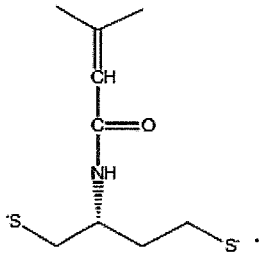
In some cases, after subjecting the reducing agent to the activation condition, the method further comprises cleaving the reducible cross-linkage.

[0016] In some cases, the reducible linkage comprises a disulfide linkage linking the nucleic acid molecule to the polymer matrix. In some cases, the nucleic acid molecule comprises a barcode sequence. In some cases, the reaction volume is provided in the aqueous interior of a droplet in a water-in-oil emulsion. In some cases, the reaction volume comprises an enzyme which can be, for example, a polymerase.

[0017] In another aspect, the disclosure provides a reducing agent comprising:



[0017a] In another aspect, the disclosure provides a method for nucleic acid amplification, comprising:

(a) providing a reagent in a reaction volume, wherein the reagent comprises a first component coupled to a second component through a reducible linkage, and wherein the reaction volume includes a template nucleic acid molecule and a stabilized reducing agent, wherein the stabilized reducing agent exhibits a lower rate of reaction with oxygen as compared to dithiothreitol and dithiobutylamine, and wherein the stabilized reducing agent is not tris(2-carboxyethyl)phosphine;

(b) using the stabilized reducing agent to cleave the reducible linkage, thereby decoupling the first component from the second component; and

(c) using the first component to subject the template nucleic acid molecule to an extension reaction to yield an extension product complementary to the template nucleic acid molecule.

[0017b] In another aspect, the disclosure provides a method for conducting a reduction reaction, comprising:

(a) providing a reagent in a reaction volume, wherein the reagent comprises a first component coupled to a second component through a reducible linkage, and wherein the reaction volume includes a reducing agent that is in an inactive state such that the reducing agent is substantially unreactive with the reducible linkage;

(b) subjecting the reducing agent to an activation condition such that the reducing agent becomes reactive with the reducible linkage; and

(c) permitting the reducing agent to react with the reducible linkage to decouple the first component from the second component,

wherein the activation condition cleaves a covalent bond of the reducing agent.

[0018] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0019] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative

Claims

1. A method for nucleic acid amplification, comprising:
   (a) providing a reagent in a reaction volume, wherein the reagent comprises a first component coupled to a second component through a reducible linkage, and wherein the reaction volume includes a template nucleic acid molecule and a stabilized reducing agent, wherein the stabilized reducing agent exhibits a lower rate of reaction with oxygen as compared to dithiothreitol and dithiobutylamine, and wherein the stabilized reducing agent is not tris(2-carboxyethyl)phosphine;
   (b) using the stabilized reducing agent to cleave the reducible linkage, thereby decoupling the first component from the second component; and
   (c) using the first component to subject the template nucleic acid molecule to an extension reaction to yield an extension product complementary to the template nucleic acid molecule.
2. The method of Claim 1, wherein the subjecting includes cycling a temperature of the reaction volume.
3. The method of Claim 1, wherein the stabilized reducing agent comprises a stabilized thiol containing compound.
4. The method of Claim 1, wherein the second component comprises a bead.
5. The method of Claim 1, wherein the first component comprises a nucleic acid molecule and the second component comprises a polymer matrix.
6. The method of Claim 5, wherein the polymer matrix comprises a hydrogel.
7. The method of Claim 5, wherein the reducible linkage comprises a disulfide linkage linking the nucleic acid molecule to the polymer matrix.
8. The method of Claim 5, wherein the nucleic acid molecule comprises a barcode sequence, and wherein in (a), the nucleic acid molecule comprises a primer that participates in the extension reaction.
9. The method of Claim 8, wherein, in (c), the extension product comprises the barcode sequence.
10. The method of Claim 1, wherein the reaction volume is provided in the aqueous interior of a droplet in a water-in-oil emulsion.

11. A method for conducting a reduction reaction, comprising:

(a) providing a reagent in a reaction volume, wherein the reagent comprises a first component coupled to a second component through a reducible linkage, and wherein the reaction volume includes a reducing agent that is in an inactive state such that the reducing agent is substantially unreactive with the reducible linkage;

(b) subjecting the reducing agent to an activation condition such that the reducing agent becomes reactive with the reducible linkage; and

(c) permitting the reducing agent to react with the reducible linkage to decouple the first component from the second component,

wherein the activation condition cleaves a covalent bond of the reducing agent.

12. The method of Claim 11, wherein, in (a), the reaction volume includes a template nucleic acid molecule.

13. The method of Claim 12, further comprising, after (c), using the first component to subject the template nucleic acid molecule to an extension reaction to yield an extension product complementary to the template nucleic acid molecule.

14. The method of Claim 11, wherein the reducing agent, in its inactive state, is a stabilized reducing agent, which stabilized reducing agent comprises a stabilized thiol containing compound.

15. The method of Claim 11, wherein the first component comprises a nucleic acid molecule, which nucleic acid molecule comprises a barcode sequence, and the second component comprises a polymer matrix.

16. The method of Claim 15, wherein the polymer matrix comprises a hydrogel.

17. The method of Claim 15, wherein the reducible linkage comprises a disulfide linkage linking the nucleic acid molecule to the polymer matrix.

18. The method of Claim 11, wherein the reaction volume is provided in the aqueous interior of a droplet in a water-in-oil emulsion.

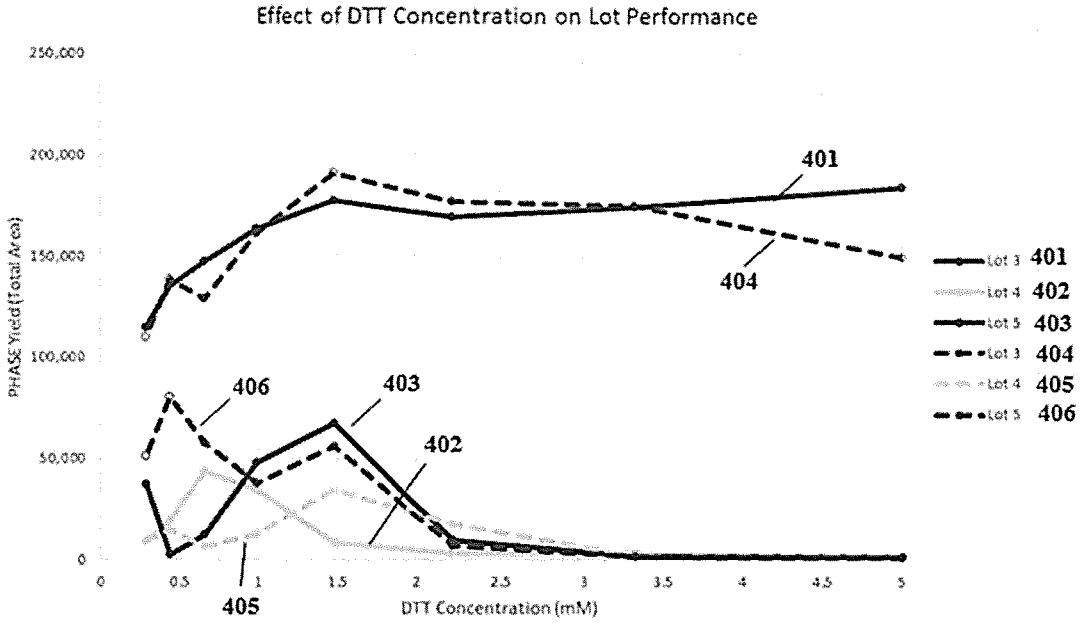

Effect of DTT Concentration on Lot Performance
PHASE Yield (Total Area)
250,000
200,000
150,000
100,000
50,000
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
DTT Concentration (mM)
401
404
406
403
402
405
Lot 3 401
Lot 4 402
Lot 5 403
Lot 3 404
Lot 4 405
Lot 5 406

FIG. 4

A

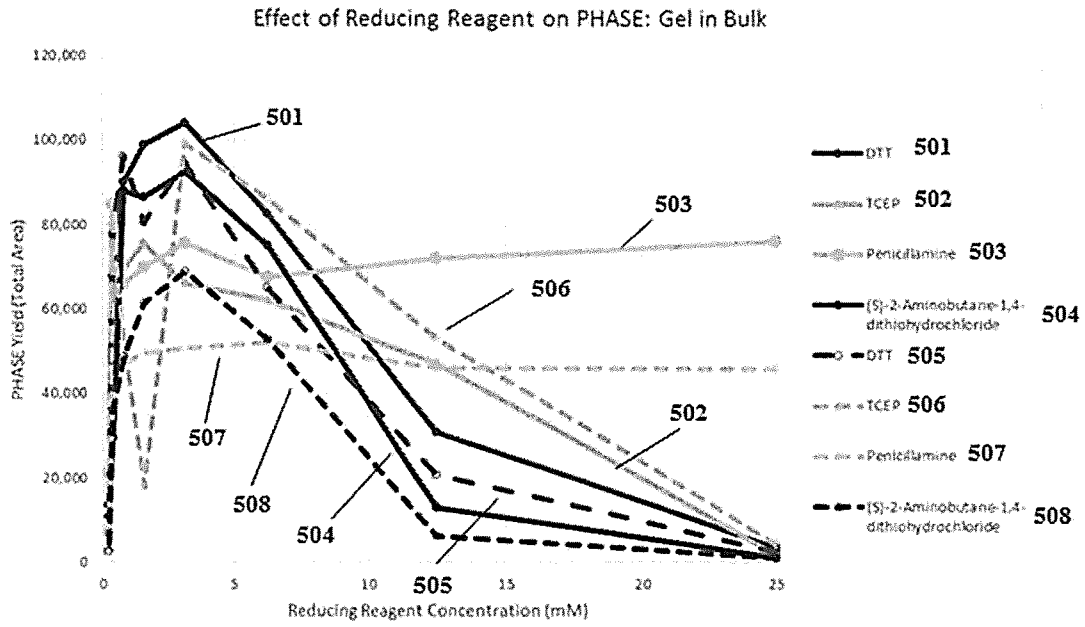

Effect of Reducing Reagent on PHASE: Gel in Bulk
PHASE Yield (Total Area)
120,000
100,000
80,000
60,000
40,000
20,000
0
0
5
10
15
20
25
Reducing Reagent Concentration (mM)
501
502
503
504
505
506
507
508
DTT 501
TCEP 502
Penicillamine 503
(S)-2-Aminobutane-1,4-dithiohydrochloride 504
DTT 505
TCEP 506
Penicillamine 507
(S)-2-Aminobutane-1,4-dithiohydrochloride 508

FIG. 5

B

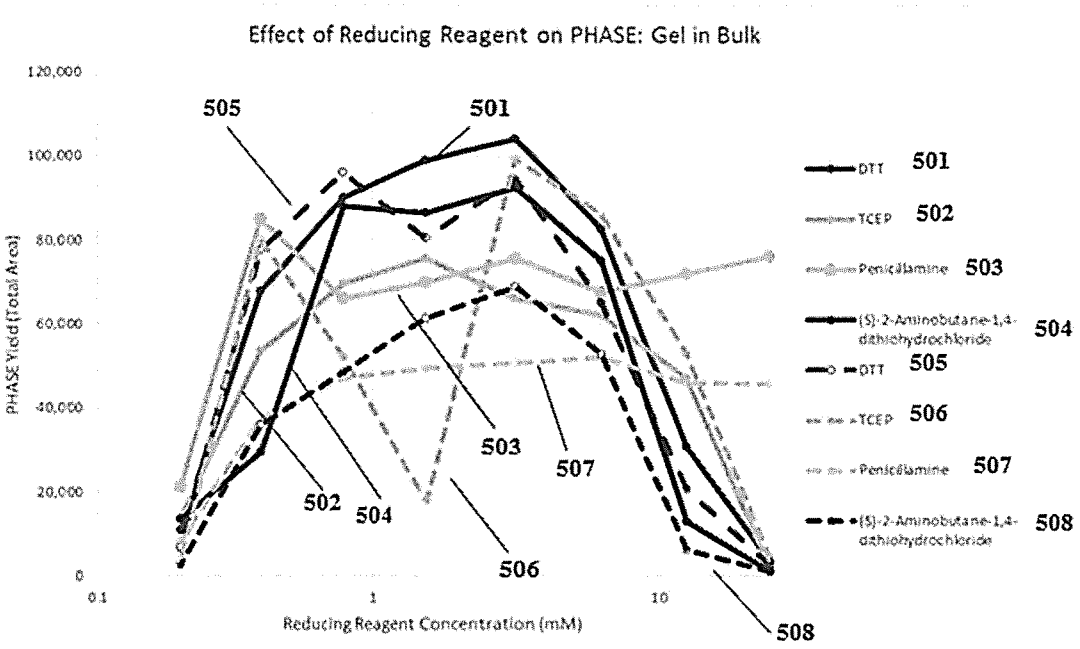

Effect of Reducing Reagent on PHASE: Gel in Bulk
PHASE Yield (Total Area)
120,000
100,000
80,000
60,000
40,000
20,000
0
0.1
1
10
Reducing Reagent Concentration (mM)
505
501
502
504
503
507
506
508
DTT 501
TCEP 502
Penicillamine 503
(S)-2-Aminobutane-1,4-dithiohydrochloride 504
DTT 505
TCEP 506
Penicillamine 507
(S)-2-Aminobutane-1,4-dithiohydrochloride 508

FIG. 5 (cont.)

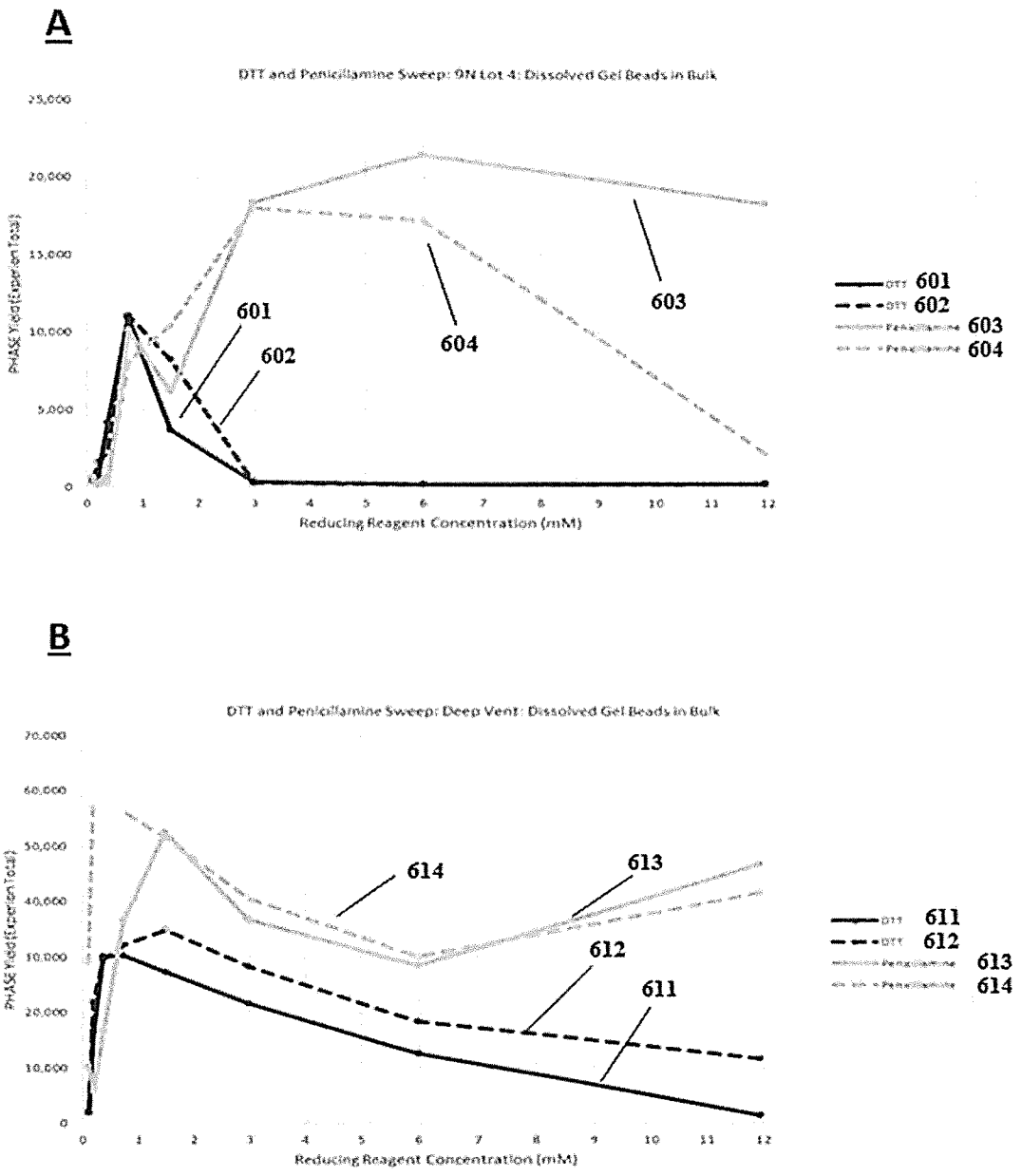

A
DTT and Penicillamine Sweep: 9N Lot 4: Dissolved Gel Beads in Bulk
PHASE Yield (Fraction Total)
Reducing Reagent Concentration (mM)
601
602
603
604
DTT 601
DTT 602
603
604
B
DTT and Penicillamine Sweep: Deep Vent: Dissolved Gel Beads in Bulk
PHASE Yield (Fraction Total)
Reducing Reagent Concentration (mM)
611
612
613
614
DTT 611
DTT 612
613
614

FIG. 6

C

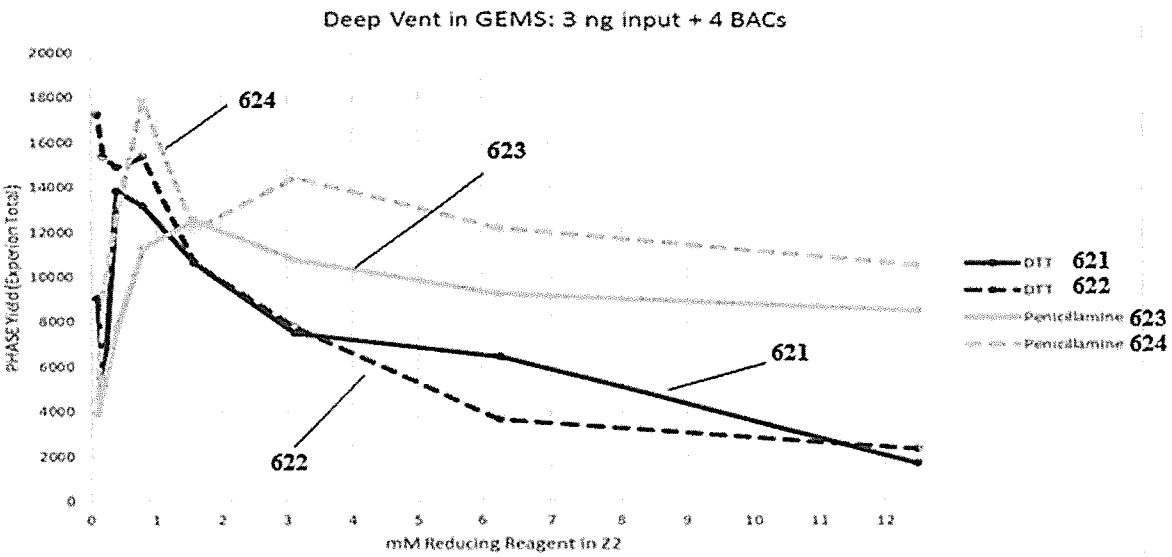

Deep Vent in GEMS: 3 ng input + 4 BACs
PHASE Yield (Experion Total)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
1
2
3
4
5
6
7
8
9
10
11
12
mM Reducing Reagent in Z2
624
623
621
622
DTT 621
DTT 622
Penicillamine 623
Penicillamine 624

FIG. 6 (cont.)

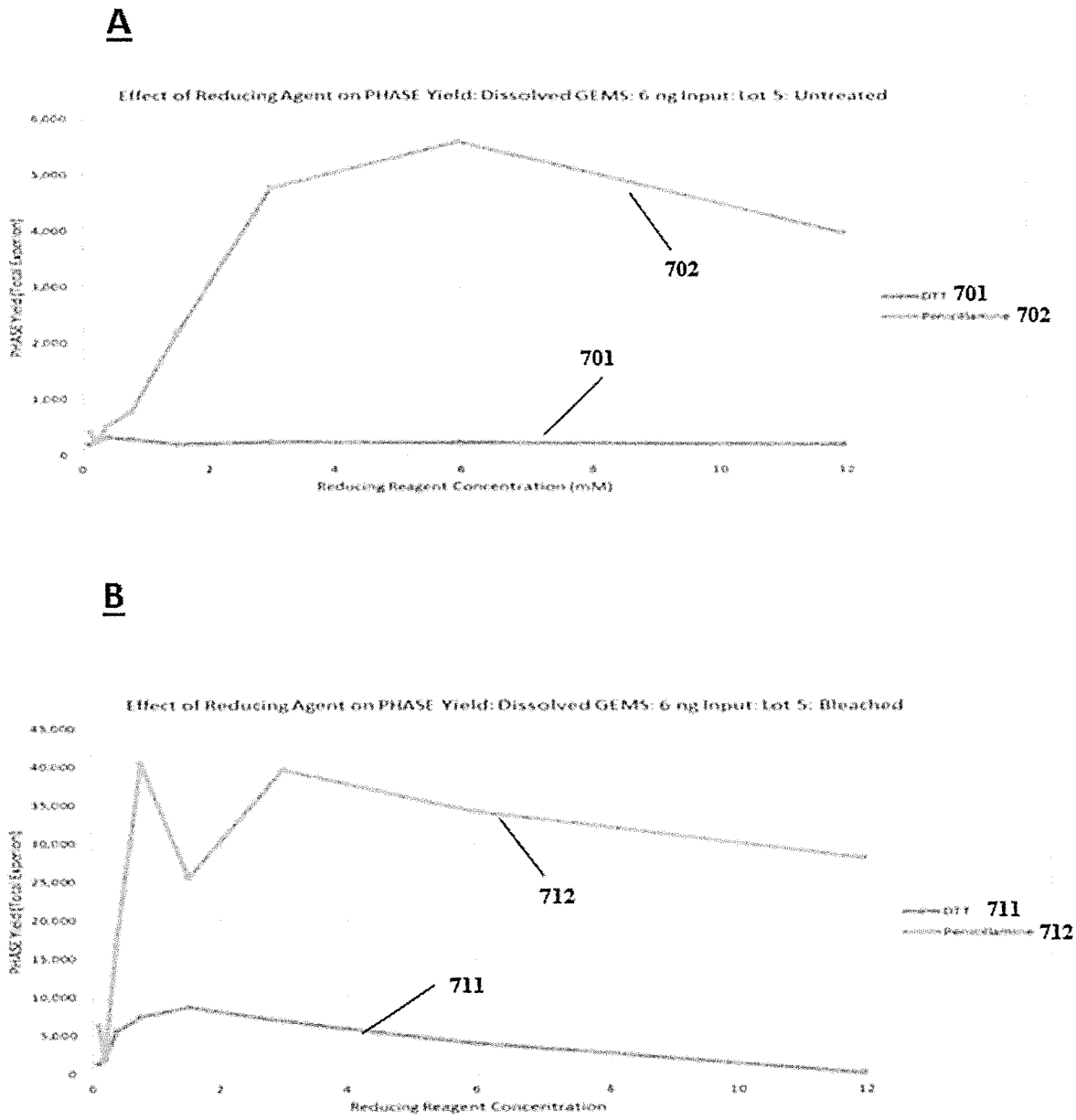

A
Effect of Reducing Agent on PHASE Yield: Dissolved GEMS: 6 ng Input: Lot 5: Untreated
PHASE Yield (Total Expansion)
Reducing Reagent Concentration (mM)
702
701
DTT 701
Penicillamine 702
B
Effect of Reducing Agent on PHASE Yield: Dissolved GEMS: 6 ng Input: Lot 5: Bleached
PHASE Yield (Total Expansion)
Reducing Reagent Concentration
712
711
DTT 711
Penicillamine 712

FIG. 7

C

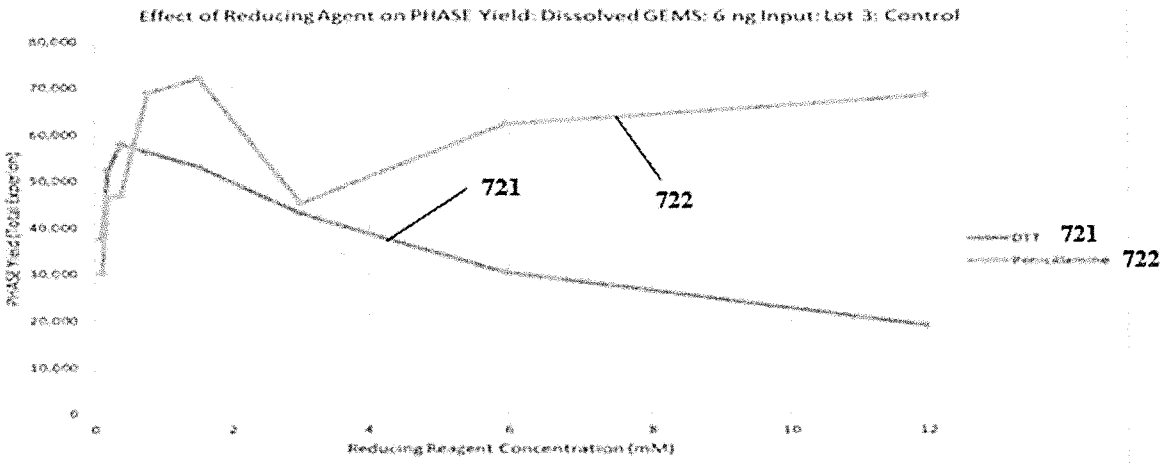
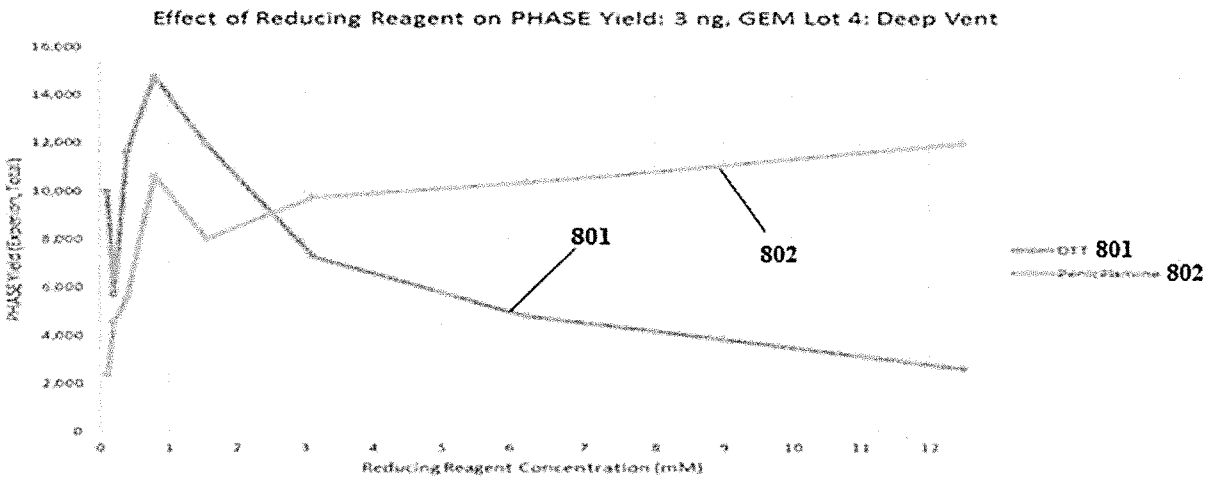

Effect of Reducing Agent on PHASE Yield: Dissolved GEMS: 6 ng Input: Lot 3: Control
PHASE Yield (Fragments)
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
0
2
4
6
8
10
12
Reducing Reagent Concentration (mM)
721
722
721
722

FIG. 7 (cont.)

A


Effect of Reducing Reagent on PHASE Yield: 3 ng, GEM Lot 4: Deep Vent
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
0
1
2
3
4
5
6
7
8
9
10
11
12
Reducing Reagent Concentration (mM)
801
802
DTT 801
802

B

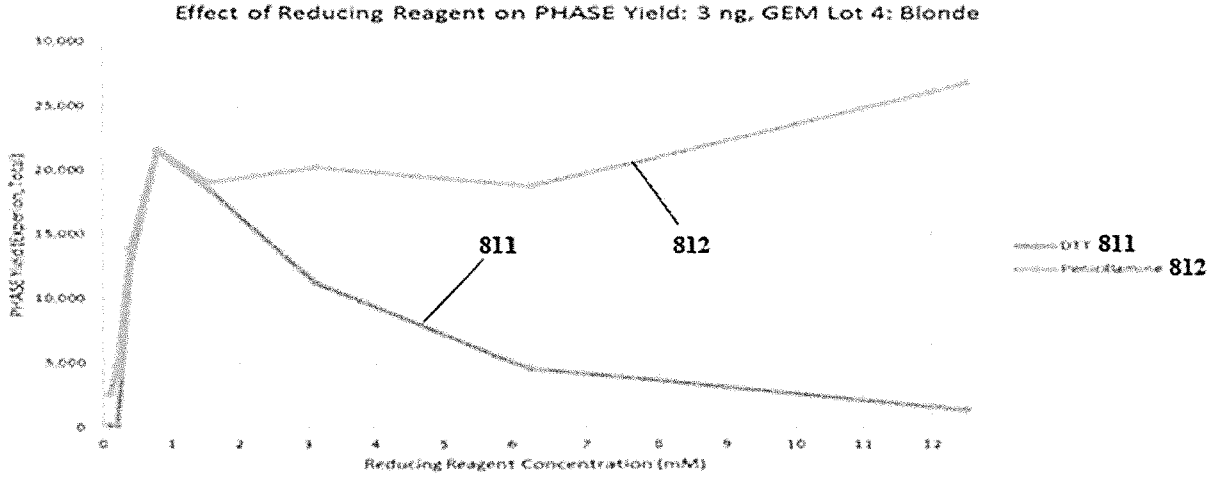

Effect of Reducing Reagent on PHASE Yield: 3 ng, GEM Lot 4: Blonde
30,000
25,000
20,000
15,000
10,000
5,000
0
0
1
2
3
4
5
6
7
8
9
10
11
12
Reducing Reagent Concentration (mM)
811
812
DTT 811
812

FIG. 8

C

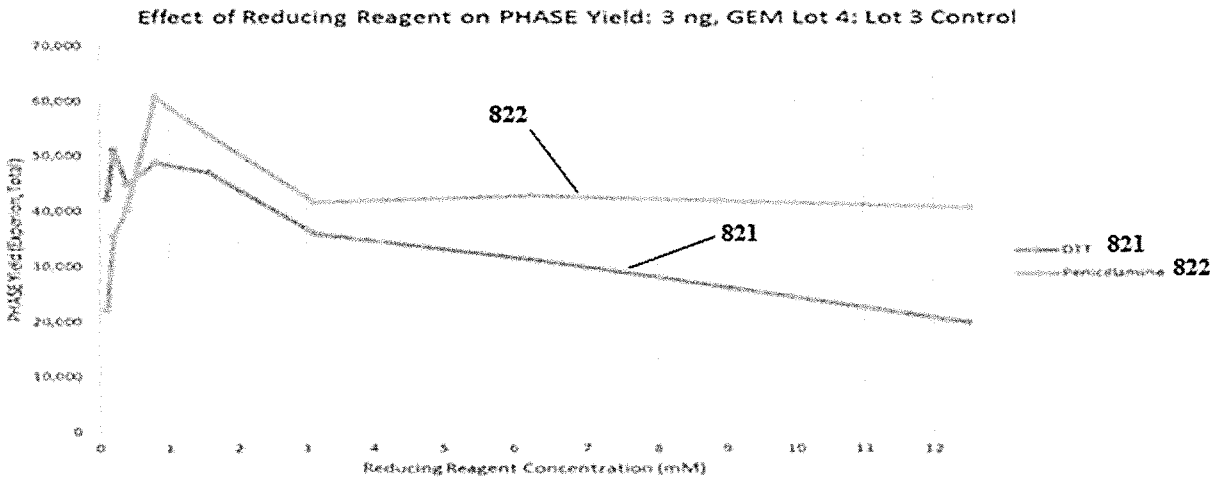
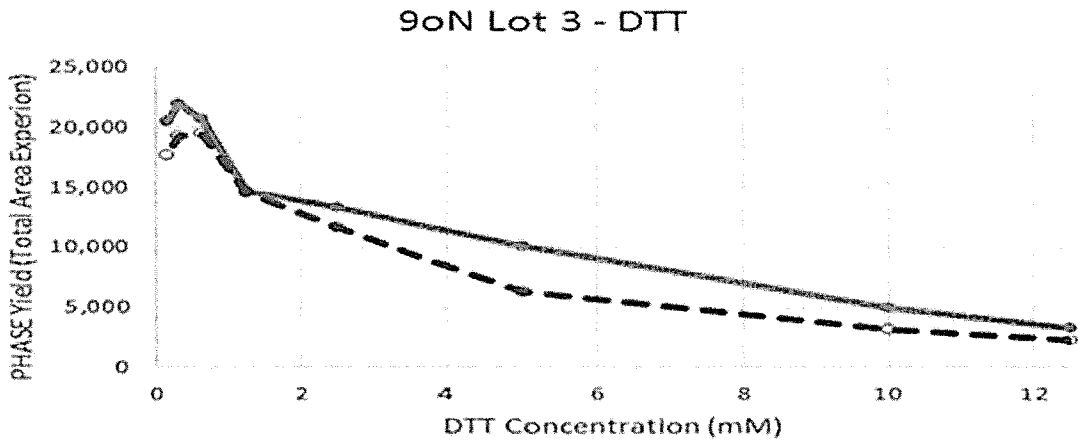

Effect of Reducing Reagent on PHASE Yield: 3 ng, GEM Lot 4: Lot 3 Control
822
821
821
822
Reducing Reagent Concentration (mM)

FIG. 8 (cont.)

A


9oN Lot 3 - DTT
PHASE Yield (Total Area Experion)
25,000
20,000
15,000
10,000
5,000
0
0
2
4
6
8
10
12
DTT Concentration (mM)

B

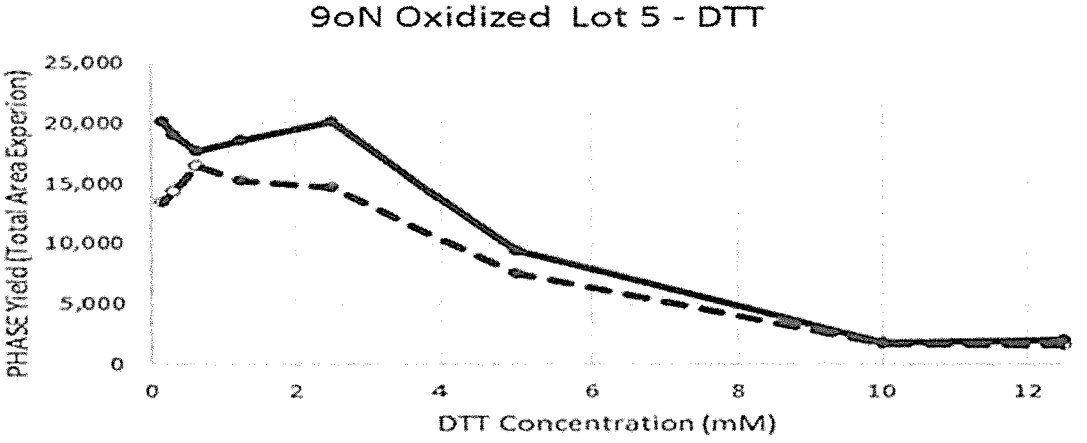

9oN Oxidized Lot 5 - DTT
PHASE Yield (Total Area Experion)
25,000
20,000
15,000
10,000
5,000
0
0
2
4
6
8
10
12
DTT Concentration (mM)

FIG. 9

C

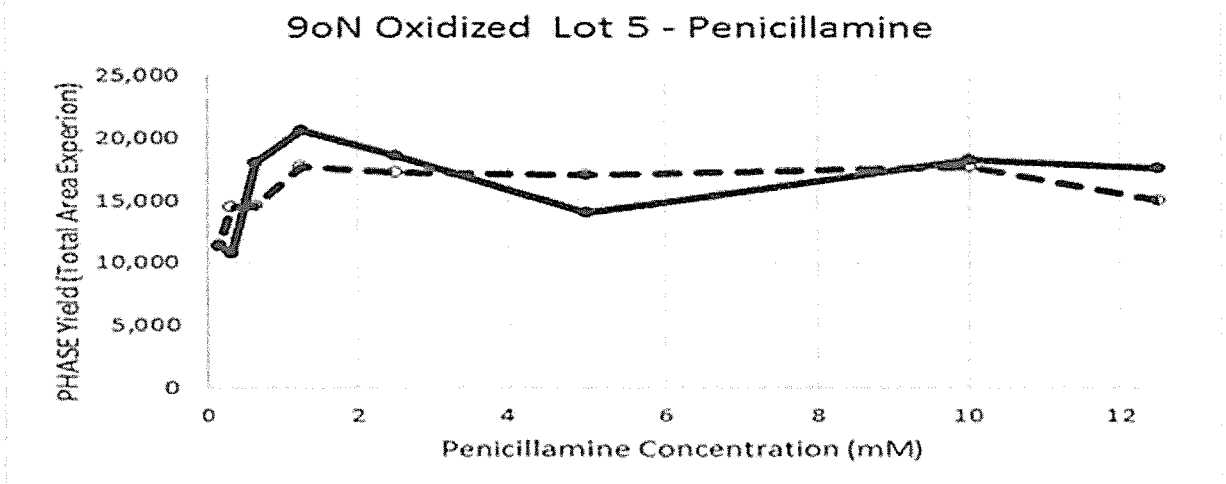

9oN Oxidized Lot 5 - Penicillamine
PHASE Yield (Total Area Experion)
25,000
20,000
15,000
10,000
5,000
0
0
2
4
6
8
10
12
Penicillamine Concentration (mM)

FIG. 9 (cont.)

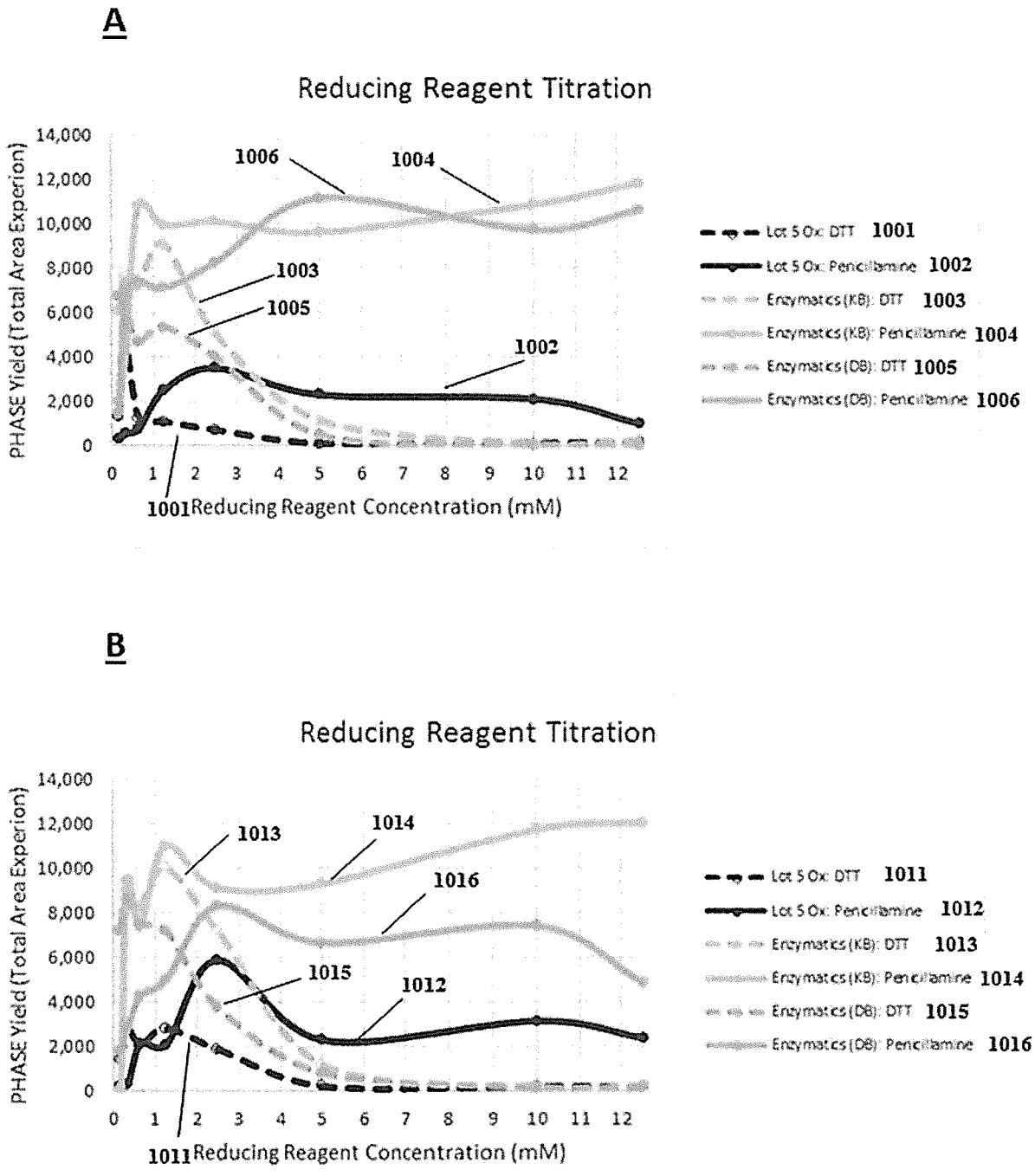

A
Reducing Reagent Titration
PHASE Yield (Total Area Experion)
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Reducing Reagent Concentration (mM)
1001
1002
1003
1004
1005
1006
Lot 5 Ox: DTT 1001
Lot 5 Ox: Penicillamine 1002
Enzymatics (KB): DTT 1003
Enzymatics (KB): Penicillamine 1004
Enzymatics (D8): DTT 1005
Enzymatics (D8): Penicillamine 1006
B
Reducing Reagent Titration
PHASE Yield (Total Area Experion)
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Reducing Reagent Concentration (mM)
1011
1012
1013
1014
1015
1016
Lot 5 Ox: DTT 1011
Lot 5 Ox: Penicillamine 1012
Enzymatics (KB): DTT 1013
Enzymatics (KB): Penicillamine 1014
Enzymatics (D8): DTT 1015
Enzymatics (D8): Penicillamine 1016

FIG. 10

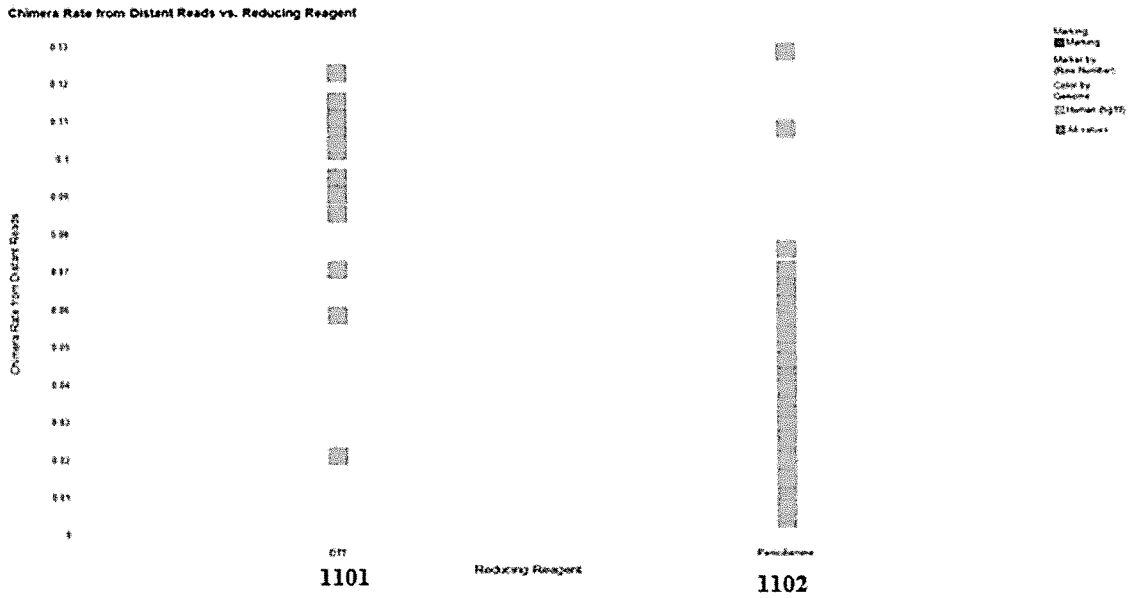

Chimera Rate from Distant Reads vs. Reducing Reagent
Chimera Rate from Distant Reads
Reducing Reagent
1101
1102

FIG. 11

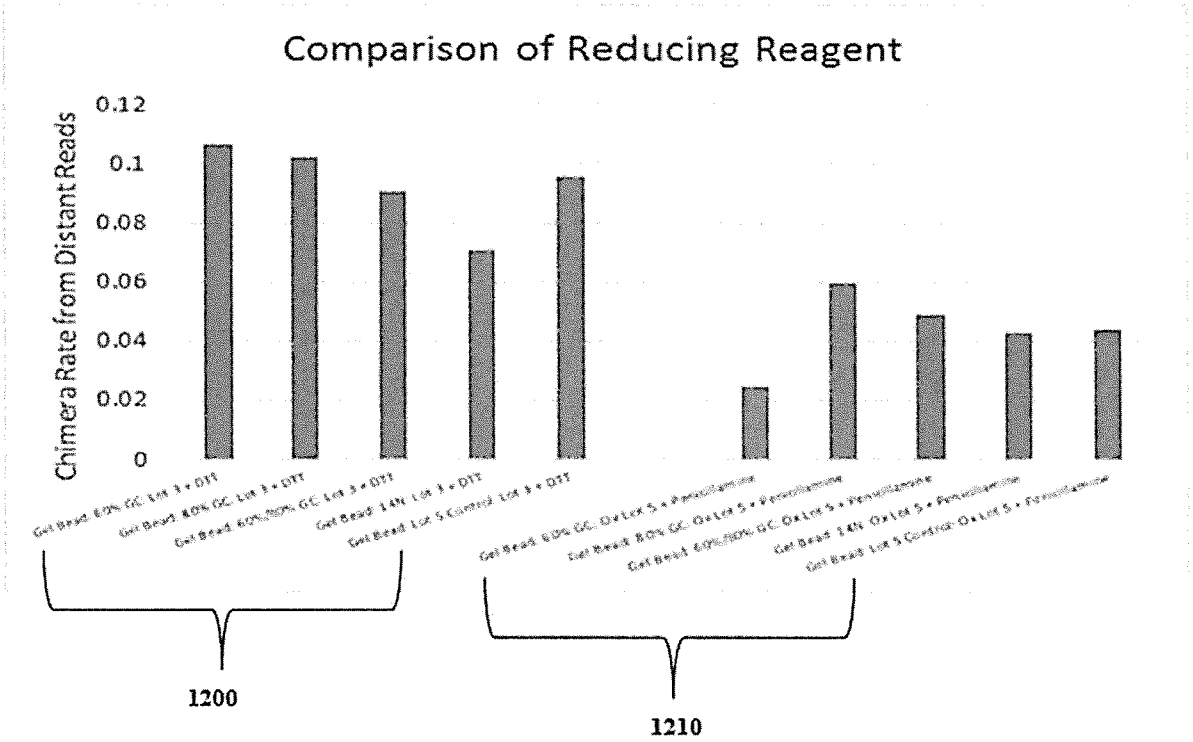

Comparison of Reducing Reagent
Chimera Rate from Distant Reads
0.12
0.1
0.08
0.06
0.04
0.02
0
1200
1210

FIG. 12