CLAIMS

1. A method for determining sequences of a plurality of target polynucleotides, comprising:

(a) contacting a solid support with sequencing primers under hybridization conditions, wherein the solid support comprises a plurality of target polynucleotides immobilized thereon; and the sequencing primers are complementary to at least a portion of the target polynucleotides;

(b) contacting the solid support with a first aqueous solution comprising DNA polymerase and one or more of four different types of nucleotides under conditions suitable for DNA polymerase-mediated primer extension, wherein each of the nucleotides comprises a 3′ blocking group comprising an unsubstituted or substituted allyl group;

(c) incorporating one type of nucleotides into the sequencing primers to produce extended copy polynucleotides;

(d) performing one or more fluorescent measurements of the extended copy polynucleotides;

(e) removing the 3′ blocking group of the incorporated nucleotides in a second aqueous solution comprising a transition metal catalyst and one or more reducing agents, the transition metal catalyst is generated from a palladium or nickel complex with a water-soluble non-reducing phosphine or a N,N-bidentate non-phosphine ligand, and wherein the one or more reducing agents comprise a boron-containing reducing agent; and

(f) washing the solid support with a third aqueous solution after the removal of the 3′ blocking group of the incorporated nucleotides.

2. The method of claim 1, further comprising: repeating steps (b) through (f) until sequences of at least a portion of the target polynucleotides are determined.

3. The method of claim 2, wherein steps (b) through (f) are repeated at least 50 times, at least 100 times, at least 150 times, at least 200 times, at least 250 times, or at least 300 times.

4. The method of claim 1, wherein the transition metal catalyst is Pd(0) catalyst, and the Pd(0) catalyst is generated from the palladium complex with the water-soluble non-reducing phosphine.

5. The method of claim 1, wherein the water-soluble non-reducing phosphine comprises tris(substituted C_1-C_6 alkyl)phosphine or tris(substituted C_6-C_{10} aryl)phosphine, or a combination thereof.

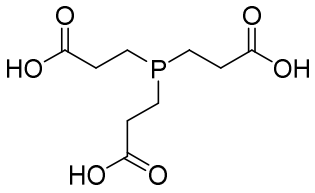
6. The method of claim 5, wherein the transition metal catalyst is a Pd(0) catalyst generated *in situ* from the palladium complex and the water-soluble non-reducing tris(substituted C_1-C_6 alkyl)phosphine.

7. The method of claim 6, wherein the palladium complex comprises $[Pd(Allyl)Cl]_2$, Na_2PdCl_4, K_2PdCl_4, Li_2PdCl_4, [Pd(Allyl)(THPP)]Cl, $[Pd(Allyl)(THPP)_2]Cl$, $Pd(CH_3CN)_2Cl_2$, $Pd(OAc)_2$, $Pd(PPh_3)_4$, $Pd(dba)_2$, $Pd(Acac)_2$, $PdCl_2(COD)$, $Pd(TFA)_2$, Na_2PdBr_4, K_2PdBr_4, $PdCl_2$, $PdBr_2$, or $Pd(NO_3)_2$, or combinations thereof.

8. The method of claim 7, wherein the palladium complex comprises $[Pd(Allyl)Cl]_2$ or Na_2PdCl_4.

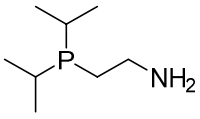
9. The method of claim 5, wherein the tris(substituted C_1-C_6 alkyl)phosphine is a tris(carboxy substituted C_1-C_6 alkyl)phosphine.

10. The method of claim 9, wherein the tris(substituted C_1-C_6 alkyl)phosphine is

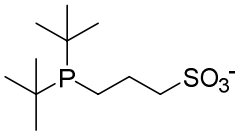


(TCEP).

11. The method of claim 5, wherein the tris(substituted C_1-C_6 alkyl)phosphine is:



(DIPA) or

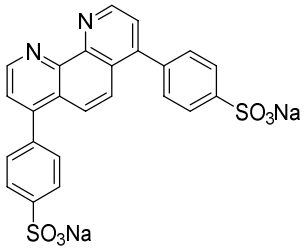


(diBuPpropSO$_3$).

12. The method of claim 1, wherein the molar ratio of the palladium complex and the water-soluble non-reducing phosphine is about 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, 1:5, 1:5.5, 1:6, 1:6.5, 1:7, 1:7.5, 1:8, 1:8.5, 1:9, 1:9.5 or 1:10.

13. The method of claim 1, wherein the transition metal catalyst is Pd(0) catalyst, and the Pd(0) catalyst is generated from the palladium complex with the N,N-bidentate non-phosphine ligand.

14. The method of claim 13, wherein the N,N-bidentate ligand is selected from the group consisting of:



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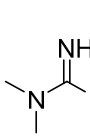

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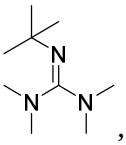

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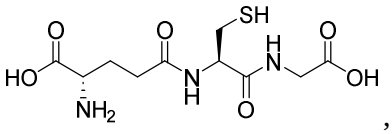

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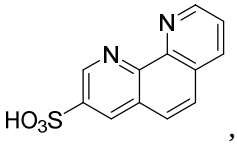

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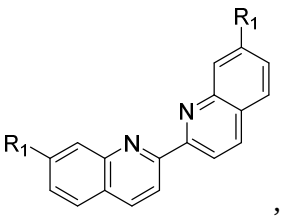

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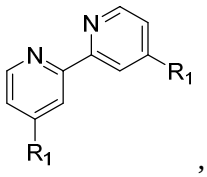

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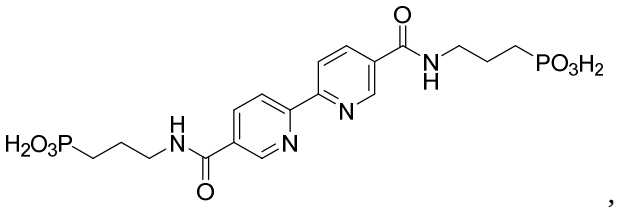
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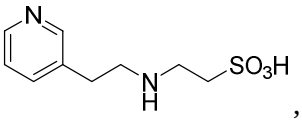
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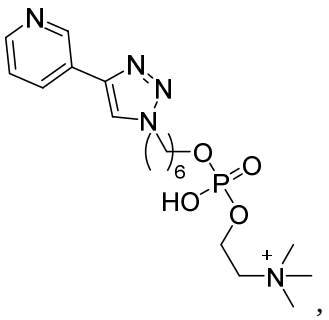
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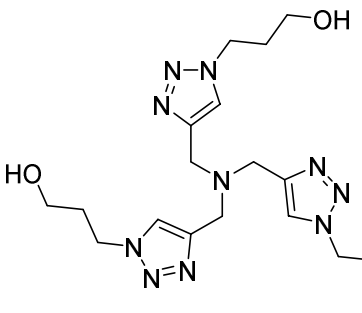
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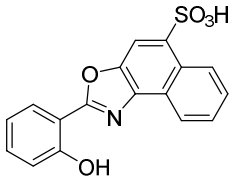
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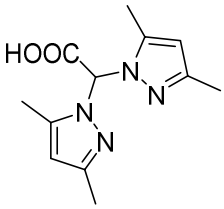
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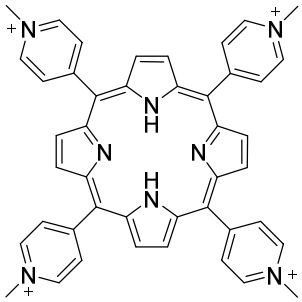
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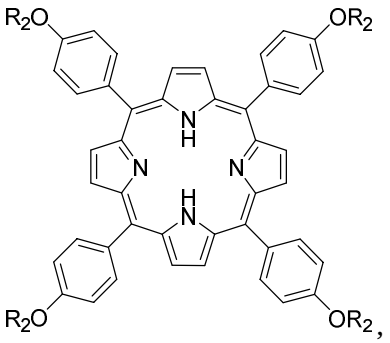
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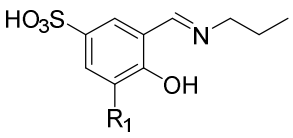
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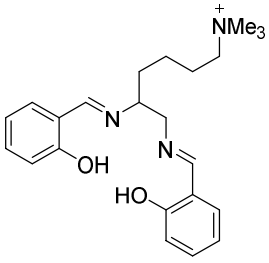
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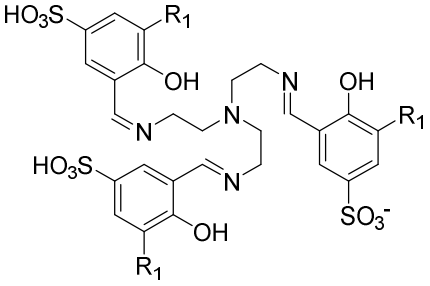
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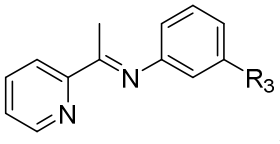
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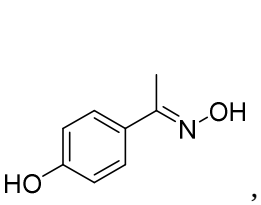
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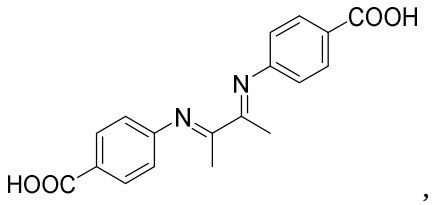
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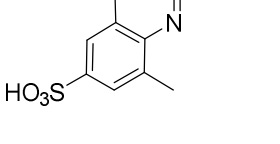
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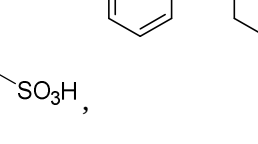
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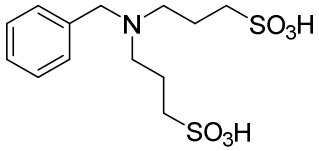
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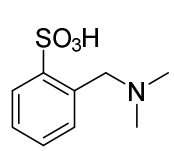
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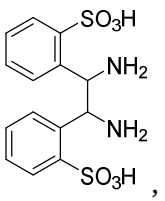
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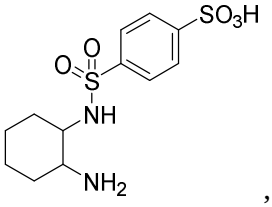
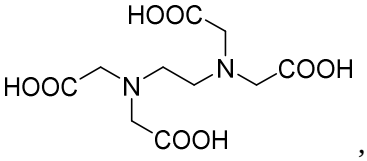
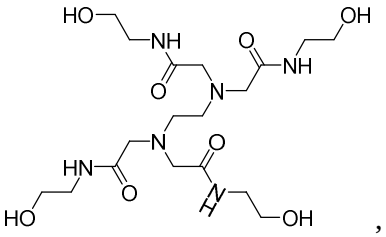
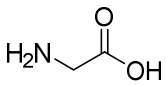
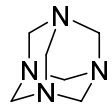
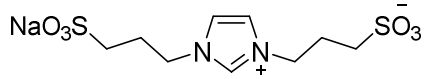
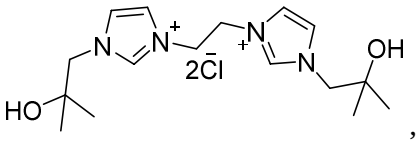
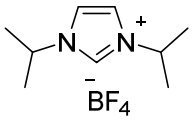
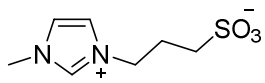
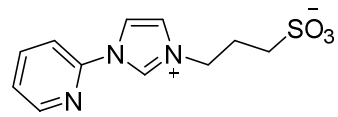
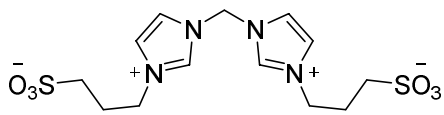
 ,  ,  ,

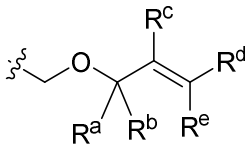
 ,  ,  ,  ,

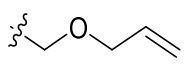
 ,  ,  , and

 , and salts thereof; wherein R_1 is independently $-SO_3H$, -COOH, $-PO_3H_2$, or $-NMe_3^+$; R_2 is $-(CH_2)_{1-3}COOH$; and R_3 is $-SO_3H$ or -COOH.

15. The method of claim 1, wherein the boron-containing reducing agent is NH_3BH_3 or $B_2(OH)_4$.

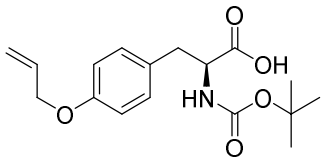
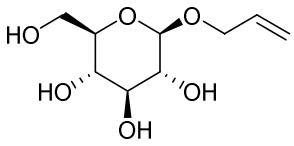
16. The method of claim 1, wherein the pH of the second aqueous solution is from about 7.0 to about 10, or from about 7.5 to about 9.5.

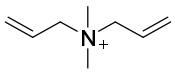
17. The method of claim 1, wherein the 3′ blocking group has the structure  attached to the 3′ oxygen of the nucleotide, wherein each of R^a, R^b, R^c, R^d and R^e is independently H, halogen, unsubstituted or substituted C_1-C_6 alkyl, or C_1-C_6 haloalkyl.

18. The method of claim 17, wherein the 3´ blocking group of the nucleotide has the structure  attached to the 3′ oxygen of the nucleotide.

19. The method claim 1, wherein the first aqueous solution or the third aqueous solution comprises at least one Pd(0) scavenger.

20. The method of claim 19, wherein the Pd(0) scavenger comprises one or more allyl moieties selected from the group consisting of –O-allyl, –S-allyl, –NR-allyl, and $-N^+RR'$-allyl, and combinations thereof, wherein R is H, unsubstituted or substituted C_1-C_6 alkyl, unsubstituted or substituted C_2-C_6 alkenyl, unsubstituted or substituted C_2-C_6 alkynyl, unsubstituted or substituted C_6-C_{10} aryl, unsubstituted or substituted 5 to 10 membered heteroaryl, unsubstituted or substituted C_3-C_{10} carbocyclyl, or unsubstituted or substituted 5 to 10 membered heterocyclyl; and R′ is H, unsubstituted C_1-C_6 alkyl or substituted C_1-C_6 alkyl.

21. The method of claim 20, wherein the Pd(0) scavenger comprising one or more –O-allyl moieties is  (N-Boc tyrosine(allyl)-OH) or  (allyl-β-D-gluocopyranoside), or a salt thereof.

22. The method of claim 20, wherein the Pd(0) scavenger comprising one or more one or more –NR-allyl or $–N^{+}RR'$-allyl moieties is  Z^{-}, wherein Z^{-} is Cl^{-} or F^{-}.

23. The method of claim 19, wherein the Pd(0) scavenger comprising one or more allyl moieties is in the first aqueous solution.

24. The method of claim 1, wherein the third aqueous solution further comprises at least one Pd(II) scavenger.

25. The method of claim 24, wherein the Pd(II) scavenger comprises L-cysteine or sodium thiosulfate.

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

an aqueous cleavage mixture comprising a transition metal catalyst and one or more reducing agents, wherein the transition metal catalyst is generated from a palladium or nickel complex with a water-soluble non-reducing phosphine or a N,N-bidentate non-phosphine ligand, and wherein the one or more reducing agents comprise a boron-containing reducing agent.