(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 January 2025 (02.01.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/003421 A1

(51) International Patent Classification:
C12Q 1/6806 (2018.01)

(21) International Application Number:
PCT/EP2024/068277

(22) International Filing Date:
28 June 2024 (28.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/511,076 29 June 2023 (29.06.2023) US

(71) Applicant: **ILLUMINA, INC.** [US/US]; 5200 Illumina Way, San Diego, California 92122 (US).

(72) Inventors: **WEEKES, Dale**; Illumina, Inc., 5200 Illumina Way, San Diego, California 92122 (US). **MILLER, Oliver**; Illumina, Inc., 5200 Illumina Way, San Diego, California 92122 (US).

(74) Agent: **MARKS & CLERK LLP**; 15 Fetter Lane, London EC4A 1BW (GB).

(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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, 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).

Published:
— *with international search report (Art. 21(3))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

WO 2025/003421 A1

(54) **Title:** METHODS AND FORMULATIONS FOR DENATURING DNA

(57) **Abstract:** Disclosed herein are formulations and methods for denaturing DNA that utilize betaine as the denaturant. In some examples, the formulations include additives other than betaine, such as, for example, dimethyl sulfoxide (DMSO) and/or diethylene glycol (DEG).

METHODS AND FORMULATIONS FOR DENATURING DNA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/511,076, filed June 29, 2023 and entitled "Methods and Formulations for Denaturing DNA," the entire contents of which are incorporated by reference herein.

FIELD

[0002] This application relates to methods and formulations for denaturing DNA.

BACKGROUND

[0003] Formamide and sodium hydroxide are currently used in a number of sequencing systems to both denature the DNA library before sequencing and de-hybridise sequencing strands during clustering/resynthesis between reads However, neither formamide nor sodium hydroxide are ideal denaturing agents. Formamide is a hazardous substance. It is a suspected carcinogen and may cause birth defects. It, therefore, necessitates specialized disposal routes, hinders efforts to reach Corporate Social Responsibility goals, and is a key complaint from customers. Also, manual denaturation by NaOH increases the hands-on time of sequencing, is a potential source of human error, and requires the customer to purchase and store additional chemicals and equipment. Operator error during denaturation is a frequent issue for customers using sodium hydroxide and is a significant cause of customer run failures. Additionally, sodium hydroxide solution is hazardous to users because of its corrosive properties and cannot be used in cartridges without additional measures such as alkaline-resistant foils.

SUMMARY

[0004] Some examples herein provide a solution formulation for denaturing double-stranded DNA (dsDNA), the solution formulation including betaine; and at least one organic solvent.

[0005] In some examples, the at least one organic solvent includes diethylene glycol (DEG).

[0006] In some examples, a concentration of betaine in the solution formulation is between about 1M and about 5M, and a concentration of DEG in the solution formulation is between about 3% and about 40%.

[0007] In some examples, the concentration of DEG in the solution formulation is between about 20% and about 40%. In some examples, the concentration of DEG in the solution formulation is between about 20% and about 30%. In some examples, the concentration of DEG in the solution formulation is about 20%.

[0008] In some examples, the concentration of betaine in the solution formulation is between about 2M and about 5M. In some examples, the concentration of betaine in the solution formulation is between about 2M and about 4M. In some examples, the concentration of betaine in the solution formulation is about 2M. In some examples, the concentration of betaine in the solution formulation is about 3M.

[0009] In some examples, the at least one organic solvent includes diethylene glycol (DEG) and dimethyl sulfoxide (DMSO).

[0010] In some examples, a concentration of betaine in the solution formulation is between about 1M and about 5M, and a concentration of the DEG in the solution formulation is between about 3% and about 40%.

[0011] In some examples, a concentration of DMSO in the solution formulation is between about 5% and about 30%. In some examples, the concentration of the DMSO in the solution formulation is between about 7% and about 12%. In some examples, the concentration of DMSO in the solution formulation is about 10%. In some examples, the concentration of DMSO in the solution formulation is between about 17% and about 22%. In some examples, the concentration of DMSO in the solution formulation is about 20%.

[0012] In some examples, the concentration of the DEG in the solution formulation is between about 5% and about 25%. In some examples, the concentration of the DEG in the solution formulation is between about 13% and about 17%. In some examples, the concentration of the DEG in the solution formulation is about 15%.

[0013] In some examples, the concentration of betaine in the solution formulation is between about 2M and about 3M. In some examples, the concentration of betaine in the solution

formulation is about 2M. In some examples, the concentration of betaine in the solution formulation is about 2.5M. In some examples, the concentration of betaine in the solution formulation is about 3M.

[0014] In some examples, the at least one organic solvent includes DMSO.

[0015] In some examples, a concentration of betaine in the solution formulation is between about 1M and about 5M. In some examples, the concentration of betaine in the solution formulation is between about 2M and about 3M. In some examples, the concentration of betaine in the solution formulation is about 2M. In some examples, the concentration of betaine in the solution formulation is about 2.5M. In some examples, the concentration of betaine in the solution formulation is about 3M.

[0016] In some examples, a concentration of DMSO in the solution formulation is between about 5% and about 30%. In some examples, the concentration of the DMSO in the solution formulation is between about 7% and about 12%. In some examples, the concentration of DMSO in the solution formulation is about 10%. In some examples, the concentration of DMSO in the solution formulation is between about 17% and about 22%. In some examples, the concentration of DMSO in the solution formulation is about 20%.

[0017] In some examples, any of the solution formulations further include a zwitterionic buffer. In some examples, the zwitterionic buffer produces a pH in the solution formulation at a range of between about 9.5 and about 11.5. In some examples, the zwitterionic buffer includes any one or more of 4-(cyclohexylamino)-1-butanesulfonic acid (CABS), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO), *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS), and 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate) (CHAPS).

[0018] Some examples herein provide a method of denaturing double-stranded DNA (dsDNA), the method including: contacting the dsDNA in a fluid-filled container with a denaturant including betaine.

[0019] In some examples, a ratio of denaturant to the dsDNA is between about 1:1 and about 3:1. In some examples, a ratio of denaturant to the dsDNA is about 1:1. In some examples, the ratio of the denaturant to the dsDNA is about 1.3:1. In some examples, the ratio of the denaturant to the dsDNA is about 1.5:1. In some examples, the ratio of the denaturant to the

dsDNA is about 2:1. In some examples, the ratio of the denaturant to the dsDNA is about 2.5:1. In some examples, the ratio of the denaturant to the dsDNA is about 3:1.

[0020] In some examples, the denaturant further includes diethylene glycol (DEG).

[0021] In some examples, the denaturant further includes dimethyl sulfoxide (DMSO).

[0022] In some examples, the denaturant further includes a zwitterionic buffer. In some examples, the zwitterionic buffer produces a pH in the fluid-filled container between about 9.5 and about 11.5.

[0023] Some examples herein provide a kit for denaturing double-stranded DNA (dsDNA), the kit including: a denaturant including betaine and diethylene glycol (DEG) in a solution; and a buffer.

[0024] In some examples, a concentration of DEG in the solution is between about 20% and about 40%.

[0025] In some examples, the denaturant further includes dimethyl sulfoxide (DMSO).

[0026] In some examples, the concentration of DEG in the solution is between about 5% and about 20%.

[0027] In some examples, a concentration of betaine in the solution is between about 1M and 5M. In some examples, the concentration of betaine in the solution is between about 2M and about 3.5M. In some examples, the concentration of betaine in the solution is about 2M. In some examples, the concentration of betaine in the solution is about 2.5M. In some examples, the concentration of betaine in the solution is about 3.0M. In some examples, the concentration of betaine in the solution is about 3.5M.

[0028] It is to be understood that any respective features/examples of each of the aspects of the disclosure as described herein may be implemented together in any appropriate combination, and that any features/examples from any one or more of these aspects may be implemented together with any of the features of the other aspect(s) as described herein in any appropriate combination to achieve the benefits as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIGs. 1A-1C show comparable sequencing metrics for denaturant formulations containing betaine v. a formamide control (LDR).

[0030] FIG. 2 shows data from a standard 2x151 sequencing run using DNA denaturant containing betaine that is DMSO-free.

[0031] FIG. 3 shows that primary metrics were not significantly different when TruSeq DNA Nano human libraries that were denatured with formulations containing betaine v. standard formamide (LDR).

[0032] FIG. 4 shows metrics for DNA denaturant formulations containing betaine that are DMSO-free compared to formulations that contain DMSO.

[0033] FIGs. 5A-5C show sequencing data obtained using a DNA denaturant formulation that contains betaine and diethylene glycol (DEG) compared to using the denaturant formamide (LDR).

[0034] FIGs. 6A and 6B show sequencing data in which DNA denaturant formulations that contained only betaine were compared to DNA denaturant formulations that contained additional components other than betaine. FIG. 6A shows data of denaturing after clustering. FIG. 6B shows data of denaturing DNA libraries.

[0035] FIGs. 7A and 7B show data of denaturing DNA libraries using various DNA denaturant formulations that contain various concentrations of DEG and DMSO, as well as other additives.

[0036] FIG. 8 shows data of denaturing DNA after clustering using various DNA denaturant formulations containing different concentrations of buffer with different pH ranges.

[0037] FIG. 9 shows data of denaturing DNA after clustering using various DNA denaturant formulations with different concentrations of betaine.

[0038] FIG. 10 shows data of denaturing DNA using various XDR formulations using a surface hybridization-based assay.

[0039] FIG. 11 shows data of GC coverage using various XDR formulations to denature DNA during a sequencing reaction.

[0040] FIG. 12 shows data of global coverage when various XDR formulations when various concentrations of betaine and DMSO were used to denature DNA during sequencing reactions.

[0041] FIG. 13 shows sequencing data when using various XDR formulations to denature DNA in a HiSeq X sequencing run.

[0042] FIG. 14 shows data of sequencing intensity of various sequencing reads using the XDR14 formulation as the denaturant.

[0043] FIG. 15 shows data of various sequencing metrics using various XDR formulations in an HiSeq X sequencing run.

[0044] FIG. 16 shows data of various sequencing metrics using various XDR formulations with and without DEG, in an HiSeq X sequencing run.

[0045] FIG. 17 shows data of various sequencing metrics using various XDR formulations with and without CAPS buffer, in an HiSeq X sequencing run.

[0046] FIG. 18 shows data of the viscosity of XDR formulations compared to LDR.

[0047] FIG. 19 shows data of the viscosity of various XDR formulations and the viscosity of most of the high-performance XDR formulations (see insert).

DETAILED DESCRIPTION

[0048] Current DNA denaturants include formamide and sodium hydroxide. Formamide is a serious health hazard and sodium hydroxide requires manual denaturation that can cause human error, in addition to being corrosive. Disclosed herein are alternative denaturing reagents (XDR formulations) that can be used to denature DNA. XDR formulations disclosed herein are not hazardous and can be used to effectively denature template DNA before sequencing, and/or to de-hybridize sequencing primers that are bound to surface DNA during sequencing.

[0049] In some examples, XDR formulations disclosed herein include betaine as well as other additive(s). In some examples, the XDR denaturants may include any one or more of a buffer, diethylene glycol (DEG), and dimethyl sulfoxide (DMSO). In some examples, the XDR denaturants include betaine and DEG. In some examples, the XDR denaturants include betaine, DEG, and a buffer. In some examples, the XDR formulations include betaine and DMSO. In some examples, the XDR formulations include betaine, DMSO, and a buffer. In some examples, the XDR formulations including betaine, DEG, and DMSO. In some examples, the XDR formulations include betaine, DEG, DMSO, and a buffer.

[0050] In some examples, any of the XDR formulations described herein can be used to denature libraries before sequencing. In some examples, any of the XDR formulations described herein can be used to de-hybridise strands in clustering and/or resynthesis, between reads. In some examples, any of the XDR formulations described herein can be used in bridge amplification. In some examples, any of the XDR formulations described herein can be used in exclusion amplification.

[0051] First, some terms used herein will be briefly explained. Then, some example compositions and example methods using the compositions will be described.

Terms

[0052] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. The use of the term "including" as well as other forms, such as "include," "includes," and "included," is not limiting. The use of the term "having" as well as other forms, such as "have," "has," and "had," is not limiting. As used in this specification, whether in a transitional phrase or in the body of the claim, the terms "comprise(s)" and "comprising" are to be interpreted as having an open-ended meaning. That is, the above terms are to be interpreted synonymously with the phrases "having at least" or "including at least." For example, when used in the context of a process, the term "comprising" means that the process includes at least the recited steps, but may include additional steps. When used in the context of a compound, composition, or device, the term "comprising" means that the compound, composition, or device includes at least the recited features or components, but may also include additional features or components

[0053] The terms "substantially," "approximately," and "about" used throughout this specification are used to describe and account for small fluctuations, such as due to variations in processing. For example, they may refer to less than or equal to ±10% of the stated amount, such as less than or equal to ±5% of the stated amount, such as less than or equal to ±2% of the stated amount, such as less than or equal to ±1% of the stated amount, such as less than or equal to ±0.5% of the stated amount, such as less than or equal to ±0.2% of the stated amount, such as less than or equal to ±0.1% of the stated amount, such as less than or equal to ±0.05% of the stated amount.

[0054] As used herein, the term "nucleotide" is intended to mean a molecule that includes a sugar and at least one phosphate group, and in some examples also includes a nucleobase. A nucleotide that lacks a nucleobase may be referred to as "abasic." Nucleotides include deoxyribonucleotides, modified deoxyribonucleotides, ribonucleotides, modified ribonucleotides, peptide nucleotides, modified peptide nucleotides, modified phosphate sugar backbone nucleotides, and mixtures thereof. Examples of nucleotides include adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), thymidine monophosphate (TMP), thymidine diphosphate (TDP), thymidine triphosphate (TTP), cytidine monophosphate (CMP), cytidine diphosphate (CDP), cytidine triphosphate (CTP), guanosine monophosphate (GMP), guanosine diphosphate (GDP), guanosine triphosphate (GTP), uridine monophosphate (UMP), uridine diphosphate (UDP), uridine triphosphate (UTP), deoxyadenosine monophosphate (dAMP), deoxyadenosine diphosphate (dADP), deoxyadenosine triphosphate (dATP), deoxythymidine monophosphate (dTMP), deoxythymidine diphosphate (dTDP), deoxythymidine triphosphate (dTTP), deoxycytidine diphosphate (dCDP), deoxycytidine triphosphate (dCTP), deoxyguanosine monophosphate (dGMP), deoxyguanosine diphosphate (dGDP), deoxyguanosine triphosphate (dGTP), deoxyuridine monophosphate (dUMP), deoxyuridine diphosphate (dUDP), and deoxyuridine triphosphate (dUTP).

[0055] As used herein, the term "nucleotide" also is intended to encompass any nucleotide analogue which is a type of nucleotide that includes a modified nucleobase, sugar and/or phosphate moiety compared to naturally occurring nucleotides. Example modified nucleobases include inosine, xanthine, hypoxanthine, isocytosine, isoguanine, 2-aminopurine, 5-methylcytosine, 5-hydroxymethyl cytosine, 2-aminoadenine, 6-methyl adenine, 6-methyl guanine, 2-propyl guanine, 2-propyl adenine, 2-thiouracil, 2-thiothymine, 2-thiocytosine, 5-

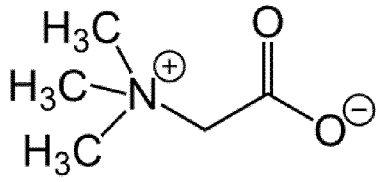
halouracil, 5-halocytosine, 5-propynyl uracil, 5-propynyl cytosine, 6-azo uracil, 6-azo cytosine, 6-azo thymine, 5-uracil, 4-thiouracil, 8-halo adenine or guanine, 8-amino adenine or guanine, 8-thiol adenine or guanine, 8-thioalkyl adenine or guanine, 8-hydroxyl adenine or guanine, 5-halo substituted uracil or cytosine, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine or the like. As is known in the art, certain nucleotide analogues cannot become incorporated into a polynucleotide, for example, nucleotide analogues such as adenosine 5'-phosphosulfate. Nucleotides may include any suitable number of phosphates, e.g., three, four, five, six, or more than six phosphates.

[0056] As used herein, the term "polynucleotide" refers to a molecule that includes a sequence of nucleotides that are bonded to one another. A polynucleotide is one nonlimiting example of a polymer. Examples of polynucleotides include deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and analogues thereof. A polynucleotide may be a single stranded sequence of nucleotides, such as RNA or single stranded DNA, a double stranded sequence of nucleotides, such as double stranded DNA, or may include a mixture of a single stranded and double stranded sequences of nucleotides. Double stranded DNA (dsDNA) includes genomic DNA, and PCR and amplification products. Single stranded DNA (ssDNA) can be converted to dsDNA and vice-versa. Polynucleotides may include non-naturally occurring DNA, such as enantiomeric DNA. The precise sequence of nucleotides in a polynucleotide may be known or unknown. The following are examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, expressed sequence tag (EST) or serial analysis of gene expression (SAGE) tag), genomic DNA, genomic DNA fragment, exon, intron, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozyme, cDNA, recombinant polynucleotide, synthetic polynucleotide, branched polynucleotide, plasmid, vector, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probe, primer or amplified copy of any of the foregoing.

[0057] The terms "polynucleotide" and "oligonucleotide" are used interchangeably herein. The different terms are not intended to denote any particular difference in size, sequence, or other property unless specifically indicated otherwise. For clarity of description the terms may be used to distinguish one species of polynucleotide from another when describing a particular method or composition that includes several polynucleotide species.

[0058] As used herein, the term "XDR", the phrase "XDR solution formulation", or the phrase "XDR formulation", each refer to a DNA denaturant formulation in which betaine is used as the primary additive to denature the DNA. In some examples, an XDR formulation includes additional component(s) such as any one or more of a buffer, di-ethylene glycol (DEG), and dimethyl sulfoxide (DMSO). Various "XDR formulations" are disclosed herein; these various "XDR formulations" are labeled, for example, as XDR1, XDR2, XDR3, etc…

[0059] As used herein, the term "betaine" is intended to refer to glycine betaine, also known as trimethylglycine (TMG), and has the following structure:



[0060] When a composition or solution herein is referred to as including a range % or a particular % of a compound, it is meant that such compound is present at that range % or particular % by volume.

[0061] As used herein, the term "LDR" refers to a denaturant that includes 99.9% formamide.

XDR Solution Formulations for Denaturing Double-Stranded DNA

[0062] Some examples herein provide a solution formulation for denaturing double-stranded DNA (dsDNA) that includes betaine. In some examples, the solution formulation de-hybridizes a primer bound to surface DNA.

[0063] In some examples, the solution formulation includes betaine. In some examples, the solution formulation includes betaine and diethylene glycol (DEG). In some examples, the solution formulation is an XDR formulation.

[0064] In some examples, a concentration of betaine in the solution formulation is between about 1M and about 5M, for example about 1M, about 1.1M, about 1.2M, about 1.3M, about 1.4M, about 1.5M, about 1.6M, about 1.7M, about 1.8M, about 1.9M, about 2M, about 2.1M, about 2.2M, about 2.3M, about 2.4M, about 2.5M, about 2.6M, about 2.7M, about 2.9M, about 3.0M, about 3.1M, about 3.2M, about 3.3M, about 3.4M, about 3.5M, about 3.6M,

about 3.7M, about 3.8M, about 3.9M, about 4.0M, about 4.1M, about 4.2M, about 4.3M, about 4.4M, about 4.5M, about 4.6M, about 4.7M, about 4.8M, about 4.9M, or about 5M. In some examples, the concentration of betaine in the solution formulation is between about 2M and about 5M. In some examples, the concentration of betaine in the solution formulation is between about 2M and about 4M. In some examples, the concentration of betaine in the solution formulation is between about 2M and about 3.5M. In some examples, the concentration of betaine in the solution formulation is less than 1M. In some examples, the concentration of betaine in the solution formulation is greater than 5M.

[0065] In some examples, a concentration of DEG in the solution formulation is between about 3% and about 40%. In some examples, the concentration of DEG in the solution formulation is between about 20% and about 40%, for example about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about, 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, or about 40%. In some examples, the concentration of DEG in the solution formulation is greater than 40%. In some examples, the concentration of DEG in the solution formulation is between about 20% and about 30%.

[0066] In some examples, the solution formulation further includes dimethyl sulfoxide (DMSO). In some examples, a concentration of DMSO in the solution formulation is between about 5% and about 30%, for example, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, or about 30%. In some examples, the concentration of the DMSO in the solution formulation is between about 7% and about 12%. In some examples, the concentration of DMSO in the solution formulation is about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15%. In some examples, the concentration of DMSO in the solution formulation is between about 17% and about 22%. In some examples, the concentration of DMSO in the solution formulation is about 17%, about 18%, about 19%, about 20%, about 21%, or about 22%.

[0067] In some examples, a concentration of the DEG is between about 5% and about 25% in any of the solution formulations containing DMSO described herein. In some examples, the concentration of DEG is about 5%, about 6%, about 7%, about 8%, about 9%, about 10%,

about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, or about 25%. In some examples, the concentration of the DEG in the solution formulation is between about 13% and about 17%. In some examples, the concentration of the DEG in the solution formulation is about 13%, about 14%, about 15%, about 16%, or about 17%.

[0068] In some examples, a concentration of betaine is between about 2M and 3M in any of the solution formulations containing DMSO described herein. In some examples, the concentration of betaine in the solution formulation is about 2M, about 2.1M, about 2.2M, about 2.3M, about 2.4M, about 2.5M, about 2.6M, about 2.7M, about 2.8M, about 2.9M, or about 3M.

[0069] In some examples, any of the solution formulations described herein further includes a buffer. In some examples, the buffer includes a zwitterionic buffer. In some examples, the zwitterionic buffer produces a pH in the solution formulation at a range of between about 9.5 and about 11.5. In some examples, the zwitterionic buffer produces a pH in the solution formulation at a range of between about 8.5 and about 12.5. In some examples, the zwitterionic buffer produces a pH in the solution formulation of about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, about 9.0, about 9.1, about 9.2, about 9.3, about 9.4, about 9.5, about 9.6, about 9.7, about 9.8. about 9.9, about 10, about 10.1, about 10.2, about 10.3, about 10.4, about 10.5, about 10.6, about 10.7, about 10.8, about 10.9, about 11, about 11.1, about 11.2, about 11.3, about 11.4, about 11.5, about 11.6, about 11.7, about 11.8, about 11.9, about 12.0, about 12.1, about 12.2, about 12.3, about 12.4, or about 12.5.

[0070] In some examples, the zwitterionic buffer includes any one or more of 4-(cyclohexylamino)-1-butanesulfonic acid (CABS), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO), *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS), and 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate) (CHAPS). In some examples, the zwitterionic buffer includes any zwitterionic buffer known in the art.

[0071] In some examples, the solution formulations described herein are any of XDR1, XDR2, XDR3, XDR4, XDR5, XDR6, XDR7, XDR8, XDR9, XDR10, XDR11, XDR12, XDR13, XDR14, XDR15, XDR16, XDR17, XDR18, XDR19, XDR20, XDR21, XDR22,

XDR23, XDR24, XDR25, XDR26, XDR27, and XDR30. The components that make up each of these XDR formulations, which are aqueous solutions, are as follows:

[0072] XDR1

- 3M betaine
- 20% DMSO
- 15% DEG

[0073] XDR2

- 3M betaine
- 15% DMSO
- 20% DEG

[0074] XDR3

- 3M betaine
- 15% DMSO
- 15% DEG

[0075] XDR4

- 2.5M betaine
- 20% DMSO
- 15% DEG

[0076] XDR5

- 2.5M betaine
- 15% DMSO

- 20% DEG

[0077] XDR6

- 2.5M betaine
- 15% DMSO
- 15% DEG

[0078] XDR7

- 3M betaine
- 20% DMSO
- 15% DEG

[0079] XDR8

- 3M betaine
- 10% DMSO
- 15% DEG

[0080] XDR9

- 3M betaine
- 15% DEG

[0081] XDR10

- 2M betaine
- 20% DMSO
- 15% DEG

[0082] XDR11

- 2M betaine
- 10% DMSO
- 15% DEG

[0083] XDR12

- 2M betaine
- 15% DMSO

[0084] XDR13

- 2.5M betaine
- 10% DMSO
- 10% DEG

[0085] XDR14

- 2.5M betaine
- 15% DMSO
- 5% DEG

[0086] XDR15

- 2.5M betaine
- 20% DMSO
- 5% DEG

[0087] XDR16

- 3M betaine
- 20% DMSO

[0088] XDR17

- 2.5M betaine
- 15% DMSO
- 10% DEG

[0089] XDR18

- 2.5M betaine
- 20% DMSO

[0090] XDR19

- 3M betaine
- 15% DMSO
- 5% DEG

[0091] XDR20

- 50mM CAPS pH 10.5
- 3M betaine
- 20% DMSO
- 5% DEG

[0092] XDR21

- 50mM CAPS pH 10.5
- 3.5M betaine
- 20% DMSO

[0093] XDR22

- 50mM CAPS pH 10.5
- 3M betaine
- 20% DMSO
- 10% DEG

[0094] XDR23

- 50mM CAPS pH 11
- 2.5M betaine
- 10% DMSO
- 20% DEG

[0095] XDR24

- 50mM CAPS pH 11
- 3M betaine
- 20% DMSO

[0096] XDR25

- 50mM CAPS pH 11
- 3M betaine
- 20% DMSO
- 5% DEG

[0097] XDR26

- 50mM CAPS pH 11
- 4M betaine

- 20% DMSO

[0098] XDR27

- 4M betaine
- 20% DMSO

[0099] XDR30

- 50mM Tris pH 9
- 4M betaine
- 20% DMSO

Methods of Denaturing Double-Stranded DNA

[0100] Some examples herein provide a method of denaturing double-stranded DNA (dsDNA), including contacting the dsDNA in a fluid-filled container with a denaturant that includes betaine. In some examples, the denaturant that includes betaine is any of the XDR formulations described herein.

[0101] In some examples, a ratio of the denaturant to the dsDNA is between about 1:1 and about 3:1. Illustratively, in some examples, a ratio of the denaturant to the dsDNA is about 1:1. In some examples, the ratio of denaturant to the dsDNA is about 1.2:1. In some examples, the ratio of the denaturant to the dsDNA is about 1.3:1. In some examples, the ratio of the denaturant to the dsDNA is about 1.4:1. In some examples, the ratio of the denaturant to the dsDNA is about 1.5:1. In some examples, the ratio of the denaturant to the dsDNA is about 1.6:1. In some examples, the ratio of the denaturant to the dsDNA is about 2:1. In some examples, the ratio of the denaturant to the dsDNA is about 2.5:1. In some examples, the ratio of the denaturant to the dsDNA is about 3:1.

[0102] In some examples, the denaturant further includes diethylene glycol (DEG). In some examples, the concentration of DEG is between about 3% and about 40%, for example, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%,

about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, or about 40%. In some examples, the concentration of DEG in the solution is less than 3%. In some examples, the concentration of DEG in the solution is greater than 40%.

[0103] In some examples, the denaturant further includes dimethyl sulfoxide (DMSO). In some examples, a concentration of DMSO in the solution formulation is between about 5% and about 30%, for example, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, or about 30%.

[0104] In some examples, the denaturant further includes a zwitterionic buffer. In some examples, the zwitterionic buffer produces a pH in the fluid-filled container between about 9.5 and about 11.5. In some examples, the zwitterionic buffer produces a pH in the solution formulation at a range of between about 8.5 and about 12.5. In some examples, the zwitterionic buffer produces a pH in the solution formulation of about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, about 9.0, about 9.1, about 9.2, about 9.3, about 9.4, about 9.5, about 9.6, about 9.7, about 9.8. about 9.9, about 10, about 10.1, about 10.2, about 10.3, about 10.4, about 10.5, about 10.6, about 10.7, about 10.8, about 10.9, about 11, about 11.1, about 11.2, about 11.3, about 11.4, about 11.5, about 11.6, about 11.7, about 11.8, about 11.9, about 12.0, about 12.1, about 12.2, about 12.3, about 12.4, or about 12.5.

[0105] In some examples, the zwitterionic buffer includes any one or more of 4-(cyclohexylamino)-1-butanesulfonic acid (CABS), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO), *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS), and 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate) (CHAPS). In some examples, the zwitterionic buffer includes any zwitterionic buffer known in the art.

[0106] Some examples herein provide a kit for denaturing double-stranded DNA (dsDNA), including a denaturant that includes betaine and diethylene glycol (DEG) in a solution; and a buffer.

[0107] In some examples, a concentration of DEG in the solution is between about 20% and about 40%. In some examples, the concentration of DEG in the solution is about 20%, about

21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, or about 40%.

[0108] In some examples, the denaturant further includes dimethyl sulfoxide (DMSO). In some examples, the concentration of DEG in the solution is between about 5% and about 20%. In some examples, the concentration of DEG in the solution is about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, or about 20%.

[0109] In some examples, any of the kits described herein include a concentration of betaine in the solution that is between about 1 M and 5 M. In some examples, the concentration of betaine in the solution is about 2 M, about 2.5 M, about 3.0 M, about 3.5 M, about 4.5 M, or about 5.0 M.

[0110] In some examples, the buffer includes a zwitterionic buffer. In some examples, the zwitterionic buffer produces a pH in the solution formulation at a range of between about 9.5 and about 11.5. In some examples, the zwitterionic buffer produces a pH in the solution formulation at a range of between about 8.5 and about 12.5. In some examples, the zwitterionic buffer produces a pH in the solution formulation of about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, about 9.0, about 9.1, about 9.2, about 9.3, about 9.4, about 9.5, about 9.6, about 9.7, about 9.8. about 9.9, about 10, about 10.1, about 10.2, about 10.3, about 10.4, about 10.5, about 10.6, about 10.7, about 10.8, about 10.9, about 11, about 11.1, about 11.2, about 11.3, about 11.4, about 11.5, about 11.6, about 11.7, about 11.8, about 11.9, about 12.0, about 12.1, about 12.2, about 12.3, about 12.4, or about 12.5.

[0111] In some examples, the zwitterionic buffer includes any one or more of 4-(cyclohexylamino)-1-butanesulfonic acid (CABS), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO), *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS), and 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate) (CHAPS). In some examples, the zwitterionic buffer includes any zwitterionic buffer known in the art.

Amplification Methods

[0112] The XDR solution formulations, the solution formulations, and the methods of denaturing double-stranded DNA described herein can be used in the context of amplification methods, such as bridge amplification and exclusion amplification. Bridge amplification and exclusion amplification can be carried out in a flow cell.

[0113] Bridge Amplification: Bridge amplification can be performed on a flow cell. Single stranded template DNA is hybridised to lawn primers in a flow cell, and a polymerase is used to extend the primer to form double-stranded DNA. The double-stranded DNA is denatured, and the original template strand of the DNA molecule is washed away. This results in a single-stranded DNA molecule being bound to the lawn primers of the flow cell. The single-stranded DNA molecule turns over and forms a "bridge" by hybridising to a nearby lawn primer that is complementary to a sequence of the single-stranded DNA molecule. Polymerase extends the hybridised primer resulting in bridge amplification of the DNA molecule and the creation of a double-stranded DNA molecule. The double-stranded DNA molecule is then denatured.

[0114] The double-stranded DNA molecule can be denatured using (i) any of the XDR solution formulations described herein, (ii) any of the solution formulations described herein, or (iii) any of the methods of denaturing double-stranded DNA described herein. Denaturing results in two copies of single-stranded templates, one of which is immobilised to the support and the other of which may be washed away.

[0115] The one strand that is immobilised on the support may be used in further bridge amplification operations so as to generate a cluster that subsequently may be sequenced. Cluster generation produces clusters of double-stranded DNA. These clusters can be denatured using (i) any of the XDR solution formulations described herein, (ii) any of the solution formulations described herein, or (iii) any of the methods of denaturing double-stranded DNA described herein.

[0116] Exclusion Amplification: Exclusion amplification methods may allow for the amplification of a single target polynucleotide per substrate region and the production of a substantially monoclonal population of amplicons in a substrate region. For example, the rate of amplification of the first captured target polynucleotide within a substrate region may be more rapid relative to much slower rates of transport and capture of target polynucleotides at

the substrate region. As such, the first target polynucleotide captured in a substrate region may be amplified rapidly and fill the entire substrate region, thus inhibiting the capture of additional target polynucleotide in the same substrate region. Alternatively, if a second target polynucleotide attaches to same substrate region after the first polynucleotide, the relatively rapid amplification of the first polynucleotide may fill enough of the substrate region to result in a signal that is sufficiently strong to perform sequencing by synthesis (e.g., the substrate region may be at least functionally monoclonal). The use of exclusion amplification may also result in super-Poisson distributions of monoclonal substrate regions; that is, the fraction of substrate regions in an array that are functionally monoclonal may exceed the fraction predicted by the Poisson distribution.

[0117] Exclusion amplification can generate clusters of double-stranded DNA. The double-strand DNA can be denatured using (i) any of the XDR solution formulations described herein, (ii) any of the solution formulations described herein, or (iii) any of the methods of denaturing double-stranded DNA described herein.

Sequencing of Template Strands

[0118] In some examples, sequencing occurs after cluster generation of DNA. In some examples, the DNA clusters are produced from bridge amplification or from exclusion amplification. In some examples, the DNA clusters are attached to the surface of a flow cell. To facilitate sequencing, it is preferable if one of the strands of DNA is removed from the surface to allow efficient hybridization of a sequencing primer to the remaining immobilised strand. Any of the solution formulations, XDR solution formulations, or any of the methods of denaturing double-stranded DNA described herein can be used to de-hybridized DNA, thereby allowing removal of a strand of DNA from the surface.

[0119] Sequence data can be obtained from both ends of a template duplex by obtaining a sequence read from one strand of the template from a primer in solution, copying the strand using immobilised primers, releasing the first strand and sequencing the second, copied strand. For example, sequence data can be obtained from both ends of the immobilised duplex by a method wherein the duplex is treated to free a 3'-hydroxyl moiety that can be used an extension primer. The extension primer can then be used to read the first sequence from one strand of the template. After the first read, the strand can be extended to fully copy all the bases up to the end of the first strand. This second copy remains attached to the surface

at the 5'-end. If the first strand is removed from the surface, the sequence of the second strand can be read. This gives a sequence read from both ends of the original fragment. The process whereby the strand is regenerated after the first read is known as “Paired-end resynthesis” or “PE resynthesis”. The typical steps of pairwise sequencing are known and have been described in WO 2008/041002, the entire contents of which are incorporated by reference herein.

[0120] Sequencing can be carried out using any suitable "sequencing-by-synthesis" technique, wherein nucleotides are added successively to the free 3' hydroxyl group, resulting in synthesis of a polynucleotide chain in the 5' to 3' direction. The nature of the nucleotide added is preferably determined after each addition. One particular sequencing method relies on the use of modified nucleotides that can act as reversible chain terminators. Such reversible chain terminators include removable 3' blocking groups. Once such a modified nucleotide has been incorporated into the growing polynucleotide chain complementary to the region of the template being sequenced there is no free 3'-OH group available to direct further sequence extension and therefore the polymerase cannot add further nucleotides. Once the nature of the base incorporated into the growing chain has been determined, the 3' block may be removed to allow addition of the next successive nucleotide. By ordering the products derived using these modified nucleotides it is possible to deduce the DNA sequence of the DNA template. Such reactions can be done in a single experiment if each of the modified nucleotides has attached thereto a different label, known to correspond to the particular base, to facilitate discrimination between the bases added at each incorporation step. Suitable labels are described in PCT application PCT/GB2007/001770, the entire contents of which are incorporated by reference herein. Alternatively, a separate reaction may be carried out containing each of the modified nucleotides added individually.

[0121] Any of the solution formulations, XDR solution formulations, or any of the methods of denaturing double-stranded DNA described herein can be used to remove sequencing primers from strands of DNA.

WORKING EXAMPLES

[0122] The examples provided below are include for illustrative purposes and are not meant to be limiting of any of the disclosures described herein.

Example 1-Methods of Testing Denaturants in Sequencing Reactions

[0123] New denaturant solutions with various XDR formulations described in Examples 2-6 below were tested during sequencing reactions. The libraries were sequenced at 2x151 cycles on a modified HiSeq X instrument and then tested on a NextSeq 2000. The sequencing was performed with standard workflows and reagents, except with new denaturant solutions (XDR formulations) replacing LDR in the cartridge, reagent plate, or reagent tubes.

[0124] As shown in the data described below in Examples 2-6 and FIGs. 1A-5B, generally, the use of various DNA denaturant formulations that contain betaine resulted in identical sequencing yield compared to using formamide as the denaturant.

Example 2-Comparable Metrics for DNA Denaturant Formulations Containing Betaine With and Without DMSO vs. Formamide

[0125] Human 450 bp libraries were sequenced as described in Example 1. DNA was denatured using different denaturant formulations containing betaine and using denaturants that utilized formamide, which functioned as a control. Sequencing metrics were performed on both Read 1 and Read 2 to determine the quality of the sequencing.

[0126] The results in FIGs. 1A-1C show that when DNA denaturant formulations are used without DMSO, DEG can be used to produce quality sequencing metrics.

[0127] The label "LDR" in each of FIGs. 1A-1C refers to a denaturant that utilized formamide (LDR). The labels "20", "10", and "5" in each of FIGs. 1A-1C refer to DNA denaturant formulations containing betaine, as described below.

Formulations with 20% DEG with DMSO and without DMSO (label "20")

Formulation with DMSO and 20% DEG:

- 50mM CAPS pH 11
- 2.5M betaine
- 20% DEG
- 10% DMSO

Formulation without DMSO and 20% DEG:

- 50mM CAPS pH 11
- 2.5M betaine
- 20% DEG

Formulations with 10% DEG with DMSO and without DMSO (label "10")

Formulation with DMSO and 10% DEG:

- 50mM CAPS pH 11
- 2.5M betaine
- 10% DEG
- 10% DMSO

Formulation without DMSO and 10% DEG:

- 50mM CAPS pH 11
- 2.5M betaine
- 10% DEG

Formulations with 5% DEG with DMSO and without DMSO (label "5")

Formulation with DMSO and 5% DEG:

- 50mM CAPS pH 11
- 2.5M betaine
- 5% DEG
- 10% DMSO

Formulation without DMSO and 5% DEG:

- 50 mM CAPS pH 11

- 2.5M betaine
- 5% DEG

Example 3-Using Denaturing Formulations Containing Betaine in Bridge-Amplification

[0128] Denaturing formulations containing betaine were tested in bridge amplification. Human 450 bp libraries were sequenced as described in Example 1.

[0129] The label "LDR" in FIG. 2 refers to a denaturant that utilized formamide as the denaturant. The label DEG BET3.5 CAPS in FIG. 2 corresponds to the below formulation:

- 50mM CAPS pH 11
- 3.5M betaine
- 20% DEG

[0130] As shown in the sequencing data in FIG. 2, relative to the LDR control, using the DEG BET3.5 CAPS denaturant resulted in a lower read 2 sequencing error rate. In addition, the DEG BET3.5 CAPS denaturant produced a pass filter percentage over 70%, which was higher than the pass filter percentage of the LDR control, which was less than 70%. This indicates that, unlike the control samples, using the BET3.5 CAPS denaturant resulted in highly pure cluster formation during bridge amplification.

Example 4-Comparing Primary Sequencing Metrics between Denaturants Containing Betaine and Denaturants that Utilize Formamide

[0131] Sequencing metrics were evaluated between denaturants containing betaine and denaturants that contain formamide. The ratios of library:denaturant:neutralizing buffer were adjusted and tested. Different formulations were tested as shown below:

LDR (control)

- 99.9% Formamide

DEG BET DMSO

- 50mM CAPS pH 11

- 2.5M betaine
- 20% DEG
- 10% DMSO

DEG30 BET

- 50mM CAPS pH 11
- 2.5M betaine
- 30% DEG

DEG BET3.5

- 50mM CAPS pH 11
- 3.5M betaine
- 20% DEG

[0132] Each of the labels "LDR", "DEG BET DMSO", "DEG30 BET", and "DEG BET 3.5" correspond to the labels on the x-axis on FIG. 3.

[0133] In FIG. 3, the ratio of 1:1:8 (library:denaturant:neutralizing buffer) was tested using each of the "LDR", "DEG BET DMSO", and "DEG30 BET" formulations. Only, the "DEG BET DMSO" formulation was used at the ratios of 1:1.5:7.5, 1:2:7, and 1:3:6.

[0134] As shown in FIG. 3, when using the DEG BEG DMSO, DEG30 BET, and DEG BET3.5 formulations, a ratio of 1:1.5:7.5 (library:denaturant:neutralizing buffer) was effective in producing good sequencing metrics. This is compared to LDR, which produced good sequencing metrics at a ratio of 1:1:8 (library:denaturant:neutralizing buffer).

Example 5-Analyzing Sequencing Data using DMSO-Free Versions of Denaturants Containing Betaine

[0135] Human 450 bp libraries were sequenced as described in Example 1. DMSO-free versions of the denaturants were tested. Different formulations were tested as shown below:

LDR (control)

- 99.9% Formamide

DEG BET DMSO

- 50mM CAPS pH 11
- 2.5M betaine
- 20% DEG
- 10% DMSO

DEG30 BET

- 50mM CAPS pH 11
- 2.5M betaine
- 30% DEG

DEG BET3.5

- 50mM CAPS pH 11
- 3.5M betaine
- 20% DEG

[0136] The "LDR", "DEG BET DMSO", "DEG30 BET", and "DEG BET3.5" labels correspond to the labels on the x-axis of FIG. 4.

[0137] The sequencing data in FIG. 4 shows that the DNA denaturant formulations without DMSO ("DEG30 BET" and "DEG BET3.5"), showed an error rate and a pass filter rate similar to that of the DNA denaturant formulation with DMSO ("DEG BET DMSO"). This data indicates that DNA denaturant formulations without DMSO can be used in sequencing reactions when the concentration of betaine or DEG is increased.

Example 6-Comparing Sequencing Performance Between a Denaturant Containing Betaine and a Denaturant that Contains Formamide, Over a 12-Month Period

[0138] Human 450 bp libraries were sequenced as described in Example 1. Sequencing metrics were tested for the reagents described below over a 12-month accelerated stability study at 60°C.

LDR

- 99.9% Formamide

DEG

- 50 mM CAPS pH11
- 2.5 M betaine
- 20% DEG
- 10% DMSO

[0139] As shown in FIG. 5A, using the DEG formulation as a denaturant resulted in the pass filter rate of read 1 that was comparable to using the "LDR" formulation As shown in FIG. 5B, using the DEG formulation as a denaturant resulted in read 1 and read 2 error rates that were comparable to the error rates when using the LDR formulation. As shown in FIG. 5C, the DEG formulation resulted in comparable sequencing metrics to that of the LDR formulation.

Example 7-Denaturing DNA using Betaine and Gradual Addition of Other Components

[0140] In this example, the following denaturants were tested:

Formamide

- 99.9% Formamide

Betaine

- 3.5M betaine

Betaine + Buffer

- 50mM CAPS pH11
- 3.5M betaine

XDR-Betaine + Buffer + DEG

- 50mM CAPS pH11
- 3.5M betaine
- 20% DEG

[0141] The labels "Formamide", "Betaine," "Betaine + Buffer", and "Betaine + Buffer + DEG" correspond to the labels on the x-axes on both FIGs. 6A and 6B.

[0142] Denaturing after clustering: HiSeq X sequencing of human 450bp libraries was performed using betaine as the denaturant along with other additives. 3.5 M betaine was used was used as a denaturant in place of formamide. The denaturant's ability to denature was measured with the 'intensity' metric (see FIG. 6A). If denaturing does not work, zero intensity will be measured using this metric.

[0143] The data shown in FIG. 6A shows successful denaturation of clusters when betaine was used as the denaturant. Adding buffer had a detrimental effect, which is shown in the "betaine + buffer" formulation. This detrimental effect is then mitigated by the addition of DEG, which is shown the "betaine + buffer + DEG" formulation. Temperature and contact time were identical across all the denaturants tested.

[0144] Denaturing libraries: HiSeq X sequencing of human 450 bp libraries was performed using betaine as the denaturant along with other additives. 3.5 M betaine was used as a denaturant in place of formamide. The ability of the denaturant to denature libraries was measured using percent GC (see FIG. 6B), which is very sensitive to incomplete denaturing. 3.5 M betaine with or without additives were used in place of formamide. The usual ratio of 1:1 (library to denaturant) was increased to 1:2 when denaturants other than formamide were used. Temperature and contact time were identical across all the denaturants tested.

[0145] As shown in FIG. 6B, the addition of buffer to denaturants containing betaine, even at high pH, reduces the ability of the formulation to denature DNA. This is evidenced by the reduction in percent GC coverage and the reduced pass filter percentage in the "betaine + buffer" formulation. Adding diethylene glycol recovers the ability of the reagent to denature DNA. This is evidenced by the increase in the percent GC coverage and the increase pass filter percentage in the "betaine + buffer + DEG" formulation relative to the "betaine + buffer" formulation. Thus, using the "betaine + buffer + DEG" formulation allows buffering of the reagent while still maintaining the ability of the reagent to denature DNA.

Example 8-Range Finding of Betaine, DEG, and DMSO

[0146] In this example, the following denaturants were tested.

[0147] Denaturants tested in FIG. 7A:

Denaturants without DMSO

5% DEG

- 50mM CAPS pH11
- 2.5M betaine
- 5% DEG

10% DEG

- 50mM CAPS pH11
- 2.5M betaine
- 10% DEG

20% DEG

- 50mM CAPS pH11
- 2.5M betaine
- 20% DEG

Denaturants with 10% DMSO

5% DEG + 10% DMSO

- 50mM CAPS pH11
- 2.5M betaine
- 20% DEG
- 10% DMSO

10% DEG + 10% DMSO

- 50mM CAPS pH11
- 2.5M betaine
- 10% DEG
- 10% DMSO

20% DEG + 10% DMSO

- 50mM CAPS pH11
- 2.5M betaine
- 20% DEG
- 10% DMSO

[0148] Denaturants tested in FIG. 7B:

Denaturants with 1.5M Betaine

5% DEG + 1.5 M Betaine

- 50mM CAPS pH11
- 1.5M betaine

- 5% DEG
- 10% DMSO

10% DEG + 1.5 M Betaine

- 50mM CAPS pH11
- 1.5M betaine
- 10% DEG
- 10% DMSO

20% DEG + 1.5 M Betaine

- 50mM CAPS pH11
- 1.5M betaine
- 20% DEG
- 10% DMSO

Denaturants with 2.0 M Betaine

5% DEG + 2.0 M Betaine

- 50mM CAPS pH11
- 2M betaine
- 5% DEG

10% DMSO10% DEG + 2.0 M Betaine

- 50mM CAPS pH11
- 2M betaine
- 10% DEG

- 10% DMSO

20% DEG + 2.0 M Betaine

- 50mM CAPS pH11
- 2M betaine
- 20% DEG
- 10% DMSO

Denaturants with 2.5 M Betaine

5% DEG + 2.5 M Betaine

- 50mM CAPS pH11
- 2.5M betaine
- 5% DEG
- 10% DMSO

10% DEG + 2.5 M Betaine

- 50mM CAPS pH11
- 2.5M betaine
- 10% DEG
- 10% DMSO

20% DEG + 2.5 M Betaine

- 50mM CAPS pH11
- 2.5M betaine
- 20% DEG

- 10% DMSO

[0149] HiSeq X sequencing of human 450 bp libraries was performed using various XDR formulations as the denaturant. The DNA was denatured after clustering. Percent GC coverage was used as the metric, as this metric is sensitive to incomplete denaturation.

[0150] In FIG. 7A denaturant formulations with and without DMSO were tested. Data shows that denaturants that contain 10% DMSO and a range of 5-20% DEG worked well (see data points in FIG. 7A in which denaturant formulations were tested that contained 10% DMSO).

[0151] In FIG. 7B denaturant formulations with various concentrations of betaine and DEG were tested. Data shows that, in the presence of 2.5M betaine, a range of 5-20% DEG works well (see data points in FIG. 7B in which denaturant formulations were tested that contained 2.5 M betaine). When the concentration of betaine is 1.5M or 2.0M issues with GC coverage start to become apparent (see data points in FIG. 7B in which denaturant formulations were tested that contained 1.5M and 2.0M betaine).

Example 9-Buffer Range Testing in XDR Formulations

[0152] In this example, the following denaturants were tested

Denaturants with 25mM Buffer Molarity

Buffer Molarity (25mM) 10.0 pH

- 25mM CAPS pH10
- 2.5M betaine
- 20% DEG
- 10% DMSO

Buffer Molarity (25mM) 11.0 pH

- 25mM CAPS pH11
- 2.5M betaine
- 20% DEG

- 10% DMSO

Denaturants with 50mM Buffer Molarity

Buffer Molarity (50mM) 10.0 pH

- 50mM CAPS pH10
- 2.5M betaine
- 20% DEG
- 10% DMSO

Buffer Molarity (50mM) 11.0 pH

- 50mM CAPS pH11
- 2.5M betaine
- 20% DEG
- 10% DMSO

[0153] HiSeq X sequencing of human 450 bp libraries was performed using XDR formulations as the denaturant. The DNA was denatured after clustering. Percent GC coverage was used as one of the sequencing metrics, as this metric is the most sensitive to incomplete denaturing. In addition, sequence intensity was tested; when there is no denaturing the sequence intensity will be zero. The data in FIG. 8 shows that across the pHs that were tested, there was no difference in the quality of the sequencing between a buffer molarity of 25 and a buffer molarity of 50.

Example 10-Testing Concentration Range of Betaine in XDR Formulations

[0154] In this example, the following denaturants were tested.

Betaine (2.5 M)

- 50 mM CAPS

- 2.5 M betaine
- 20% DEG
- 10% DMSO

Betaine (3.0 M)

- 50 mM CAPS
- 3.0 M betaine
- 20% DEG
- 10% DMSO

[0155] HiSeq X sequencing of human 450 bp libraries was performed using various XDR formulations as the denaturant. The DNA was denatured after clustering. Percent GC coverage was used as the metric, as this metric is sensitive to incomplete denaturing. The data in FIG. 9 shows that a denaturant formulation with 2.5 M betaine had a slight decrease in GC coverage relative to a denaturant formulation with 3.0 M betaine.

Example 11-HybE Assay on XDR Conditions

[0156] HybE is a surface-based assay that quantifies how much of a DNA library hybridized to the surface relative to the input concentration via a qPCR readout. In each of the tested formulations, a pH of 10.4 was used. As shown in FIG. 10, as measured by percent hybridization, betaine, DMSO, and DEG were each effective in denaturing DNA and they did not impact the subsequent efficiency of the library hybridization to the surface. The data in FIG. 10 suggests that the XDR formulations tested were as effective in denaturing DNA as the LDR control.

Example 12-Exploring the Effects of DMSO using a Mini-DOE

[0157] A mini high-powered DOE was generated using JMP software. The mini high-powered DOE was used to explore the effects of DMSO on library denaturation. The purpose of the assay was to determine whether formulations containing betaine that have

lower concentrations of DMSO were still effective in denaturing DNA. Percent GC coverage was used as the metric, as this metric is sensitive to incomplete denaturing.

[0158] The data in FIG. 11 shows that the formulations containing betaine performed slightly worse in terms of GC coverage than the LDR formulation. Additionally, the data suggests that there is a slight bias toward shorter inserts. The data in FIG. 11 also show that each of the formulations containing betaine performed similarly, except for the formulation that contained no DMSO.

Example 13-Testing XDR Formulations using the NextSeq 2000

[0159] A 3-plex TruSeq DNA Nano human 450 bp DNA library was run on a NextSeq 2000 P3 kit 2x151+8+8. Analysis was performed using DRAGEN 3.9.5 Fluente orchestrated (N+3 shown are data from each index, only 1 run per condition).

[0160] As shown in FIG. 12, low betaine or low DMSO concentrations in the denaturant formulations showed lower GC coverage compared to LDR formulations. Also, as shown in FIG. 12, high betaine and high DMSO in the denaturant formulations show similar performance to LDR; however, the denaturant formulations did show lower percent GC coverage relative to the LDR formulations. Further, as shown in FIG. 12, resynthesis at 60°C showed no improvement with either the formulations containing betaine or LDR formulations. As shown in FIG. 14 and Table 1, the XDR14 formulation produced quality sequencing metrics.

Table 1.

Level	Yield Total (G)	Projected Total Yield (G)	Aligned (%)	Error Rate (%)	Intensity Cycle 1	X > = Q30	% > = Q 30 (last 10 cycles)
Read 1	205.73	205.73	0.00	NaN	148	92.34	87.71
Read 2 (I)	9.60	9.60	0.00	NaN	156	59.96	59.96
Read 3 (I)	9.59	9.59	0.00	NaN	123	79.05	79.05
Read 4	205.64	205.64	0.00	NaN	92	77.15	68.79
Non-Indexed Total	411.37	411.37	0.00	NaN	120	84.75	78.25
Total	430.56	430.56	0.00	NaN	130	84.07	74.65

Example 14-Using HiSeq X with XDR Formulations

[0161] Various denaturing formulations containing betaine were tested using HiSeq X. FIG. 13 shows that increasing the concentration of DMSO (*e.g.*, XDR14) can improve the GC percentage. In addition, FIG. 13 shows that increasing the XDR formulation to library ratio improves underperforming conditions. Further, FIG. 13 shows that increase the denaturing temperature to 85°C provides more robust denaturing when compared to using a denaturing temperature of 65°C.

[0162] FIG. 15 shows that some of the XDR formulations give similar resynthesis percentage, PF and occupancy as the LDR control, as well as GC coverage and error rates. Further, FIG. 15 shows that XDR formulations that have betaine at a concentration of 3M improve sequencing metrics, relative to lower concentrations of betaine.

[0163] XDR formulations that contained no DEG were also tested using the HiSeq X. The purpose of testing XDR formulations without DEG was to mitigate high viscosities in certain

XDR formulations. FIG. 16 shows that the XDR20 and XDR21 formulations produced robust sequencing metrics.

[0164] XDR formulations that contained different buffers were also tested using the HiSeq X. FIG. 17 shows that the XDR26 (an XDR formulation that contains CAPS pH 11 buffer) outperformed XDR27 and XDR30, which contain PW1 and Tris pH 9 buffers, respectively.

Example 15-Testing for Viscosity in XDR Formulations

[0165] Using a viscometer, XDR formulations were tested to determine their viscosity. FIG. 18 shows that XDR formulations have a significantly higher viscosity than LDR. FIG. 19 shows that the XDR formulations with the highest viscosity generally correlated with the XDR formulations that performed the best in sequencing reactions.

Additional Comments

[0166] While various illustrative examples are described above, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the disclosure. The appended claims are intended to cover all such changes and modifications that fall within the true spirit and scope of the examples provided herein.

[0167] It is to be understood that any respective features/examples of each of the aspects of the disclosure as described herein may be implemented together in any appropriate combination, and that any features/examples from any one or more of these aspects may be implemented together with any of the features of the other aspect(s) as described herein in any appropriate combination to achieve the benefits as described herein.

WHAT IS CLAIMED IS:

1. A solution formulation for denaturing double-stranded DNA (dsDNA), the solution formulation comprising:

betaine; and

at least one organic solvent.

2. The solution formulation of claim 1, wherein the at least one organic solvent comprises diethylene glycol (DEG).

3. The solution formulation of claim 2, wherein a concentration of betaine in the solution formulation is between about 1M and about 5M, and a concentration of DEG in the solution formulation is between about 3% and about 40%.

4. The solution formulation of claim 3, wherein the concentration of DEG in the solution formulation is between about 20% and about 40%.

5. The solution formulation of claim 4, wherein the concentration of DEG in the solution formulation is between about 20% and about 30%.

6. The solution formulation of any one of claims 3-5, wherein the concentration of DEG in the solution formulation is about 20%.

7. The solution formulation of any one of claims 1-6, wherein the concentration of betaine in the solution formulation is between about 2M and about 5M.

8. The solution formulation of any one of claims 1-7, wherein the concentration of betaine in the solution formulation is between about 2M and about 4M.

9. The solution formulation of any one of claims 1-8, wherein the concentration of betaine in the solution formulation is about 2M.

10. The solution formulation of any one of claims 1-8, wherein the concentration of betaine in the solution formulation is about 3M.

11. The solution formulation of claim 1, wherein the at least one organic solvent comprises diethylene glycol (DEG) and dimethyl sulfoxide (DMSO).

12. The solution formulation of claim 11, wherein a concentration of betaine in the solution formulation is between about 1M and about 5M, and a concentration of the DEG in the solution formulation is between about 3% and about 40%.

13. The solution formulation of claim 11 or claim 12, wherein a concentration of DMSO in the solution formulation is between about 5% and about 30%.

14. The solution formulation of claim 13, wherein the concentration of the DMSO in the solution formulation is between about 7% and about 12%.

15. The solution formulation of claim 14, wherein the concentration of DMSO in the solution formulation is about 10%.

16. The solution formulation of claim 13, wherein the concentration of DMSO in the solution formulation is between about 17% and about 22%.

17. The solution formulation of claim 16, wherein the concentration of DMSO in the solution formulation is about 20%.

18. The solution formulation of any one of claims 11-17, wherein the concentration of the DEG in the solution formulation is between about 5% and about 25%.

19. The solution formulation of claim 18, wherein the concentration of the DEG in the solution formulation is between about 13% and about 17%.

20. The solution formulation of claim 19, wherein the concentration of the DEG in the solution formulation is about 15%.

21. The solution formulation of any one of claims 11-20, wherein the concentration of betaine in the solution formulation is between about 2M and about 3M.

22. The solution formulation of claim 21, wherein the concentration of betaine in the solution formulation is about 2M.

23. The solution formulation of claim 21, wherein the concentration of betaine in the solution formulation is about 2.5M.

24. The solution formulation of claim 21, wherein the concentration of betaine in the solution formulation is about 3M.

25. The solution formulation of claim 1, wherein the at least one organic solvent comprises DMSO.

26. The solution formulation of claim 25, wherein a concentration of betaine in the solution formulation is between about 1M and about 5M.

27. The solution formulation of claim 26, wherein the concentration of betaine in the solution formulation is between about 2M and about 3M.

28. The solution formulation of claim 27, wherein the concentration of betaine in the solution formulation is about 2M.

29. The solution formulation of claim 27, wherein the concentration of betaine in the solution formulation is about 2.5M.

30. The solution formulation of claim 27, wherein the concentration of betaine in the solution formulation is about 3M.

31. The solution formulation of any one of claims 25-30, wherein a concentration of DMSO in the solution formulation is between about 5% and about 30%.

32. The solution formulation of claim 31, wherein the concentration of the DMSO in the solution formulation is between about 7% and about 12%.

33. The solution formulation of claim 31, wherein the concentration of DMSO in the solution formulation is about 10%.

34. The solution formulation of claim 31, wherein the concentration of DMSO in the solution formulation is between about 17% and about 22%.

35. The solution formulation of claim 31, wherein the concentration of DMSO in the solution formulation is about 20%.

36. The solution formulation of any one of claims 1-35, further comprising a zwitterionic buffer.

37. The solution formulation of claim 36, wherein the zwitterionic buffer produces a pH in the solution formulation at a range of between about 9.5 and about 11.5.

38. The solution formulation of claim 36 or claim 37, wherein the zwitterionic buffer comprises any one or more of 4-(cyclohexylamino)-1-butanesulfonic acid (CABS), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO), *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS), and 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate) (CHAPS).

39. A method of denaturing double-stranded DNA (dsDNA), the method comprising:
contacting the dsDNA in a fluid-filled container with a denaturant comprising betaine.

40. The method of claim 39, wherein a ratio of denaturant to the dsDNA is about 1:1.

41. The method of claim 39, wherein the ratio of the denaturant to the dsDNA is about 1.3:1.

42. The method of claim 39, wherein the ratio of the denaturant to the dsDNA is about 1.5:1.

43. The method of claim 39, wherein the ratio of the denaturant to the dsDNA is about 2:1.

44. The method of claim 39, wherein the ratio of the denaturant to the dsDNA is about 2.5:1.

45. The method of claim 39, wherein the ratio of the denaturant to the dsDNA is about 3:1.

46. The method of any one of claims 39-45, wherein the denaturant further comprises diethylene glycol (DEG).

47. The method of any one of claims 39-46, wherein the denaturant further comprises dimethyl sulfoxide (DMSO).

48. The method of any one of claims 39-47, wherein the denaturant further comprises a zwitterionic buffer.

49. The method of claim 48, wherein the zwitterionic buffer produces a pH in the fluid-filled container between about 9.5 and about 11.5.

50. A kit for denaturing double-stranded DNA (dsDNA), the kit comprising:
a denaturant comprising betaine and diethylene glycol (DEG) in a solution; and
a buffer.

51. The kit of claim 50, wherein a concentration of DEG in the solution is between about 20% and about 40%.

52. The kit of claim 50 or claim 51, wherein the denaturant further comprises dimethyl sulfoxide (DMSO).

53. The kit of any one of claims 50 to 52, wherein the concentration of DEG in the solution is between about 5% and about 20%.

54. The kit of any one of claims 50-53, wherein a concentration of betaine in the solution is between about 1M and 5M.

55. The kit of claim 54, wherein the concentration of betaine in the solution is about 2M.

56. The kit of claim 54, wherein the concentration of betaine in the solution is about 2.5M.

57. The kit of claim 54, wherein the concentration of betaine in the solution is about 3.0M.

58. The kit of claim 54, wherein the concentration of betaine in the solution is about 3.5M.

59. The kit of any one of claims 50 to 53, wherein the concentration of betaine in the solution is between about 2M and about 3.5M.

60. The method of claim 39, wherein a ratio of denaturant to the dsDNA is between about 1:1 and about 3:1.

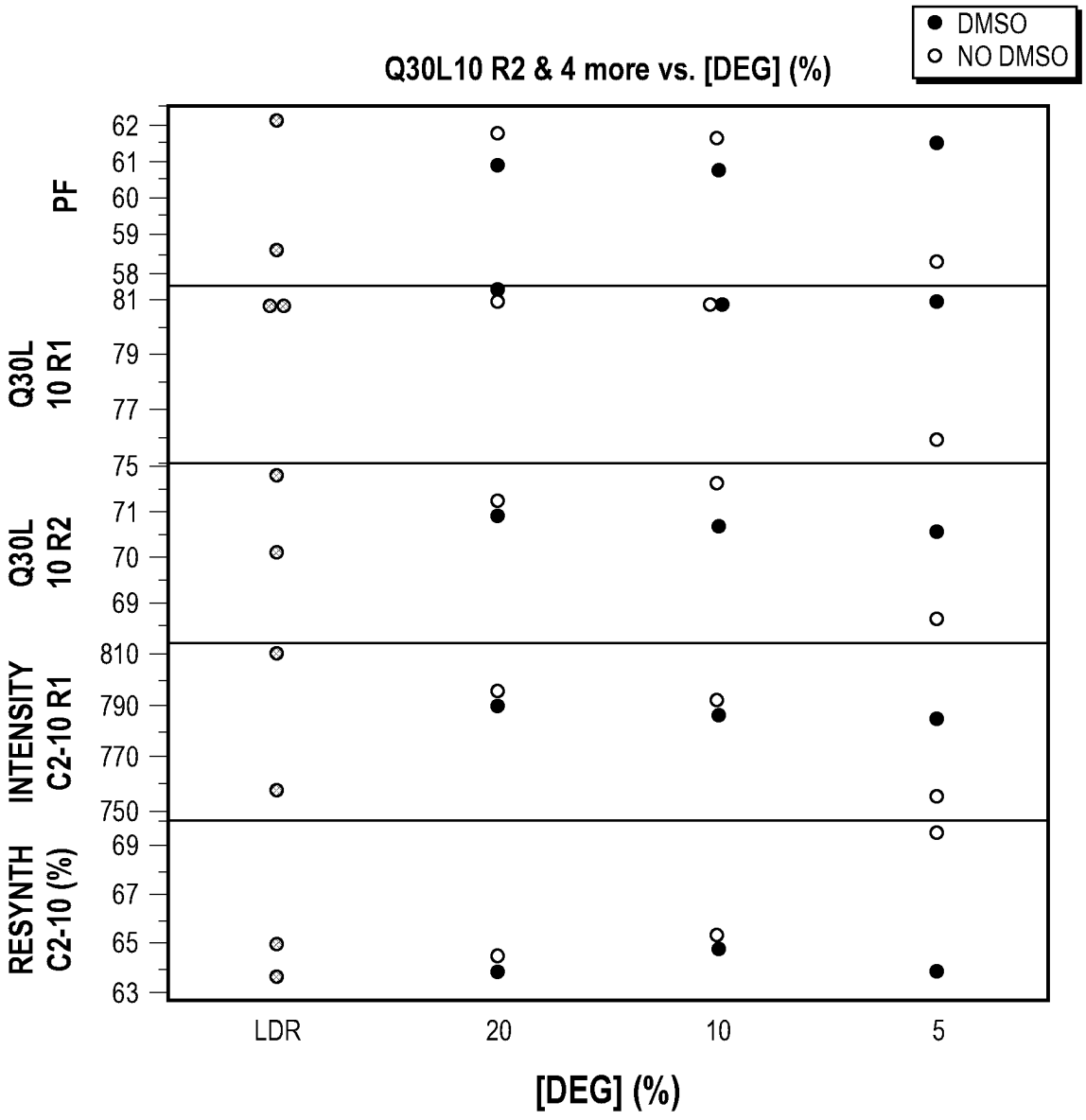

DMSO
NO DMSO
Q30L10 R2 & 4 more vs. [DEG] (%)
PF
Q30L 10 R1
Q30L 10 R2
INTENSITY C2-10 R1
RESYNTH C2-10 (%)
62
61
60
59
58
81
79
77
75
71
70
69
810
790
770
750
69
67
65
63
LDR
20
10
5
[DEG] (%)

FIG. 1A

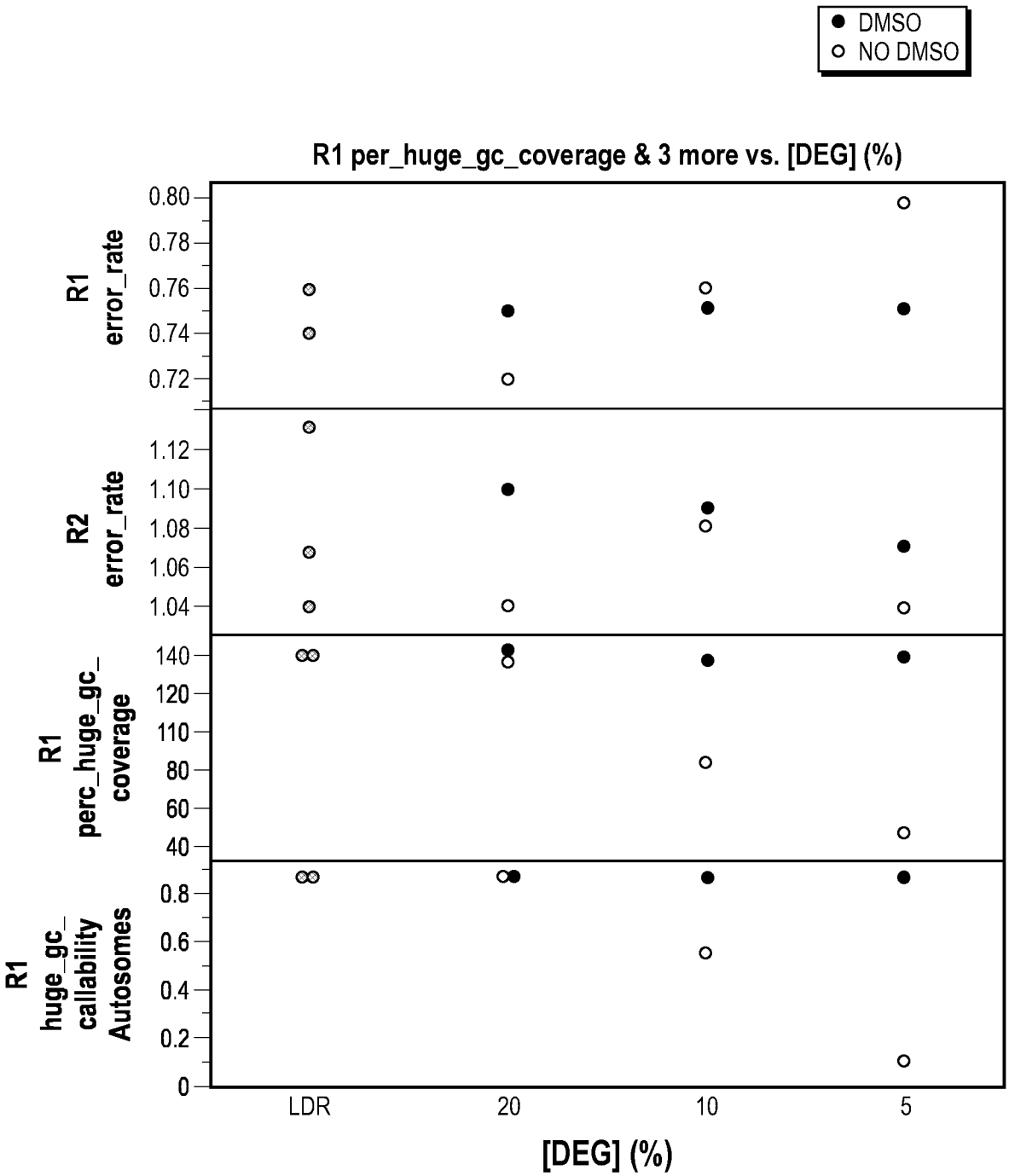

DMSO
NO DMSO
R1 per_huge_gc_coverage & 3 more vs. [DEG] (%)
R1 error_rate
0.80
0.78
0.76
0.74
0.72
R2 error_rate
1.12
1.10
1.08
1.06
1.04
R1 perc_huge_gc_ coverage
140
120
110
80
60
40
R1 huge_gc_ callability Autosomes
0.8
0.6
0.4
0.2
0
LDR
20
10
5
[DEG] (%)

FIG. 1B

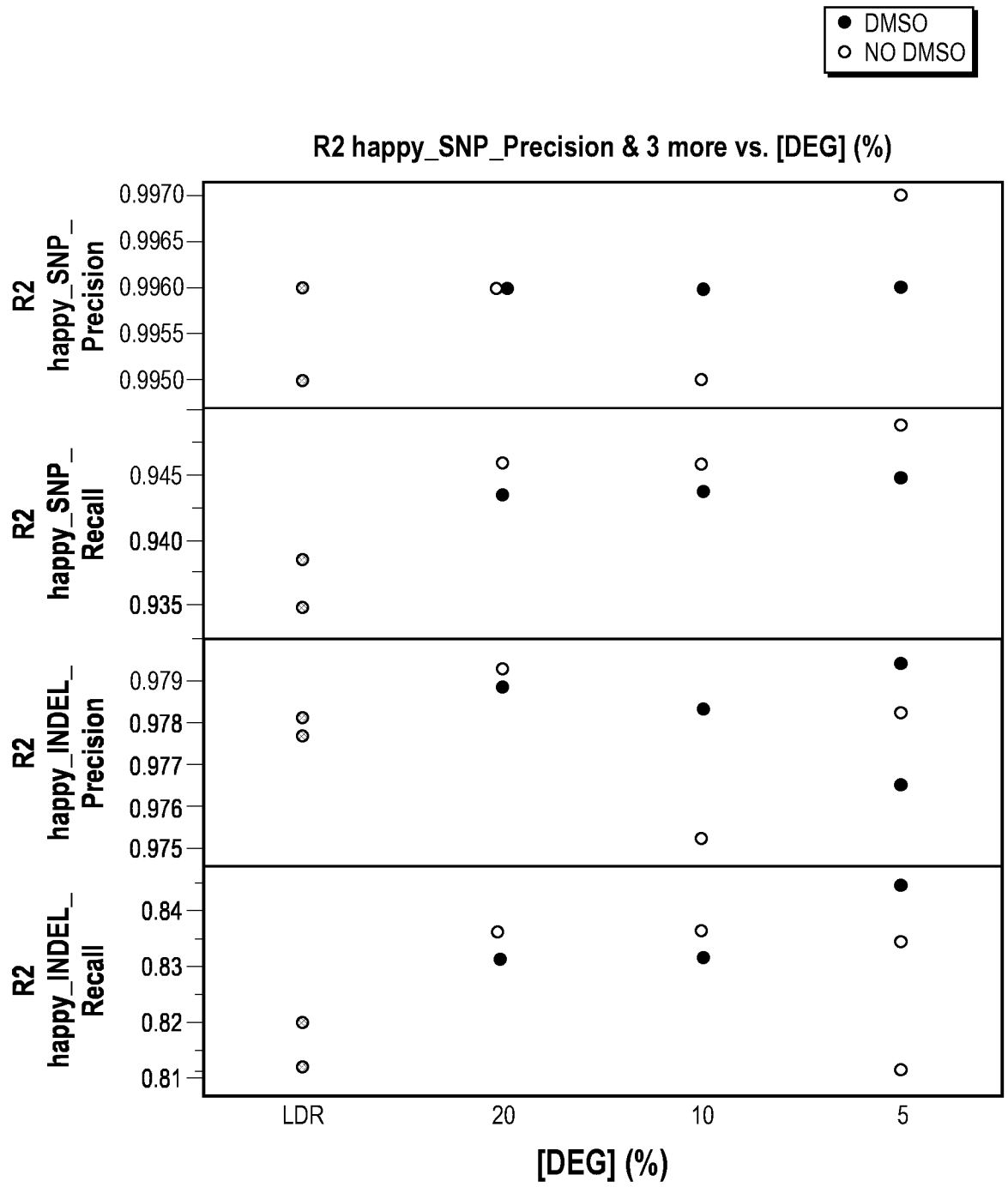

DMSO
NO DMSO
R2 happy_SNP_Precision & 3 more vs. [DEG] (%)
R2 happy_SNP_Precision
0.9970
0.9965
0.9960
0.9955
0.9950
R2 happy_SNP_Recall
0.945
0.940
0.935
R2 happy_INDEL_Precision
0.979
0.978
0.977
0.976
0.975
R2 happy_INDEL_Recall
0.84
0.83
0.82
0.81
LDR
20
10
5
[DEG] (%)

FIG. 1C

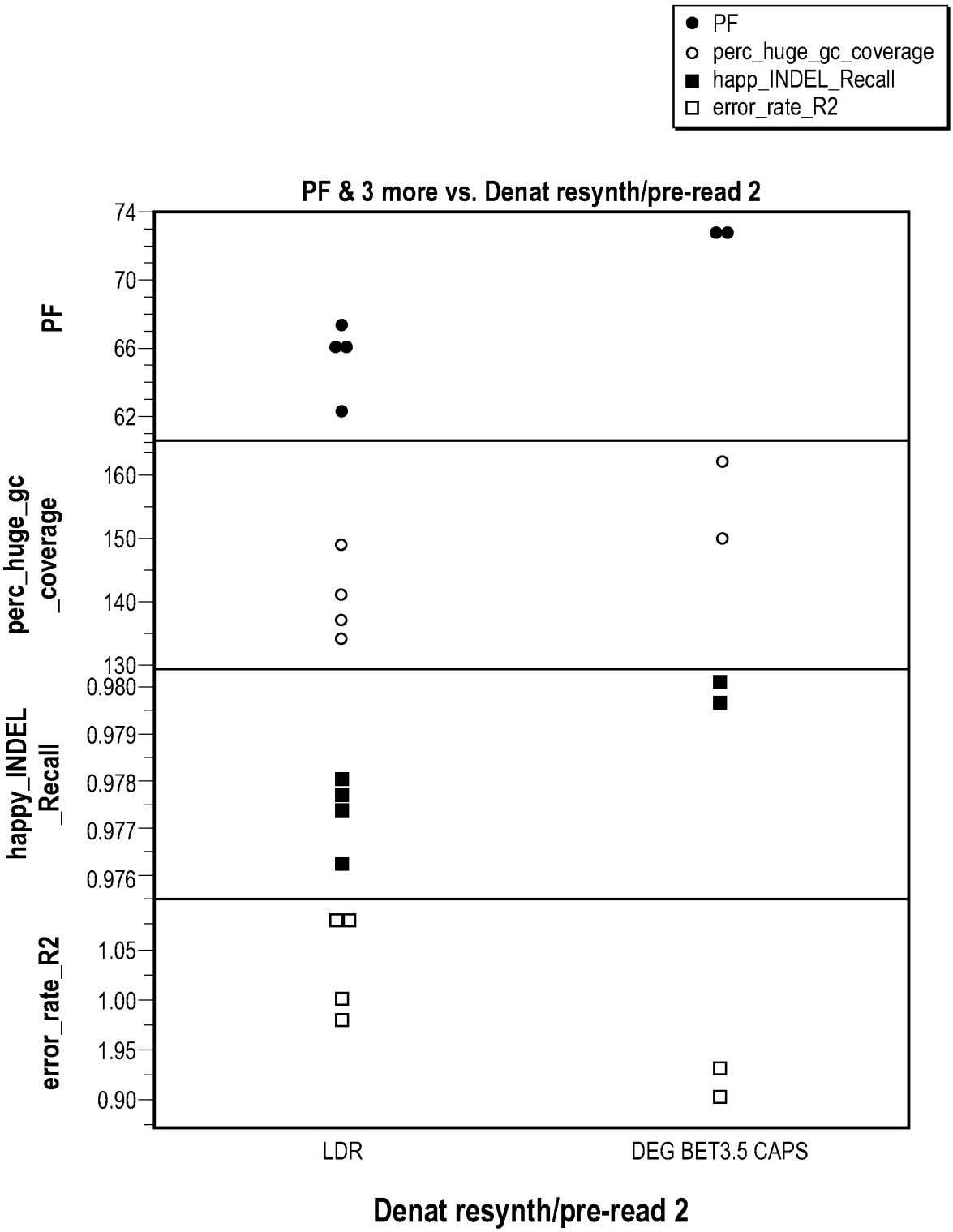

PF
perc_huge_gc_coverage
happ_INDEL_Recall
error_rate_R2
PF & 3 more vs. Denat resynth/pre-read 2
PF
74
70
66
62
perc_huge_gc
_coverage
160
150
140
130
happy_INDEL
_Recall
0.980
0.979
0.978
0.977
0.976
error_rate_R2
1.05
1.00
1.95
0.90
LDR
DEG BET3.5 CAPS
Denat resynth/pre-read 2

FIG. 2

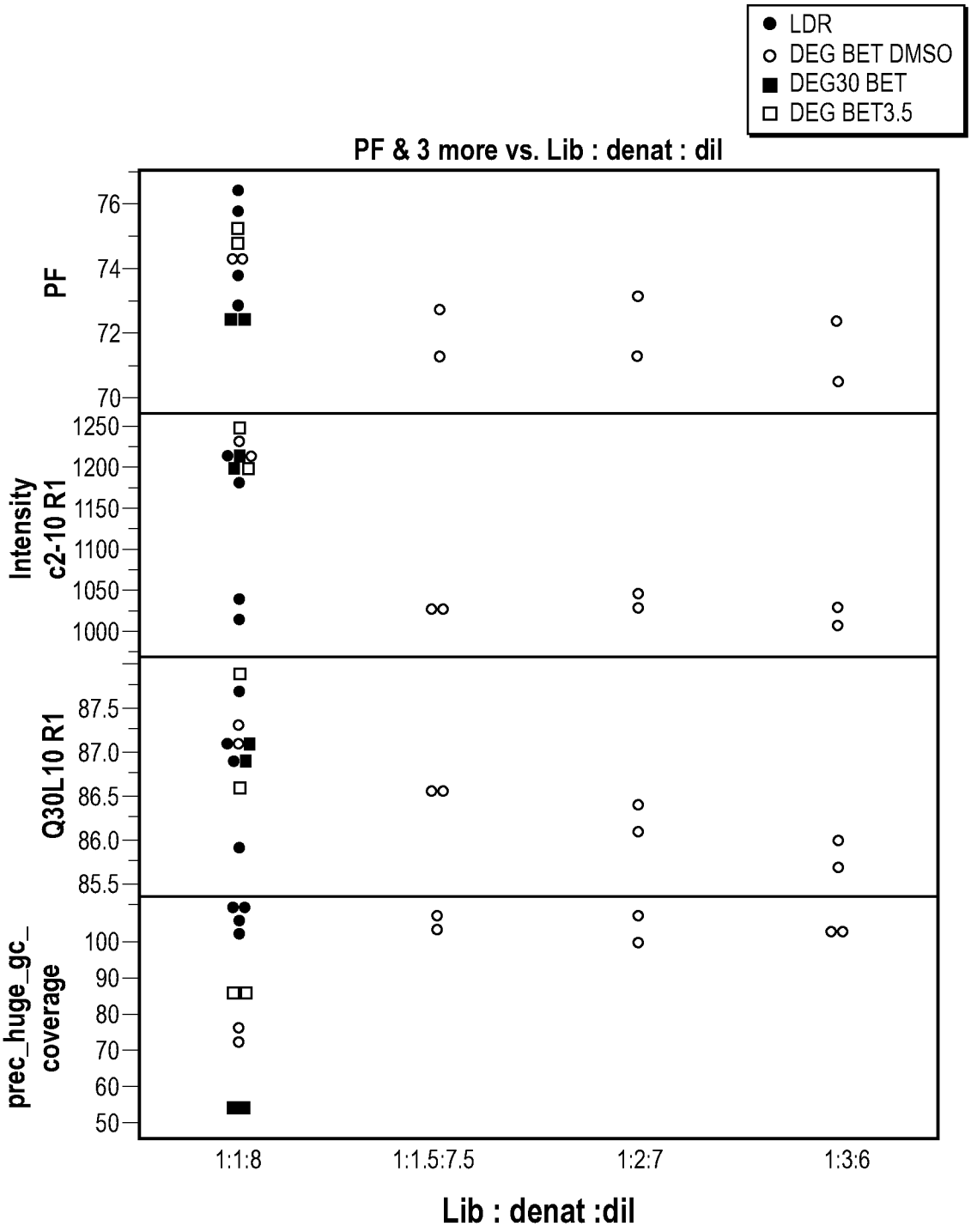

LDR
DEG BET DMSO
DEG30 BET
DEG BET3.5
PF & 3 more vs. Lib : denat : dil
PF
76
74
72
70
Intensity c2-10 R1
1250
1200
1150
1100
1050
1000
Q30L10 R1
87.5
87.0
86.5
86.0
85.5
prec_huge_gc_ coverage
100
90
80
70
60
50
1:1:8
1:1.5:7.5
1:2:7
1:3:6
Lib : denat :dil

FIG. 3

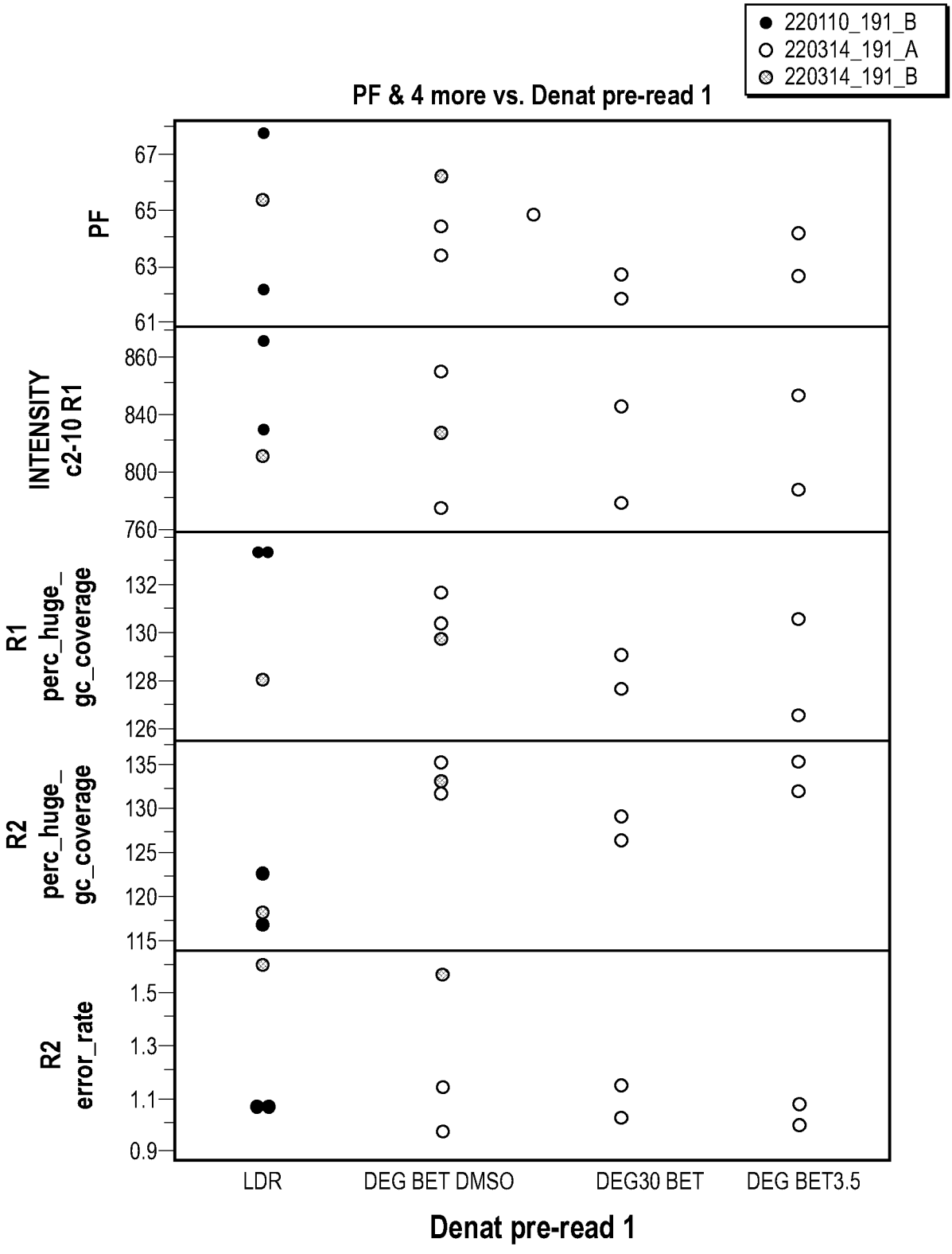

220110_191_B
220314_191_A
220314_191_B
PF & 4 more vs. Denat pre-read 1
PF
67
65
63
61
INTENSITY c2-10 R1
860
840
800
760
R1 perc_huge_ gc_coverage
132
130
128
126
R2 perc_huge_ gc_coverage
135
130
125
120
115
R2 error_rate
1.5
1.3
1.1
0.9
LDR
DEG BET DMSO
DEG30 BET
DEG BET3.5
Denat pre-read 1

FIG. 4

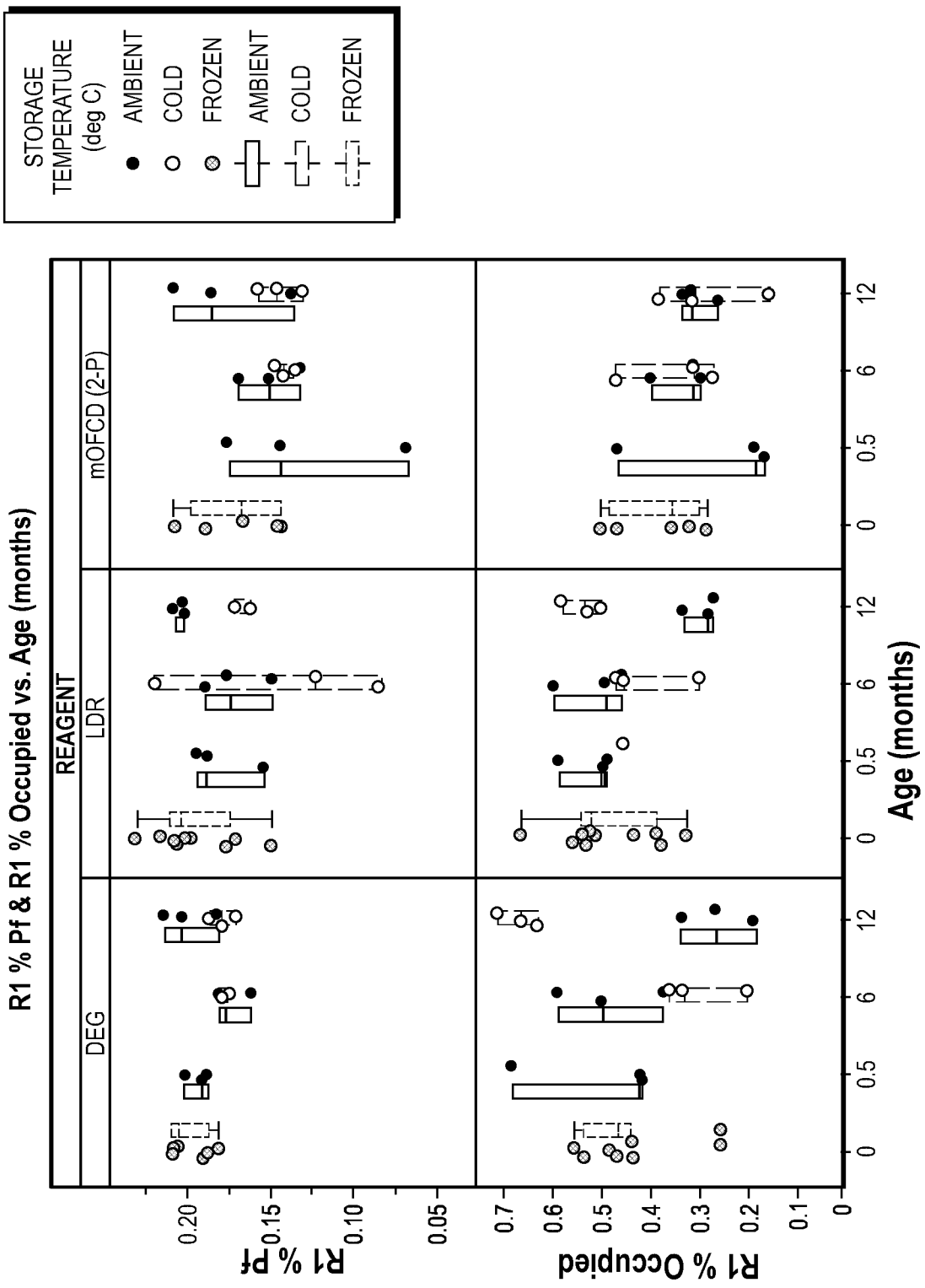

R1 % Pf & R1 % Occupied vs. Age (months)
REAGENT
DEG
LDR
mOFCD (2-P)
STORAGE TEMPERATURE (deg C)
AMBIENT
COLD
FROZEN
R1 % Pf
R1 % Occupied
Age (months)

FIG. 5A

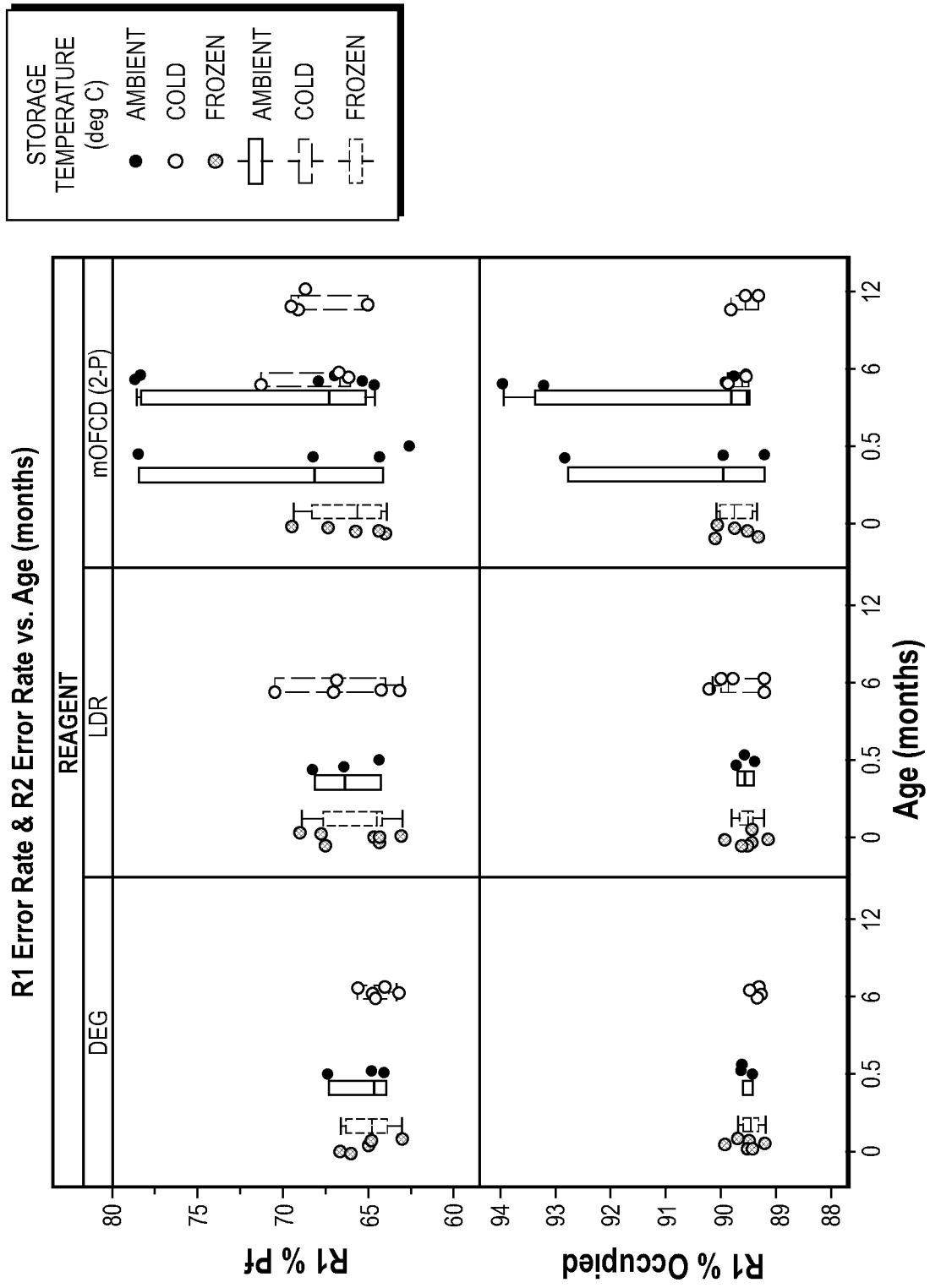

R1 Error Rate & R2 Error Rate vs. Age (months)
REAGENT
DEG
LDR
mOFCD (2-P)
R1 % Pf
R1 % Occupied
Age (months)
STORAGE TEMPERATURE (deg C)
AMBIENT
COLD
FROZEN
AMBIENT
COLD
FROZEN

FIG. 5B

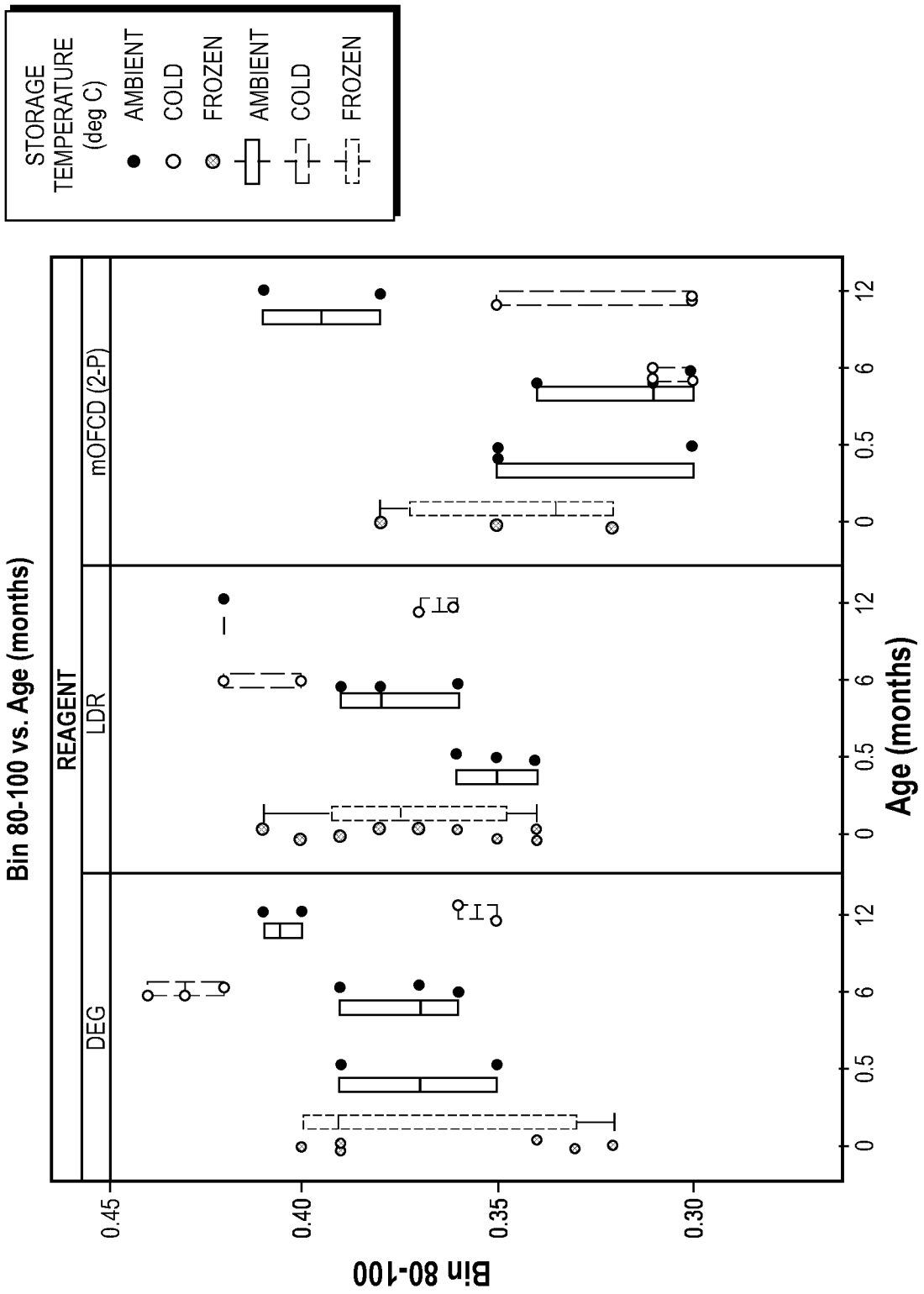

Bin 80-100 vs. Age (months)
REAGENT
DEG
LDR
mOFCD (2-P)
Bin 80-100
0.45
0.40
0.35
0.30
0
0.5
6
12
Age (months)
STORAGE TEMPERATURE (deg C)
AMBIENT
COLD
FROZEN
AMBIENT
COLD
FROZEN

FIG. 5C

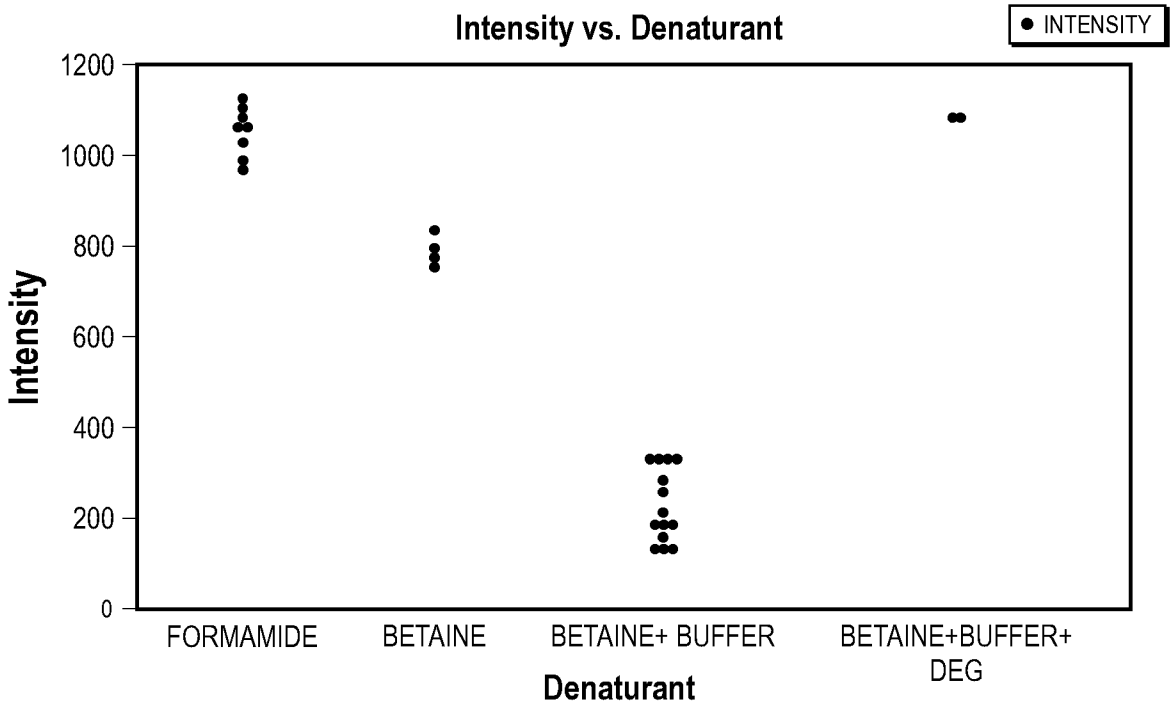

Intensity vs. Denaturant
INTENSITY
Intensity
1200
1000
800
600
400
200
0
FORMAMIDE
BETAINE
BETAINE+ BUFFER
BETAINE+BUFFER+ DEG
Denaturant

FIG. 6A

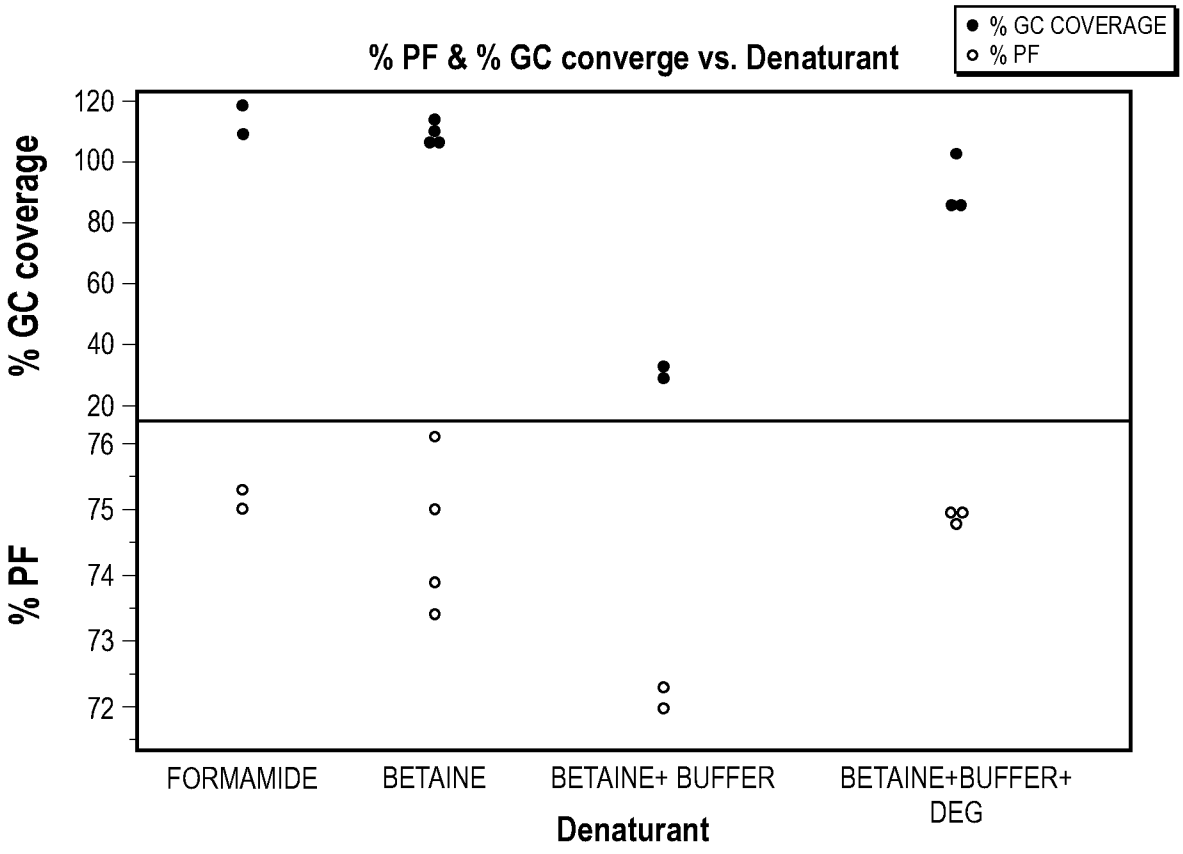

% PF & % GC converge vs. Denaturant
% GC COVERAGE
% PF
% GC coverage
120
100
80
60
40
20
% PF
76
75
74
73
72
FORMAMIDE
BETAINE
BETAINE+ BUFFER
BETAINE+BUFFER+ DEG
Denaturant

FIG. 6B

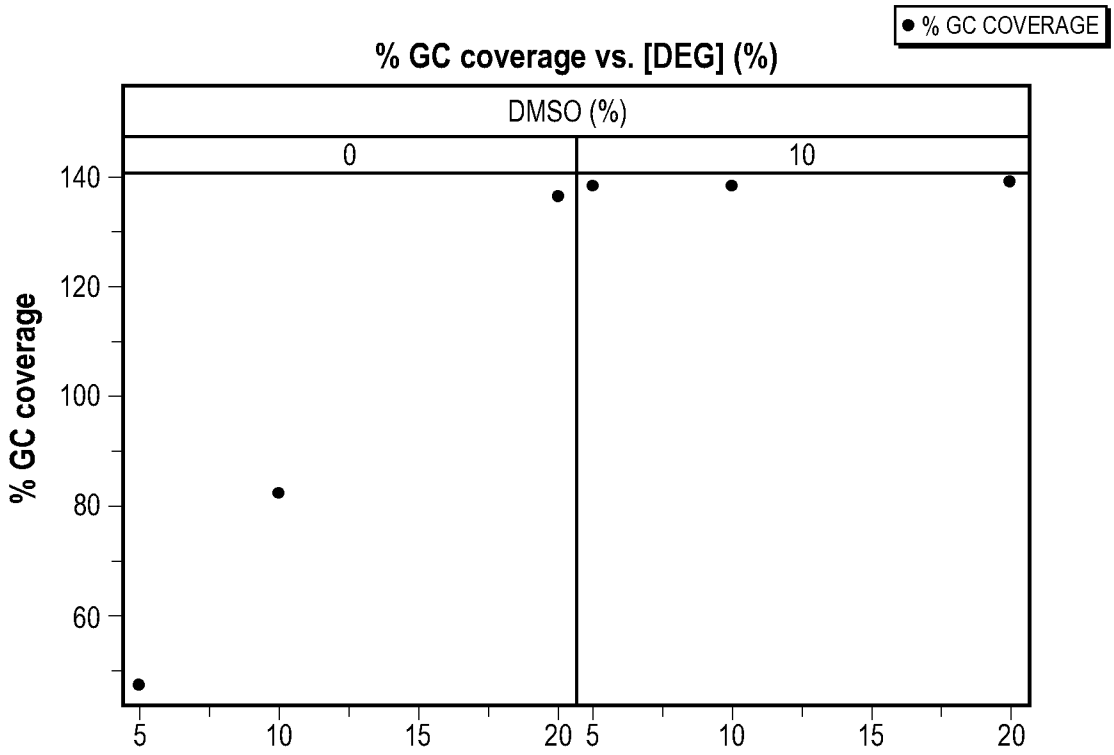

% GC COVERAGE
% GC coverage vs. [DEG] (%)
DMSO (%)
0
10
% GC coverage
140
120
100
80
60
5
10
15
20

FIG. 7A

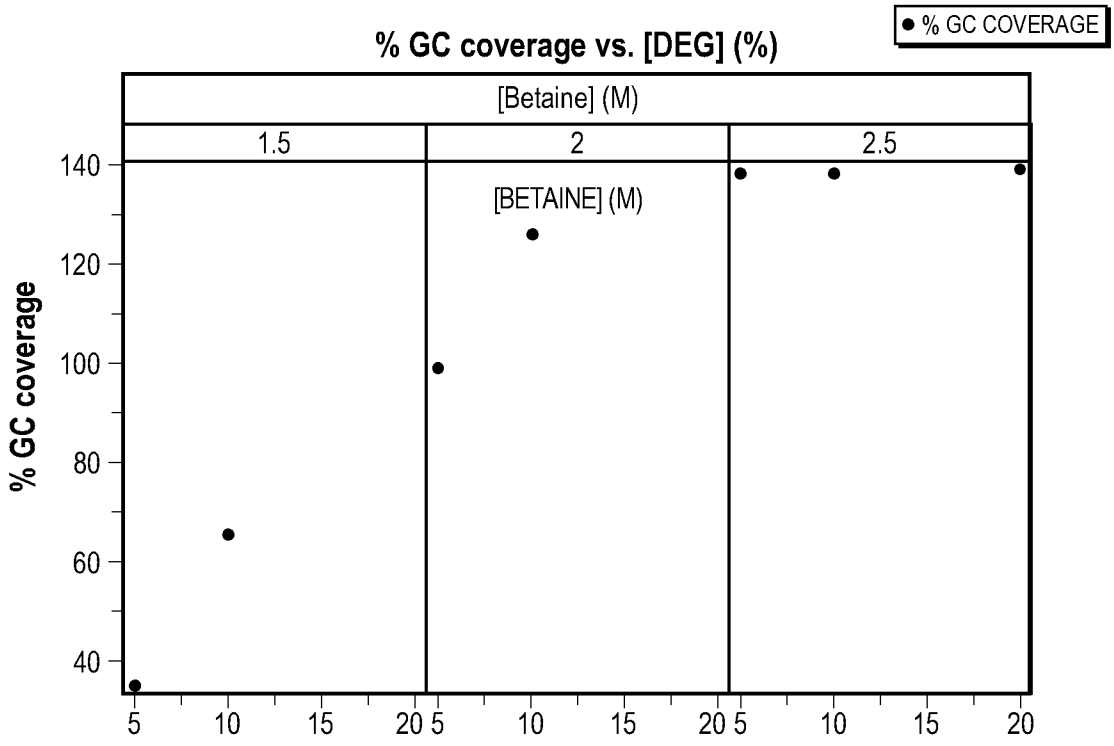

% GC COVERAGE
% GC coverage vs. [DEG] (%)
[Betaine] (M)
1.5
2
2.5
[BETAINE] (M)
% GC coverage
140
120
100
80
60
40
5
10
15
20

FIG. 7B

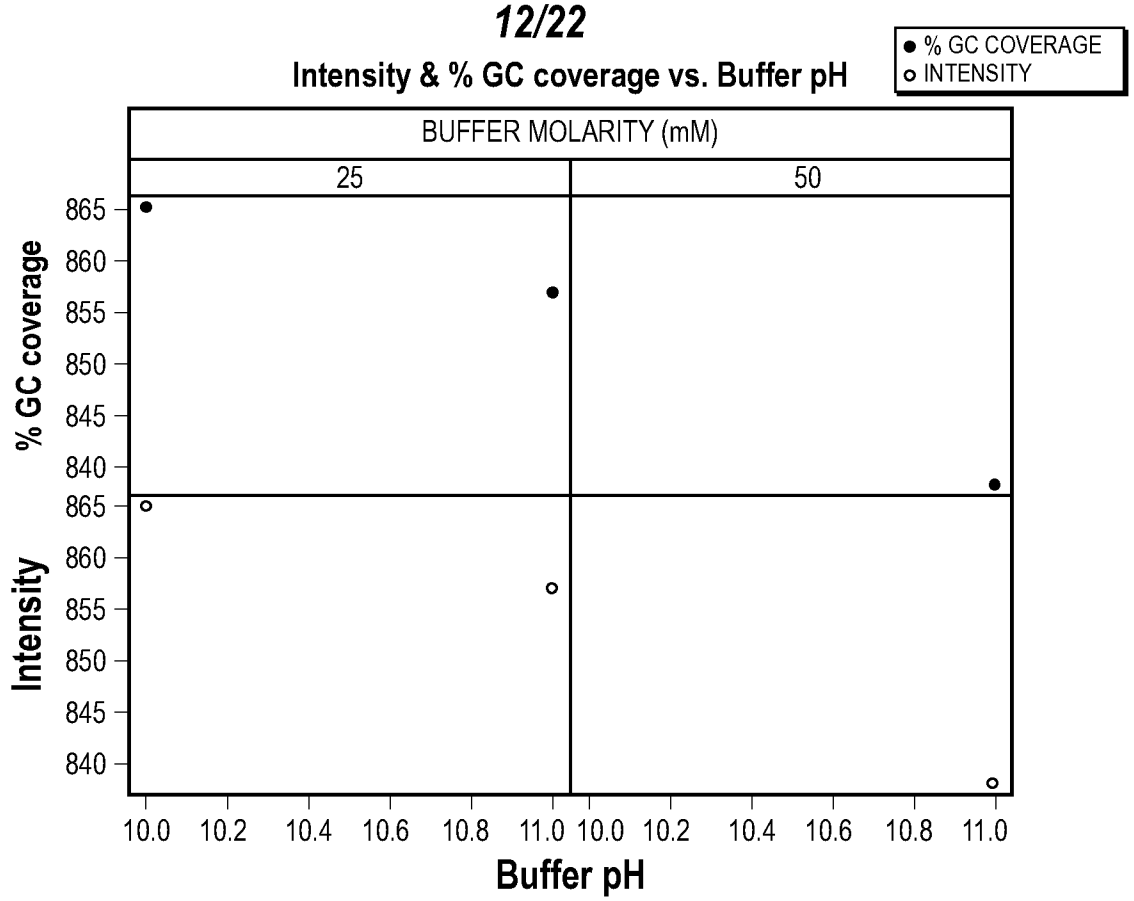

Intensity & % GC coverage vs. Buffer pH
% GC COVERAGE
INTENSITY
BUFFER MOLARITY (mM)
25
50
% GC coverage
Intensity
865
860
855
850
845
840
10.0
10.2
10.4
10.6
10.8
11.0
Buffer pH

FIG. 8

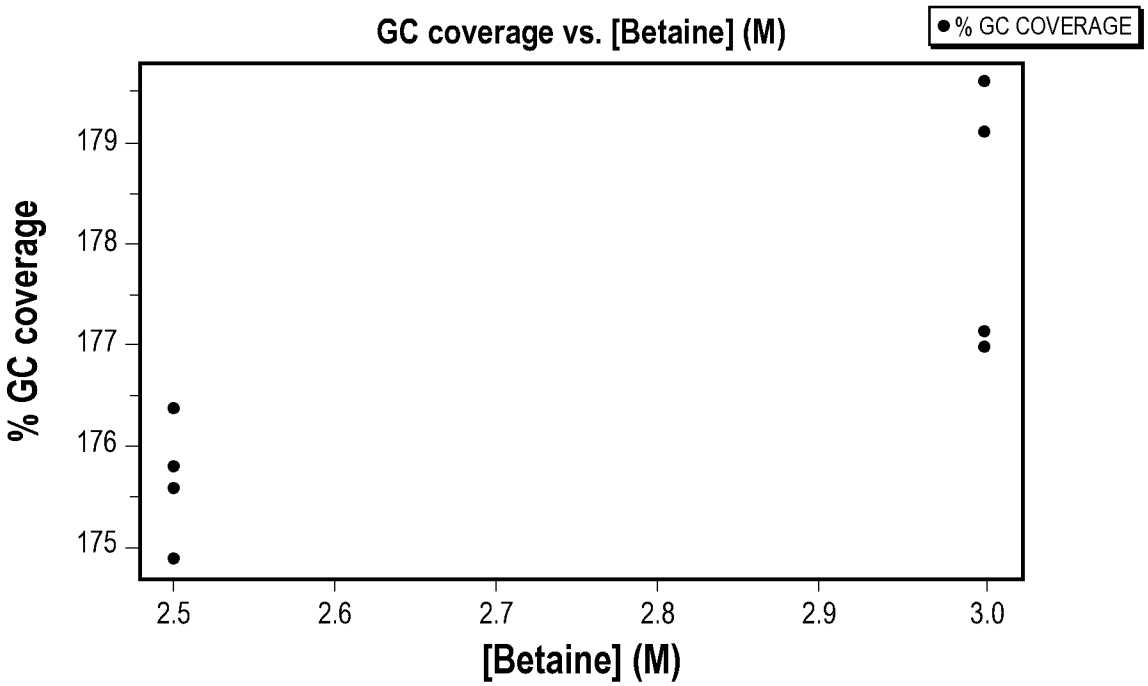

GC coverage vs. [Betaine] (M)
% GC COVERAGE
% GC coverage
179
178
177
176
175
2.5
2.6
2.7
2.8
2.9
3.0
[Betaine] (M)

FIG. 9

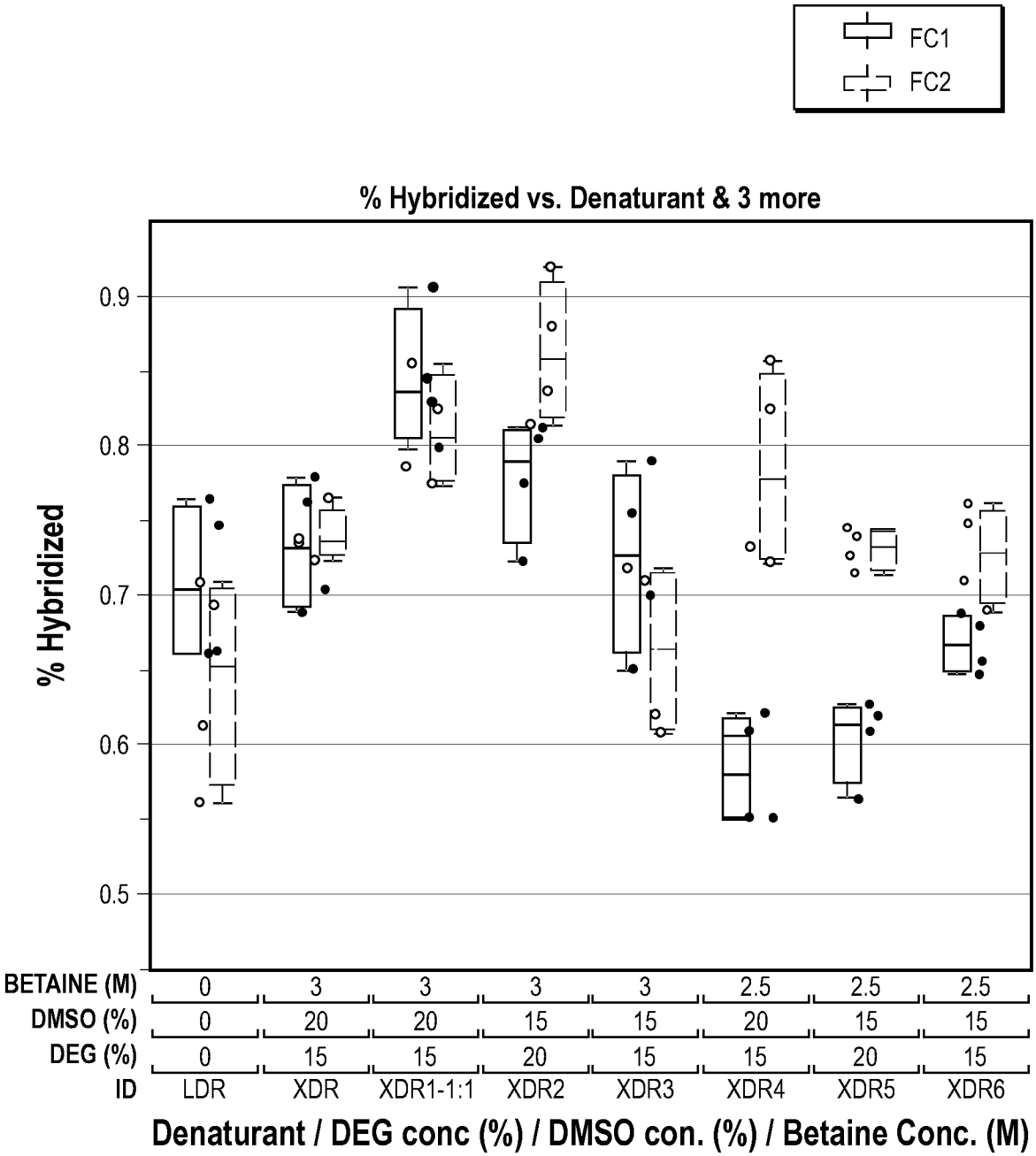

FC1
FC2
% Hybridized vs. Denaturant & 3 more
% Hybridized
0.9
0.8
0.7
0.6
0.5
BETAINE (M) 0 3 3 3 3 2.5 2.5 2.5
DMSO (%) 0 20 20 15 15 20 15 15
DEG (%) 0 15 15 20 15 15 20 15
ID LDR XDR XDR1-1:1 XDR2 XDR3 XDR4 XDR5 XDR6
Denaturant / DEG conc (%) / DMSO con. (%) / Betaine Conc. (M)

FIG. 10

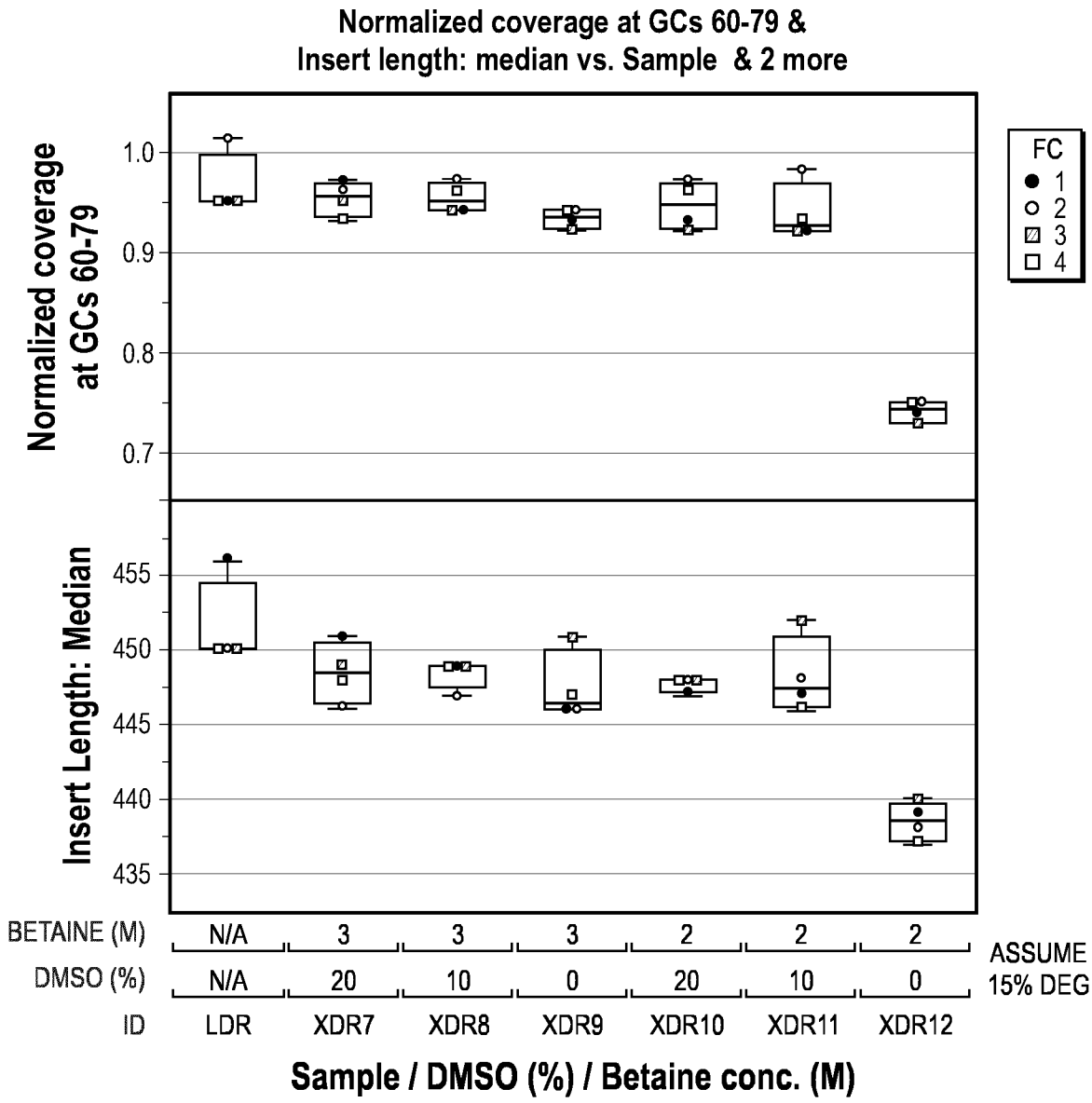
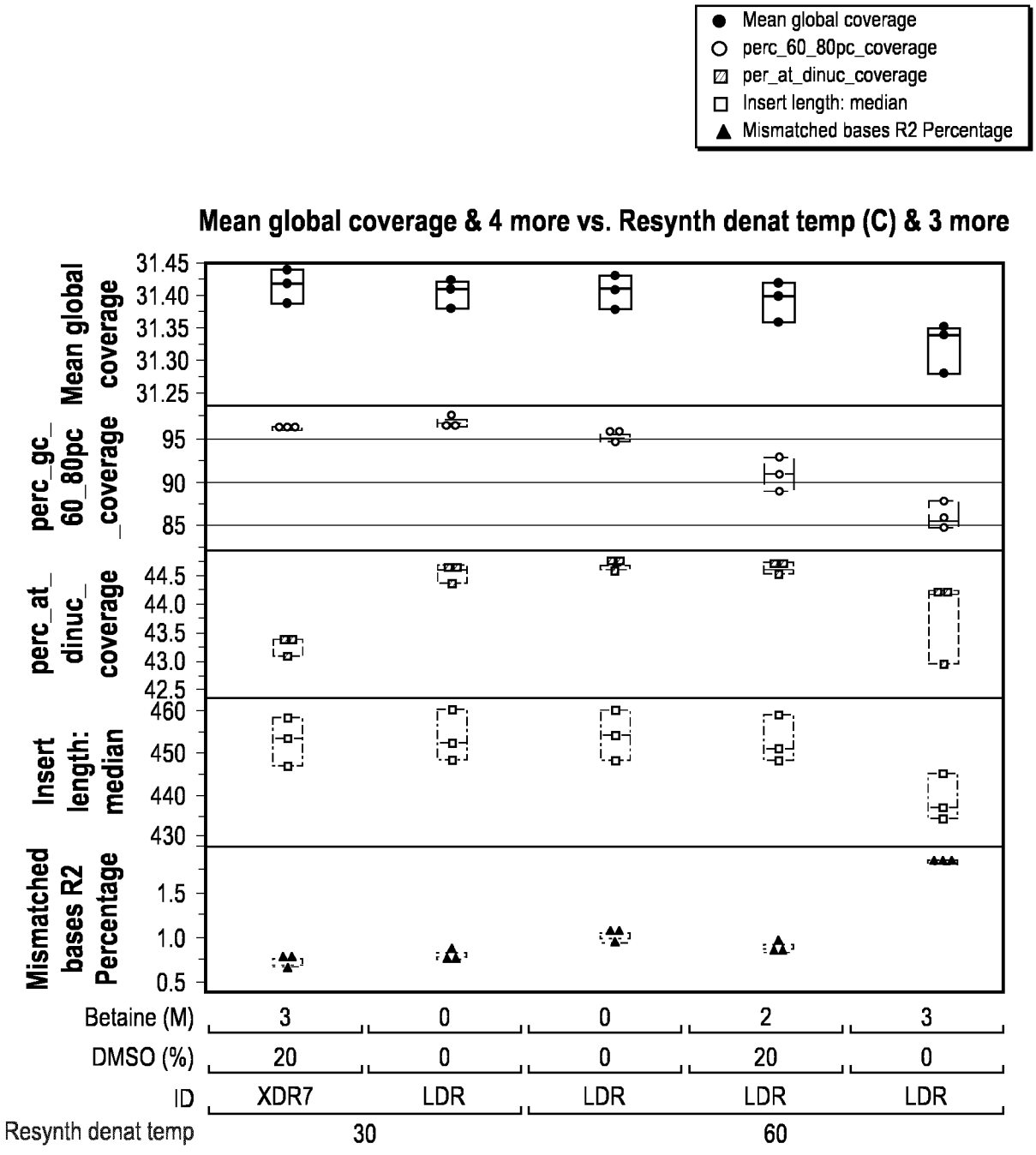

Normalized coverage at GCs 60-79 &
Insert length: median vs. Sample & 2 more
Normalized coverage at GCs 60-79
1.0
0.9
0.8
0.7
Insert Length: Median
455
450
445
440
435
FC
1
2
3
4
BETAINE (M) N/A 3 3 3 2 2 2
DMSO (%) N/A 20 10 0 20 10 0
ID LDR XDR7 XDR8 XDR9 XDR10 XDR11 XDR12
ASSUME 15% DEG
Sample / DMSO (%) / Betaine conc. (M)

FIG. 11



Resynth denat temp (C) / Denaturant / DMSO (%) / Betaine (M) ordered by perc_gc_60_80pc_coverage (decending)

FIG. 12

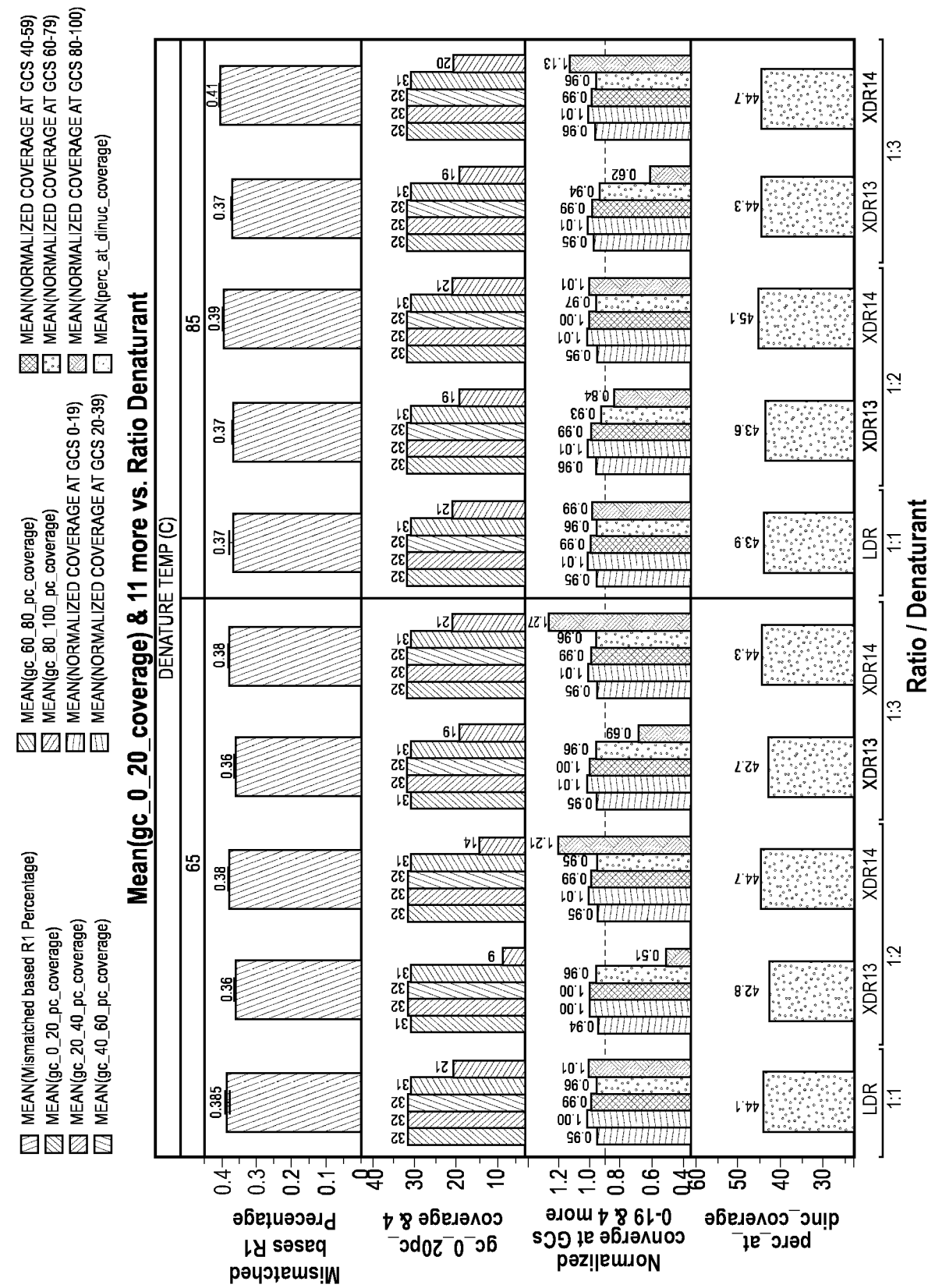

MEAN(Mismatched based R1 Percentage)
MEAN(gc_0_20_pc_coverage)
MEAN(gc_20_40_pc_coverage)
MEAN(gc_40_60_pc_coverage)
MEAN(gc_60_80_pc_coverage)
MEAN(gc_80_100_pc_coverage)
MEAN(NORMALIZED COVERAGE AT GCS 0-19)
MEAN(NORMALIZED COVERAGE AT GCS 20-39)
MEAN(NORMALIZED COVERAGE AT GCS 40-59)
MEAN(NORMALIZED COVERAGE AT GCS 60-79)
MEAN(NORMALIZED COVERAGE AT GCS 80-100)
MEAN(perc_at_dinuc_coverage)
Mean(gc_0_20_coverage) & 11 more vs. Ratio Denaturant
DENATURE TEMP (C)
65
85
Mismatched bases R1 Percentage
gc_0_20pc_ coverage & 4
Normalized converge at GCs 0-19 & 4 more
perc_at_ dinc_coverage
LDR
XDR13
XDR14
1:1
1:2
1:3
Ratio / Denaturant

FIG. 13

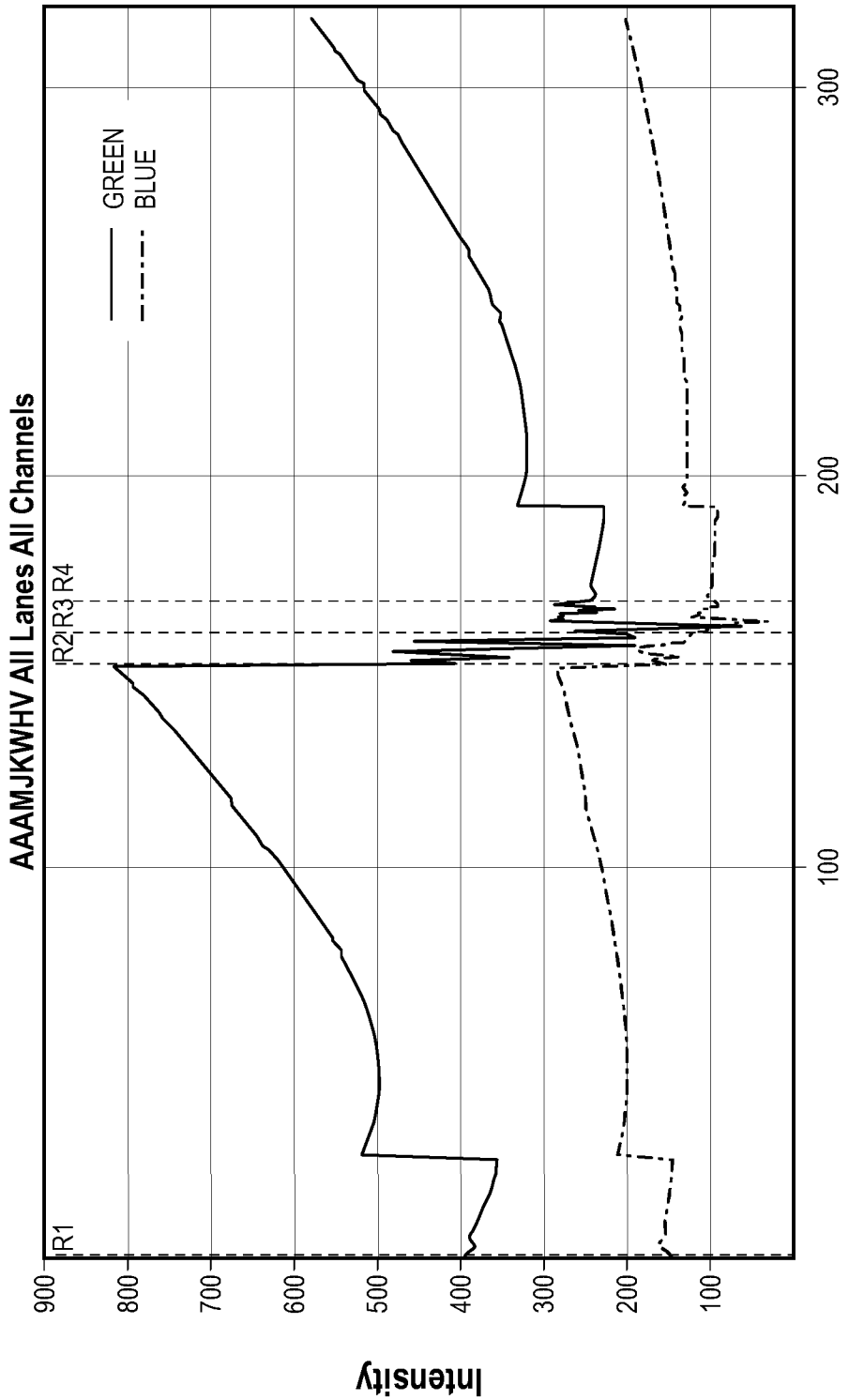

AAAMJKWHV All Lanes All Channels
GREEN
BLUE
R1
R2
R3
R4
Intensity
Cycle
900
800
700
600
500
400
300
200
100
100
200
300

FIG. 14

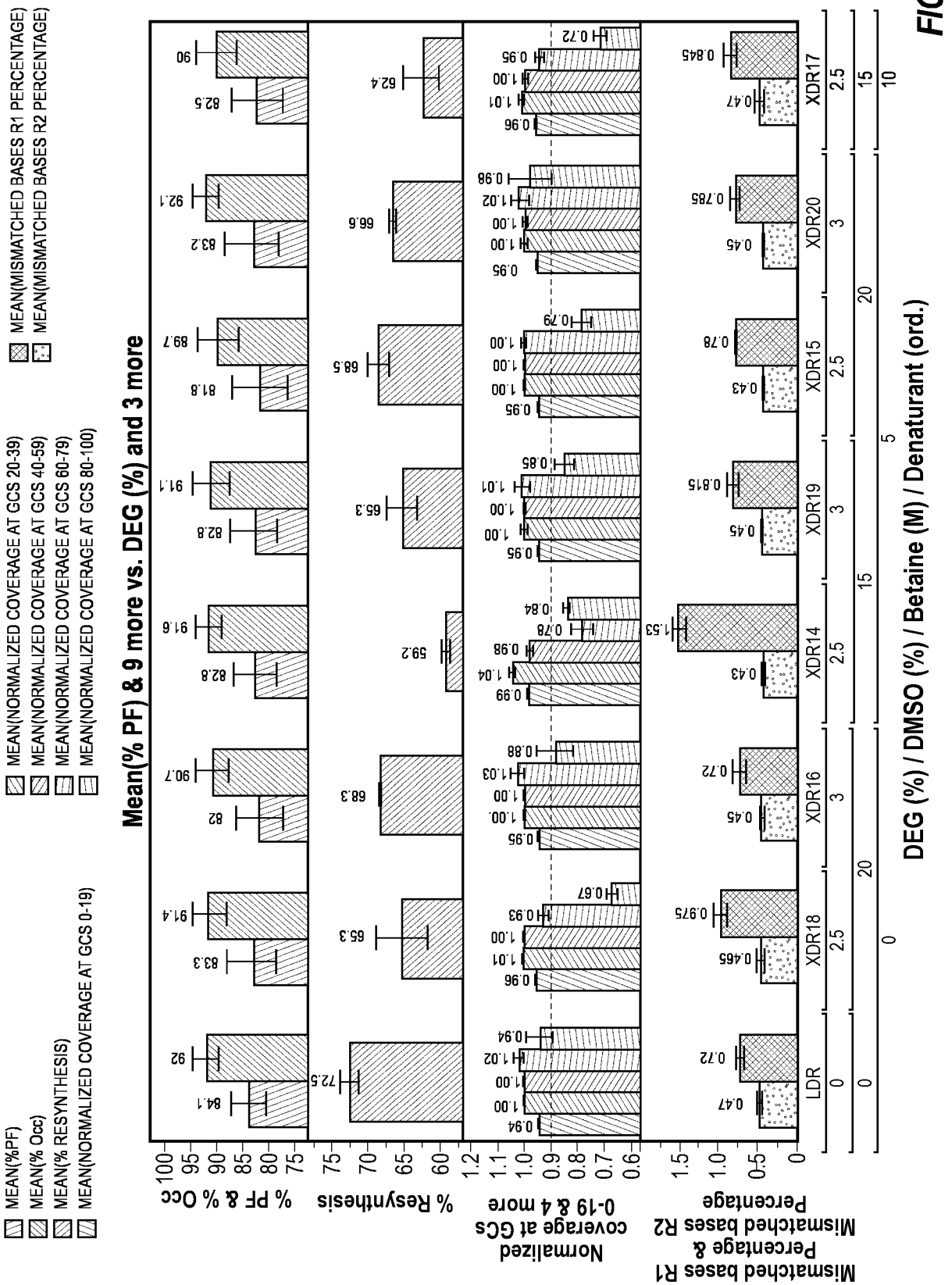

MEAN(%PF)
MEAN(% Occ)
MEAN(% RESYNTHESIS)
MEAN(NORMALIZED COVERAGE AT GCS 0-19)
MEAN(NORMALIZED COVERAGE AT GCS 20-39)
MEAN(NORMALIZED COVERAGE AT GCS 40-59)
MEAN(NORMALIZED COVERAGE AT GCS 60-79)
MEAN(NORMALIZED COVERAGE AT GCS 80-100)
MEAN(MISMATCHED BASES R1 PERCENTAGE)
MEAN(MISMATCHED BASES R2 PERCENTAGE)
Mean(% PF) & 9 more vs. DEG (%) and 3 more
% PF & % Occ
% Resynthesis
Normalized coverage at GCs 0-19 & 4 more
Mismatched bases R1 Percentage & Mismatched bases R2 Percentage
LDR
XDR18
XDR16
XDR14
XDR19
XDR15
XDR20
XDR17
DEG (%) / DMSO (%) / Betaine (M) / Denaturant (ord.)

FIG. 15

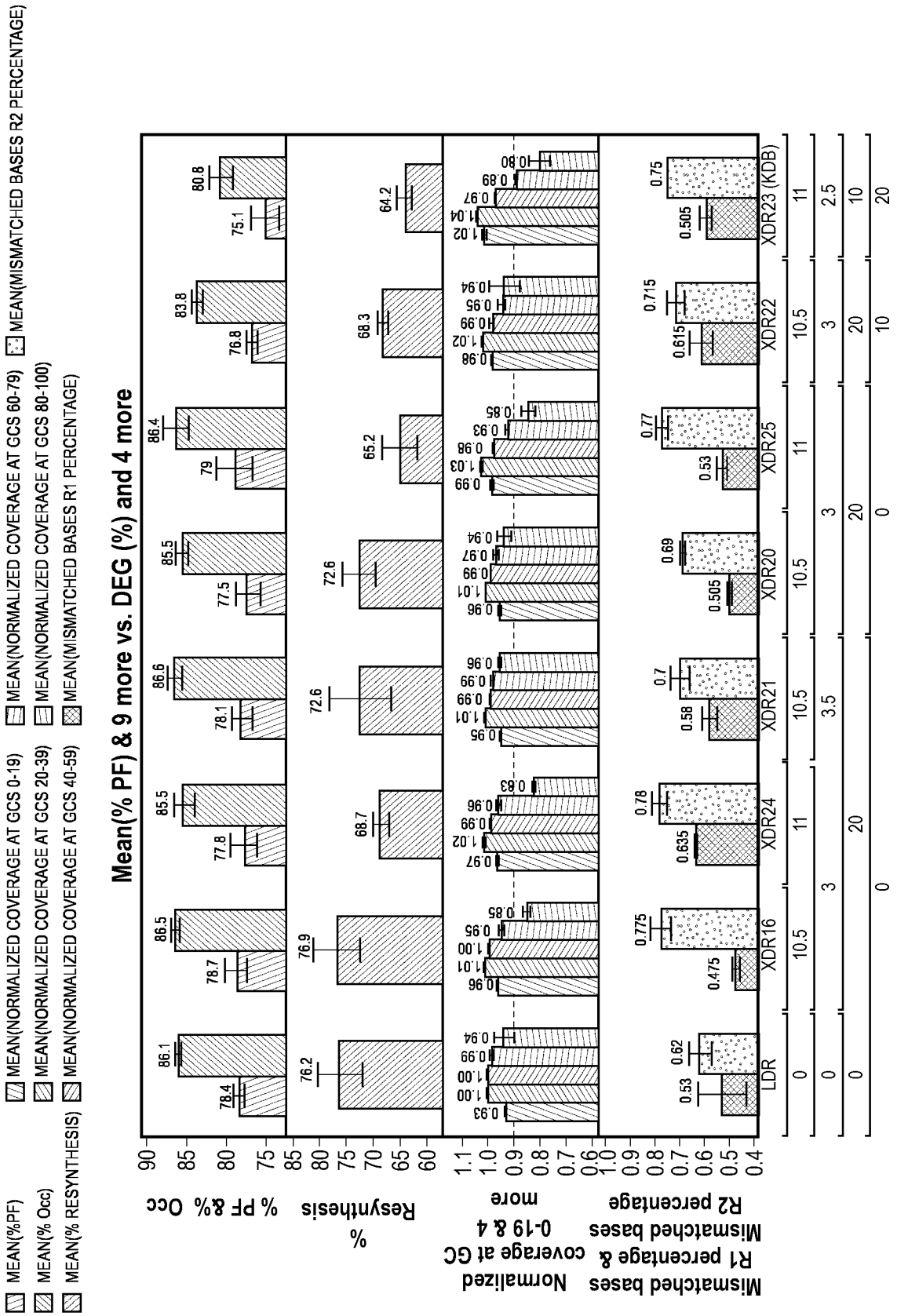

MEAN(%PF)
MEAN(% Occ)
MEAN(% RESYNTHESIS)
MEAN(NORMALIZED COVERAGE AT GCS 0-19)
MEAN(NORMALIZED COVERAGE AT GCS 20-39)
MEAN(NORMALIZED COVERAGE AT GCS 40-59)
MEAN(NORMALIZED COVERAGE AT GCS 60-79)
MEAN(NORMALIZED COVERAGE AT GCS 80-100)
MEAN(MISMATCHED BASES R1 PERCENTAGE)
MEAN(MISMATCHED BASES R2 PERCENTAGE)
Mean(% PF) & 9 more vs. DEG (%) and 4 more
% PF &% Occ
Resynthesis %
Normalized coverage at GC 0-19 & 4 more
Mismatched bases R1 percentage & Mismatched bases R2 percentage
LDR
XDR16
XDR24
XDR21
XDR20
XDR25
XDR22
XDR23 (KDB)
DEG (%) / DMSO (%) / Betaine (M) / CAPS pH / Denaturant

FIG. 16

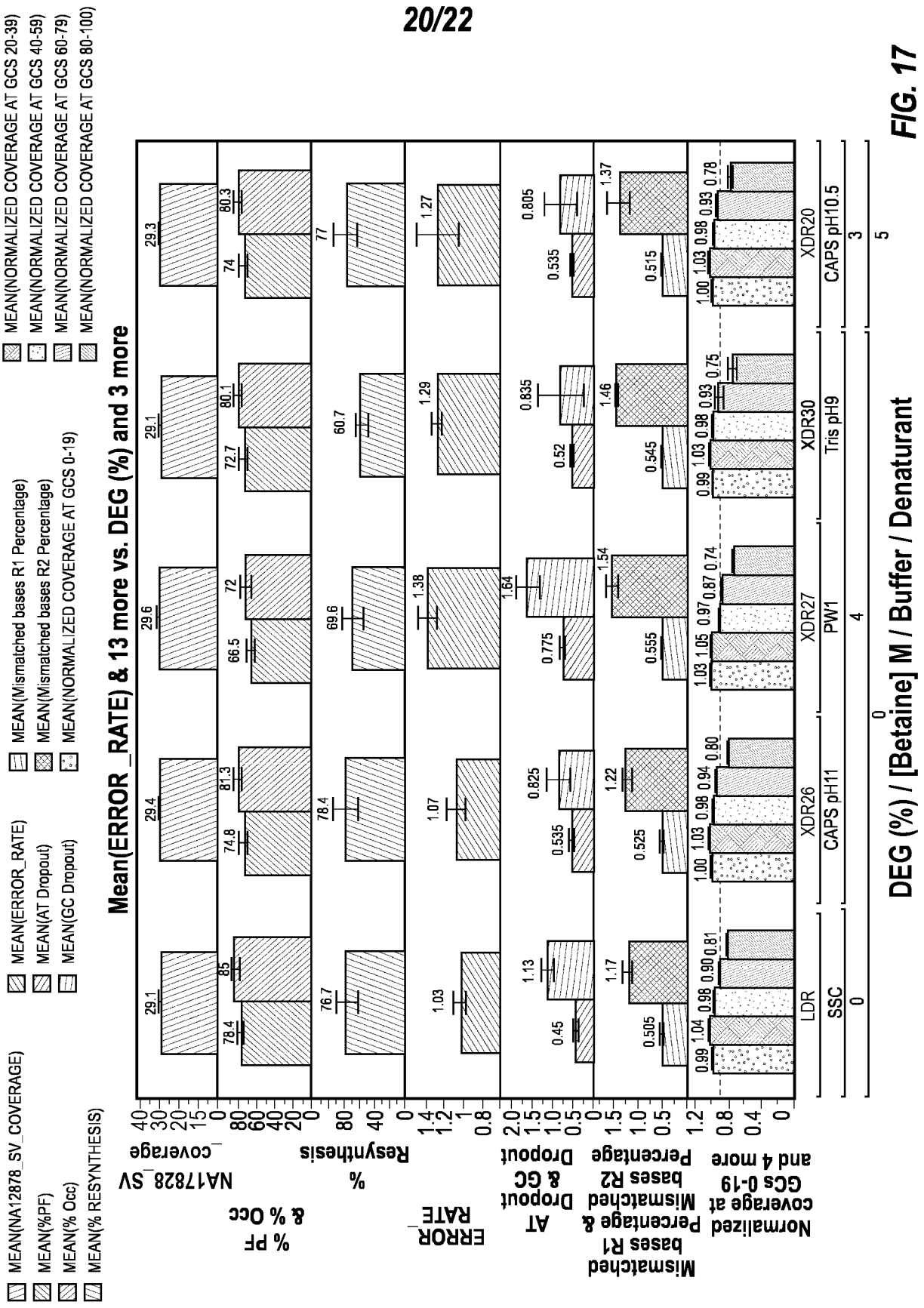
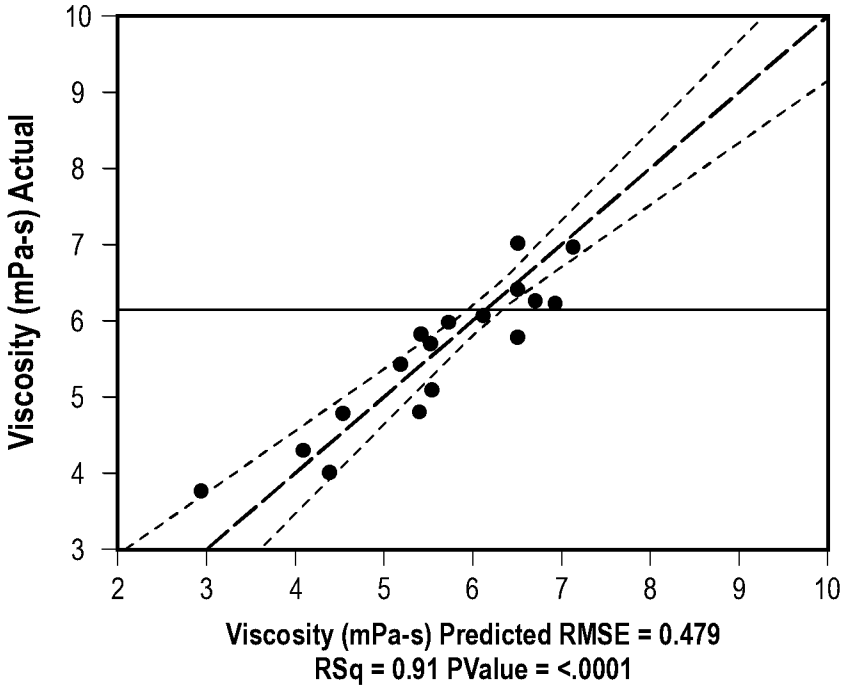

MEAN(NA12878_SV_COVERAGE)
MEAN(%PF)
MEAN(% Occ)
MEAN(% RESYNTHESIS)
MEAN(ERROR_RATE)
MEAN(AT Dropout)
MEAN(GC Dropout)
MEAN(Mismatched bases R1 Percentage)
MEAN(Mismatched bases R2 Percentage)
MEAN(NORMALIZED COVERAGE AT GCS 0-19)
MEAN(NORMALIZED COVERAGE AT GCS 20-39)
MEAN(NORMALIZED COVERAGE AT GCS 40-59)
MEAN(NORMALIZED COVERAGE AT GCS 60-79)
MEAN(NORMALIZED COVERAGE AT GCS 80-100)
Mean(ERROR_RATE) & 13 more vs. DEG (%) and 3 more
NA12878_SV_coverage
% PF & % Occ
% Resynthesis
ERROR_RATE
AT Dropout & GC Dropout
Mismatched bases R1 Percentage & Mismatched bases R2 Percentage
Normalized coverage at GCs 0-19 and 4 more
LDR
SSC
XDR26
CAPS pH11
XDR27
PW1
XDR30
Tris pH9
XDR20
CAPS pH10.5
DEG (%) / [Betaine] M / Buffer / Denaturant

FIG. 17



Effect Summary

Source	LogWorth	PValue
BETAINE (M)	9.447	0.00000
DEG (%)	7.943	0.00000
DMSO (%)	6.194	0.00000

FIG. 18

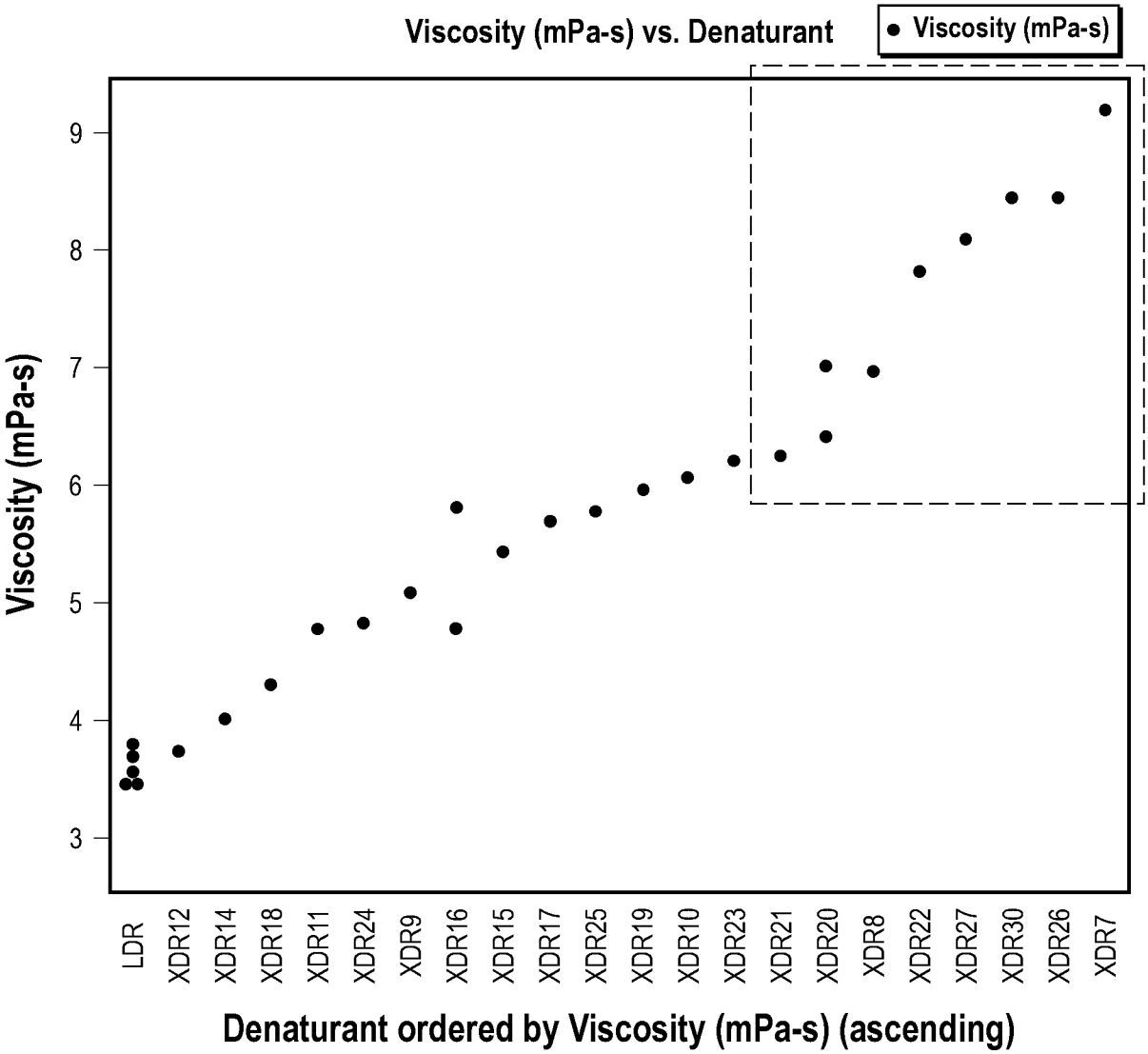

Viscosity (mPa-s) vs. Denaturant
Viscosity (mPa-s)
Viscosity (mPa-s)
9
8
7
6
5
4
3
LDR
XDR12
XDR14
XDR18
XDR11
XDR24
XDR9
XDR16
XDR15
XDR17
XDR25
XDR19
XDR10
XDR23
XDR21
XDR20
XDR8
XDR22
XDR27
XDR30
XDR26
XDR7
Denaturant ordered by Viscosity (mPa-s) (ascending)

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/068277

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6806
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/209970 A1 (LATHAM GARY J [US]) 19 August 2010 (2010-08-19) paragraphs [0012], [0050] - [0051], [0054], [0039] abstract the whole document	1-60
X	JP 4 761265 B2 (KONAN GAKUEN) 31 August 2011 (2011-08-31) claim 5 paragraphs [0008], [0188] abstract the whole document	1-60
A	US 2007/037139 A1 (TOMONO JUN [JP] ET AL) 15 February 2007 (2007-02-15) abstract the whole document	1-60

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2024	30/09/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Helliot, Bertrand

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/068277

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99/09213 A1 (TAKARA SHUZO CO [JP]; YAMAMOTO JUNKO [JP] ET AL.) 25 February 1999 (1999-02-25) abstract the whole document	1-60
A	US 2005/202490 A1 (MAKAROV VLADIMIR L [US] ET AL) 15 September 2005 (2005-09-15) abstract the whole document	1-60
A	CHAKRABARTI RAJ ET AL: "The enhancement of PCR amplification by low molecular-weight sulfones", GENE, vol. 274, no. 1-2, 1 January 2001 (2001-01-01), pages 293-298, XP093205668, DOI: 10.1016/s0378-1119(01)00621-7 abstract the whole document	1-60
A	HENKE WOLFGANG ET AL: "Betaine improves the PCR amplification of GC-rich DNA sequences", NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, GB, vol. 25, no. 19, 1 January 1997 (1997-01-01), pages 3957-3958, XP002171292, ISSN: 0305-1048, DOI: 10.1093/NAR/25.19.3957 abstract the whole document	1-60

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/068277

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010209970 A1	19-08-2010	AU 2010213981 A1	18-08-2011
		BR PI1008778 A2	06-09-2016
		CN 102395686 A	28-03-2012
		EP 2396426 A1	21-12-2011
		IL 214326 A	31-03-2015
		KR 20110120923 A	04-11-2011
		RU 2011137526 A	20-03-2013
		US 2010209970 A1	19-08-2010
		WO 2010093552 A1	19-08-2010
		ZA 201105632 B	25-04-2012
JP 4761265 B2	31-08-2011	JP 4761265 B2	31-08-2011
		JP 2009096766 A	07-05-2009
US 2007037139 A1	15-02-2007	CN 1820081 A	16-08-2006
		EP 1621638 A1	01-02-2006
		JP 4496166 B2	07-07-2010
		JP WO2004099446 A1	13-07-2006
		KR 20060015550 A	17-02-2006
		TW 200506052 A	16-02-2005
		US 2007037139 A1	15-02-2007
		WO 2004099446 A1	18-11-2004
WO 9909213 A1	25-02-1999	AT E320507 T1	15-04-2006
		CN 1274389 A	22-11-2000
		DE 69833864 T2	21-12-2006
		EP 1004676 A1	31-05-2000
		JP 4104285 B2	18-06-2008
		KR 20010022902 A	26-03-2001
		WO 9909213 A1	25-02-1999
US 2005202490 A1	15-09-2005	CA 2559209 A1	29-09-2005
		CA 2902980 A1	29-09-2005
		EP 1725682 A1	29-11-2006
		EP 2290106 A1	02-03-2011
		EP 2380993 A1	26-10-2011
		HK 1100960 A1	05-10-2007
		HK 1162611 A1	31-08-2012
		US 2005202490 A1	15-09-2005
		US 2013309668 A1	21-11-2013
		US 2018030527 A1	01-02-2018
		WO 2005090607 A1	29-09-2005