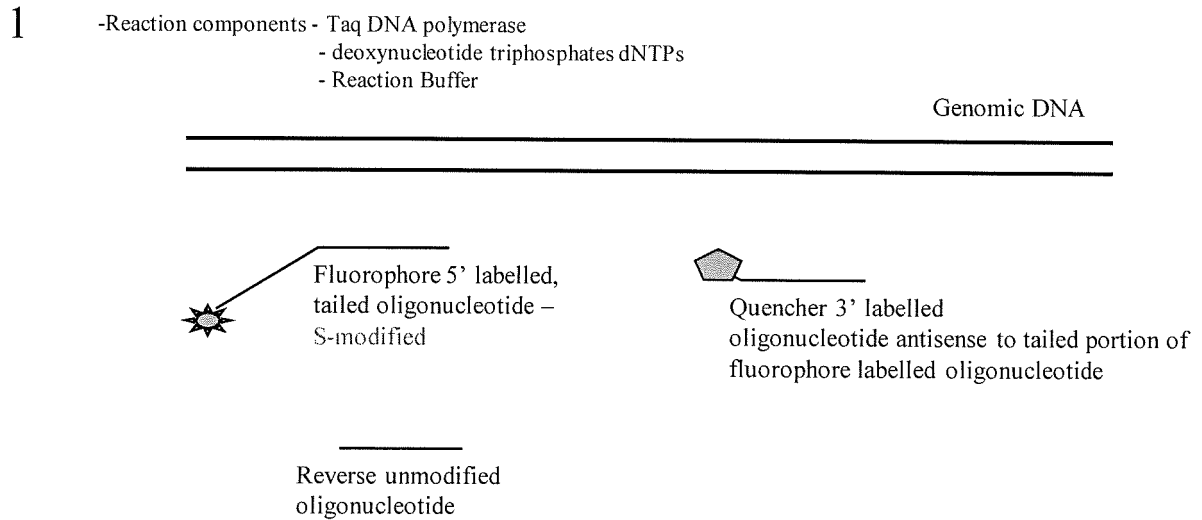
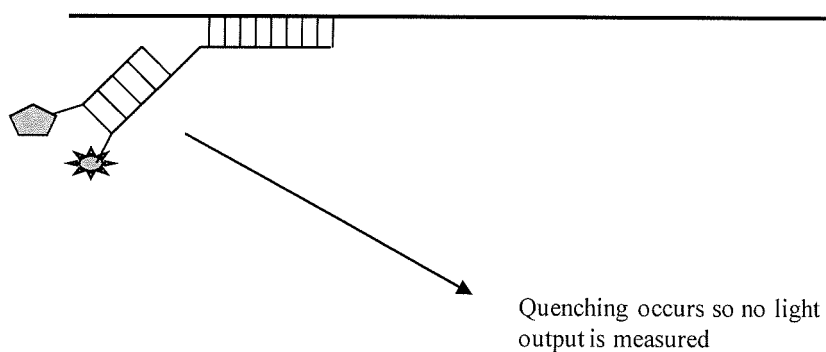


Figure 1 – Direct detection of DNA sequence



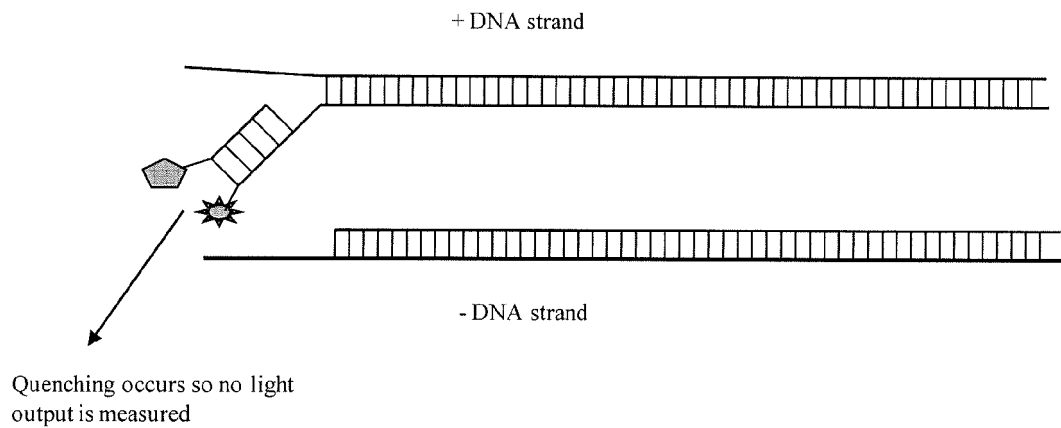
2

1st round of thermal cycling – forward tailed primer hybridises to genomic DNA and quencher oligonucleotide is hybridised to fluorophore-labelled tail portion of the oligonucleotide. Reverse primer hybridises to genomic DNA (not shown)



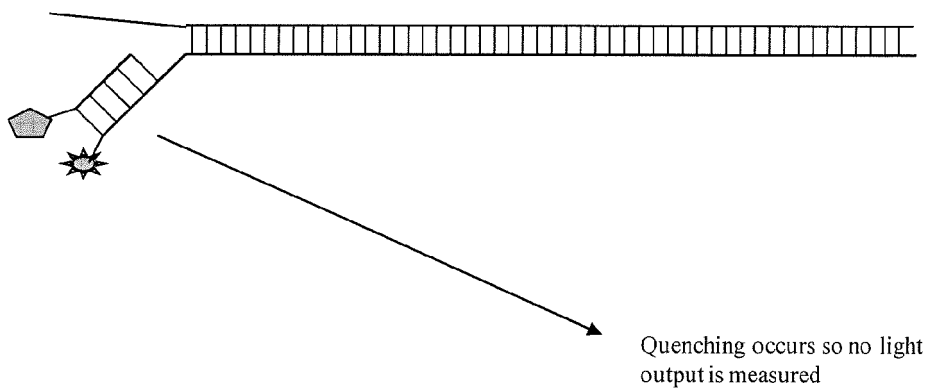
3

DNA synthesis occurs copying genomic DNA incorporating the tail sequence into the synthesised strand



4

Synthesised strand DNA is copied inclusive of primer tail. Reverse strand is also primed and copied from more tailed primer (not shown)



- 5 DNA synthesis is initiated from fluorescent labelled primer

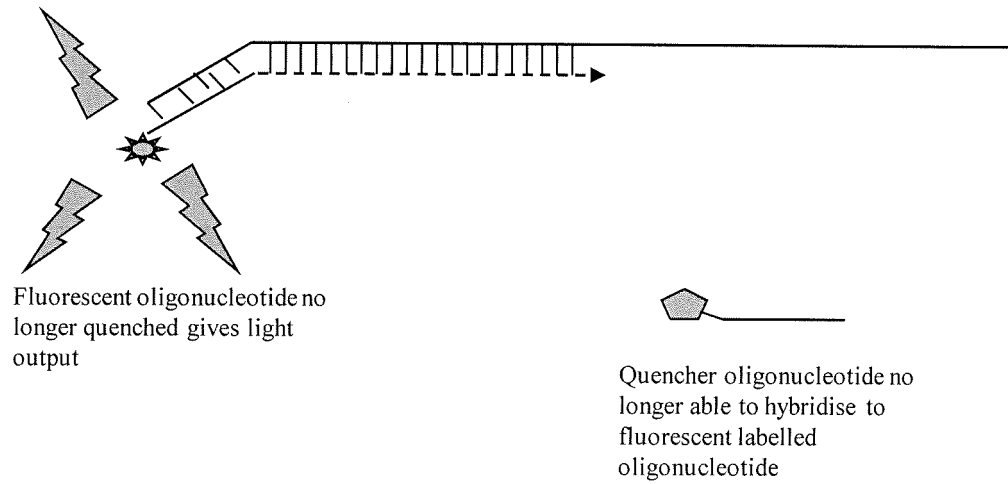
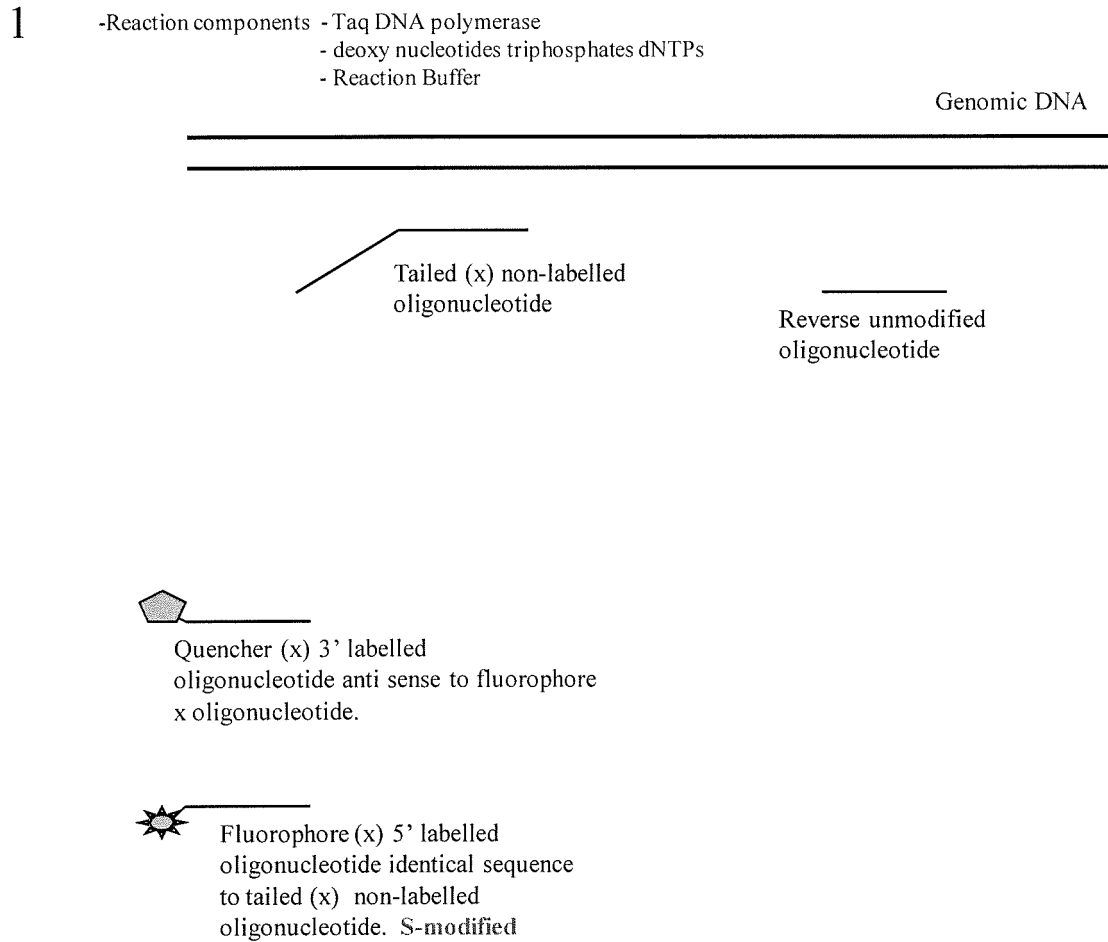
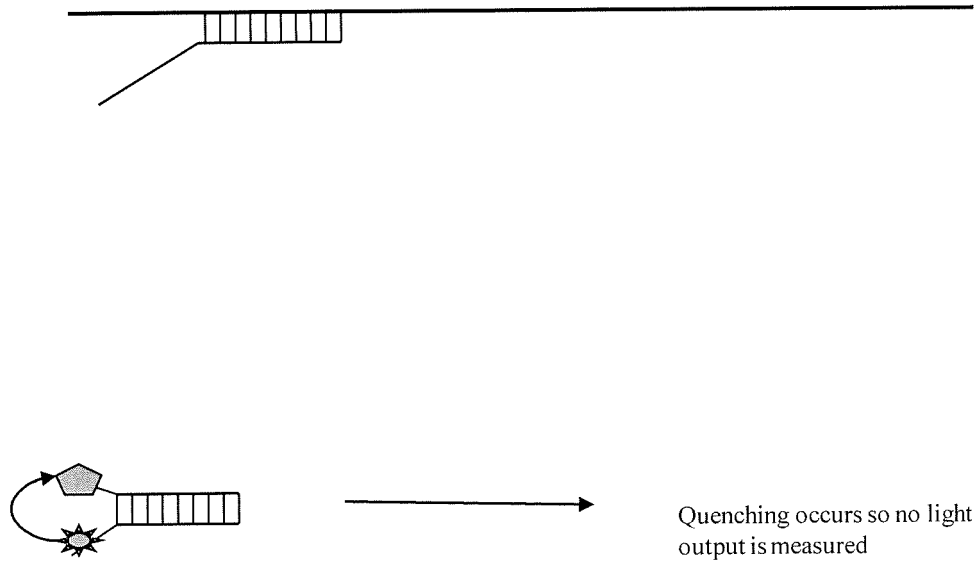


Figure 2 – Indirect (real-time) detection of DNA sequence



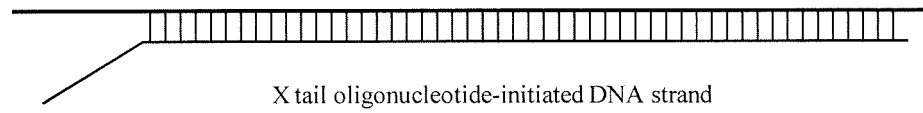
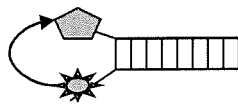
2

1st round of thermal cycling – the forward, tailed primer hybridises to genomic DNA and the quencher oligonucleotide hybridises to its complementary fluorophore-labelled oligonucleotide. The reverse primer hybridises to genomic DNA (not shown).



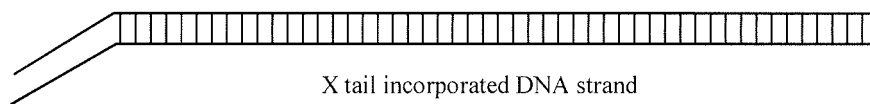
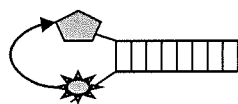
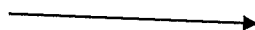
3

DNA synthesis occurs from the oligonucleotide copying genomic DNA incorporating the x tail sequence into the synthesised strand. The reverse strand is not shown.



Quenching occurs so no light output is measured

- 4 Synthesised strand DNA is copied inclusive of primer tails. Reverse strand is also primed and copied from more tailed primer (not shown)



Quenching occurs so no light output is measured

- 5 DNA synthesis is initiated from x-fluorescent ly-labelled primer

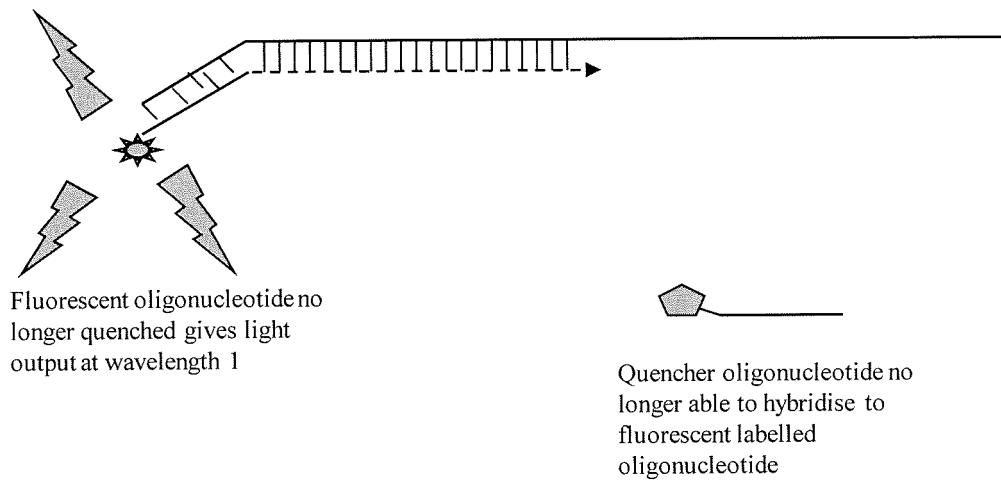
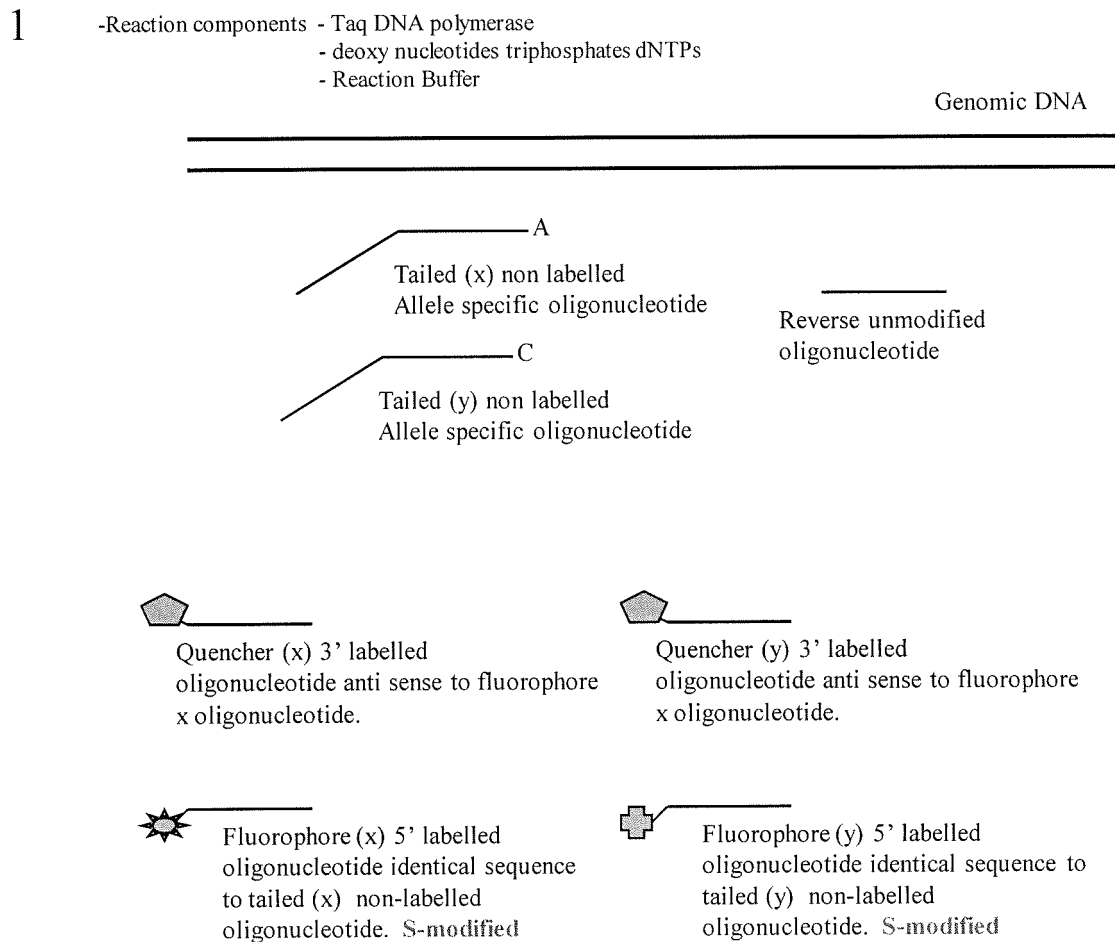
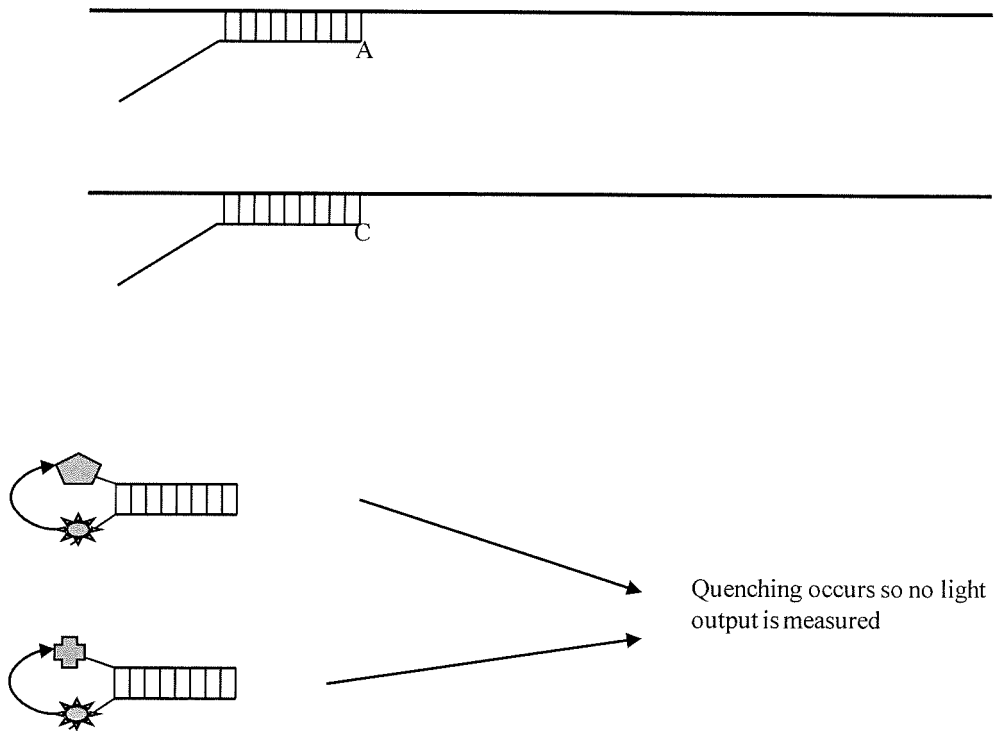


Figure 3 – Indirect (endpoint) detection of DNA sequence - SNP Genotyping



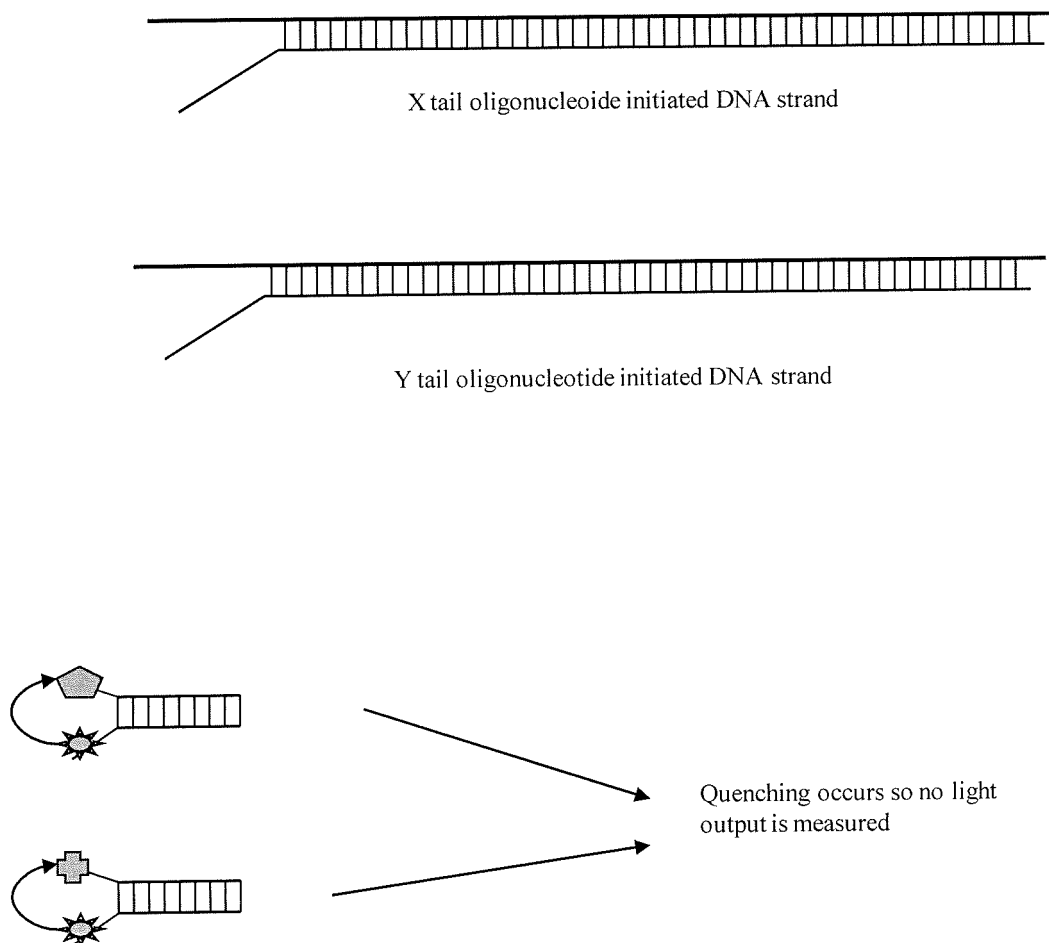
2

1st round of thermal cycling – depending on genotype of DNA under test either or both forward tailed primer hybridise to genomic DNA and quencher oligonucleotides are hybridised to their complementary fluorophore labelled oligonucleotide. Reverse primer hybridises to genomic DNA (not shown). Example shows heterozygous individual.

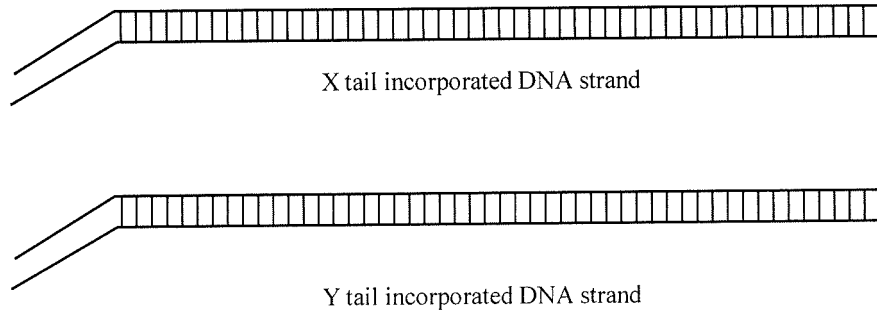
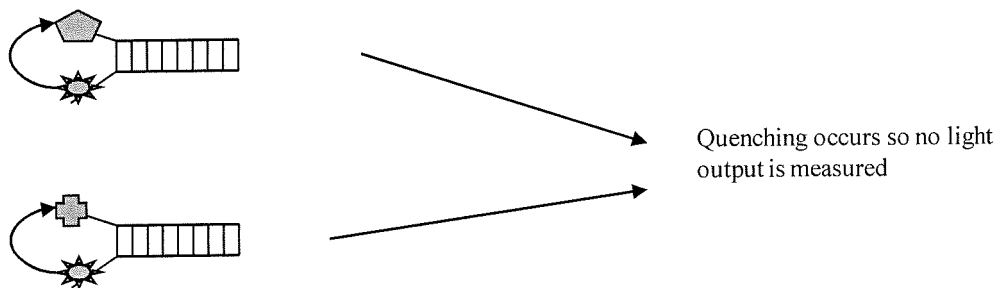


3

DNA synthesis occurs from both allele-specific oligonucleotides copying genomic DNA incorporating the x and y tail sequences into the synthesised strands. The reverse strand is not shown.



- 4 Synthesised strand DNA is copied inclusive of primer tails. Reverse strand is also primed and copied from more tailed allele specific primer (not shown)



- 5 DNA synthesis is initiated from both x and y fluorescent labelled primers

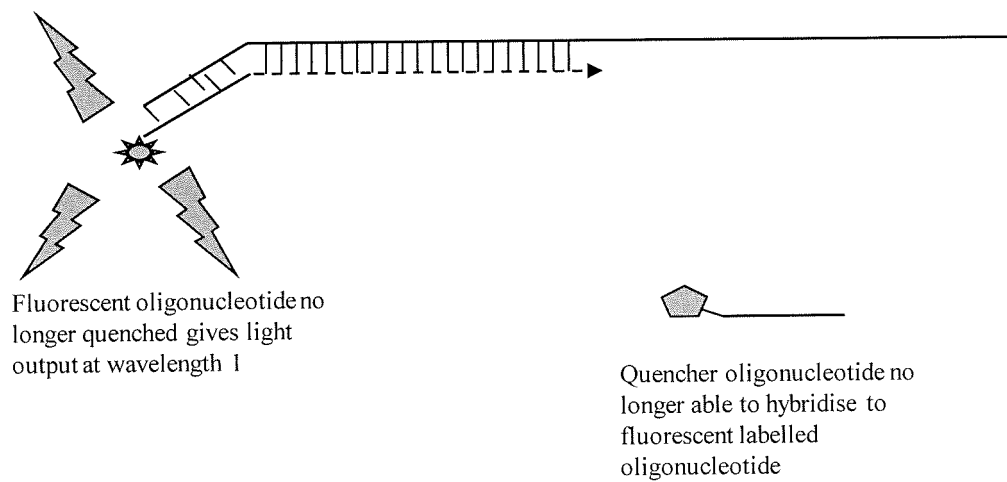
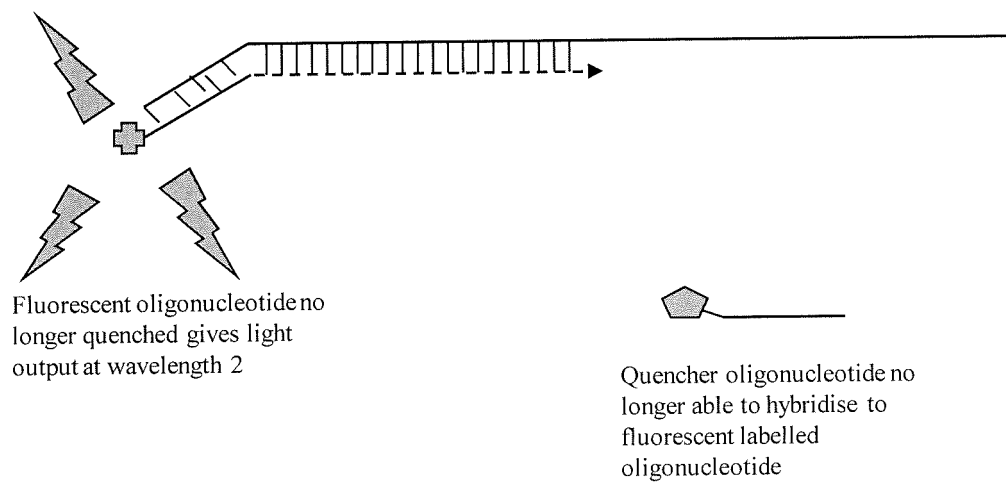


Figure 4

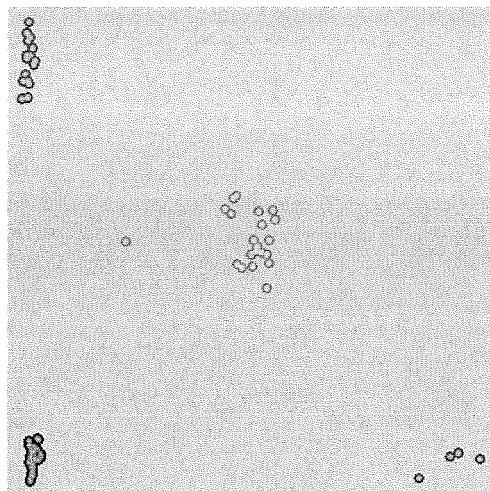
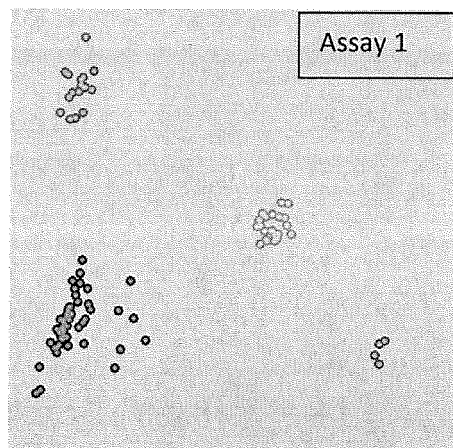
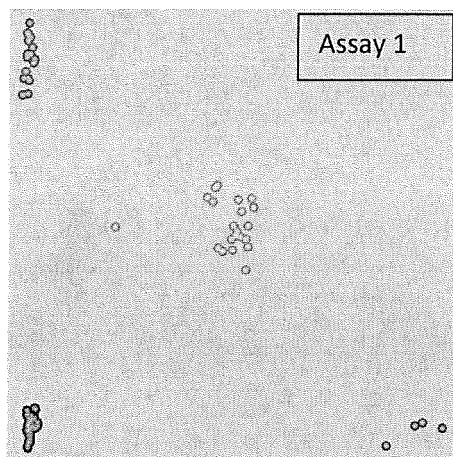


Figure 5 - Differently modified fluorescent oligos with , standard (non-S modified) quenchers

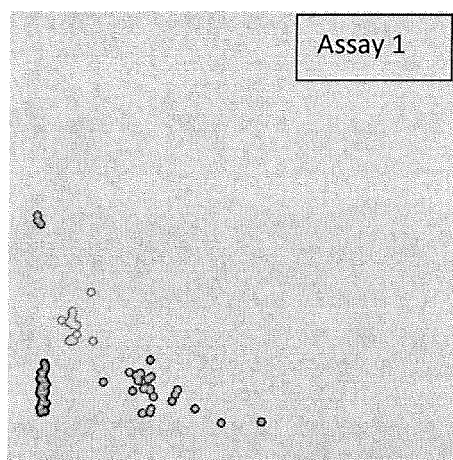
Standard (non-phosphorothioate modified) fluorescently-labelled primers:



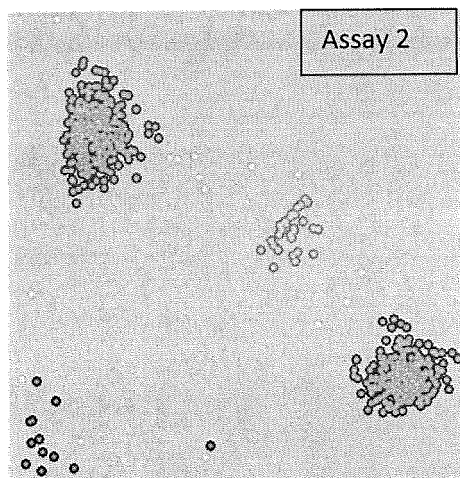
Semi-S phosphorothioate-modified fluorescently-labelled primers:



Full-S phosphorothioate-modified fluorescently-labelled primers:



Standard (non-phosphorothioate modified) fluorescently-labelled primers:



Semi-S phosphorothioate-modified fluorescently-labelled primers:

