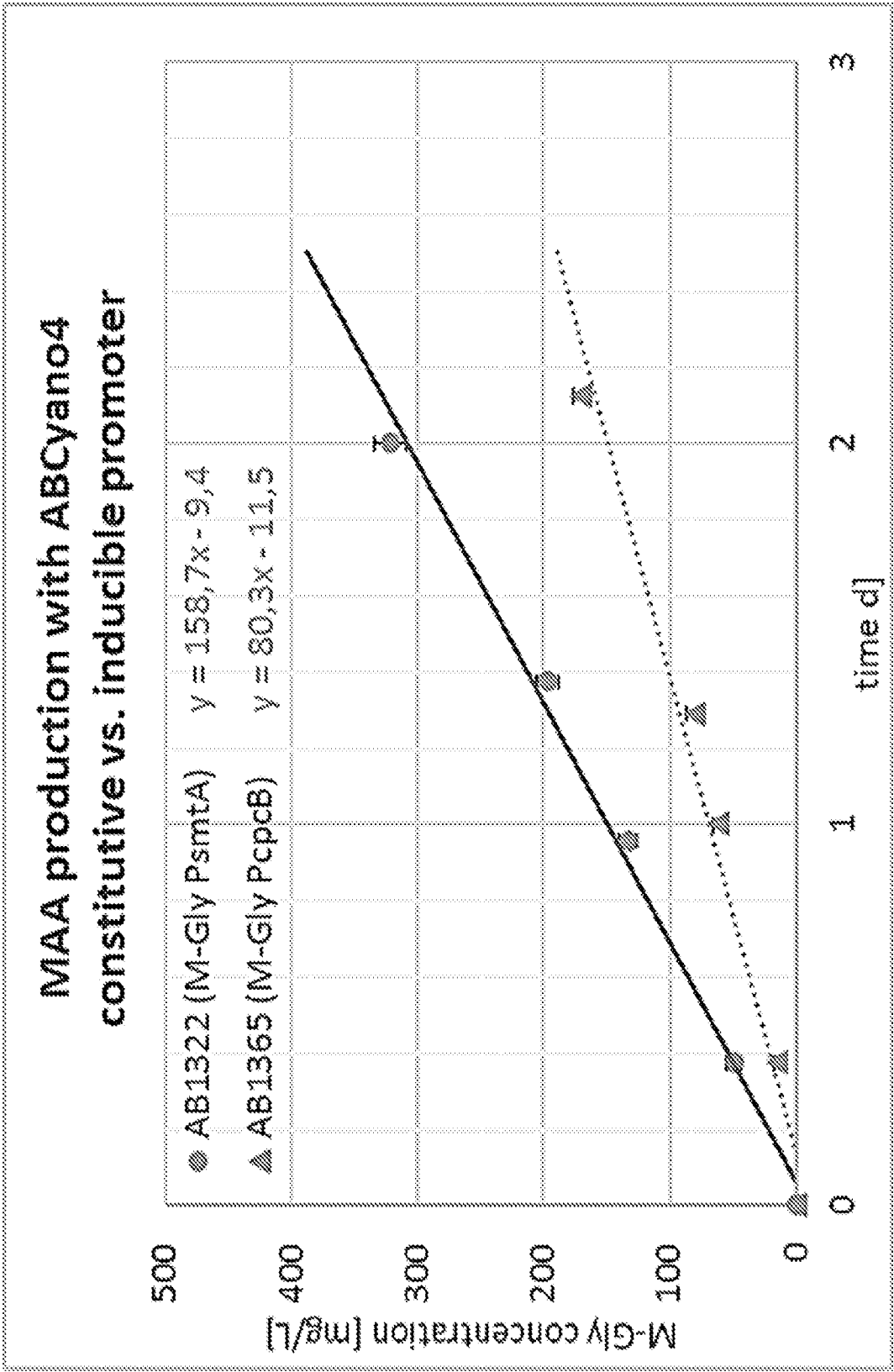


FIG. 35A

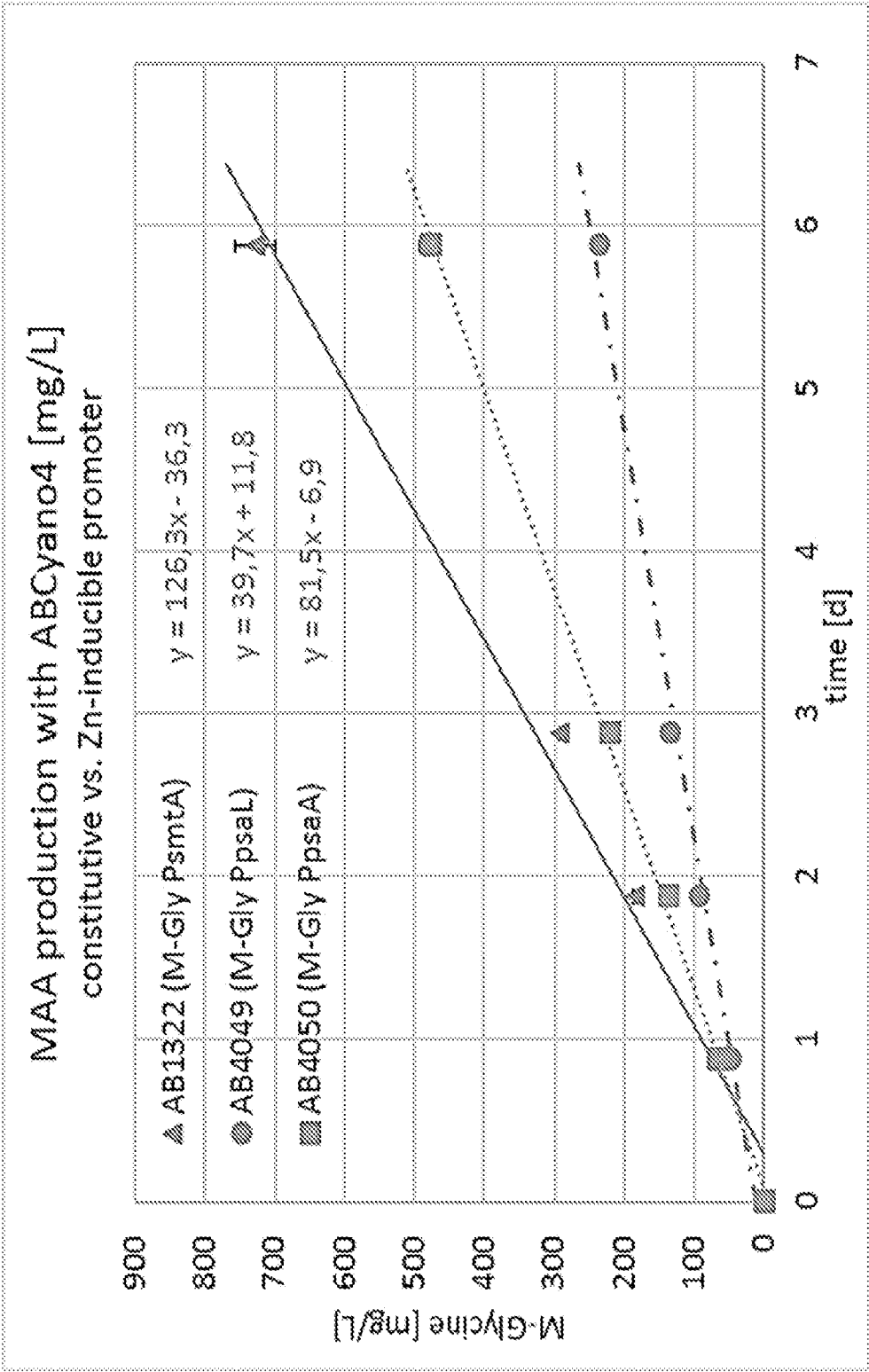


FIG. 35B

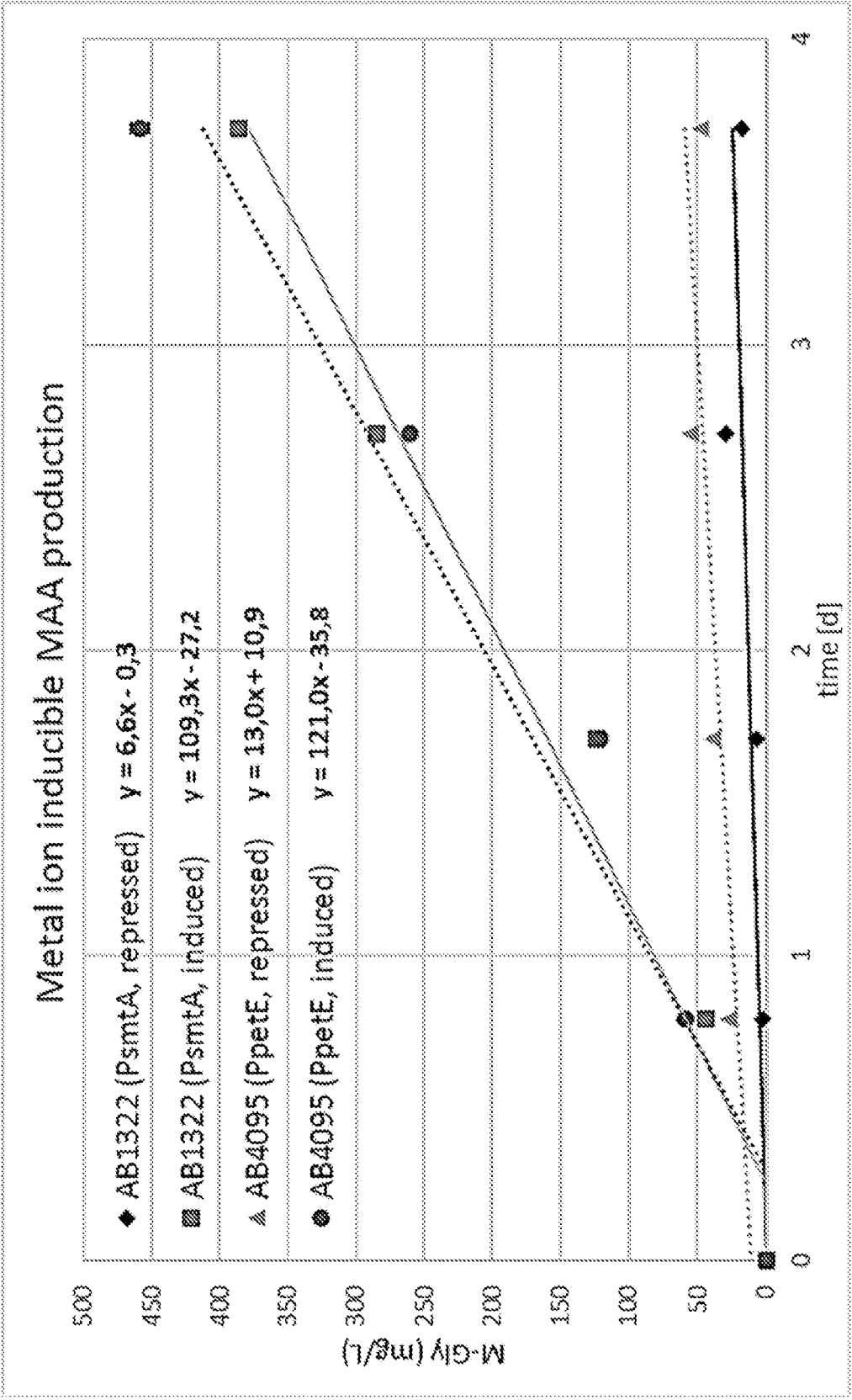


FIG. 35C

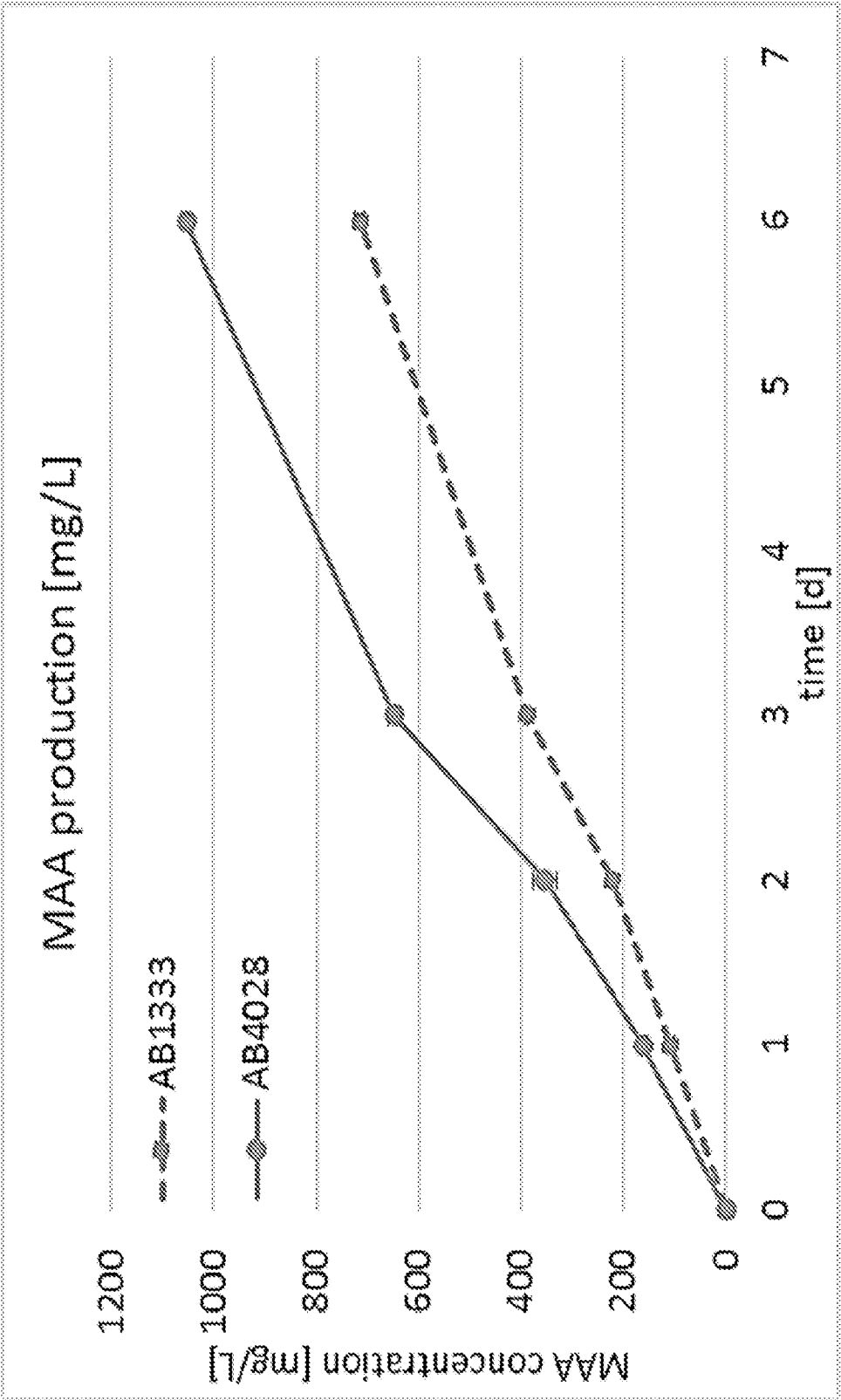


FIG. 36A

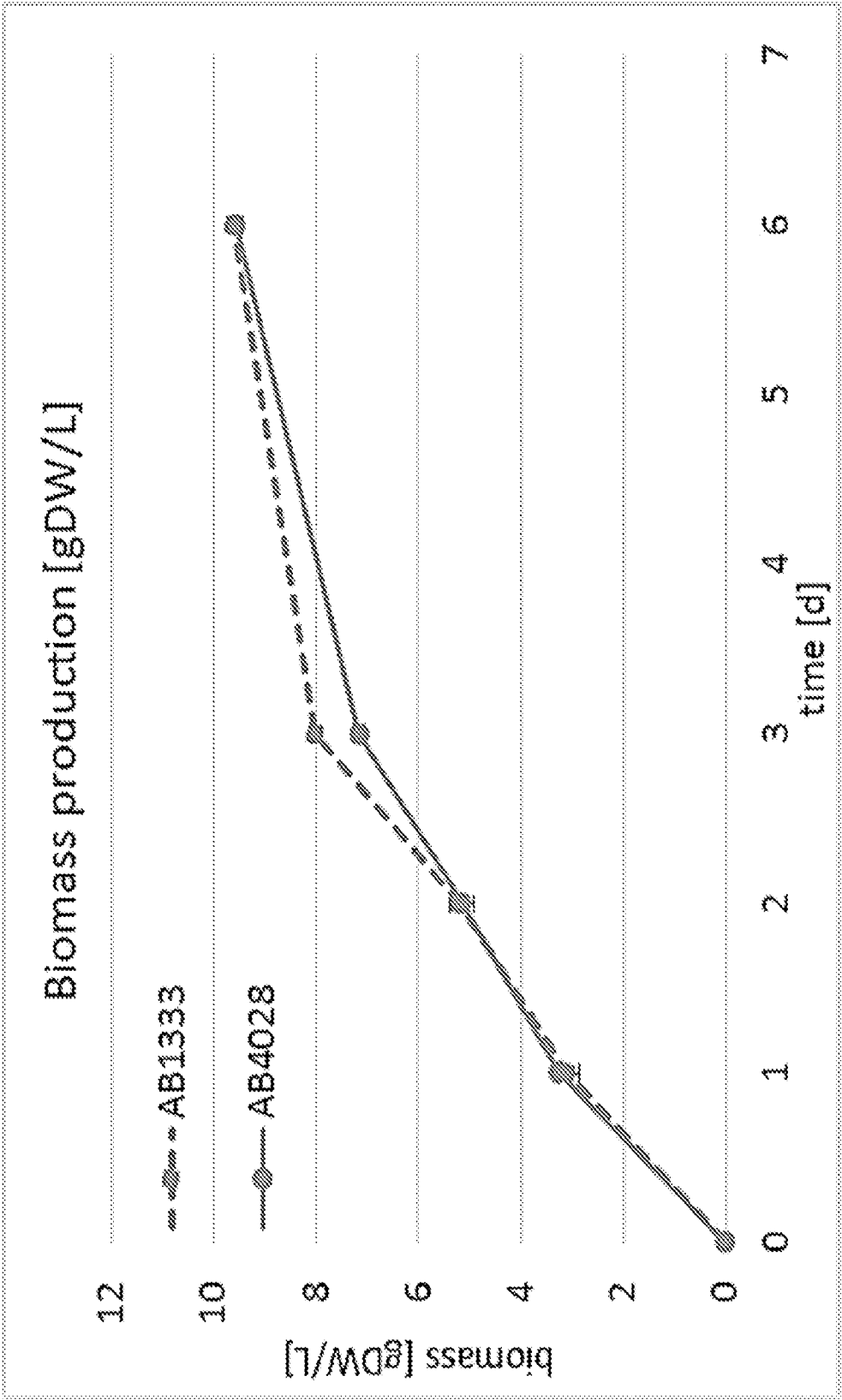


FIG. 36B

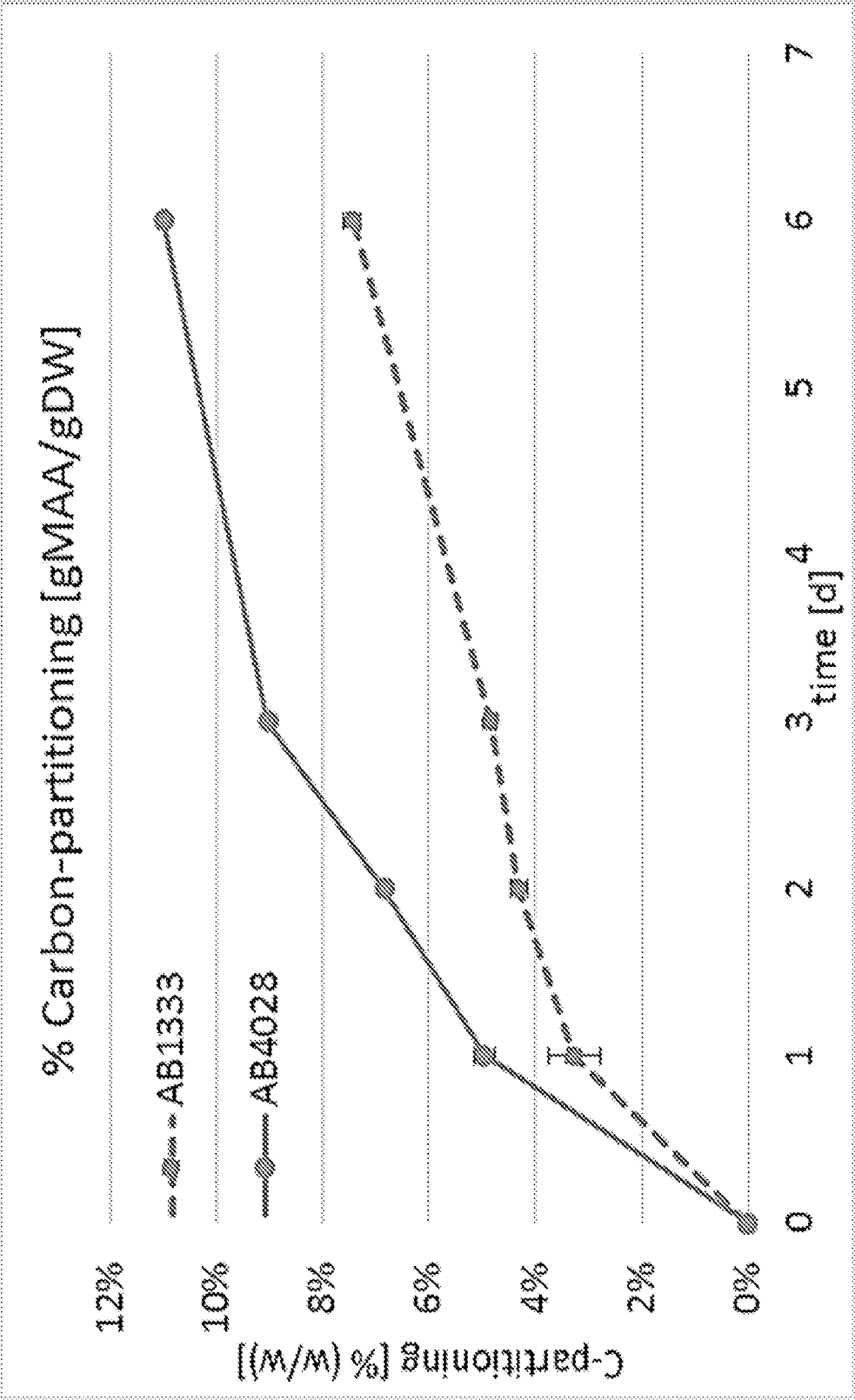


FIG. 36C

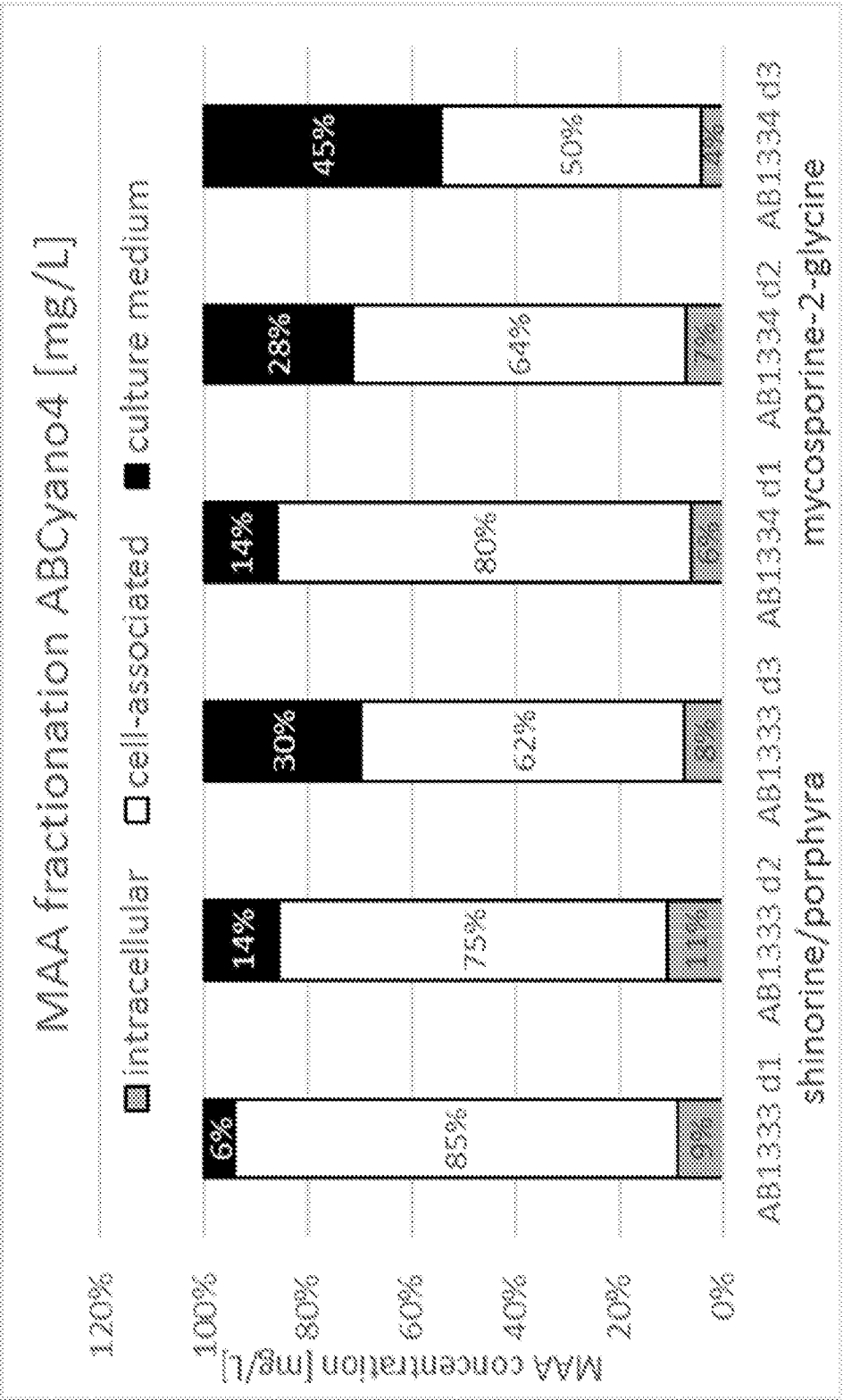


FIG. 37

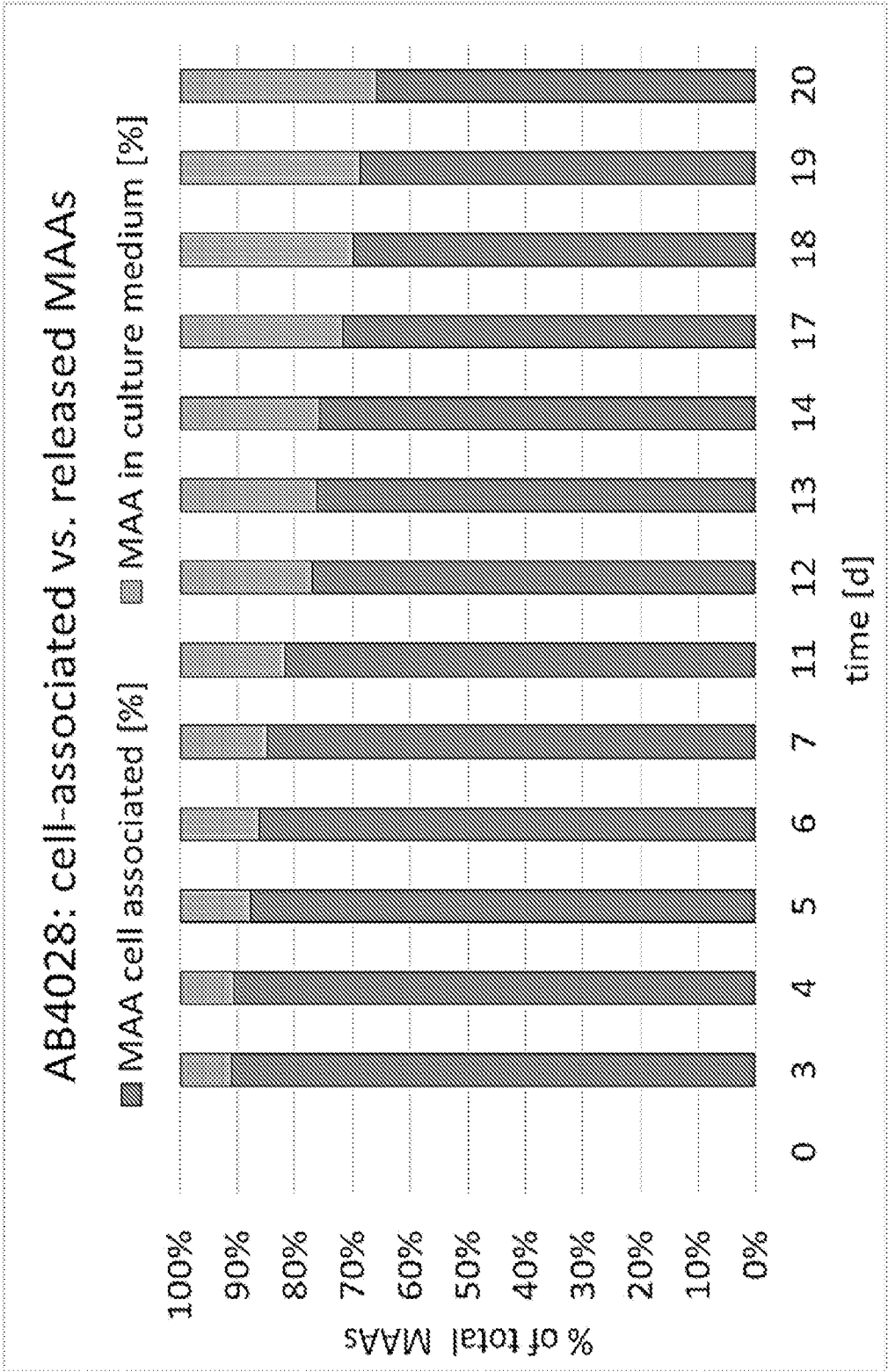
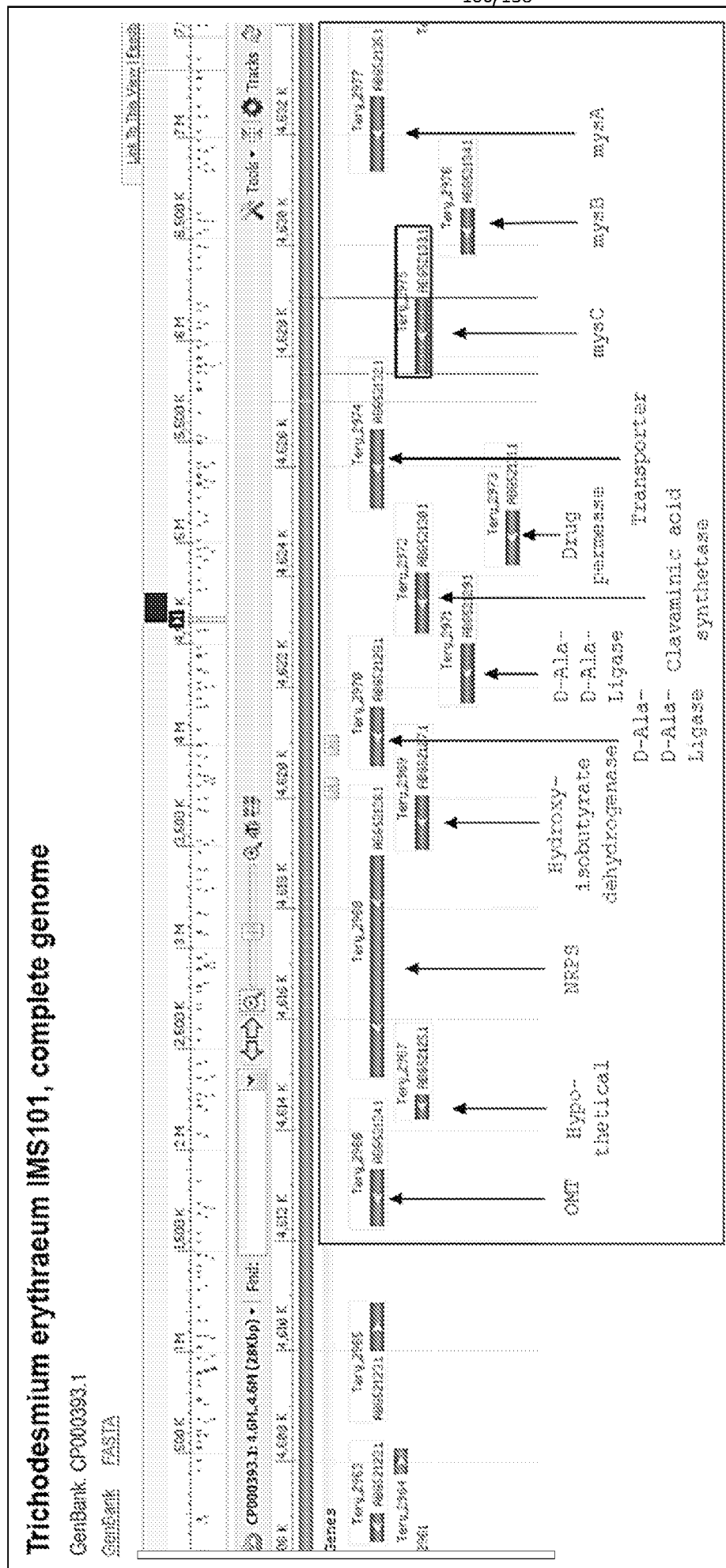


FIG. 38



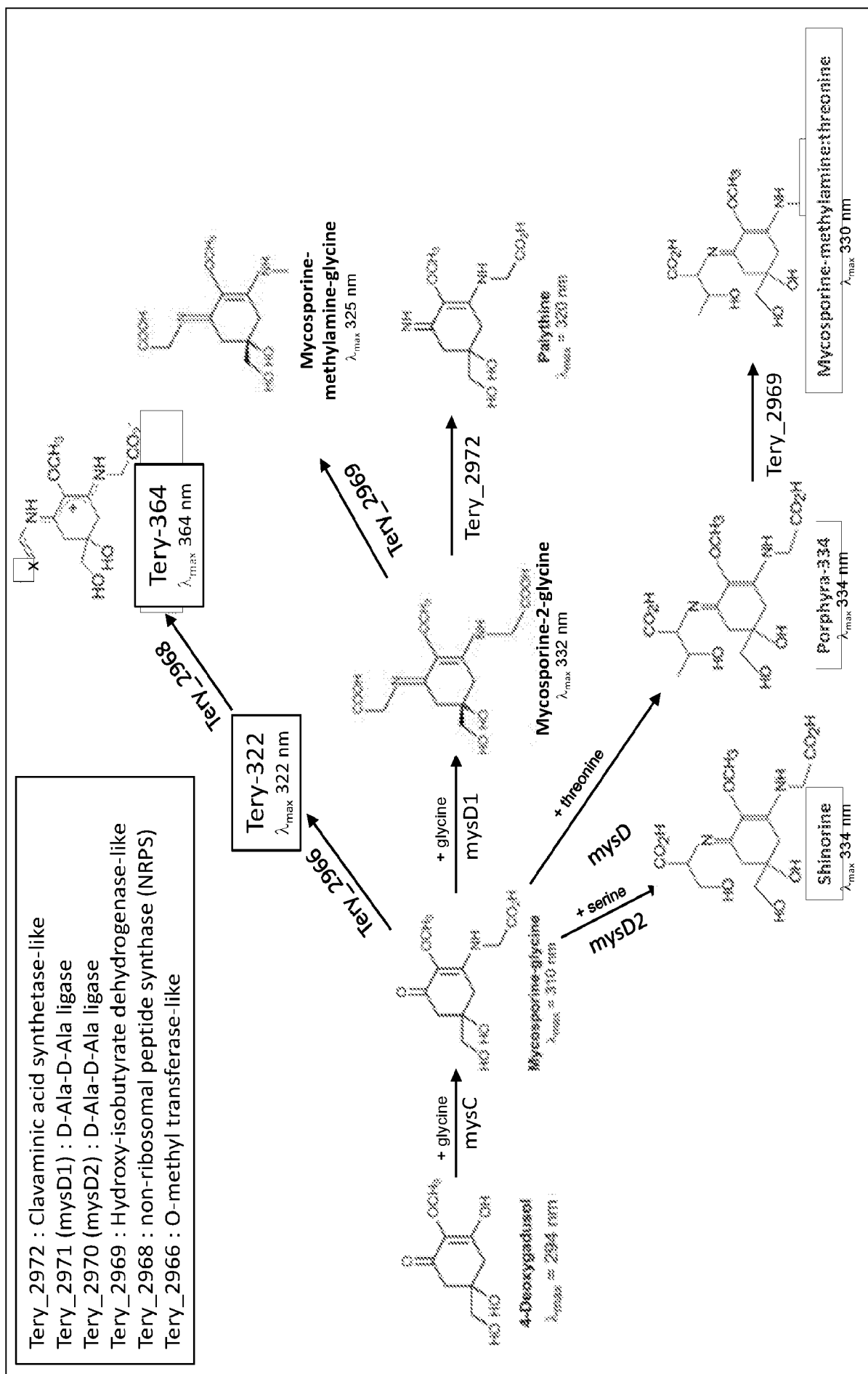


FIG. 40

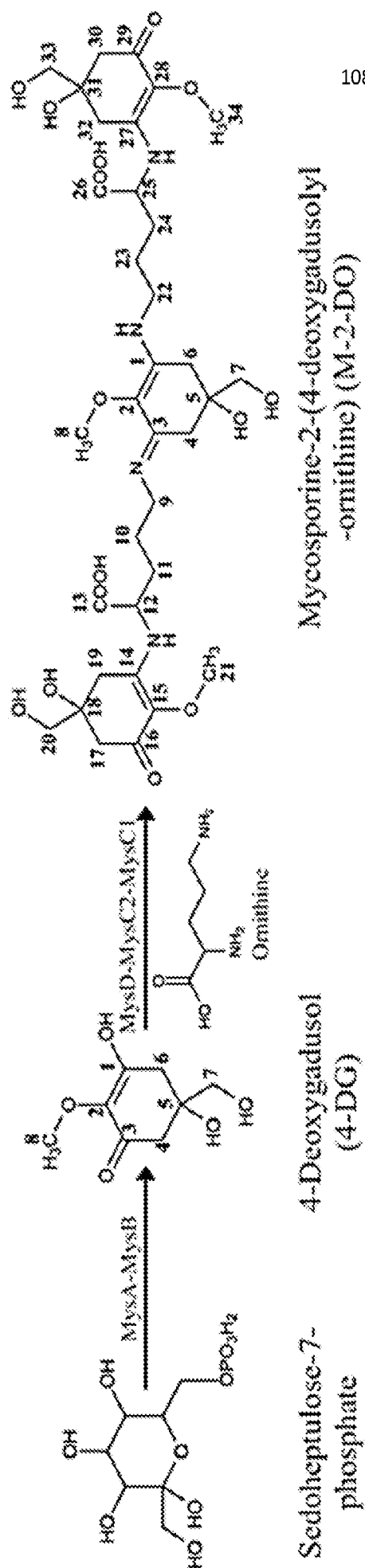


FIG. 41

1 10 20 30 40 50 60 70 80 90 100 110 120 130

1 130 140 150 160 170 180 190 200 210 220 230 240 250 260

1 270 280 290 300 310 320 330 340 350 360 370 380 390

1 400 410 420 430 440 450 460 470 480 490 500 510 520

1 530 540 550 560 570 580 590 600 610 620 630 640 650

1 660 670 680 690 700 710 720 730 740 750 760 770 780

1 790 800 810 820 830 840 850 860 870 880 890 900 910

1 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040

1 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170

1 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300

1 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430

1 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560

1 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690

1 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820

1 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920 1930 1940 1950

1 1960 1970 1980 1990 2000 2010 2020 2030 2040 2050 2060 2070 2080

1 2090 2100 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200 2210

1 2220 2230 2240 2250 2260 2270 2280 2290 2300 2310 2320 2330 2340

1 2350 2360 2370 2380 2390 2400 2410 2420 2430 2440 2450 2460 2470

1 2480 2490 2500 2510 2520 2530 2540 2550 2560 2570 2580 2590 2600

1 2610 2620 2630 2640 2650 2660 2670 2680 2690 2700 2710 2720 2730

1 2740 2750 2760 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860

1 2870 2880 2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990

1 3000 3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120

1 3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240 3250

1 3260 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360 3370 3380

1 3390 3400 3410 3420 3430 3440 3450 3460 3470 3480 3490 3500 3510

1 3520 3530 3540 3550 3560 3570 3580 3590 3600 3610 3620 3630 3640

1 3650 3660 3670 3680 3690 3700 3710 3720 3730 3740 3750 3760 3770

1 3780 3790 3800 3810 3820 3830 3840 3850 3860 3870 3880 3890 3900

1 3910 3920 3930 3940 3950 3960 3970 3980 3990 4000 4010 4020 4030

1 4040 4050 4060 4070 4080 4090 4100 4110 4120 4130 4140 4150 4160

1 4170 4180 4190 4200 4210 4220 4230 4240 4250 4260 4270 4280 4290

1 4300 4310 4320 4330 4340 4350 4360 4370 4380 4390 4400 4410 4420

1 4430 4440 4450 4460 4470 4480 4490 4500 4510 4520 4530 4540 4550

1 4560 4570 4580 4590 4600 4610 4620 4630 4640 4650 4660 4670 4680

1 4690 4700 4710 4720 4730 4740 4750 4760 4770 4780 4790 4800 4810

1 4820 4830 4840 4850 4860 4870 4880 4890 4900 4910 4920 4930 4940

1 4950 4960 4970 4980 4990 5000 5010 5020 5030 5040 5050 5060 5070

1 5080 5090 5100 5110 5120 5130 5140 5150 5160 5170 5180 5190 5200

1 5210 5220 5230 5240 5250 5260 5270 5280 5290 5300 5310 5320 5330

1 5340 5350 5360 5370 5380 5390 5400 5410 5420 5430 5440 5450 5460

1 5470 5480 5490 5500 5510 5520 5530 5540 5550 5560 5570 5580 5590

1 5600 5610 5620 5630 5640 5650 5660 5670 5680 5690 5700 5710 5720

1 5730 5740 5750 5760 5770 5780 5790 5800 5810 5820 5830 5840 5850

1 5860 5870 5880 5890 5900 5910 5920 5930 5940 5950 5960 5970 5980

1 5990 6000 6010 6020 6030 6040 6050 6060 6070 6080 6090 6100 6110

1 6120 6130 6140 6150 6160 6170 6180 6190 6200 6210 6220 6230 6240

1 6250 6260 6270 6280 6290 6300 6310 6320 6330 6340 6350 6360 6370

1 6380 6390 6400 6410 6420 6430 6440 6450 6460 6470 6480 6490 6500

1 6510 6520 6530 6540 6550 6560 6570 6580 6590 6600 6610 6620 6630

1 6640 6650 6660 6670 6680 6690 6700 6710 6720 6730 6740 6750 6760

1 6770 6780 6790 6800 6810 6820 6830 6840 6850 6860 6870 6880 6890

1 6900 6910 6920 6930 6940 6950 6960 6970 6980 6990 7000 7010 7020

1 7030 7040 7050 7060 7070 7080 7090 7100 7110 7120 7130 7140 7150

1 7160 7170 7180 7190 7200 7210 7220 7230 7240 7250 7260 7270 7280

1 7290 7300 7310 7320 7330 7340 7350 7360 7370 7380 7390 7400 7410

1 7420 7430 7440 7450 7460 7470 7480 7490 7500 7510 7520 7530 7540

1 7550 7560 7570 7580 7590 7600 7610 7620 7630 7640 7650 7660 7670

1 7680 76

FIG. 42A

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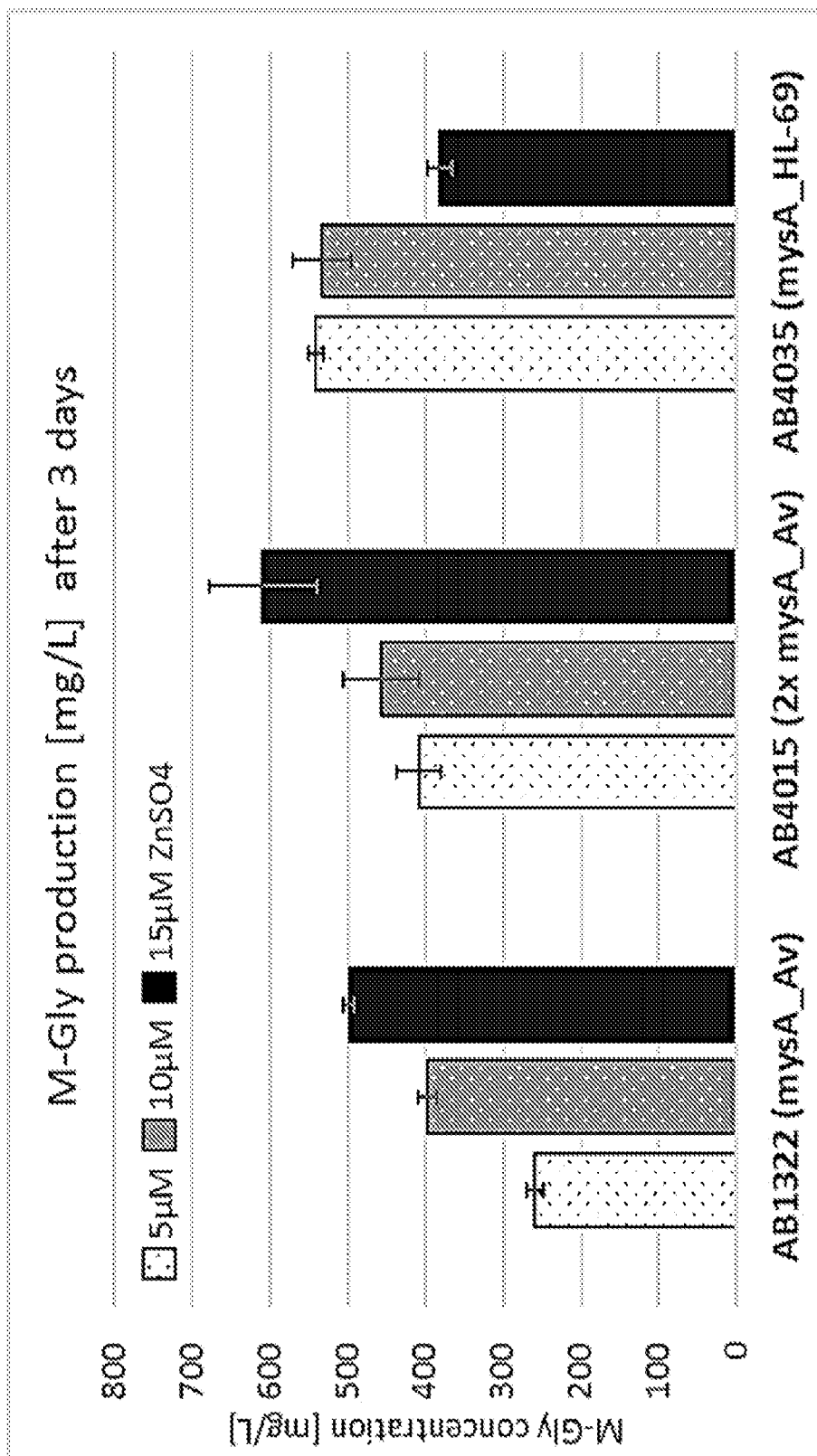


FIG. 42B

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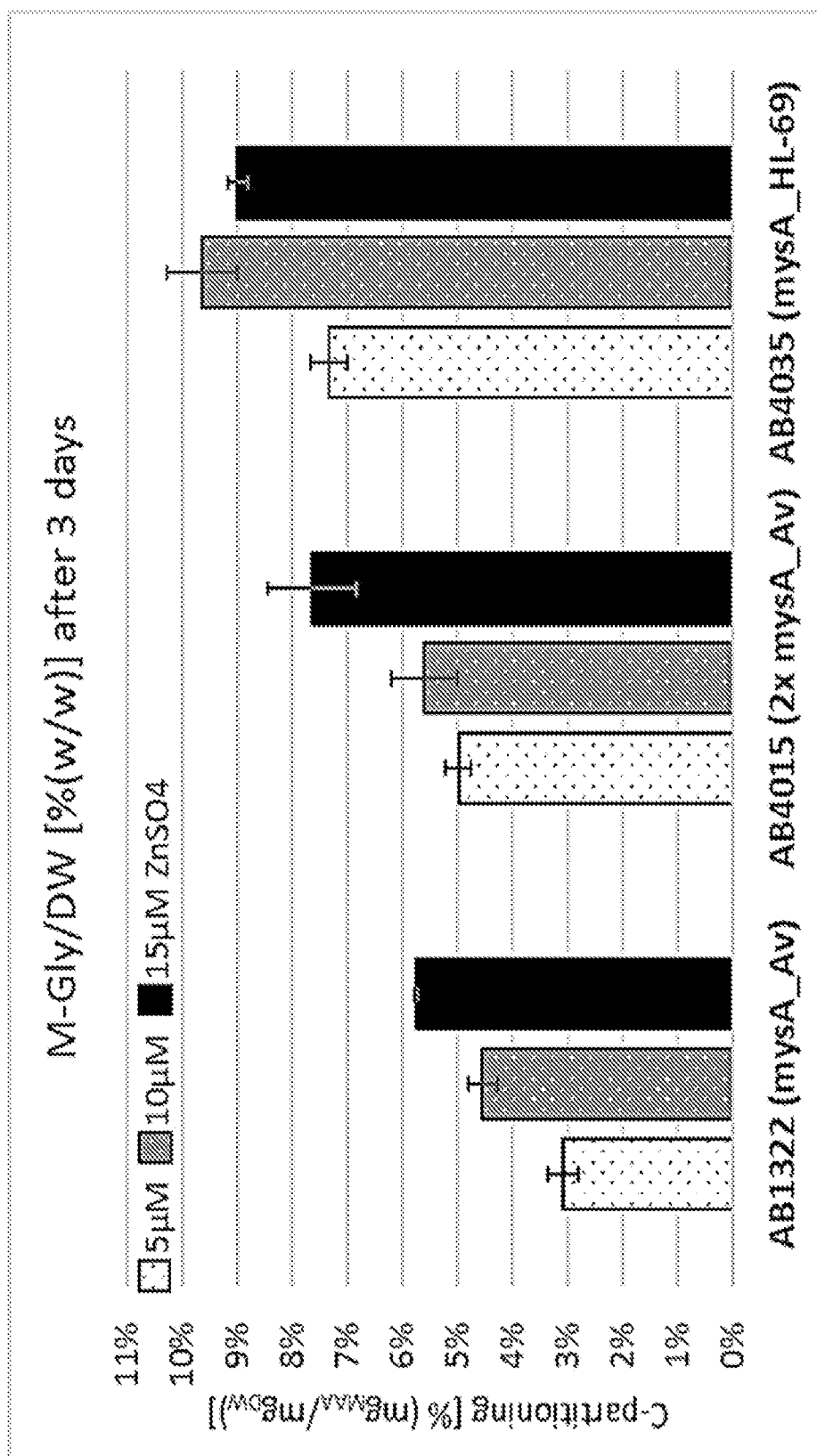


FIG. 42C

Outer ring = forward strand
Inner ring = reverse strand

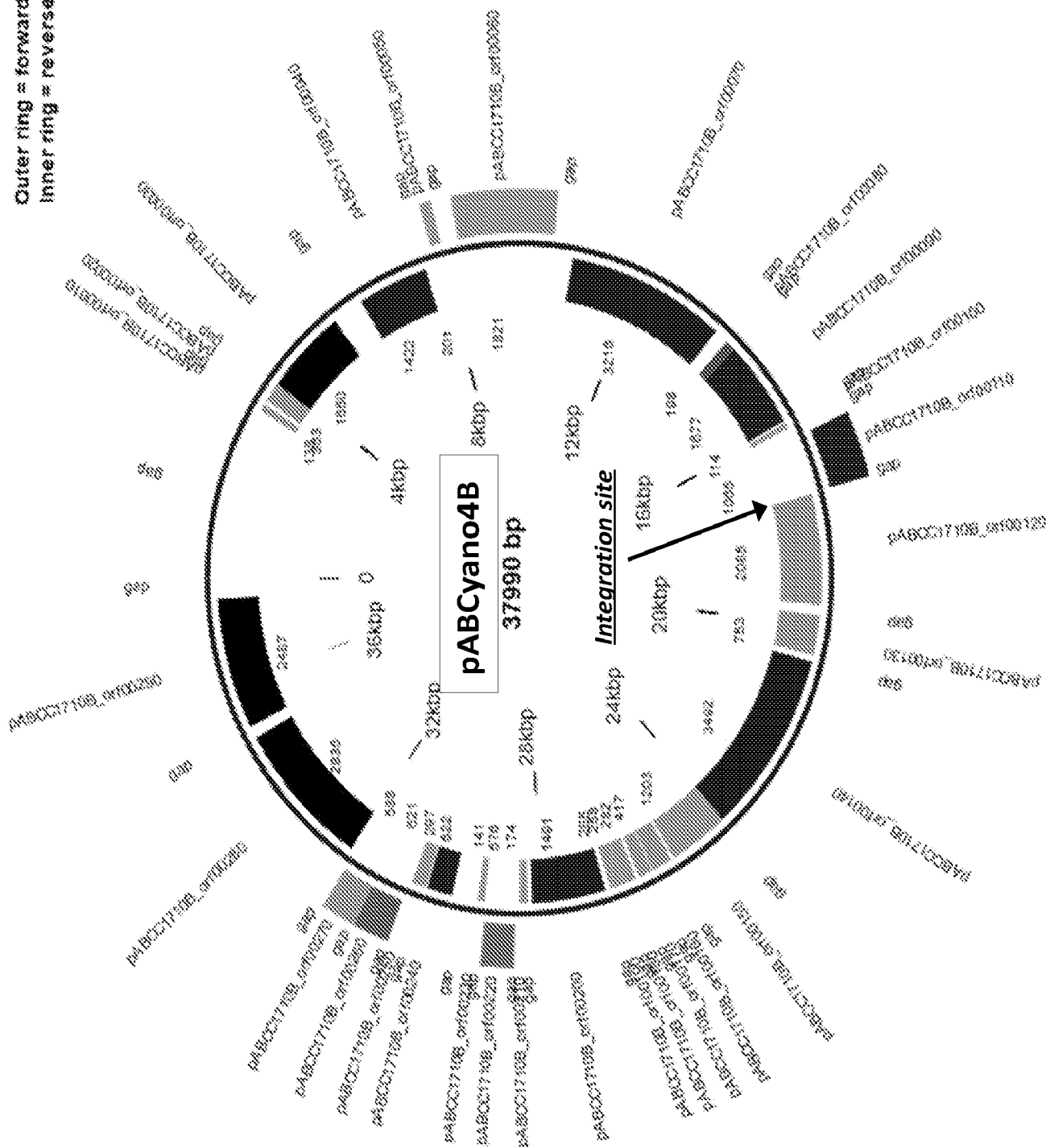


FIG. 43

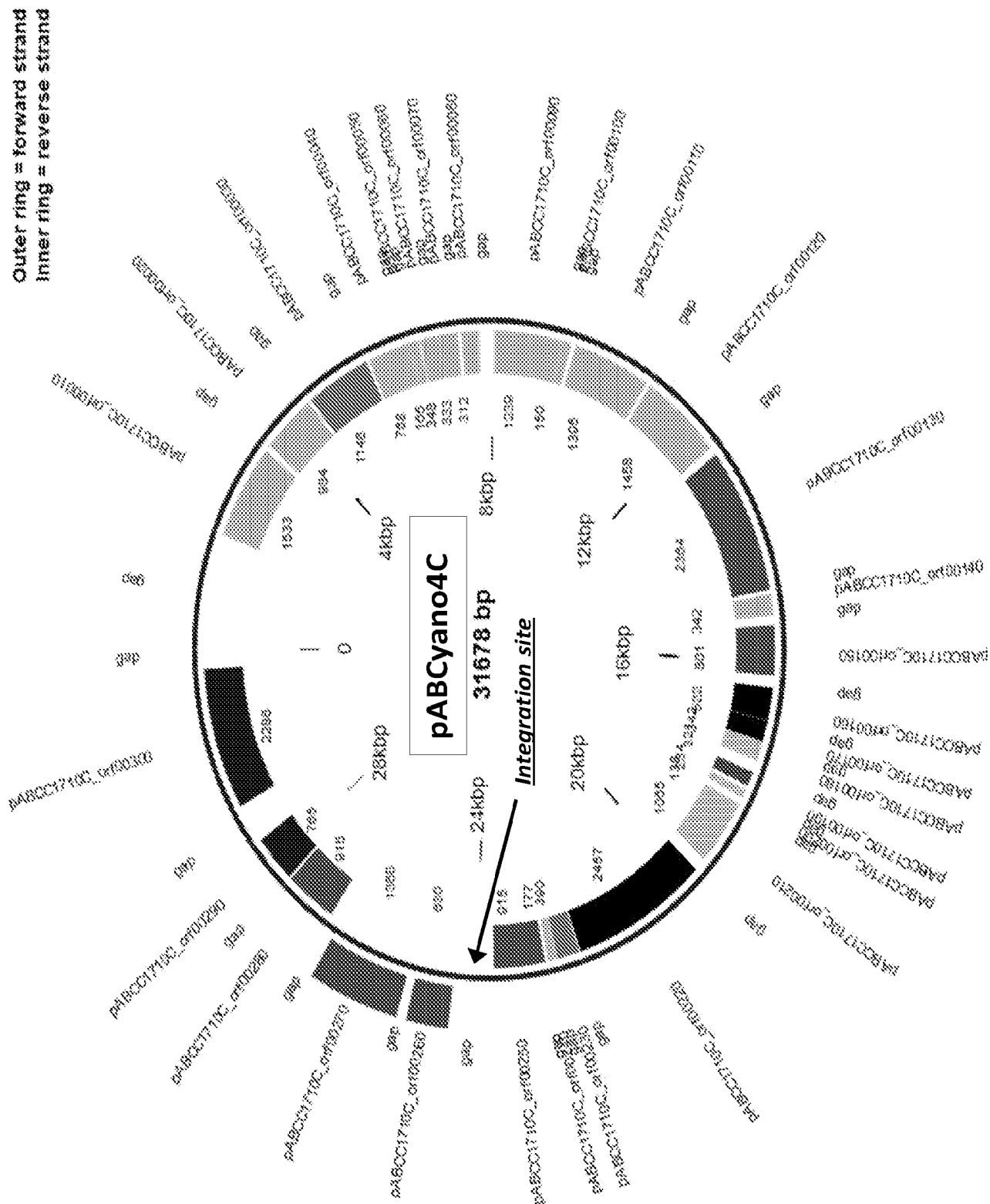


FIG. 44

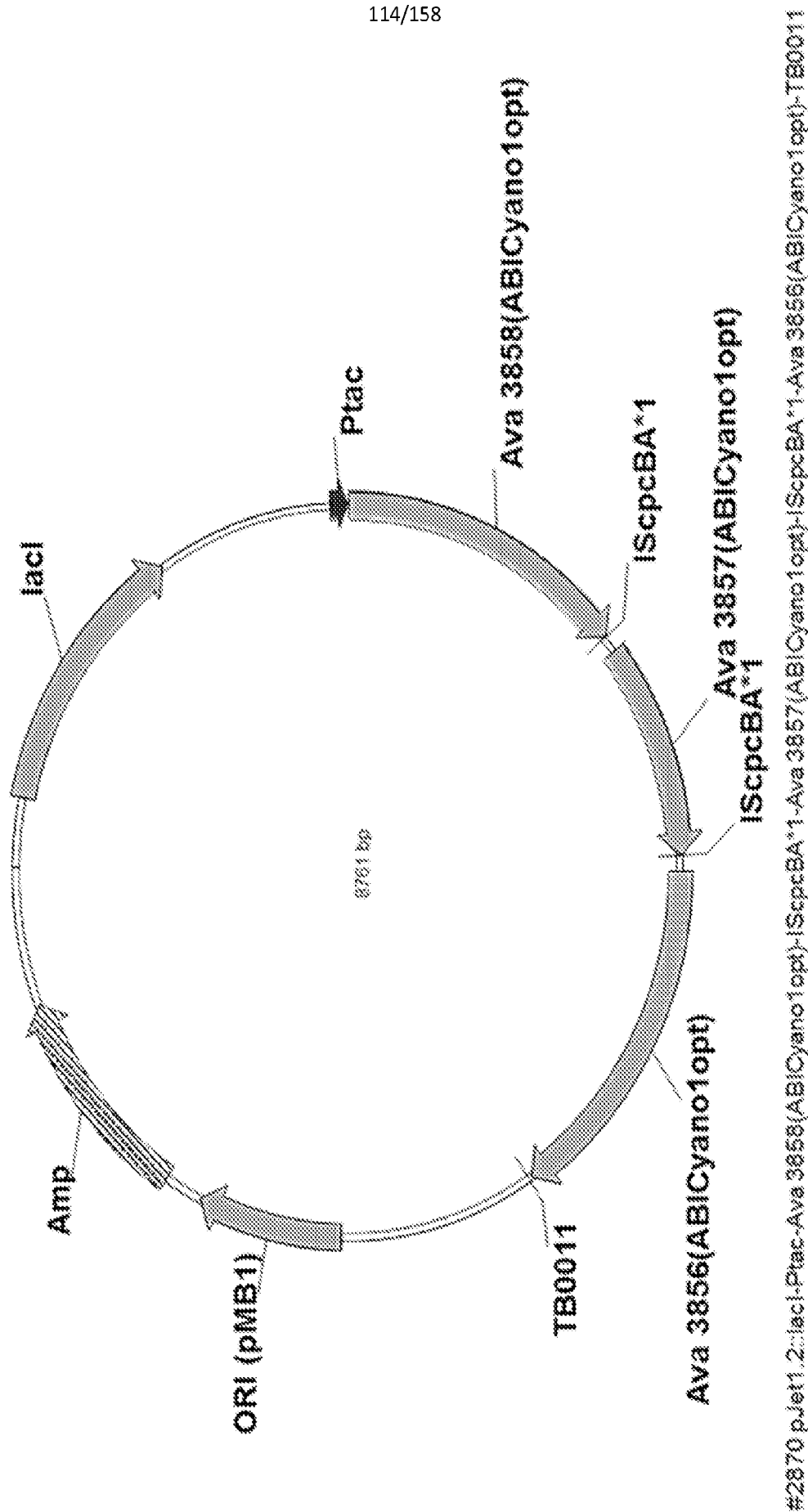


FIG. 45A

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```

ID      #2870\pJet1.2::lacI-Ptac-Ava_3858 (ABICyano1opt) - IScpcBA*1 -
Ava_3857 (ABICyano1opt) - IScpcBA*1 - Ava_3856 (ABICyano1opt) - TB0011 standard;
circular DNA;      ; 8763 BP.
CC      This file is created by Vector NTI
CC      http://www.invitrogen.com/
CC      VNTDATE|-11916978|
CC      VNTDBDATE|-11916978|
CC      LSOWNER|
CC      VNTAUTHORNAME|Frank Uliczka|
CC      VNTAUTHOREML|frank.uliczka@algenol.com|
CC      VNTTOAUTHORNAME|Anne Neuenfeldt|
FH      Key      Location/Qualifiers
FH
FT      misc_feature      5277..6134
FT                          /vntifkey="21"
FT                          /label=Amp
FT      terminator      3668..3713
FT                          /vntifkey="43"
FT                          /label=TB0011
FT      CDS      79..1308
FT                          /vntifkey="4"
FT                          /label=Ava_3858 (ABICyano1opt)
FT      misc_feature      1309..1373
FT                          /vntifkey="21"
FT                          /label=IScpcBA*1
FT      CDS      1374..2213
FT                          /vntifkey="4"
FT                          /label=Ava_3857 (ABICyano1opt)

```

FIG. 45A (continued)

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FT	misc_feature	2214..2278
FT		/vntifkey="21"
FT		/label=IScpCBA*1
FT	CDS	2279..3655
FT		/vntifkey="4"
FT		/label=Ava_3856(ABICyanolopt)
FT	rep_origin	4500..5114
FT		/vntifkey="33"
FT		/label=ORI\ (pMB1)
FT	CDS	6930..8012
FT		/vntifkey="4"
FT		/label=lacI
FT	promoter	1..74
FT		/vntifkey="30"
FT		/label=Ptac

FIG. 45A (continued)

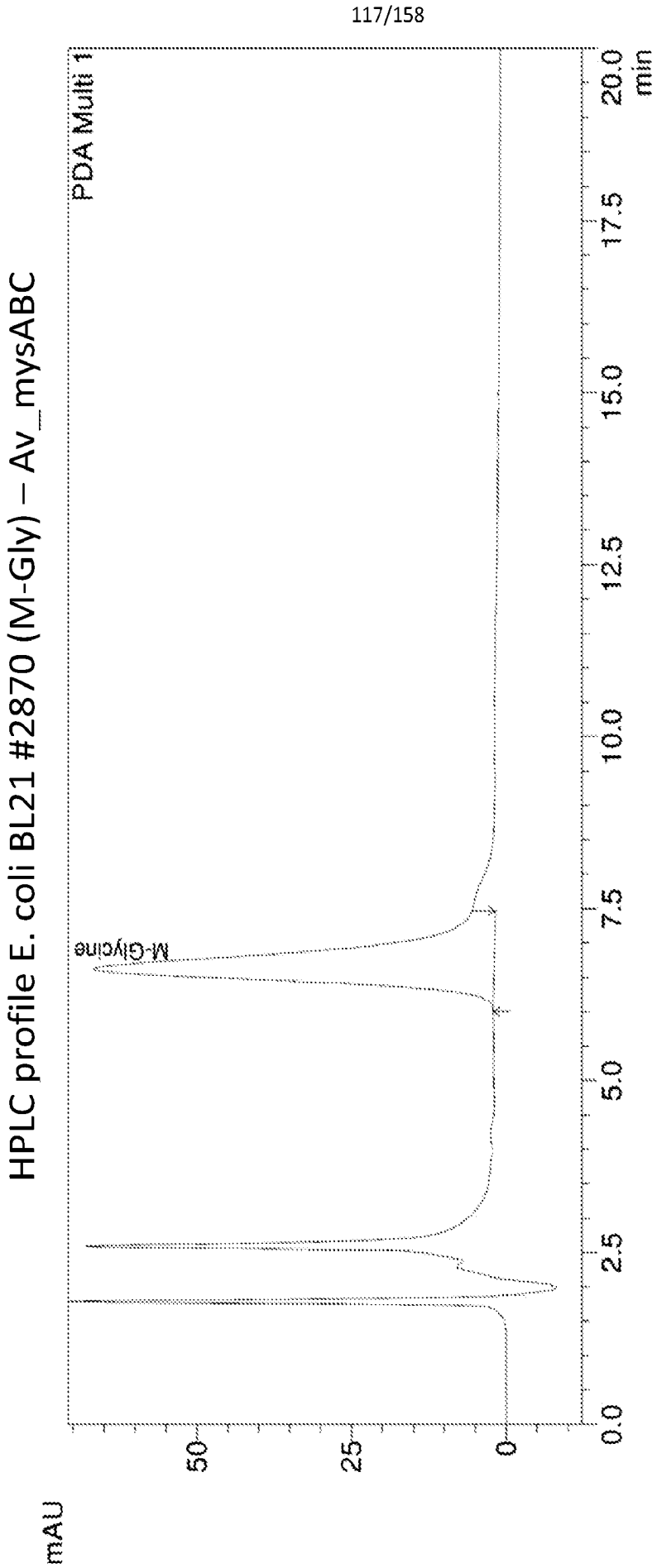


FIG. 45B

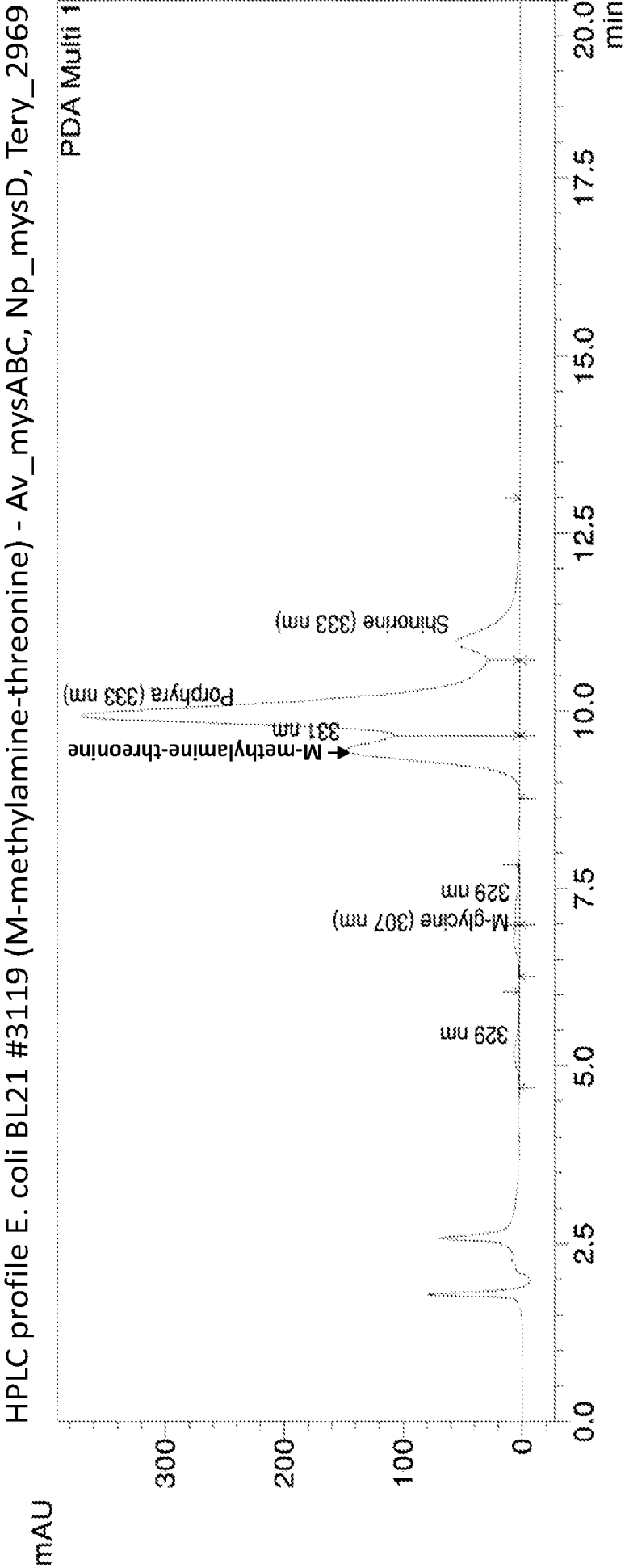


FIG. 46

HPLC profile E. coli BL21 #31117 (Palythine) - Av_mysABC, Tery_mysD1, Tery_2972

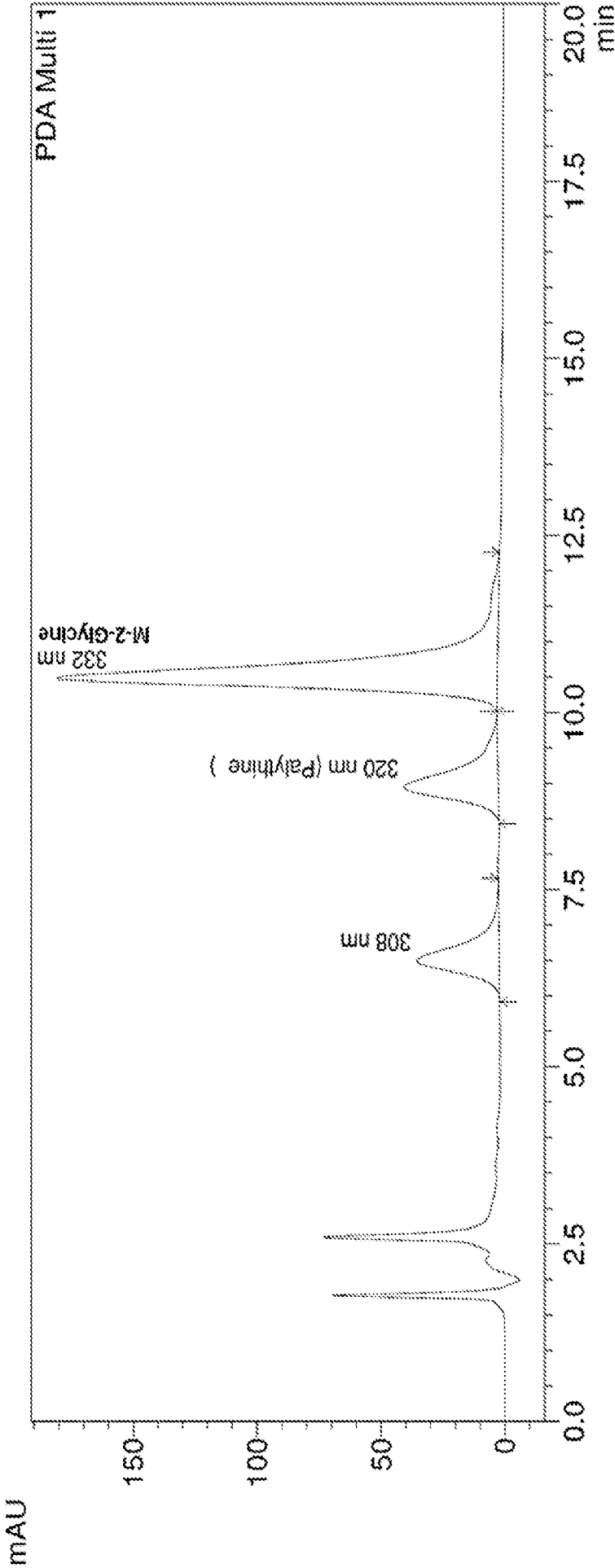


FIG. 47

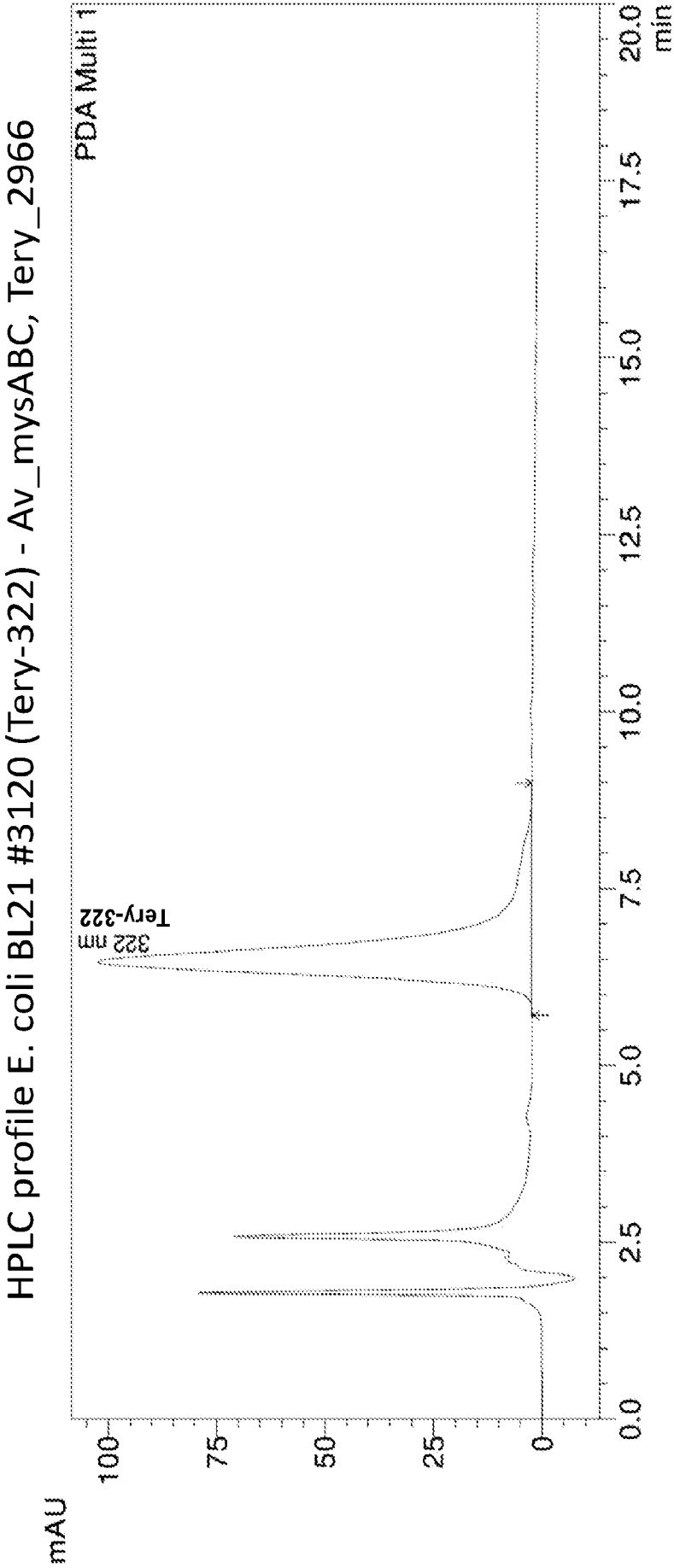


FIG. 48

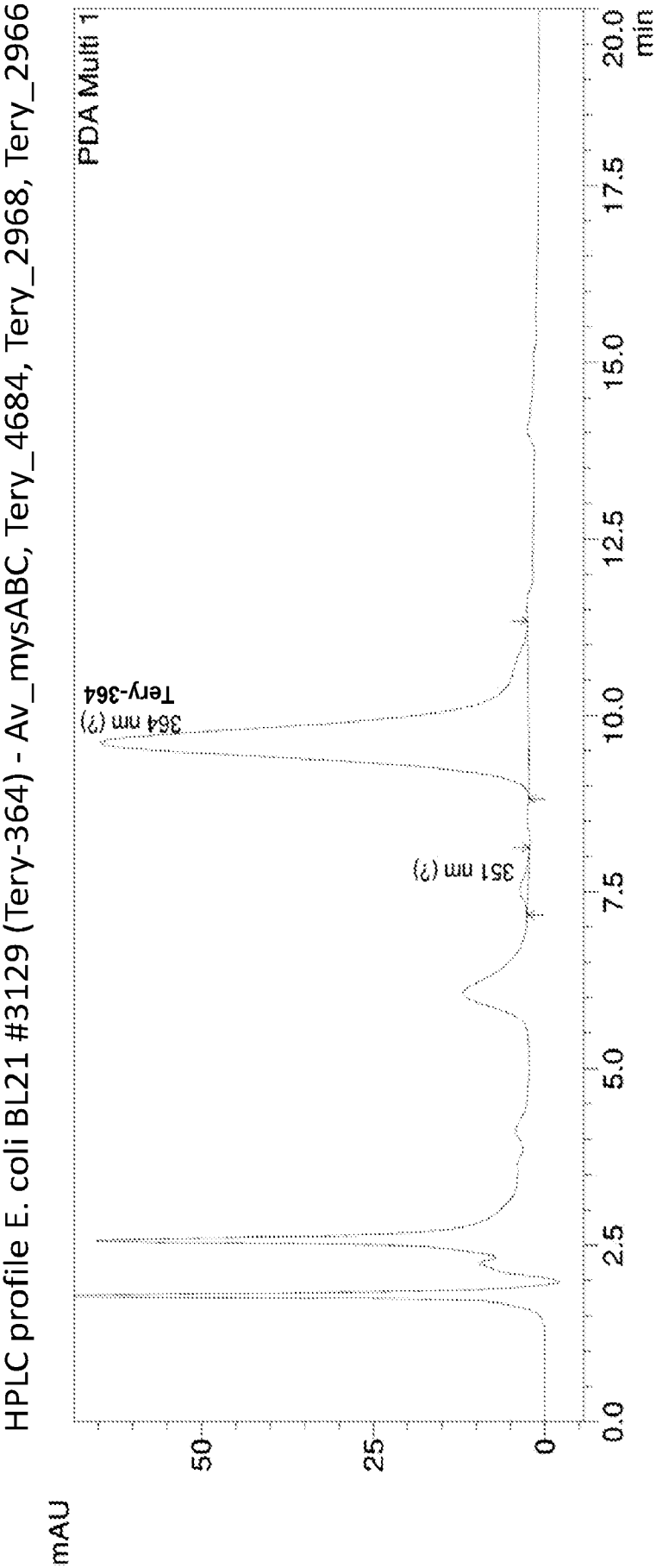


FIG. 49

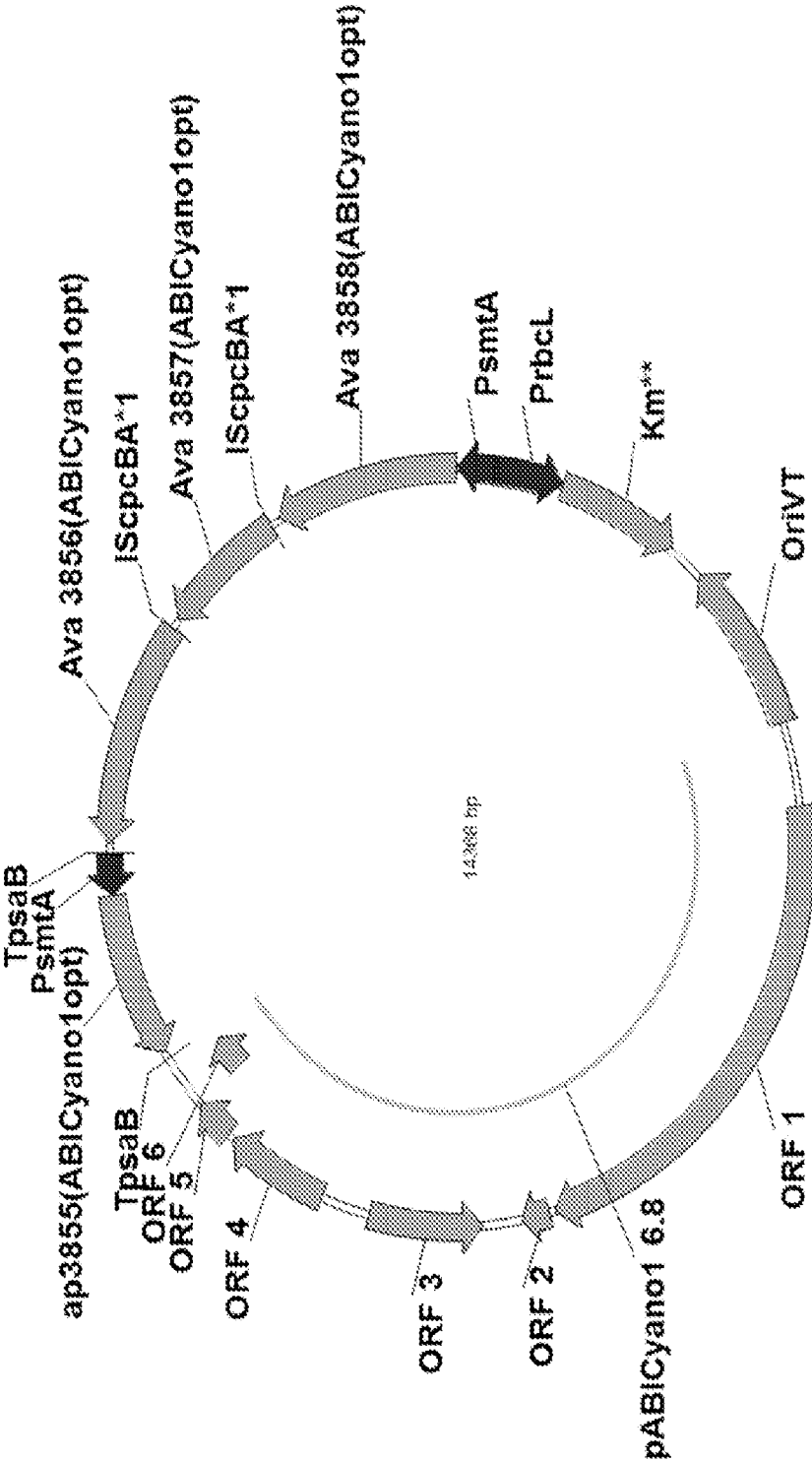
Sequence alignment of Tery_2966 (O-methyltransferase) homologs from different cyanobacterial species

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
IMS101														
NIES-2100														
TS821														
K2W001														
PCC7335														
Consensus														
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
IMS101														
NIES-2100														
TS821														
K2W001														
PCC7335														
Consensus														
	261	270	280	290	300	310	320	330	340	350	355			
IMS101														
NIES-2100														
TS821														
K2W001														
PCC7335														
Consensus														

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IMS101 Trichodesmium erythraeum IMS101
NIES-2100 Calothrix sp. NIES2100
TS821 Crococcidiopsis sp. TS821
K2W001 Euhalothece sp. KZN001
PCC7335 Synechococcus sp. PCC7335
Consensus

FIG. 50



#2693 pAB1 6.8::PsmA-Ava 3856(ABICyano1opt)-IScpcBA⁺1-Ava 3857(ABICyano1opt)-TpsaB-PsmA-ap3855(ABICyano1opt)

FIG. 51

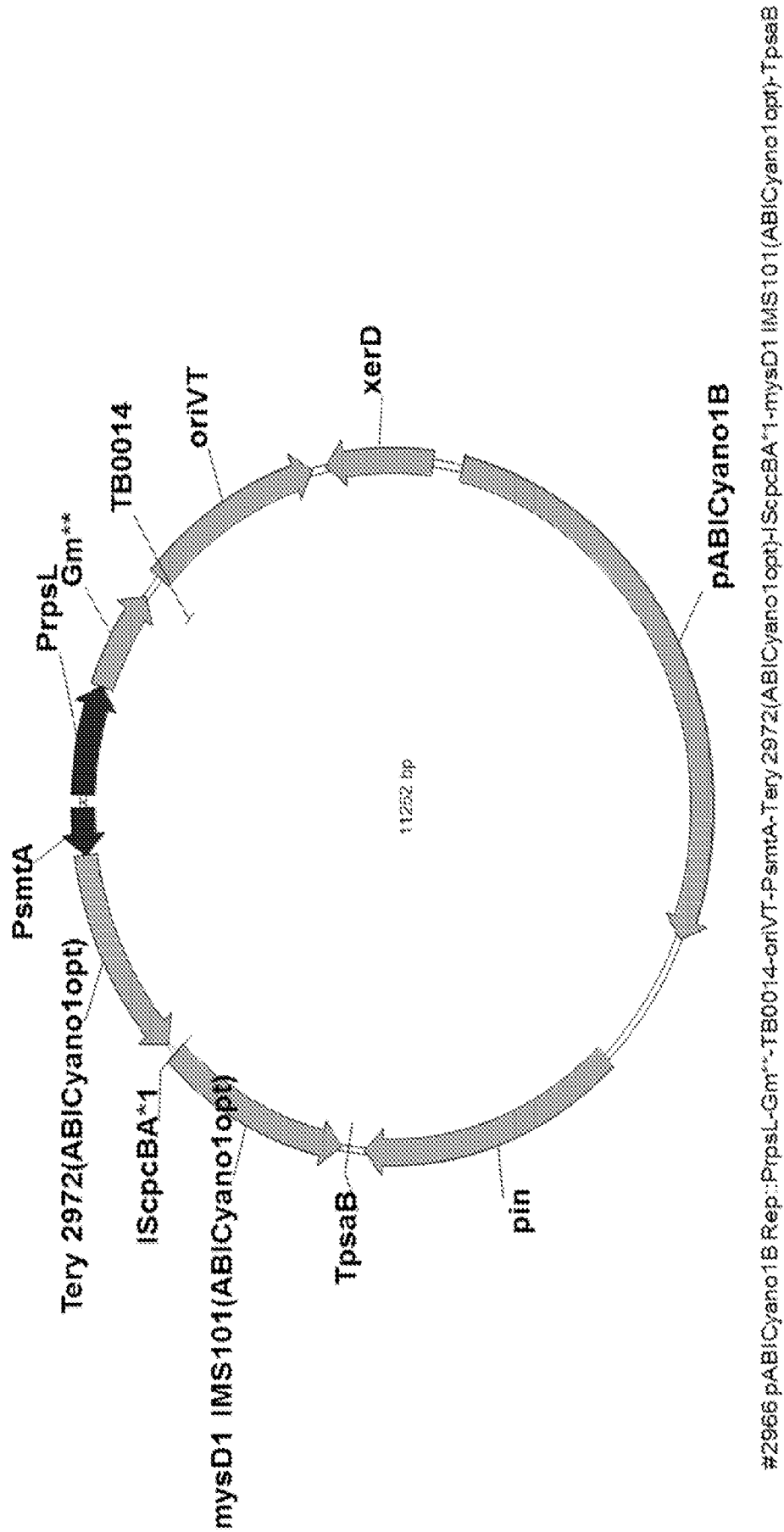


FIG. 52

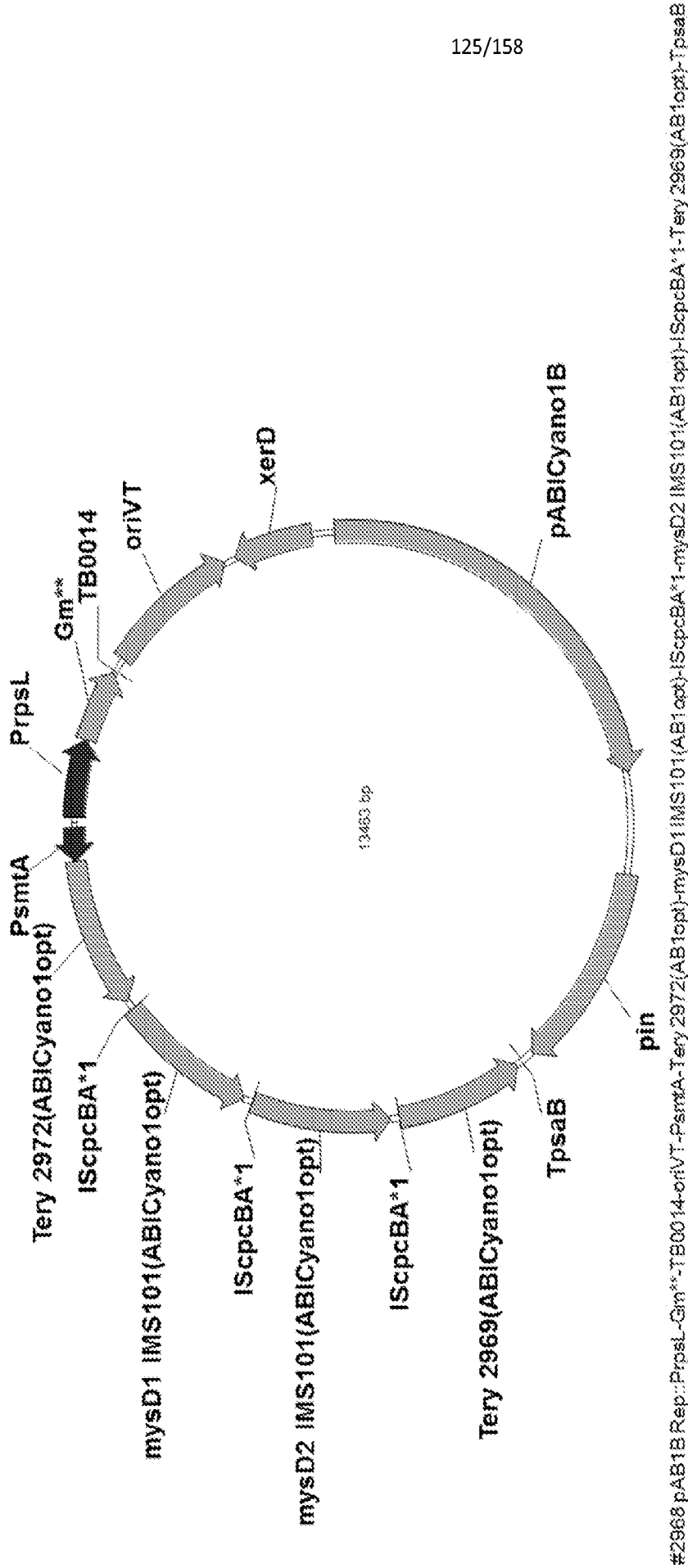


FIG. 53

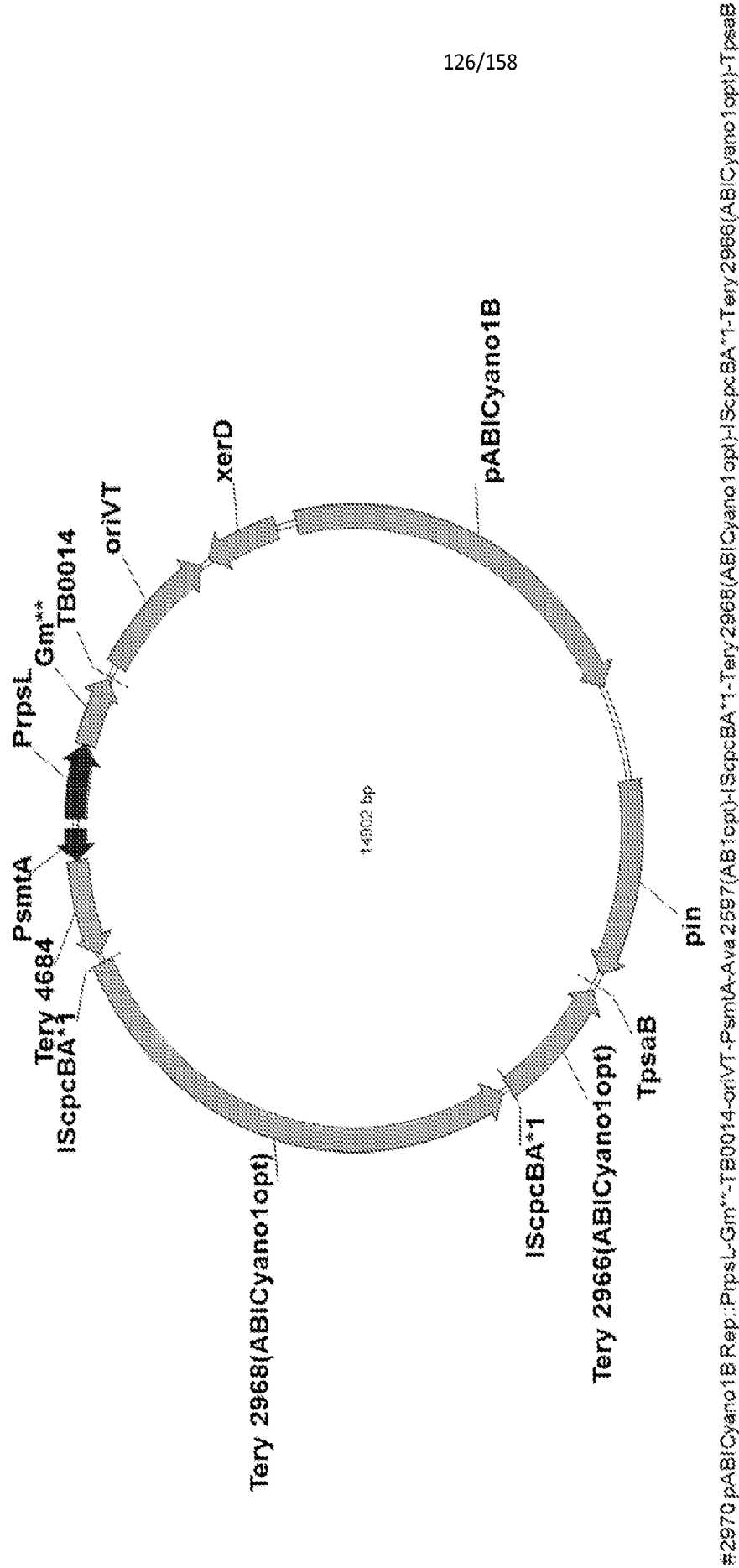


FIG. 54

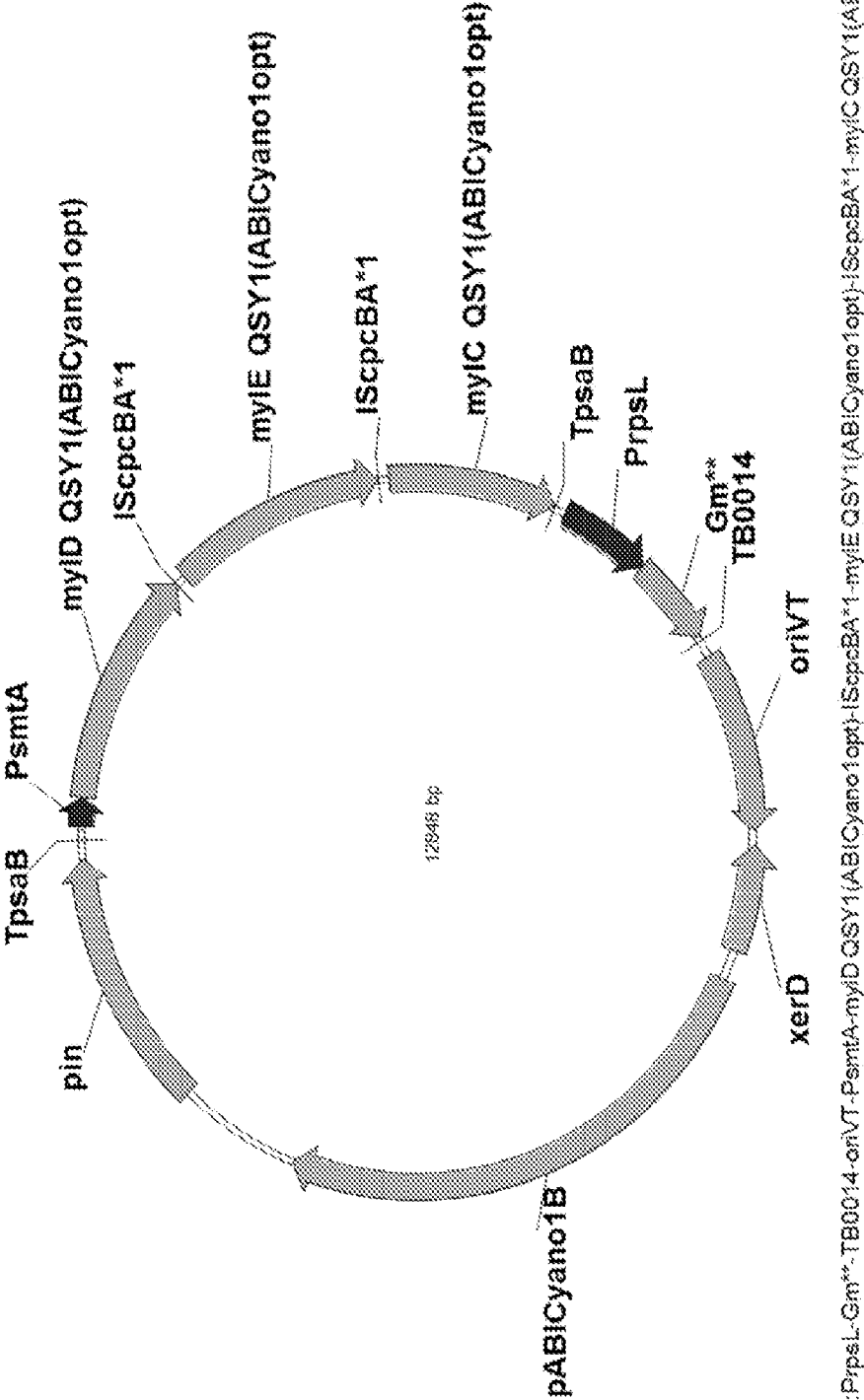


FIG. 55

#3049 pABICyano1B Rep.:PrpsL-Gm^{res}-TB0014-oriVT-PsmTA-myD QSY1(ABICyano1opt)-IScpCBA*1-myIE QSY1(ABICyano1opt)-IScpCBA*1-myIC QSY1(ABICyano1opt)-TpsaB

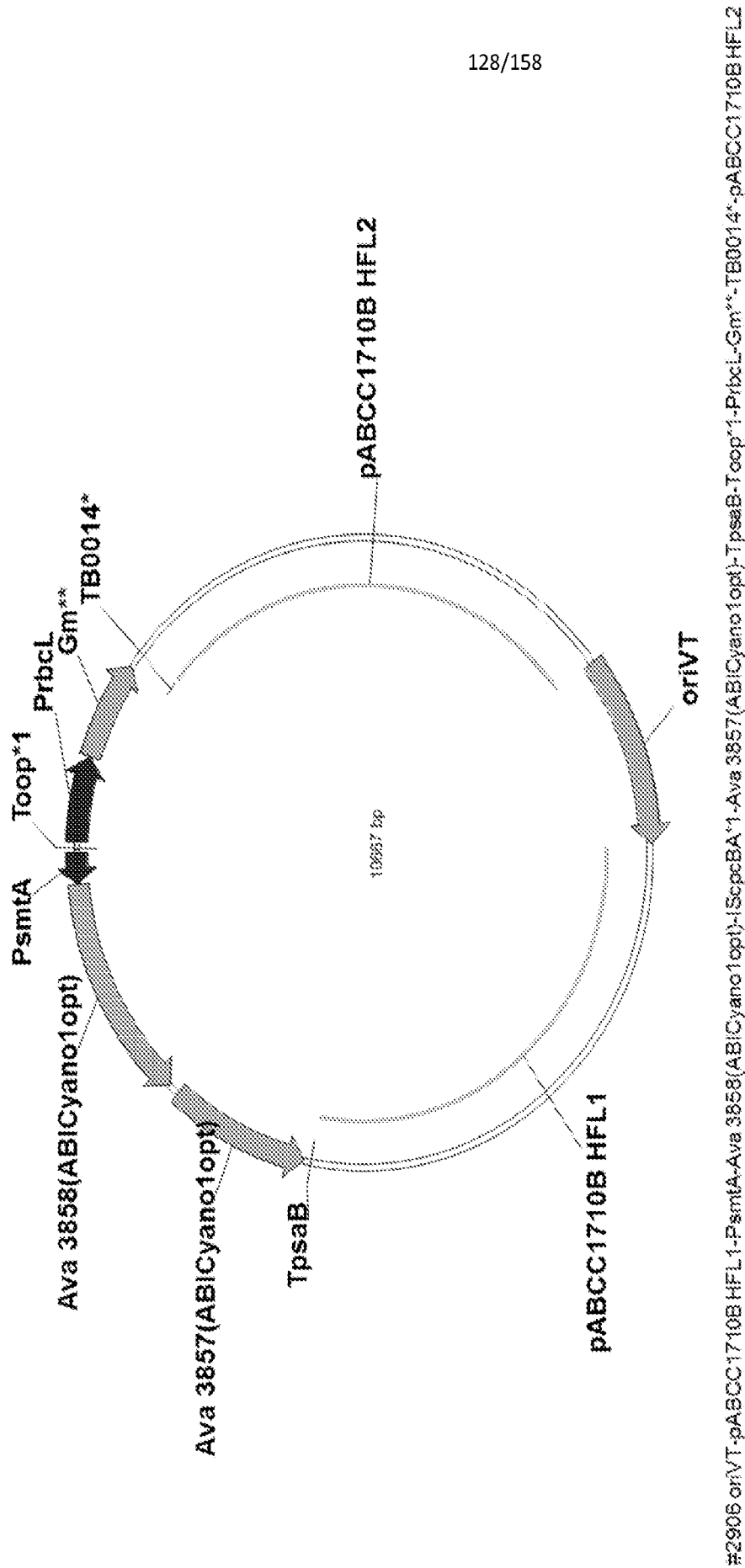


FIG. 56

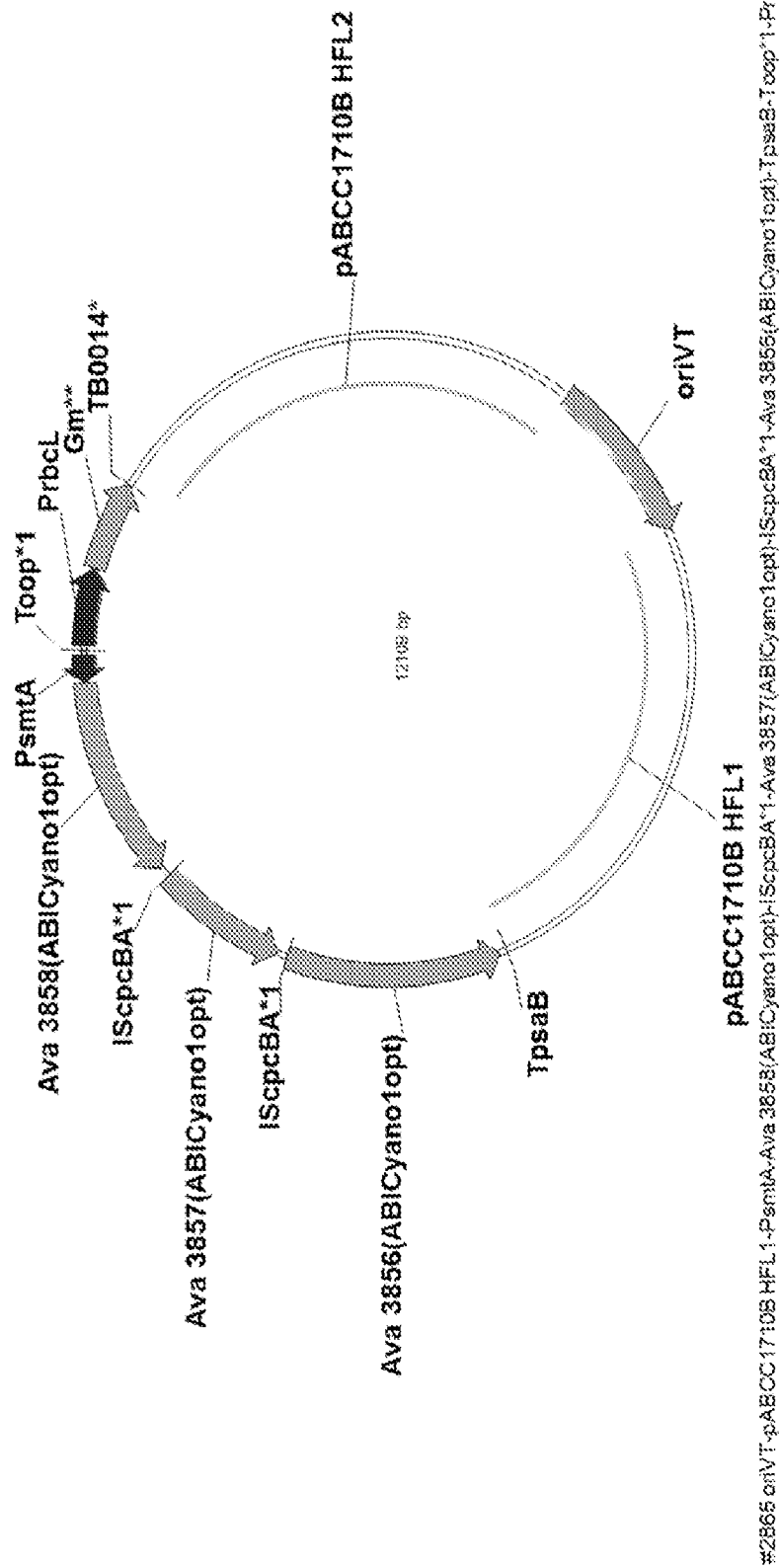


FIG. 57

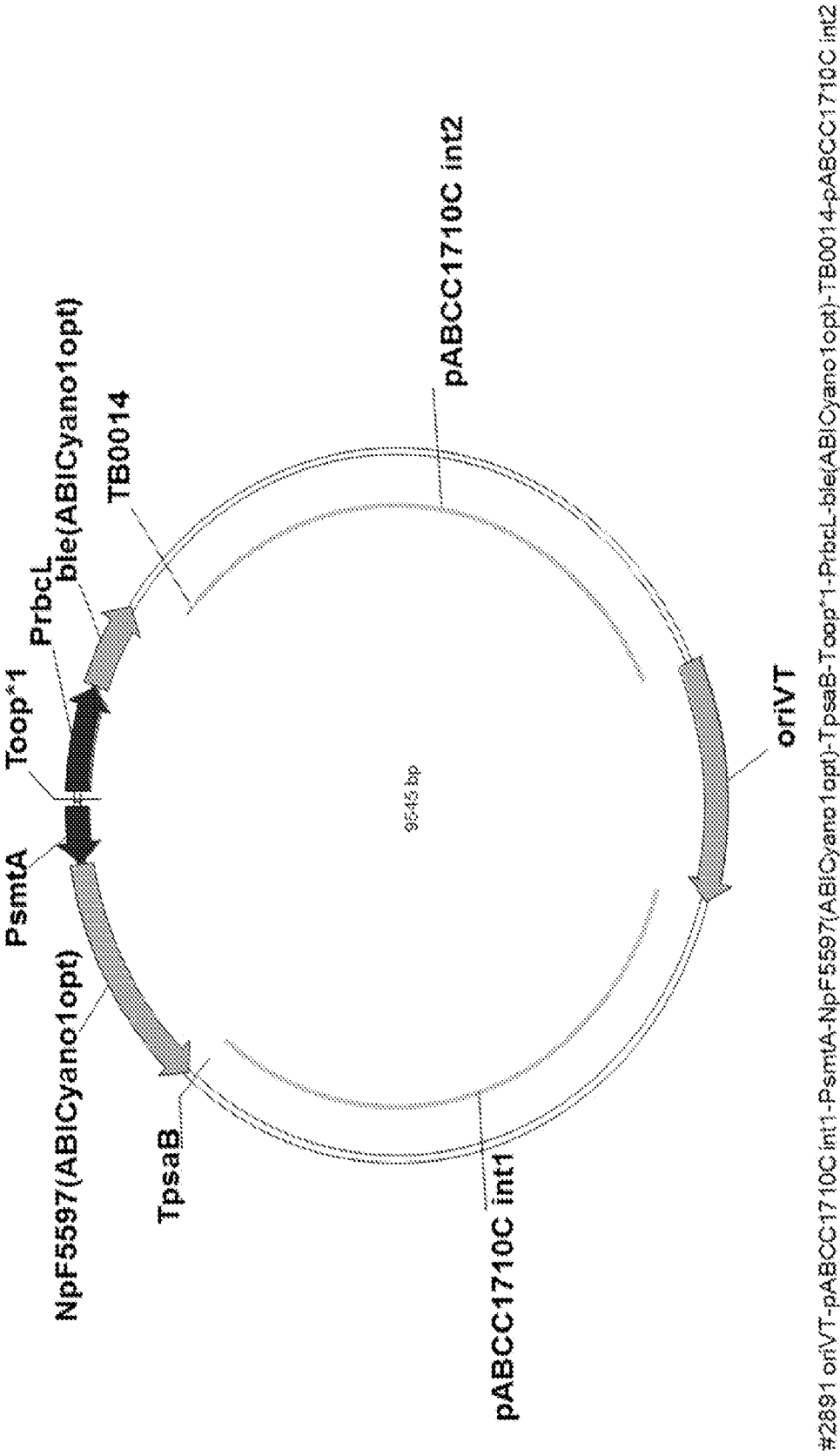


FIG. 58

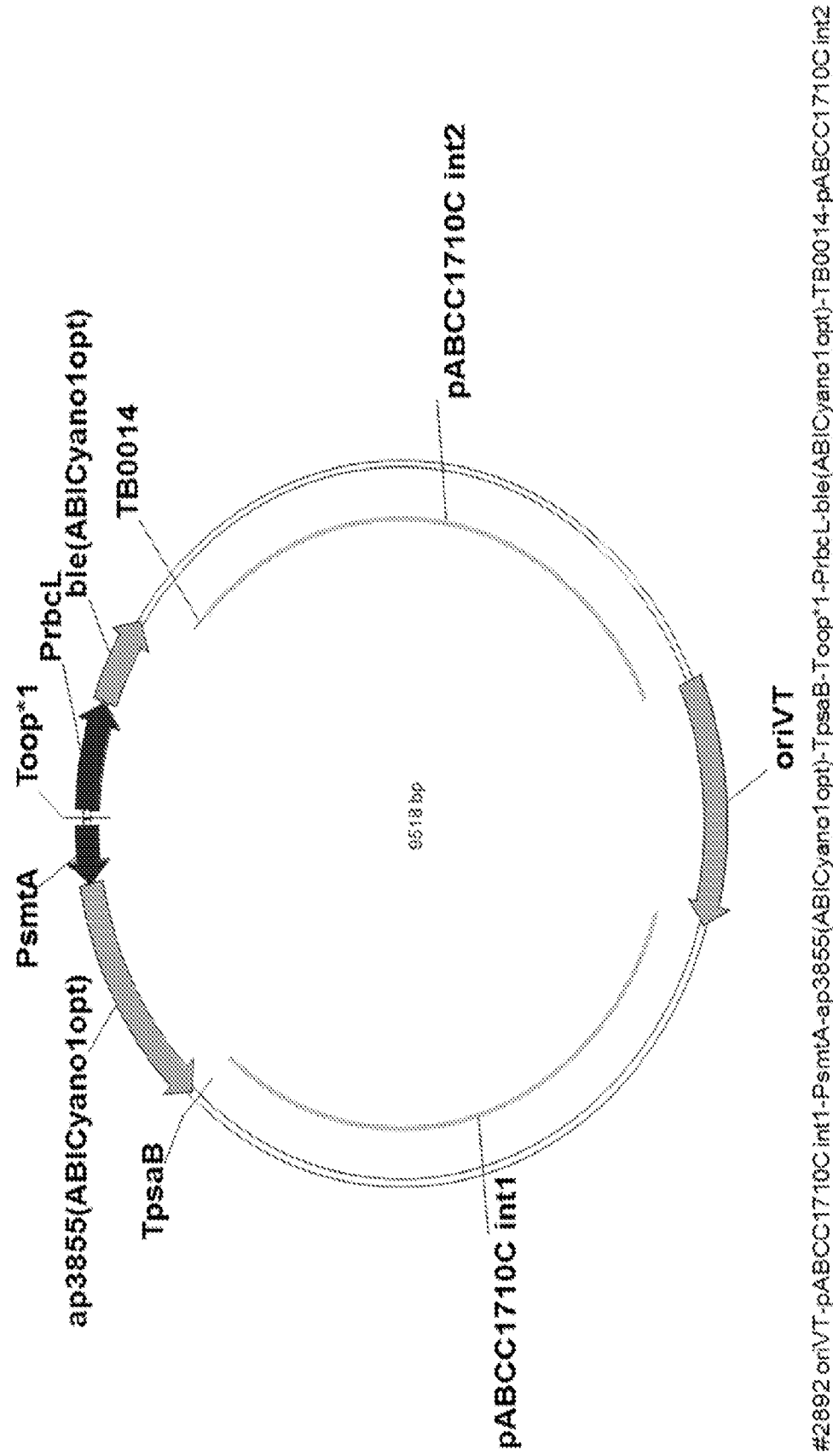


FIG. 59

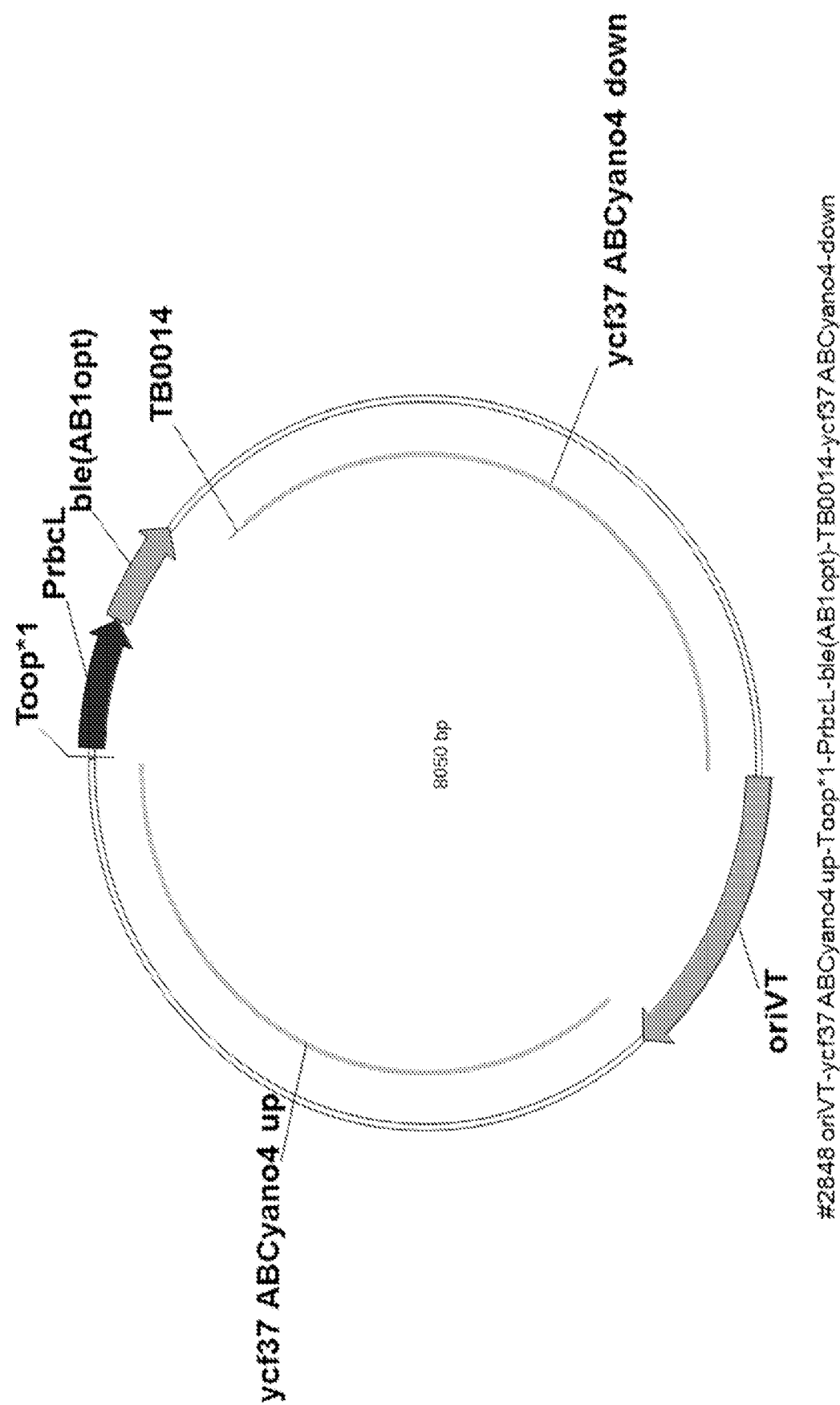


FIG. 60

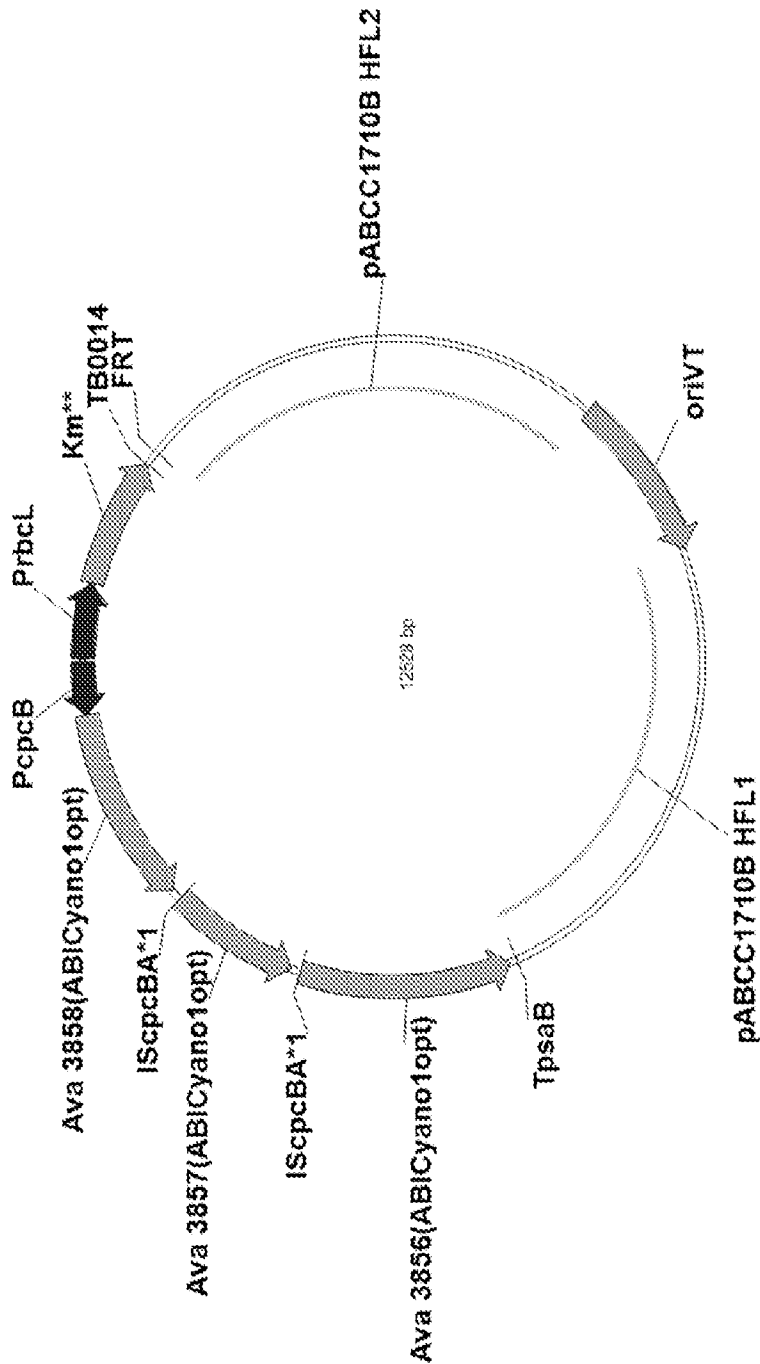


FIG. 61

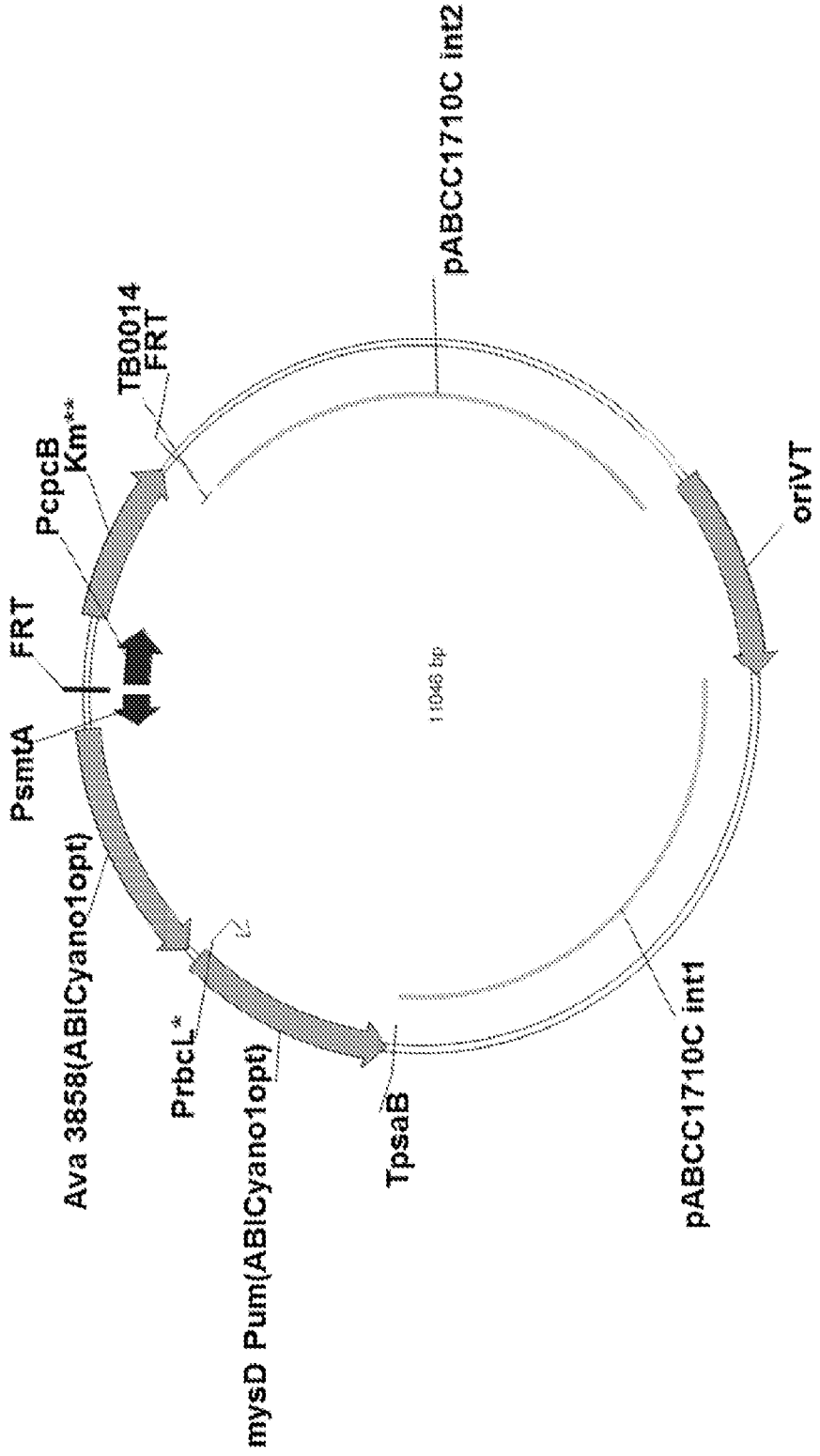


FIG. 62

#2992 oriVT-pABCC1710C int1 HFL1-PsmA-Ava 3858(ABICyano1opt)-PrbcL*-mysD Pum(ABICyano1opt)-TpsaB-FRT-PcpcB-Km^{rs}-TB0014-FRT-FRT-pABCC1710C int2

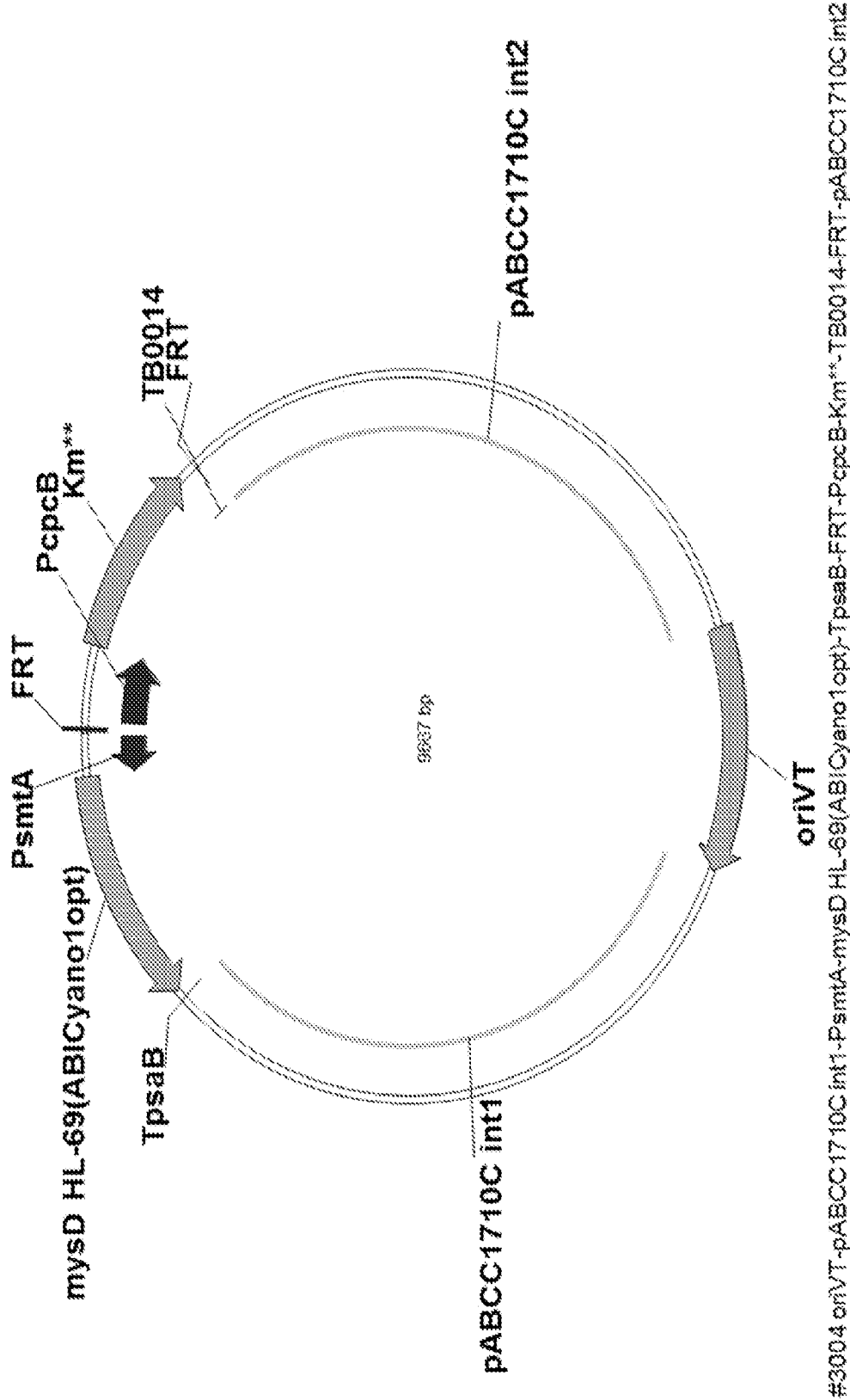


FIG. 63

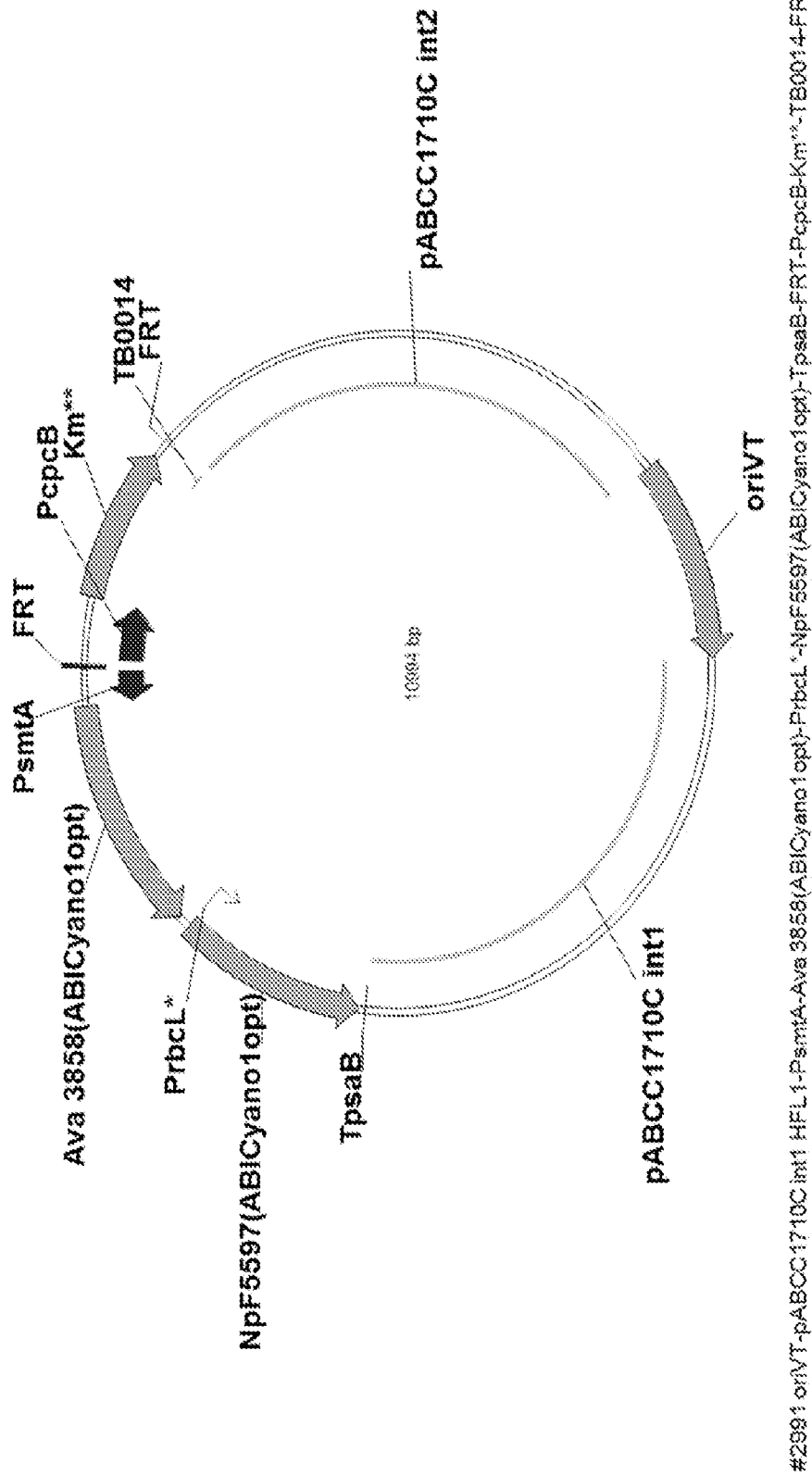


FIG. 64

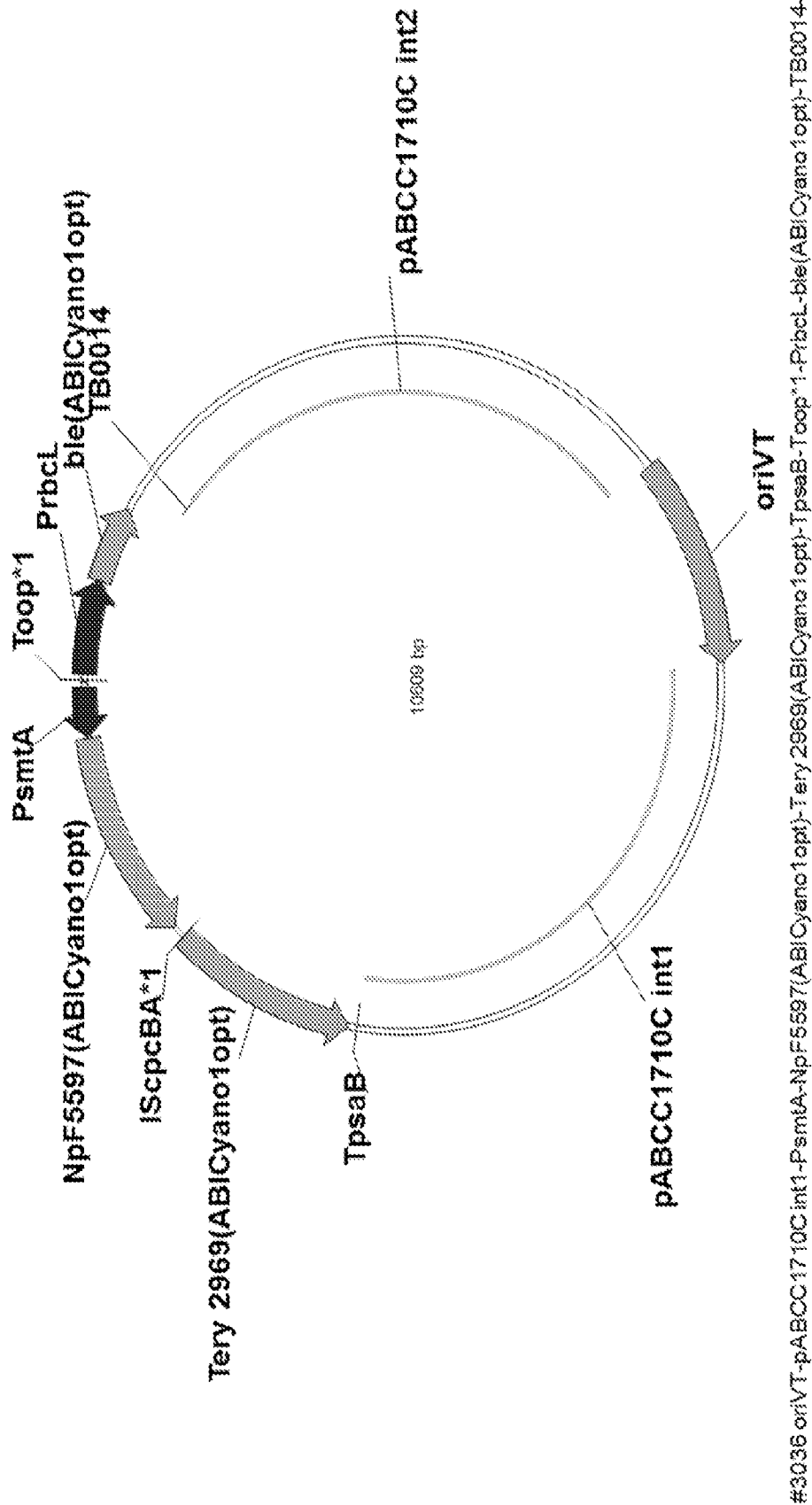


FIG. 65

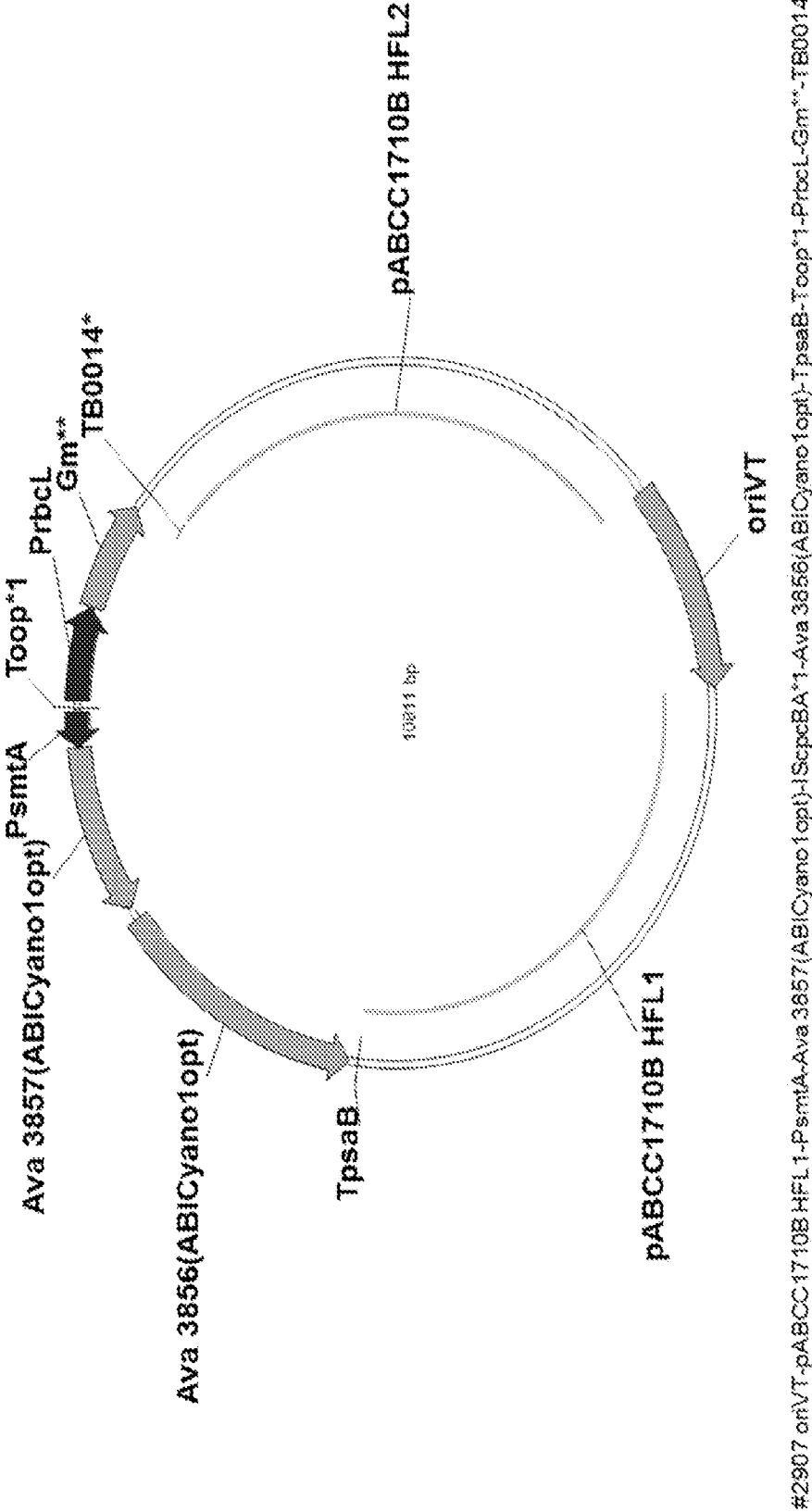


FIG. 66

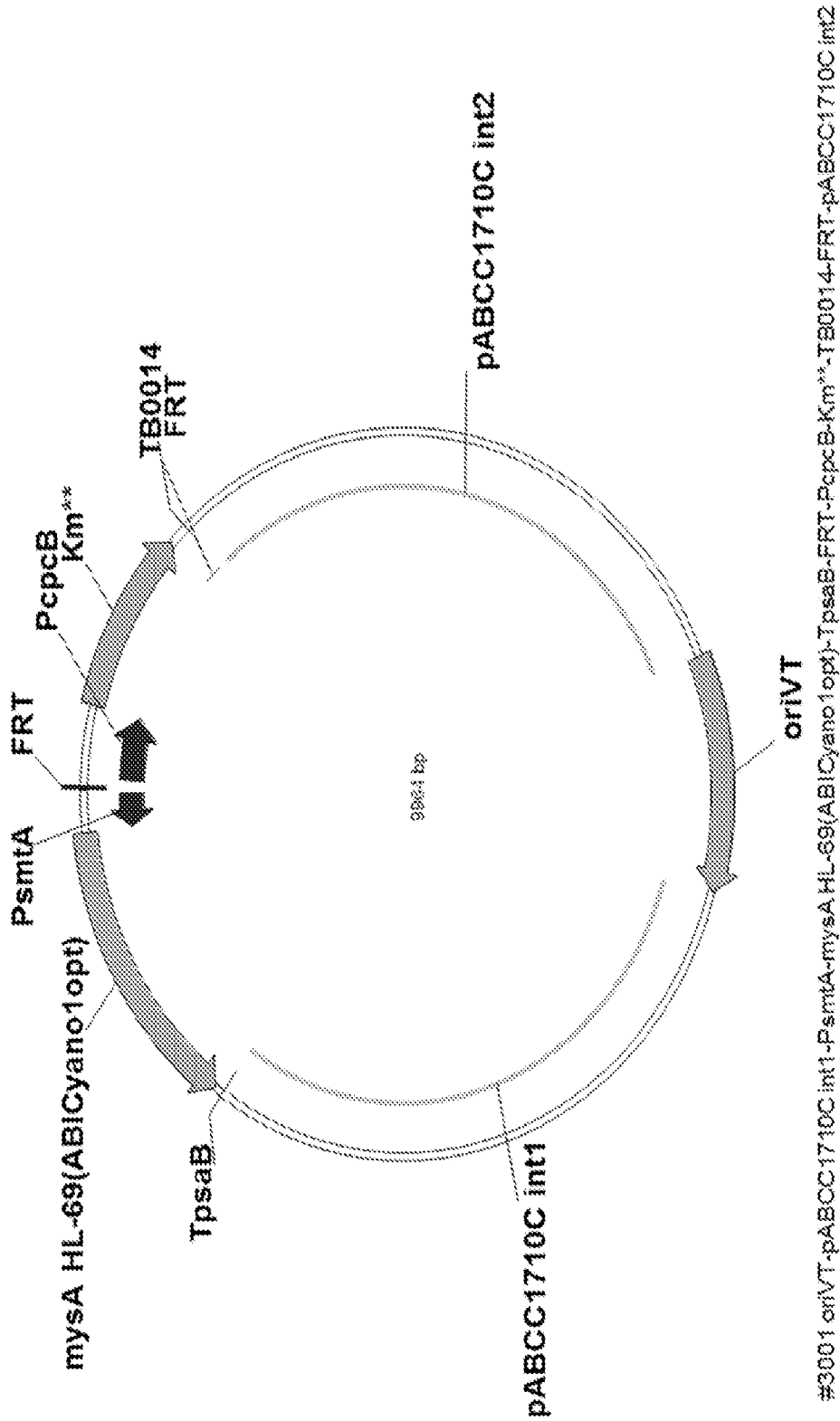


FIG. 67

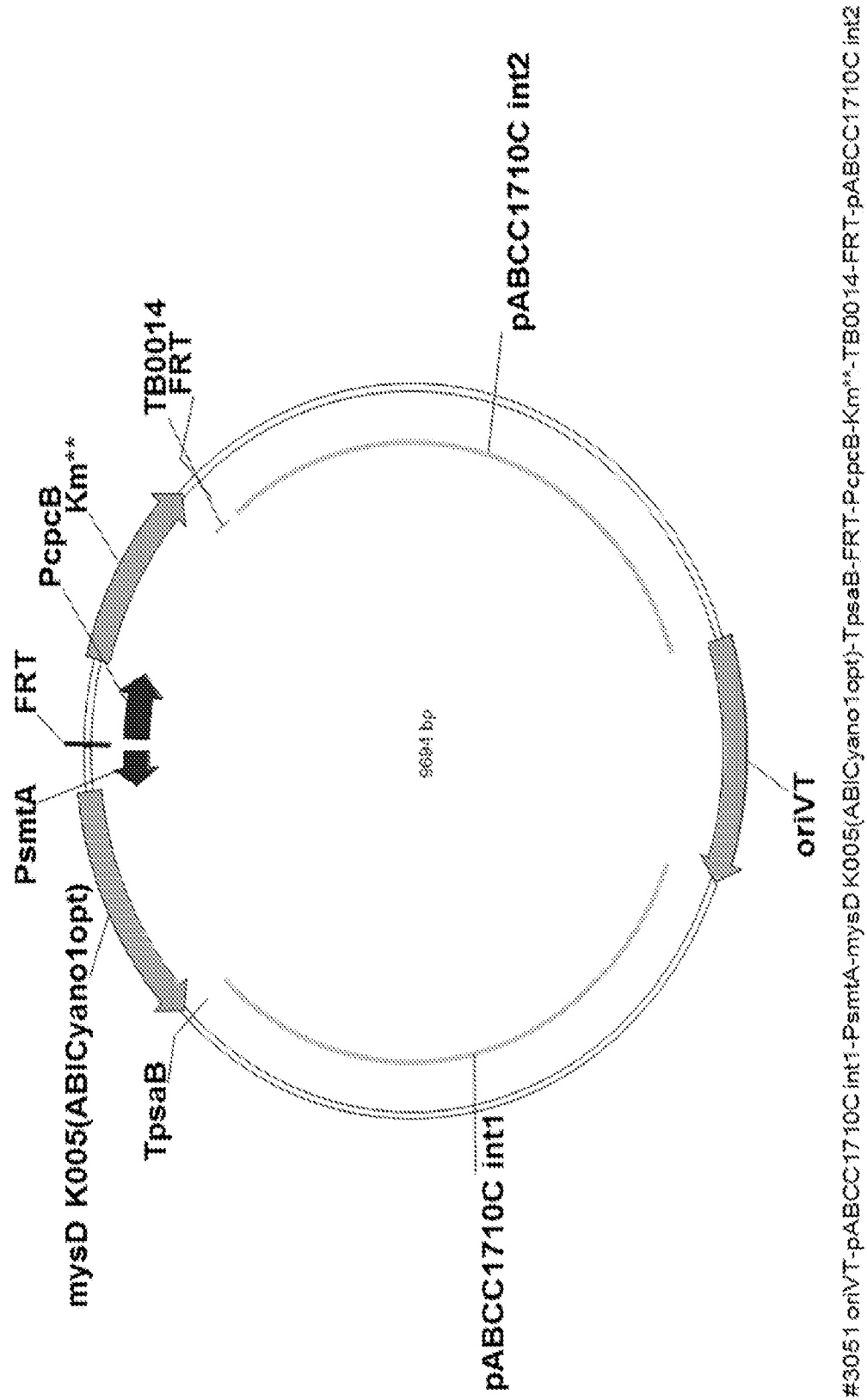


FIG. 68

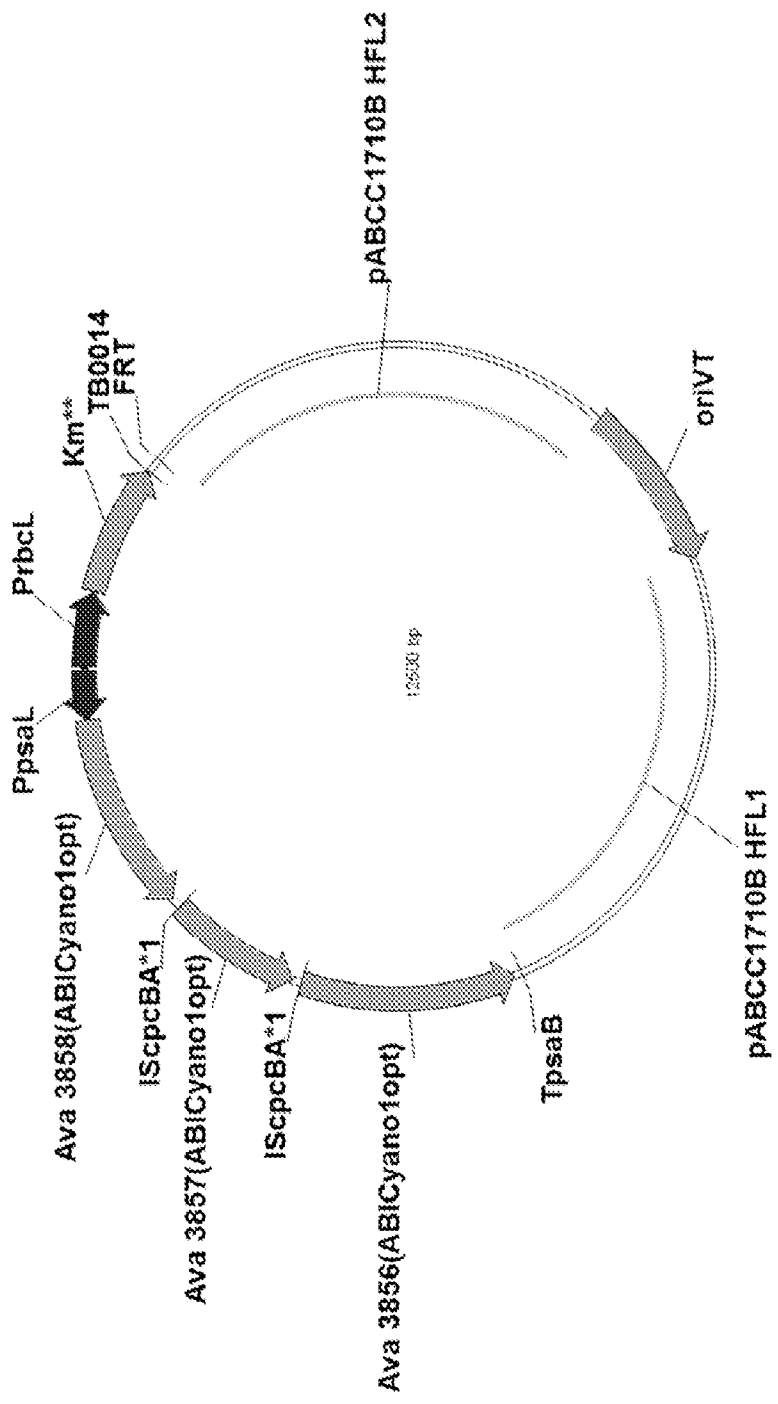


FIG. 69

#3038 oriVT-pABCC1710B HFL1-PpsaL-Ava 3858(ABICyano1opt)-IScpcBA*1-Ava 3857(ABICyano1opt)-IScpcBA*1-Ava 3856(ABICyano1opt)-TpsaB-Tpocp*1-Prbcl-Km**-TB0014-pABCC1710B HFL2

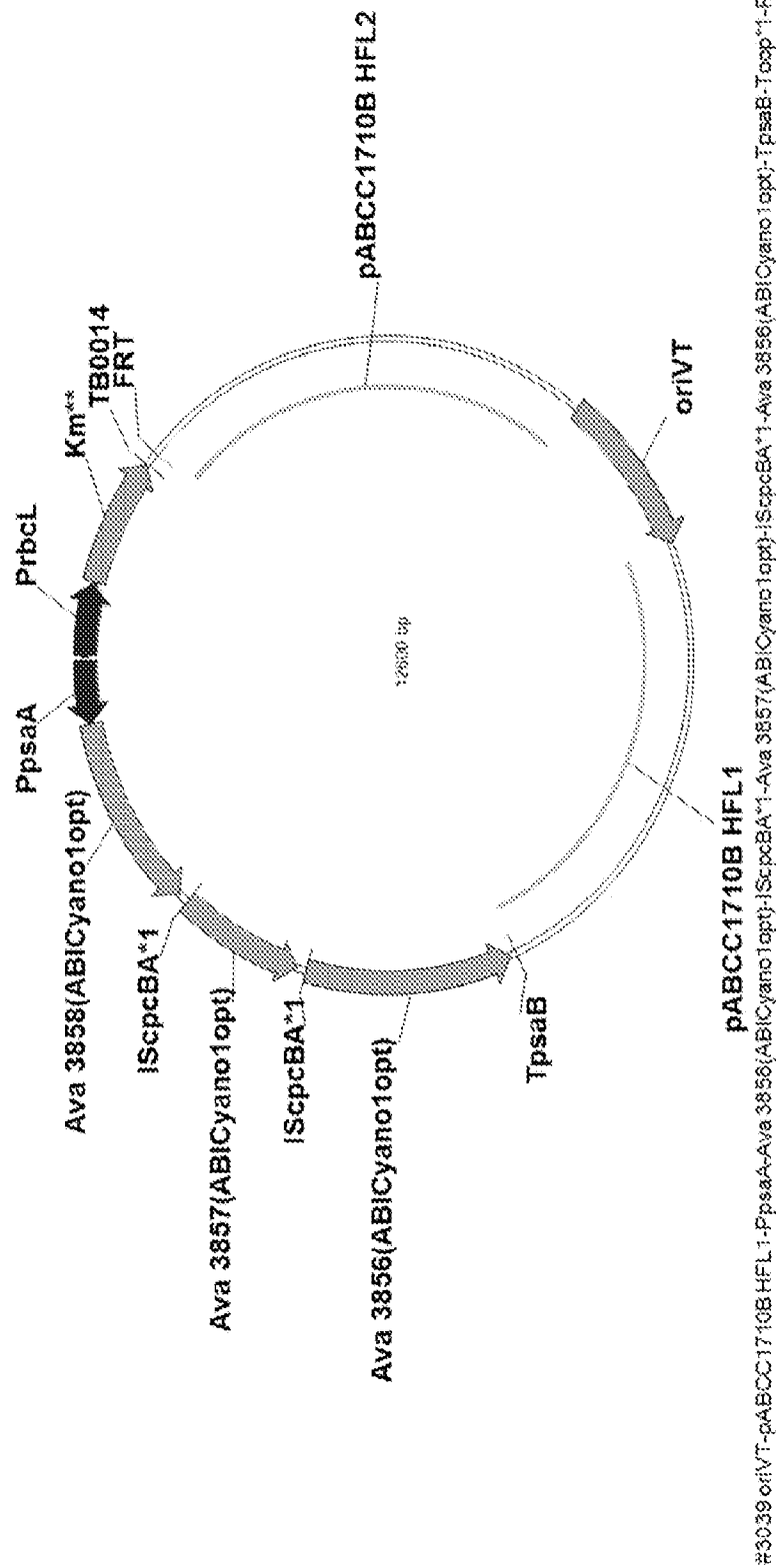


FIG. 70

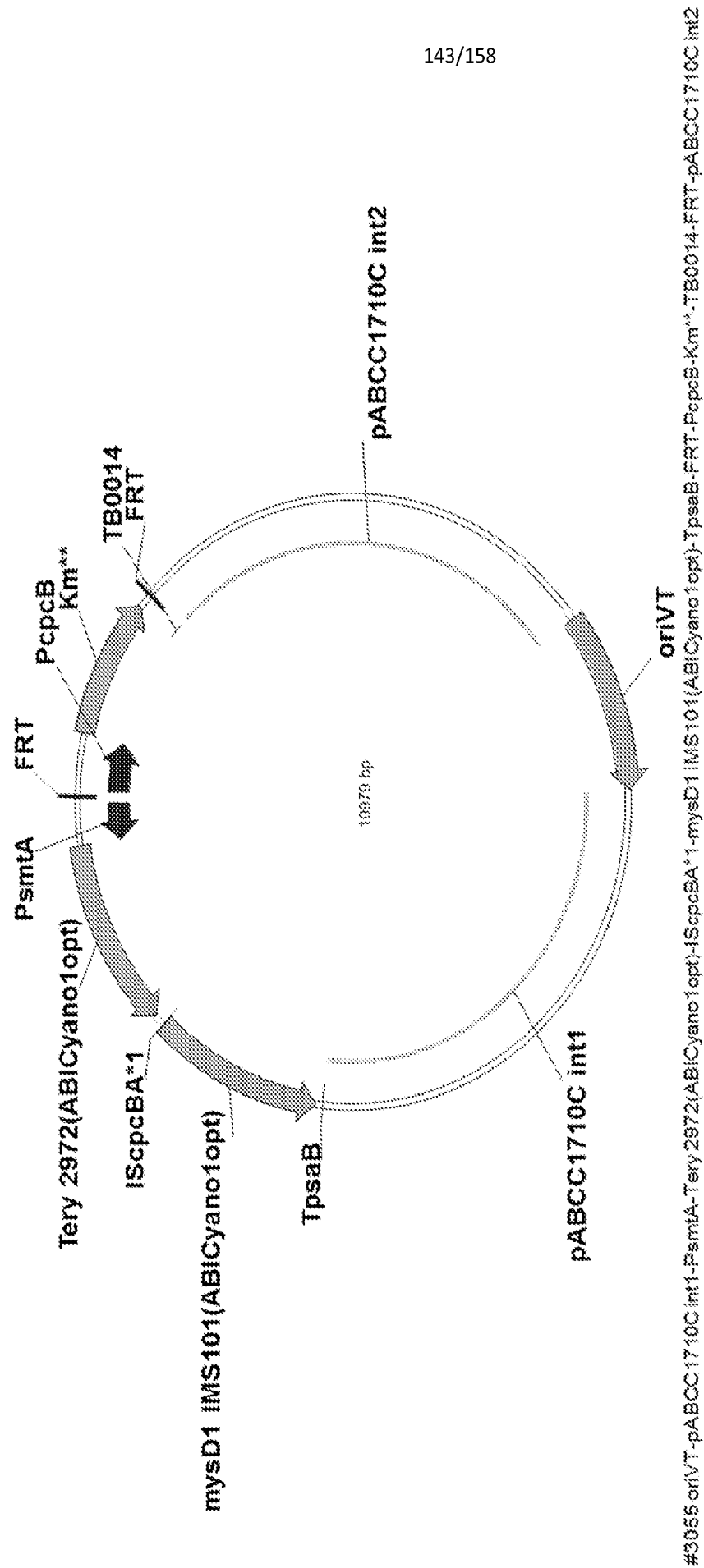


FIG. 71

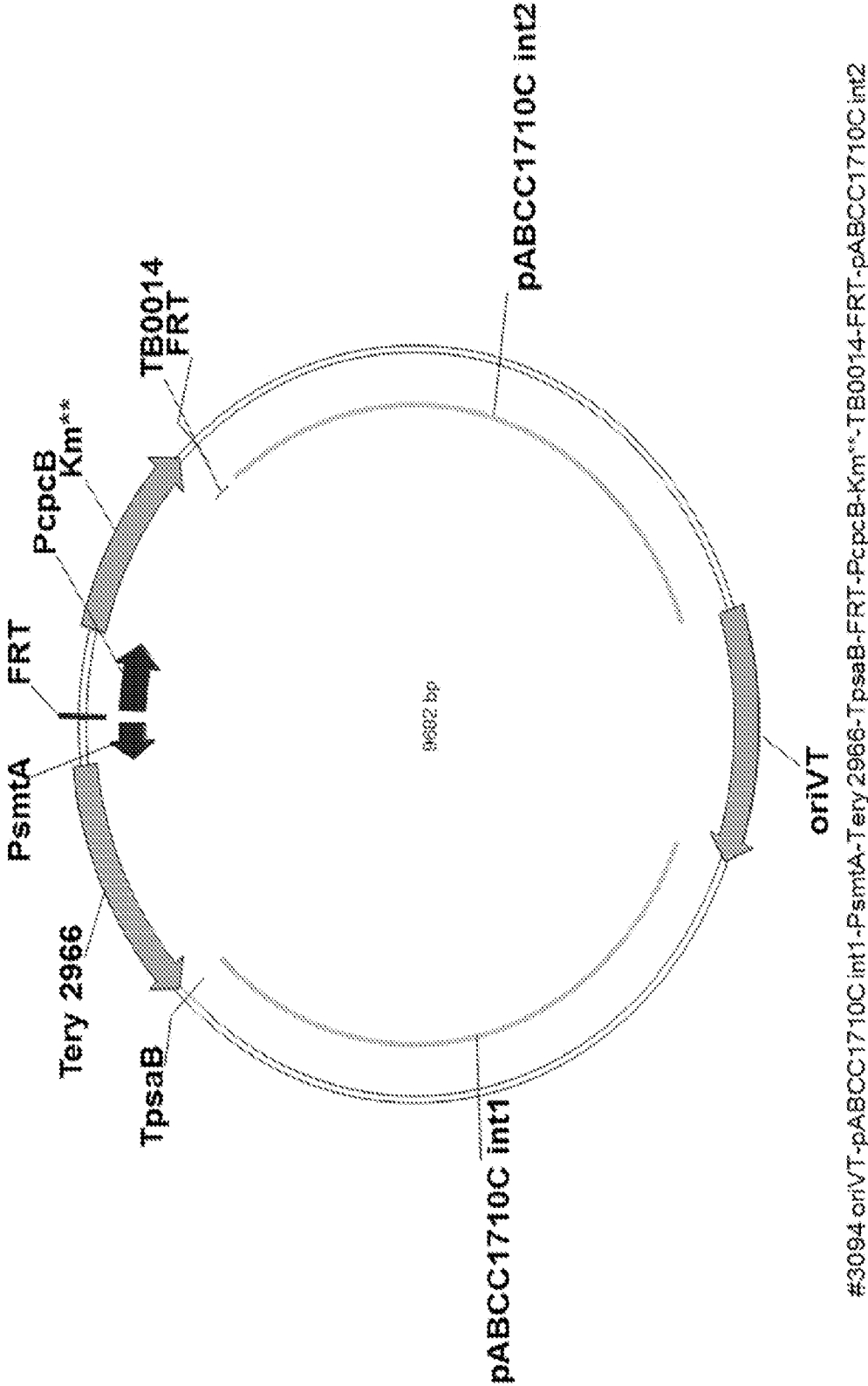


FIG. 72

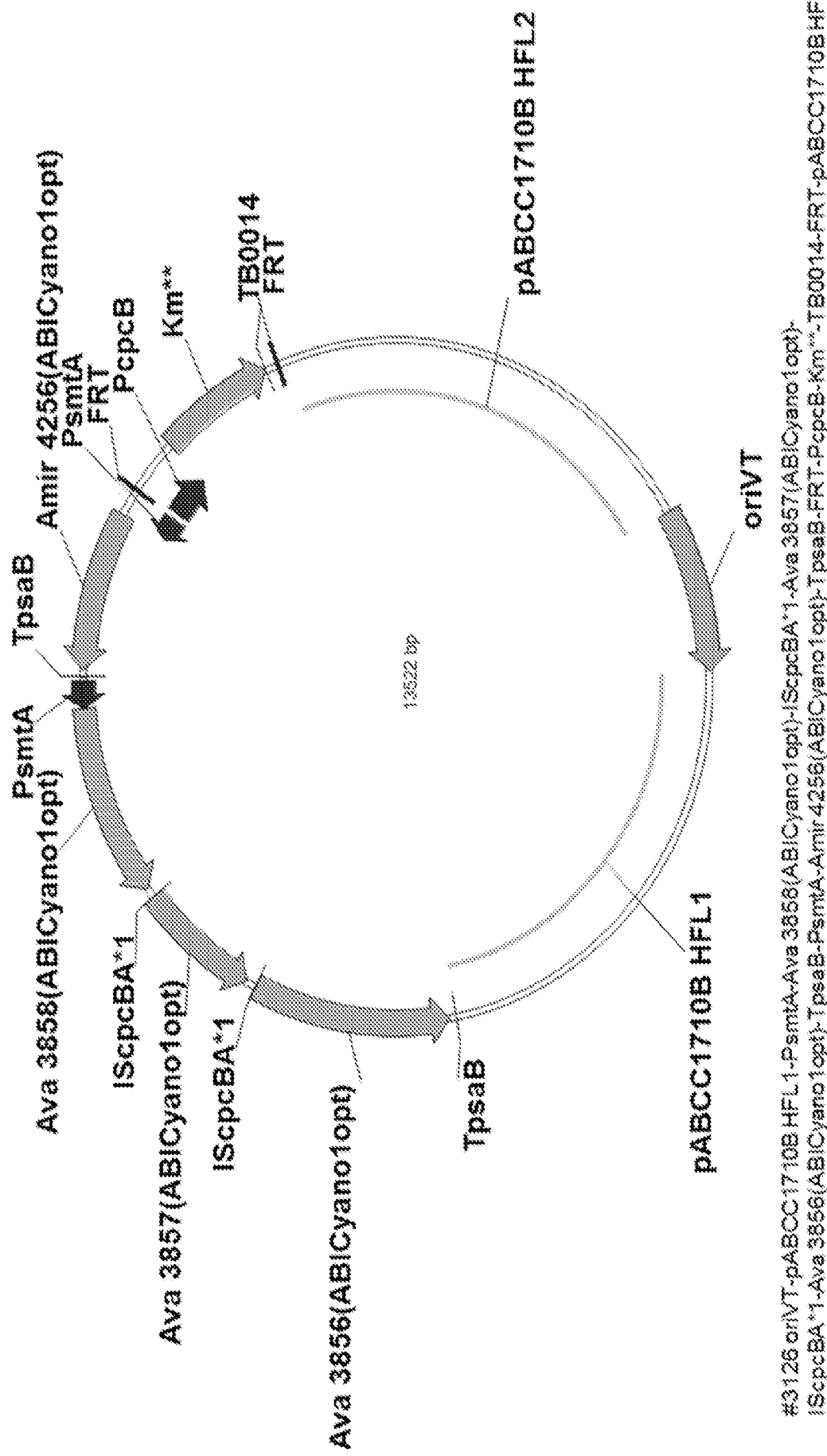


FIG. 73

#3126 oriVT-pABCC1710B HFL1-PsmA-Ava 3858(ABICyano1opt)-IScpBA*1-Ava 3857(ABICyano1opt)-IScpBA*1-Ava 3856(ABICyano1opt)-TpsaB-PsmA-Amir 4256(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710B HFL2

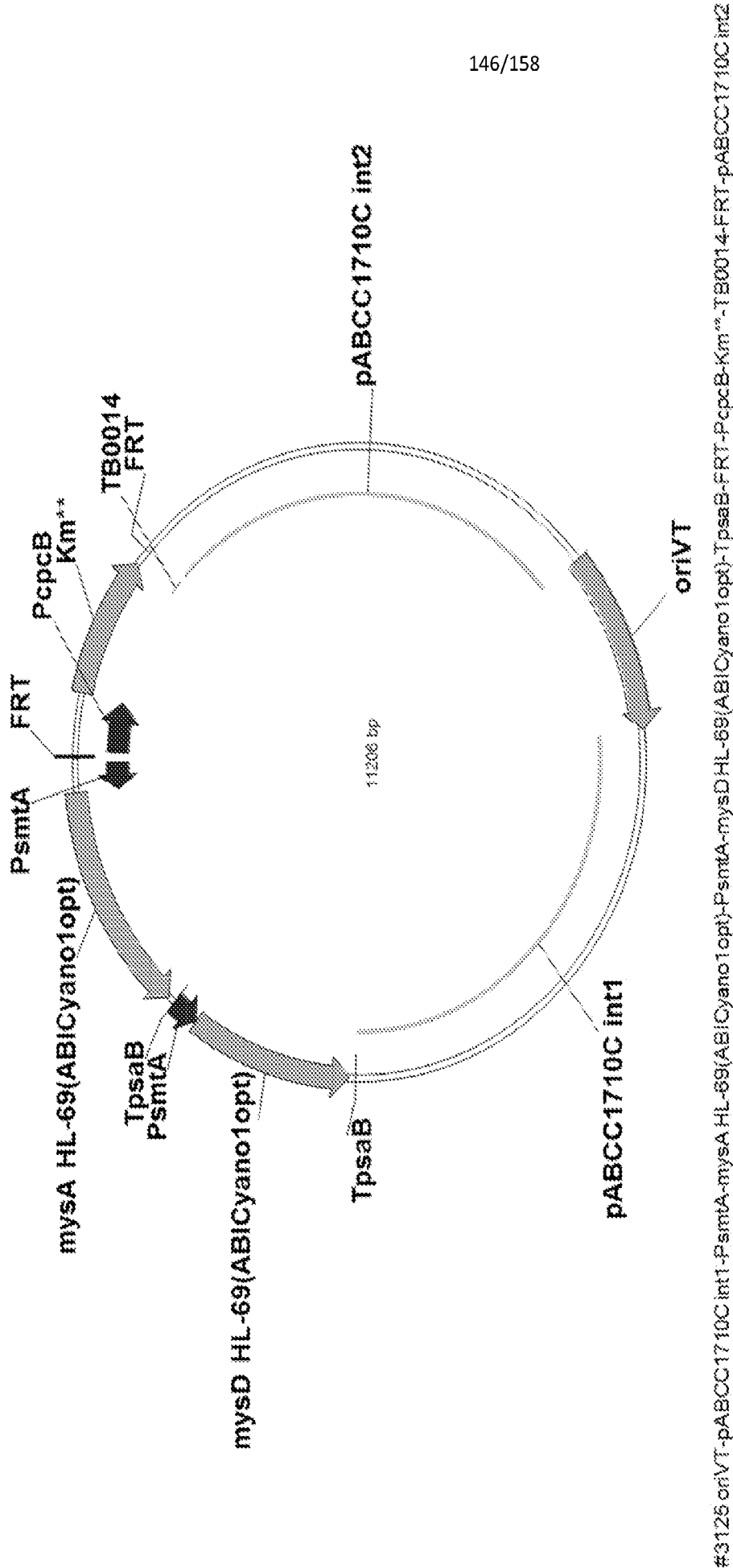


FIG. 74

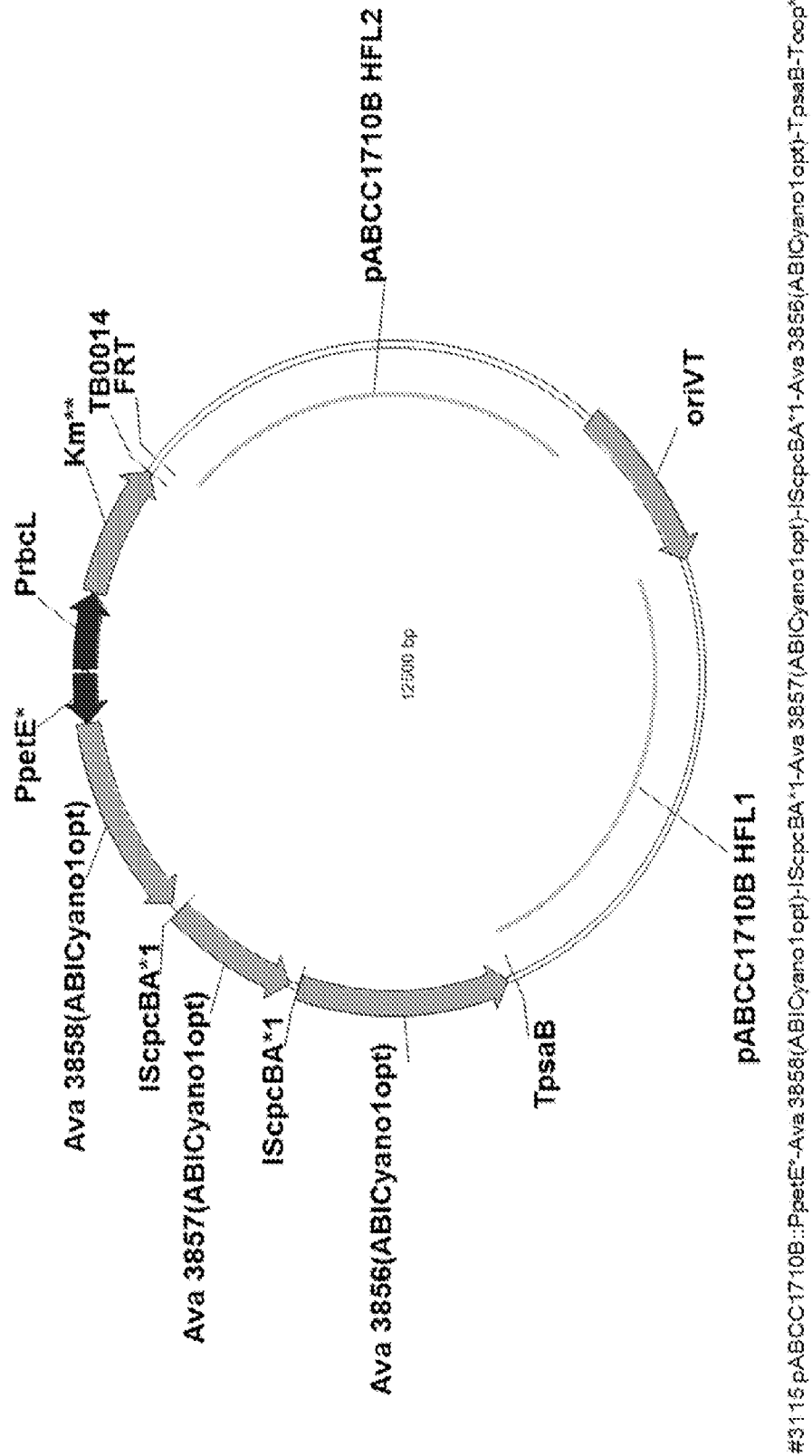


FIG. 75

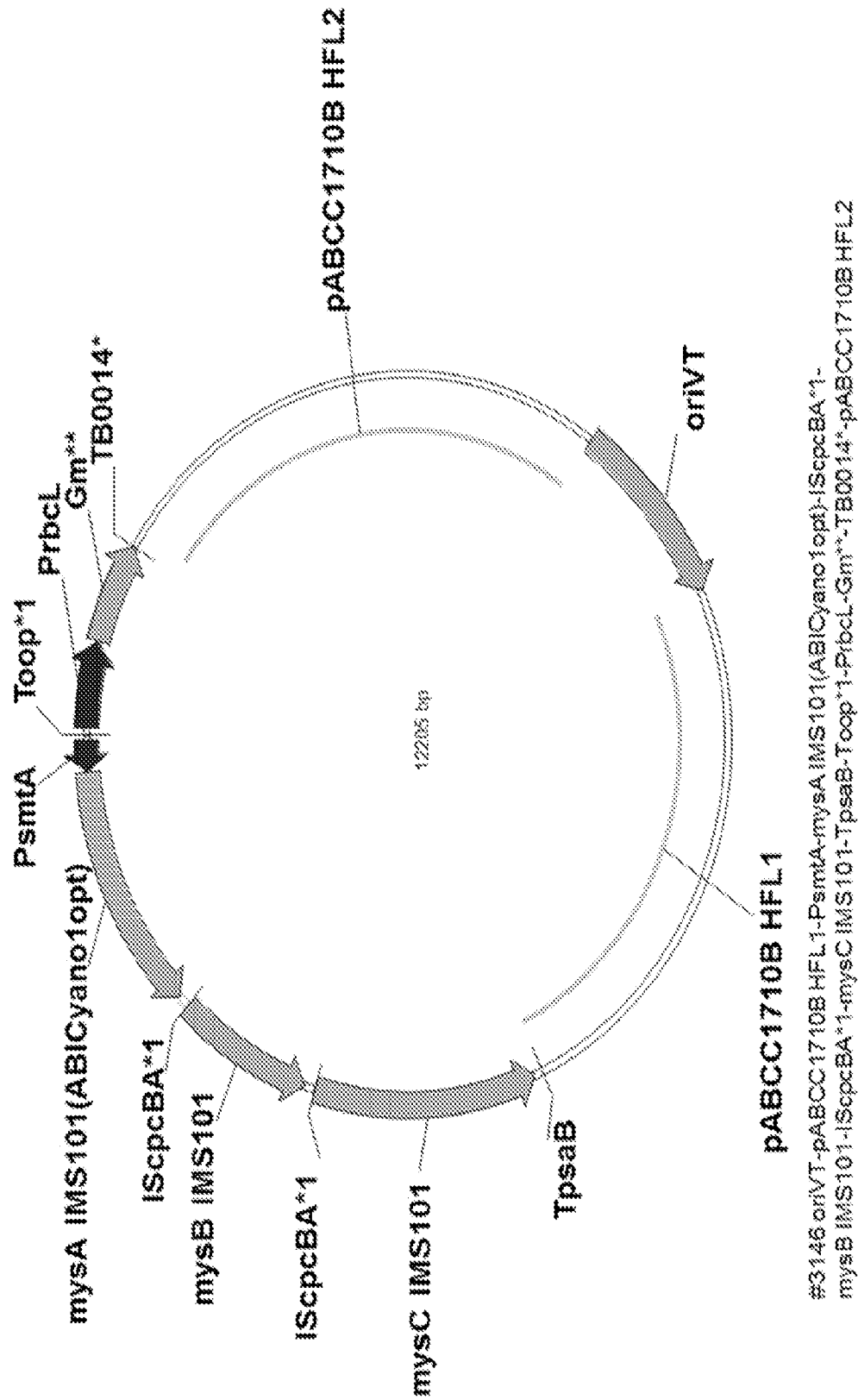


FIG. 76

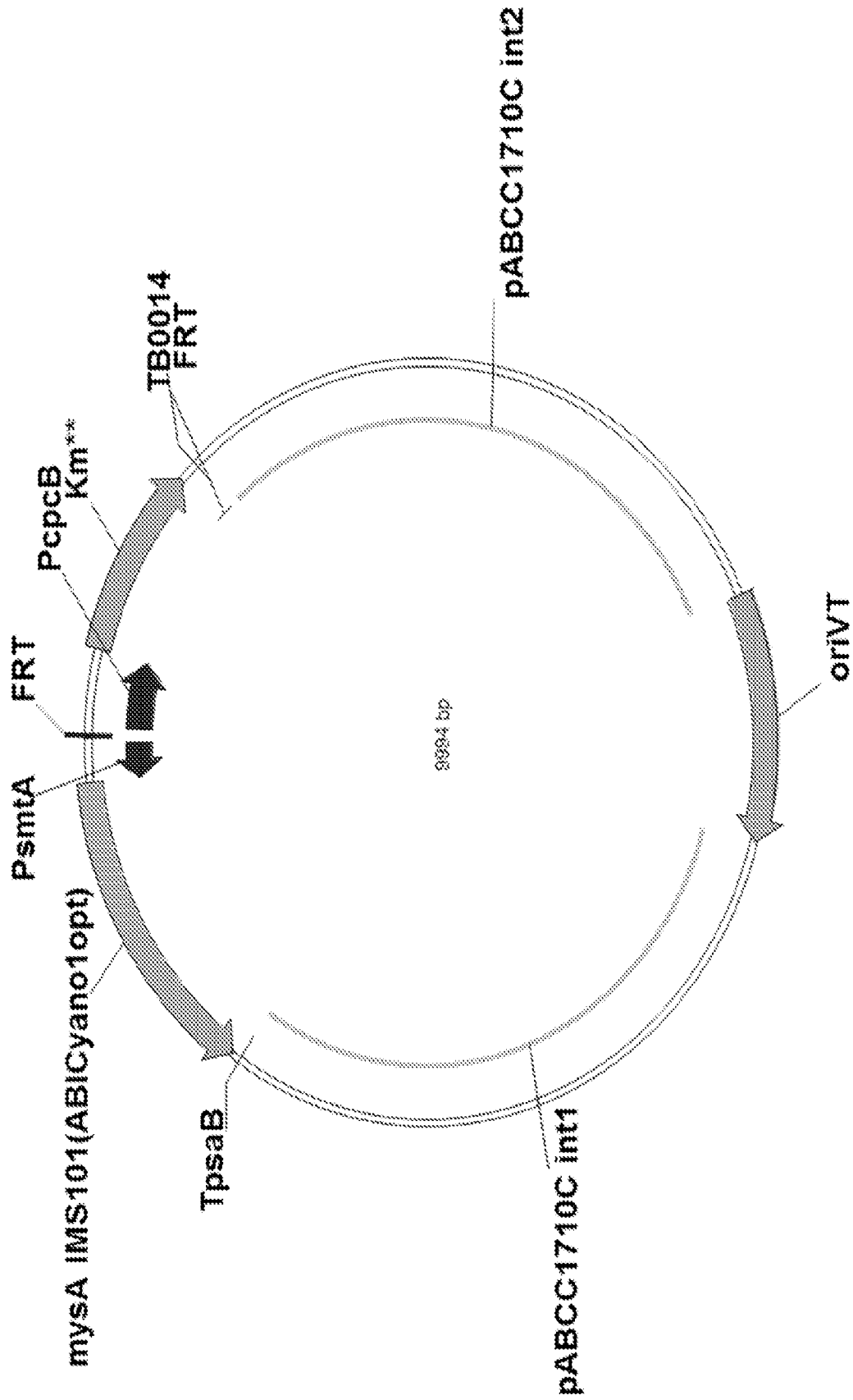


FIG. 77

#3002 oriVT-pABCC1710C int1-PsmTA-mysA IMS101(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

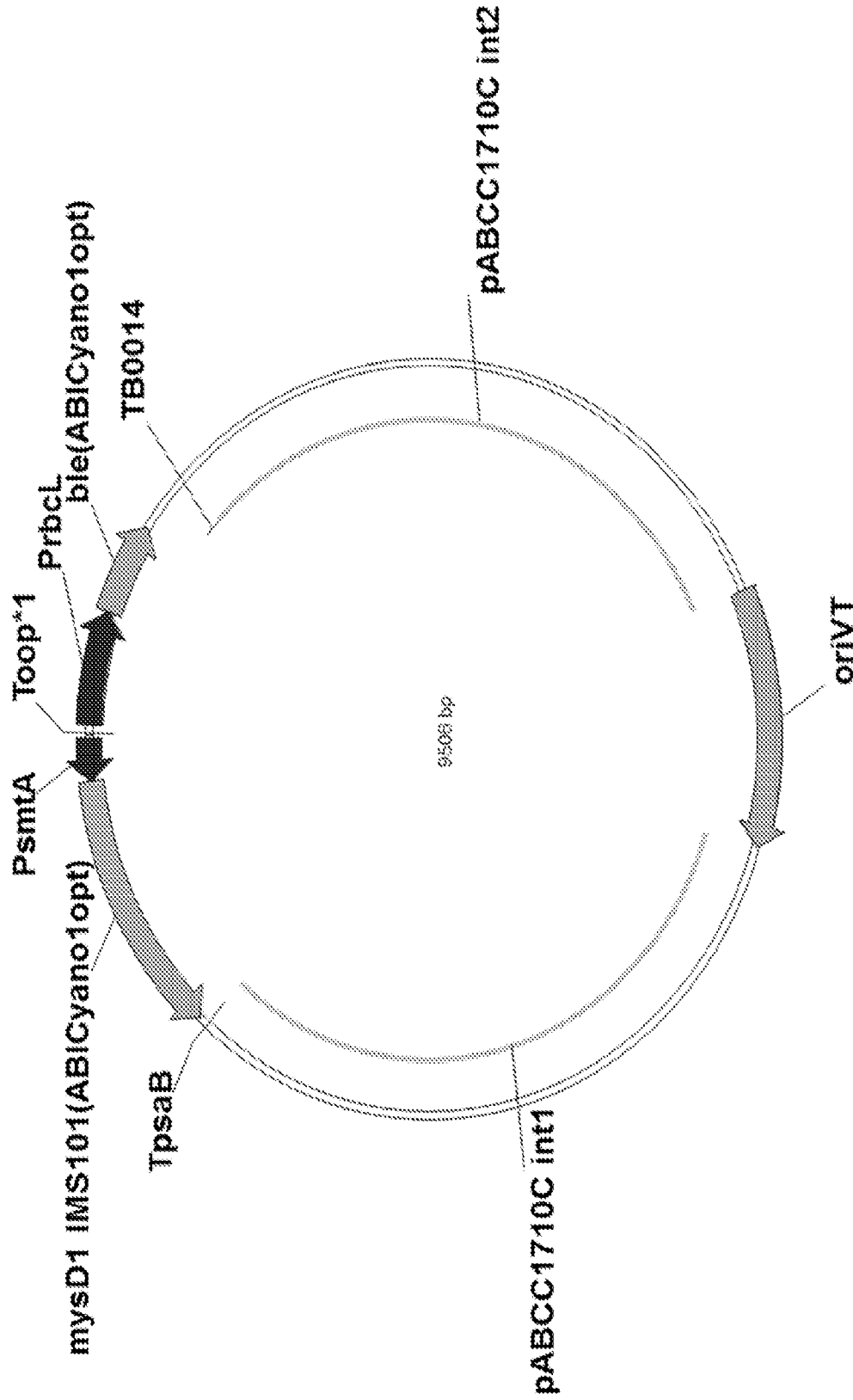
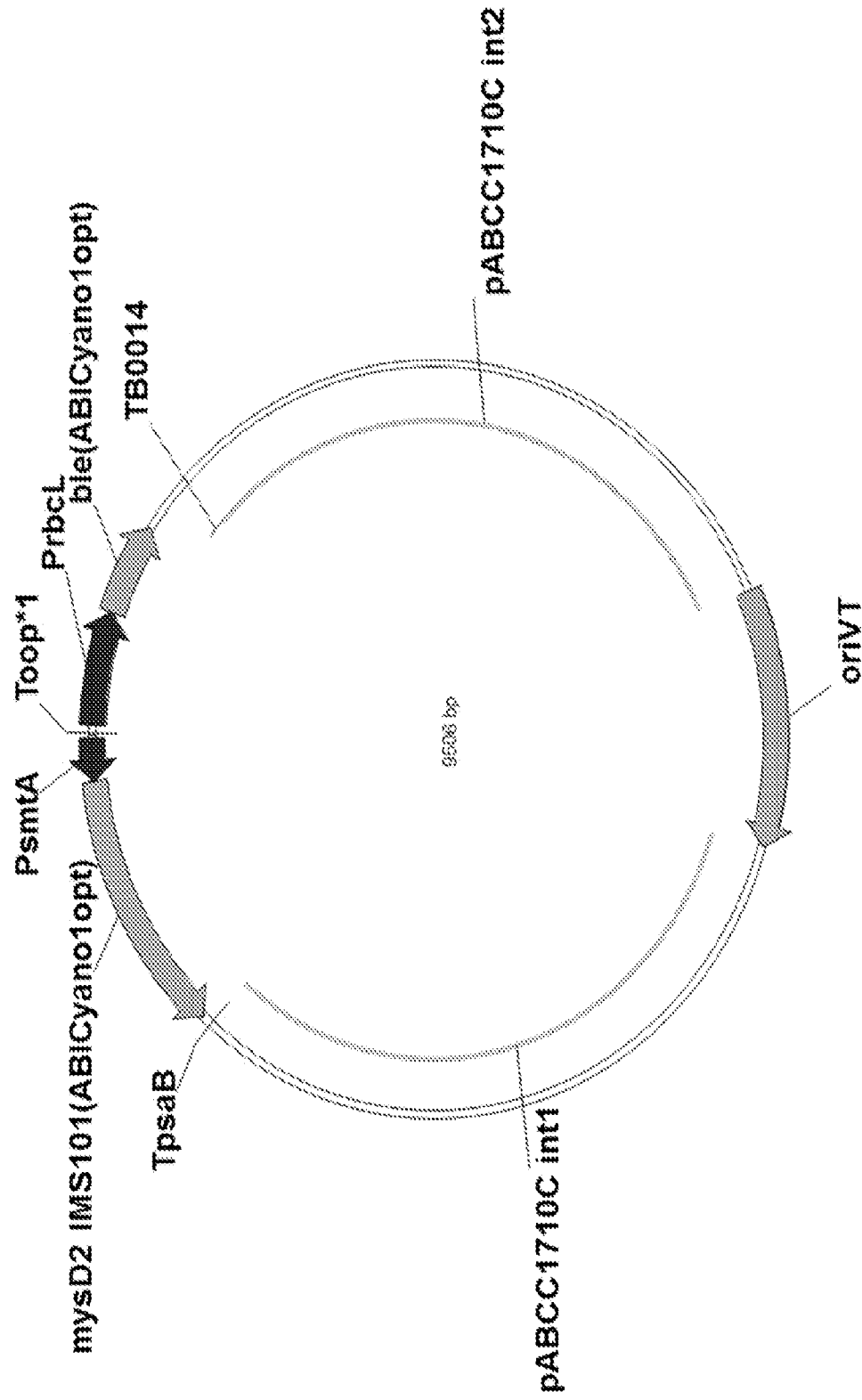


FIG. 78

#3034 oriVT-pABCC1710C int1-PsmtA-mysD1 IMS101(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710C int2



#3035 oriVT-pABCC1710C int1-PsmtA-mysD2 IMS101(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710C int2

FIG. 79

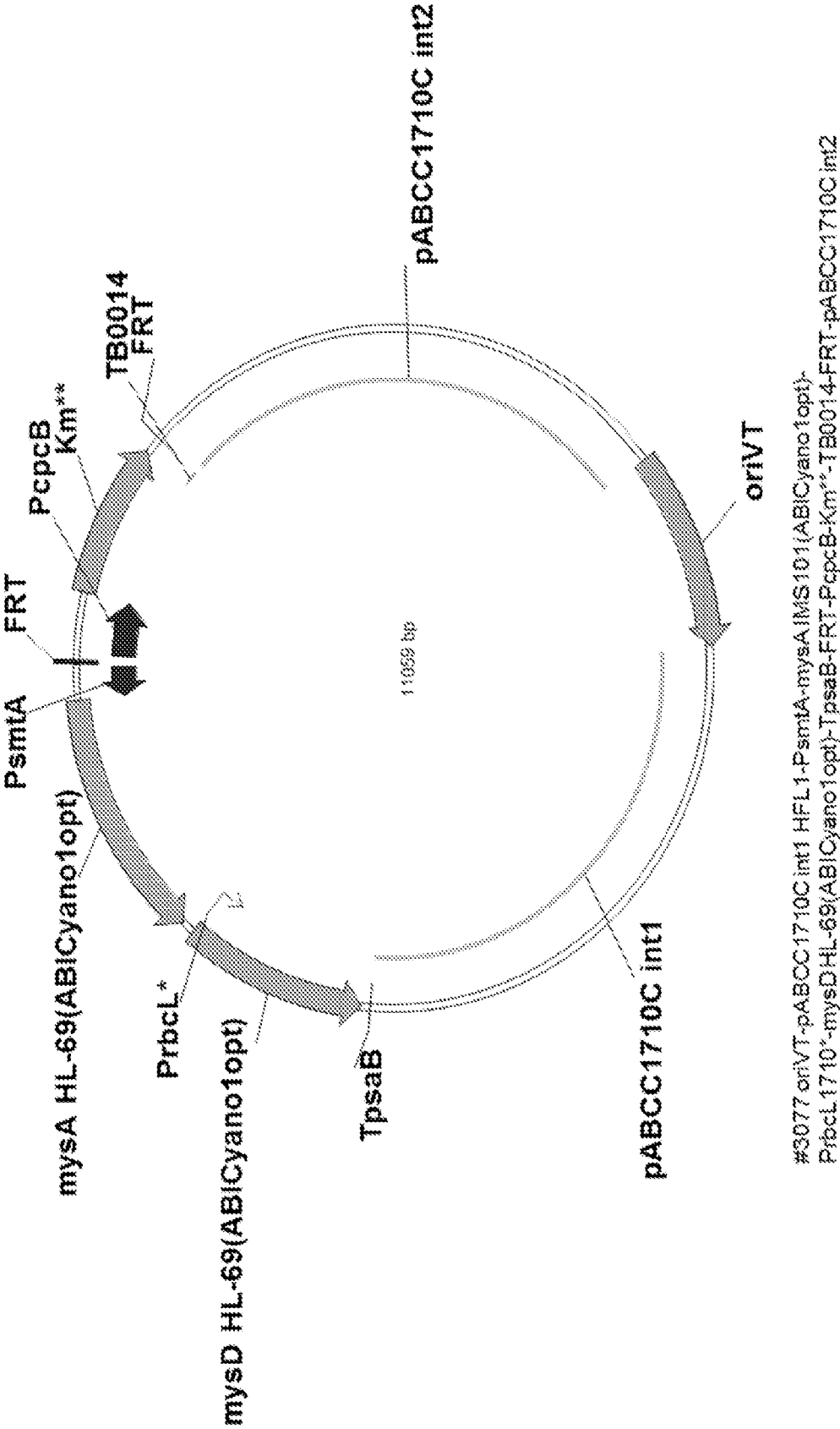


FIG. 80

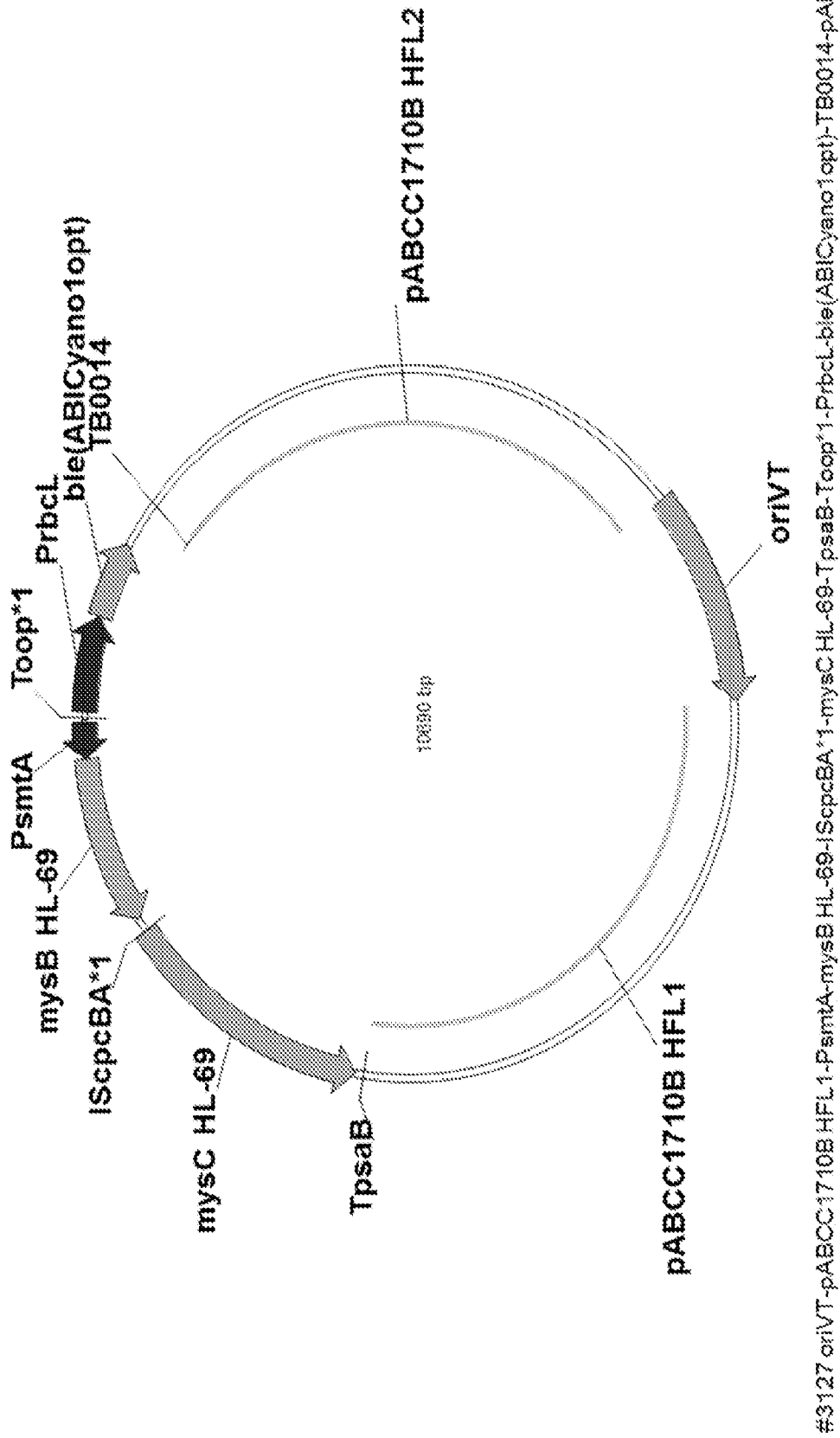


FIG. 81

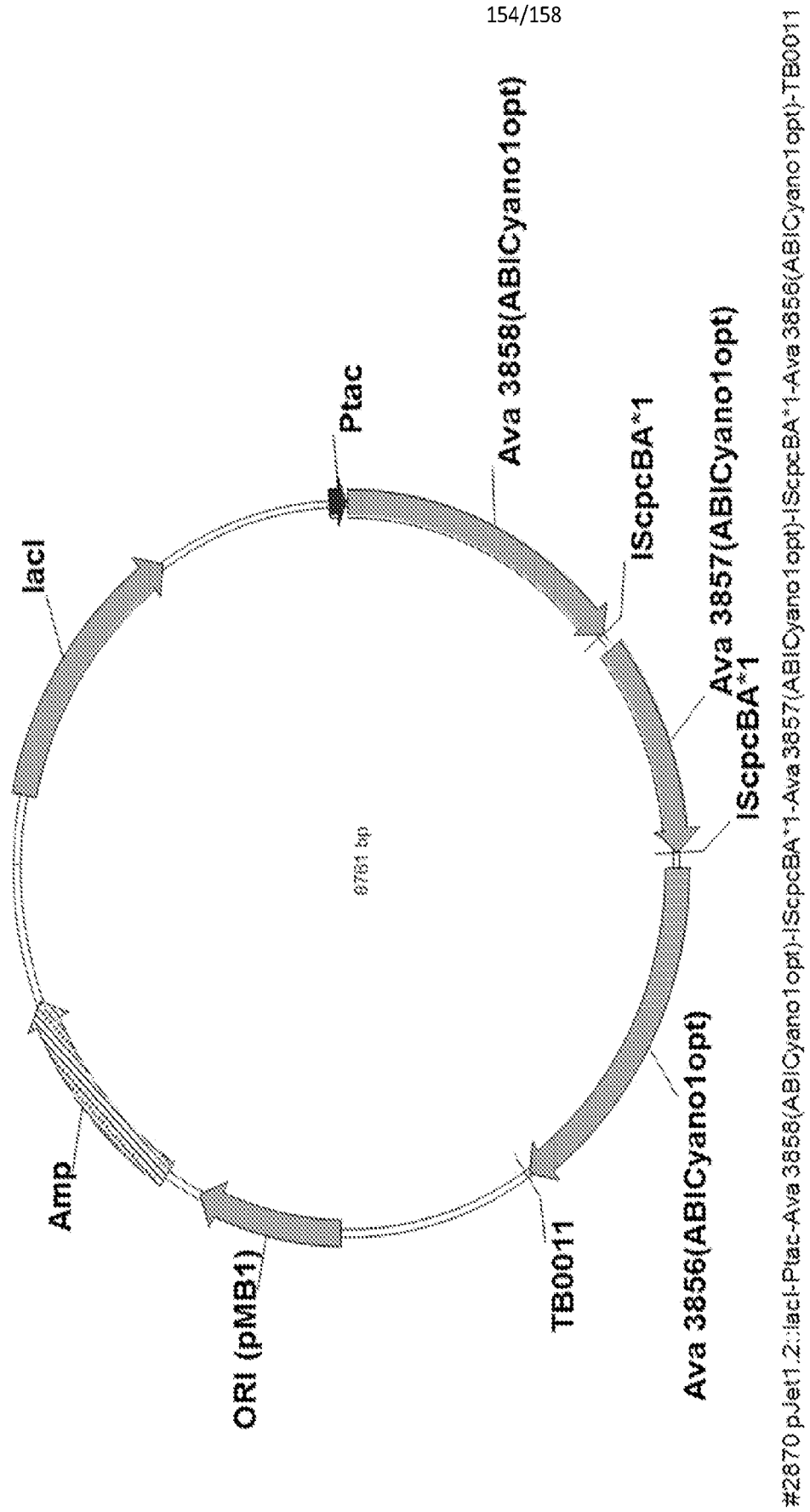


FIG. 82

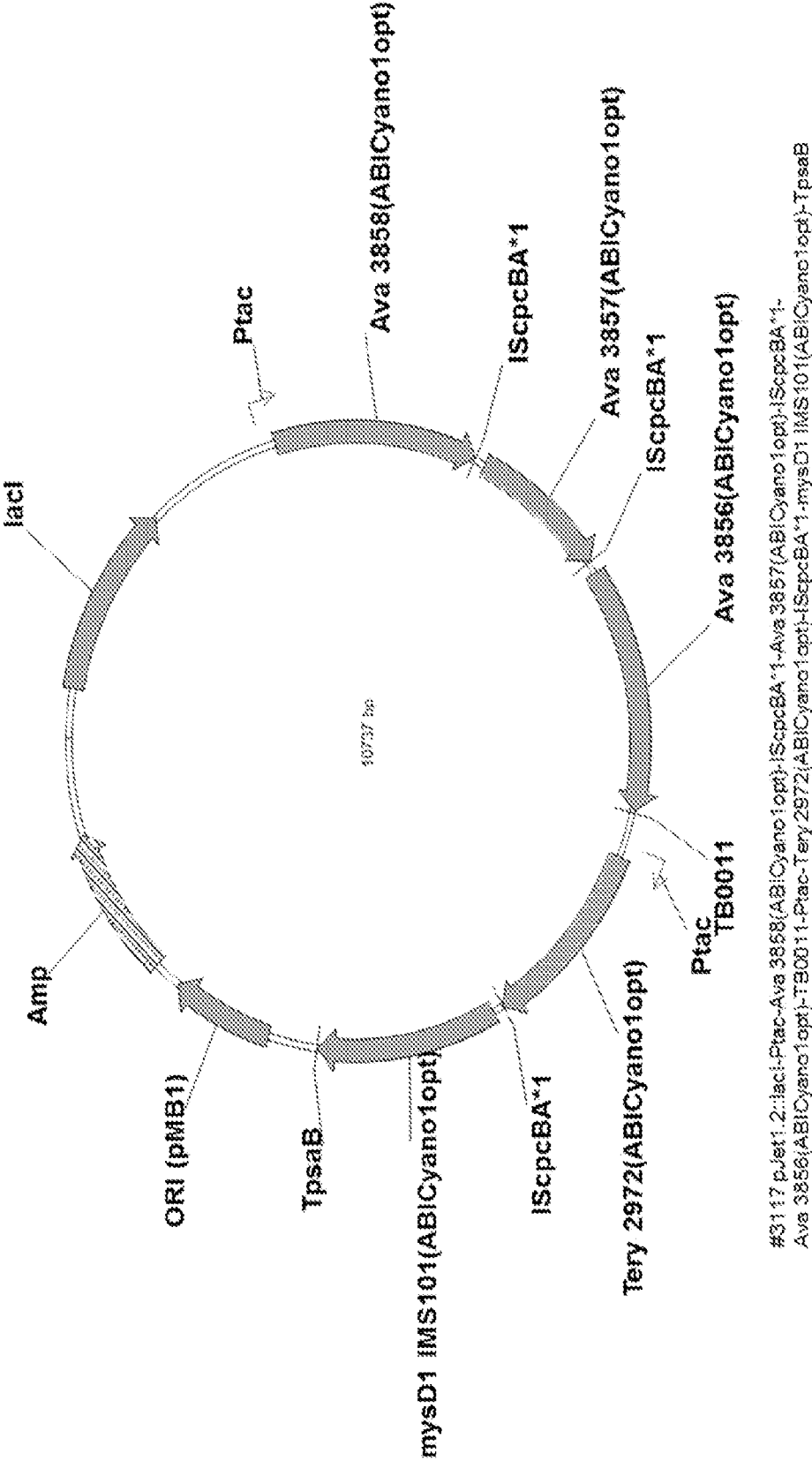


FIG. 83

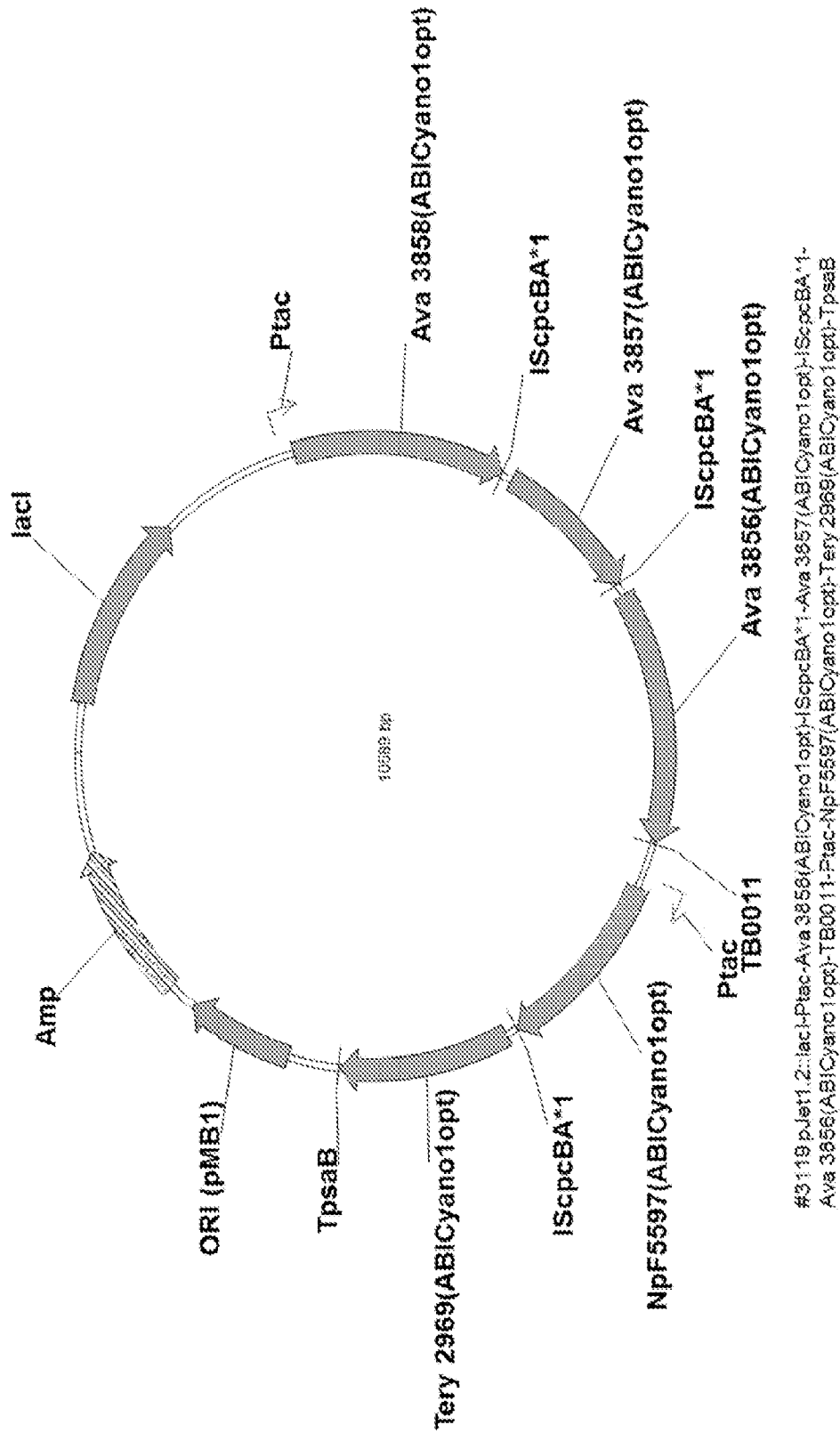


FIG. 84

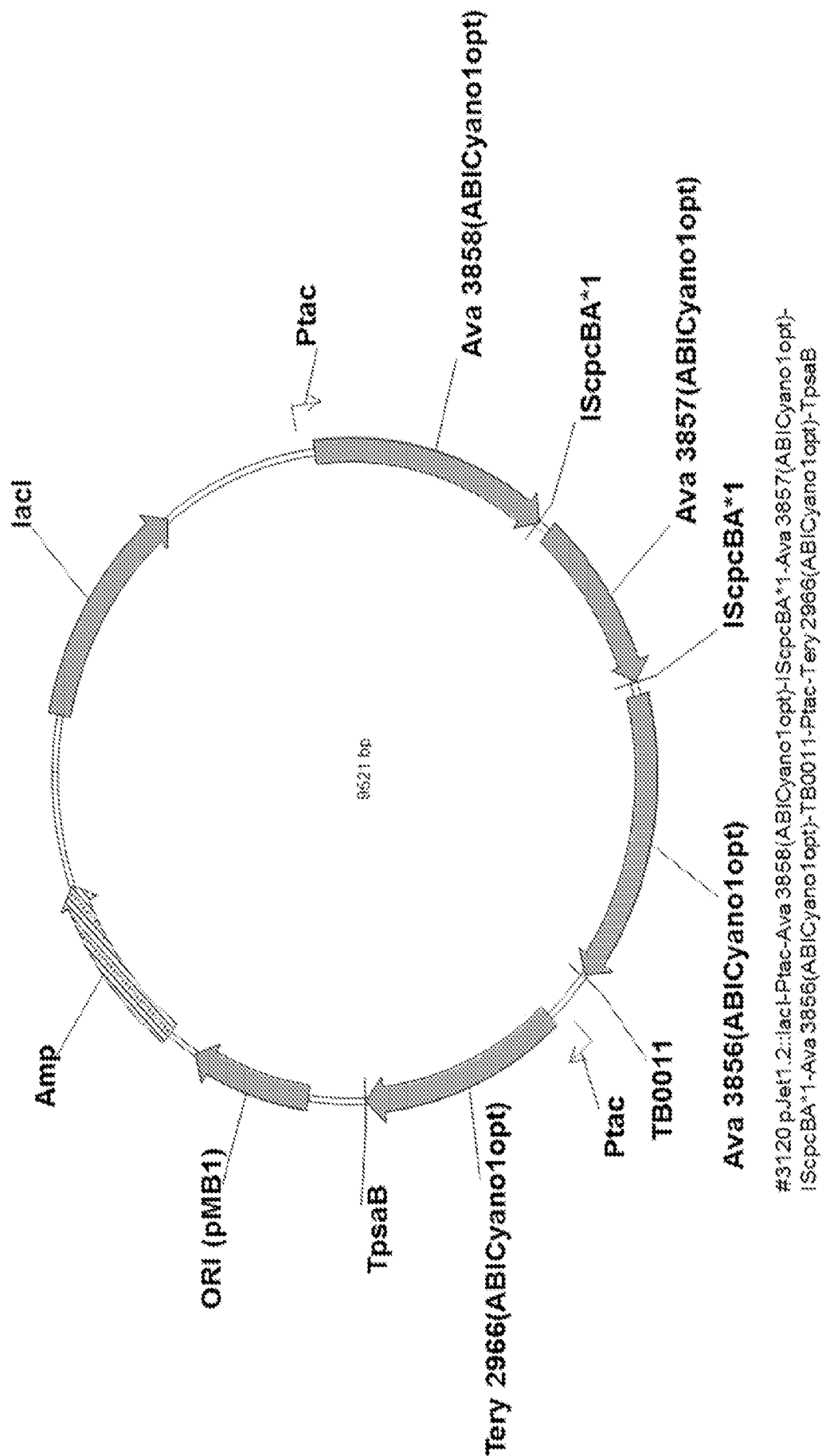


FIG. 85

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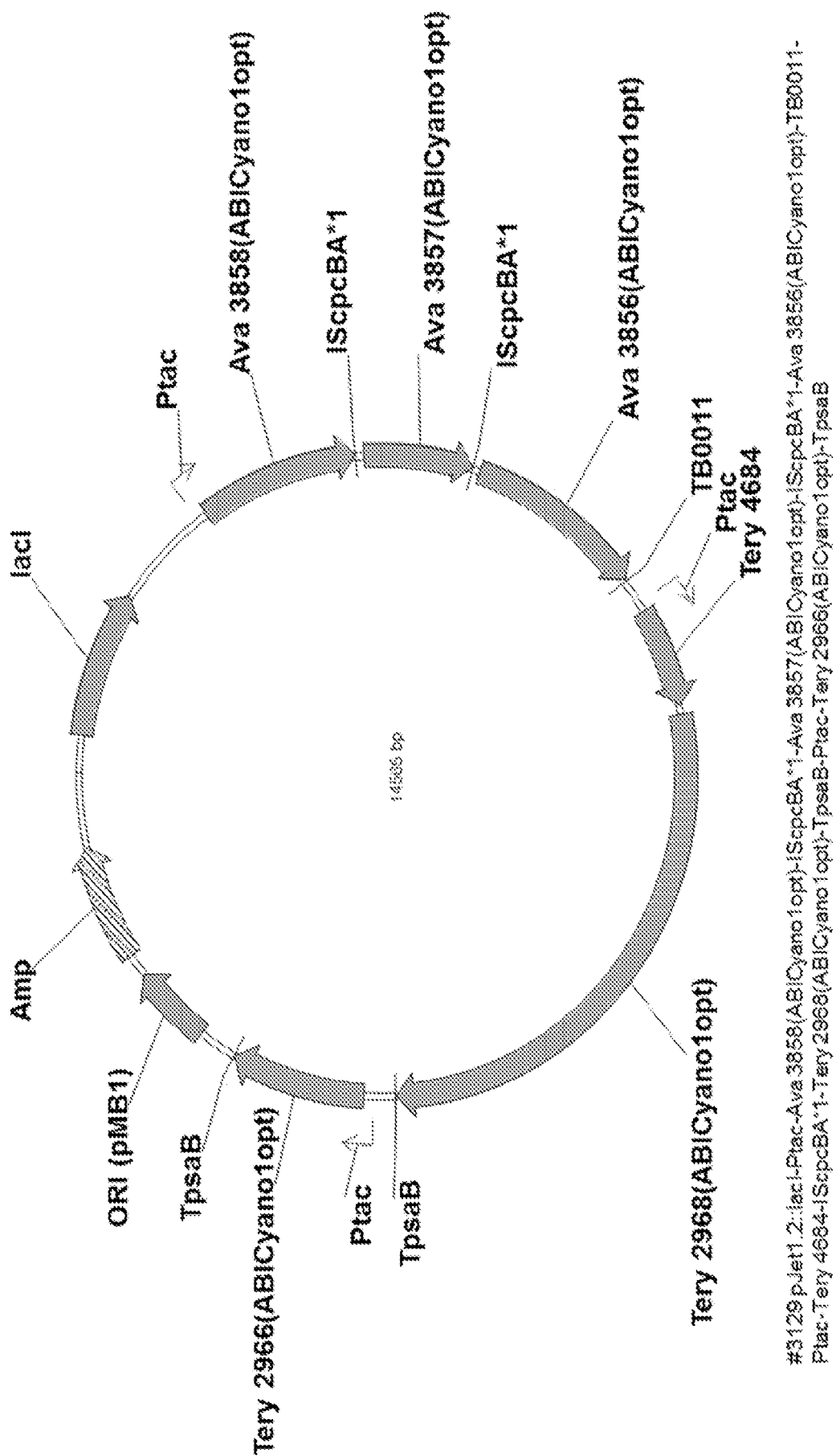


FIG. 86