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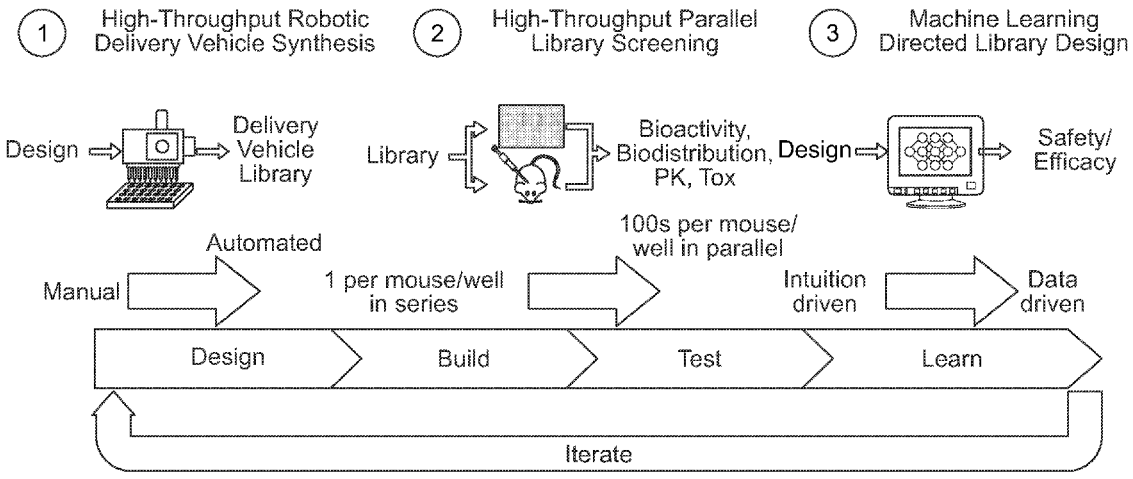
(71) Applicant: **BATELLE MEMORIAL INSTITUTE** [US/US]; 505 King Avenue, Columbus, Ohio 43201 (US).

(72) Inventors: **DUONG, Anthony D.**; 2769 Minerva Lake Road, Columbus, Ohio 43231 (US). **HUK, Danielle J.**; 2234 Adidas Ct, Hilliard, Ohio 43026 (US). **GUPTA, Cherry**; 1919 Inchcliff Road, Columbus, Ohio 43221 (US). **SIMS JR., Kenneth R.**; 2716 Red Robin Way, Delaware, Ohio 43015 (US). **KOERIS, Michael S.**; 27 Angelou Street, Irvine, California 92617 (US). **SHANK, Zachary R.**; 1975 Grewell Road, London, Ohio 43140 (US). **COLBERT, Ashlee J.**; 7721 Lerner Dr., Blacklick, Ohio 43004 (US). **MCCUE, Andrea D.**; 2741 Fishinger Rd, Columbus, Ohio 43201 (US). **SCHMITZ, Emma K.**; 1328 Hunter Ave Apt. A., Columbus, Ohio 43201 (US). **HILLRICH, Caleb T.**; 2899 Neil Ave Apt 372B, Columbus, Ohio 43202 (US). **MILLER, Shannon D.**; 5354 Dexter Ave, Hilliard, Ohio 43026 (US). **HOY, Joanna L.**; 369 South Terrace Avenue, Columbus, Ohio 43204 (US).

(74) Agent: **LARSEN, Joshua P.**; Barnes & Thornburg LLP, 11 South Meridian Street, Indianapolis, Indiana 46204 (US).

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The DBTL cycle for non-viral gene delivery development based on three pillars: automated synthesis, high throughput testing, and machine learning design.

Fig. 1

(57) Abstract: The disclosure relates to barcoded polymer nanoparticles for in vivo screening and for in vivo therapeutic delivery, and methods therefor. More particularly, the invention relates to polymer nanoparticles, such as reversible addition-fragmentation chain transfer (RAFT) polymer compositions, associated with polynucleotide barcodes, for therapeutic delivery, and for high throughput in vivo screening of drug delivery nanoparticles.

[Continued on next page]

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RAPID DESIGN, BUILD, TEST, AND LEARN TECHNOLOGIES FOR IDENTIFYING AND USING NON-VIRAL CARRIERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 63/172,069 filed on April 7, 2021, the entire disclosure of which is incorporated herein by reference.

FIELD OF THE DISCLOSURE

[0002] The disclosure relates to barcoded polymer nanoparticles for *in vivo* screening and for *in vivo* therapeutic delivery, and methods therefor. More particularly, the invention relates to polymer nanoparticles, such as reversible addition-fragmentation chain transfer (RAFT) polymer compositions, associated with polynucleotide barcodes, for therapeutic delivery, and for high throughput *in vivo* screening of drug delivery nanoparticles.

BACKGROUND

[0003] Genetic medicines (including gene therapy, gene silencing, splicing regulators, and nuclease based gene editors) are poised to produce revolutionary treatments, including vaccines, infectious disease treatments, antimicrobial treatments, antiviral treatments, and most notably, genetic disease treatments. However, the *in vivo* delivery of these genetic medicine payloads to the specific tissues and cells that need to be treated, while avoiding tissues and cells that can reduce the efficacy or safety of the genetic medicine, poses a significant challenge. Additional challenges include the ability to deliver large genetic payloads or multiple payloads. Adeno-associated viruses (AAVs) are the most widely used tool for genetic medicine delivery, but AAVs are not able to deliver large genetic payloads or multiple payloads (such as the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system), and they sometimes trigger unwanted immune responses, including the generation of anti-AAV antibodies, a cell mediated response. Some of the immune responses caused by AAV in patients are potentially fatal immune responses.

[0004] Therapeutics based on the CRISPR/Cas9 system have an exceptional potential to treat a number of genetic diseases due to the capability of this system for precise and programmable

gene editing. Gene editing and repair using the CRISPR/Cas9 system has two main mechanisms, including non-homologous end joining (NHEJ) which repairs the site of cut by inducing random indel mutation, and homology-directed repair (HDR), which repairs the cut site based on a pre-existing template. Because a pre-designed template can be used for HDR-directed repair, therapies based on this mechanism can be tailored to cure a large number of different genetic diseases. However, the main challenge is that HDR repair requires the delivery of CRISPR/Cas9, small guide RNA (sgRNA) and a donor DNA strand at the same time to a particular location. This requirement becomes particularly limiting for *in vivo* applications because ensuring co-delivery of multiple large molecules to the same targeted location is currently not feasible. For example, the Cas9 enzyme sequence and guide RNA complex is too large to fit into AAVs.

[0005] Thus, there is a need for effective non-viral delivery systems, including gene delivery systems. The current state-of-the-art non-viral gene delivery systems, such as liposomes, have many drawbacks such as poor biocompatibility and the inability to easily engineer or functionalize them. Additional concerns are that such non-viral gene delivery systems are easily degraded by various enzymes as they pass through intracellular or intercellular compartments, and these systems have not been able to package multiple large payloads.

[0006] The inventors have designed barcoded polymer nanoparticle (e.g., a polymer derived from a controlled living/radical polymerization such as a RAFT polymer) delivery compositions. These compositions have the advantage of being biocompatible, non-toxic, and can be programmed in many ways. For example, the barcoded polymer nanoparticle delivery compositions can be programmed to have functional groups that enable them to evade early degradation, that enable them to evade immune responses, and that enable intracellular imaging and controlled delivery of therapeutic genes and other therapeutic molecules. Thus, these non-viral delivery compositions can enhance the stability, safety, and/or efficacy of genetic medicine payloads and other payloads by providing immune evasion, tissue-directed intracellular delivery, and the ability to deliver large genetic payloads or multiple payloads.

[0007] The present disclosure combines these non-viral delivery compositions with rapid design, build, test, and learn (DBTL) technologies that will vastly accelerate gene delivery and address the disadvantages that exist in limited gene delivery vehicles. In addition to hastening editing therapies of today to transition through clinicals, it is anticipated that these

technologies will enable the general delivery of larger more molecularly diverse genetic payloads, and other payloads, which will in turn, continue to improve treatments for genetic diseases and other diseases.

SUMMARY

[0008] In some aspects, the disclosure provides for a composition comprising a non-viral delivery vehicle comprising one or more nanoparticle forming polymers, and a nucleic acid construct.

[0009] In some aspects, the disclosure provides for a method of *in vivo* screening for a desired polymer nanoparticle for use as a delivery vehicle, the method comprising (a) preparing a library comprising two or more types of polymer nanoparticles, wherein each polymer nanoparticle is associated with a nucleic acid construct comprising a different polynucleotide barcode, (b) administering the library to an animal, (c) removing cells or tissues from the animal, (d) isolating the nucleic acid constructs from the cells or the tissues of the animal, (e) detecting the nucleic acid constructs in the cells or the tissues of the animal, and (f) identifying the desired polymer nanoparticle for use as a delivery vehicle.

[0010] In some aspects, the disclosure provides for a method of treating a patient with a disease, the method comprising administering to the patient the polymer nanoparticle identified in the *in vivo* screening methods described herein, wherein the polymer nanoparticle further comprises a drug payload, such as a polynucleotide or a protein payload or a small molecule therapeutic payload, and treating the disease in the patient.

[0011] The following clauses, and combinations thereof, provide various additional illustrative aspects of the invention described herein. The various embodiments described in any other section of this patent application, including the section titled "DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS" and the "EXAMPLES" are applicable to any of the following embodiments of the invention described in the numbered clauses below.

[0012] 1. A composition comprising:

[0013] a. a non-viral delivery vehicle comprising one or more nanoparticle forming polymers, and

[0014] b. a nucleic acid construct.

[0015]

[0016] 2. The composition of clause 1, wherein the non-viral delivery vehicle comprises a polymer nanoparticle.

[0017]

[0018] 3. The composition of clause 1 or 2, wherein the nucleic acid constructis associated with the non-viral delivery vehicle via an electrostatic interaction.

[0019]

[0020] 4. The composition of any one of clauses 1 to 3, wherein the nucleic acid construct is associated with the non-viral delivery vehicle by an electrostatic interaction of a positively charged polymer segment of the one or more nanoparticle forming polymers and a negatively charged polynucleotide segment of the nucleic acid construct.

[0021]

[0022] 5. The composition of any one of clauses 1 to 3, wherein the nucleic acid construct is associated with the non-viral delivery vehicle by a high affinity, non-covalent bond interaction between a biotin molecule on the 5' and/or the 3' end of the nucleic acid construct and a molecule that binds to biotin on the one or more nanoparticle forming polymers.

[0023]

[0024] 6. The composition of any one of clauses 1 to 3, wherein the nucleic acid construct is associated with the non-viral delivery vehicle by a covalent bond between a carboxy terminated polymer segment and the nucleic acid construct, wherein the nucleic acid construct comprises a primary amine on the 5' and/or the 3' end.

[0025]

[0026] 7. The composition of any one of the preceding clauses, wherein the nucleic acid construct comprises:

[0027] a. two primer binding segments; and

[0028] b. one or more unique polynucleotide barcodes between the two primer binding segments.

[0029]

[0030] 8. The composition of clause 7, wherein the primer binding segments range in length from about 15 base pairs to about 30 base pairs.

[0031]

[0032] 9. The composition of clause 7 or 8, wherein the primer binding segments are a universal primer binding set.

[0033]

[0034] 10. The composition of any one of clauses 7 to 9, wherein the one or more polynucleotide barcodes comprise unique sequences of 6-20 nucleotides in length.

[0035]

[0036] 11. The composition of clause 10, wherein the polynucleotide barcodes further comprise a hamming distance of at least 2-6 bases between any two unique polynucleotide barcode sequences.

[0037]

[0038] 12. The composition of any one of the preceding clauses, wherein the nucleic acid construct further comprises from about 6 to about 12 random bases at the 3' end of the polynucleotide barcode.

[0039]

[0040] 13. The composition of clause 12, wherein the about 6 to about 12 random bases at the 3' end of the polynucleotide barcode are for bioinformatic removal of PCR duplicates.

[0041]

[0042] 14. The composition of any one of the preceding clauses, wherein the nucleic acid construct ranges in length from about 42 nucleotides to about 210 nucleotides.

[0043]

[0044] 15. The composition of any one of the preceding clauses, wherein the one or more nanoparticle forming polymers comprise monomer units compatible with a controlled living/radical polymerization.

[0045]

[0046] 16. The composition of any one of the preceding clauses, wherein the one or more nanoparticle forming polymers are prepared by reversible-deactivation radical polymerization, atom transfer radical polymerization (ATRP), reversible addition fragmentation chain transfer polymerization (RAFT), iodine-transfer polymerization (ITP), selenium-centered radical-mediated polymerization, telluride-mediated polymerization (TERP), stibine-mediated polymerization, or ring-opening polymerization.

[0047]

[0048] 17. The composition of any one of the preceding clauses, wherein the one or more nanoparticle forming polymers are prepared from monomers containing a vinyl group.

[0049]

[0050] 18. The composition of any one of the preceding clauses, wherein the one or more nanoparticle forming polymers are prepared using a chain transfer agent such as those used in reversible addition-fragmentation chain transfer (RAFT).

[0051]

[0052] 19. The composition of any one of the preceding clauses, wherein the one or more nanoparticle forming polymers are RAFT block copolymers comprising

[0053] a. a first terminus comprising a first capping unit derived from a first chain transfer agent in a RAFT copolymerization process;

[0054] b. a first block prepared from one or more monomer units covalently attached to the first reactive functional unit, and having a molecular weight (M_n) in the range of about 1 kDa to about 500 kDa and a degree of polymerization in the range of about 10 to about 2500;

[0055] c. optionally a second block prepared from one or more monomer units covalently attached to the first block, and having a molecular weight (M_n)in the range of about 1 kDa to about 500 kDa and a degree of polymerization in the range of about 20 to about 2000; and

[0056] d. a second terminus comprising a second capping unit derived from a first or a second chain transfer agent.

[0057]

[0058] 20. The composition of clause 19, wherein the non-viral delivery vehicle has one or more of an overall molecular weight (M_n) in the range of about 25 kDa to about 60 kDa, an overall degree of polymerization in the range of about 700 to about 900, a target size in the range of about of about 10 to about 60 nm, and a maximum corona-to-core ratio (CCR) of about 1.5 to about 3.5.

[0059]

[0060] 21. The composition of clause 19 or 20, wherein the first block is prepared from one or more monomer units selected from the group consisting of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, and methyl methacrylate.

[0061]

[0062] 22. The composition of clause 19 or 20, wherein the first block is prepared from one of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, or methyl methacrylate.

[0063]

[0064] 23. The composition of any one of clauses 19 to 22, wherein the second block is prepared from one or more monomer units selected from the group consisting of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, and methyl methacrylate.

[0065]

[0066] 24. The composition of any one of clauses 19 to 23, wherein the second block is a random copolymer prepared from two different monomer units independently selected from the group consisting of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, and methyl methacrylate.

[0067]

[0068] 25. The composition of any one of clauses 19 to 23, wherein the second block is a random copolymer prepared from three different monomer units independently selected from the group consisting of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, and methyl methacrylate.

[0069]

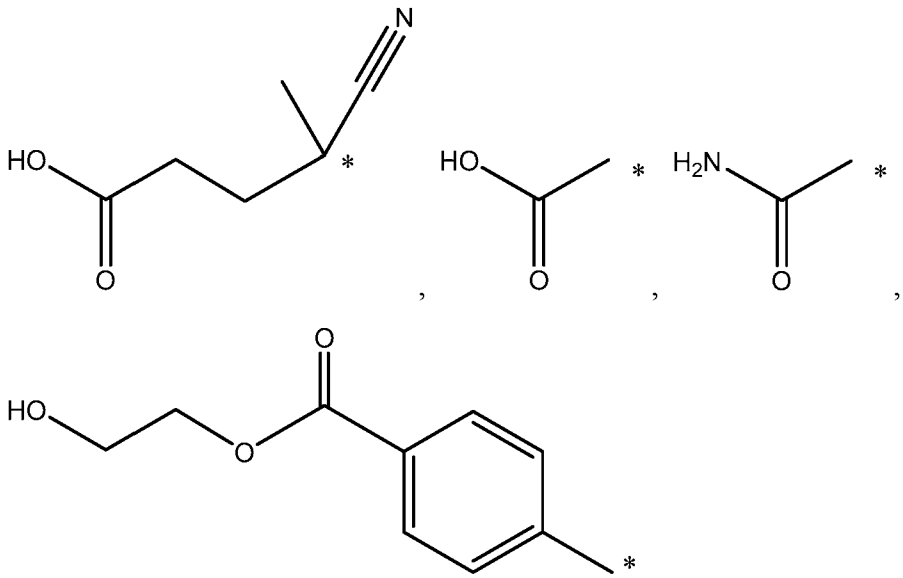
[0070] 26. The composition of any one of clauses 19 to 25, wherein the second block is a random copolymer prepared from 2-dimethylaminoethyl acrylate, butyl methacrylate, and propyl acrylic acid; or 2-dimethylaminoethyl acrylate and butyl methacrylate; or 2-dimethylaminoethyl acrylate, butyl methacrylate, and ethyl acrylic acid.

[0071]

[0072] 27. The composition of any one of clauses 19 to 26, wherein each chain transfer agent is independently selected from the group consisting of bis(carboxymethyl)trithiocarbonate, bis(2-amino-2-oxoethyl) trithiocarbonate, bis[4-(2-hydroxyethoxycarbonyl)benzyl] trithiocarbonate, 4-cyano-4-(ethylsulfanylthiocarbonyl) sulfanylvpentanoic acid, 4-cyano-4-((phenylcarbonothioyl)thio)pentanoic acid, and 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid.

[0073]

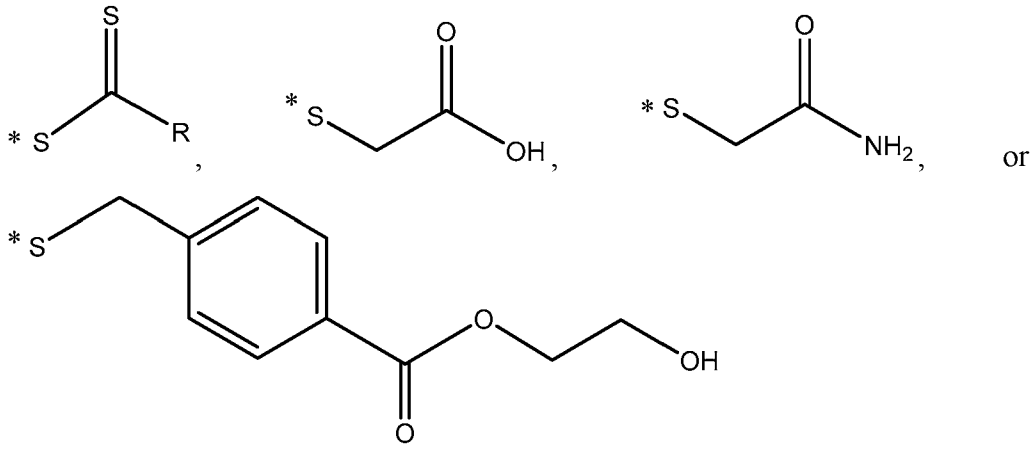
[0074] 28. The composition of any one of clauses 19 to 27, wherein the first capping unit is of the formula

[0075] 

[0076] wherein * represents a point of covalent attachment to the first block.

[0077]

[0078] 29. The composition of any one of clauses 19 to 28, wherein the second capping unit is of the formula

[0079] 

[0080] wherein * represents a point of covalent attachment to the second block, and R is $-SC_2$-C_{12} alkyl or C_6H_5,

[0081]

[0082] 30. A method of *in vivo* screening for a desired polymer nanoparticle for use as a delivery vehicle, the method comprising (a) preparing a library comprising two or more types of polymer nanoparticles, wherein each polymer nanoparticle is associated with a nucleic acid construct comprising a different

polynucleotide barcode, (b) administering the library to an animal, (c) removing cells or tissues from the animal, (d) isolating the nucleic acid constructs from the cells or the tissues of the animal, (e) detecting the nucleic acid constructs in the cells or the tissues of the animal, and (f) identifying the desired polymer nanoparticle for use as a delivery vehicle.

[0083]

[0084] 31. The method of clause 30 wherein the polymer nanoparticle associated with the nucleic acid construct is according to any one of clauses 1 to 29.

[0085]

[0086] 32. The method of clause 30 or 31 wherein the nucleic acid construct is detected by a method selected from the group consisting of the polymerase chain reaction (PCR), isothermal amplification, sequencing, or a combination thereof, to obtain nucleotide sequence data.

[0087]

[0088] 33. The method of any one of clauses 30 to 32, wherein the polymer nanoparticle is loaded with a payload.

[0089]

[0090] 34. The method of clause 33, wherein the payload is a luminescent molecule.

[0091]

[0092] 35. The method of clause 34, wherein the luminescence is used to track the biodistribution or cell uptake via imaging.

[0093]

[0094] 36. The method of any one of clauses 30 to 35, wherein the administration to the animal is via an intramuscular, an intravenous, an intraperitoneal, an oral, or a pulmonary route.

[0095]

[0096] 37. The method of any one of clauses 30 to 36, wherein the nucleic acid construct is isolated from the cells and the tissues by mixing with a first organic compound and incubating the organic phase with an aqueous phase of the cell or tissue sample, separating the organic phase from the aqueous phase, mixing

the organic phase with a second organic compound, incubating the mixture, precipitating the nucleic acid construct from the mixture, removing the organic phase by evaporation, and resuspending the nucleic acid construct in an aqueous composition.

[0097]

[0098] 38. The method of clause 37, wherein the organic phase comprises phenol chloroform.

[0099]

[00100] 39. The method of clause 37, wherein the nucleic acid construct is separated from cationic material in the cells or tissues by titrating the aqueous composition of the nucleic acid contstruct to a pH of greater than 7.4.

[00101]

[00102] 40. The method of clause 30, wherein the nucleic acid construct is separated from material in the cells or tissues by binding the nucleic acid construct with a molecule with a binding affinity to the nucleic acid construct greater than the binding affinity to the cell or tissue material.

[00103]

[00104] 41. The method of clause 30, wherein the nucleic acid construct is separated from material in the cells or tissues via size exclusion chromatography.

[00105]

[00106] 42. The method of clause 30, wherein the nucleic acid construct is separated from material in the cells or tissues via dialysis or diafiltration.

[00107]

[00108] 43. The method of clause 30, wherein the nucleic acid construct is separated from material in the cells or tissues via filtration.

[00109]

[00110] 44. The method of clause 30, wherein the nucleic acid construct is separated from material in the cells or tissues by digesting proteins using an enzyme.

[00111]

[00112] 45. The method of clause 44 wherein the enzyme is Proteinase K.

[00113]

[00114] 46. The method of clause 30, wherein the nucleic acid constructs associated with the polymer nanoparticles are detected by first diluting the isolated nucleic acid constructs by a factor of at least 1000 times, and then amplifying the nucleic acid constructs by PCR using primers.

[00115]

[00116] 47. The method of clause 46, wherein the primers from the PCR step are enzymatically digested prior to detection of amplicons.

[00117]

[00118] 48. The method of clause 32, wherein the nucleotide sequence data is converted to fast Q files; and the fast Q files are mapped to known polynucleotide barcodes and the polynucleotide barcodes are enumerated.

[00119]

[00120] 49. A method of treating a patient with a disease, the method comprising administering to the patient the polymer nanoparticle identified in the *in vivo* screening method of any one of clauses 30 to 48, wherein the polymer nanoparticle further comprises a payload, and treating the disease in the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[00121] Fig. 1 is a schematic diagram showing a simplified flow diagram illustrating a DBTL cycle for non-viral gene delivery development based on automated synthesis, high throughput testing, and machine learning design.

[00122] Fig. 2 is a schematic diagram showing an automated multiplexed synthesis of a large, diverse library of PNPs, with various size, charge and hydrophobicity to generate data for gene editing, cytotoxicity, and inflammation.

[00123] Fig. 3(a)-3(b) are schematic diagrams showing graph neural network architecture (3(a)) and Zeta potential prediction from SMILES input (3(b)).

[00124] Fig. 4 is an illustration of one representative example of a nucleic acid construct of the present disclosure, showing the length (in base pairs (bp)) of the primer binding segments (20 bp and 21 bp in the construct shown), the polynucleotide barcode (8 bp

in the construct shown), and the random sequence fragment (7 bp in the construct shown) of the present disclosure.

[00125] Figs. 5(a)-5(c) are a schematic drawing of nucleic acid construct labeling reaction methods using electrostatic loading reaction (Fig. 5(a)), avidin-streptavidin conjugation (Fig. 5(b)), and direct amidification (Fig. 5(c)).

[00126] Fig. 6 is an e-gel showing amplification of nucleic acid constructs electrostatically bound to polymer nanoparticles. The presence of the double band in the samples with nucleic acid construct confirms that the barcodes were attached to the PNP. The absence of the double band in the not test control (NTC) validates the positive result.

[00127] Fig. 7 is a series of e-gels showing DNA barcode amplification from a pooled sample of 96 unique barcodes, each attached to a prototype PNP. Each frame in the figure is one row of a 48 channel gel electrophoresis. The first column of each gel is a DNA latter, the bottom band of which is ~100 bases. The bright band in each column near the 100 bp mark indicates amplicons coming from the barcodes.

[00128] Fig. 8. is an e-gel showing DNA barcode amplification from a pooled sample of 96 unique barcodes, attached to a prototype PNP, extracted after being spiked into a culture of HEK-293 cells. Each frame in the figure is one row of a 48 channel gel electrophoresis. The first column of each gel is a D=NA latter, the bottom band of which is ~100 bases. The bright band in each column near the 100 bp mark indicates amplicons coming from the barcodes.

[00129] Fig. 9 is an e-gel showing DNA barcode amplification from 10 unique of PNPs with unique barcodes, after being spiked into HEK-293 cells. The presence of the double band is evidence of barcode amplification, present in the positive control sample known to have the barcodes, and not observed in the no test control (NTC) sample, which was phosphate buffered saline only.

[00130] Fig. 10 is a series of images showing each of the 10 unique barcoded PNPs from Fig. 7 were loaded with a plasmid expressing a fluorescent TdTomato protein. The loaded PNPs were each dosed into HEK-293 cells. After 48 hours, the cells were imaged via fluorescent microscopy with a Texas Red filter, and the images are shown above.

[00131] Fig. 11(a)-Fig. 11(e) show flow cytometry scatter plots depicting cell event distribution of HEK293T cells treated with a representative PNP carrying a td-tomato encoding fluorescent cargo plasmid (Figs. 11(a)-11(c)), and heat maps depicting Transfection

efficiency and viability of a library of 88 diverse PNPs (PNP Library Transfection Efficiency (Fig. 11(d)) and PNP Library Viability (Fig. 11(e))). In Fig. 11(a), the area under the curve denoted by the bar in the graph accounts for 84.17% of the cells.

[00132] Figs. 12(a)-12(d) show gel images of PCR amplified barcodes extracted from indicated mouse tissues (Figs. 12(a) and 12(b)); sequencing data demonstrating the ability to detect all 96 individual barcodes from a single mouse organ, where (a) denotes low dose and (b) denotes high dose (Fig. 12(c)); and a graph depicting relative abundance of each barcode in a single organ (Fig. 12(d)).

[00133] Fig. 13 is an electrophoresis gel showing a band corresponding to barcode (BC) amplicons produced from PCR performed on samples of PNPs with barcodes attached at various molar ratios of PNP to BC (i.e. moles of polymer divided by moles of barcode). The presence of the band for all barcoded PNP samples confirms that the barcode can be detected via PCR on PNPs labeled with barcodes at ratios of anywhere from 20:1 to 10,000:1 (moles PNP:moles BC).

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[00134] The invention relates to barcoded polymer nanoparticles for *in vivo* screening and for *in vivo* therapeutic delivery, and methods therefor. More particularly, the invention relates to polymer nanoparticles, such as a controlled living/radical polymerization products, such as reversible addition-fragmentation chain transfer (RAFT) polymer compositions, associated with polynucleotide barcodes, for therapeutic delivery, and for high throughput *in vivo* screening of drug delivery nanoparticles. In one embodiment, the payload can be a nucleic acid of 3 kB or more, or any other suitable payload, such as another polynucleotide or a protein or a small molecule therapeutic or a luminescent molecule.

[00135] The invention relates to the use of barcoded polymer nanoparticle compositions (e.g., a polymer nanoparticle derived from a controlled living/radical polymerization process, such as RAFT copolymers) as a platform with a high degree of tunability in structure and function, opportunities to protect payloads from adverse reactions or degradation by the immune system, and passive cell targeting via surface charge, or particle size. These delivery systems also lend themselves to computer-aided design, and they have suitable pathways to

robust, commercial scale manufacturing processes with higher yields and fewer purification steps than viral delivery composition manufacturing processes.

[00136] In one embodiment a composition comprising a polymer nanoparticle (e.g., a polymer nanoparticle derived from a controlled living/radical polymerization process, such as RAFT polymer) associated with a nucleic acid construct is provided. In another embodiment, a method of *in vivo* screening to identity a desired polymer nanoparticle (e.g., a polymer nanoparticle derived from a controlled living/radical polymerization process, such as RAFT polymer) associated with a nucleic acid construct for use as a delivery vehicle is provided. In another embodiment, a method of treating a patient with a disease is provided comprising administering to the patient the polymer nanoparticle identified in the screening method.

[00137] In one embodiment, the method of *in vivo* screening for a desired polymer nanoparticle for use as a delivery vehicle comprises, (a) preparing a library comprising two or more types of polymer nanoparticles, wherein each polymer nanoparticle is associated with a nucleic acid construct comprising a different polynucleotide barcode, (b) administering the library to an animal, (c) removing cells or tissues from the animal, (d) isolating the nucleic acid constructs from the cells or tissues of the animal, (e) detecting the nucleic acid constructs in the cells or tissue of the animal, and (f) identifying the desired polymer nanoparticle for use as a delivery vehicle. In various embodiments, the nucleic acid construct can be detected by, for example, the polymerase chain reaction (PCR), isothermal amplification, or sequencing the nucleic acids in the cells or tissues of the animal.

[00138] In another embodiment, a method of treating a patient with a disease is provided, comprising administering to the patient the polymer nanoparticle identified in the *in vivo* screening method, wherein the polymer nanoparticle further comprises a drug payload, such as a polynucleotide or a protein payload, or a small molecule therapeutic or luminescent molecule payload, and treating the disease in the patient.

[00139] In various embodiments, any suitable route for administration of the library of polymer nanoparticles associated with nucleic acid constructs for the method of *in vivo* screening for the polymer nanoparticle associated with a nucleic acid construct, or for the method of treatment can be used including parenteral administration. Suitable routes for such parenteral administration include intravenous, intraarterial, intraperitoneal, intrathecal, epidural, intracerebroventricular, intraurethral, intrasternal, intracranial, intratumoral,

intramuscular and subcutaneous delivery. In one embodiment, means for parenteral administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques. In other embodiments, oral or pulmonary routes of administration can be used.

[00140] In one aspect, libraries of barcoded polymer nanoparticles can be pooled and concentrated before administration to the animal of the nucleic acid constructs associated with the polymer nanoparticles. Methods for library preparation and for sequencing are described in Green and Sambrook, "Molecular Cloning: A Laboratory Manual", 4th Edition, Cold Spring Harbor Laboratory Press, (2012), incorporated herein by reference.

[00141] In various embodiments, cell or tissue samples may be analyzed for the presence of the polymer nanoparticle associated with the nucleic acid constructs described herein. The samples can be any tissue, cell, or fluid sample from an animal, for example, selected from the group consisting of urine, nasal secretions, nasal washes, inner ear fluids, bronchial lavages, bronchial washes, alveolar lavages, spinal fluid, bone marrow aspirates, sputum, pleural fluids, synovial fluids, pericardial fluids, peritoneal fluids, saliva, tears, gastric secretions, stool, reproductive tract secretions, lymph fluid, whole blood, serum, plasma, or any tissue or cell sample from an animal. Exemplary tissue or cell samples include brain tissue or cells, muscle tissue or cells, skin tissue or cells, heart tissue or cells, kidney tissue or cells, stomach tissue or cells, liver tissue or cells, urinary tract tissue or cells, gastrointestinal tract tissue or cells, head or neck tissue or cells, lung tissue or cells, reproductive tract tissue or cells, pancreatic tissue or cells, or any other tissue or cell type from an animal.

[00142] In one illustrative aspect for removing cells or tissues from the animal and isolating the nucleic acid constructs from the cells or tissues of the animal, the nucleic acid constructs are removed from cells or tissues of the animal. In various embodiments, nucleic acid constructs (e.g., DNA or RNA) obtained from the tissues or cells of the animal can be removed by rupturing the cells and isolating the nucleic acid constructs from the lysate. Techniques for rupturing cells and for isolation of nucleic acids are well-known in the art, and removal techniques include homogenization, such as by using a bead-beating technique. In other embodiments, the nucleic acid constructs may be isolated by rupturing cells using a detergent or a solvent, such as phenol-chloroform. In another aspect, the nucleic acid constructs may be separated from the lysate by physical methods including, but not limited to,

centrifugation, dialysis, diafiltration, filtration, size exclusion, pressure techniques, digestion of proteins with Proteinase K, or by using a substance with an affinity for nucleic acids such as, for example, beads that bind nucleic acids.

[00143] In one illustrative embodiment, the nucleic acid constructs are removed from cells or tissues by treating with a mixture of an organic phase (e.g., phenol chloroform) and an aqueous phase (e.g., water). The organic phase (e.g., phenol chloroform) is isolated and the nucleic acid construct can be precipitated by raising the pH, for example, to pH 7.4. The organic phase (e.g., phenol chloroform) can be evaporated and the nucleic acid constructs can be suspended in water and diluted to appropriate concentrations for PCR and/or sequencing. In one embodiment, the isolated nucleic acid constructs are suspended in either water or a buffer after sufficient washing.

[00144] In other embodiments, commercial kits are available for isolation of the nucleic acid constructs, such as Qiagen™, Nuclisensm™, Wizard™ (Promega), QiaAmp 96 DNA Extraction Kit™ and a Qiacube HT™ instrument, and Promegam™. Methods for preparing nucleic acids for PCR and/or sequencing are also described in Green and Sambrook, "Molecular Cloning: A Laboratory Manual", 4th Edition, Cold Spring Harbor Laboratory Press, (2012), incorporated herein by reference.

[00145] The polynucleotide barcodes can be detected by using, for example, the polymerase chain reaction (PCR), isothermic amplification, sequencing, and/or imaging. The polymerase chain reaction (PCR) has been developed to analyze nucleic acids in a laboratory. PCR evolved over the last decade into a new generation of devices and methods known as Next Generation Sequencing (NGS). NGS provides faster detection and amplification of nucleic acids at a cheaper price. The NGS devices and methods allow for rapid sequencing as the nucleic acids are amplified in massively parallel, high-throughput platforms.

[00146] In one illustrative aspect, the nucleic acid constructs can be sequenced, to detect the polynucleotide barcodes using any suitable sequencing method including Next Generation Sequencing (e.g., using Illumina, ThermoFisher, or PacBio or Oxford Nanopore Technologies sequencing platforms), sequencing by synthesis, pyrosequencing, nanopore sequencing, or modifications or combinations thereof can be used. In one embodiment, the sequencing can be amplicon sequencing. In another embodiment, the sequencing can be whole genome sequencing. In another embodiment, the sequencing can be exome/targeted

hybridization sequencing. Methods for sequencing nucleic acids are also well-known in the art and are described in Sambrook et al., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press, incorporated herein by reference.

[00147] In one aspect, the nucleic acid construct can comprise a polynucleotide barcode and the barcode comprises a unique sequence not present in any known genome for identification of the polynucleotide barcode. In another embodiment, a set of different nucleic acid constructs with different polynucleotide barcodes (e.g., 88 or 96 different polynucleotide barcodes) can be used to allow for multiplexing of samples on one sequencing run.

[00148] In various embodiments, the polynucleotide barcodes can be from about 5 to about 35 base pairs in length, about 5 to about 34 base pairs in length, about 5 to about 33 base pairs in length, about 5 to about 32 base pairs in length, about 5 to about 31 base pairs in length, about 5 to about 30 base pairs in length, about 5 to about 29 base pairs in length, about 5 to about 28 base pairs in length, about 5 to about 27 base pairs in length, about 5 to about 26 base pairs in length, about 5 to about 25 base pairs in length, about 5 to about 24 base pairs in length, about 5 to about 23 base pairs in length, about 5 to about 22 base pairs in length, about 5 to about 21 base pairs in length, about 5 to about 20 base pairs in length, about 5 to about 19 base pairs in length, about 5 to about 18 base pairs in length, about 5 to about 17 base pairs in length, about 5 to about 16 base pairs in length, about 5 to about 15 base pairs in length, about 5 to 14 base pairs in length, about 5 to 13 base pairs in length, about 5 to 12 base pairs in length, about 5 to 11 base pairs in length, about 5 to 10 base pairs in length, about 5 to 9 base pairs in length, about 5 to 8 base pairs in length, about 6 to 10 base pairs in length, about 7 to 10 base pairs in length, about 8 to 10 base pairs in length, or about 6 to about 20 base pairs in length.

[00149] Various embodiments of polynucleotide barcodes are shown below in Table 1 (labeled "Polynucleotide Barcodes"). These polynucleotide barcodes can be used in the nucleic acid constructs alone or in combinations of, for example, two or more polynucleotide barcodes, three or more polynucleotide barcodes, four or more polynucleotide barcodes, etc. In the embodiment where more than one polynucleotide barcode is used, the hamming distance between the polynucleotide barcodes can be about 2 to about 6 nucleotides, or any suitable number of nucleotides can form a hamming distance, or no nucleotides are present between the polynucleotide barcodes.

Table 1

Polynucleotide Barcodes	Polynucleotide Barcodes	Polynucleotide Barcodes
GCTACATAAT	AGCAGTCCCG	CAAAATAGCG
ATGTTACACA	TTTGGGCTGT	GAAGAAGAAG
TGGGGCCCAA	CTCACGATCT	CACCCGCACG
TAGTTTATCC	TGGCGCATAC	ACGATGCCCG
ACCCCGTCTT	GCAATTGAAA	CCTACTACAC
CCGGCCATCA	TCGGGAGACG	ATTGAAACAA
GAGCTTGCTC	CCCGGCGAAA	GACCGAAGAT
ACGTTCTATA	TGATGCGGAA	ACGGCCTGAA
TACAGCAAAA	AACTGAGGCG	AGGGGAGGTC
GTTAGGTGGT	CATATTATTT	CAATCAACTT
GGAGACCGAC	AAAAGTCATT	GGACAACCGA
TGGCCCCTTG	AAGCGGTGAG	TCCCTAAGGC
TGGCCGTAAG	AAGGTAATCA	GTTCTACACG
CGTTCGTCAA	CTGACACTTA	ACTAACCAGT
CGGACGTGGA	CTGTTTTCTA	GAAGCTGGAT
AGAGGGGGCA	CACATGGCAG	GGAACCATGG
GTTCAGGTCG	TTCAATCCGG	CTCTACCTGG
CTCGCAAGAG	TGTCCGGCAT	TAATGCCTGC
GCAACGACTT	TGGTACCGTG	TAAAGGCAAT
GCCATCCATC	AAGAGATATT	CGCCTGGGAA
TTCCGAGCAG	GATGTACTAC	TCTTGGGGAA
CTTCTGGACA	GAAATGGAAT	AGAGAGAGAG
AACATTAGAC	TTAAAATACT	GCGTTGGCGC
AAGCAATAGT	TGACCGGAAC	TTACGACAGA
AGGGTAAGAC	GTCGCCGCAA	GGAACTCTTA
CGTTGTCTTG	TAGGATACCG	GATTGTGGAG
TTTCCCCGCC	AGTCCAATTG	GGGCACTGAT
CGAATGGATC	GGGGGCTATA	AGACGCACCA
CATCACTTGC	ACCTTCAGTT	CCAATTATAA
CTCTCGCACT	ATGGCAAGTA	TAGAGACGCA
GTTCACGTGC	AGAATGTTTT	CCTCTTGTCG
AATAAGCCTG	AGTTCGTTTG	GAGGAAGCTC
GTTAACAATT	CACTACTGAC	AGTCCCGAGT
ATTCAGATCC	GATCAAGAGC	TGCTTGCAGT
CCTGCTGATT	ATTTATCGAG	CCCACTTCCC
CTTGGTCATA	CCTTTTTCCA	CGTTGCCGCG
TCTTCCTGTT	GCACAGAGGT	CCCCTGGTTC

ACTGCCATGG	TGATCTGAAT	ACGACCAATA
CATGTATAGT	GTTGGAGGGA	CTTAGGGTTC
GGTAGCGGCA	TTTTGAAGGT	AAACATATCA
TCACTCTAAC	TAAGTCCTAA	GGGTCGTAGA
AAGGTGCACC	GGTGTTAGGG	CTCCGTAGCG
AATGCTCGTT	TGTATGCACC	CTGGTCATAA
TGTCTAGAAA	CCGTGCCATT	TTGACAGATC
CTGCCTGCCT	GAAATCACCC	GAGTAAAGTC
ACTATAAAAG	TTTGCACGTG	ATATGGGCTT
TAGTATCGAG	CGTCTGTTTT	TACAACTACT
ATCGCAGTCC	CTACACCACA	AATTCAGCCG
TCATCAGAAC	TGCTACAGGG	GATTGTACTA
TCCTAGACGC	GGGAATATAT	TCGTAATGCG
GCCGGGCGGG	TCATGTATTT	CGATAACTGC
GCCCAGAAGA	TCTCCGTTTA	AACTTGGCGG
CTTAGAGCTG	TACCTCTCGC	CGTGGATGTA
GTCTGCGCTT	GCTTCAACCG	CCTTCCCGAA
CGCCGTCCTT	ATGAAGCTAC	CTAAACCCGT
TTTATCTGCT	CGGTACAACT	CAACATTCCC
TGCTTCGGAG	GTGTGGTCGT	CTTACCCTCT
GGGGAGAATG	GGGGTCATGT	GGAAAGTTCT
GTGGTAAGTG	AGGCAGCCCA	CGGATTGGCT
GAAATTAGTA	CAAGCACGAT	AATGTAGGGC
GCTATCCTAA	TCAAATGGAT	AATGAATCGC
ATCTGTACGA	GGACTGAATA	ATCATACACC
AGTTCGGGGC	CCGTAGACGT	AGTTGGGCAG
CGAGTCTGTC	CGGCGTACCG	AGAAGAAGGG
ATCCTACGCA	GGCGGCGCCC	GCGTGCGCTA
ATGGTGGATA	AGACTTGATC	CCCCGATAAA
CCTCTAACTA	ACCTTGCACA	TACCAAGTGC
ATAGCTGCAC	TAAGGTGAGT	TGTGTTTTCG
GACAGAATTT	TTGTTGTTTC	CCCAGATGTC
CAATTGGCAT	GAGGGAATAC	GCGAGCTTCC
TCTAGTAGAC	CTCGTACGCG	GTGTCACGTA
TTATTCATGG	CCGCGGTTTA	ATAGGCCGAG
TTGGCAACCG	TTAAAGTTAA	GAGCTACCAG
CATAATACAT	GCATATGGGT	CGCGGCGGAG
ACAGACTCAC	AGTCTGAGCC	TCTTGCACGA
GCGATGCTGC	TGTCGGTTCG	TGCCCTAAAG
CATCTTTGCC	GGTCTCAACC	TTGCGCTTTG
GTGACTCCAG	GTAACGGCAT	CATATAAAGG

GGACGAGTCT	ACACTGAGAA	AATAGCGAAT
TAGTGGCGTG	CCCAACGTCG	TACGCTAAGG
AACGCAGCTT	AAGAAACTGC	ACTTAGTTCG
AGAACAGGTG	ACCAGCCCAC	CGTGCGGAAC
AGGCTATGTT	TGTAGTTACT	ACCCGATTCG
CCTGGATCTT	GGCTAGAGGC	TGCAGAGTTT
CTAGCCGGCC	GTTCGGCAGA	GAATCATTAG
ACCAGTTATC	CCAAAATAGA	AGTACACTGG
ACGTTATAGC	CCCATATAAC	TTGTGCGGTT
TCGAGTTTGA	GTCACTACCG	ATGACATGCA
TGAAGCGAGC	GTAGTGTGGC	TTCTCGGACG
GACTGGCGAA	CAATCTCATA	AGATTGAAGA
GATGGACCTA	CCATGTTATA	GGCGGACTGT
GTCCACAACG	TAAGCAGTGG	TTTATGGTAA
CCTCCCCAGA	TCGGCGGCTA	CAGTAGGGTG
TTATGACGCC	TATTAAATGC	GACAGGCAAG
CTTGATCCGT	GTCGCCATTA	GATGTGTCGT
AATGCGCAAT	GGCGTCGTTC	ACTTGACGGA
GTACCCCTCA	CTAGTAGATA	AAGTCCGAAA
CGACAGCTCG	TCGTCAGTAT	TGGGTGTAGG
TGACCTGGCT	GGGGTATCGG	ACTTACCGCG
TTCATAGCCC	TGCTCTGCCA	CTGTGCACCC
CCCAAGAGAA	TGCCGTAACT	ATTGCTCTCT
AAACGAAGTA	CGGTACAGGC	CAGAAGACAA
GACGTTTACA	TCCTAATTTG	TTACGCTATA
GATCGATTTG	TCTTTCTGGA	ACGTGGAAAT
CACTGTCACC	CCGCGACTTG	TGAGGCTGGT
TGTGAGAGTT	ACCTATAGCG	ATTATGAGAT
GACGTAACCT	GCCGGCACCT	GACTTGTAGT
CAGACTCTGC	TTTGATAGGC	TCGCTGAGGA
TATGCCAATA	ACTGTGAGCT	CCCAACTCTA
ACAGGTGATG	TTATCGTTCA	GATAGGGAGG
GTCATCGCGT	ACTAGTGGCC	TAGAAATCAG
TCTTATAAAC	CCTCCGTGGT	GTCGCTAGAA
GTGTAGACTG	TTAGGGTATG	AAAATAGAAA
AAACAACCGG	GAATCAGGCG	GCTCCTGGGT
ATCCTGTACC	GGCTGACCAA	CGCGCTCGCG
TTATAAGAAT	TGCCAGACCG	GGCAAACGCA
ATAAGTAGGC	TCCCTACGCG	TTTACTACCT
TCTCGTAAGG	TCCGCTGGAG	ATCCTAAACT
GATCCGCCGC	GGATCAAAAC	CTCCGTATGT

TGTCAGGTTT	TTCACCTCAC	TATCGTCCAG
TCCGAAGCCC	GACACACGGC	GCCGGCGGTA
TCCATGTCCA	TGGGCGATTA	TGCTCCATTT
GTGATGGTAC	TAAGATCTTC	TGGCTGTTGT
CTCCACATAC	CTCCGACTAC	TACTGCGCAA
TTCGGATGAG	GGGCCATCAT	TATACGGCTT
ACGACATCGC	TCAGGCCAGA	GGTTATTACC
GAGATGCACA	CTTGTGGGGC	ATCAGGAGGA
TTTGTATGGC	AGATAGTCTG	CTATTGCCAG
CTTTTCTAGA	GCGTCAAAGT	ACGTACACAC
AGTCTAATCA	ACGAAAATTT	CAGCCTAGCT
GACTTAGCCA	GAGTCTGGTG	GAAAAACAAC
TATCACAGTA	ATCGAGCGAC	CGTTCAGTTA
AAGCTCGAGT	GGTCCTCAGA	CAATCAGAAT
TGTTACGACA	TGATTTTGTC	GGGCTACTCT
AAGGATAGTC	GCATTTCTCA	CCCCATTGGG
GCACTTAGCC	GCATGCCAGT	TAGGGAACGG
GAGGGATCCG	ATTAGACGAC	CAGCTGATAC
ATTCTAGAAG	AAAGCCCATA	ATTCCTGTGA
GATAACTGAT	CACTACATTC	TCAGAGCCGT
ATCTGACTGT	CACGGTTTCT	CATGAAAAGC
CAAAGCGAAC	CCCACCAGTG	TGACCTGTGA
GAAATTGCGA	CTCACTTGTC	GCATTAGCAG
GGGTCCAGTC	GATAGACTCT	GACAGAACCA
ATCAGGTAGC	ATTTCCATTT	TCCAGTATAT
GAAAGGTCCT	ATATGTGGCC	TGTTCCGCTA
GGCTACCACA	CGGGACGAAC	GATATCCATT
TTATTGCTGA	AGAACCGTGA	CATATGGACC
CGCCGCGTTT	TAGTGTACTG	GATATAGTAA
TTTTCAAAAG	AACTAATCGA	CACCTTTTTT
CTGGGCTAAA	CGAAGTGACG	AGCTTGCGGG
CCCGATGAGA	CGGAGCCTCG	CGCACAGGGA
TGGGAAATAT	ATCACACGAG	TCTGGGTGCT
GTACGAGCGG	CGACGAGTTC	TGAGTCGTTT
GCGTGCAGCT	GCTTCCCGTG	TTACAATGTG
AGTCTGCGGA	GATTCATACC	CTTGCAAACA
TAACTATTTA	GAGAGAAGCG	TGTCGAGCTG
GAGTTGCCGG	GAAGTGGCCT	ACTTTAACCT
CAGCCCGGCG	GGACGACGCC	ATATAAGTGC
TCACCTACAT	TAGGGTCTCA	GGAAGGGCGT
AGTGGCTAAC	AACTACAGGT	TTTGACTTGA

AGAATGTGAG	GTGGCCTGTG	GTATAAACGG
TAGTTTCGCA	CTTTACCAGC	TAACCGGATG
CTTCATTTCT	CGCGTTACTG	TTCTCATCAG
GCCATGATAT	TTGCTCCCGT	CTCGGTTACG
ACGGCAAATC	CATCAAACAA	ATATGGTTCT
ATCGATAGTA	GCTTTATGAT	CGCCCCCGAA
CCTAAAGGCA	CTGCATACTG	ACCTCGATCG
TACGAGCGGT	GGTGGCTCAG	CTCGAATAAT
TTTGTCGTCG	GGACGATCAA	GCCCGAGCTT
TACAAGCTTG	CCGACTGGTG	AACAGTCAAC
GACCAACACG	GGAACAACCG	CTGGAACCTC
GAACGACGAA	GAACGAGACC	AATAACGGGG
TCGGAACGCA	CACCAAGAAA	ACGCCCCACT
ATCCGGTGGT	ATGCATTACC	GGCAACATGA
TAAAACGTAG	GTATCATGCC	GCTATTTCGC
TATGTGAGCC	AGTAGATGTT	TTCCACTTTA
GAGGCATCGA	CTCTAGATGT	GCCGATGGAT
GAATGGGTGG	GCTACTTGTG	AAGTTGGTAA
AACGACACAA	TATGAAACGT	CACTAGCTAG
GTACGATGCA	CCTCGTTGAT	ACATGCCCCT
AGAAGGCGCC	CTAGAGCCAT	TTCATTACTC
CCGCAATGGA	TAGAGTTATA	GGTTTAATAT
TACGGATTTT	AACGAGAGGC	CCTGCAGTGA
GTCGTTAGCT	GGTCTACCGT	TCTTTAAGTT
GGACTAGGGC	GCCCCCTCAC	TGGCGATCGA
ATTGGTATTC	CATAGGAATT	CTTTTTAGCT
ATCCCAGAGA	TCCGGCTCGT	CCCAGTCTCT
GTCCCAGCTC	TGAGAGTCGG	AAATGTTTCG
CACGAGGAAT	CGTAGAAATA	ATATAAGACG
TACAATTGCA	CTTTACATGA	TCACTTTACA
ATTCCTGAAT	GAGCGCCGTC	CCTGGCGCCC
TAGCGAGGCG	GGCTCTCGGC	GGATTACTGG
CTGGATGGGC	AGAGCTTGTT	GAATGATCTT
GCGACGGCCA	AATCAGCCAC	GCTCGGATCG
ACCTGCACAA	AGAAGAGCCA	CAGCTGCGAG
CATGACAGAC	TCGTATGAGT	ACCCTTACTA
TTACCAACGT	TTCTTCCTCG	AGGTGAAACT
CAGGTGTGTG	ACACAAAAGC	CGAATTTGAT
CGAGGGACGG	CGCGGGACCC	CGCTGTGCGG
CGTCTCGGTA	GTCGCGACAC	TTACCGCACC
TAAGCTATCT	CCGGAGGAAA	GGAATCTTAA

TACTCCCCTA	CGGCGTATGA	CTCAACACCC
TTATATTCAT	TAGGCATTCT	CGTGCCCTTG
AGCGATCTGC	AAAGGAGGGA	GCAGGCTCGA
TCTTCTGATC	ACCTTTACGG	ACCAACGAAG
ATAGTTCCCA	CTACCGTTAA	CCTGTAATTT
TTTACGGGTG	GAGCTTCGCC	GGGTGGGATG
GTGTCCCCTG	GCCATAGAAG	TTGCTCACCG
GCGGGGGTCG	TTTAGCGTAT	TTACGACCAC
CATTGATCTA	GCAAACAGAT	TTTTCTAACC
AGGGACGGTG	TAGGTCATGG	GCTTTAGATA
CAGTTACTTT	CTCTAACAGA	CACGTATTGG
CCATACTTCC	GGCTCATGAA	AAATATCTCC
ATCAGAATTA	CAATGTCTCA	GCTGGAAAAC
AAACTAGGCA	TGATCGTATT	GAGCGCATTA
AATGTCGTTG	GCGCTTTTCA	GTGGAGGGGT
CACATGGGTC	AAGATTATAT	TCCACTGGGA
GGTCGCTGGT	ACTAGCTGAC	CAATAGCGGA
ACTGTATTAC	GGTGAGCTCA	CATCTAGTTT
CCGAGACGCG	CGCTTTCGCT	GAAGTTCCGG
ACTCCAACCC	TGATTCAAAA	AGCGAGATTC
ATATTACAAG	ACTGAACAGG	TTAAGGTCGG
CCATGGATAG	ATTCGAGCTA	AATGGTTAGG
CCGTCTCAAT	TGTAGGCTAA	CGTTATTATA
GATCGTCGGG	ACAAAGCTTT	ACGGAAAGGA
TCTTGTTTTG	GCCCGAGGGA	CCTTGTCCCG
AATATTGCTC	GCCCGCTGGG	ATACTTTTTT
AACGTCGTCT	ACCCCGCTGA	CTGGGTCTGG
AATATTTTTG	CTTATGCCCT	AACCATTGCG
CGTAACGTGC	CCGCCATAGC	AGACCGGGCC
GCGTGGTTAT	CTTAATGATT	TGGGACACAC
CAAAACATTA	CAGTCCACAA	TGCGCAGTTG
CGTATCCTGA	ATGGACGGAC	CGTTCGCCTT
TCGCTTACAA	CGGCCTCTCG	TCTCACTCGT
TCCATTGTGT	TAGTCGCCAT	ACACCGACGT
GCCCCCATTC	GTTGATCTTC	TTCAGCCCCT
TGACGTCTAT	ACTTGCCAAG	AGGCGACTAA
TGGGCCGAGG	ATGACTGGTT	TGCTATCAAG
AAGTGTCAAG	TGTCGTAGGA	GTCCAGTAGC
GACAGTAGAG	AGCAAACACG	CGTGTGGGCG
CGCAGCCATC	TACTGATGAA	GTGGTTCTCC
GAGGCAGAAC	GTATCCCATA	GCAGCCGACG

GTTGAAATTG	TAGCCAGGTT	GCTGTCCACG
ATCTGATAAA	CGTGTGGCGA	CGACACTCAT
AGCTGTCTCT	ATCGAATTGC	CATGGCACCT
TTTTAGGTTA	CCCCAATATT	TGTGACGTGT
TATCTGTCCG	CCCGTTTCTC	TTTGGACTAA
AAAACATATG	TCCGCATCTA	TTCATGCCCG
GTAAAGAAGA	CAAGCCTCAT	TTGATCGTGG
TCGACGTGCA	TTTCAATCCC	TAGCATAGGA
TAGATCTTAA	CCTTCCCATC	GTAGTTGCAA
CACTGGTCAC	AGGTACAAGA	GGGACAGCTA
ATTCTGATGT	GTGTAATGGA	AAACCCCCAA
ATGGCCCTGA	AAACTGAGCT	ACTCTCACAA
GGTGATGAGA	ATCTCTGCCC	ATCATTGCCA
CACCGTGGGG	CGACATTTGC	CCAGTTTGCG
GCTTGCTCGG	TGTGAACCCG	ACATTAGTCA
CCAGTTGAAC	TGACACCCCA	CTCCAGGGTA
CGTCTGTACC	TAGGCCAAAG	GAAGGGCCAA
CCAACGCGGC	GAAATTGTAG	CAGTCTCCCC
ACGTGATCGA	GCGTCTGATT	GAGACATTCC
CCATCGAATC	TCTCATTGTT	AACGGTGTTG
CGGTGTCTGC	CTGACATCTC	AGCATTATCA
AAACCACCTC	GTATCCAGTG	CTATACCGAG
TCAATGTTCC	GATGGCCGTT	AACTGGATCA
TTCGACATGT	TCACCCTCTC	GTCTTGTCGG
AGGCACGATA	GGCACTATTC	GACGAGCCGC
CACGAGATCA	AAATAACTGT	GGAACACTGT
CATGCTGGGG	CAGCTCCATT	TAAATGCGTT
TACCATGGTT	CTCTTGACTC	GCGAACACAG
TTGCCCATAT	TTTCCTATAC	TTCTCTCAAC
TGCACATTCG	CCATACCCGA	GTCGTACTGA
GTTATGTTGG	TCGCCGAGCG	TGTGGCGTAA
TGAGTTATGA	CGCTGAAGCC	TGAGCGGCGT
GATGGCCCCC	TCTGGCCCCA	CCTCGTGAAC
GATGGGTTAC	GCTACATTGA	GAGCAATGAA
AGCTACGTTG	CGCATCATAA	CGAGACCTAA
ACCCCATGCA	GCAAAGGGCC	AACTGAGCGC
TACTACCGTT	AACGGCGCAG	TAAAGCTCGT
TCGCTTCTAC	CGACTGACAT	CTCTTTACGT
CTGGCAGTGC	ATGACAGGGC	CCCCGTGGAA
TCTATATATA	CAAGTTCTCC	TCGGTTCGTC
GGATTAGTTC	TCGCCGCTTT	CTGCTTACAC

GTGTTACGCT	ATGCCGGAAA	ACACCGTAAT
TCGACTCCGT	GCGGTTACTA	CCTGGTCGGC
GGTAGCAGGC	GACATTACAA	GGTTATTTGG
TATTGGATTC	CAGAGAGGGC	GCAACTGAGT
GTTCGATCGA	GCACCGCCTC	ATAAGGCCTC
ATATTAATAT	CGGTCCGAGC	CGTGCGAAGG
AGAACGATTG	TGTCCGGTGC	GTCACACACT
GTAAAGTGTA	GGTCGGTTGC	CATACGGCAA
CCCATGTGCC	GCTCAGCTAA	GAACTGCCCA
GTGGCCTCGC	AGCAGTTCGT	AATATGTGAA
GACACTAGGA	AAATCGATGA	CCGATCCTGT
ATATTCTGAC	GCTCGGTATG	CAAAGAGCCT
TAAGTAGACG	CCCGCCGCGG	TAACTTAGAG
TAACGGTCTA	GTGTGATAGG	CAGCATGTAG
TAGTTTCATT	TTGGACTCCA	CCCCATGCAG
TTGGATCCGA	TGCTTATCTA	TCTGAACCAC
CGTGACAACC	CAAAAGGCGT	GCGTGCAAAA
CGCGCTCAGA	TAGGGGGCCT	GCTAGTACCG
CGTTCTTAAT	AAGTATTAAT	TTTCCCGCGC
ACAAGAGTTT	GTTTAGCCCG	CCTTAGTAGG
AGGGTTATAG	CGCTAATATG	TTGTGTCTTG
ACCACGACTC	ACAACACGTT	GCAACGAAGC
GTACTCGGGG	AGAGATGCTC	TGAAACCCTT
ACAAATATCT	TGCCTGATAT	TTCTACGATC
GATCGGGGTG	CTTGTAAGTA	ATTAAAGGTG
ATGTAACTCC	CATATTGCCG	TATCTAACGG
ATGAAGAAGC	CTTAGAAAGT	AGTGCTCCTG
ATGTATTGTC	ATGTTGTATT	CCGTCCCTCT
TGCATTGGAA	CGCATTGAAG	CTAACGAGCG
GCGGACGATC	TTATGTTGGT	AAGTCCGGCT
CCGTACTTGA	TCGCCTCAGA	GGCGTATAAG
TTTGCCCCCG	TTCGTTGAGG	AGATATTAGG
ACCTCACGCG	GGTGCCGGGC	TCCTAACAGC
ATTAAGGGGC	ACCATTGTAA	GAGGATACGC
CGTGGACATG	TTGATTGTCA	CGCTCTTTAA
TTAGCCCTTC	CGGCTCACCT	ACCGGCAGGC
CGAGAGTTTG	CTATCACATG	GCTAAAATCT
TGCATCCTCT	GTAGACAGAA	GCCGTTGACG
TGCGATTCCG	CCTTTACCAA	GGAGTTGTTG
TTATTACGTT	GCACATCGAC	TACTTGAGAA
TGATGTGGTT	TCTCACTTTC	CGGGTGCGCT

GGGCGTCAAT	TTCGAGTACT	AAAAGCGTCT
CCCTTGAAAT	TAGAAGAGCA	GTAAAGATAG
TCTTTGGGGC	AACCCCACCA	GCCTGGTCAG
ACCGGCAGGC	CTGTATCAGT	GGCAAAAAGG
GCTAAAATCT	ACATAATGAG	ACCCTTCTCT
GCCGTTGACG	AGCCTTCCGC	TCACATAGTG
GGAGTTGTTG	CAGTGCTTTT	TCGTCTGTGC
TACTTGAGAA	TAGTCCGTGT	TGCTCGGATC
CGGGTGCGCT	CGGAATCGGT	GGCGTATAAG
AAAAGCGTCT	CTTGCGGAGA	AGATATTAGG
GTAAAGATAG	AAAAATTTGG	TCCTAACAGC
GCCTGGTCAG	TGTTTTCCGC	GAGGATACGC
GGCAAAAAGG	ATGCTAGGCG	CGCTCTTTAA
ACCCTTCTCT	GACTAATTTC	GGCGTATAAG
TCACATAGTG	CTGTAGTAAC	AGATATTAGG
TCGTCTGTGC	CGGATGACTT	TCCTAACAGC
TGCTCGGATC	TCAGAGTGGA	GAGGATACGC

[00150] In another embodiment, a random sequence fragment can be linked to the 5' and/or the 3' end of the polynucleotide barcode and the random sequence fragment can, for example, be used for bioinformatic removal of PCR duplicates. The random sequence fragment can also be used to add length to the nucleic acid construct and can serve as a marker for bioinformatic analysis to identify the beginning or the end of the polynucleotide barcode after sequencing. In another embodiment, the nucleic acid construct comprises at least a first and a second random sequence fragment, and the first random sequence fragment can be linked to the 5' end of the polynucleotide barcode and the second random sequence fragment can be linked to the 3' end of the polynucleotide barcode. In another embodiment, one or at least one random sequence fragment is linked to the 5' and/or the 3' end of the polynucleotide barcode. In one aspect, the random sequence fragments can be extended as needed to make the nucleic acid construct longer for different applications such as whole genome sequencing where short inserts may be lost.

[00151] In various embodiments, the random sequence fragments can be from about 5 to about 20 base pairs in length, about 5 to about 19 base pairs in length, about 5 to about 18 base pairs in length, about 5 to about 17 base pairs in length, about 5 to about 16 base pairs in length, about 5 to about 15 base pairs in length, about 5 to about 14 base pairs in length, about

5 to about 13 base pairs in length, about 5 to about 12 base pairs in length, about 5 to about 11 base pairs in length, about 5 to about 10 base pairs in length, about 5 to about 9 base pairs in length, about 5 to about 8 base pairs in length, about 6 to about 10 base pairs in length, about 7 to about 10 base pairs in length, or about 8 to about 10 base pairs in length.

[00152] In another illustrative aspect, the polynucleotide barcode may be flanked by primer binding segments (i.e., directly or indirectly linked to the polynucleotide barcode) so that the nucleic acid construct comprising the polynucleotide barcode can be amplified during a polymerase chain reaction (PCR) and/or sequencing protocol. In one aspect, the primer binding segments can be useful for binding to one or more universal primers or a universal primer set. In one illustrative embodiment, the universal primers can contain overhang sequences that enable attachment of index adapters for sequencing. In this aspect, the primers can be any primers of interest. In this embodiment, the first primer binding segment can be linked at its 3' end to the 5' end of a first random sequence fragment and the second primer binding segment can be linked at its 5' end to the 3' end of a second random sequence fragment with the polynucleotide barcode between the random sequence fragments. In another embodiment, the first primer binding segment can be linked at its 3' end to the 5' end of the polynucleotide barcode and the second primer binding segment can be linked at its 5' end to the 3' end of a random sequence fragment (see Fig. 1 for an example) linked to the 3' end of the polynucleotide barcode. In another embodiment, the first primer binding segment can be linked at its 3' end to the 5' end of a random sequence fragment and the second primer binding segment can be linked at its 5' end to the 3' end of the polynucleotide barcode where the polynucleotide barcode is linked at its 5' end to the 3' end of the random sequence fragment. In yet another embodiment, the first primer binding segment can be linked at its 3' end to the 5' end of the polynucleotide barcode and the second primer binding segment can be linked at its 5' end to the 3' end of the polynucleotide barcode.

[00153] In embodiments where primer binding segments are included in the nucleic acid construct, the primer binding segments can range in length from about 15 base pairs to about 30, from about 15 base pairs to about 29 base pairs, from about 15 base pairs to about 28 base pairs, from about 15 base pairs to about 26 base pairs, from about 15 base pairs to about 24 base pairs, from about 15 base pairs to about 22 base pairs, from about 15 base pairs to about 20 base pairs, 16 base pairs to about 28 base pairs, from about 16 base pairs to about

26 base pairs, from about 16 base pairs to about 24 base pairs, from about 16 base pairs to about 22 base pairs, from about 16 base pairs to about 20 base pairs, 17 base pairs to about 28 base pairs, from about 17 base pairs to about 26 base pairs, from about 17 base pairs to about 24 base pairs, from about 17 base pairs to about 22 base pairs, from about 17 base pairs to about 20 base pairs, 18 base pairs to about 28 base pairs, from about 18 base pairs to about 26 base pairs, from about 18 base pairs to about 24 base pairs, from about 18 base pairs to about 22 base pairs, or from about 18 base pairs to about 20 base pairs.

[00154] An exemplary sequence of a nucleic acid construct is shown below. The /5AmMC6/ is a 5' amine modification for attachment to the polymer nanoparticle. The *'s are phosphorothioate bond modifications for stability. The A*G*A*CGTGTGCTCTTCCGATCT sequence is the 5' primer binding segment sequence. The GCTACATAAT is an exemplary barcode polynucleotide sequence. The N's represent the random sequence fragment. The AGATCGGAAGAGCGTCG*T*G*T is the 3' primer binding segment sequence.

[00155] /5AmMC6/A*G*A*CGTGTGCTCTTCCGATCTGCTACATAATNNNNNNN NNNAGATCGGAAGAGCGTCG*T*G*T

[00156]

In all of the various embodiments described above, the entire nucleic acid construct can range in length from about 30 base pairs to about 240 base pairs, about 30 base pairs to about 230 base pairs, about 30 base pairs to about 220 base pairs, about 30 base pairs to about 210 base pairs, about 30 base pairs to about 200 base pairs, about 30 base pairs to about 190 base pairs, about 30 base pairs to about 180 base pairs, about 30 base pairs to about 170 base pairs, about 30 base pairs to about 160 base pairs, about 30 base pairs to about 150 base pairs, about 30 base pairs to about 140 base pairs, about 30 base pairs to about 130 base pairs, about 30 base pairs to about 120 base pairs, from about 30 base pairs to about 110 base pairs, from about 30 base pairs to about 100 base pairs, from about 30 base pairs to about 90 base pairs, from about 30 base pairs to about 80 base pairs, from about 30 base pairs to about 70 base pairs, from about 30 base pairs to about 60 base pairs, from about 30 base pairs to about 50 base pairs, from about 30 base pairs to about 40 base pairs, 40 base pairs to about 120 base pairs, from about 40 base pairs to about 110 base pairs, from about 40 base pairs to about 100 base pairs, from about 40 base pairs to about 90 base pairs, from about 40 base pairs to about

80 base pairs, from about 40 base pairs to about 70 base pairs, from about 40 base pairs to about 60 base pairs, from about 40 base pairs to about 50 base pairs, 50 base pairs to about 120 base pairs, from about 50 base pairs to about 110 base pairs, from about 50 base pairs to about 100 base pairs, from about 50 base pairs to about 90 base pairs, from about 50 base pairs to about 80 base pairs, from about 50 base pairs to about 70 base pairs, from about 50 base pairs to about 60 base pairs, or about 42 base pairs to about 210 base pairs.

[00157] The nucleic acid constructs are associated with the polymer nanoparticles, and exemplary polymer nanoparticle to nucleic acid construct ratio ranges are about 20:1 to about 10000:1, about 20:1 to about 9000:1, about 20:1 to about 8000:1, about 20:1 to about 7000:1, about 20:1 to about 6000:1, about 20:1 to about 5000:1, about 20:1 to about 4000:1, about 20:1 to about 3000:1, about 20:1 to about 2000:1, about 20:1 to about 1000:1, about 20:1 to about 900:1, about 20:1 to about 800:1, about 20:1 to about 700:1, about 20:1 to about 600:1, about 20:1 to about 500:1, about 20:1 to about 400:1, about 20:1 to about 300:1, about 20:1 to about 200:1, or about 20:1 to about 100:1.

[00158] In one illustrative aspect, the barcoded polymer nanoparticles may be used as delivery vehicles according to the present disclosure. In some embodiments, the non-viral delivery vehicle comprises one or more nanoparticle forming polymers. In some embodiments, the non-viral delivery vehicle comprises polymer nanoparticles. In some embodiments, the non-viral delivery vehicle is not a lipid based system. In some embodiments, the non-viral delivery vehicle comprises polymer nanoparticles made from controlled living/radical polymerization processes. It will be appreciated that the identity of the monomer units is not particularly limited so long as the monomer units being used are compatible with a controlled living/radical polymerization, such as reversible-deactivation radical polymerization, atom transfer radical polymerization (ATRP), reversible addition fragmentation chain transfer polymerization (RAFT), iodine-transfer polymerization (ITP), selenium-centered radical-mediated polymerization, telluride-mediated polymerization (TERP), stibine-mediated polymerization, ring-opening polymerization, or like polymerization processes. In some embodiments, the polymer nanoparticles may be made by RAFT copolymerization to synthesize a diverse set of block copolymers, and to screen their ability to form complexes with a payload. In one aspect, polymer nanoparticles (e.g., RAFT copolymers) may be produced by chemically bonding a payload to a constituent polymer, such as by the grafting of

the payload onto RAFT copolymers using chain transfer agents, and subsequently assembling the polymers into a delivery vehicle.

[00159] In various embodiments, payloads may be combined with the polymer nanoparticles compositions using any or all of covalent bonds, electrostatic interactions, and ligand affinity interactions. In one aspect, covalent bonding methods include the use of EDC/NHS to form stable amide bonds between the payload and the polymer nanoparticles for improved stability (both "on the shelf" and *in vivo*), ease of separation and extraction, and sensitive detection. In another illustrative aspect, electrostatic bonding methods include the use of cationic polymer nanoparticles that electrostatically complex with the payload. In another embodiment, ligand affinity bonding includes the use of ligands such as avidin and biotin, both covalently bonded to the polymer nanoparticles and the payload via EDC/NHS chemistry to yield the stable combination of the payload and the polymer nanoparticles.

[00160] It will be appreciated that RAFT polymerization is generally known in the art. Suitable reagents, monomers, and conditions for RAFT polymerization previously investigated can be used in the copolymers, methods, and compositions described herein, such as those described in United States Patent Nos. 9,006,193, 9,464,300, and 9,476,063, the disclosures of each of which are incorporated by reference in their entirety.

[00161] Chain transfer agents (CTAs) useful in connection with the present disclosure are known in the art. The identity of the CTA is not particularly limited. It will be appreciated that chain transfers steps that form the basis of RAFT polymerization involve a reversible transfer of a functional chain end-group (typically a thiocarbonylthio group, Z−C(=S)S−R) between chains and the propagating radicals. The overall process is comprised of the insertion of monomers between the R- and Z−C(=S)S-groups of a RAFT agent (CTA), which form the α and ω end-group of the majority of the resulting polymeric chains. Suitable CTAs for use in connection with the present disclosure include but are not limited to trithiocarbonates (Z = S-alkyl), dithiobenzoates (Z = Ph), dithiocarbamate (Z = N-alkyl), xanthates (Z = O-alkyl), and the like. (See, Sébastien Perrier, *Macromolecules* 2017 *50* (19), 7433-7447) In some embodiments, RAFT copolymerization may be achieved using chain transfer agents (CTAs) containing one or more terminal carboxyl groups in order to obtain carboxy terminated polymers with ends available for bonding to the payload via the methods described above. In this embodiment, when the resulting mono or di-carboxy terminated polymer is dispersed in a

low pH (e.g., a pH of less than 6) buffer, both ends of the polymer are exposed and available for labeling via EDC/NHS chemistry. In this embodiment, when the polymer is transferred to a physiological pH (~ pH 7), the core blocks self-assemble, encapsulating the payload in the hydrophobic core, to be released and exposed upon acidification in the endosomal compartment of a cell. In some embodiments, the first or second chain transfer agent can be selected from the group consisting of bis(carboxymethyl)trithiocarbonate, bis(2-amino-2-oxoethyl) trithiocarbonate, bis[4-(2-hydroxyethoxycarbonyl)benzyl] trithiocarbonate, 4-cyano-4-(ethylsulfanylthiocarbonyl) sulfanylvpentanoic acid, 4-cyano-4-((phenylcarbonothioyl)thio)pentanoic acid, and 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid, 4-cyano-4-(thiobenzoylthio)pentanoic acid, 2-cyano-2-propyl benzodithioate, cyanomethyl methyl(phenyl)carbamodithioate, 2-cyano-2-propyl dodecyl trithiocarbonate, 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid, cyanomethyl dodecyl trithiocarbonate, 2-cyano-2-propyl 4-cyanobenzodithioate, and the like.

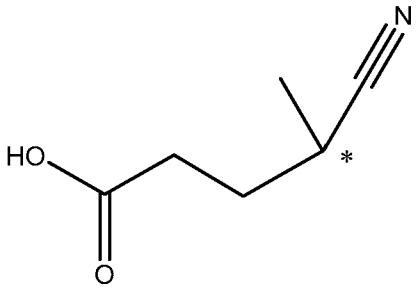
[00162] It will be apprectiated that RAFT useful in connection with the present disclosure can be of a variety of polymer compositions. For example, RAFT polymers useful in connection with the present disclosure can be a randon block polymer comprising a single polymer block, or a diblock RAFT copolymer comprising two polymer blocks, or a triblock RAFT copolymer comprising three polymer blocks, or further numbers of blocks can be used. The skilled person will readily appreciate that the the preparation of block polymers by RAFT polymerization is known in the art and that such polymerization processes can be applied to the present disclosure. (See, Goby, et. al., Nat. Commun. 4:2505 doi: 10.1038/ncomms3505 (2013))

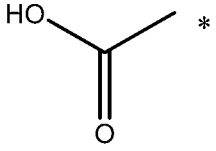
[00163] In some embodiments, RAFT copolymers as prepared herein can be described by the following structure:

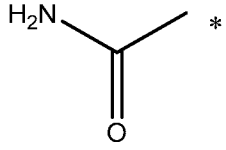
$$\text{CTACap-[Block 1]}_m\text{-[Block 2]}_n\text{-CTACap}$$

where each CTACap is a capping unit derived from the chain transfer agent(s) used in the process for preparing the RAFT copolymer. The CTA used for preparing each of Block 1 and Block 2 can be the same or different. In some embodiments, the CTA used to prepare each of Block 1 and Block 2 is the same (e.g. macroCTA). In some embodiments, the CTA used to prepare each of Block 1 and Block 2 is different. In some embodiments, the CTA used to

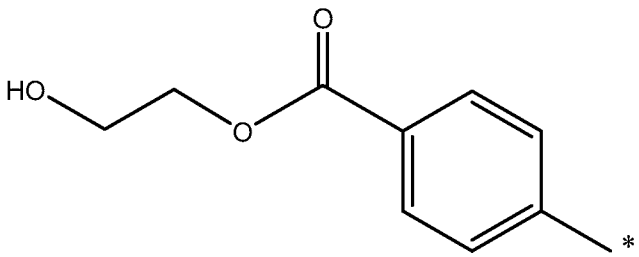
prepare one or both of Block 1 and Block 2 comprises a functional group for the covalent attachment of a biomolecule, drug, or label to the RAFT copolymer. In some embodiments, the covalent attachment can be via an ester or an amide bond. In some embodiments, the covalent attachment can be via EDC-NHS chemistry. In some embodiments, the first capping unit is of the formula



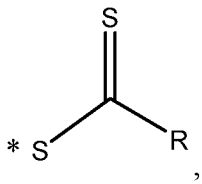
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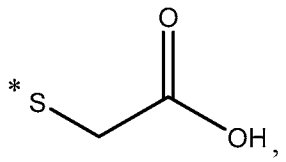
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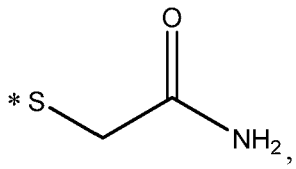
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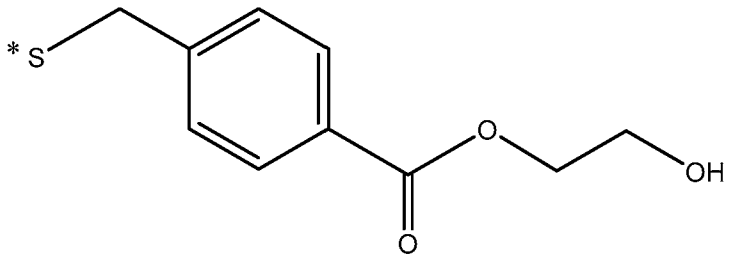
wherein * represents a point of covalent attachment to the first block. In some embodiments, the second capping unit is of the formula



,


,


, or



wherein * represents a point of covalent attachment to the second block, and R is $-SC_2$-C_{12} alkyl or C_6H_5,

[00164] In some embodiments, the RAFT copolymer can be associated with a DNA molecule, in particular a nucleic acid construct of the present disclosure, via several methods

including, electrostatic interaction, high affinity, non-covalent bond, avidin-streptavidin conjugation, or by direct covalent attachment through, for example, an amide bond. In some embodiments, the RAFT copolymer can be associated with a DNA molecule, in particular a nucleic acid construct of the present disclosure, via electrostatic interaction complexed with a biological molecule. In some embodiments, the RAFT copolymer can be associated with a DNA molecule, in particular a nucleic acid construct of the present disclosure, via electrostatic interaction complexed with a biological molecule. In some embodiments, the RAFT copolymer can be associated with a DNA molecule, in particular a nucleic acid construct of the present disclosure, via a high affinity, non-covalent bond, avidin-streptavidin conjugation. In some embodiments, the RAFT copolymer can be associated with a DNA molecule, in particular a nucleic acid construct of the present disclosure, by direct covalent attachment through, for example, an amide bond.

[00165] As shown in Figs. 5(a)-5(e), the PNPs described herein can be associated with a nucleic acid construct of the present disclosure via electrostatic interaction, avidin-streptavidin conjugation, or by direct covalent attachment. Briefly, as shown in Figs. 5(a)-5(e), the labels provided in the figure are as follows: 001. Polymer nanoparticle (PNP) with positively charged corona in the case of electrostatic loading. 002. Nucleic acid constructs with negative charges due to the phosphate groups. 003. Electrostatically loaded PNP-nucleic acid construct complex. 004. Carboxylate group on the terminal end of the polymer chains in the corona of the PNP. 005. Primary amine group on the 5' end of the amine terminated nucleic acid construct. 006. Phosphate group on the 3' end of the nucleic acid construct. 007. Amide bond formed in the direct amidification reaction between the amine terminal nucleic acid construct and the carboxylate terminated PNP. 008. Primary amine on the biotin bonding protein such as avidin. 010. Amide bond formed between the carboxylate group on the terminal end of the polymer chains in the corona of the PNP and the primary amine on the biotin bonding protein such as avidin. 011. Nucleic acid construct with a biotin functional group on the 5' terminus. 012. Electrostatic coupling reaction that occurs when positively charged PNPs are mixed with negatively charged nucleic acid constructs. 013. Direct amidification reaction that is carried out via an EDA-NHC reaction between the carboxylate group on the terminal end of the polymer chains in the corona of the PNP and the primary amine on the amine terminated nucleic acid constructs. 014 Direct amidification reaction that is carried out via an EDA-NHC

reaction between the carboxylate group on the terminal end of the polymer chains in the corona of the PNP and the primary amine on the biotin bonding protein such as avidin. 015. Coupling of the biotin on the 5' end of the nucleic acid construct and the avidin conjugated to the carboxylate terminus on the corona of the PNPs.

[00166] In some embodiments, each of Block 1 and Block 2 can comprise one or more monomer units polymerized using a RAFT polymerization process. It will be appreciated that the identity of the monomer units is not particularly limited so long as the monomer units being used are compatible with a controlled living/radical polymerization, such as reversible-deactivation radical polymerization, atom transfer radical polymerization (ATRP), reversible addition fragmentation chain transfer polymerization (RAFT), iodine-transfer polymerization (ITP), selenium-centered radical-mediated polymerization, telluride-mediated polymerization (TERP), stibine-mediated polymerization, ring-opening polymerization, or like polymerization processes. Suitable monomer units include but are not limited to 2-dimethylaminoethyl acrylate (DMAEMA), 2-(diethylamino) ethyl methacrylate (DEAEMA), 2-(diisopropylamino) ethyl methacrylate (DPAEMA), butyl methacrylate (BMA), ethyl acrylic acid (EAA), propyl acrylic acid (PAA), (hydroxyethyl)methacrylate (HEMA), methyl methacrylate (MMA), Acrylic acid (AA), Acetoacetanilide (AAA), 4-Aminobenzonitrile (ABN), 9-Anthracenylmethyl acrylate (ACMA), 9-Anthracenylmethyl methacrylate (ACMMA), Aminoethyl methacrylate (AEM), 2-(2-aminoethylamino) ethyl methacrylate (AEAEMA), 4-(2-Acryloxyethoxy)-2-hydroxybenzophenone (AEHBP), 2-Aminoethyl methacrylate (AEMA), N-(2-Aminoethyl) methacrylamide (AEMAA), 3-amino-2-hydroxypropyl methacrylate (AEAHPMA), 3-aminopropyl methacrylamide (AHPMA), Allyl methacrylate (ALMA), Acrylamide (AM), Amidoamine (AMA), 3-Methacryl amido-3-methylbutanoic acid (AMBA), 2-Allyloxybenzaldehyde (AOBA), [2-(Acryloyloxy)ethyl]trimethylammonium chloride (AOETMA), 3-(Acryloyloxy)-2-hydroxypropyl methacrylate (AOHOPMA), 4-Aminophenethyl alcohol (APA), N-(3-Aminopropyl)methacrylamide (APMA), 5-(3-(Amino)-propoxy)-2-nitrobenzyl methacrylate (APNBMA), N-[N′-(2- aminoethyl)-2-aminoethyl]aspartamide (Asp(DET)), 2-Azidoethyl Methacrylate (AzEMA), 2,2′-Bithiophene (2-2-BTP), tert-Butyl acrylate (BA), Bromoacetaldehyde diethyl acetal (BAADA), N-(t-BOC-aminopropyl)methacrylamide (BAPMAA), tert-Butyl 2-bromoacrylate (BBA), 4-Butylbenzoyl chloride (BBC), 1,3-Butadiene (BD), 2-Butyl-2-ethyl-1,3-propanediol (BEPD), Di-tert-butyl

iminodiacetate (BIDA), 3-(Bromomethyl)-5-((trimethylsilyl)ethynyl)benzenesulfonyl fluoride (BMTMSEBS), 2-(Benzyloxy)ethanol (BOE), 4-tert-Butoxystyrene (BOS), Branched polyethyleneimine (BPEI), 3-Bromo-5-((trimethylsilyl)ethynyl)benzenesulfonyl fluoride (BTMSEBS), ε-Caprolactone (CAP), Carboxybetaine methacrylate (CBMA), 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (CEDPCPP), N-Cyclohexylmaleimide (CHMI), 3-Chloro-2-hydroxypropyl methacrylate (CHPMA), Dodecyl acrylate (DA), N,N-Diallylacrylamide (DAA), Diallylmethylamine (DAMA), Diallyldimethylammonium chloride (DADMAC), 2,5-Diaminopyridine (DAP), 5,5′-Dibromo-4,4′-didodecyl-2,2′-bithiophene (DBDDBT), 5,5′-Dibromo-4,4′-ditetradecyl-2,2′-bithiophene (DBDTBT), 2,5-Dibromo-3-hexylthiophene (DBHTP), Dichloromethylvinylsilane (DCMVS), 2-(Diethylamino)ethanethiol hydrochloride (DEAET), 2-(Diethylamino)ethyl methacrylate (DEAEMA), Diethyl oxalpropionate (DEOP), DL-Lactide (DLL), N,N-dimethylamino-2-ethylmethacrylate) (DMA), N-[3-(N,N-dimethylamino)propyl]-methacrylamide (DMAPMA), [2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide (DMAPS), N,N′-dimethylbutylamine (DMBA), N,N′-dimethylethanolamine (DMEA), N,N-dimethylamino-2-ethylacrylate or 2-(dimethylamino)ethyl acrylate (DMAEA), 1-(Dimethylamino)pyrrole (DMAP), 4,5-Dimethoxy-2-nitrobenzyl alcohol (DMONBA),3,4-Dimethoxythiophene (DMOT), 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 2-deoxy-2-methacrylamido glucopyranose (DOMAAG), 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,2-Dioleoyl-3-trimethylammonium Propane (DOTAP), 1,2-Diphytanoyl-sn-glycero-3-phosphoethanolamine (DPyPE), 3-Dodecylthiophene (3-DT), N-[2-(2-pyridyldithio)]ethyl methacrylamide (DTEMA), Ethyl acrylic acid (EAA), Ethyl 2-(bromomethyl)acrylate (EBMA), Ethyl 1-cyano-1-cyclopropanecarboxylate (ECCPC), 3,4-Ethylenedioxythiophene (EDOT), Ethylene glycol dimethacrylate (EGDMA), Ethylene glycol phenyl ether acrylate (EGPEA), Ethyl methacrylate (EMA), 3-(Fluorosulfonyl)-5-((trimethylsilyl)ethynyl)benzoic acid (3FTMSEBA), 3-Formyl-5-((trimethylsilyl)ethynyl)benzenesulfonyl fluoride (3FTMSEBS), 4-Formyl-2-((trimethylsilyl)ethynyl)benzenesulfonyl fluoride (4FTMSEBS), 5-Fluoro-2,3-thiophenedicarboxaldehyde (5FTPDCA), N-acetyl-D-galactose (GalNAc), N-acetyl-D-glucose (GlcNAc), Glycidyl methacrylate (GMA), Glycosylphosphatidylinositol (GPI), 5,7-

Hexadecadiynoic acid (HDDA), 2-Hydroxyethyl methacrylate (HEMA), 1,1,1,3,3,3-Hexafluoroisopropyl acrylate (HFIPA), 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA), 4-Hydroxybutyl acrylate (HOBA), 2-(4-Hydroxyphenylazo)benzoic acid (HPABA), 2,2,3,3,4,4,4-Heptafluorobutyl acrylate (HPFBA), N-(2-Hydroxypropyl)-Methacrylamide (HPMA), 2,2,3,4,4,4-Hexafluorobutyl acrylate (HXFBA), Isobornyl acrylate (IBA), 4-Iodobenzoyl chloride (IBC), Isobornyl methacrylate (IBMA), N-(Isobutoxymethyl)acrylamide (IBMAA), Isodecyl acrylate (IDA), Maleic anhydride (MA), [2-(Methacryloyloxy)ethyl]trimethylammonium chloride (MAETMA), Methacryloyl-L-Lysine (MAL), Methacryloxysuccinimide (MAS), Methacrylamidotrehalose (MAT), Methyl heptadecanoate (MHD), 1-Methyl-1H-indole-3-carbaldehyde (MICA), 2- N-Methylmaleimide (MMI), Methyl-5-norbornene-2,3-dicarboxylic anhydride (MNDCA), Methoxyethyl methacrylate (MOEMA), 2-(2-Methoxyethoxy)ethyl methacrylate (MOEOEMA), 3-Methyl-3-oxetanemethanol (MOM), 2-Methacryloyloxyethyl phosphorylcholine (MPC), Methyl 4-vinylbenzoate (MVB), 2-Naphthyl acrylate (NA), N-(Acryloxy)succinimide (NAS), o-Nitrobenzyl methacrylate (NBMA), N-Hydroxysuccinimide (NHS), N-(Methacryloxy)succinimide methacrylate (NHSMA), N-Isopropylacrylamide (NIPAM), 2-Naphthyl methacrylate (NMA), N-(Methacryloyloxy)succinimide (NMS), N-(n-Octadecyl)acrylamide (NODAA), 4-Nitro-N-propylbenzylamine hydrochloride (NPBAHC), Oligoethylene glycol methacrylate (OEGMA), Oligoethylenimine (OEI), 3-Phenylthiophene (3-PTP), Poly(N-methyl 4-vinylpyridine iodide) (P4VPQ), Poly(2-aminoethylmethacrylamide) (PAEMA), N-(N′-{N″-[N‴-(2-aminoethyl)-2-aminoethyl]-2-aminoethyl}-2-aminoethyl)-aspartamide (Asp(TEP)), 4-Pentenoic anhydride (PAN), Pentabromobenzyl acrylate (PBBA), Pentabromobenzyl methacrylate (PBBMA), Pentabromophenyl acrylate (PBPA), Pentabromophenyl methacrylate (PBPMA), Poly(ε-caprolactone) (PCL), trans-2-Phenylcyclopropyl isocyanate (PCPI), 1,5-Pentanediol (PD), 2-Phenyl-1,3-dioxan-5-ol (PDO), Pyridyl disulfide ethyl methacrylate (PDSEMA), Poly(ethylene glycol) (PEG), Poly(ethylene glycol) acrylate (PEGA), Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, Poly(ethylene glycol) ethyl ether methacrylate (PEGEEMA), Poly(ethylene glycol) methacrylate (PEGMA), Pentaethylenehexamine (PEHA), Poly(ethylenimine) (PEI), Pentaerythritol tetraacrylate (PETA), Pentafluorophenyl (PFP), Pentafluorophenyl acrylate (PFPA), Pentafluorophenyl methacrylate (PFPMA), Poly(glutamic acid) (PGA), Poly-

(glycoamidoamine) (PGAA), Poly(glycidylbutylamine) (PGBA), Poly(glycidyl methacrylate) functionalized with ethanolamine (PGEA), Poly(glycidyl methacrylate) (PGMA), Poly(N-(2-Hydroxypropyl)methacrylamide) (PHPMA), Poly(lactic acid) (PLA), Poly(L-glutamate), (PLG), Poly(lactic-co-glycolic acid) (PLGA,), Poly(L-lysine) (PLL), Poly(L-lactic acid) (PLLA), Poly(lauryl methacrylate) (PLMA), Poly(methacrylic acid) (PMAA), Poly-(2-deoxy-2-methacrylamido glucopyranose) (PMAG), Poly-(methyl methacrylate) (PMMA), Poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), Poly[N-(3-(methacryloylamino) propyl)-N,N-dimethyl-N-(3-sulfopropyl) ammonium hydroxide] (PMPD), Poly(n-butyl acrylate) (PnBA), Poly(n-butyl methacrylate) (PnBMA), Poly(N-isopropyl acrylamide) (PNIPAM), Poly(oligoethylene glycol methacrylate) (POEGMA), Poly(propylene glycol) (PPG), Poly(propylenimine) (PPI), 3,4-Propylenedioxythiophene (ProDOT), Poly(styrene) (PS), Poly(sodium 4-styrenesulfonate) (PSS), Poly(tributyl-(4-vinylbenzyl)phosphonium chloride) (PTBP), Poly(triethyl-(4-vinylbenzyl)phosphonium chloride) (PTEP), Poly((2-trimethylamino)ethyl metacrylate chloride) (PTMAEMA), Poly((vinylbenzyl) trimethylammonium) (PVBTMA), Poly(2-vinyl-4,4-dimethylazlactone) (PVDMA), Poly(N-ethyl-4-vinylpyridinium bromide) (PVP), Quaternized Poly-DMAEMA (QPDMAEMA), Sulfobetaine methacrylate (SBMA), 3-Sulfopropyl methacrylate potassium salt (SPMAP), Thiocholesterol (TC), Thiophene-2,5-diboronic acid bis(pinacol) ester (TDABP), Triethylene glycol dimethacrylate (TEGDMA), Trifluoroethylene (TFE), 2,2,2-Trifluoroethyl acrylate (TFEA), 2,2,2-Trifluoroethyl methacrylate (TFEMA), Tetrahydrofurfuryl acrylate (THFA), Triallylisocyanurate (TIC), Trimethylene Carbonate (TMC), 4,4′-Trimethylenedipiperidine (TMDP), Trimethylolpropane tris(3-mercaptopropionate) (TMPTMP), (Trimethylsilyl)methacrylate (TMSMA), Triphenylcarbenium pentachlorostannate (TPCPCS), 3-Vinylbenzaldehyde (3VBA), 4-Vinylpyridinium chloride (4VP), Vinyl acrylate (VA), Vinyl acetate (VAT), 4-Vinylbenzoic acid (VBA), (Vinylbenzyl)trimethylammonium chloride (VBTAC), 1-Vinylimidazole (VI), 1-Vinyl-2-pyrrolidone (VP), m-Xylylenediamine (XDA), Zinc stearate (ZS), and the like.

[00167] In some embodiments, the monomer units used to make Block 1 and/or Block 2 of RAFT copolymers as described herein are selected from the group consisting of 2-(dimethylamino) ethyl acrylate (DMAEEA), 2-(diethylamino) ethyl methacrylate (DEAEEA), 2-(diisopropylamino) ethyl methacrylate (DIEAMA), butyl methacrylate (BMA), ethyl acrylic

acid (EAA), propyl acrylic acid (PAA), (hydroxyethyl)methacrylate, and methyl methacrylate (MMA).

[00168] In some embodiments, the RAFT copolymers provided herein can be described by the formula:

$$CTACap\text{-}[(B1M1)_{m\text{-}B1M2\text{-}B1M3}\text{-}(B1M2)_{\text{-}m\text{-}B1M1\text{-}B1M3}\text{-}(B1M3)_{\text{-}m\text{-}B1M1\text{-}B1M2}]_m\text{-}[(B2M1)_{n\text{-}B2M2\text{-}B2M3}\text{-}(B2M2)_{\text{-}n\text{-}B2M1\text{-}B2M3}\text{-}(B2M3)_{\text{-}n\text{-}B2M1\text{-}B2M2}]_n\text{-}CTACap$$

[00169] For example, a RAFT copolymer as described herein having a single monomer in Block 1 of 25 units and 3 different monomers in Block 2 having an average monomer unit ratio of 20:10:5 for a total n of 35, can be described by the general formula

$$CTACap\text{-}[B1M1]_{25}\text{-}[(B2M1)_{20}\text{-}(B2M2)_{10}\text{-}(B2M3)_5]\text{-}CTACap$$

[00170] It will be further appreciated that the polymers prepared using a RAFT polymerization are random polymers having a distribution of units and hence molecular weights. Therefore, the cartoon representation of Block 2 in the example above is a random copolymer comprising 35 monomer units of B2M1, B2M2, and B2M3 in the ratio described above.

[00171] In another illustrative embodiment, the polymer nanoparticle composition can be coated with one or more polymers to protect the compositions from immune responses or to enhance endosomal escape. In one embodiment, the one or more polymers comprise polyethylene glycol. In another embodiment, the one or more polymers comprise polyethylene glycol poly-L-lysine. In yet another embodiment, the one or more polymers comprise polyethylenimine. In an additional embodiment, the one or more polymers comprise polyethylene glycol poly-L-lysine and polyethylenimine.

[00172] It will be appreciated that tuning the parameters and properties of the RAFT copolymers described herein can be advantageous to their use in the compositions and methods as described herein. A ccordingly, the methods for preparing RAFT copolymers either in singleton or in library format as described herein are capable of providing particular parameters and properties of the RAFT copolymers.

[00173] In some embodiments, a RAFT block polymer as described herein has one or more of an overall molecular weight (M_n) (i.e. the total of all blocks) in the range of about 1 kDa to about 1000 kDa, or about 2 kDa to about 500 kDa, or about 2 kDa to about 160 kDa, and overall degree of polymerization in the range of about 10 to about 3500, or about 20 to

about 2500, or about 30 to about 900, a size in the range of about of about 10 nm to about 10000 nm, and a maximum corona-to-core ratio (CCR) of about 1 to about 4. In some embodiments, the overall molecular weight (M_n) in the range of about 30 kDa to about 120 kDa, about 40 kDa to about 110 kDa about 50 kDa to about 100 kDa, about 60 kDa to about 90 kDa, about 40 kDa to about 80 kDa, and about 40 kDa to about 60 kDa. In some embodiments, the overall degree of polymerization in the range of about 40 to about 850, about 60 to about 800, about 100 to about 700, about 200 to about 600, or about 300 to about 500. In some embodiments, the size is in the range of about of about 10 nm to about 10000 nm, or about 20 nm to about 5000 nm, or about 50 nm to about 3000 nm, or about 20 nm to about 1000 nm, or about 50 nm to about 1000 nm, or about 30 nm to about 500 nm, or about 200 nm to about 2000 nm, or about 100 nm to about 5000 nm, or about 100 nm to about 500 nm, or about 10 nm to about 50 nm, about 15 nm to about 45 nm, about 20 nm to about 40 nm, or about 25 nm to about 35 nm. In some embodiments, the maximum corona-to-core ratio (CCR) is less than 4, or less than 3, about 1 to about 3.8, about 1.2 to about 3.5, about 1.5 to about 3, about 1.5 to about 2.5, or about 1 to about 2.

[00174] In some embodiments, a first block can be prepared from one or more monomer units and have a molecular weight (M_n) in the range of about 1 kDa to about 500 kDa, or about 10 kDa to about 200 kDa, or about 10 kDa to about 160 kDa, or about 1 kDa to about 80 kDa, and a degree of polymerization in the range of about 10 to about 3500, or about 10 to about 2500, or about 20 to about 2000, or about 50 to about 1200, or about 50 to about 1000. In some embodiments, a first block molecular weight (M_n) can be in the range of about 1 kDa to about 500 kDa, or about 2 kDa to about 400 kDa, or about 5 kDa to about 200 kDa, or about 10 kDa to about 160 kDa, or about 15 kDa to about 100 kDa, or about 25 kDa to about 60 kDa, or about 30 kDa to about 55 kDa, about 30 kDa to about 50 kDa, or about 30 kDa to about 40 kDa, and the like. In some embodiments, the first block degree of polymerization is in the range of about 30 to about 350, about 50 to about 300, about 70 to about 250, about 80 to about 240, about 100 to about 200, and the like.

[00175] In some embodiments, the second block can be prepared from one or more monomer units, and can have a molecular weight (M_n)in the range of about 1 kDa to about 500 kDa, or about 10 kDa to about 200 kDa, or about 10 kDa to about 160 kDa, or about 1 kDa to about 80 kDa, and a degree of polymerization in the range of about 10 to about 3500, or about

10 to about 2500, or about 20 to about 2000, or about 50 to about 1200, or about 50 to about 1000. In some embodiments, the second block molecular weight (M_n) is in the range of about 10 kDa to about 70 kDa, about 15 kDa to about 65 kDa, about 20 kDa to about 60 kDa, about 25 kDa to about 55 kDa, about 30 kDa to about 50 kDa, about 35 kDa to about 45 kDa, about 5 kDa to about 15 kDa, and the like. In some embodiments, the second block degree of polymerization is in the range of about 3 to about 2500; or about 20 to about 2000, or about 30 to about 1500, or about 40 to about 1200, or about 10 to about 500, or about 12 to about 450, or about 20 to about 400, or about 25 to about 350, or about 50 to about 300, or about 100 to about 250, or about 150 to about 200, or about 5 to about 50, or about 5 to about 20, and the like.

[00176] In some embodiments, a third, fourth, or subsequent block can be prepared from one or more monomer units, and each can have a molecular weight (M_n) in the range of about 1 kDa to about 500 kDa, or about 10 kDa to about 200 kDa, or about 10 kDa to about 160 kDa, or about 1 kDa to about 80 kDa, and a degree of polymerization in the range of about 10 to about 3500, or about 10 to about 2500, or about 20 to about 2000, or about 50 to about 1200, or about 50 to about 1000. In some embodiments, the third, fourth, or subsequent block molecular weight (M_n) is in the range of about 10 kDa to about 70 kDa, about 15 kDa to about 65 kDa, about 20 kDa to about 60 kDa, about 25 kDa to about 55 kDa, about 30 kDa to about 50 kDa, about 35 kDa to about 45 kDa, about 5 kDa to about 15 kDa, and the like. In some embodiments, the third, fourth, or subsequent block degree of polymerization is in the range of about 3 to about 2500; or about 20 to about 2000, or about 30 to about 1500, or about 40 to about 1200, or about 10 to about 500, or about 12 to about 450, or about 20 to about 400, or about 25 to about 350, or about 50 to about 300, or about 100 to about 250, or about 150 to about 200, or about 5 to about 50, or about 5 to about 20, and the like.

[00177] In some embodiments, a single chain transfer agent can be used in the RAFT polymerization process in connection with the present disclosure. In some embodiments, for a block polymer having more than one block, one or more single chain transfer agents can be used in the RAFT polymerization process in connection with the present disclosure. In some embodiments, for a block polymer having two blocks, a first chain transfer agent and a second chain transfer agent (which can be the same or different) can be used at each step of the RAFT polymerization process in connection with the present disclosure. In some embodiments, for a

block polymer having three blocks, a first chain transfer agent, a second chain transfer agent, and a third chain transfer agent (which can be the same or different) can be used at each step of the RAFT polymerization process in connection with the present disclosure.

[00178] It will be appreciated that a variety of solvents can be used in the RAFT polymerization method steps and purification steps described herein. Suitable solvents include, but are not limited to, 2-Chloroethanol, Acetic Acid (Glacial), Acetone, Acetonitrile, Acetophenone, Aniline, Benzaldehyde, Benzyl Acetate, Carbon disulfide, Cyclohexane, Cyclohexanol, Di(ethylene glycol), Di(propylene glycol), Diacetone alcohol, Diethyl ether, Dimethylsulfoxide, Ethanol, Ethyl acetate, Ethylene glycol, Formaldehyde (37% solution), Formamide, Formic acid, Formic acid (96%), HexaneIsobutanol, Isopropanol, Isopropyl acetate, Isopropyl ether, m-Cresol, Methanol, Methyl acetate, Methyl ethyl ketone, Mineral Oil, N,N-Dimethylformamide, n-Butanol, n-Octane, n-Propanol, Propylene glycol, Pyridine, t-Butanol, Tetrahydrofuran, Trifluoroacetic acid, water, and the like, and combinations thereof.

[00179] In some embodiments, the one or more nanoparticle forming polymers are RAFT block copolymers comprising

[00180] a. a first terminus comprising a first capping unit derived from a first chain transfer agent in a RAFT copolymerization process;

[00181] b. a first block prepared from one or more monomer units covalently attached to the first reactive functional unit, and having a molecular weight (M_n) in the range of about 1 kDa to about 500 kDa and a degree of polymerization in the range of about 10 to about 2500;

[00182] c. optionally a second block prepared from one or more monomer units covalently attached to the first block, and having a molecular weight (M_n)in the range of about 1 kDa to about 500 kDa and a degree of polymerization in the range of about 10 to about 2500;

[00183] d. optionally a third block prepared from one or more monomer units covalently attached to the first and/or second blocks, and having a molecular weight (M_n)in the range of about 1 kDa to about 500 kDa and a degree of polymerization in the range of about 10 to about 2500;

[00184] e. optionally a fourth block prepared from one or more monomer units covalently attached to the first, second, and/or third blocks, and having a molecular weight

(M_n)in the range of about 1 kDa to about 500 kDa and a degree of polymerization in the range of about 10 to about 2500;

[00185] f. optionally a fifth block prepared from one or more monomer units covalently attached to the first, second, third, and/or fourth blocks, and having a molecular weight (M_n)in the range of about 1 kDa to about 500 kDa and a degree of polymerization in the range of about 10 to about 2500; and

[00186] g. a second terminus comprising a second capping unit derived from a first, a second, a third, or a fourth chain transfer agent.

[00187] Illustrative payloads for the polymer nanoparticle described herein can include any one or a combination of compositions selected from the group comprising: nucleic acids (e.g., DNA or RNA), pDNA, oligodeoxyribonucleic acids (ODNs), dsDNA, ssDNA, antisense oligonucleotides, antisense RNA, siRNA, messenger RNA, guide RNA (e.g., small guide RNA), ribonucleoproteins, donor DNA strands used in the CRISPR/Cas9 system, and enzymes, such as CRISPR-associated enzymes, e.g., Cas9, enzymes used in other gene editing systems, such as ZFNs, custom designed homing endonucleases, TALENS systems, other gene editing endonucleases, and reverse transcriptase.

[00188] In another aspect, the present disclosure rapidly identifies top candidates using a machine learning model. In the illustrative embodiment, a graph neural network (GNN) is used for this process. Polymers can be characterized at three scales: monomer, block, and full polymer. Monomers combine to form blocks, and blocks combine to form full polymers. Polymer properties are dependent on characteristics of the polymer at all three scales. The relationships between monomers, blocks, and polymers can be captured with a directed graph. Information can then be shared between nodes in the graph to create a numerical representation of the full polymer at all three scales: monomer, block, and polymer. These numerical representations can then be used in a neural network to prediction properties of the polymer. The use of a GNN for polymer property prediction in the illustrative embodiment provides two primary benefits: first, the graph can model polymer characteristics at all three scales which is important for accurate prediction; second, the graph provides a flexible modeling structure that can accommodate several polymer structures.

[00189] The machine learning model is first trained on a combination of public data and preliminary testing data, supplemented with the large data sets described above. The

illustrative embodiment involves a three-loop deep learning cycle to accelerate high-throughput characterization and screening for PNPs. The three deep learning loops characterize the PNP physical properties, *in vitro* bioactivity, and *in vivo* bioactivity, respectively. Each loop utilizes a GNN deep learning model (*see* Figure 3a) to characterize the candidate PNPs. The GNN takes the simplified molecular-input line-entry system (SMILES) strings defining the monomers as an input (the nodes of the graph), and the edges of the graph define the relationship between the monomers and how they combine to form the PNP. The edges of the graph also allow additional information about the polymer (e.g., ratios of monomers and degree of polymerization) to be incorporated into the GNN.

[00190] The presently disclosed architecture offers at least three distinct advantages. First, the deep learning model is not dependent on polymer fingerprinting. Rather, the deep learning model will learn an appropriate numerical embedding from the SMILES strings. Second, the graph allows the model the flexibility to represent various families of PNPs with ease. Third, using SMILES strings as inputs allows the limited training dataset to be augmented with enumerated SMILES strings, increasing the amount of training data available and improving the model performance. Testing of this GNN architecture has shown impressive ability to predict zeta potential (*see* Figure 3b), a critical characteristic for non-viral gene delivery vehicles. Once trained, these deep learning models will be used to prioritize the synthesis and characterization of candidate PNPs in the high-throughput system to meet the requirements of a bioactivity. Iterative data can be used to fine-tune the models in an active learning cycle to improve future performance.

[00191] In some embodiments, data augmentation may be performed to artificially increase the size and variety of the data used to train the machine learning model (and, consequently, increase model performance). Deep learning models require relatively large datasets for training and can over-fit to small datasets. As discussed above, the GNN takes the SMILES strings defining the monomers as an input. A monomer (a building block of a polymer) has a single canonical SMILES string, but it also has multiple alternative SMILES string representations. SMILES enumeration can be performed to generate these alternative forms from the canonical SMILES string and, thus, increase the size of the training data set many times over. The neural network model is then able to leverage this increase in data size and variety of representations to improve performance.

[00192] In other embodiments, a modified Transformer model (rather than a GNN) may be used to predict polymer properties (and, thus, rapidly identify top candidates for non-viral carriers for delivering base editing proteins, among other applications). The modified Transformer model exploits relative positional information of inputs to create numerical embeddings for monomer string inputs. These numerical embeddings can then be used in deep learning and statistical models for polymer property prediction. Additionally, the Transformer model is more computationally efficient compared to many other deep learning architectures that can process sequential data. The original Transformer architecture consists of an encoding and decoding architecture. The encoder takes an input sequence of data and outputs a high dimensional embedding, while the decoder takes the high dimensional embedding as an input and tries to predict the original or similar sequence to the one input into the encoder. The present disclosure does not need to predict a sequential output, so it only uses the encoding portion of the Transformer to predict polymer properties, both physical and *in-vitro/in-vivo*.

[00193] In yet another aspect, an illustrative embodiment of the present disclosure allows for the selection the top candidates for PNP-mediated delivery of the SOD1-targeting CBE in a mouse model of ALS. Functional gene editing tests in a microglial cell line stably expressing EGFP or SOD1 can then be performed using these top candidates. Moreover, the efficacy and safety PNP-mediated CBE delivery can be assessed in the G93A-SOD1 mouse model of ALS. Prior success of CBE base editors for slowing ALS progression in mice shows a likelihood that they can also lead to clinical translation of a novel ALS gene editing therapy.

[00194] While certain illustrative embodiments have been described in detail in the drawings and the foregoing description, such an illustration and description is to be considered as exemplary and not restrictive in character, it being understood that only illustrative embodiments have been shown and described and that all changes and modifications that come within the spirit of the disclosure are desired to be protected. There exist a plurality of advantages of the present disclosure arising from the various features of the apparatus, systems, and methods described herein. It will be noted that alternative embodiments of the apparatus, systems, and methods of the present disclosure may not include all of the features described, yet still benefit from at least some of the advantages of such features. Those of

ordinary skill in the art may readily devise their own implementations of the apparatus, systems, and methods that incorporate one or more of the features of the present disclosure.

EXAMPLES

[00195] EXAMPLE 1

[00196] Barcode design

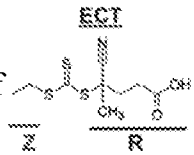
[00197] The nucleic acid constructs used in this example comprised a unique portion comprising 8-10 nucleotides in the center of the polynucleotide, the unique portion further constrained by the requirement of a hamming distance of at least 3 bases from any other barcodes to be pooled. Directly on the 3' end of the barcode, 7-10 random bases are included for bioinformatic removal of PCR duplicates. This central sequence is flanked by universal primer annealing sites containing overhangs for the addition of index adapters during sequencing library preparation. Fig. 4 shows a representative illustration of these barcodes. These nucleic acid constructs were designed with either a biotin functional group or an amine functional group on the 5' end.

[00198] EXAMPLE 2

[00199] Polymer Nanoparticle Synthesis

[00200] A diblock copolymer was synthesized as described in PCTapp349529(21477779.1) using reversible addition-fragmentation chain transfer (RAFT) polymerization with reagents and amounts listed in Table 2. Block 1 reagents were combined in a round bottom flask, purged with argon, and heated to 60 oC for 6 hours using a heating mantle. The reaction product was purified using four 80:20 pentane:ether precipitation washes and centrifugation cycles and dried in vacuo. The Block 1 product was used as the macroRAFT agent for Block 2, and the calculated reagent volumes (as calculated based on theoretical molecular weight information for Block 1) were combined in a round bottom for the Block 2 reaction. The reaction mixture was argon purged before being heated at 60 oC for 24 hours. The reaction product was purified using the same purification process and dried in vacuo. The resulting polymer was dialyzed in deionized water for 4 days with multiple water changes each day. Finally, the dialyzed material was lyophilized for 4 days and stored at room temperature for experimental use.

Table 2. Reagents and amounts used to synthesize barcoded PNPs

Block 1			
Reagent	Purpose	Lot 0001 Amount	Lot 0002 Amount
2-dimethylaminoethyl acrylate (DMAEMA)	Monomer	15999.6 mg	32000.0 mg
(4-cyano-4-(((ethylthio)carbonothioyl)thio)pentanoicacid) (ECT)	Chain transfer agent	76.9 mg	153.7 mg
Azobisisobutyronitrile (AIBN)	Initiator	9.5 mg	19.05 mg
Dimethylformamide (DMF)	Solvent	24131.1 mg	48229.8 mg
Block 1 Reaction Yield	% Yield	31.75%	39.55%
Block 2			
Reagent	Purpose	Lot 0001 Amount	Lot 0002 Amount
2-dimethylaminoethyl acrylate (DMAEMA)	Monomer	713.0 mg	2110.6 mg
butyl methacrylate (BMA)	Monomer	1934.4 mg	5727.6 mg
propyl acrylic acid (PAA)	Monomer	518.2 mg	1540.1 mg
Block 1 macroRAFT agent, meaning ECT+DMAEMA. The ECT end groups (R & Z) were still present to perform their function, but they were on the end of the p(DMAEMA) polymer synthesized as block 1. For reference, here is ECT  R & Z groups on either side of onyl group.	Macro Chain transfer agent	1528.4 mg	4526.2 mg

Azobisisobutyronitrile (AIBN)	Initiator	1.7 mg	4.93 mg
Dimethylformamide (DMF)	Solvent	7045.0 mg	20828.8 mg
Block 2 Reaction Yield	% Yield	73.33%	70.99%

[00201] EXAMPLE 3:

[00202] Composition Example 1 (CE1): Electrostatic attachment of nucleic acid constructs (containing DNA barcodes) to polymer nanoparticles

[00203] RAFT copolymers were synthesized according to the methods above and the reagents listed in Table 2. The polymer was dispersed in phosphate buffered saline at a concentration of ~5 mg/ml. For electrostatic loading (Fig. 5a), nucleic acid constructs (according to the design shown in Fig. 4 including polynucleotide barcodes) were dissolved in tris EDTA buffer at a concentration of ~ 100 μM (~1.9 mg/mL). These stock solutions were mixed together with PBS to produce a solution with a final concentration of 0.05 mg/mL polymer and 0.00389 mg/mL nucleic acid construct. They were incubated at room temperature for at least 30 minutes to allow the positively charged polymer to associate with the negatively charged nucleic acid constructs. The sample was transferred to an amicon ultra-4 centrifuge tube (MWCO 30 kDa). (Max 3.5 mL/Tube) and centrifuged at 4,000 x g for 15 minutes to remove any unbound nucleic acid constructs. The filtrate (containing unbound nucleic acid constructs) was discarded and sterile PBS was added to the retentate to final concentration of 0.05 mg/mL polymer and 0.00389 mg/mL nucleic acid constructs. The electrostatically bound nucleic acid constructs were amplified via PCR, using primers designed to bind to the universal primer binding segments on the nucleic acid constructs. The amplicons were detected via gel electrophoresis on agarose gel. The presence of a double band (Fig. 6) in a lane indicates the presence of amplicons, showing that nucleic acid constructs were bound to the PNPs.

[00204] EXAMPLE 4:

[00205] Composition Example 2 (CE2): Nucleic acid construct conjugation to PNPs via avidin-biotin linkage

[00206] RAFT copolymers made using CTAs that contain at least one carboxyl terminal group were further functionalized with avidin (Fig. 5b). A RAFT copolymer was transferred

into a MES buffer at ~12 mg/mL. The sample was sonicated for 30 minutes. EDC reagent and Sulfo-NHS reagent was added to the polymer at a molar ratio of 10:1 and 25:1 respectively, reagent to PNP. The sample was incubated for at least 10 minutes at room temperature to allow the reaction to occur. The reaction volume was filtered through a membrane with a molecular weight cut off of 30 kDa via centrifuge at ~3000 x g for ~15 minutes. The filtrate was discarded, and sterile PBS was added to the retentate to reconstitute to 10 mg/mL polymer. Avidin (36.9 mg) was added to the reaction and incubated for 15 minutes at room temperature. The sample was transferred to an amicon ultra-4 centrifuge tube (MWCO 30 kDa). (Max 3.5 mL/Tube) and centrifuged at 4,000 x g for 15 minutes. The filtrate was discarded and sterile PBS was added to the retentate to final concentration of 8 mg/mL polymer. A nucleic acid constructs with biotin attached to the 5' end was added to the avidin functionalized polymer at a molar ratio of 10 moles of polymer to 1 mole of nucleic acid constructs. The sample was incubated for at least 15 minutes. The sample was transferred to an amicon ultra-4 centrifuge tube (MWCO 30 kDa, Max 3.5 mL/Tube) and centrifuged at 4,000 x g for 15 minutes to remove any unbound nucleic acid constructs. The filtrate (containing unbound nucleic acid constructs) was discarded and sterile PBS was added to the retentate to final concentration of 8 mg/mL polymer. The conjugated nucleic acid constructs were amplified via PCR, using primers designed to bind to the universal primer binding segments on the nucleic acid constructs. The amplicons were detected via gel electrophoresis on agarose gel. The presence of a double band (Figs. 6 and 7) in a lane indicates the presence of amplicons, showing that nucleic acid constructs were bound to the PNPs.

[00207] To test the range of PNP to barcode ratios that can be used in the reduction to practice of nucleic acid constructs labeled PNPs, the method above was used to attach the nucleic acid constructs to PNPs using the avidin-biotin linkage (Fig. 5a), varying the PNP to nucleic acid constructs ratio from as low as 20:1 to as high as 10,000 to 1 (moles polymer to moles nucleic acid constructs). Fig. 13 shows a gel electrophoresis graph with bands corresponding to the amplicons from nucleic acid constructs produced from a PCR reaction on the nucleic acid constructs with these various PNP to nucleic acid constructs ratios, indicating that these ratios are in the useable range for the reduction to practice of the nucleic acid constructs PNP composition.

[00208] The avidin-biotin conjugation method was used to attach 96 unique barcodes to 96 aliquots of the polymer described in Table 2, yielding 96 aliquots of the same polymer in which the population of nanoparticles in each aliquot has a unique barcode attached. These 96 aliquots were pooled by combining the aliquots in volumetrically equivalent amounts into a single vial, yielding a dispersion of 96 distinct populations of barcoded PNPs, in which all populations comprised a polymer micelle formed from the polymer described in Table 2 and a unique barcode from the population of 96 unique barcodes.

[00209] The pooled sample of avidin-biotin conjugated nucleic acid construct-PNPs were spiked into HEK-293T cells. The cells were seeded in 96 well plates at 20,000 cells per well, in 100 μL of media and left to adhere overnight. Twenty-four hours after seeding, the pooled sample of PNPs with 96 unique barcodes were added at a dose of ~0.024 mg/mL PNP in each well and placed in an incubator at 37°C overnight. The next day, barcodes extracted from the samples, using the QIAamp 96 DNA extraction kit and a Qiacube HT instrument according to the manufacturer's protocol. The conjugated barcodes were amplified via PCR, using primers designed to bind to the universal primer binding sequences on the barcodes. The amplicons were detected via gel electrophoresis on agarose gel. The presence of a double band (Fig. 8) in a lane indicates the presence of amplicons, showing that nucleic acid constructs were bound to the PNPs.

[00210] The pooled sample of PNPs with 96 unique barcodes conjugated via avidin-biotin linkage, was administered to mice (using the in vivo screening protocol described below). Twenty-four hours after dosing, the mice were sacrificed and the tissues were analyzed for the presence of the barcoded PNPs. PCR was used to amplify the barcodes from the tissue samples and agilent fragment analysis was used to detect the presence of nucleic acid constructs-PNPs, with a dark band matching the positive control as the indicator of the presence of nucleic acid constructs-PNPs (Fig. 12a). This experiment reduced to practice the ability to label PNPs with unique nucleic acid constructs, administer them to mice, and then detect their biodistribution via PCR. We further used deep sequencing techniques to distinguish all 96 unique barcodes in the liver tissue. Library preparations, sequencing and sequence analysis were performed as described below. All 96 unique barcodes were detected in a way that was countable and distinguishable from the others (Fig. 12c and Fig. 12d). This shows that our compositions allow not only the detection of uniquely barcoded PNPs in mouse tissue 24 hours after dosing, but it

also allows us to quantitatively distinguish each uniquely barcoded PNP from all other uniquely barcoded PNPs in the animal tissue.

[00211] EXAMPLE 5:

[00212] Preparation of RAFT Copolymers for Polymer Nanoparticle Libraries

[00213] Briefly, Block 1 reagents (monomer(s), chain transfer agent, initiator, and solvent) were combined in wells of a polypropylene 96-well u-shaped bottom microplate (Greiner Bio-One), in polypropylene 96 well cluster tubes (Corning), in polypropylene Eppendorf microcentrifuge tubes (Sigma-Aldrich), or in polypropylene 50 mL or 15 mL conical tubes (VWR) and placed in a VWR 1400E Sheldon vacuum oven. A 20 mL glass vial was filled with approximately 10-15 mL of solvent (e.g., dimethylformamide), and the vial was placed in the oven to provide a source for atmosphere saturation. The oven was purged with argon at ~3 L/min for approximately 45 minutes and heated to between 60 °C and 75 °C for 6-24 hours. Upon completion of the reaction, acetone was added to the wells or tubes to prevent polymer solidification and the wells or tubes were sealed and left at room temperature overnight. The next day, the reaction product solutions were transferred to 1.5 mL Eppendorf tubes (if necessary) and purified via at least three precipitation washes using an appropriate purification solvent solution (e.g., 80:20 pentane:ether, isopropyl alcohol, methanol, etc.) and centrifugation cycles and dried *in vacuo*. The Block 1 product was used as the macroRAFT agent for Block 2, and the calculated reagent volumes (as calculated based on theoretical or actual molecular weight information for Block 1) were combined in a polypropylene 96-well u-shaped bottom microplate (Greiner Bio-One), in polypropylene 96 well cluster tubes (Corning), in polypropylene Eppendorf microcentrifuge tubes (Sigma-Aldrich), or in polypropylene 50 mL or 15 mL conical tubes (VWR) for the Block 2 reaction. The reaction mixtures were placed in a VWR 1400E Sheldon vacuum oven, which was argon purged at ~3 L/min for approximately 45 minutes before being heated to between 60 °C and 75 °C for 6-24 hours. The reaction products were purified using the same purification process as used for Block 1 library materials and dried *in vacuo*. The resulting polymers were resuspended in either acetone or chloroform and aliquoted as needed for experimental use (these transfer solvents evaporated prior to material use), stored in a dry state at room temperature, or dissolved in deionized water, frozen, and lyophilized prior to experimental use. Size was measured using a Wyatt Technology DynaPro Plate Reader III. Molecular weights for Block 1 materials were measured using a

DynaPro Plate Reader III. Nanoparticle sizes above the DynaPro Plate Reader III molar mass capability threshold prevented measurement of Block 2 molecular weights for these polymer libraries. All molecular weights for high-throughput polymer libraries are reported as weight average molecular weight (M_w).

[00214] A summary of the reagents, amounts, and reaction conditions used to synthesize Block 1 and Block 2 of a pilot PNP library of 96 PNPs are shown in Tables 3 and 4, respectively, below. PNPs 22, 61, and 89-96 were used as 10 unique PNPs for HEK cell studies. PNPs 1-88 were used as unique PNPs for flow cytometry studies. Table 3 Abbreviations: ACVA, 4,4′-Azobis(4-cyanovaleric acid); AIBN, Azobisisobutyronitrile; BMA, butyl methacrylate; CTP, 4-Cyano-4-(thiobenzoylthio)pentanoic acid; DMAEMA, dimethylaminoethyl methacrylate; DMF, N,N-Dimethylformamide; ECT, 4-Cyano-4-[(ethylsulfanylthiocarbonyl)sulfanyl]pentanoic acid; MMA, methyl methacrylate. Table 4 Abbreviations: ACVA, 4,4′-Azobis(4-cyanovaleric acid); AIBN, Azobisisobutyronitrile; BMA, butyl methacrylate; CTP, 4-Cyano-4-(thiobenzoylthio)pentanoic acid; DMAEMA, dimethylaminoethyl methacrylate; DMF, N,N-Dimethylformamide; ECT, 4-Cyano-4-[(ethylsulfanylthiocarbonyl)sulfanyl]pentanoic acid; HEMA, 2-Hydroxyethyl methacrylate; MMA, methyl methacrylate. Table 5 Abbreviations: PDI, polydispersity index.

[00215] Static Light Scattering (SLS) and Dynamic Light Scattering (DLS) measurements to determine Block 1 molar mass and PNP size (e.g., diameter) were determined using a DynaPro Plate Reader III by Wyatt Technology. Data acquisition and handling were made with DYNAMICS software. SLS and DLS data were obtained under the following conditions:

[00216] SOLVENT: Water

[00217] TEMPERATURE: 25 °C

[00218] SAMPLE VOLUME: 200 μL

[00219] DATA ACQUISITION SETTINGS: 5 acquisitions of 5 seconds per acquisition

Table 3. Summary of Block 1 Reagents and Reaction Conditions Used in Pilot PNP Library

PNP	Block 1 Reagents, Purpose, Amounts, and Reaction Conditions

	Monomer and Amount (mol)		Chain Transfer Agent (CTA) and Amount (mol)		Initiator and Amount (mol)		Solvent Amount (mol)		Time (min)	Temp (C)	Block 1 Rxn % Yield
1	DMAEMA	2.49E-04	CTP	2.49E-06	ACVA	2.49E-06	DMF	2.04E-03	363	75	56.4
2	DMAEMA	2.49E-04	CTP	1.09E-06	ACVA	1.09E-06	DMF	2.04E-03	363	75	62.1
3	DMAEMA	2.49E-04	CTP	8.54E-07	ACVA	8.55E-07	DMF	2.04E-03	363	75	59.2
4	DMAEMA	2.49E-04	CTP	7.00E-07	ACVA	7.01E-07	DMF	2.04E-03	363	75	57.9
5	DMAEMA	2.49E-04	CTP	5.18E-07	ACVA	5.16E-07	DMF	2.04E-03	363	75	66.7
6	DMAEMA	2.49E-04	CTP	4.61E-07	ACVA	4.57E-07	DMF	2.04E-03	363	75	66.6
7	DMAEMA	2.49E-04	CTP	4.13E-07	ACVA	4.08E-07	DMF	2.04E-03	363	75	61.5
8	DMAEMA	2.49E-04	CTP	3.65E-07	ACVA	3.68E-07	DMF	2.04E-03	363	75	50.6
9	DMAEMA	2.49E-04	CTP	3.36E-07	ACVA	3.37E-07	DMF	2.04E-03	363	75	57.2
10	DMAEMA	2.49E-04	CTP	3.07E-07	ACVA	3.12E-07	DMF	2.04E-03	363	75	56.7
11	DMAEMA	2.49E-04	CTP	1.52E-06	ACVA	1.52E-06	DMF	2.04E-03	363	75	74.3
12	DMAEMA	2.49E-04	CTP	1.09E-06	ACVA	1.09E-06	DMF	2.04E-03	363	75	73.0
13	DMAEMA	2.49E-04	CTP	8.54E-07	ACVA	8.55E-07	DMF	2.04E-03	363	75	68.1
14	DMAEMA	2.49E-04	CTP	7.00E-07	ACVA	7.01E-07	DMF	2.04E-03	363	75	64.6
15	DMAEMA	2.49E-04	CTP	5.18E-07	ACVA	5.16E-07	DMF	2.04E-03	363	75	69.5
16	DMAEMA	2.49E-04	CTP	4.61E-07	ACVA	4.57E-07	DMF	2.04E-03	363	75	57.0
17	DMAEMA	2.49E-04	CTP	4.13E-07	ACVA	4.08E-07	DMF	2.04E-03	363	75	53.4
18	DMAEMA	2.49E-04	CTP	3.65E-07	ACVA	3.68E-07	DMF	2.04E-03	363	75	66.4
19	DMAEMA	2.49E-04	CTP	3.36E-07	ACVA	3.37E-07	DMF	2.04E-03	363	75	55.0
20	DMAEMA	2.49E-04	CTP	2.49E-06	ACVA	2.49E-06	DMF	2.04E-03	363	75	66.6
21	DMAEMA	2.49E-04	CTP	1.52E-06	ACVA	1.52E-06	DMF	2.04E-03	363	75	75.4
22	DMAEMA	2.49E-04	CTP	1.09E-06	ACVA	1.09E-06	DMF	2.04E-03	363	75	73.6

23	DMAEMA	2.49E-04	CTP	8.54E-07	ACVA	8.55E-07	DMF	2.04E-03	363	75	65.1
24	DMAEMA	2.49E-04	CTP	7.00E-07	ACVA	7.01E-07	DMF	2.04E-03	363	75	71.4
25	DMAEMA	2.49E-04	CTP	5.95E-07	ACVA	5.94E-07	DMF	2.04E-03	363	75	60.7
26	DMAEMA	2.49E-04	CTP	5.18E-07	ACVA	5.16E-07	DMF	2.04E-03	363	75	66.6
27	DMAEMA	2.49E-04	CTP	4.61E-07	ACVA	4.57E-07	DMF	2.04E-03	363	75	55.2
28	DMAEMA	2.49E-04	CTP	4.13E-07	ACVA	4.08E-07	DMF	2.04E-03	363	75	55.7
29	DMAEMA	2.49E-04	CTP	3.65E-07	ACVA	3.68E-07	DMF	2.04E-03	363	75	51.4
30	DMAEMA	2.49E-04	CTP	3.36E-07	ACVA	3.37E-07	DMF	2.04E-03	363	75	53.3
31	DMAEMA	2.49E-04	CTP	3.07E-07	ACVA	3.12E-07	DMF	2.04E-03	363	75	47.2
32	DMAEMA	2.49E-04	CTP	1.52E-06	ACVA	1.52E-06	DMF	2.04E-03	363	75	88.7
33	DMAEMA	2.49E-04	CTP	1.09E-06	ACVA	1.09E-06	DMF	2.04E-03	363	75	72.0
34	DMAEMA	2.49E-04	CTP	8.54E-07	ACVA	8.55E-07	DMF	2.04E-03	363	75	65.0
35	DMAEMA	2.49E-04	CTP	5.95E-07	ACVA	5.94E-07	DMF	2.04E-03	363	75	69.4
36	DMAEMA	2.49E-04	CTP	4.61E-07	ACVA	4.57E-07	DMF	2.04E-03	363	75	46.1
37	DMAEMA	2.49E-04	CTP	4.13E-07	ACVA	4.08E-07	DMF	2.04E-03	363	75	47.5
38	DMAEMA	2.49E-04	CTP	3.36E-07	ACVA	3.37E-07	DMF	2.04E-03	363	75	52.3
39	DMAEMA	2.49E-04	CTP	3.07E-07	ACVA	3.12E-07	DMF	2.04E-03	363	75	55.9
40	DMAEMA	2.49E-04	CTP	2.49E-06	ACVA	2.49E-06	DMF	2.04E-03	363	75	82.3
41	DMAEMA	2.49E-04	CTP	8.54E-07	ACVA	8.55E-07	DMF	2.04E-03	363	75	76.5
42	DMAEMA	2.49E-04	CTP	7.00E-07	ACVA	7.01E-07	DMF	2.04E-03	363	75	71.7
43	DMAEMA	2.49E-04	CTP	4.13E-07	ACVA	4.08E-07	DMF	2.04E-03	363	75	54.9
44	DMAEMA	2.49E-04	CTP	3.65E-07	ACVA	3.68E-07	DMF	2.04E-03	363	75	54.3
45	DMAEMA	2.49E-04	CTP	3.36E-07	ACVA	3.37E-07	DMF	2.04E-03	363	75	54.6
46	DMAEMA	2.49E-04	CTP	3.07E-07	ACVA	3.12E-07	DMF	2.04E-03	363	75	55.0

47	DMAEMA	2.49E-04	CTP	1.09E-06	ACVA	1.09E-06	DMF	2.04E-03	363	75	93.5
48	DMAEMA	2.49E-04	CTP	8.54E-07	ACVA	8.55E-07	DMF	2.04E-03	363	75	79.7
49	DMAEMA	2.49E-04	CTP	5.18E-07	ACVA	5.16E-07	DMF	2.04E-03	363	75	56.9
50	DMAEMA	2.49E-04	CTP	4.61E-07	ACVA	4.57E-07	DMF	2.04E-03	363	75	52.0
51	DMAEMA	2.49E-04	CTP	4.13E-07	ACVA	4.08E-07	DMF	2.04E-03	363	75	55.8
52	DMAEMA	2.49E-04	CTP	3.36E-07	ACVA	3.37E-07	DMF	2.04E-03	363	75	51.8
53	DMAEMA	2.49E-04	CTP	3.07E-07	ACVA	3.12E-07	DMF	2.04E-03	363	75	53.1
54	DMAEMA	2.49E-04	CTP	1.52E-06	ACVA	1.52E-06	DMF	2.04E-03	363	75	86.5
55	DMAEMA	2.49E-04	CTP	8.54E-07	ACVA	8.55E-07	DMF	2.04E-03	363	75	72.9
56	DMAEMA	2.49E-04	CTP	7.00E-07	ACVA	7.01E-07	DMF	2.04E-03	363	75	71.5
57	DMAEMA	2.49E-04	CTP	5.95E-07	ACVA	5.94E-07	DMF	2.04E-03	363	75	59.9
58	DMAEMA	2.49E-04	CTP	5.18E-07	ACVA	5.16E-07	DMF	2.04E-03	363	75	63.7
59	DMAEMA	2.49E-04	CTP	4.13E-07	ACVA	4.08E-07	DMF	2.04E-03	363	75	52.0
60	DMAEMA	2.49E-04	CTP	3.65E-07	ACVA	3.68E-07	DMF	2.04E-03	363	75	54.9
61	DMAEMA	2.49E-04	CTP	3.36E-07	ACVA	3.37E-07	DMF	2.04E-03	363	75	55.3
62	DMAEMA	2.49E-04	CTP	3.07E-07	ACVA	3.12E-07	DMF	2.04E-03	363	75	42.2
63	DMAEMA	2.49E-04	CTP	1.52E-06	ACVA	1.52E-06	DMF	2.04E-03	363	75	82.9
64	DMAEMA	2.49E-04	CTP	1.09E-06	ACVA	1.09E-06	DMF	2.04E-03	363	75	71.2
65	DMAEMA	2.49E-04	CTP	5.95E-07	ACVA	5.94E-07	DMF	2.04E-03	363	75	60.4
66	DMAEMA	2.49E-04	CTP	5.18E-07	ACVA	5.16E-07	DMF	2.04E-03	363	75	64.9
67	DMAEMA	2.49E-04	CTP	3.65E-07	ACVA	3.68E-07	DMF	2.04E-03	363	75	54.0
68	DMAEMA	2.49E-04	CTP	3.36E-07	ACVA	3.37E-07	DMF	2.04E-03	363	75	44.2
69	DMAEMA	2.49E-04	CTP	3.07E-07	ACVA	3.12E-07	DMF	2.04E-03	363	75	52.4
70	DMAEMA	2.49E-04	CTP	2.49E-06	ACVA	4.97E-07	DMF	2.04E-03	363	75	52.5

71	DMAEMA	2.49 E-04	CTP	1.52E-06	ACVA	3.03 E-07	DMF	2.04 E-03	363	75	46.6
72	DMAEMA	2.49 E-04	CTP	1.09E-06	ACVA	2.19 E-07	DMF	2.04 E-03	363	75	40.2
73	DMAEMA	2.49 E-04	CTP	8.54E-07	ACVA	1.71 E-07	DMF	2.04 E-03	363	75	37.7
74	DMAEMA	2.49 E-04	CTP	5.18E-07	ACVA	1.03 E-07	DMF	2.04 E-03	363	75	27.3
75	DMAEMA	2.49 E-04	CTP	4.61E-07	ACVA	9.05 E-08	DMF	2.04 E-03	363	75	26.7
76	DMAEMA	2.49 E-04	CTP	4.13E-07	ACVA	8.21 E-08	DMF	2.04 E-03	363	75	25.1
77	DMAEMA	2.49 E-04	CTP	3.65E-07	ACVA	7.37 E-08	DMF	2.04 E-03	363	75	23.0
78	DMAEMA	2.49 E-04	CTP	3.36E-07	ACVA	6.74 E-08	DMF	2.04 E-03	363	75	22.8
79	DMAEMA	2.49 E-04	CTP	2.49E-06	ACVA	4.97 E-07	DMF	2.04 E-03	363	75	52.7
80	DMAEMA	2.49 E-04	CTP	1.52E-06	ACVA	3.03 E-07	DMF	2.04 E-03	363	75	47.6
81	DMAEMA	2.49 E-04	CTP	1.09E-06	ACVA	2.19 E-07	DMF	2.04 E-03	363	75	39.3
82	DMAEMA	2.49 E-04	CTP	8.54E-07	ACVA	1.71 E-07	DMF	2.04 E-03	363	75	37.1
83	DMAEMA	2.49 E-04	CTP	7.00E-07	ACVA	1.41 E-07	DMF	2.04 E-03	363	75	34.0
84	DMAEMA	2.49 E-04	CTP	5.95E-07	ACVA	1.18 E-07	DMF	2.04 E-03	363	75	31.8
85	DMAEMA	2.49 E-04	CTP	5.18E-07	ACVA	1.03 E-07	DMF	2.04 E-03	363	75	25.1
86	DMAEMA	2.49 E-04	CTP	4.61E-07	ACVA	9.05 E-08	DMF	2.04 E-03	363	75	25.7
87	DMAEMA	2.49 E-04	CTP	4.13E-07	ACVA	8.21 E-08	DMF	2.04 E-03	363	75	23.9
88	DMAEMA	2.49 E-04	CTP	7.00E-07	ACVA	1.41 E-07	DMF	2.04 E-03	363	75	27.7
89	DMAEMA	2.49 E-04	CTP	8.54E-07	ACVA	1.71 E-07	DMF	2.04 E-03	363	75	32.8
90	DMAEMA	2.49 E-04	CTP	3.65E-07	ACVA	7.37 E-08	DMF	2.04 E-03	363	75	21.0
91	DMAEMA	2.53 E-04	CTP	8.42E-07	ACVA	8.44 E-07	DMF	2.03 E-03	360	70	103.4
92	MMA	3.95 E-04	CTP	1.32E-06	AIBN	2.63 E-07	DMF	2.04 E-03	360	70	61.2
93	BMA	2.80 E-04	ECT	9.32E-07	AIBN	1.86 E-07	DMF	2.01 E-03	360	70	32.4
94	DMAEMA	2.53 E-04	ECT	8.43E-07	AIBN	8.44 E-07	DMF	2.03 E-03	360	65	101.0

95	MMA	3.96 E-04	ECT	1.32E-06	ACVA	1.32 E-07	DMF	2.04 E-03	360	65	13.7
96	BMA	2.80 E-04	CTP	9.31E-07	AIBN	9.32 E-07	DMF	2.01 E-03	360	65	32.1

Table 4. Summary of Block 2 Reagents and Reaction Conditions Used in Pilot PNP Library

PNP	Block 2 Reagents, Purpose, Amounts, and Reaction Conditions										
	Monomer and Amount (mol)		Macro Chain Transfer Agent (mCTA) and Amount (mol)		Initiator and Amount (mol)		Solvent and Amount (mol)		Time (min)	Temp (C)	Diblock Post-Lyophilization % Yield
1	HEMA	2.54 E-05	p(DMAEMA), (006-A1 Block 1)	1.27 E-06	ACVA	1.27 E-06	DMF	8.00 E-04	1440	64	65.3
2	HEMA	2.70 E-05	p(DMAEMA), (006-A3 Block 1)	1.33 E-06	ACVA	1.33 E-06	DMF	8.33 E-04	1440	64	85.7
3	HEMA	2.38 E-05	p(DMAEMA), (006-A4 Block 1)	1.19 E-06	ACVA	1.19 E-06	DMF	7.25 E-04	1440	64	77.4
4	HEMA	1.91 E-05	p(DMAEMA), (006-A5 Block 1)	9.60 E-07	ACVA	9.60 E-07	DMF	5.36 E-04	1440	64	56.6
5	HEMA	2.15 E-05	p(DMAEMA), (006-A7 Block 1)	1.08 E-06	ACVA	1.08 E-06	DMF	5.92 E-04	1440	64	83.8
6	HEMA	2.07 E-05	p(DMAEMA), (006-A8 Block 1)	1.03 E-06	ACVA	1.03 E-06	DMF	5.54 E-04	1440	64	87.4
7	HEMA	1.99 E-05	p(DMAEMA), (006-A9	9.78 E-07	ACVA	9.78 E-07	DMF	5.32 E-04	1440	64	85.5

			Block 1)								
8	HEMA	1.51 E-05	p(DMA EMA), (006-A10 Block 1)	7.57 E-07	ACVA	7.56 E-07	DMF	3.99 E-04	1440	64	95.6
9	HEMA	1.75 E-05	p(DMA EMA), (006-A11 Block 1)	8.71 E-07	ACVA	8.71 E-07	DMF	4.56 E-04	1440	64	87.4
10	HEMA	1.51 E-05	p(DMA EMA), (006-A12 Block 1)	7.48 E-07	ACVA	7.49 E-07	DMF	3.62 E-04	1440	64	90.2
11	HEMA	7.07 E-05	p(DMA EMA), (006-B2 Block 1)	1.55 E-06	ACVA	1.56 E-06	DMF	2.67 E-03	1440	64	73.3
12	HEMA	4.53 E-05	p(DMA EMA), (006-B3 Block 1)	9.94 E-07	ACVA	9.95 E-07	DMF	1.58 E-03	1440	64	71.6
13	HEMA	5.96 E-05	p(DMA EMA), (006-B4 Block 1)	1.31 E-06	ACVA	1.31 E-06	DMF	2.22 E-03	1440	64	70.3
14	HEMA	5.16 E-05	p(DMA EMA), (006-B5 Block 1)	1.12 E-06	ACVA	1.12 E-06	DMF	1.87 E-03	1440	64	87.8
15	HEMA	5.24 E-05	p(DMA EMA), (006-B7 Block 1)	1.15 E-06	ACVA	1.15 E-06	DMF	1.90 E-03	1440	64	85.3
16	HEMA	3.02 E-05	p(DMA EMA), (006-B8	6.62 E-07	ACVA	6.60 E-07	DMF	1.01 E-03	1440	64	86.3

			Block 1)								
17	HEMA	2.94 E-05	p(DMA EMA), (006-B9 Block 1)	6.35 E-07	ACVA	6.35 E-07	DMF	9.77 E-04	1440	64	90.9
18	HEMA	3.18 E-05	p(DMA EMA), (006-B10 Block 1)	6.93 E-07	ACVA	6.92 E-07	DMF	1.02 E-03	1440	64	84.3
19	HEMA	2.94 E-05	p(DMA EMA), (006-B11 Block 1)	6.50 E-07	ACVA	6.49 E-07	DMF	9.96 E-04	1440	64	86.3
20	HEMA	1.45 E-04	p(DMA EMA), (006-C1 Block 1)	2.03 E-06	ACVA	2.03 E-06	DMF	5.86 E-03	1440	64	74.8
21	HEMA	1.12 E-04	p(DMA EMA), (006-C2 Block 1)	1.57 E-06	ACVA	1.57 E-06	DMF	4.41 E-03	1440	64	79.8
22	HEMA	1.02 E-04	p(DMA EMA), (006-C3 Block 1)	1.44 E-06	ACVA	1.44 E-06	DMF	4.03 E-03	1440	64	82.6
23	HEMA	9.06 E-05	p(DMA EMA), (006-C4 Block 1)	1.27 E-06	ACVA	1.27 E-06	DMF	3.55 E-03	1440	64	82.9
24	HEMA	6.83 E-05	p(DMA EMA), (006-C5 Block 1)	9.54 E-07	ACVA	9.53 E-07	DMF	2.56 E-03	1440	64	86.9
25	HEMA	6.52 E-05	p(DMA EMA), (006-C6 Block 1)	9.16 E-07	ACVA	9.17 E-07	DMF	2.50 E-03	1440	64	94.4

26	HEMA	6.91 E-05	p(DMA EMA), (006-C7 Block 1)	9.62 E-07	ACVA	9.63 E-07	DMF	2.61 E-03	1440	64	89.5
27	HEMA	6.36 E-05	p(DMA EMA), (006-C8 Block 1)	8.91 E-07	ACVA	8.92 E-07	DMF	2.44 E-03	1440	64	97.0
28	HEMA	5.01 E-05	p(DMA EMA), (006-C9 Block 1)	6.99 E-07	ACVA	6.99 E-07	DMF	1.86 E-03	1440	64	90.0
29	HEMA	4.93 E-05	p(DMA EMA), (006-C10 Block 1)	6.95 E-07	ACVA	6.96 E-07	DMF	1.86 E-03	1440	64	90.1
30	HEMA	5.40 E-05	p(DMA EMA), (006-C11 Block 1)	7.51 E-07	ACVA	7.53 E-07	DMF	2.02 E-03	1440	64	90.4
31	HEMA	1.99 E-05	p(DMA EMA), (006-C12 Block 1)	2.83 E-07	ACVA	2.82 E-07	DMF	6.23 E-04	1440	64	82.4
32	HEMA	1.87 E-04	p(DMA EMA), (006-D2 Block 1)	1.92 E-06	ACVA	1.92 E-06	DMF	7.52 E-03	1440	64	73.9
33	HEMA	9.69 E-05	p(DMA EMA), (006-D3 Block 1)	9.94 E-07	ACVA	9.95 E-07	DMF	3.77 E-03	1440	64	83.0
34	HEMA	1.12 E-04	p(DMA EMA), (006-D4 Block 1)	1.15 E-06	ACVA	1.15 E-06	DMF	4.45 E-03	1440	64	81.5

35	HEMA	1.09 E-04	p(DMA EMA), (006-D6 Block 1)	1.12 E-06	ACVA	1.12 E-06	DMF	4.31 E-03	1440	64	85.9
36	HEMA	6.28 E-05	p(DMA EMA), (006-D8 Block 1)	6.48 E-07	ACVA	6.46 E-07	DMF	2.45 E-03	1440	64	94.7
37	HEMA	5.56 E-05	p(DMA EMA), (006-D9 Block 1)	5.74 E-07	ACVA	5.74 E-07	DMF	2.15 E-03	1440	64	96.7
38	HEMA	3.42 E-05	p(DMA EMA), (006-D11 Block 1)	3.54 E-07	ACVA	3.53 E-07	DMF	1.21 E-03	1440	64	90.7
39	HEMA	3.73 E-05	p(DMA EMA), (006-D12 Block 1)	3.88 E-07	ACVA	3.89 E-07	DMF	1.33 E-03	1440	64	76.5
40	HEMA	1.80 E-04	p(DMA EMA), (006-E1 Block 1)	1.46 E-06	ACVA	1.47 E-06	DMF	7.27 E-03	1440	64	81.5
41	HEMA	1.08 E-04	p(DMA EMA), (006-E4 Block 1)	8.80 E-07	ACVA	8.81 E-07	DMF	4.23 E-03	1440	64	80.6
42	HEMA	1.32 E-04	p(DMA EMA), (006-E5 Block 1)	1.07 E-06	ACVA	1.07 E-06	DMF	5.28 E-03	1440	64	81.7
43	HEMA	6.04 E-05	p(DMA EMA), (006-E9 Block 1)	4.94 E-07	ACVA	4.92 E-07	DMF	2.32 E-03	1440	64	92.0

44	HEMA	4.29 E-05	p(DMAEMA), (006-E10 Block 1)	3.52 E-07	ACVA	3.53 E-07	DMF	1.58 E-03	1440	64	82.2
45	HEMA	6.52 E-05	p(DMAEMA), (006-E11 Block 1)	5.32 E-07	ACVA	5.32 E-07	DMF	2.52 E-03	1440	64	81.3
46	HEMA	5.72 E-05	p(DMAEMA), (006-E12 Block 1)	4.66 E-07	ACVA	4.67 E-07	DMF	2.17 E-03	1440	64	70.6
47	HEMA	3.47 E-04	p(DMAEMA), (006-F3 Block 1)	2.34 E-06	ACVA	2.34 E-06	DMF	1.44 E-02	1440	64	76.5
48	HEMA	1.83 E-04	p(DMAEMA), (006-F4 Block 1)	1.23 E-06	ACVA	1.23 E-06	DMF	7.42 E-03	1440	64	77.4
49	HEMA	1.29 E-04	p(DMAEMA), (006-F7 Block 1)	8.68 E-07	ACVA	8.67 E-07	DMF	5.22 E-03	1440	64	80.4
50	HEMA	1.20 E-04	p(DMAEMA), (006-F8 Block 1)	8.09 E-07	ACVA	8.10 E-07	DMF	4.87 E-03	1440	64	81.1
51	HEMA	1.06 E-04	p(DMAEMA), (006-F9 Block 1)	7.13 E-07	ACVA	7.14 E-07	DMF	4.24 E-03	1440	64	75.3
52	HEMA	2.62 E-05	p(DMAEMA), (006-F11 Block 1)	1.79 E-07	ACVA	1.78 E-07	DMF	8.80 E-04	1440	64	83.2

53	HEMA	9.45 E-05	p(DMAEMA), (006-F12 Block 1)	6.36 E-07	ACVA	6.35 E-07	DMF	3.77 E-03	1440	64	77.4
54	HEMA	3.09 E-04	p(DMAEMA), (006-G2 Block 1)	1.77 E-06	ACVA	1.77 E-06	DMF	1.28 E-02	1440	64	78.8
55	HEMA	2.27 E-04	p(DMAEMA), (006-G4 Block 1)	1.30 E-06	ACVA	1.30 E-06	DMF	9.33 E-03	1440	64	77.3
56	HEMA	2.40 E-04	p(DMAEMA), (006-G5 Block 1)	1.38 E-06	ACVA	1.38 E-06	DMF	9.90 E-03	1440	64	75.0
57	HEMA	1.54 E-04	p(DMAEMA), (006-G6 Block 1)	8.86 E-07	ACVA	8.85 E-07	DMF	6.30 E-03	1440	64	82.7
58	HEMA	1.17 E-04	p(DMAEMA), (006-G7 Block 1)	6.72 E-07	ACVA	6.71 E-07	DMF	4.68 E-03	1440	64	83.9
59	HEMA	9.14 E-05	p(DMAEMA), (006-G9 Block 1)	5.26 E-07	ACVA	5.24 E-07	DMF	3.66 E-03	1440	64	74.6
60	HEMA	9.93 E-05	p(DMAEMA), (006-G10 Block 1)	5.70 E-07	ACVA	5.71 E-07	DMF	3.97 E-03	1440	64	N/A
61	HEMA	8.66 E-05	p(DMAEMA), (006-G11 Block 1)	4.97 E-07	ACVA	4.96 E-07	DMF	3.43 E-03	1440	64	72.8

62	HEMA	6.36 E-05	p(DMA EMA), (006-G12 Block 1)	3.65 E-07	ACVA	3.64 E-07	DMF	2.51 E-03	1440	64	65.1
63	HEMA	3.78 E-04	p(DMA EMA), (006-H2 Block 1)	1.89 E-06	ACVA	1.89 E-06	DMF	1.57 E-02	1440	64	70.6
64	HEMA	3.50 E-04	p(DMA EMA), (006-H3 Block 1)	1.75 E-06	ACVA	1.75 E-06	DMF	1.46 E-02	1440	64	74.1
65	HEMA	1.87 E-04	p(DMA EMA), (006-H6 Block 1)	9.35 E-07	ACVA	9.35 E-07	DMF	7.69 E-03	1440	64	70.8
66	HEMA	1.78 E-04	p(DMA EMA), (006-H7 Block 1)	8.90 E-07	ACVA	8.92 E-07	DMF	7.28 E-03	1440	64	81.8
67	HEMA	1.16 E-04	p(DMA EMA), (006-H10 Block 1)	5.78 E-07	ACVA	5.78 E-07	DMF	4.67 E-03	1440	64	81.9
68	HEMA	7.23 E-05	p(DMA EMA), (006-H11 Block 1)	3.60 E-07	ACVA	3.60 E-07	DMF	2.86 E-03	1440	64	83.0
69	HEMA	8.50 E-05	p(DMA EMA), (006-H12 Block 1)	4.23 E-07	ACVA	4.25 E-07	DMF	3.35 E-03	1440	64	70.6
70	HEMA	3.75 E-05	p(DMA EMA), (007-A1 Block 1)	1.88 E-06	ACVA	3.75 E-07	DMF	1.92 E-03	1440	64	55.7

71	HEMA	1.72 E-05	p(DMA EMA), (007-A2 Block 1)	8.63 E-07	ACVA	1.72 E-07	DMF	7.77 E-04	1440	64	71.1
72	HEMA	1.99 E-05	p(DMA EMA), (007-A3 Block 1)	9.99 E-07	ACVA	2.00 E-07	DMF	9.65 E-04	1440	64	76.6
73	HEMA	1.30 E-05	p(DMA EMA), (007-A4 Block 1)	6.51 E-07	ACVA	1.30 E-07	DMF	5.74 E-04	1440	64	80.3
74	HEMA	6.12 E-06	p(DMA EMA), (007-A7 Block 1)	3.07 E-07	ACVA	6.14 E-08	DMF	2.28 E-04	1440	64	73.5
75	HEMA	3.34 E-06	p(DMA EMA), (007-A8 Block 1)	1.68 E-07	ACVA	3.35 E-08	DMF	7.03 E-05	1440	64	72.3
76	HEMA	5.40 E-06	p(DMA EMA), (007-A9 Block 1)	2.71 E-07	ACVA	5.42 E-08	DMF	1.97 E-04	1440	64	66.6
77	HEMA	5.01 E-06	p(DMA EMA), (007-A10 Block 1)	2.52 E-07	ACVA	5.03 E-08	DMF	1.86 E-04	1440	64	73.6
78	HEMA	5.24 E-06	p(DMA EMA), (007-A11 Block 1)	2.61 E-07	ACVA	5.21 E-08	DMF	1.96 E-04	1440	64	77.6
79	HEMA	7.27 E-05	p(DMA EMA), (007-B1 Block 1)	1.59 E-06	ACVA	3.18 E-07	DMF	3.94 E-03	1440	64	45.1

80	HEMA	3.45 E-05	p(DMA EMA), (007-B2 Block 1)	7.54 E-07	ACVA	1.51 E-07	DMF	1.76 E-03	1440	64	67.5
81	HEMA	2.10 E-05	p(DMA EMA), (007-B3 Block 1)	4.58 E-07	ACVA	9.17 E-08	DMF	1.02 E-03	1440	64	66.6
82	HEMA	3.26 E-05	p(DMA EMA), (007-B4 Block 1)	7.13 E-07	ACVA	1.43 E-07	DMF	1.71 E-03	1440	64	52.8
83	HEMA	1.84 E-05	p(DMA EMA), (007-B5 Block 1)	4.02 E-07	ACVA	8.06 E-08	DMF	9.00 E-04	1440	64	76.9
84	HEMA	2.63 E-05	p(DMA EMA), (007-B6 Block 1)	5.76 E-07	ACVA	1.15 E-07	DMF	1.37 E-03	1440	64	60.8
85	HEMA	1.81 E-05	p(DMA EMA), (007-B7 Block 1)	3.96 E-07	ACVA	7.92 E-08	DMF	9.29 E-04	1440	64	70.2
86	HEMA	2.14 E-05	p(DMA EMA), (007-B8 Block 1)	4.67 E-07	ACVA	9.35 E-08	DMF	1.11 E-03	1440	64	69.4
87	HEMA	1.64 E-05	p(DMA EMA), (007-B9 Block 1)	3.58 E-07	ACVA	7.17 E-08	DMF	8.34 E-04	1440	64	79.0
88	HEMA	8.48 E-05	p(DMA EMA), (007-E5 Block 1)	6.90 E-07	ACVA	1.38 E-07	DMF	4.76 E-03	1440	64	62.9
89	HEMA	1.45 E-04	p(DMA EMA), (007-F4	9.74 E-07	ACVA	1.95 E-07	DMF	8.18 E-03	1440	64	49.4

			Block 1)								
90	HEMA	5.07 E-05	p(DMAEMA), (007-E10 Block 1)	4.12 E-07	ACVA	8.24 E-08	DMF	2.82 E-03	1440	64	46.5
91	MMA	3.17 E-04	p(DMAEMA), (009-A10 Block 1)	1.06 E-06	ACVA	1.06 E-06	DMF	9.27 E-04	1440	60	30.5
92	DMAEMA	8.08 E-04	p(MMA), (009-E8 Block 1)	2.69 E-06	AIBN	5.39 E-07	DMF	1.72 E-03	1440	60	61.9
93	DMAEMA	9.92 E-04	p(BMA), (009-G2 Block 1)	3.31 E-06	AIBN	6.61 E-07	DMF	2.11 E-03	1440	60	74.4
94	BMA	7.75 E-04	p(DMAEMA), (011-B1 Block 1)	2.58 E-06	AIBN	2.58 E-06	DMF	1.74 E-03	1440	60	17.3
95	DMAEMA	1.25 E-04	p(MMA), (011-E6 Block 1)	4.16 E-07	ACVA	4.17 E-08	DMF	2.66 E-04	1440	60	21.2
96	DMAEMA	2.71 E-04	p(BMA), (011-G7 Block 1)	9.04 E-07	AIBN	9.04 E-07	DMF	5.78 E-04	1440	60	53.6

Table 5. Summary of Pilot PNP Library Characterization Data

PNP	Block 1 DynaPro Result (molar mass, kDa)	DynaPro DLS Z-Avg Result (size, nm)	Size PDI (goal < 0.3)
1	16.3	386.9	Multimodal
2	16.8	574.3	Multimodal
3	17.9	331.8	0.121
4	21.6	345.1	0.152

5	22.3	355.8	0.483
6	23.3	379.7	0.268
7	22.7	365.7	0.219
8	23.9	317.8	0.040
9	24.1	296.2	0.137
10	27.2	323.9	0.254
11	17.4	1017.8	0.166
12	26.9	1368.9	Multimodal
13	18.7	450.2	0.087
14	20.9	389.2	0.152
15	21.9	291.5	0.111
16	31.2	361	0.243
17	30.3	317.7	0.201
18	35.0	345.3	0.114
19	30.7	267.1	0.137
20	12.1	1383	0.238
21	17.7	459	0.301
22	18.8	436.7	0.314
23	18.6	448	0.492
24	26.7	422.8	0.368
25	24.1	333	0.214
26	25.1	275.6	Multimodal
27	22.8	318.3	0.219
28	28.9	342	0.557
29	27.0	222.5	Multimodal
30	26.5	251.3	0.48
31	62.1	207.3	0.548
32	17.0	522.5	0.435
33	26.4	477.6	Multimodal
34	20.8	369.9	0.237
35	22.9	321.4	0.276
36	26.4	282.4	0.229
37	30.4	319.1	0.351
38	54.2	262.3	0.202
39	53.1	315.5	0.171
40	20.8	730.2	Multimodal
41	31.9	379.8	0.332
42	24.3	309.2	0.237
43	40.9	317.8	0.338
44	56.5	1186.1	0.271
45	37.7	386.4	0.153
46	43.2	279.6	0.138

47	14.7	413.3	0.29
48	23.6	351	0.218
49	24.2	266.7	0.524
50	24.0	249.3	0.401
51	29.0	269.9	0.371
52	105.1	271.2	0.074
53	30.4	302.5	0.145
54	18.0	375.2	Multimodal
55	20.5	440.4	0.496
56	19.0	296.3	0.109
57	24.8	277.1	0.475
58	34.7	299.6	0.393
59	36.0	269	0.351
60	34.9	264.8	0.181
61	40.5	353.1	0.138
62	41.3	230.5	0.151
63	16.2	354.5	Multimodal
64	15.0	355.5	0.334
65	23.7	332.6	0.231
66	26.6	253.4	0.217
67	34.6	294.3	0.151
68	44.3	275.2	0.207
69	45.6	404.8	0.287
70	9.4	2023.5	0.020
71	18.3	473.2	0.521
72	13.5	549.2	0.153
73	19.7	568	Multimodal
74	30.0	262	0.353
75	53.7	229.4	Multimodal
76	31.3	230.7	0.444
77	30.3	387.4	Multimodal
78	29.5	266.7	Multimodal
79	11.4	1155.7	Multimodal
80	21.3	619.2	0.198
81	28.9	337.7	0.053
82	17.5	336	0.13
83	28.8	90.5	Multimodal
84	19.0	251.3	0.164
85	21.1	360.6	Multimodal
86	18.8	225.3	Multimodal
87	22.4	168	Multimodal
88	13.1	298.6	0.217

89	11.0	281	0.314
90	16.3	300	0.503
91	29.0	214	0.293
92	9.1	14.2	0.412
93	3.9	21.2	Multimodal
94	15.6	432.8	Multimodal
95	13.2	268.9	Multimodal
96	14.2	657.2	0.356

[00220] EXAMPLE 6:

[00221] Composition Example 3 (CE3): Nucleic acid constructs conjugation to PNPs direct amidification with an amine terminal nucleic acid construct

[00222] RAFT copolymers made using CTAs that contain at least one carboxyl terminal group were further functionalized with amine terminal DNA barcodes. A RAFT copolymer was transferred into a MES buffer at ~12 mg/mL. The sample was sonicated for 30 minutes. EDC reagent and Sulfo-NHS reagent was added to the polymer at a molar ratio of 10:1 and 25:1 respectively, reagent to PNP. The sample was incubated for at least 10 minutes at room temperature to allow the reaction to occur. The reaction volume was filtered through a membrane with a molecular weight cut off of 30 kDa via centrifuge at ~3000 x g for ~15 minutes. The filtrate was discarded, and sterile PBS was added to the retentate to reconstitute to 10 mg/mL polymer. A nucleic acid constructs with a primary amine group attached to the 5' end was added to polymer and the sample was incubated for at least 15 minutes. The sample was transferred to an amicon ultra-4 centrifuge tube (MWCO 30 kDa, Max 3.5 mL/Tube) and centrifuged at 4,000 x g for 15 minutes to remove any unbound nucleic acid constructs. The filtrate (containing unbound nucleic acid constructs) was discarded and sterile PBS was added to the retentate to final concentration of 8 mg/mL polymer. The conjugated barcodes were amplified via PCR, using primers designed to bind to the primer binding segments on the nucleic acid constructs. The amplicons were detected via gel electrophoresis on agarose gel. The presence of a double band (Fig. 9) in a lane indicates the presence of amplicons, showing that nucleic acid constructs were bound to the PNPs.

[00223] The direct amidification method was used to attach 10 unique barcodes to 10 unique PNPs. The 10 unique PNPs were prepared according to the reagents shown in EXAMPLE 5.

[00224] Subsequently, the direct amidification method was used to attach 10 unique DNA barcodes to each PNP, giving each a unique label. The nucleic acid construct-PNPs were loaded with a pDNA encoding for the expression of tdTomato red fluorescent protein, and then used to treat HEK-293T cells at ~0.024 mg/mL PNP, 24 hours after seeding in a 96 well plate at 20,000 cells per well. They were left to incubate for another 48 hours, at which time, expression of the payload was observed via fluorescence microscopy using a Texas Red filter set (Fig. 10). This provides evidence that the nucleic acid constructs-PNPs are capable of being taken up by mammalian cells and delivering a payload, as indicated by the red fluorescent images.

[00225] The direct amidification method was then used to attach 88 unique barcodes to 88 unique PNPs. The 88 unique PNPs were prepared according to the reagents shown in EXAMPLE 5. The amidification method was then used to attach 88 unique DAN barcodes to each PNP giving each a unique label. The nucleic acid construct-PNPs were loaded with a pDNA encoding for the expression of tdTomato red fluorescent protein, and then used to treat HEK-293T cells at ~0.024 mg/mL PNP, 24 hours after seeding in a 96 well plate at 20,000 cells per well. They were left to incubate for another 48 hours, at which time, expression of the payload was measured via flow cytometry using a Cytoflex (Figs. 11(a)-(c)). This provided evidence that some of the nucleic acid construct-PNPs were able to be taken up by mammalian cells. The cells were also given a live/dead stain using zombie dye, and cell viability was measured via flow cytometry (Figs. 11(d)-(e)) showing that the PNPs were of relatively low cytotoxicity, with cell viability numbers of greater than 75% for the vast majority of PNPs.

[00226] EXAMPLE 7:

[00227] Methods for screening nucleic acid construct-PNPs in vitro and in vivo.

[00228] The pooled sample of polymer nanoparticles produced from the recipe in Table 2, with 96 unique barcodes attached via avidin-biotin linkange, was used for measuring cell uptake and cytotoxicity in vitro in HEK293T cells, and for measuring biodistribution in vivo administration in mice. nucleic acid construct-PNPs were formulated in a sterile saline solution and stored at 4°C for up to 1 month prior to in vivo dosing. Cell uptake efficiency and cytotoxicity are assessed in vitro using HEK293T cells with 0.024 mg/mL PNP at 250 ng or 150 ng/well pDNA treatment concentrations. Cell uptake was demonstrated by fluorescence microscopy. Animals are assigned to dose groups using a stratified randomization program designed to maintain similar group mean body weights by sex. Animals are administered either

a control or test article via a single bolus intravenous tail-vein injections. Tested doses ranged from 0-150 mg/kg, with adverse clinical events being observed in 35% of animals at 150 mg/kg. Blood and tissue are collected from all animals and snap frozen in liquid nitrogen.

[00229] EXAMPLE 8:

[00230] Extraction of nucleic acid construct-PNPs from in vitro biological samples.

[00231] 10-20 mg of tissues are placed in a Tris-EDTA lysis buffer and homogenized using the TissueLyser bead-beating system and a 5mm stainless steel bead. Homogenization is carried out at 25Hz in 5-7 minute intervals until solution is homogenous in appearance. Proteinase K is then added to the lysate for protein digestion and incubated in a Thermomixer at 55°C for 2-4 hours. DNA is extracted from the tissue lysate using the QIAamp 96 DNA extraction kit and a Qiacube HT instrument according to the manufacturer's protocol. Concentration and purity of isolated samples is determined using a NanoDrop.

[00232] EXAMPLE 9:

[00233] PCR Amplification of Polynucleotide Barcodes in Nucleic Acid Constructs.

[00234] Polymerase chain reaction is used to produce amplicons from extracted nucleic acid constructs. PCR is performed using a single set of universal primers that anneal to the universal amplification sites on the barcode, thereby amplifying all unique barcodes within a sample in a single reaction. Positive amplification of barcode(s) within a sample is determined using electrophoresis (agarose gel or bioanalyzer) indicated by the presence of a band at ~120 bp.

[00235] EXAMPLE 10:

[00236] Library Preparation and Sequencing of Nucleic Acid Constructs.

[00237] Sequencing libraries are prepared from the amplicons generated during first stage PCR amplification. Our universal primers also contain overhang sequences that enable attachment of Index Adapters for sequencing. Illumina Unique Dual Indexes are annealed to the overhangs on the amplicon by PCR. Individual indexed libraries are then pooled in equal amounts and purified using a NucleoSpin Gel and PCR Clean-up kit according the manufacturer's protocols. The molar concentration of the final sequencing library is determined using a Qubit dsDNA High Sensitivity Assay kit and Qubit Fluorometer. The library is spiked with 2% PhiX, diluted to 1.8 pM and loaded onto a High Output 300 cycle NextSeq sequencing cardrige. Paired end sequencing is performed using a NextSeq550 instrument.

[00238] EXAMPLE 11:

[00239] Sequence Analysis and Bioinformatics.

[00240] Merged reads from each Sample ID are demultiplexed into PE FASTQ files, and merged into a single file. The merged reads are processed to identify those containing both the 5' and 3' flanking adapters. Trimmed reads are then downselected for sequences containing the correct barcode length. Barcode counts are generated from these downselected sequences and tagged according to whether they are spiked or random. Barcode counts are then normalized to the number of FASTQ reads in the sample.

[00241] HYPOTHETICAL EXAMPLE:

[00242] By way of example, in one illustrative embodiment, the presently disclosed rapid DBTL technologies may be used to develop a gene therapy for forms of amyotrophic lateral sclerosis (ALS) caused by toxic, gain-of-function mutations in superoxide dismutase 1 (SOD1). This gene therapy may involve delivering a CRISPR base-editing protein via a non-viral gene delivery vehicle to inactivate the production of mutant SOD1 protein in microglia, a cell type that modulates the progression of the disease but remains refractory to efficient viral transduction. This will enable safe and efficient therapeutic "hit-and-run" editing for ALS.

[00243] An exemplary disease that can be treated with the methods described herein is ALS. ALS is a rapidly progressive, paralytic, and invariably fatal disorder characterized by the selective loss of motor neurons in the spinal cord and brain. Though most cases of ALS are sporadic, dominantly inherited mutations in SOD1 (a ubiquitously expressed metalloenzyme that normally converts superoxide anions into oxygen and hydrogen peroxide) account for up to 20% of all inherited or familial forms of ALS. Base editors are a recently emerged gene-editing modality capable of introducing targeted single-base substitutions in DNA without the requirement for a double-strand break (DSB). Base editors consist of fusions of a catalytically impaired Cas9 nuclease variant, known as a Cas9 nickase, with a nucleobase deaminase enzyme. This example will rely on the ability of base editors, specifically cytidine base editors (CBEs), to catalyze C > T base transitions at CGA, CAG or CAA triplets in a target gene sequence, which creates an in-frame stop codon that triggers the degradation of a target mRNA by nonsense-mediated decay – a surveillance mechanism used by cells to prevent the formation of truncated proteins. Using this method, SOD1 will be inactivated in a manner that does not require a DSB and does not rely on the stochastic and mutagenic NHEJ repair pathway, thus overcoming two of the major limitations facing the clinical implementation of CRISPR-Cas9

for ALS. Thus, while first generation CRISPR is considered the "cut and paste" of gene editing, base editors are considered to be an "eraser and pencil" function, allowing for precise single base edits to a genome, opening new mechanisms for revolutionary ALS treatments. However, innovations in gene delivery have significantly lagged innovations in technologies for gene editing itself. Thus, efficient delivery of base editing systems to the specific cell types involved in driving the progression of ALS represents a key limitation impeding its safe and efficient implementation for treatment of the disorder. Non-viral delivery vehicles will be used to address many of these limitations.

[00244] In this illustrative example, the rapid DBTL technologies can iterate through hundreds of diverse polymer nanoparticle candidates, using automated high-throughput synthesis of diverse polymer nanoparticle libraries, parallel *in vitro* and *in vivo* screens of barcoded libraries, and a machine learning algorithm to analyze the large data sets and predict new libraries for rapid iteration. Figure 1 presents a simplified flow diagram illustrating a DBTL cycle for non-viral gene delivery development based on automated synthesis, high throughput testing, and machine learning design.

[00245] HYPOTHETICAL EXAMPLE:

[00246] In one aspect, a library of 100s of polymer nanoparticles (PNPs) encapsulating CBE mRNA can be screened in a high throughput *in vitro* and *in vivo* platform. In the illustrative embodiment, over 500 PNPs are synthesized and uniquely labeled and tracked via DNA barcoding. A highly versatile PNP platform based on reversible addition-fragmentation chain transfer (RAFT) polymerization will be used due to its flexibility, reproducibility, and scalability. *See* K. Sims et al., "Rigor and reproducibility in polymer nanoparticle synthesis and characterization," *Rsc Advances* **2020,** *10* (5), 2513-2518 (incorporated herein by reference). As shown in Figure 2, the RAFT polymerization platform can be used to generate highly monodisperse PNPs with a diverse variety of sizes, charges and chemical make-up. The PNPs can be functionalized to attach cell penetrating peptides to enable higher order functionality and protection to both the vehicle and the cargo. In some embodiments, PNPs may be labeled with quantum dots and other biomarkers via avidin-biotin conjugation. *See* A. Duong et al., "Scalable, Semicontinuous Production of Micelles Encapsulating Nanoparticles via Electrospray," *Langmuir* 2014**,** *30* (14), 3939-3948. (incorporated herein by reference). This combination of microglia non-viral delivery vehicles with base editing payloads is highly innovative because it has the potential to lead to a new therapy for ALS. Moreover, the

DBTL technologies of the present disclosure are generalizable to enable the creation of advanced non-viral delivery vehicles capable of accessing the other cell types involved in ALS.

[00247] After library synthesis, as described above, these PNPs can then be rapidly tested *in vitro* in a microglial cell line for toxicity, inflammation, and mRNA delivery efficiency via GFP expression. In parallel, the biodistribution and toxicity of the entire library can be assessed using loaded nanoparticles delivered via an intrathecal injection to the cerebrospinal fluid (CSF) of the G93A-SOD1 mouse model of ALS using an mRNA encoding a bioluminescent luciferase that can be tracked via *in vitro* imaging system (IVIS). This screen should result in three large data sets including particle physical characteristics, *in vitro* bioactivity, and *in vivo* biodistribution and toxicity, which, taken together, will provide the basis for an informed design of a novel non-viral delivery vehicle library which will be synthesized in a second iteration. This novel library can then be tested for functional gene editing tests in a microglial cell line modified to express a mutant SOD1 protein. The PNPs can be loaded with mRNA encoding CBE designed to inactivate GFP and SOD1, detected by fluorescence measurement and sequencing.

WHAT IS CLAIMED IS:

1. A composition comprising:
   a. a non-viral delivery vehicle comprising one or more nanoparticle forming polymers, and
   b. a nucleic acid construct.

2. The composition of claim 1, wherein the non-viral delivery vehicle comprises a polymer nanoparticle.

3. The composition of claim 1, wherein the nucleic acid constructis associated with the non-viral delivery vehicle via an electrostatic interaction.

4. The composition of claim 1, wherein the nucleic acid construct is associated with the non-viral delivery vehicle by an electrostatic interaction of a positively charged polymer segment of the one or more nanoparticle forming polymers and a negatively charged polynucleotide segment of the nucleic acid construct.

5. The composition of claim 1, wherein the nucleic acid construct is associated with the non-viral delivery vehicle by a high affinity, non-covalent bond interaction between a biotin molecule on the 5' and/or the 3' end of the nucleic acid construct and a molecule that binds to biotin on the one or more nanoparticle forming polymers.

6. The composition of claim 1, wherein the nucleic acid construct is associated with the non-viral delivery vehicle by a covalent bond between a carboxy terminated polymer segment and the nucleic acid construct, wherein the nucleic acid construct comprises a primary amine on the 5' and/or the 3' end.

7. The composition of claim 1, wherein the nucleic acid construct comprises:
   a. two primer binding segments; and

b. one or more unique polynucleotide barcodes between the two primer binding segments.

8. The composition of claim 7, wherein the primer binding segments range in length from about 15 base pairs to about 30 base pairs.

9. The composition of claim 7, wherein the primer binding segments are a universal primer binding set.

10. The composition of claim 7, wherein the one or more polynucleotide barcodes comprise unique sequences of 6-20 nucleotides in length.

11. The composition of claim 10, wherein the polynucleotide barcodes further comprise a hamming distance of at least 2-6 bases between any two unique polynucleotide barcode sequences.

12. The composition of claim 1, wherein the nucleic acid construct further comprises from about 6 to about 12 random bases at the 3' end of the polynucleotide barcode.

13. The composition of claim 12, wherein the about 6 to about 12 random bases at the 3' end of the polynucleotide barcode are for bioinformatic removal of PCR duplicates.

14. The composition of claim 1, wherein the nucleic acid construct ranges in length from about 42 nucleotides to about 210 nucleotides.

15. The composition of claim 1, wherein the one or more nanoparticle forming polymers comprise monomer units compatible with a controlled living/radical polymerization.

16. The composition of claim 1, wherein the one or more nanoparticle forming polymers are prepared by reversible-deactivation radical polymerization, atom transfer radical polymerization (ATRP), reversible addition fragmentation chain transfer polymerization (RAFT), iodine-

transfer polymerization (ITP), selenium-centered radical-mediated polymerization, telluride-mediated polymerization (TERP), stibine-mediated polymerization, or ring-opening polymerization.

17. The composition of claim 1, wherein the one or more nanoparticle forming polymers are prepared from monomers containing a vinyl group.

18. The composition of claim 1, wherein the one or more nanoparticle forming polymers are prepared using a chain transfer agent such as those used in reversible addition-fragmentation chain transfer (RAFT).

19. The composition of claim 1, wherein the one or more nanoparticle forming polymers are RAFT block copolymers comprising

a. a first terminus comprising a first capping unit derived from a first chain transfer agent in a RAFT copolymerization process;

b. a first block prepared from one or more monomer units covalently attached to the first reactive functional unit, and having a molecular weight (M_n) in the range of about 1 kDa to about 200 kDa and a degree of polymerization in the range of about 10 to about 2500;

c. optionally a second block prepared from one or more monomer units covalently attached to the first block, and having a molecular weight (M_n)in the range of about 1 kDa to about 200 kDa and a degree of polymerization in the range of about 20 to about 2000; and

d. a second terminus comprising a second capping unit derived from a first or a second chain transfer agent.

20. The composition of claim 19, wherein the non-viral delivery vehicle has one or more of an overall molecular weight (M_n) in the range of about 25 kDa to about 60 kDa, an overall degree of polymerization in the range of about 700 to about 900, a target size in the range of about of about 10 to about 60 nm, and a maximum corona-to-core ratio (CCR) of about 1.5 to about 3.5.

21. The composition of claim 19, wherein the first block is prepared from one or more monomer units selected from the group consisting of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, and methyl methacrylate.

22. The composition of claim 19, wherein the first block is prepared from one of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, or methyl methacrylate.

23. The composition of claim 19, wherein the second block is prepared from one or more monomer units selected from the group consisting of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, and methyl methacrylate.

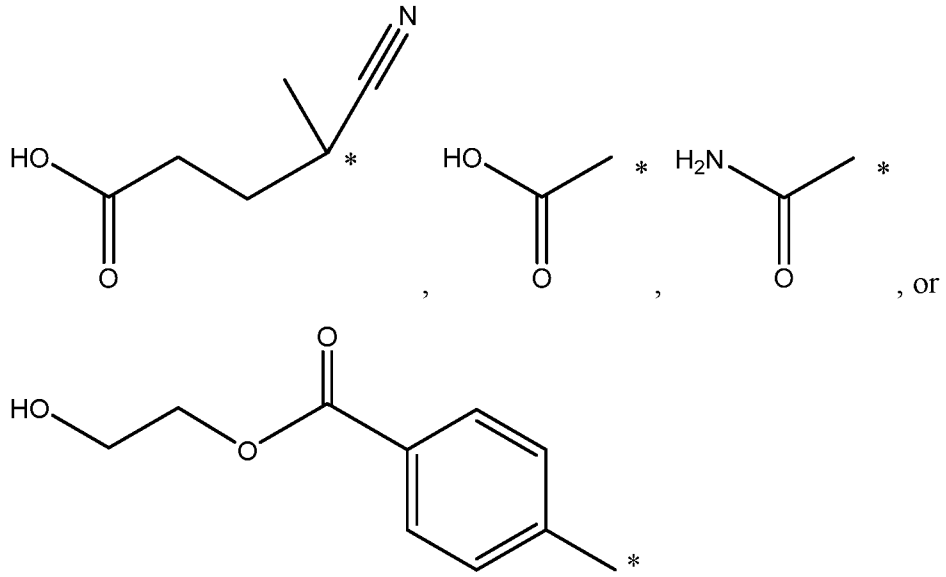
24. The composition of claim 19, wherein the second block is a random copolymer prepared from two different monomer units independently selected from the group consisting of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, and methyl methacrylate.

25. The composition of claim 19, wherein the second block is a random copolymer prepared from three different monomer units independently selected from the group consisting of 2-dimethylaminoethyl acrylate, 2-(diethylamino) ethyl methacrylate, 2-(diisopropylamino) ethyl methacrylate, butyl methacrylate, ethyl acrylic acid, propyl acrylic acid, (hydroxyethyl)methacrylate, and methyl methacrylate.

26. The composition of claim 19, wherein the second block is a random copolymer prepared from 2-dimethylaminoethyl acrylate, butyl methacrylate, and propyl acrylic acid; or 2-dimethylaminoethyl acrylate and butyl methacrylate; or 2-dimethylaminoethyl acrylate, butyl methacrylate, and ethyl acrylic acid.

27. The composition of claim 19, wherein each chain transfer agent is independently selected from the group consisting of bis(carboxymethyl)trithiocarbonate, bis(2-amino-2-oxoethyl) trithiocarbonate, bis[4-(2-hydroxyethoxycarbonyl)benzyl] trithiocarbonate, 4-cyano-4-(ethylsulfanylthiocarbonyl) sulfanylvpentanoic acid, 4-cyano-4-((phenylcarbonothioyl)thio)pentanoic acid, and 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid.

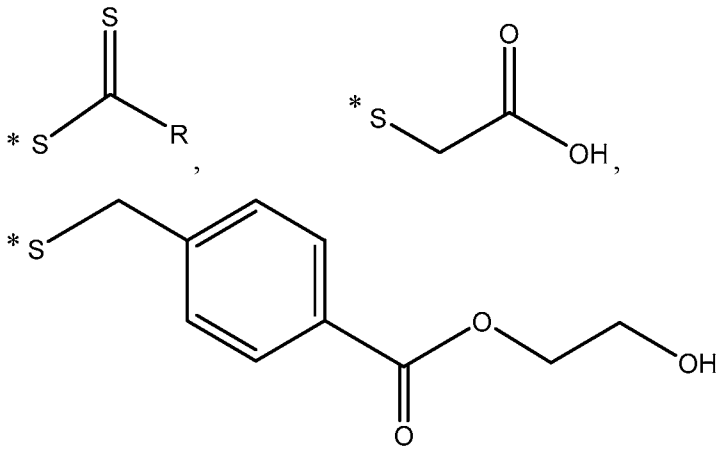
28. The composition of claim 19, wherein the first capping unit is of the formula



, , , or

wherein * represents a point of covalent attachment to the first block.

29. The composition of claim 19, wherein the second capping unit is of the formula



or

wherein * represents a point of covalent attachment to the second block, and R is $-SC_2$-C_{12} alkyl or C_6H_5,

30. A method of *in vivo* screening for a desired polymer nanoparticle for use as a delivery vehicle, the method comprising (a) preparing a library comprising two or more types of polymer nanoparticles, wherein each polymer nanoparticle is associated with a nucleic acid construct comprising a different polynucleotide barcode, (b) administering the library to an animal, (c) removing cells or tissues from the animal, (d) isolating the nucleic acid constructs from the cells or the tissues of the animal, (e) detecting the nucleic acid constructs in the cells or the tissues of the animal, and (f) identifying the desired polymer nanoparticle for use as a delivery vehicle.

31. The method of claim 30 wherein the polymer nanoparticle associated with the nucleic acid construct is according to any one of claims 1 to 29.

32. The method of claim 30 wherein the nucleic acid construct is detected by a method selected from the group consisting of the polymerase chain reaction (PCR), isothermal amplification, sequencing, or a combination thereof, to obtain nucleotide sequence data.

33. The method of claim 30, wherein the polymer nanoparticle is loaded with a payload.

34. The method of claim 33, wherein the payload is a luminescent molecule.

35. The method of claim 34, wherein the luminescence is used to track the biodistribution or cell uptake via imaging.

36. The method of claim 30, wherein the administration to the animal is via an intramuscular, an intravenous, an intraperitoneal, an oral, or a pulmonary route.

37. The method of claim 30, wherein the nucleic acid construct is isolated from the cells and the tissues by mixing with a first organic compound and incubating the organic phase with an aqueous phase of the cell or tissue sample, separating the organic phase from the aqueous phase, mixing the organic phase with a second organic compound, incubating the mixture, precipitating the nucleic acid construct from the mixture, removing the organic phase by evaporation, and resuspending the nucleic acid construct in an aqueous composition.

38. The method of claim 37, wherein the organic phase comprises phenol chloroform.

39. The method of claim 37, wherein the nucleic acid construct is separated from cationic material in the cells or tissues by titrating the aqueous composition of the nucleic acid contstruct to a pH of greater than 7.4.

40. The method of claim 30, wherein the nucleic acid construct is separated from material in the cells or tissues by binding the nucleic acid construct with a molecule with a binding affinity to the nucleic acid construct greater than the binding affinity to the cell or tissue material.

41. The method of claim 30, wherein the nucleic acid construct is separated from material in the cells or tissues via size exclusion chromatography.

42. The method of claim 30, wherein the nucleic acid construct is separated from material in the cells or tissues via dialysis or diafiltration.

43. The method of claim 30, wherein the nucleic acid construct is separated from material in the cells or tissues via filtration.

44. The method of claim 30, wherein the nucleic acid construct is separated from material in the cells or tissues by digesting proteins using an enzyme.

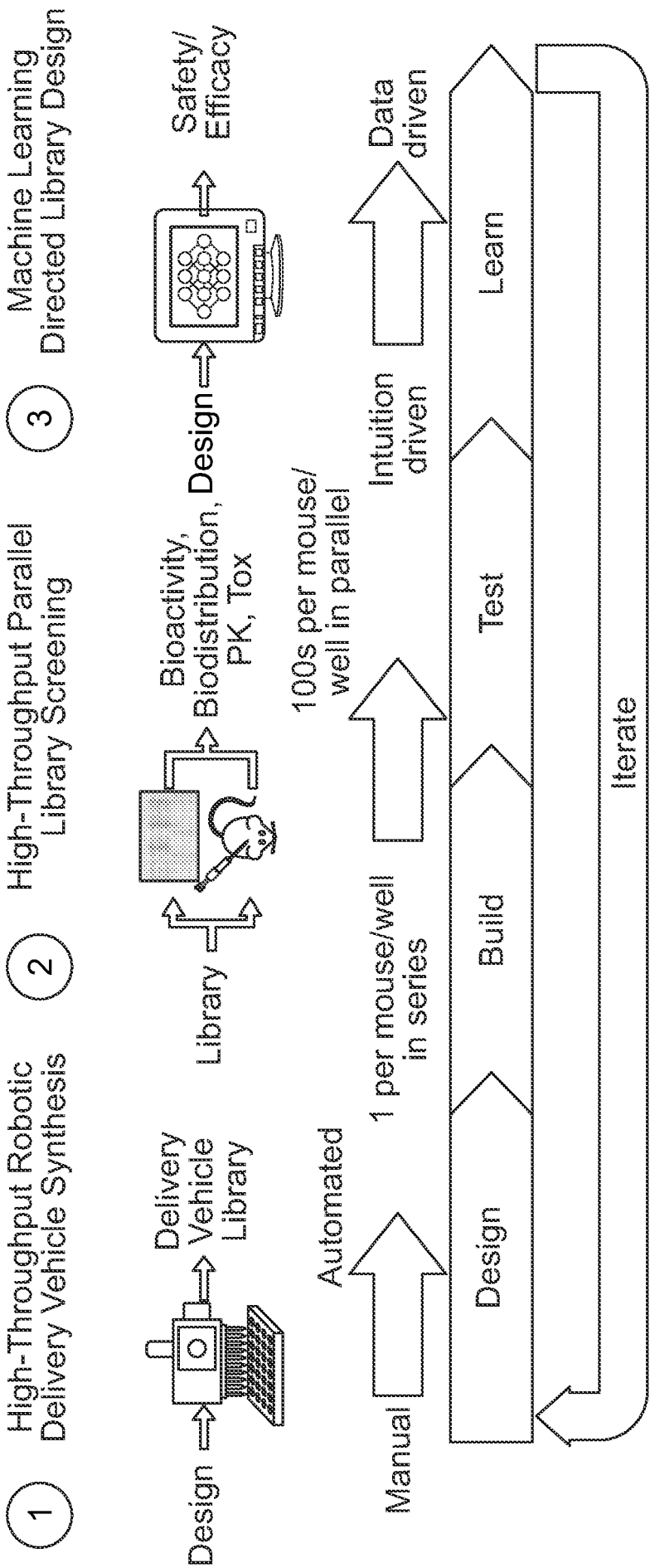
45. The method of claim 44 wherein the enzyme is Proteinase K.

46. The method of claim 30, wherein the nucleic acid constructs associated with the polymer nanoparticles are detected by first diluting the isolated nucleic acid constructs by a factor of at least 1000 times, and then amplifying the nucleic acid constructs by PCR using primers.

47. The method of claim 46, wherein the primers from the PCR step are enzymatically digested prior to detection of amplicons.

48. The method of claim 32, wherein the nucleotide sequence data is converted to fast Q files; and the fast Q files are mapped to known polynucleotide barcodes and the polynucleotide barcodes are enumerated.

49. A method of treating a patient with a disease, the method comprising administering to the patient the polymer nanoparticle identified in the *in vivo* screening method of claim 30, wherein the polymer nanoparticle further comprises a payload, and treating the disease in the patient.



The DBTL cycle for non-viral gene delivery development based on three pillars: automated synthesis, high throughput testing, and machine learning design.

Fig. 1

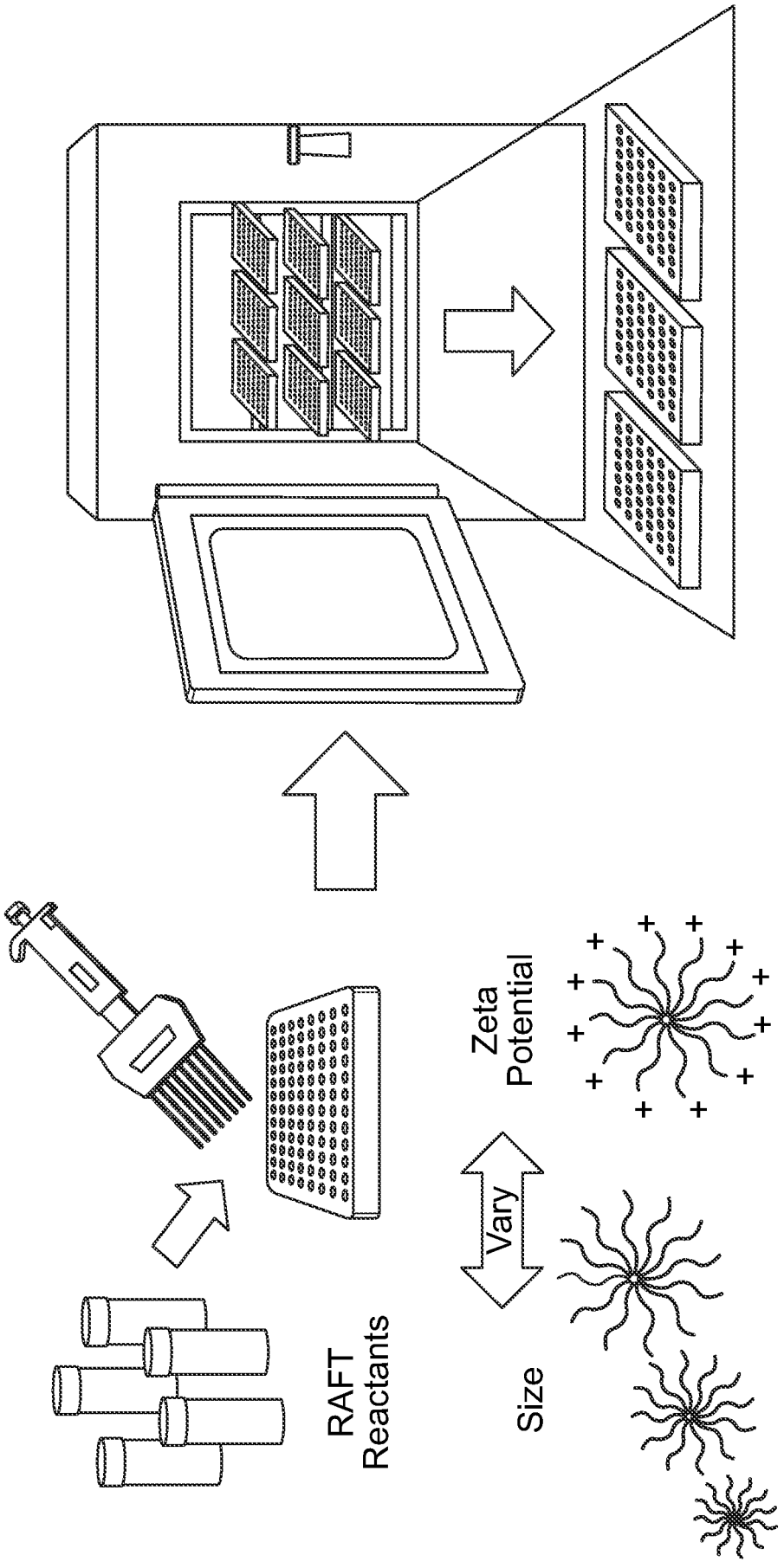


Fig. 2

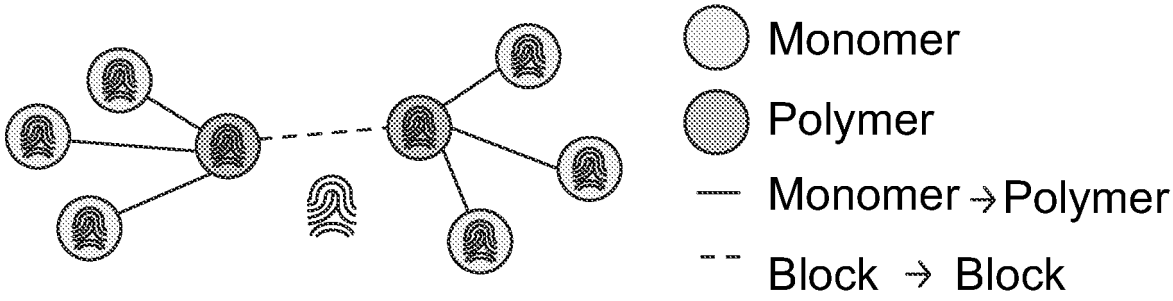

Monomer
Polymer
Monomer → Polymer
Block → Block

Fig. 3A

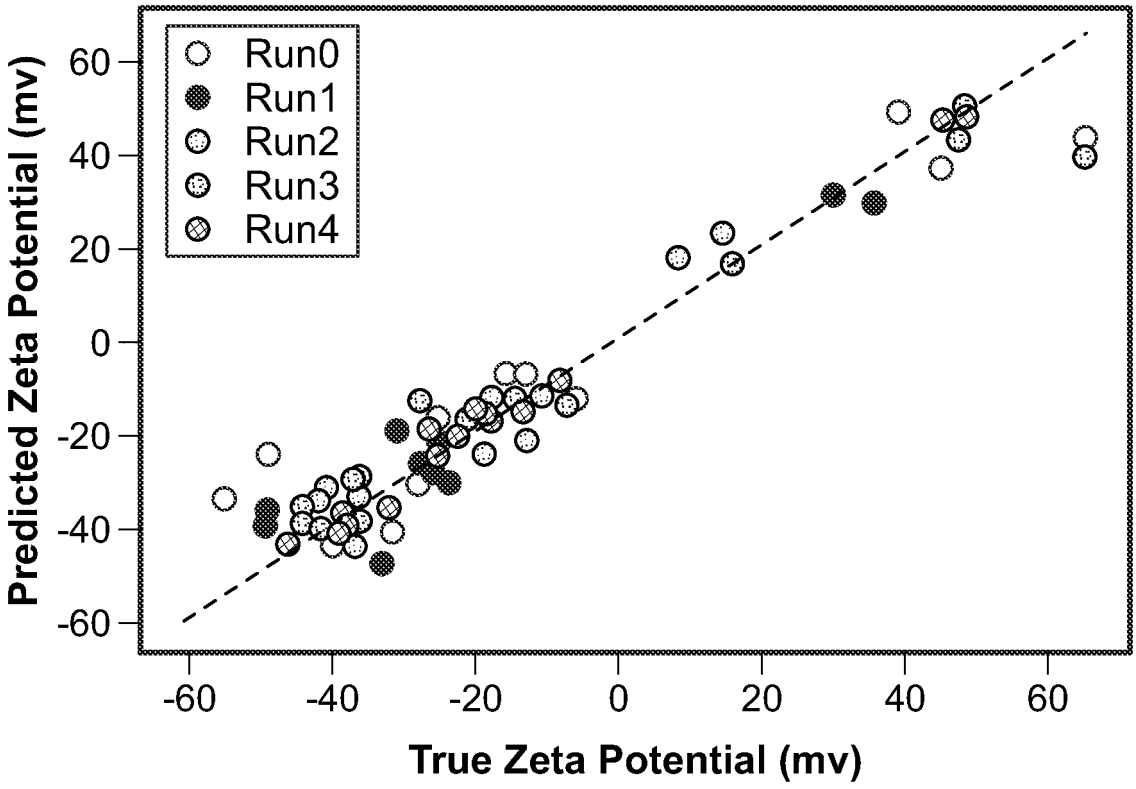

Run0
Run1
Run2
Run3
Run4
Predicted Zeta Potential (mv)
True Zeta Potential (mv)
60
40
20
0
-20
-40
-60
-60
-40
-20
0
20
40
60

Fig. 3B

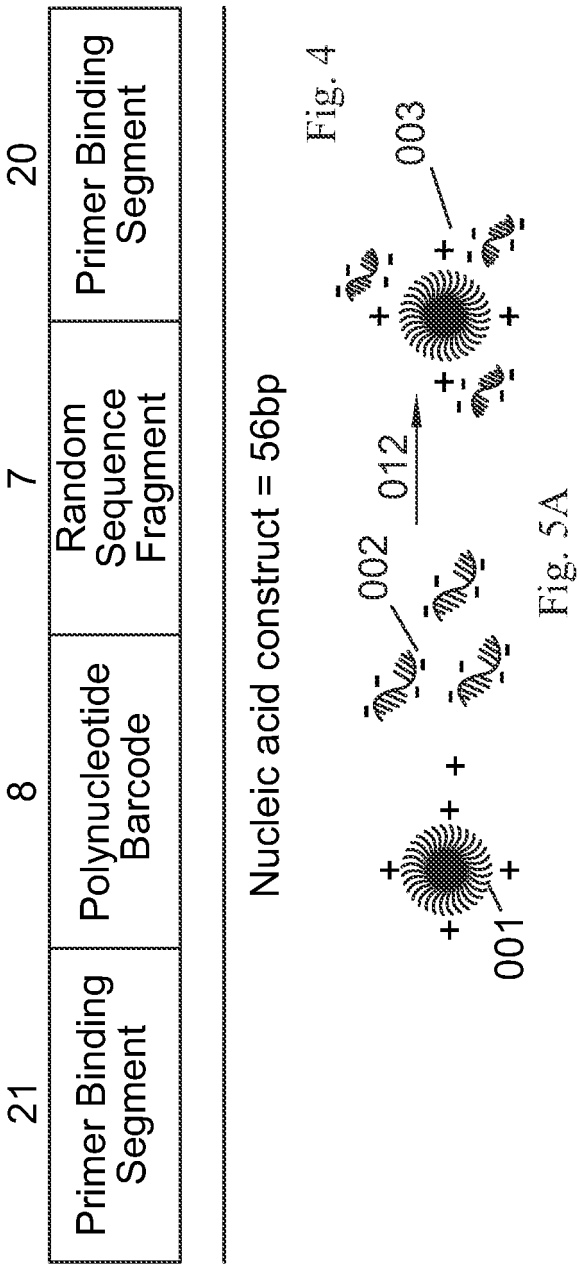


Fig. 4

Fig. 5A

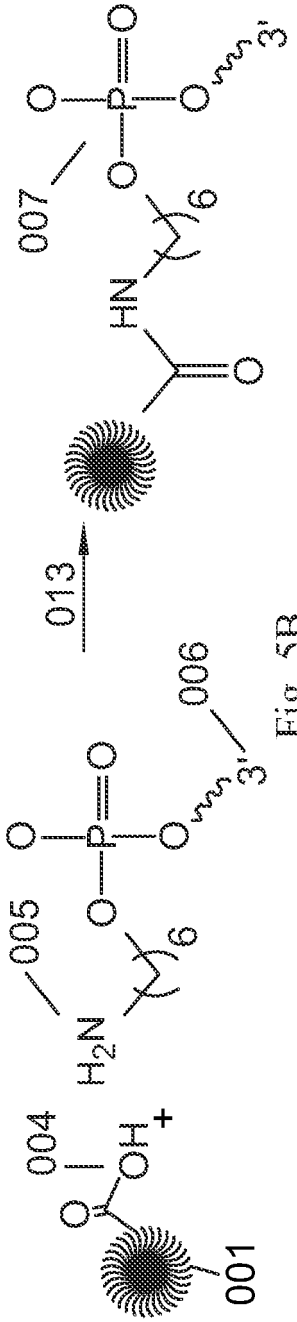


Fig. 5B

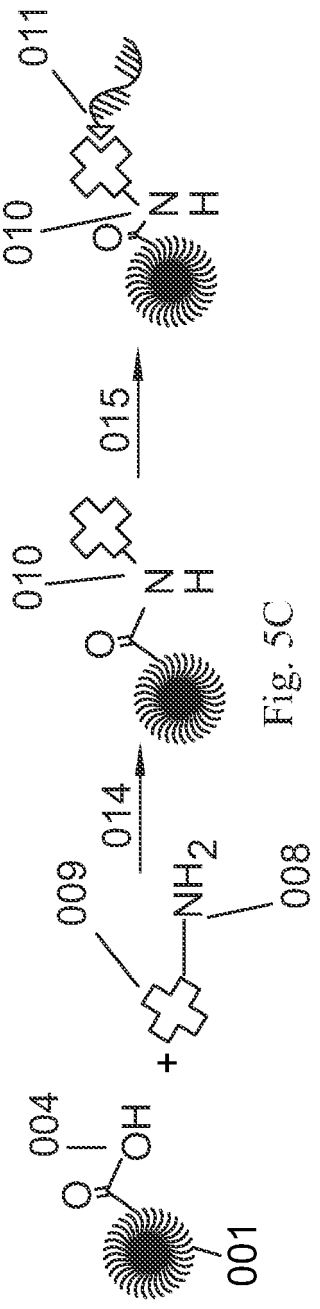


Fig. 5C

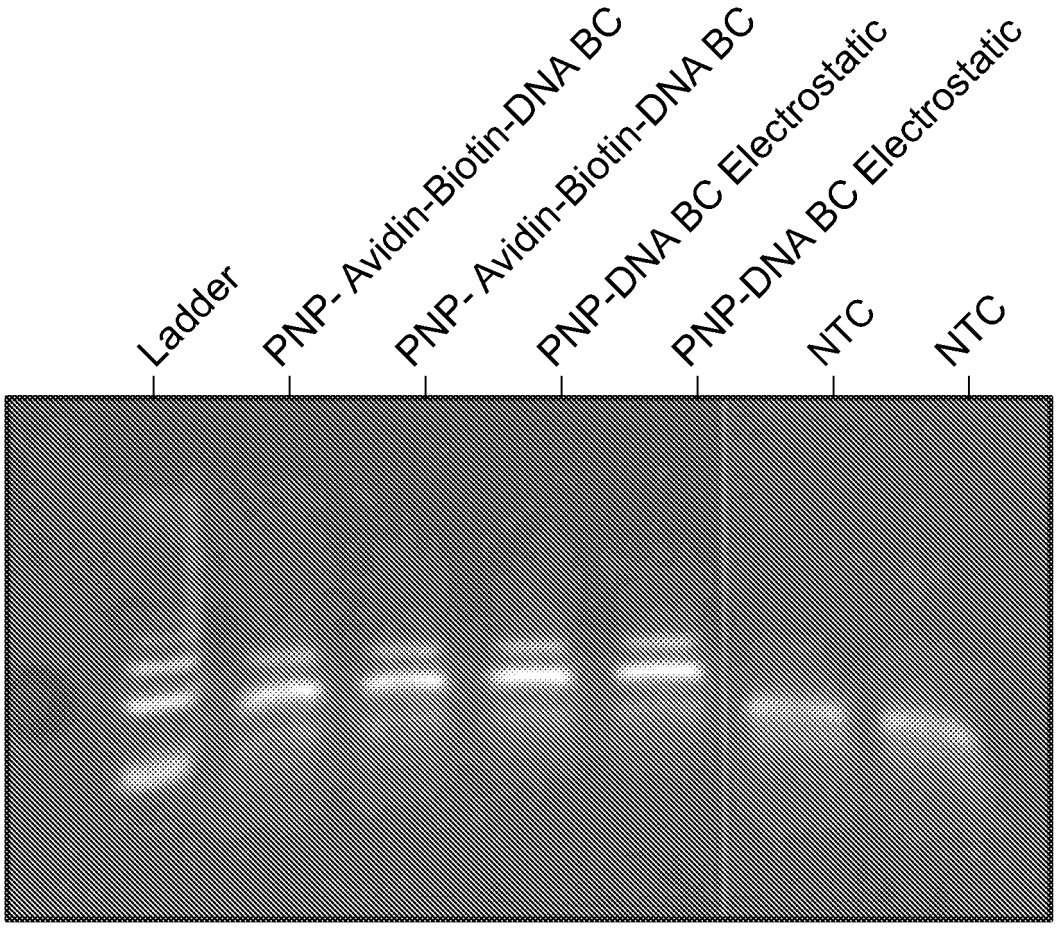

Ladder
PNP- Avidin-Biotin-DNA BC
PNP- Avidin-Biotin-DNA BC
PNP-DNA BC Electrostatic
PNP-DNA BC Electrostatic
NTC
NTC

Fig. 6

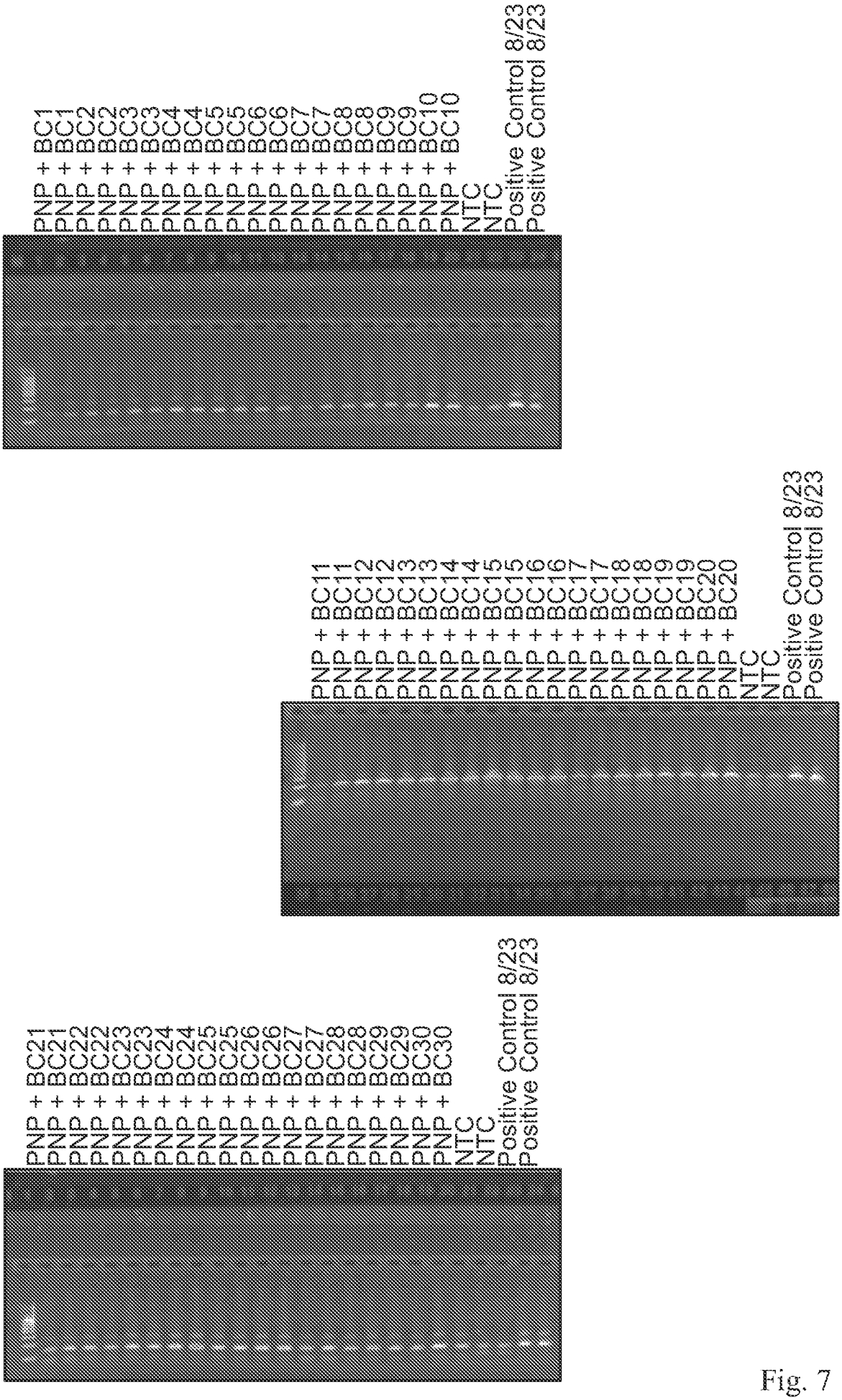

PNP + BC1
PNP + BC1
PNP + BC2
PNP + BC2
PNP + BC3
PNP + BC3
PNP + BC4
PNP + BC4
PNP + BC5
PNP + BC5
PNP + BC6
PNP + BC6
PNP + BC7
PNP + BC7
PNP + BC8
PNP + BC8
PNP + BC9
PNP + BC9
PNP + BC10
PNP + BC10
NTC
NTC
Positive Control 8/23
Positive Control 8/23
PNP + BC11
PNP + BC11
PNP + BC12
PNP + BC12
PNP + BC13
PNP + BC13
PNP + BC14
PNP + BC14
PNP + BC15
PNP + BC15
PNP + BC16
PNP + BC16
PNP + BC17
PNP + BC17
PNP + BC18
PNP + BC18
PNP + BC19
PNP + BC19
PNP + BC20
PNP + BC20
NTC
NTC
Positive Control 8/23
Positive Control 8/23
PNP + BC21
PNP + BC21
PNP + BC22
PNP + BC22
PNP + BC23
PNP + BC23
PNP + BC24
PNP + BC24
PNP + BC25
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PNP + BC26
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PNP + BC27
PNP + BC27
PNP + BC28
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Positive Control 8/23
Positive Control 8/23

Fig. 7

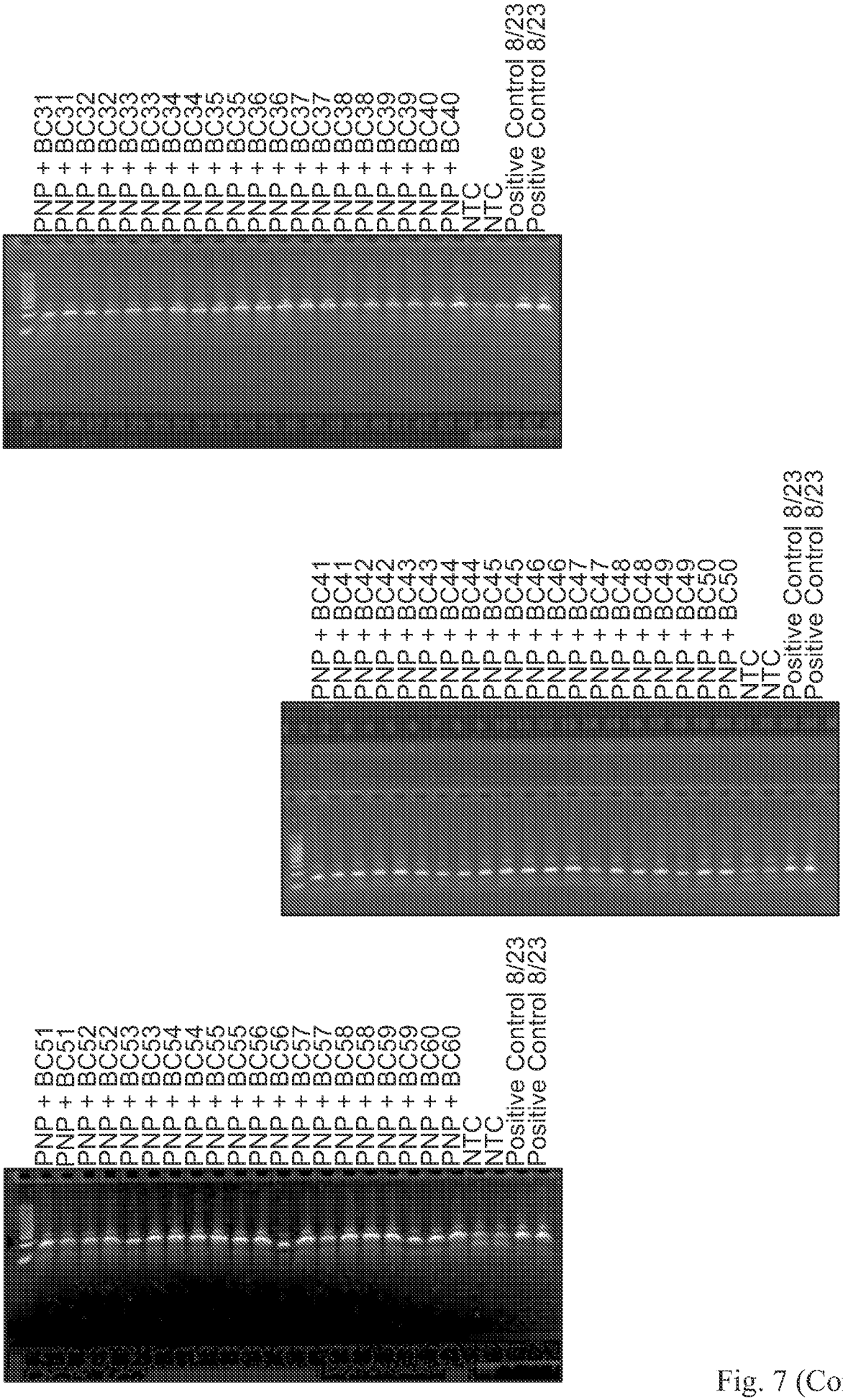

PNP + BC31
PNP + BC31
PNP + BC32
PNP + BC32
PNP + BC33
PNP + BC33
PNP + BC34
PNP + BC34
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PNP + BC35
PNP + BC36
PNP + BC36
PNP + BC37
PNP + BC37
PNP + BC38
PNP + BC38
PNP + BC39
PNP + BC39
PNP + BC40
PNP + BC40
NTC
NTC
Positive Control 8/23
Positive Control 8/23
PNP + BC41
PNP + BC41
PNP + BC42
PNP + BC42
PNP + BC43
PNP + BC43
PNP + BC44
PNP + BC44
PNP + BC45
PNP + BC45
PNP + BC46
PNP + BC46
PNP + BC47
PNP + BC47
PNP + BC48
PNP + BC48
PNP + BC49
PNP + BC49
PNP + BC50
PNP + BC50
NTC
NTC
Positive Control 8/23
Positive Control 8/23
PNP + BC51
PNP + BC51
PNP + BC52
PNP + BC52
PNP + BC53
PNP + BC53
PNP + BC54
PNP + BC54
PNP + BC55
PNP + BC55
PNP + BC56
PNP + BC56
PNP + BC57
PNP + BC57
PNP + BC58
PNP + BC58
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NTC
Positive Control 8/23
Positive Control 8/23

Fig. 7 (Cont)

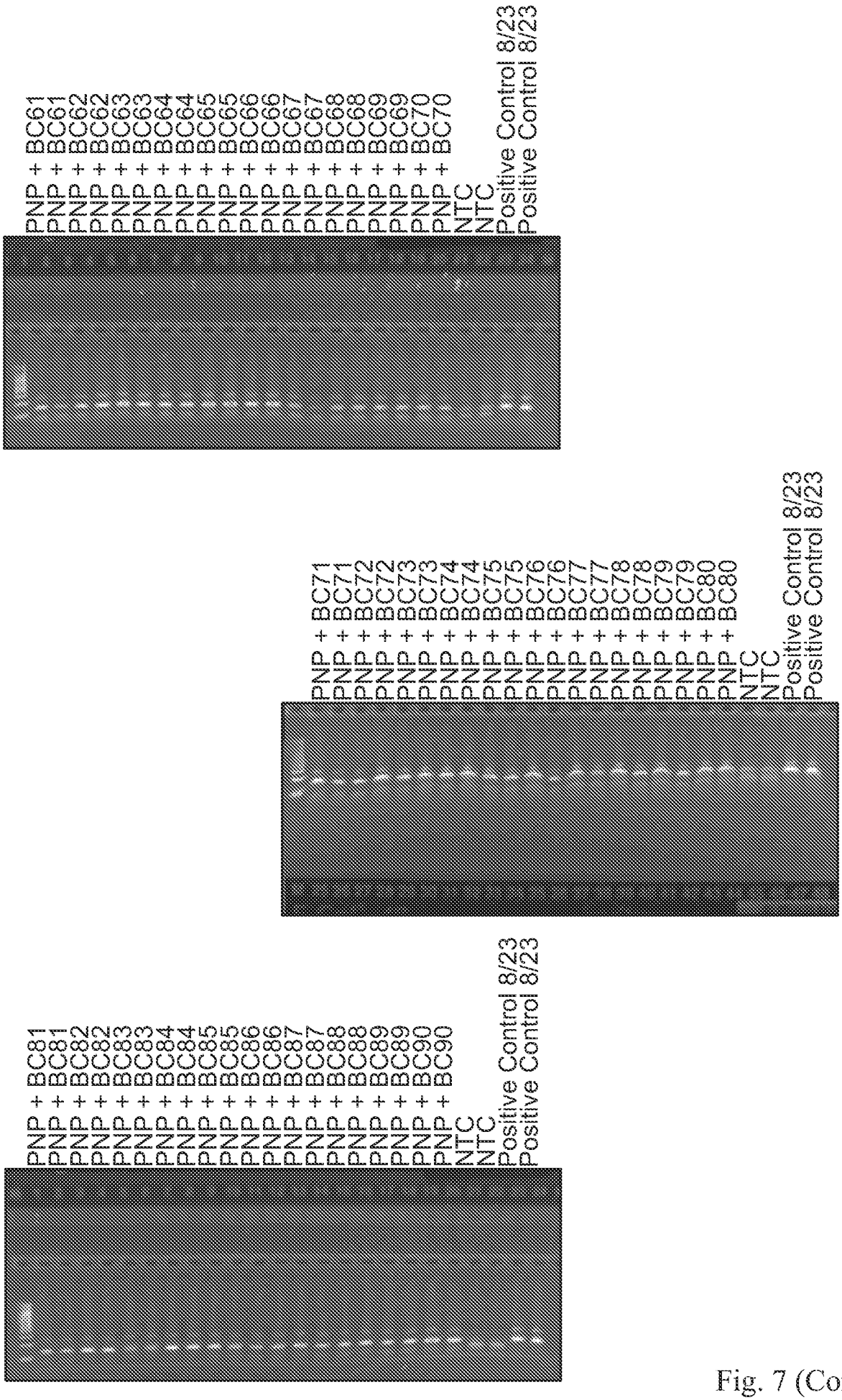

PNP + BC61
PNP + BC61
PNP + BC62
PNP + BC62
PNP + BC63
PNP + BC63
PNP + BC64
PNP + BC64
PNP + BC65
PNP + BC65
PNP + BC66
PNP + BC66
PNP + BC67
PNP + BC67
PNP + BC68
PNP + BC68
PNP + BC69
PNP + BC69
PNP + BC70
PNP +
NTC
NTC
Positive Control 8/23
Positive Control 8/23
PNP + BC71
PNP + BC71
PNP + BC72
PNP + BC72
PNP + BC73
PNP + BC73
PNP + BC74
PNP + BC74
PNP + BC75
PNP + BC75
PNP + BC76
PNP + BC76
PNP + BC77
PNP + BC77
PNP + BC78
PNP + BC78
PNP + BC79
PNP + BC79
PNP + BC80
PNP +
NTC
NTC
Positive Control 8/23
Positive Control 8/23
PNP + BC81
PNP + BC81
PNP + BC82
PNP + BC82
PNP + BC83
PNP + BC83
PNP + BC84
PNP + BC84
PNP + BC85
PNP + BC85
PNP + BC86
PNP + BC86
PNP + BC87
PNP + BC87
PNP + BC88
PNP + BC88
PNP + BC89
PNP + BC89
PNP + BC90
PNP +
NTC
NTC
Positive Control 8/23
Positive Control 8/23

Fig. 7 (Cont)

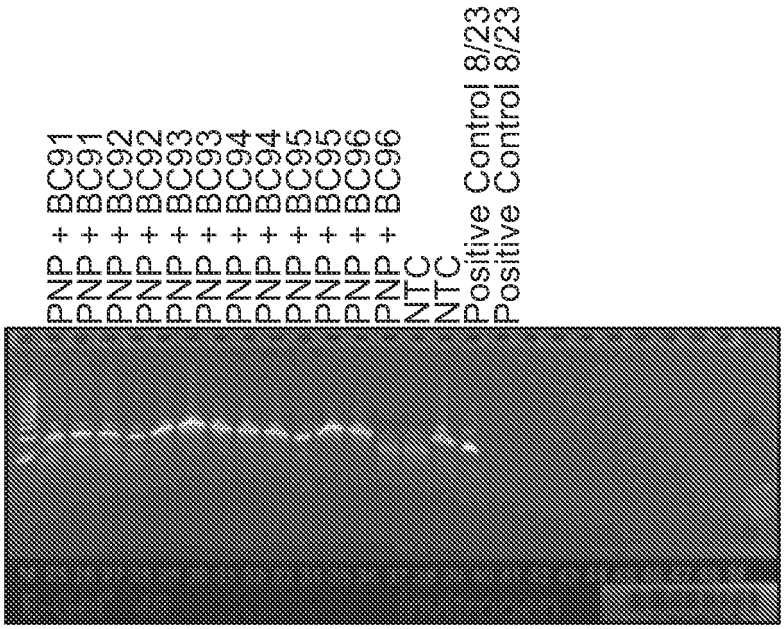

PNP + BC91
PNP + BC91
PNP + BC92
PNP + BC92
PNP + BC93
PNP + BC93
PNP + BC94
PNP + BC94
PNP + BC95
PNP + BC95
PNP + BC96
PNP + BC96
NTC
NTC
Positive Control 8/23
Positive Control 8/23

Fig. 7 (Cont)

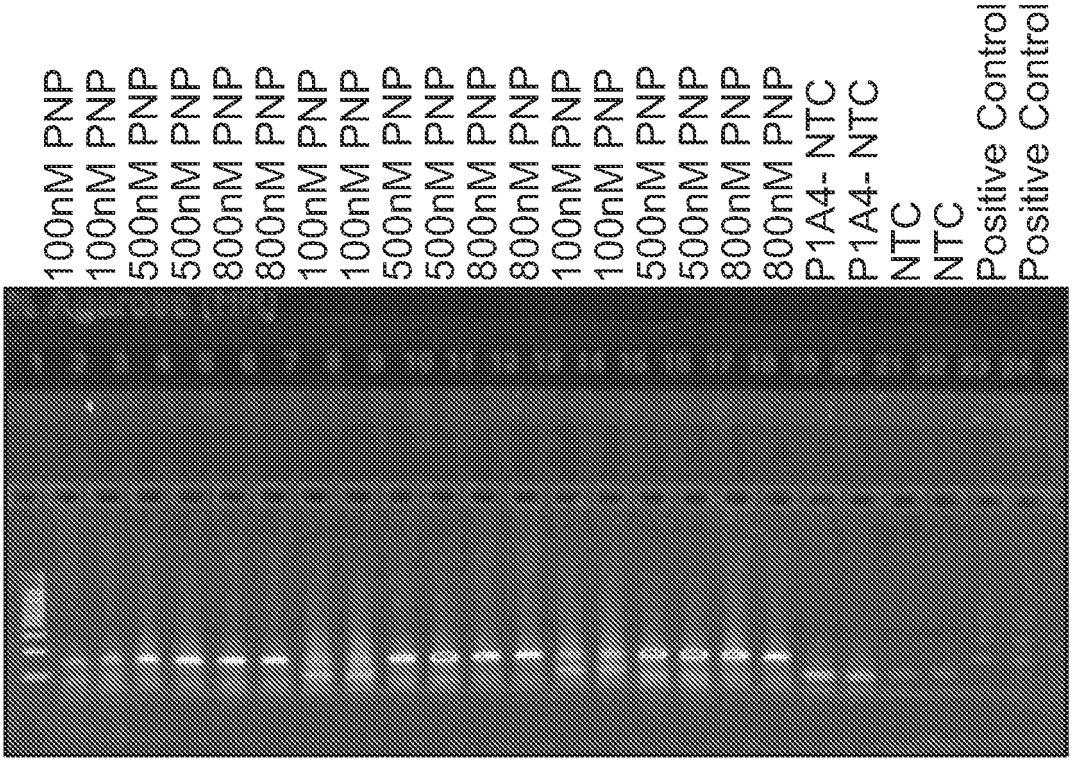

100nM PNP
100nM PNP
500nM PNP
500nM PNP
800nM PNP
800nM PNP
100nM PNP
100nM PNP
500nM PNP
500nM PNP
800nM PNP
800nM PNP
100nM PNP
100nM PNP
500nM PNP
500nM PNP
800nM PNP
800nM PNP
P1A4- NTC
P1A4- NTC
NTC
NTC
Positive Control
Positive Control

Fig. 8

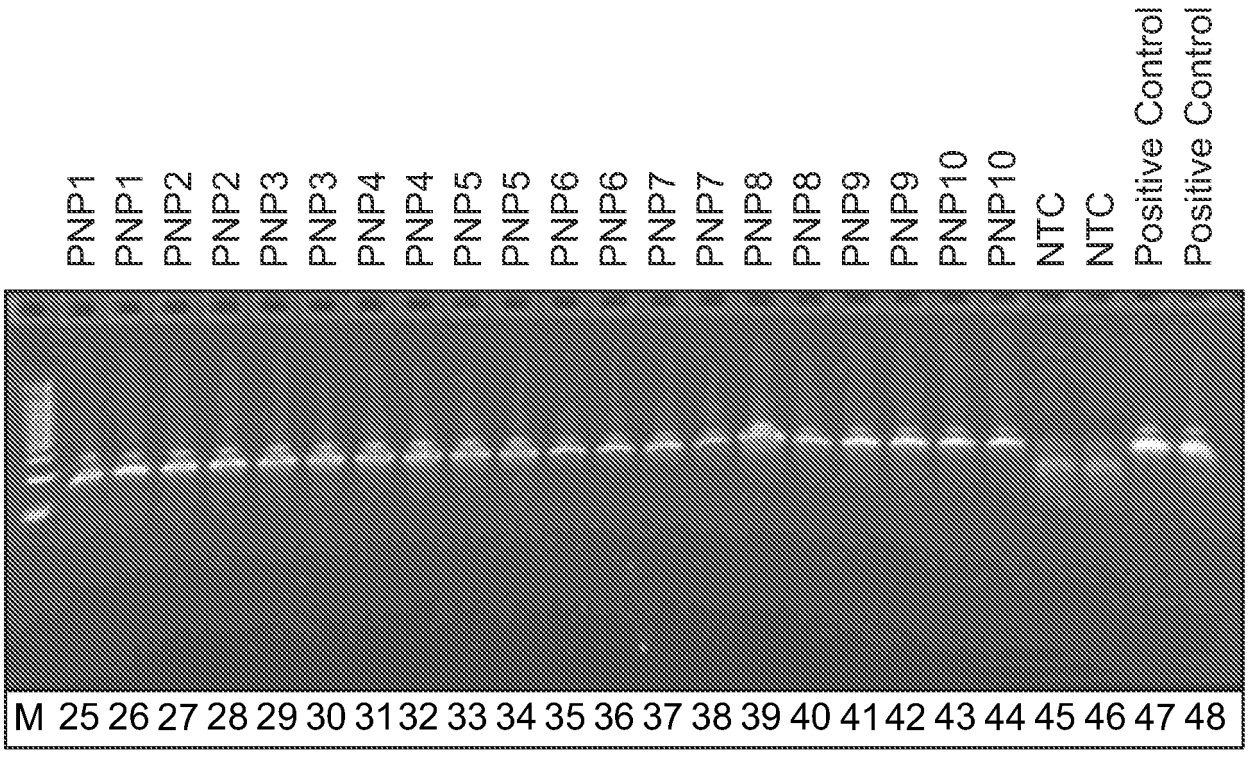

PNP1
PNP1
PNP2
PNP2
PNP3
PNP3
PNP4
PNP4
PNP5
PNP5
PNP6
PNP6
PNP7
PNP7
PNP8
PNP8
PNP9
PNP9
PNP10
PNP10
NTC
NTC
Positive Control
Positive Control
M 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48

Fig. 9

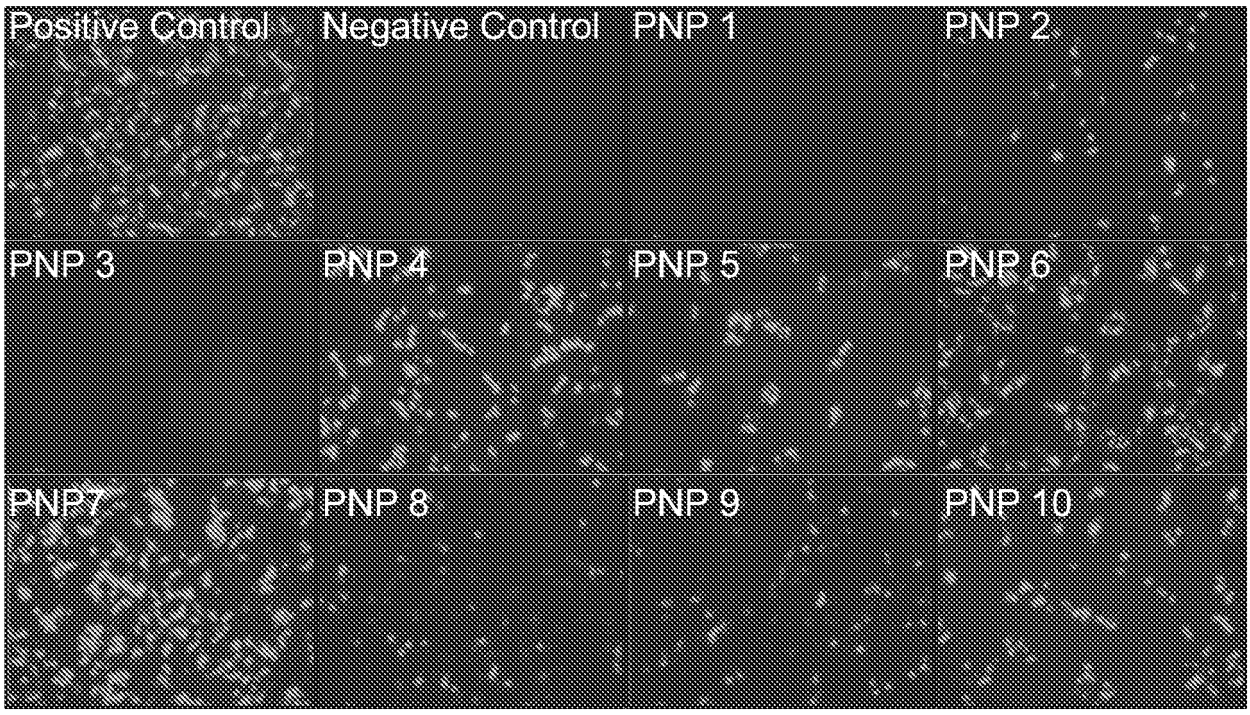

Positive Control
Negative Control
PNP 1
PNP 2
PNP 3
PNP 4
PNP 5
PNP 6
PNP7
PNP 8
PNP 9
PNP 10

Fig. 10

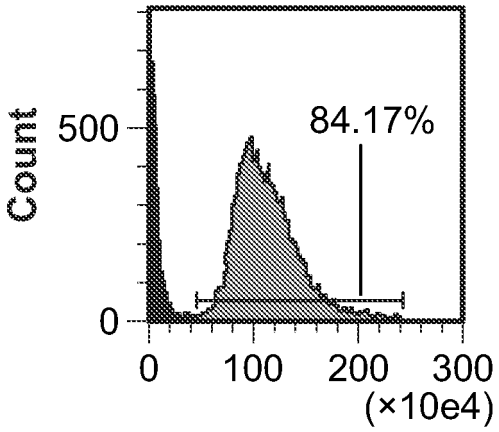


Fig. 11A

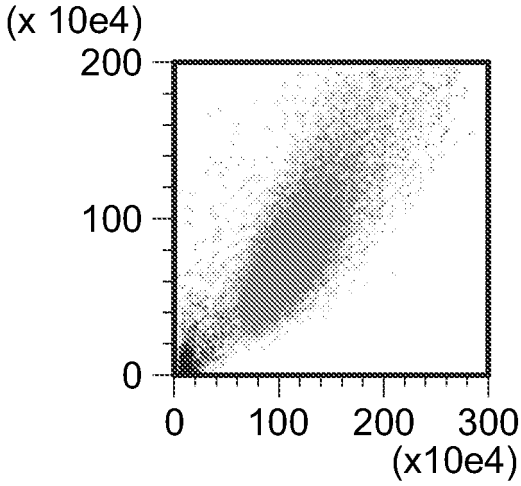


Fig. 11B

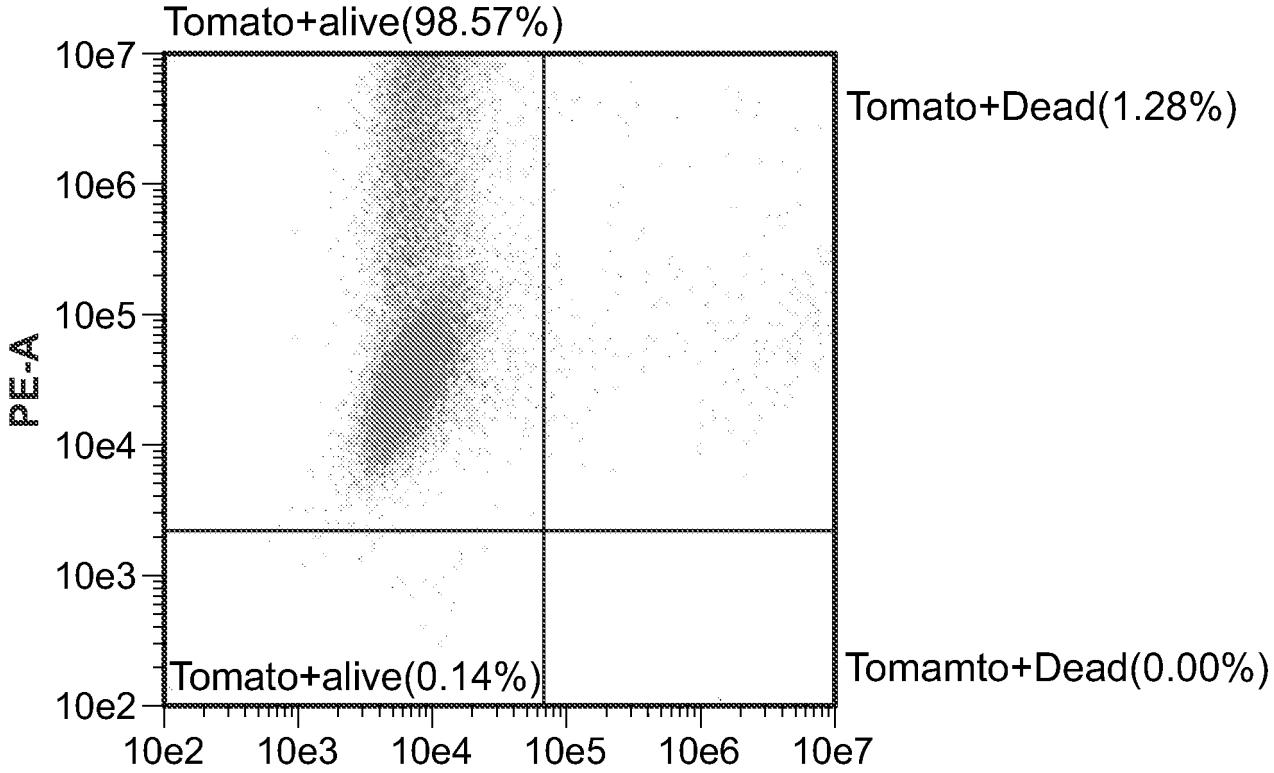


Fig. 11C

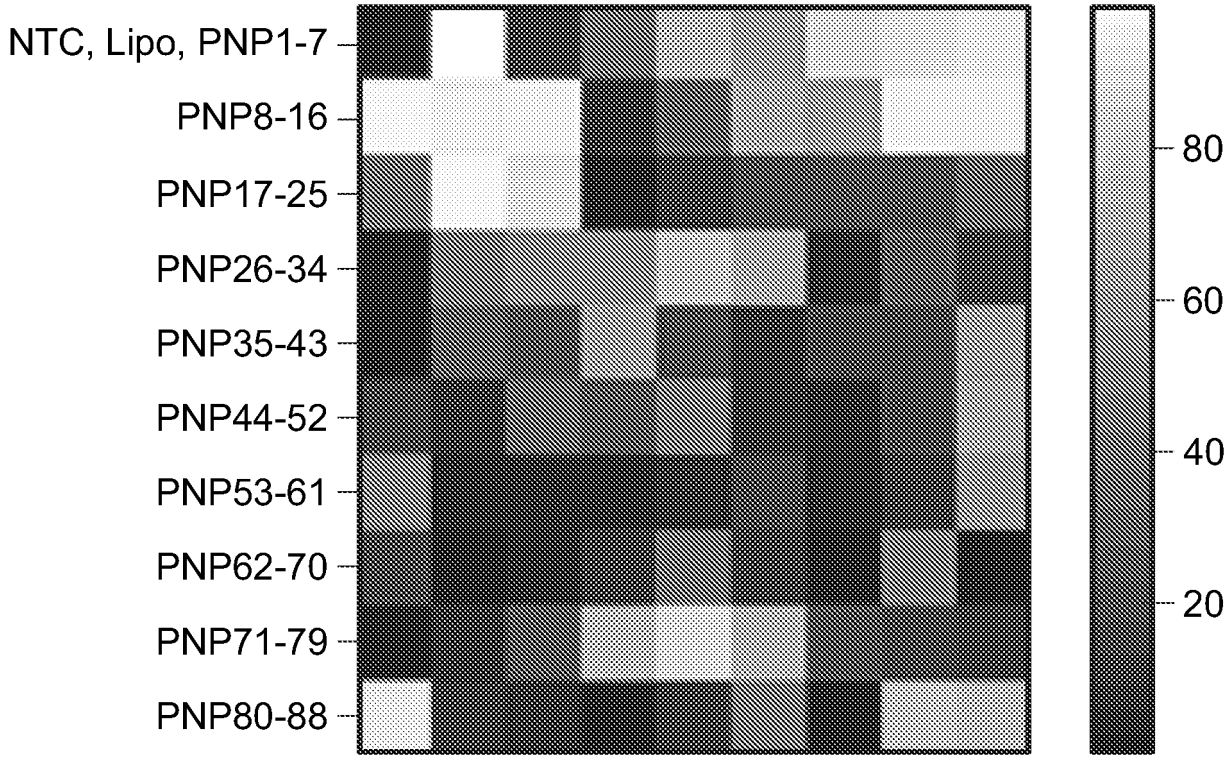


Fig. 11D

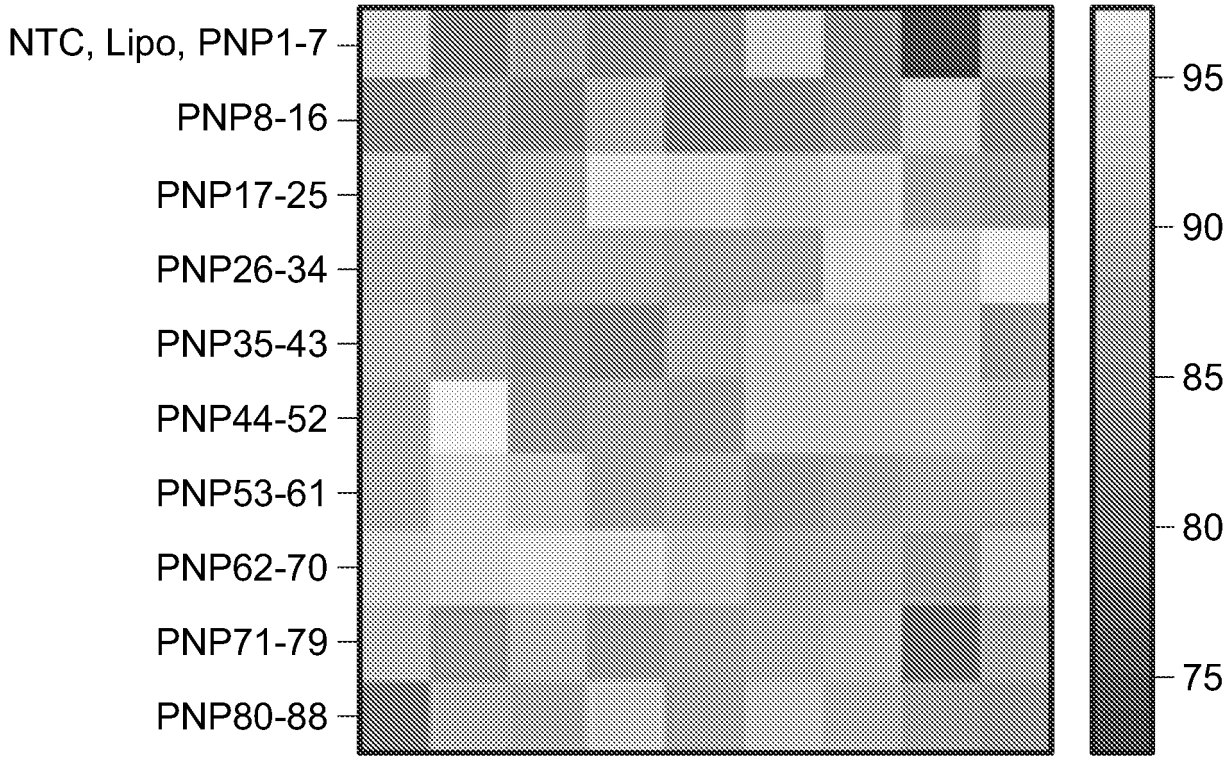


Fig. 11E

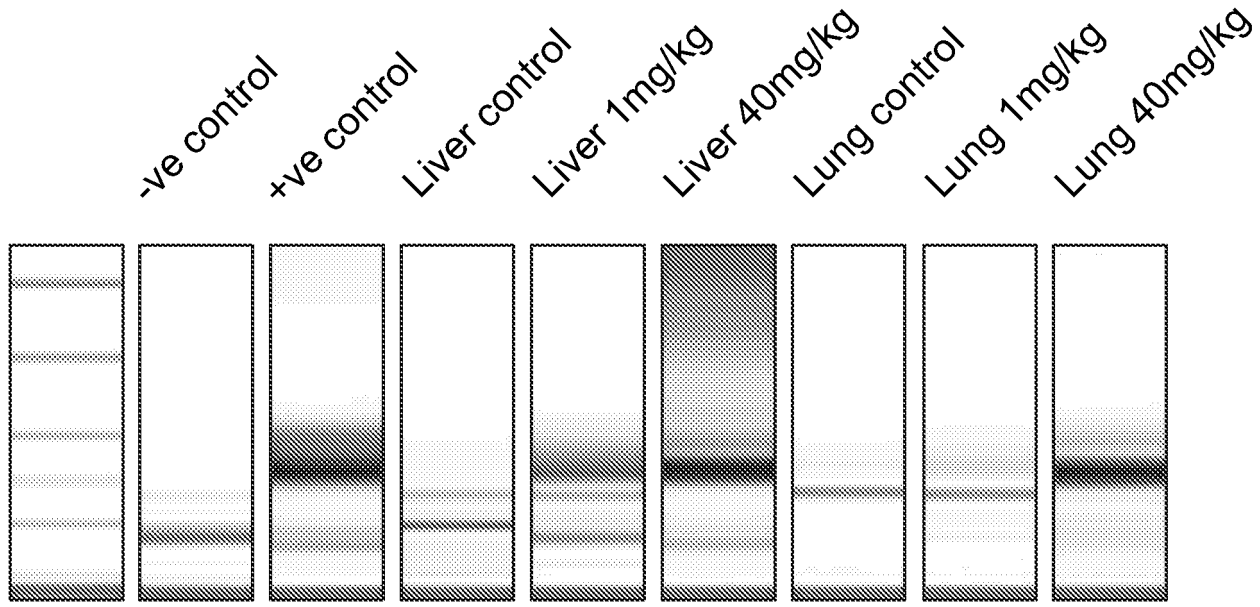


Fig. 12A

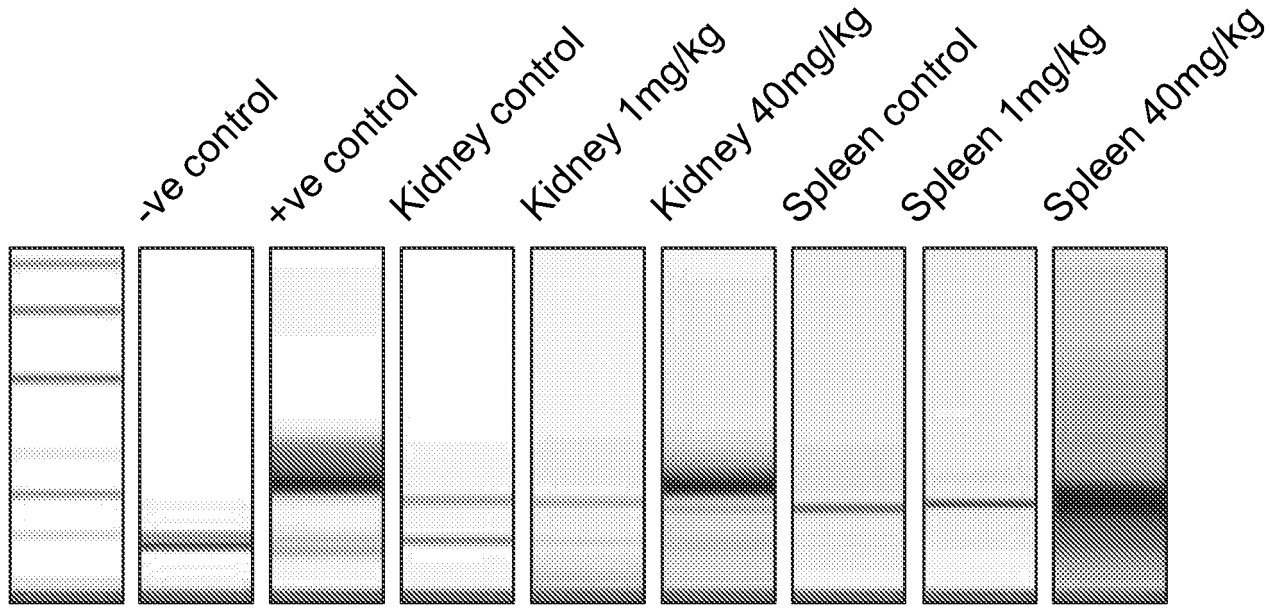


Fig. 12B

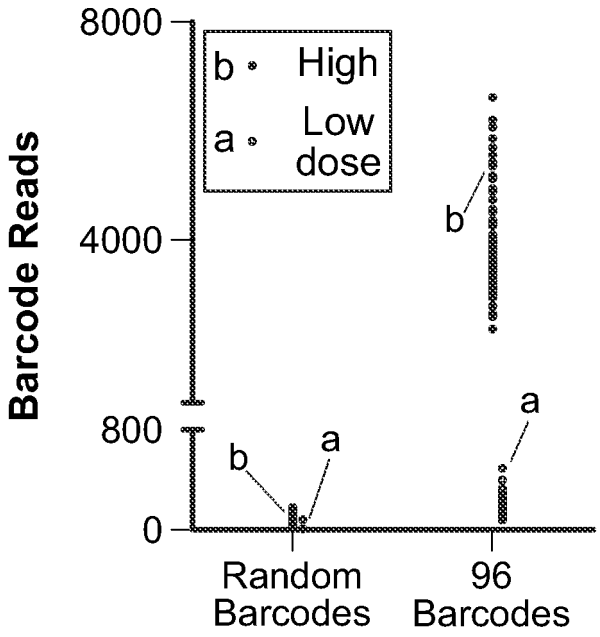


Fig. 12C

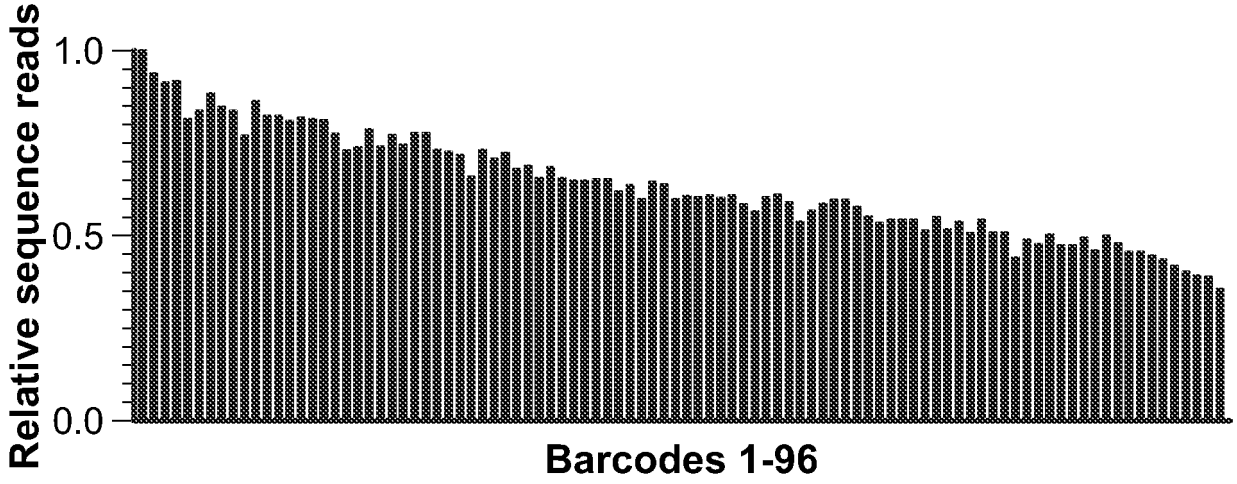


Fig. 12D

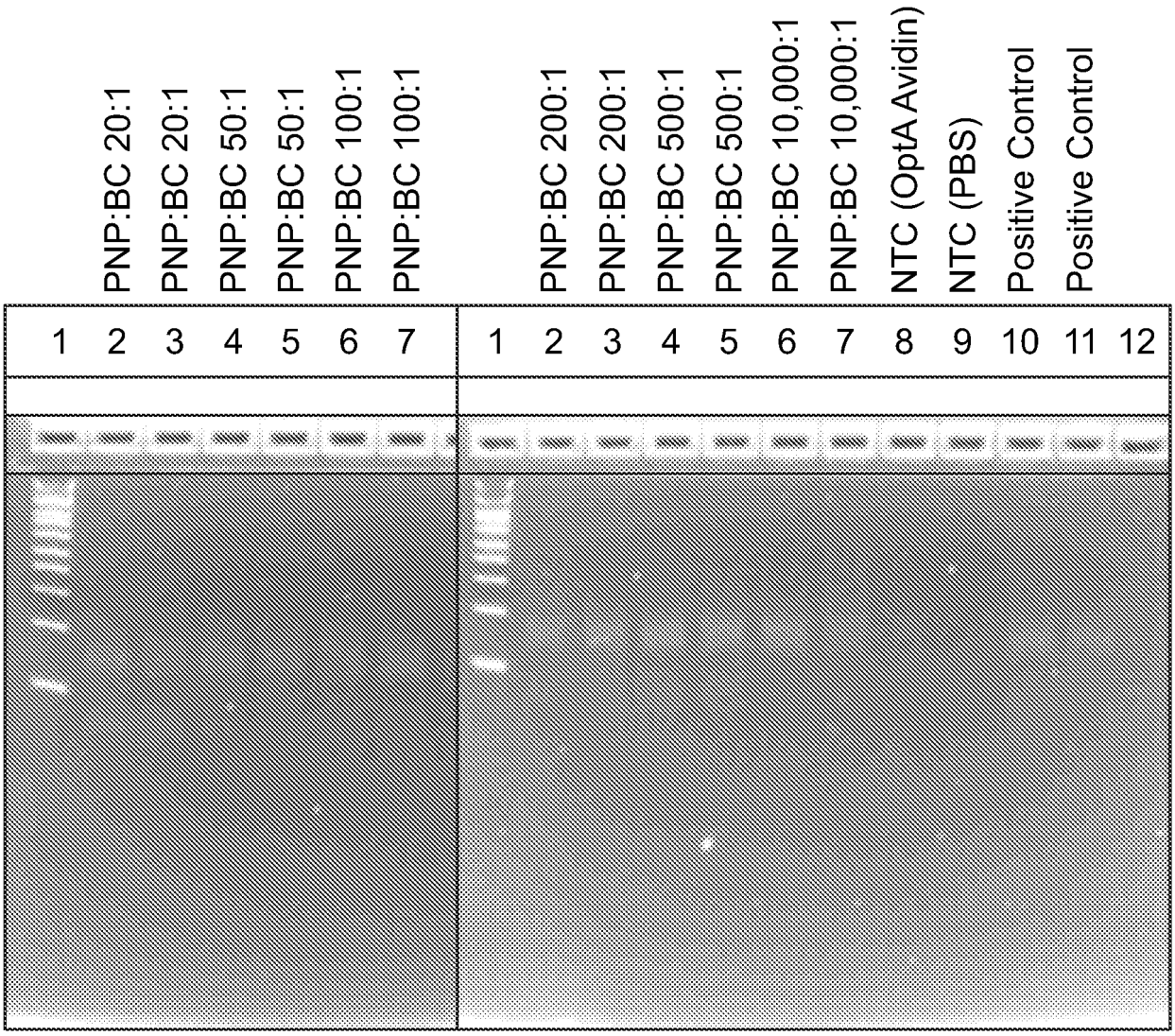

PNP:BC 20:1
PNP:BC 20:1
PNP:BC 50:1
PNP:BC 50:1
PNP:BC 100:1
PNP:BC 100:1
PNP:BC 200:1
PNP:BC 200:1
PNP:BC 500:1
PNP:BC 500:1
PNP:BC 10,000:1
PNP:BC 10,000:1
NTC (OptA Avidin)
NTC (PBS)
Positive Control
Positive Control
1 2 3 4 5 6 7
1 2 3 4 5 6 7 8 9 10 11 12

Fig. 13