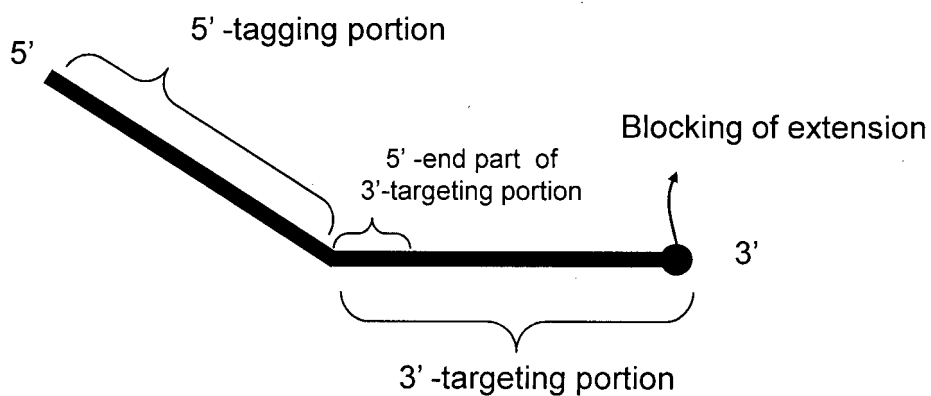


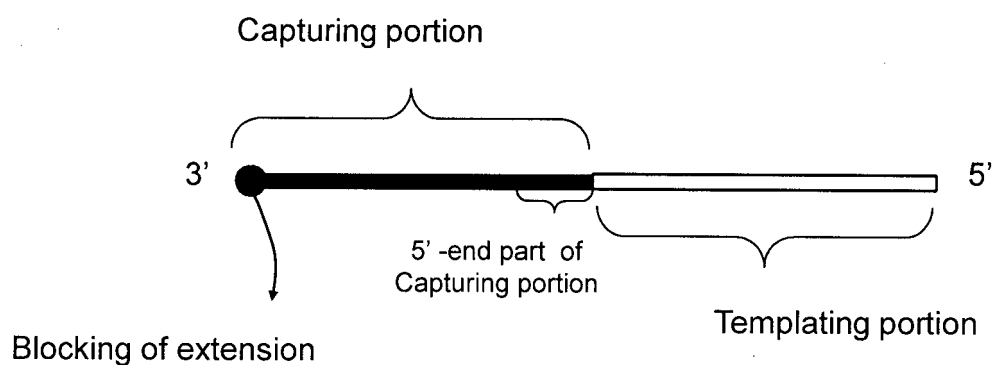
1/16

Fig. 1

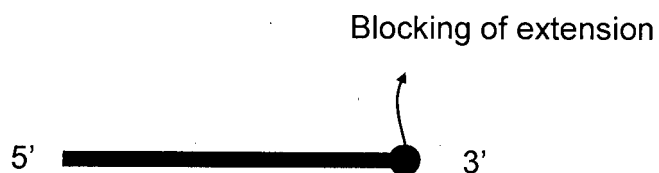
A. Probing and Tagging Oligonucleotide (PTO)



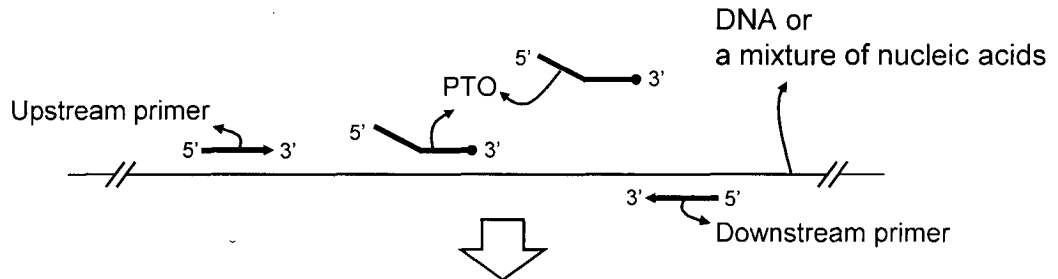
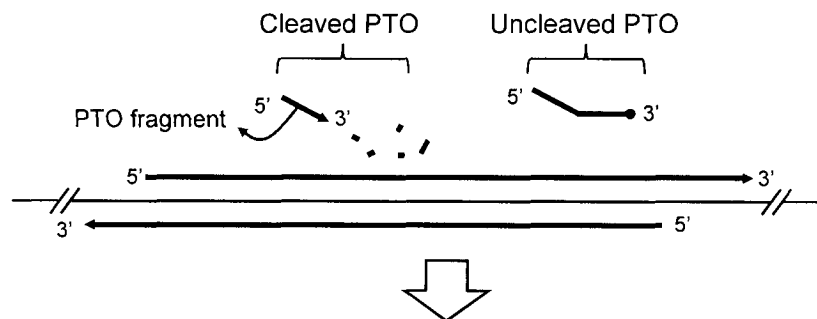
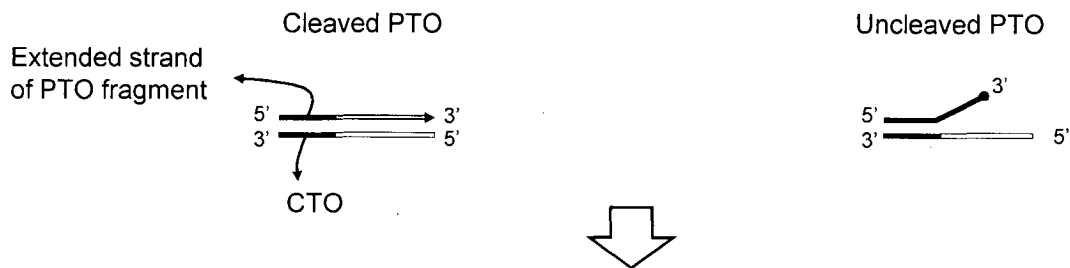
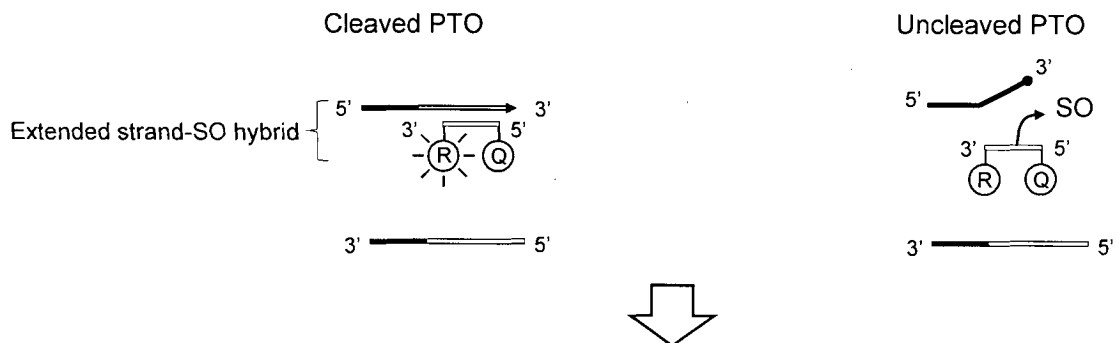
B. Capturing and Templating Oligonucleotide (CTO)



C. Signaling Oligonucleotide (SO)



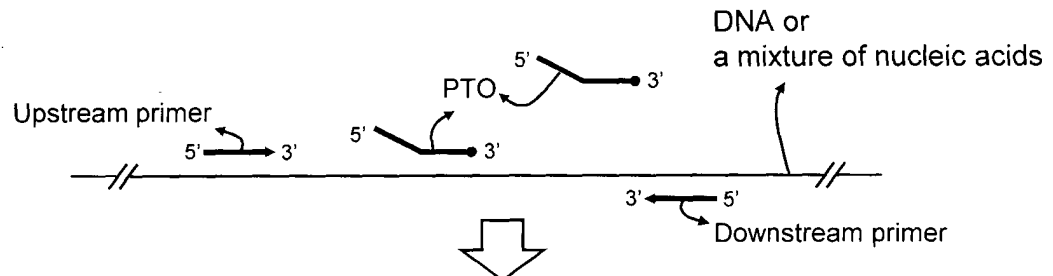
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Fig. 2**A. Hybridization****B. Primer extension & Cleavage of PTO****C. Hybridization of PTO fragment to CTO and Extension****D. Hybridization of SO to extended strand & Detection****E. (Optional) Melting analysis**

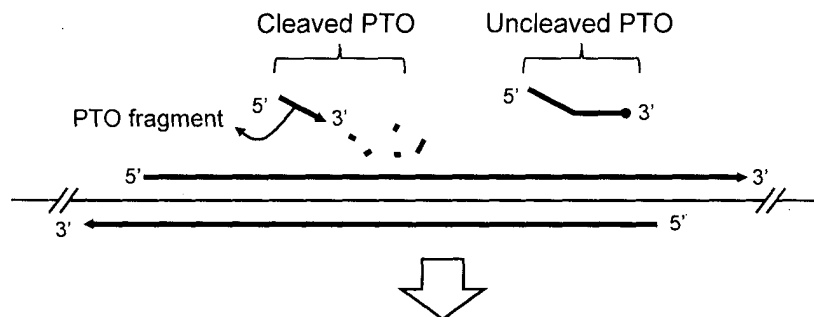
3/16

Fig. 3

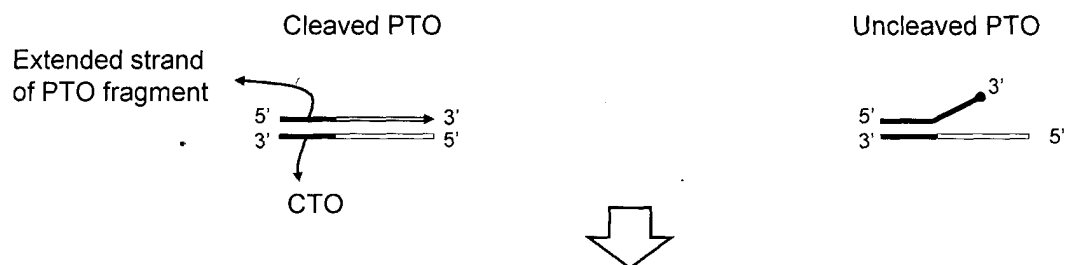
A. Hybridization



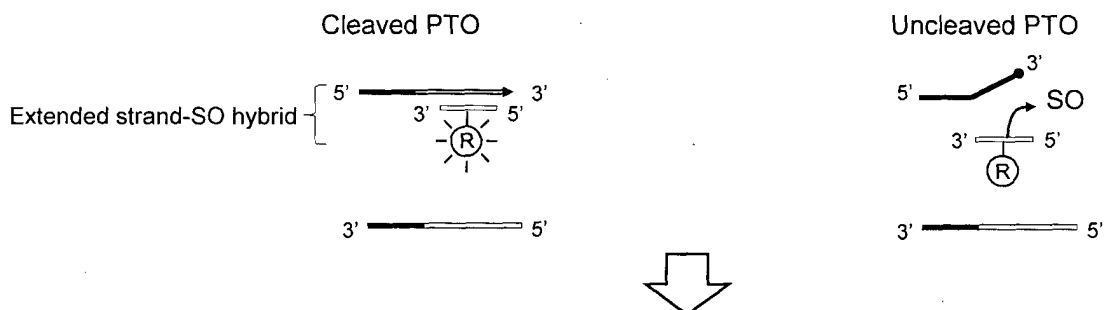
B. Primer extension & Cleavage of PTO



C. Hybridization of PTO fragment to CTO and Extension

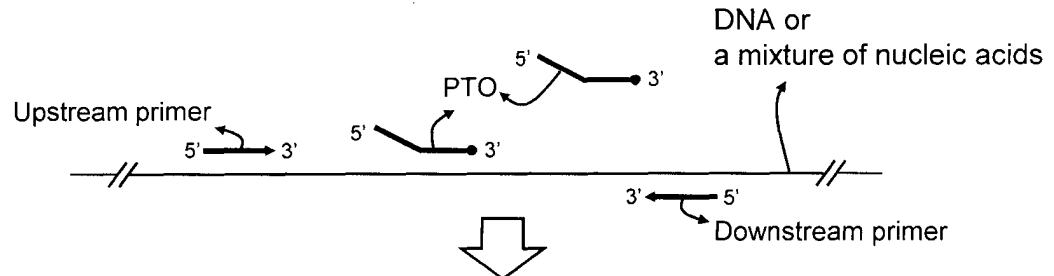
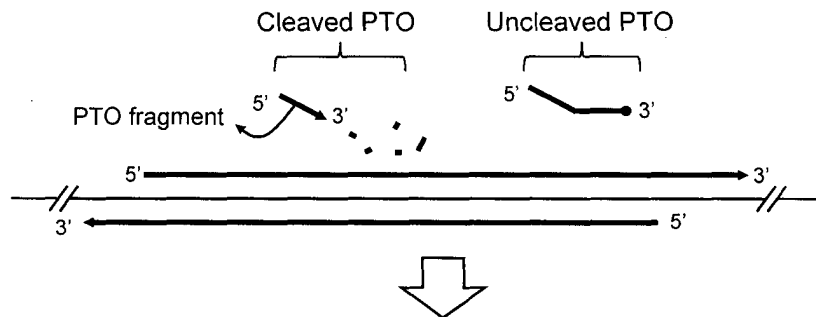
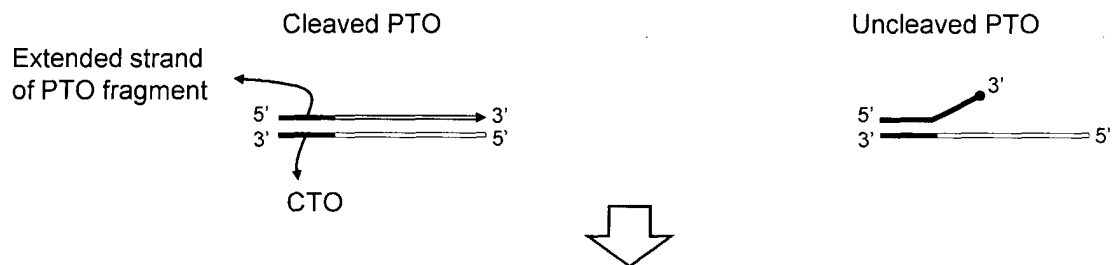
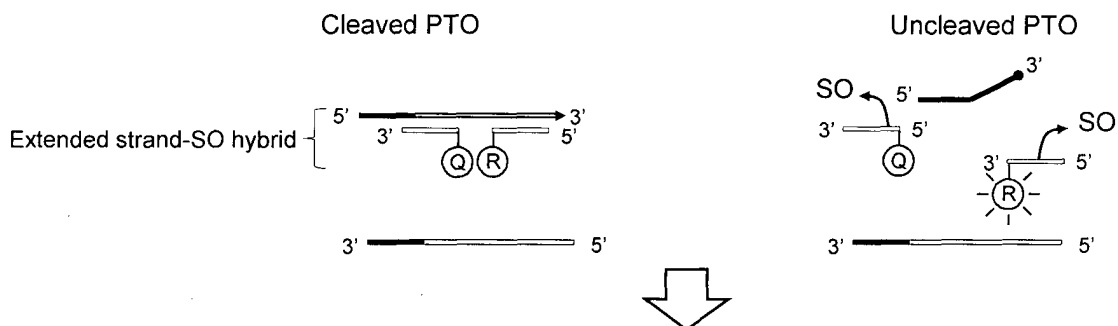


D. Hybridization of SO to extended strand & Detection

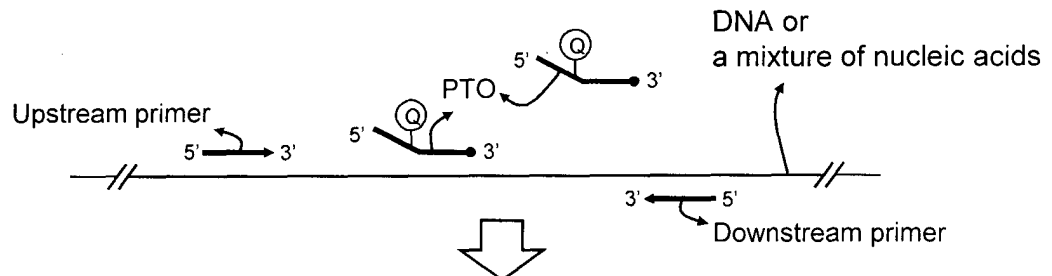
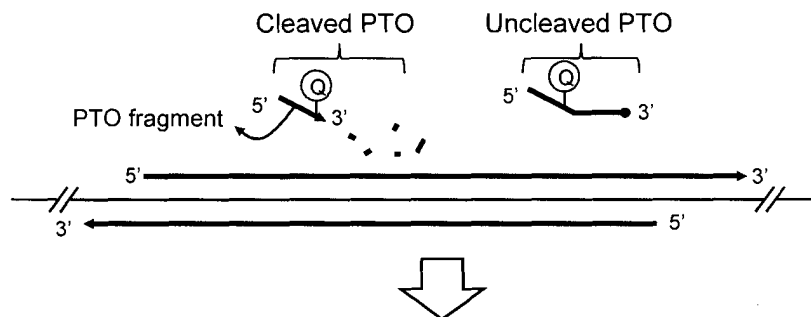
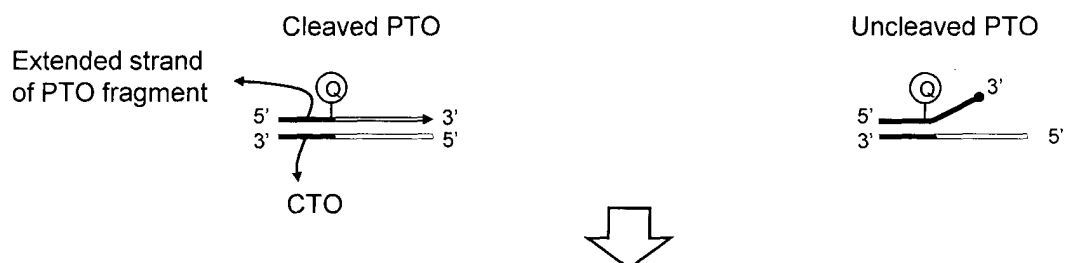
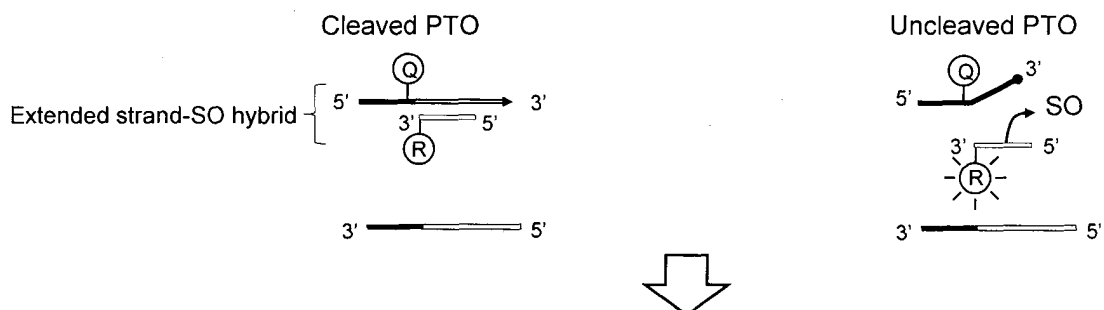


E. (Optional) Melting analysis

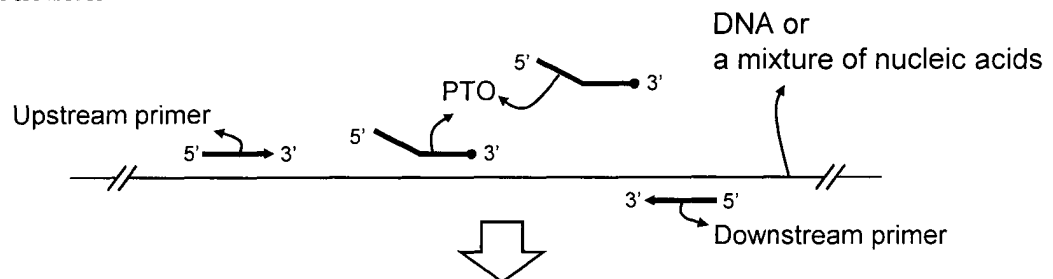
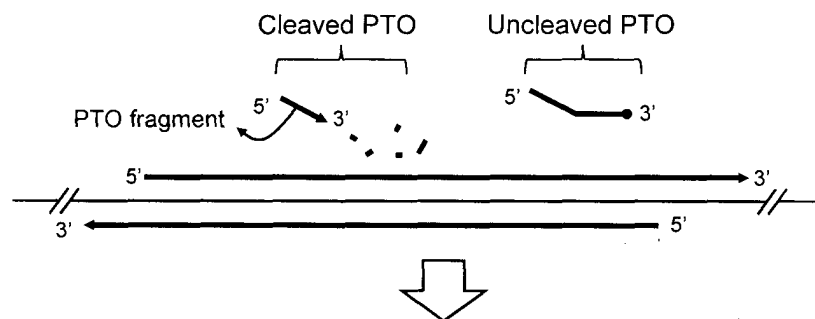
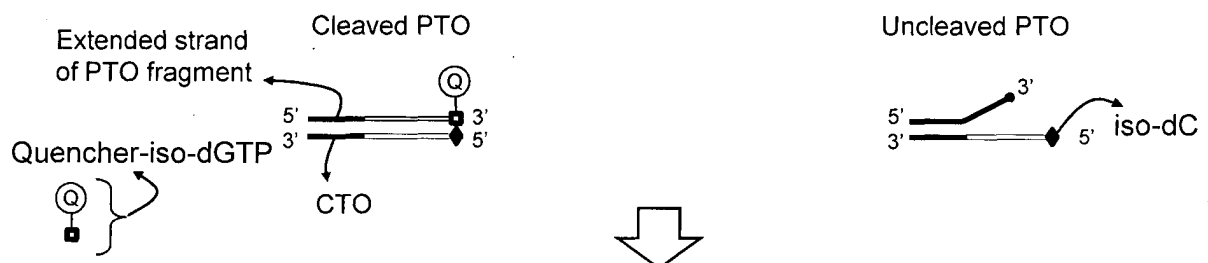
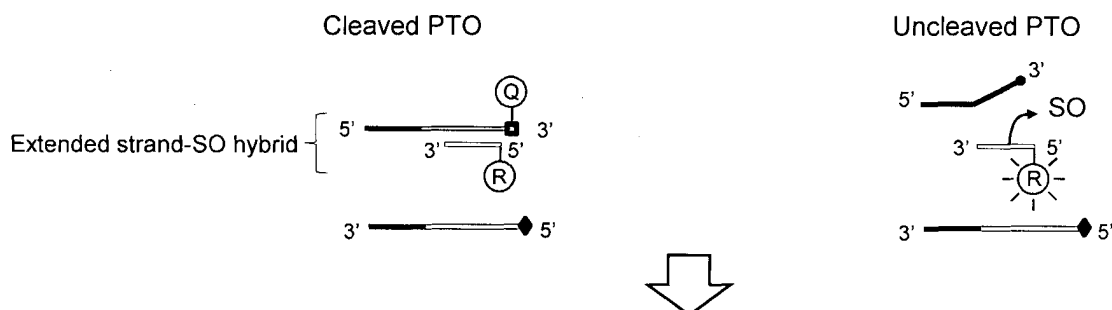
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Fig. 4**A. Hybridization****B. Primer extension & Cleavage of PTO****C. Hybridization of PTO fragment to CTO and Extension****D. Hybridization of SO to extended strand & Detection****E. (Optional) Melting analysis**

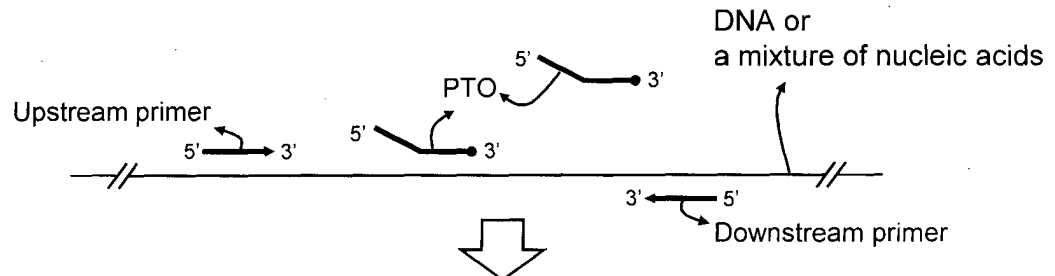
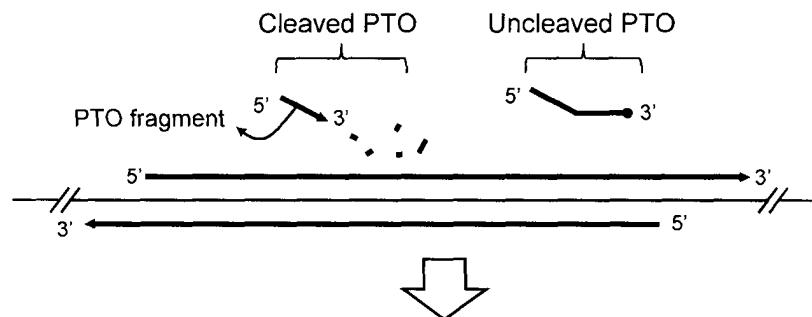
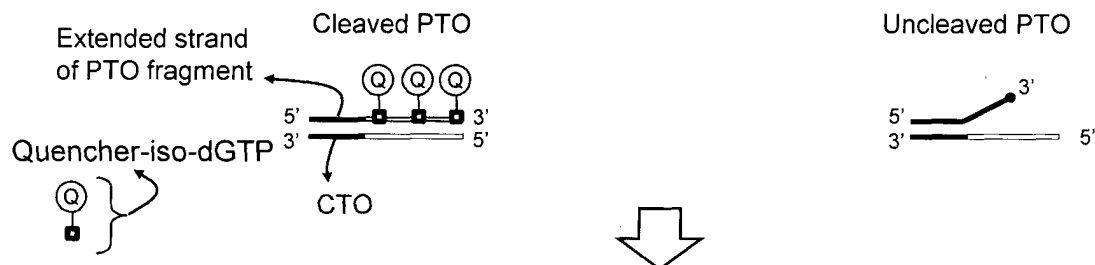
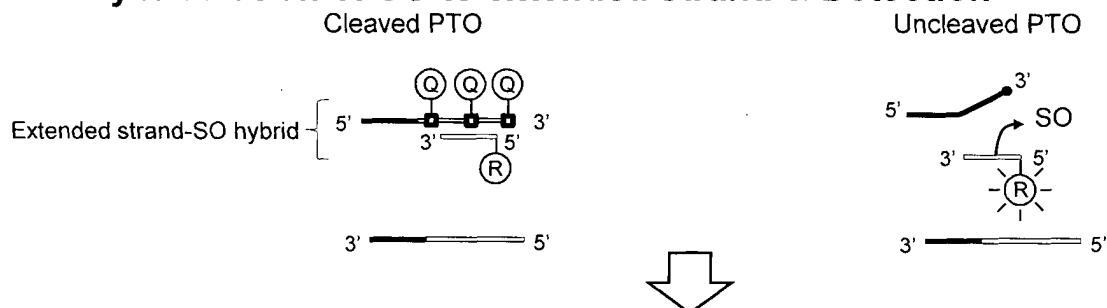
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Fig. 5**A. Hybridization****B. Primer extension & Cleavage of PTO****C. Hybridization of PTO fragment to CTO and Extension****D. Hybridization of SO to extended strand & Detection****E. (Optional) Melting analysis**

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Fig. 6**A. Hybridization****B. Primer extension & Cleavage of PTO****C. Hybridization of PTO fragment to CTO and Extension****D. Hybridization of SO to extended strand & Detection****E. (Optional) Melting analysis**

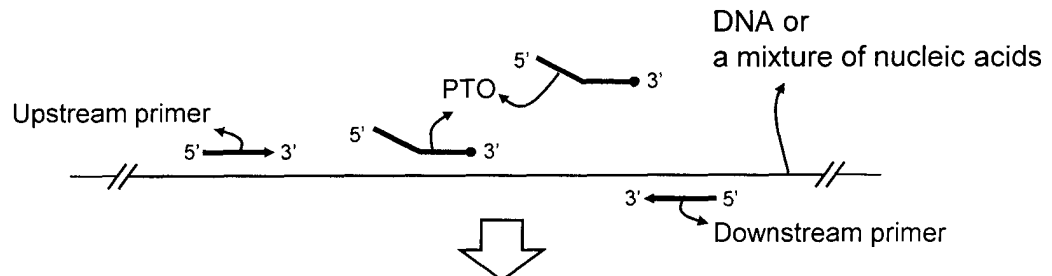
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Fig. 7**A. Hybridization****B. Primer extension & Cleavage of PTO****C. Hybridization of PTO fragment to CTO and Extension****D. Hybridization of SO to extended strand & Detection****E. (Optional) Melting analysis**

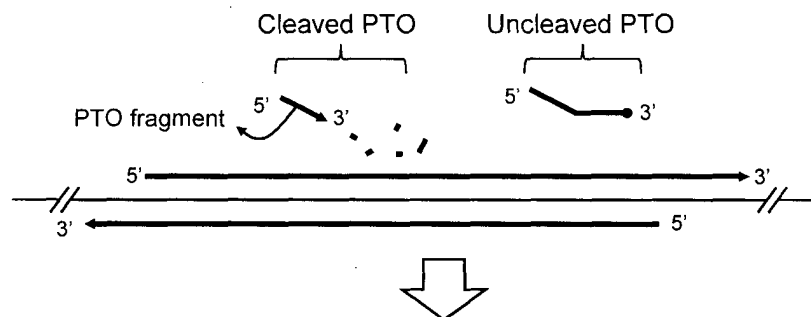
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Fig. 8

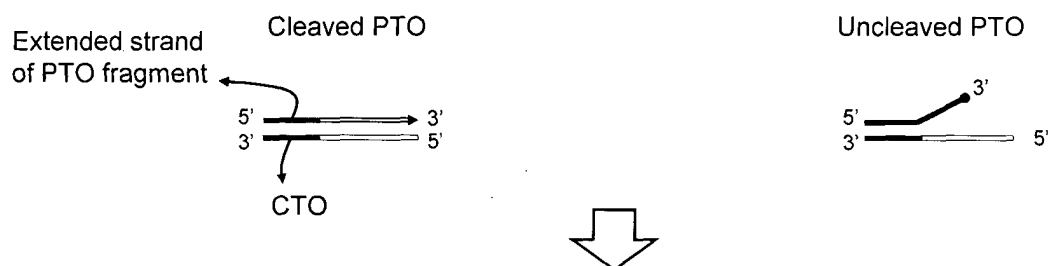
A. Hybridization



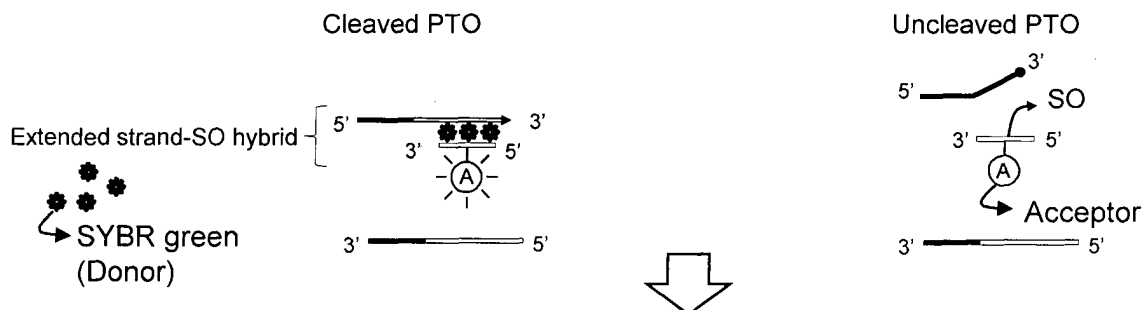
B. Primer extension & Cleavage of PTO



C. Hybridization of PTO fragment to CTO and Extension

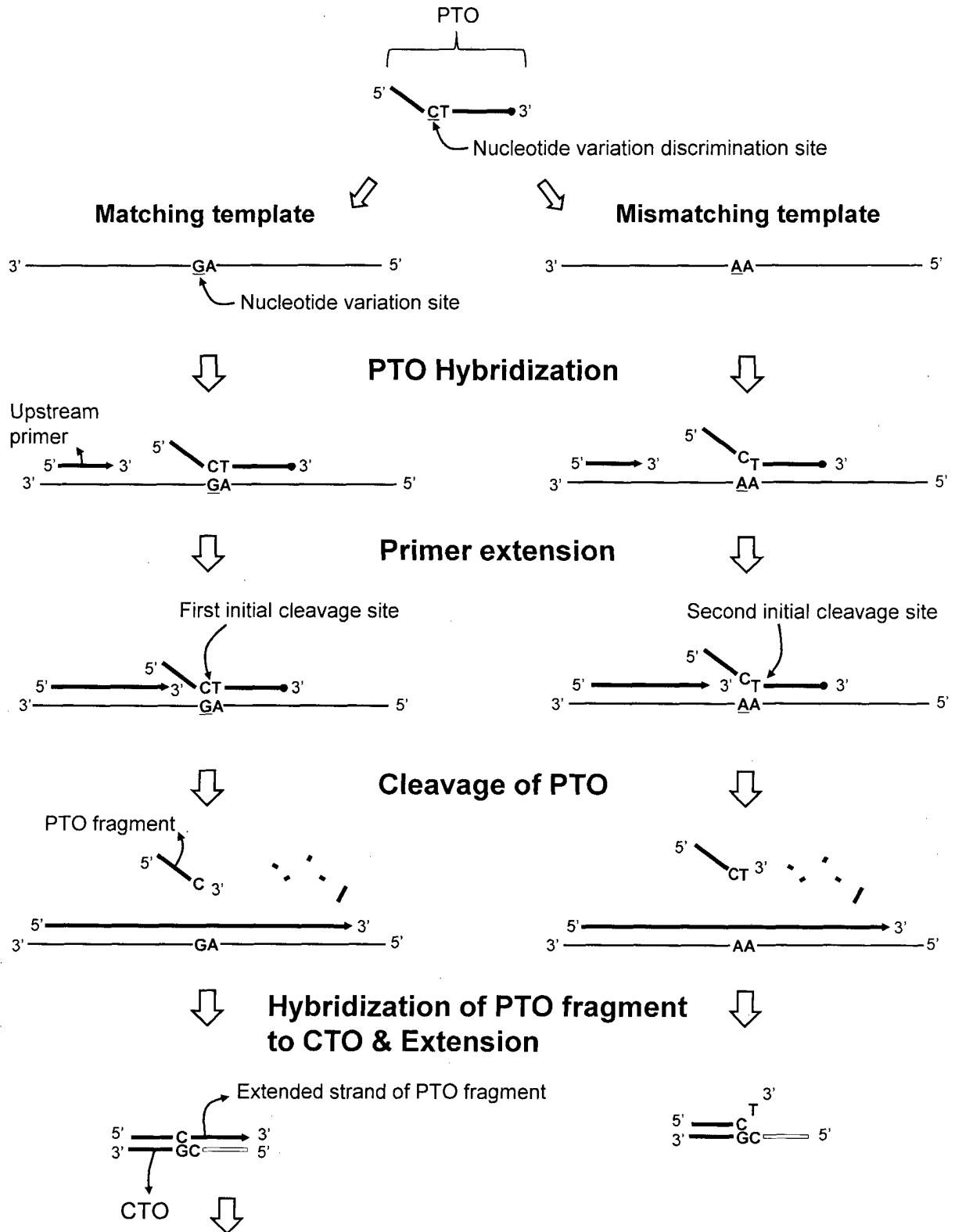


D. Hybridization of SO to extended strand & Detection

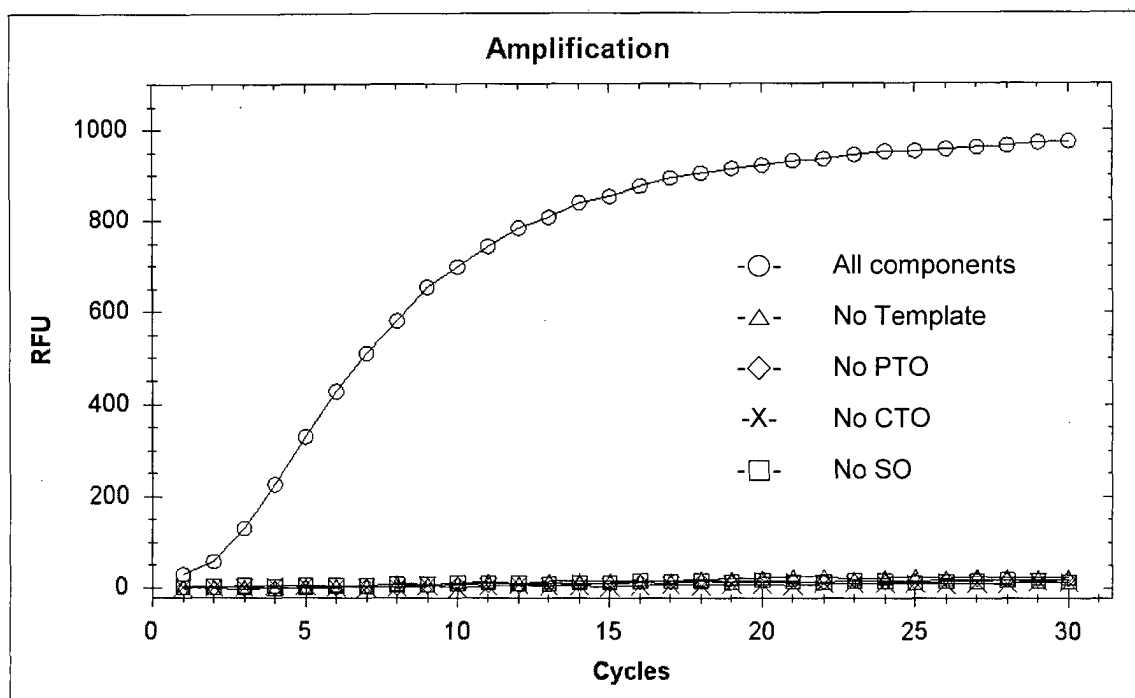


E. (Optional) Melting analysis

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Fig. 9**Hybridization of SO to extended strand & Detection**

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Fig. 10A

Template ¹⁾	Upstream Primer	PTO ²⁾	CTO ³⁾	SO ⁴⁾	Ct
+	+	+	+	+	1.72
-	+	+	+	+	-
+	+	-	+	+	-
+	+	+	-	+	-
+	+	+	+	-	-

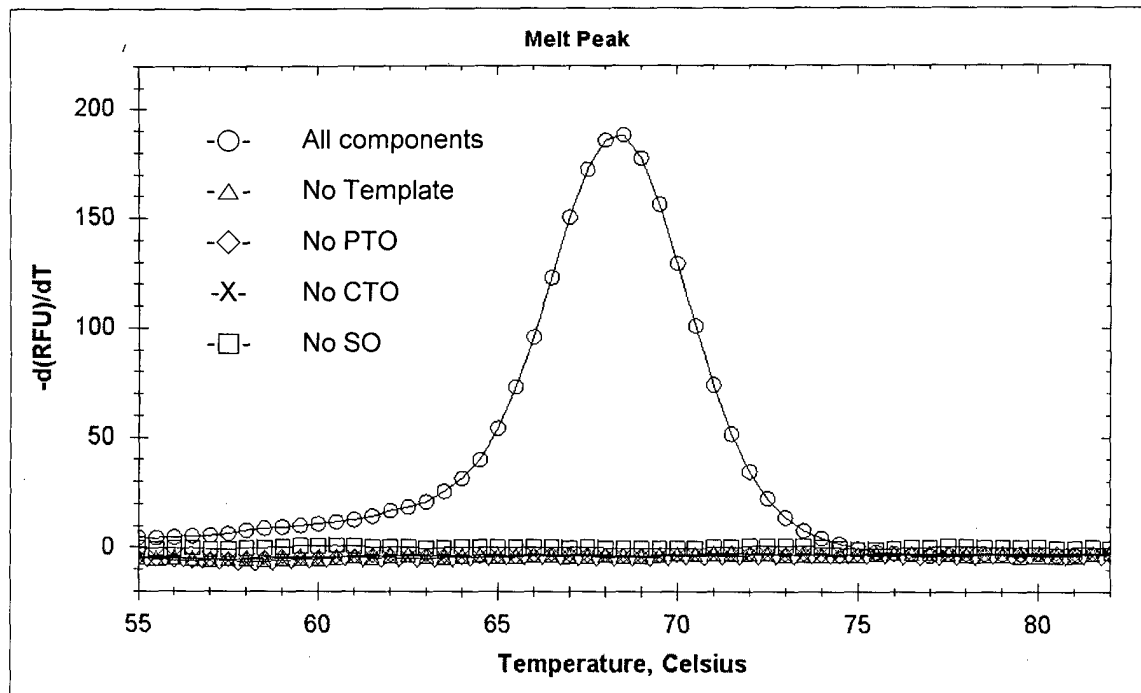
¹⁾ Template is a synthetic oligonucleotide for *Neisseria gonorrhoeae* gene.

²⁾ PTO (Probing and Tagging Oligonucleotide) is blocked with a carbon spacer at its 3'-end.

³⁾ CTO (Capturing and Templating Oligonucleotide) is blocked with a carbon spacer at its 3'-end.

⁴⁾ SO (Signaling Oligonucleotide) has a fluorescent molecule at its 5'-end and a quencher molecule at its 3'-end.

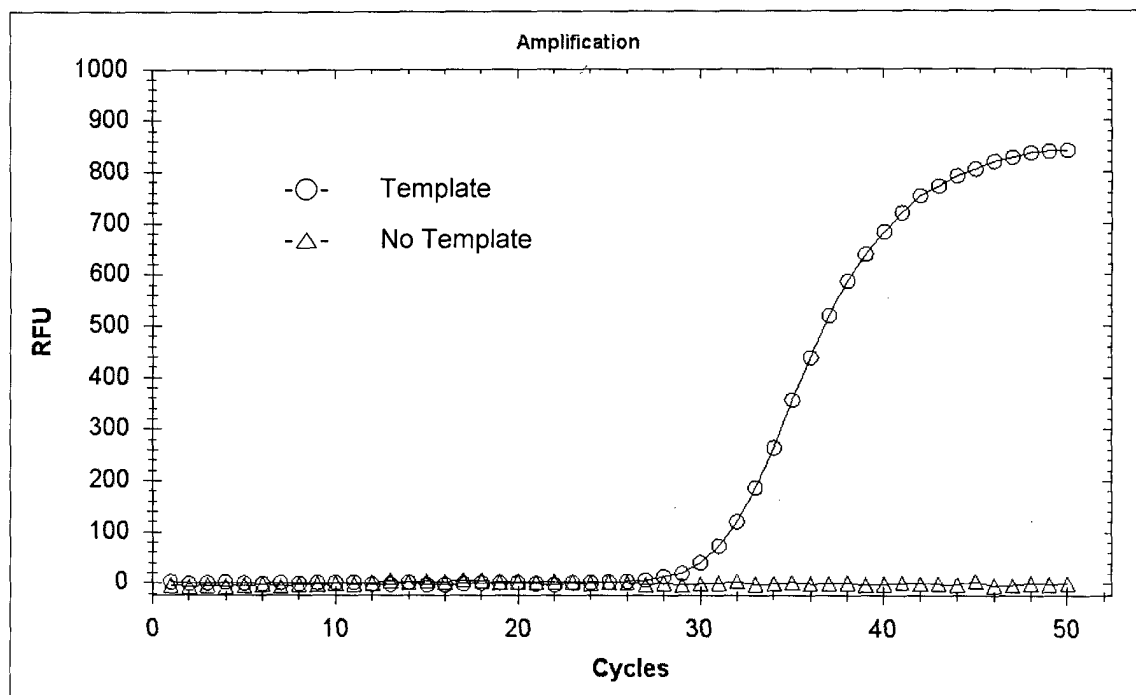
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Fig. 10B

Template ¹⁾	Upstream Primer	PTO ²⁾	CTO ³⁾	SO ⁴⁾	Tm ⁵⁾
+	+	+	+	+	68.5
-	+	+	+	+	-
+	+	-	+	+	-
+	+	+	-	+	-
+	+	+	+	-	-

- ¹⁾ Template is a synthetic oligonucleotide for *Neisseria gonorrhoeae* gene.
- ²⁾ PTO (Probing and Tagging Oligonucleotide) is blocked with a carbon spacer at its 3'-end.
- ³⁾ CTO (Capturing and Templating Oligonucleotide) is blocked with a carbon spacer at its 3'-end.
- ⁴⁾ SO (Signaling Oligonucleotide) has a fluorescent molecule at its 5'-end and a quencher molecule at its 3'-end.
- ⁵⁾ Tm represents melting temperature of the extended strand-SO hybrid.

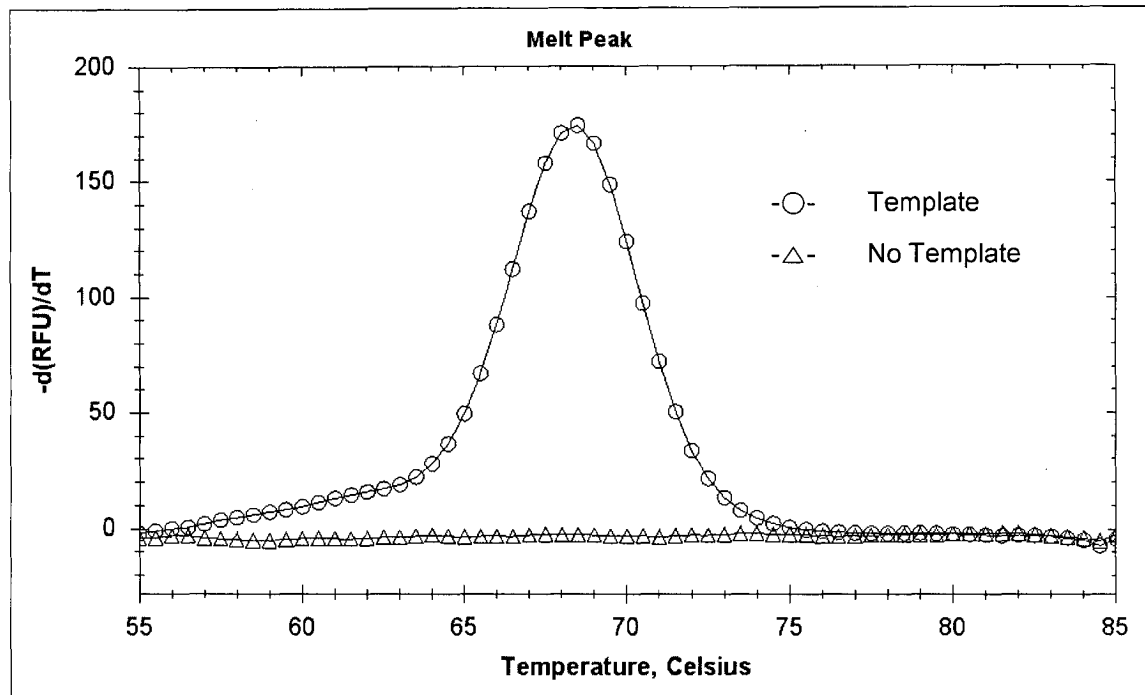
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Fig. 11A

Template ¹⁾	Primers ²⁾	PTO ³⁾	CTO ⁴⁾	SO ⁵⁾	Ct
+	+	+	+	+	30.34
-	+	+	+	+	-

- ¹⁾ Template is a genomic DNA of *Neisseria gonorrhoeae*.
- ²⁾ Primers are a pair of primers for PCR.
- ³⁾ PTO (Probing and Tagging Oligonucleotide) is blocked with a carbon spacer at its 3'-end.
- ⁴⁾ CTO (Capturing and Templating Oligonucleotide) is blocked with a carbon spacer at its 3'-end.
- ⁵⁾ SO (Signaling Oligonucleotide) has a fluorescent molecule at its 5'-end and a quencher molecule at its 3'-end.

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Fig. 11B

Template ¹⁾	Primers ²⁾	PTO ³⁾	CTO ⁴⁾	SO ⁵⁾	T _m ⁶⁾
+	+	+	+	+	68.5
-	+	+	+	+	-

¹⁾ Template is a genomic DNA of *Neisseria gonorrhoeae*.

²⁾ Primers are a pair of primers for PCR.

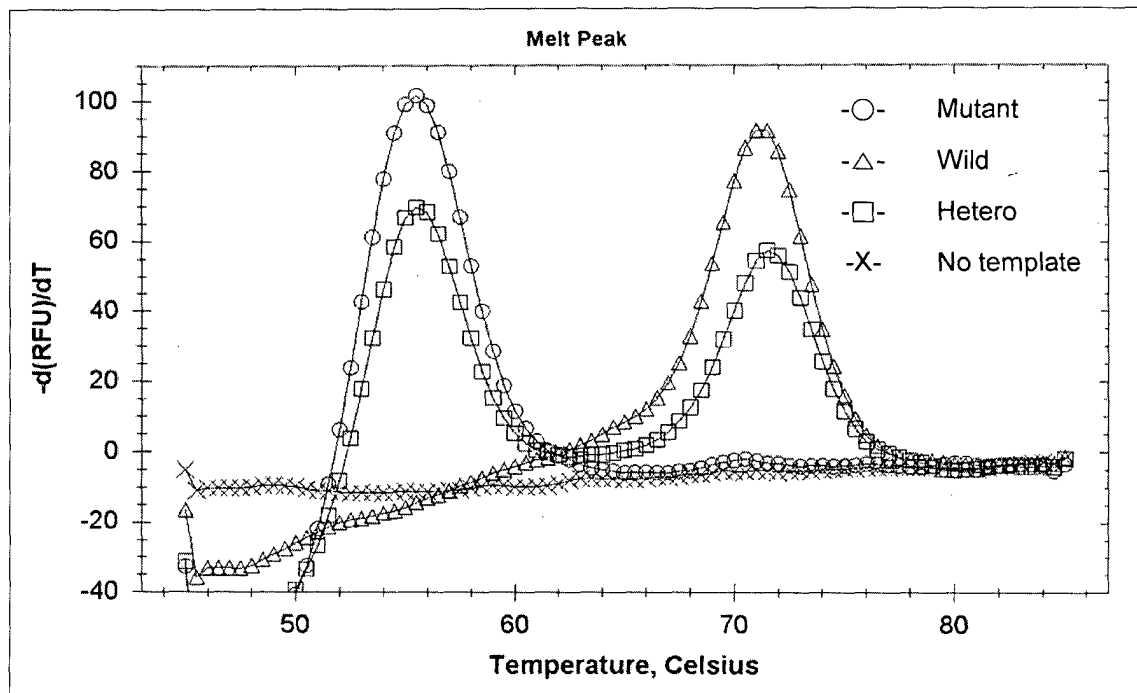
³⁾ PTO (Probing and Tagging Oligonucleotide) is blocked with a carbon spacer at its 3'-end.

⁴⁾ CTO (Capturing and Templating Oligonucleotide) is blocked with a carbon spacer at its 3'-end.

⁵⁾ SO (Signaling Oligonucleotide) has a fluorescent molecule at its 5'-end and a quencher molecule at its 3'-end.

⁶⁾ T_m represents melting temperature of the extended strand-SO hybrid.

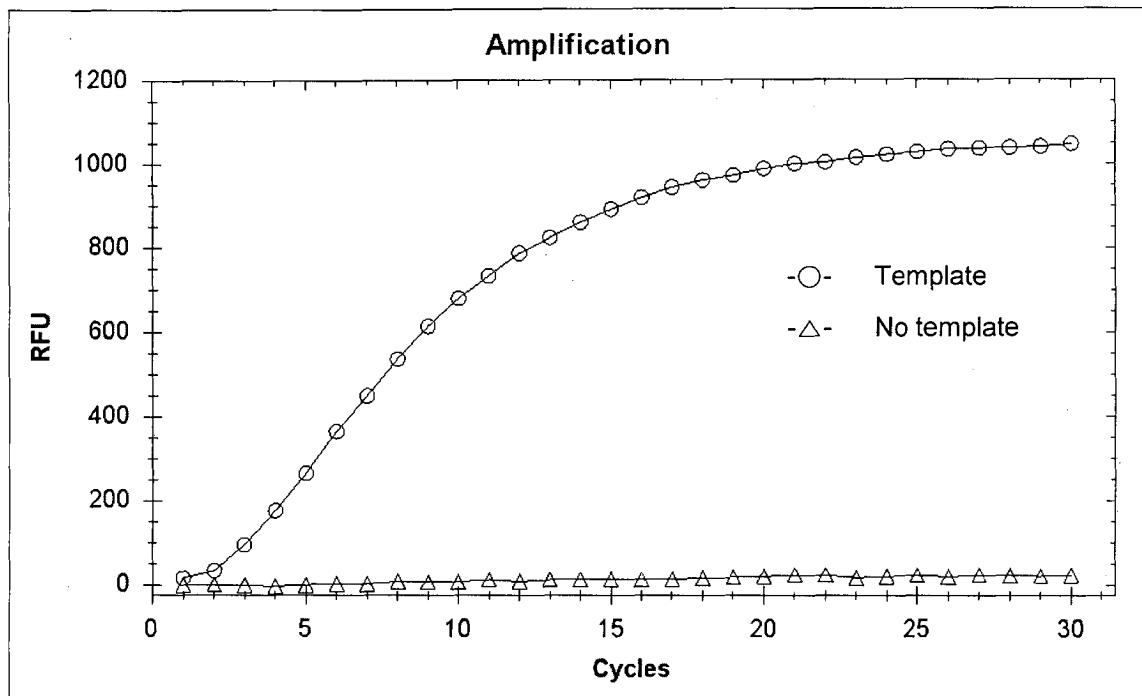
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Fig. 12

Template ¹⁾	Primers ²⁾	PTOs ³⁾	CTOs ⁴⁾	SO ⁵⁾	T _m ⁶⁾
Mutant	+	+	+	+	55.5
Wild	+	+	+	+	71.0
Hetero	+	+	+	+	55.5, 71.0
-	+	+	+	+	-

- ¹⁾ Template is MTHFR(C677T) wild-type (C), mutant-type (T), or hetero-type (C/T) human genomic DNA.
- ²⁾ Primers are a pair of primers for PCR.
- ³⁾ PTOs (Probing and Tagging Oligonucleotide) include a PTO for detecting wild-type template and a PTO for mutant-type and are blocked with a carbon spacer at its 3'-end.
- ⁴⁾ CTOs (Capturing and Templating Oligonucleotide) include a CTO for detecting wild-type template and a CTO for mutant-type and are blocked with a carbon spacer at its 3'-end.
- ⁵⁾ SO (Signaling Oligonucleotide) has a quencher molecule at its 5'-end and a fluorescent molecule at its 3'-end.
- ⁶⁾ T_m represents melting temperature of the extended strand-SO hybrid.

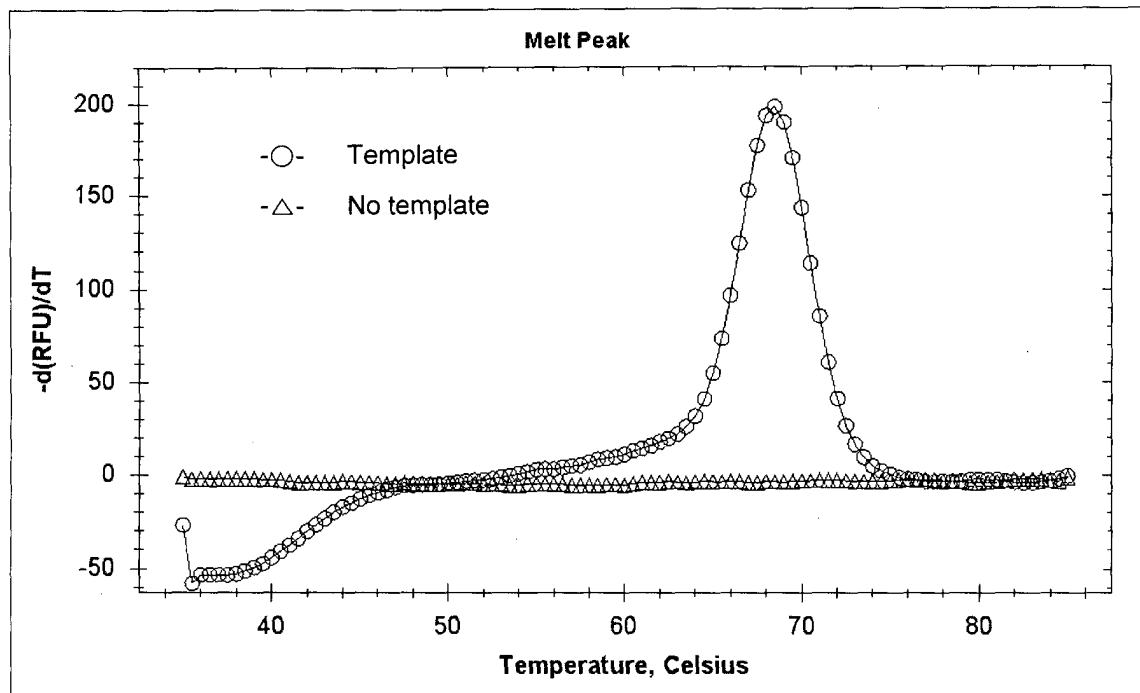
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Fig. 13A

Template ¹⁾	PTO ²⁾	CTO ³⁾	SO ⁴⁾	Ct
+	+	+	+	3.10
-	+	+	+	-

- 1) Template is a synthetic oligonucleotide for *Neisseria gonorrhoeae* gene.
- 2) PTO (Probing and Tagging Oligonucleotide) is blocked with a carbon spacer at its 3'-end.
- 3) CTO (Capturing and Templating Oligonucleotide) is blocked with a carbon spacer at its 3'-end.
- 4) SO (Signaling Oligonucleotide) has a fluorescent molecule at its 5'-end and a quencher molecule at its 3'-end.

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Fig. 13B

Template ¹⁾	PTO ²⁾	CTO ³⁾	SO ⁴⁾	Tm ⁵⁾
+	+	+	+	68.5
-	+	+	+	-

- ¹⁾ Template is a synthetic oligonucleotide for *Neisseria gonorrhoeae* gene.
- ²⁾ PTO (Probing and Tagging Oligonucleotide) is blocked with a carbon spacer at its 3'-end.
- ³⁾ CTO (Capturing and Templating Oligonucleotide) is blocked with a carbon spacer at its 3'-end.
- ⁴⁾ SO (Signaling Oligonucleotide) has a fluorescent molecule at its 5'-end and a quencher molecule at its 3'-end.
- ⁵⁾ Tm represents melting temperature of the extended strand-SO hybrid.