

CLAIMS

1. A method of sequencing a plurality of different target polynucleotides, comprising:
 - (a) contacting a solid support with an incorporation mixture comprising DNA polymerase and one or more of four different types of nucleotides, wherein the solid support comprises a plurality of different target polynucleotides immobilized thereon, and sequencing primers that are complementary and hybridized to at least a portion of the target polynucleotides;
 - (b) incorporating one type of nucleotides into the sequencing primers to produce extended copy polynucleotides, wherein each of the four types of nucleotides comprises a 3' blocking group;
 - (c) imaging and performing one or more fluorescent measurements of the extended copy polynucleotides; and
 - (d) removing the 3' blocking groups of the incorporated nucleotides in an aqueous cleavage solution comprising an active palladium catalyst;wherein the aqueous cleavage solution comprises one or more additives for improving thermal or oxidative stability of the active palladium catalyst, and wherein the one or more additives comprise one or more water soluble macrocycles.
2. The method of claim 1, wherein the active palladium catalyst is Pd(0).
3. The method of claim 2, wherein the Pd(0) is formed in situ from a Pd(II) complex and one or more water soluble phosphines.
4. The method of claim 3, wherein:
 - (i) the Pd(II) complex comprises [Pd(Allyl)Cl]₂, Na₂PdCl₄, K₂PdCl₄, Li₂PdCl₄, [Pd(Allyl)(THP)]Cl, [Pd(Allyl)(THP)₂]Cl, Pd(CH₃CN)₂Cl₂, Pd(OAc)₂, Pd(PPh₃)₄, Pd(dba)₂, Pd(Acac)₂, PdCl₂(COD), Pd(TFA)₂, Na₂PdBr₄, K₂PdBr₄, PdCl₂, PdBr₂, or Pd(NO₃)₂, or combinations thereof; optionally wherein the Pd(II) complex comprises [Pd(Allyl)Cl]₂ or Na₂PdCl₄; and/or
 - (ii) wherein the one or more water soluble phosphines comprise tris(hydroxypropyl)phosphine (THP), tris(hydroxymethyl)phosphine (THMP), 1,3,5-triaza-7-phosphaadamantane (PTA), bis(p-sulfonatophenyl)phenylphosphine dihydrate potassium salt, tris(carboxyethyl)phosphine (TCEP), or triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt, or combinations thereof; optionally wherein the one or more water soluble phosphines comprise THP.
5. The method of any one of claims 1 to 4, wherein:
 - (i) the one or more water soluble macrocycles comprise water soluble cyclodextrins, or optionally substituted analogs, salts or hydrates thereof; optionally wherein:

(a) the water soluble cyclodextrins or the analogs, salts or hydrates thereof comprise β -cyclodextrin, γ -cyclodextrin, or substituted analogs or salts thereof, or combinations thereof; or

(b) the substituted analogs of the water soluble cyclodextrin are independently substituted with one or more substituents selected from the group consisting of sulfonate, sulfo, hydroxy, carboxyl, succinyl, C1-C6 alkyl, C1-C6 alkyl substituted with sulfo, sulfonate, carboxyl, carboxylate or hydroxy, (C1-C6 alkyl)-C(=O)-, -C(=O)CH₃, -C(=O)Ph and a hydroxy protecting group, and combination thereof; and/or

(ii) the one or more water soluble cyclodextrins or the substituted analogs, salts or hydrates thereof are selected from the group consisting of sulfonated β -cyclodextrin, (2-hydroxypropyl)- β -cyclodextrin, methyl- β -cyclodextrin, acetyl- β -cyclodextrin, (2-hydroxyethyl)- β -cyclodextrin, triacetyl- β -cyclodextrin, heptakis(2,3,6-tri-O-methyl)- β -cyclodextrin, succinyl- β -cyclodextrin, heptakis(2,3,6-tri-O-benzoyl)- β -cyclodextrin, carboxymethyl- β -cyclodextrin, β -cyclodextrin hydrate, γ -cyclodextrin hydrate, (2-hydroxypropyl)- γ -cyclodextrin, and salts and combinations thereof, optionally

wherein the one or more water soluble cyclodextrins comprise sulfonated β -cyclodextrin, or a salt thereof; or

(iii) the one or more water soluble macrocycles comprise water soluble calixarenes, or optionally substituted analogs, salts or hydrates thereof; optionally

wherein the water soluble calixarenes or optionally substituted analogs, salts or hydrates thereof are selected from the group consisting of 4-sulfocalix[4]arene, 4-sulfocalix[6]arene hydrate, and 4-sulfothiacalix[4]arene sodium salt, and combinations thereof; or

(iv) the one or more water soluble macrocycles comprise water soluble cucurbiturils, or optionally substituted analogs, salts or hydrates thereof; optionally

wherein the water soluble cucurbiturils or optionally substituted analogs, salts or hydrates thereof are selected from the group consisting of cucurbit[5]uril hydrate, cucurbit[6]uril hydrate, cucurbit[7]uril hydrate, and cucurbit[8]uril hydrate, and combinations thereof.

6. The method of any one of claims 1 to 5, wherein the aqueous cleavage solution further comprises one or more oxygen scavengers and/or phosphine reducing agents, optionally wherein:

(i) the one or more oxygen scavengers comprise sodium sulfite, sodium bisulfite, or sodium metabisulfite, or combinations thereof; or

(ii) the one or more phosphine reducing agents comprise borohydrides, boranes, or silatrane, or combinations thereof.

7. The method of any one of claims 1 to 6, wherein:

(i) the one or more additives in the aqueous cleavage solution prevent or reduce the formation of palladium clusters; and/or

(ii) the one or more additives in the aqueous cleavage solution prevent or reduce the oxidation and/or thermal degradation of the active Pd catalyst.

8. The method of any one of claims 1 to 7, wherein:

(i) the method further comprising (e) washing the solid support with an aqueous wash solution, optionally

wherein steps (a) to (e) are repeated at least 50, 100, 150, 200, 250 or 300 cycles to determine the target polynucleotide sequences; and/or

(ii) the aqueous wash solution comprises at least one Pd(II) scavenger; and/or

(iii) the incorporation mixture and/or the aqueous wash solution further comprises at least one Pd(0) scavenger.

9. The method of any one of claims 1 to 8, wherein the one or more of four different types of nucleotides comprises A, C, G and T or U, and at least one type of nucleotides also carries a detectable label; optionally

wherein the aqueous cleavage solution also removes the detectable label.

10. A method for improving the stability of a composition comprising an active palladium catalyst, comprising:

mixing an aqueous composition comprising a Pd(0) catalyst with one or more additives for improving thermal or oxidative stability of the active palladium catalyst, wherein the one or more additives comprise one or more water soluble macrocycles.

11. The method of claim 10, wherein:

(i) the Pd(0) is formed in situ from a Pd(II) complex and one or more water soluble phosphines; optionally

wherein the Pd(II) complex comprises [Pd(Allyl)Cl]₂, Na₂PdCl₄, K₂PdCl₄, Li₂PdCl₄, [Pd(Allyl)(THP)]Cl, [Pd(Allyl)(THP)₂]Cl, Pd(CH₃CN)₂Cl₂, Pd(OAc)₂, Pd(PPh₃)₄, Pd(dba)₂, Pd(Acac)₂, PdCl₂(COD), Pd(TFA)₂, Na₂PdBr₄, K₂PdBr₄, PdCl₂, PdBr₂, or Pd(NO₃)₂, or combinations thereof; and/or

(ii) the one or more water soluble phosphines comprise tris(hydroxypropyl)phosphine (THP), tris(hydroxymethyl)phosphine (THMP), 1,3,5-triaza-7-phosphaadamantane (PTA), bis(p-sulfonatophenyl)phenylphosphine dihydrate potassium salt, tris(carboxyethyl)phosphine (TCEP), or triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt, or combinations thereof.

12. The method of any one of claims 10 to 11, wherein:

(i) the one or more water soluble macrocycles comprise water soluble cyclodextrins, or optionally substituted analogs, salts or hydrates thereof; optionally wherein the water soluble

cyclodextrins or the optionally substituted analogs, salts or hydrates thereof comprise β -cyclodextrin, γ -cyclodextrin, or substituted analogs or salts thereof, or combination thereof; further optionally

wherein the substituted analogs of the water soluble cyclodextrins are independently substituted with one or more substituents selected from the group consisting of sulfonate, sulfo, hydroxy, carboxyl, succinyl, C1-C6 alkyl, C1-C6 alkyl substituted with sulfo, sulfonate, carboxyl, carboxylate or hydroxy, (C1-C6 alkyl)-C(=O)-, -C(=O)CH₃, -C(=O)Ph, and a hydroxy protecting group, and combinations thereof; and/or

(ii) the one or more water soluble cyclodextrins or the substituted analogs, salts or hydrates thereof are selected from the group consisting of sulfonated β -cyclodextrin, (2-hydroxypropyl)- β -cyclodextrin, methyl- β -cyclodextrin, acetyl- β -cyclodextrin, (2-hydroxyethyl)- β -cyclodextrin, triacetyl- β -cyclodextrin, heptakis(2,3,6-tri-O-methyl)- β -cyclodextrin, succinyl- β -cyclodextrin, heptakis(2,3,6-tri-O-benzoyl)- β -cyclodextrin, carboxymethyl- β -cyclodextrin, β -cyclodextrin hydrate, γ -cyclodextrin hydrate, (2-hydroxypropyl)- γ -cyclodextrin, and salts and combinations thereof; and/or

(iii) the one or more water soluble macrocycles comprise water soluble calixarenes, or optionally substituted analogs, salts or hydrates thereof; optionally

wherein the water soluble calixarenes or optionally substituted analogs, salts or hydrates thereof are selected from the group consisting of 4-sulfocalix[4]arene, 4-sulfocalix[6]arene hydrate, and 4-sulfothiacalix[4]arene sodium salt, and combinations thereof.

13. The method of any one of claims 10 to 11, wherein:

(i) the one or more water soluble macrocycles comprise water soluble cucurbiturils, or optionally substituted analogs, salts or hydrates thereof, optionally

wherein the water soluble cucurbiturils or optionally substituted analogs, salts or hydrates thereof are selected from the group consisting of cucurbit[5]uril hydrate, cucurbit[6]uril hydrate, cucurbit[7]uril hydrate, and cucurbit[8]uril hydrate, and combinations thereof; and/or

(ii) the aqueous composition further comprises one or more oxygen scavengers and/or phosphine reducing agents, optionally wherein:

(a) the one or more oxygen scavengers comprise sodium sulfite, sodium bisulfite, or sodium metabisulfite, or combinations thereof; or

(b) the one or more phosphine reducing agents comprise borohydrides, boranes, or silatrane, or combinations thereof; and/or

(iii) the one or more additives in the aqueous composition prevent or reduce the formation of palladium clusters; and/or

(iv) the one or more additives in the aqueous composition prevent or reduce the oxidation and/or thermal degradation of the active Pd(0) catalyst.

14. A kit for use with a sequencing apparatus, comprising:

an aqueous cleavage mixture comprising an active Pd(0) catalyst; and

one or more additives for improving thermal or oxidative stability of the active Pd(0) catalyst, and wherein the one or more additives comprise one or more water soluble macrocycles.

15. The kit of claim 14, wherein the Pd(0) is formed in situ from a Pd(II) complex and one or more water soluble phosphines; optionally

(i) wherein the Pd(II) complex comprises [Pd(Allyl)Cl]₂, Na₂PdCl₄, K₂PdCl₄, Li₂PdCl₄, [Pd(Allyl)(THP)]Cl, [Pd(Allyl)(THP)₂]Cl, Pd(CH₃CN)₂Cl₂, Pd(OAc)₂, Pd(PPh₃)₄, Pd(dba)₂, Pd(Acac)₂, PdCl₂(COD), Pd(TFA)₂, Na₂PdBr₄, K₂PdBr₄, PdCl₂, PdBr₂, or Pd(NO₃)₂, or combinations thereof; optionally

wherein the Pd(II) complex comprises [Pd(Allyl)Cl]₂ or Na₂PdCl₄; and/or

(ii) wherein the one or more water soluble phosphines comprise tris(hydroxypropyl)phosphine (THP), tris(hydroxymethyl)phosphine (THMP), 1,3,5-triaza-7-phosphaadamantane (PTA), bis(p-sulfonatophenyl)phenylphosphine dihydrate potassium salt, tris(carboxyethyl)phosphine (TCEP), or triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt, or combinations thereof; optionally

wherein the one or more water soluble phosphines comprise THP.

16. The kit of any one of claims 14 to 15, wherein:

(i) water soluble macrocycles comprise water soluble cyclodextrins, or optionally substituted analogs, salts or hydrates thereof; optionally wherein:

(a) the water soluble cyclodextrins or the optionally substituted analogs, salts or hydrates thereof comprise β-cyclodextrin, γ-cyclodextrin, or substituted analogs or salts thereof, or combinations thereof; or

(b) the substituted analogs of the water soluble cyclodextrins are independently substituted with one or more substituents selected from the group consisting of sulfonate, sulfo, hydroxy, carboxyl, succinyl, C1-C6 alkyl, C1-C6 alkyl substituted with sulfo, sulfonate, carboxyl, carboxylate, or hydroxy, (C1-C6 alkyl)-C(=O)-, -C(=O)CH₃, -C(=O)Ph and a hydroxy protecting group, and combinations thereof; and/or

(ii) the one or more water soluble cyclodextrins or the substituted analogs, salts or hydrates thereof are selected from the group consisting of sulfonated β-cyclodextrin, (2-hydroxypropyl)-β-cyclodextrin, methyl-β-cyclodextrin, acetyl-β-cyclodextrin, (2-hydroxyethyl)-β-cyclodextrin, triacetyl-β-cyclodextrin, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin, succinyl-β-cyclodextrin, heptakis(2,3,6-tri-O-benzoyl)-β-cyclodextrin, carboxymethyl-β-cyclodextrin, β-cyclodextrin

hydrate, γ -cyclodextrin hydrate, (2-hydroxypropyl)- γ -cyclodextrin, and salts and combinations thereof; optionally

wherein the one or more water soluble cyclodextrins comprise sulfonated β -cyclodextrin, or a salt thereof; and/or

(iii) the aqueous cleavage solution further comprises one or more oxygen scavengers and/or phosphine reducing agents; optionally

wherein the one or more oxygen scavengers and/or phosphine reducing agents comprise sodium sulfite, sodium bisulfite, sodium metabisulfite, silatrane, borohydrides, boranes, or combinations thereof.

17. The kit of any one of claims 14 to 16, further comprising an incorporation mixture, wherein the incorporation mixture comprising a DNA polymerase and one or more of four different types of nucleotides, each having a 3' blocking group, optionally wherein:

(a) the 3' blocking group comprises an optionally substituted allyl group; optionally

(b) the 3' blocking group has the structure attached to the 3' oxygen of the nucleotide.

18. The kit of claim 17, wherein the incorporation mixture further comprises at least one Pd(0) scavenger.

19. The kit of any one of claims 14 to 18, further comprising an aqueous wash solution, or a composition that is reconstitutable into an aqueous wash solution; optionally

wherein the aqueous wash solution comprises at least one Pd(II) scavenger.

20. A cartridge for use with a sequencing apparatus, comprising a plurality of chambers, wherein one or more of the plurality of chambers is for use with a kit according to any one of claims 14 to 19.