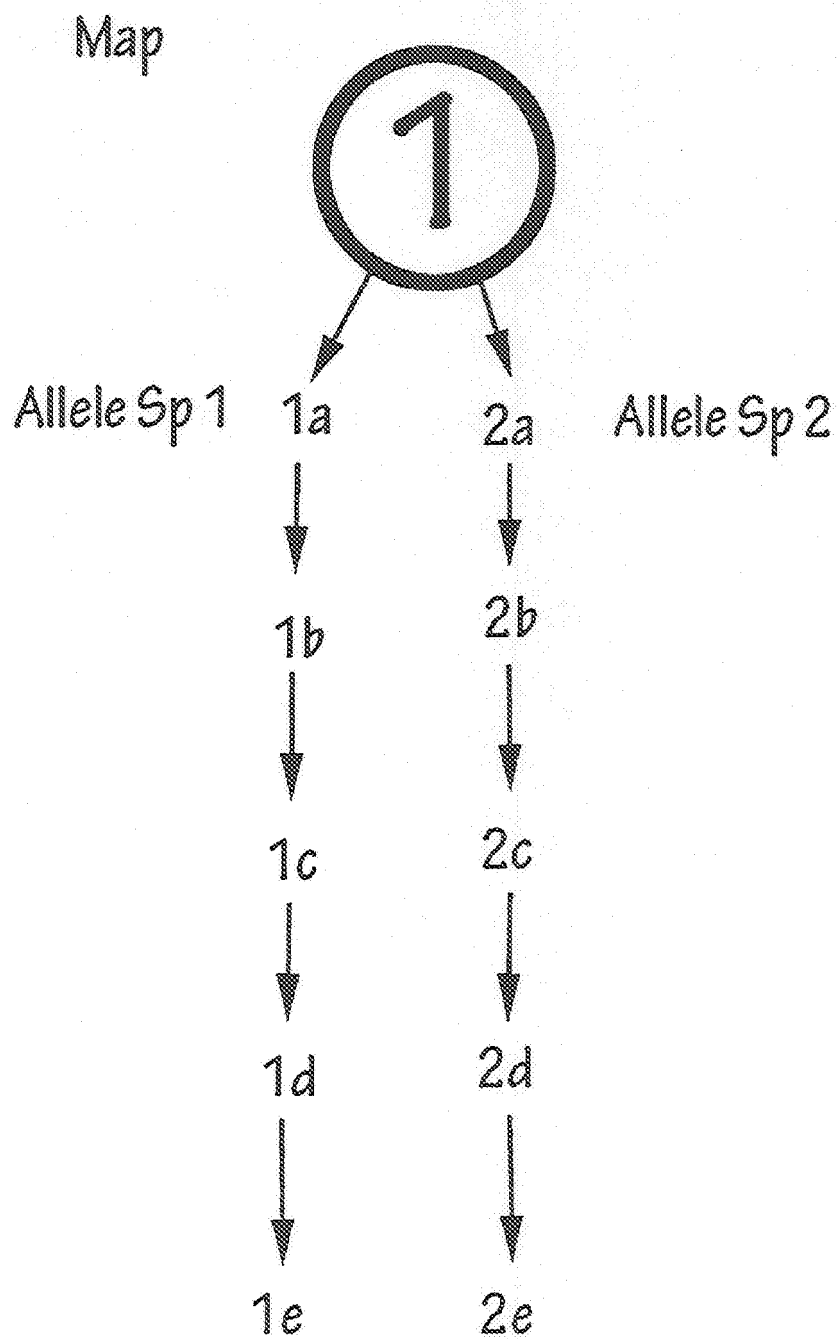


Figure 1



1

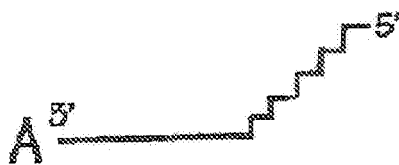
SNP ————— [A/G] —————

There are two possible outcomes
 Parallel for this SNP or

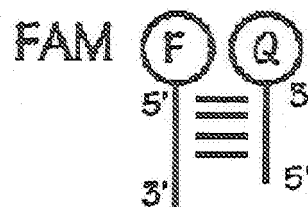
————— A —————
 ————— G —————

The primers involved in this reaction are as follows:

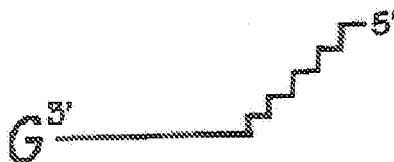
Allele specific primer 1



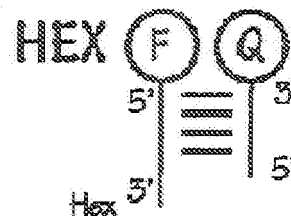
Respective Fluor/Quencher



Allele specific Primer 2



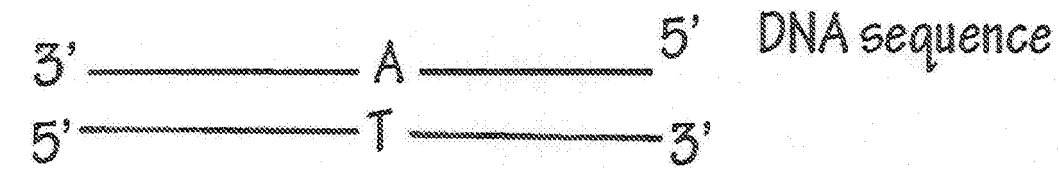
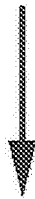
Respective Fluor/Quencher



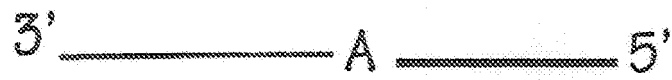
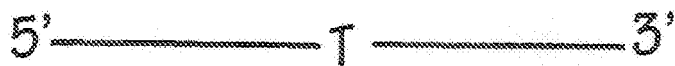
Common Primer 5' ————— 3'

1a

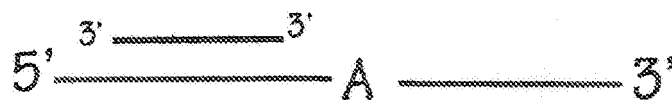
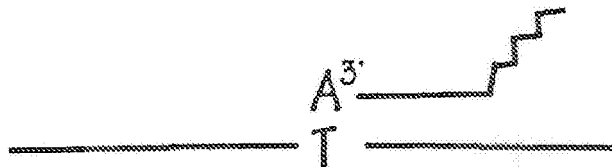
Allele Specific Primer 1



This is denatured at 94°C

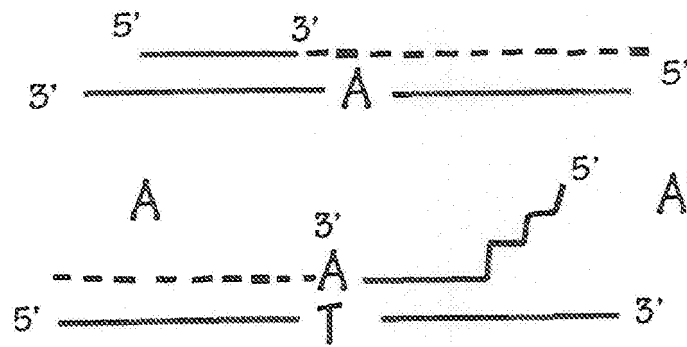


The primers then bind at 57°C

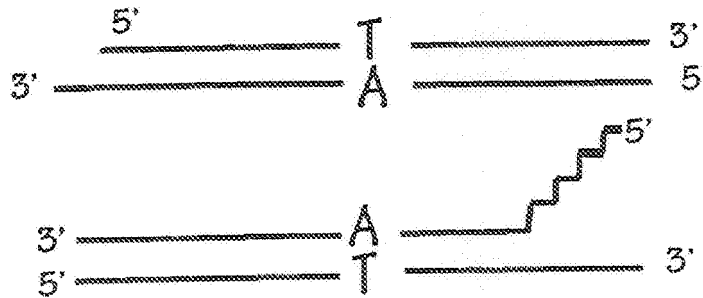


Then primers extend

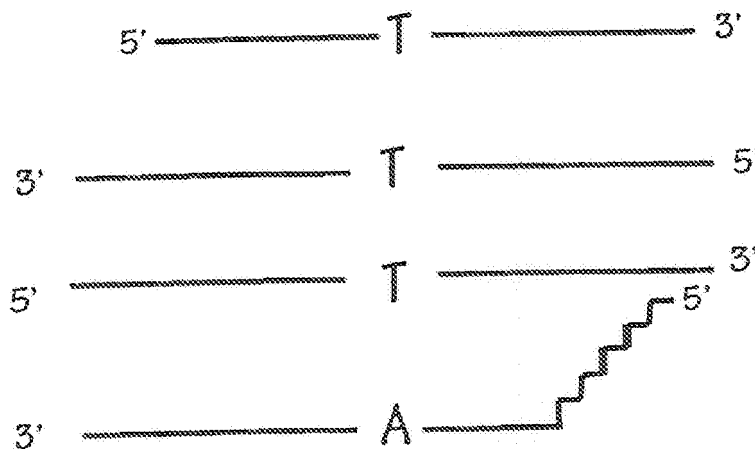
1b



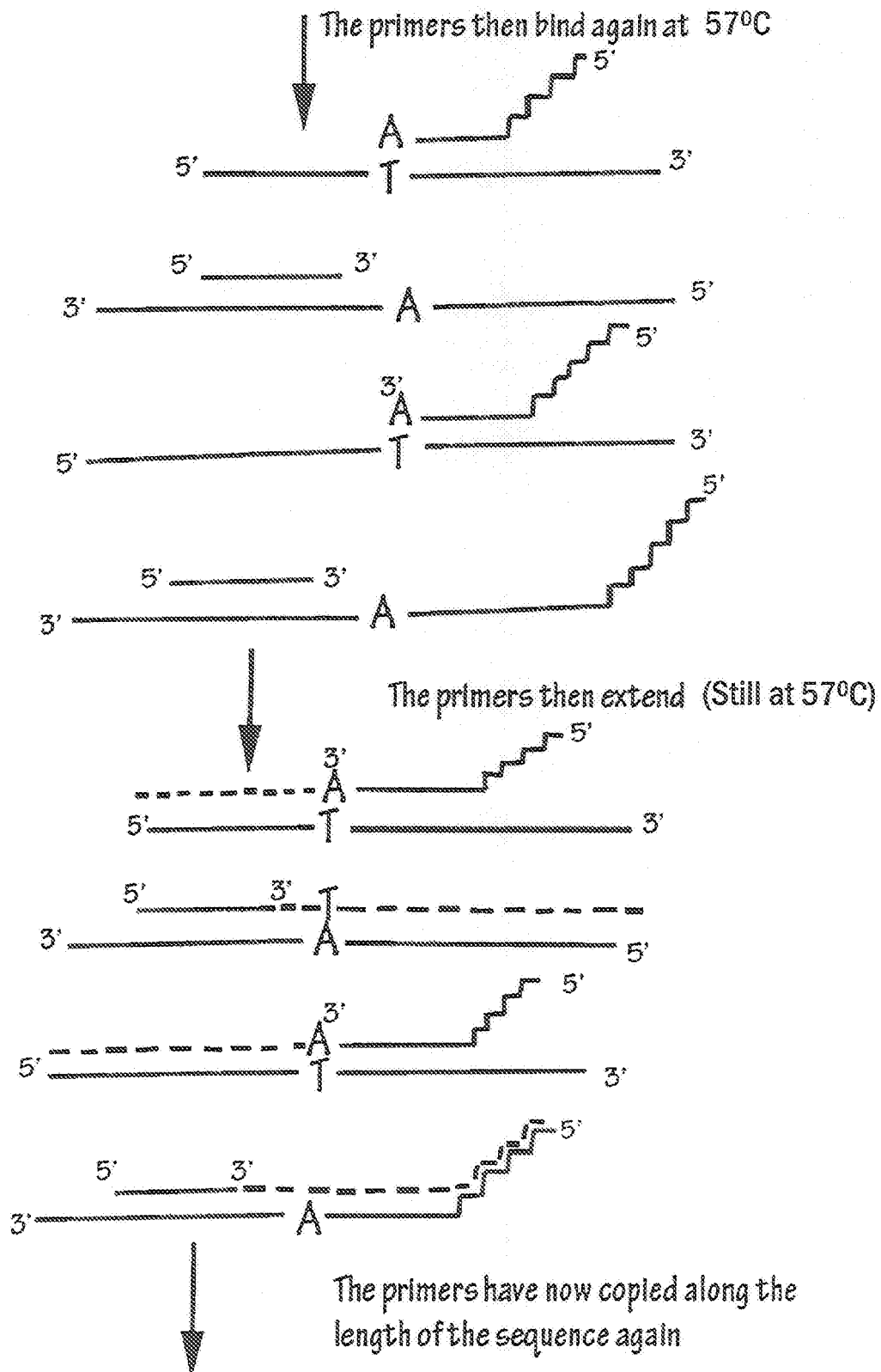
The primers have copied along the
length of the sequence
Still at 57°C



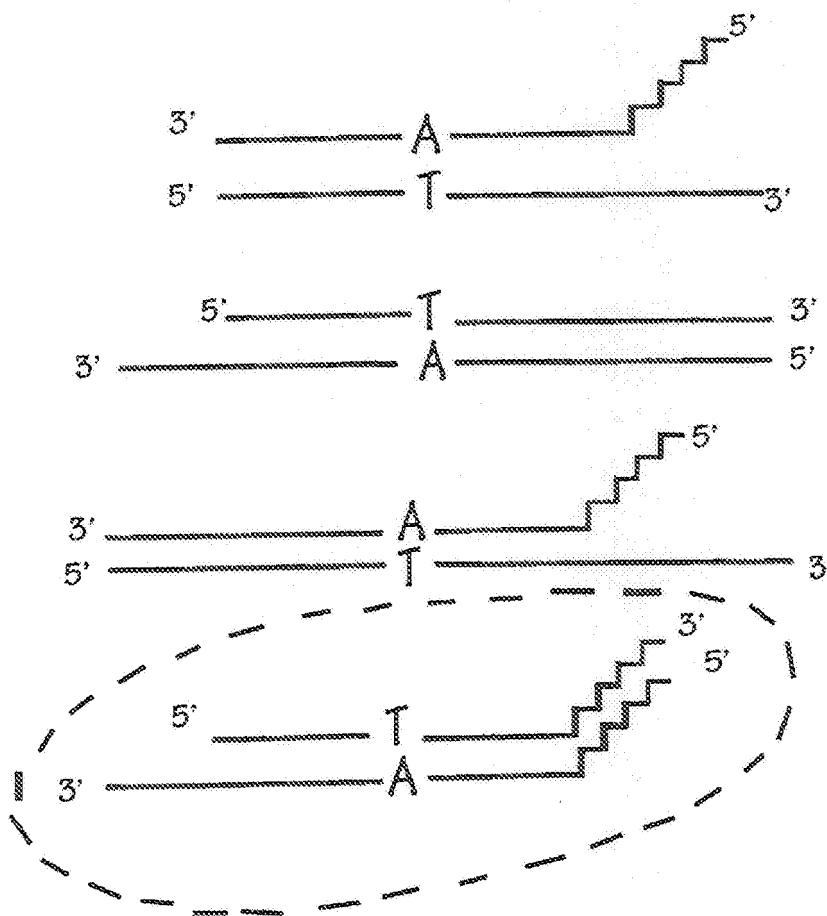
Then placed again in the 94°C
both strands denature



10:



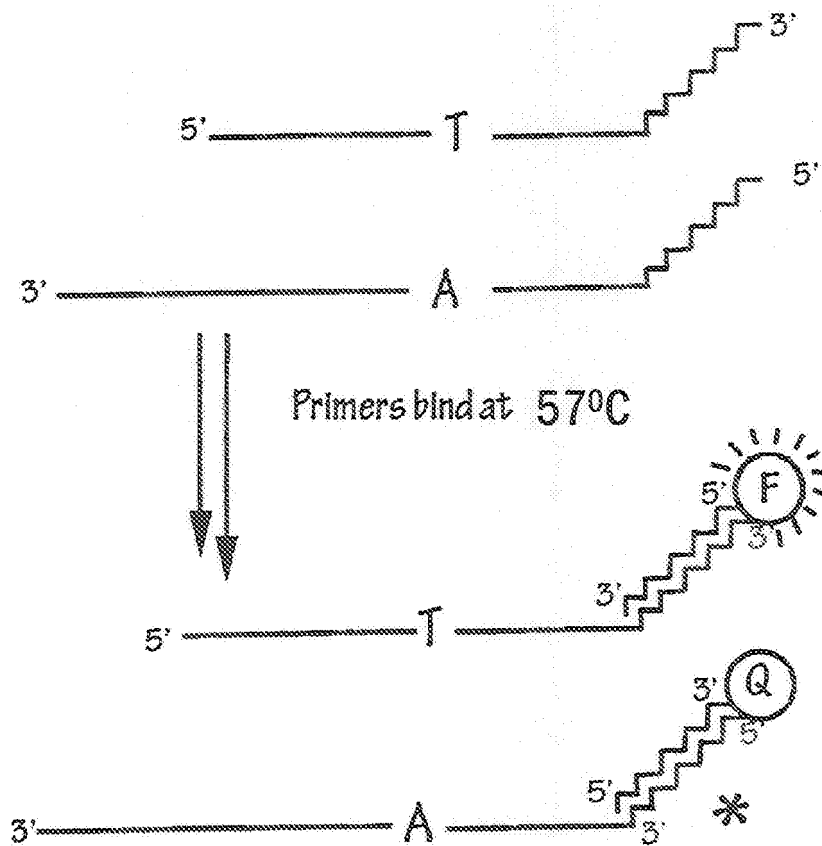
1d



At this point the most important part
is the extension of the common primer
along the allele specific primer to create
a reverse compliment of the tail sequence

Although in the next stage all denature at 94°C it is this
last scenario with the reverse compliment of the tail
sequence that is illustrated below

1e

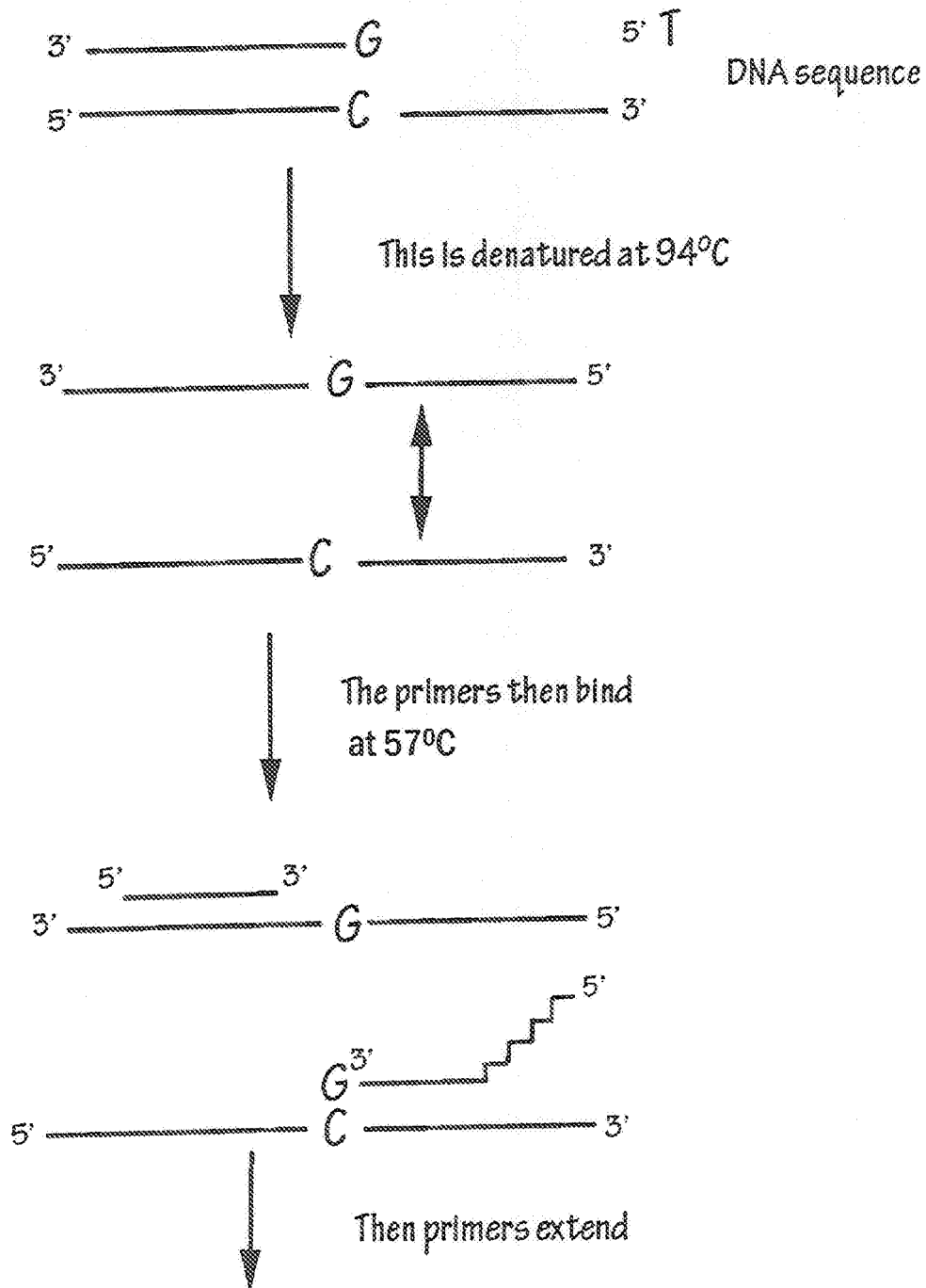


The complementary fluorescent primer is then able to bind leaving it free from the quenching effect of the quencher and able to fluoresce when excited.

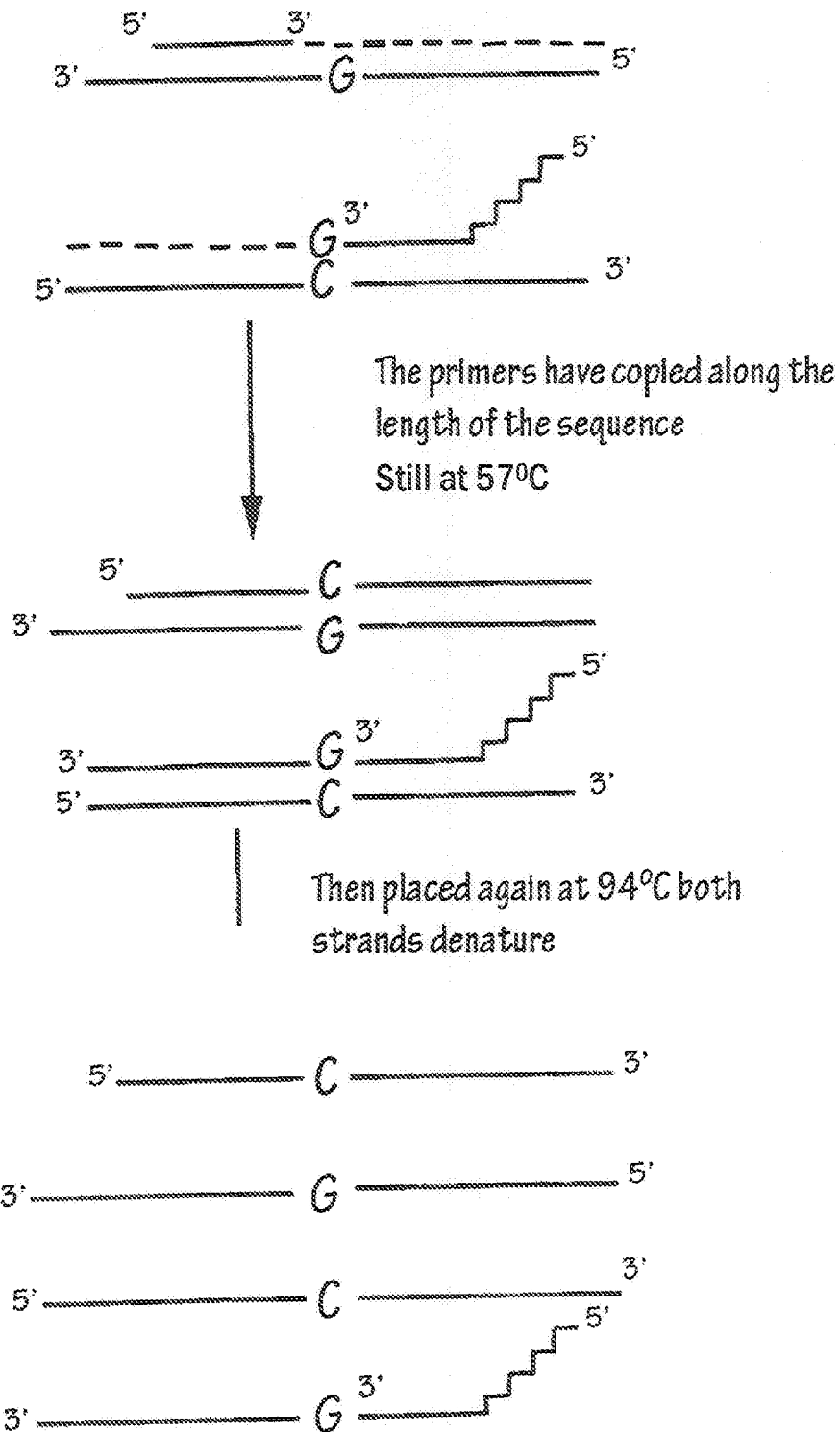
* N.B. Although the quencher can bind it is unable to extend in a 3' to 5' direction

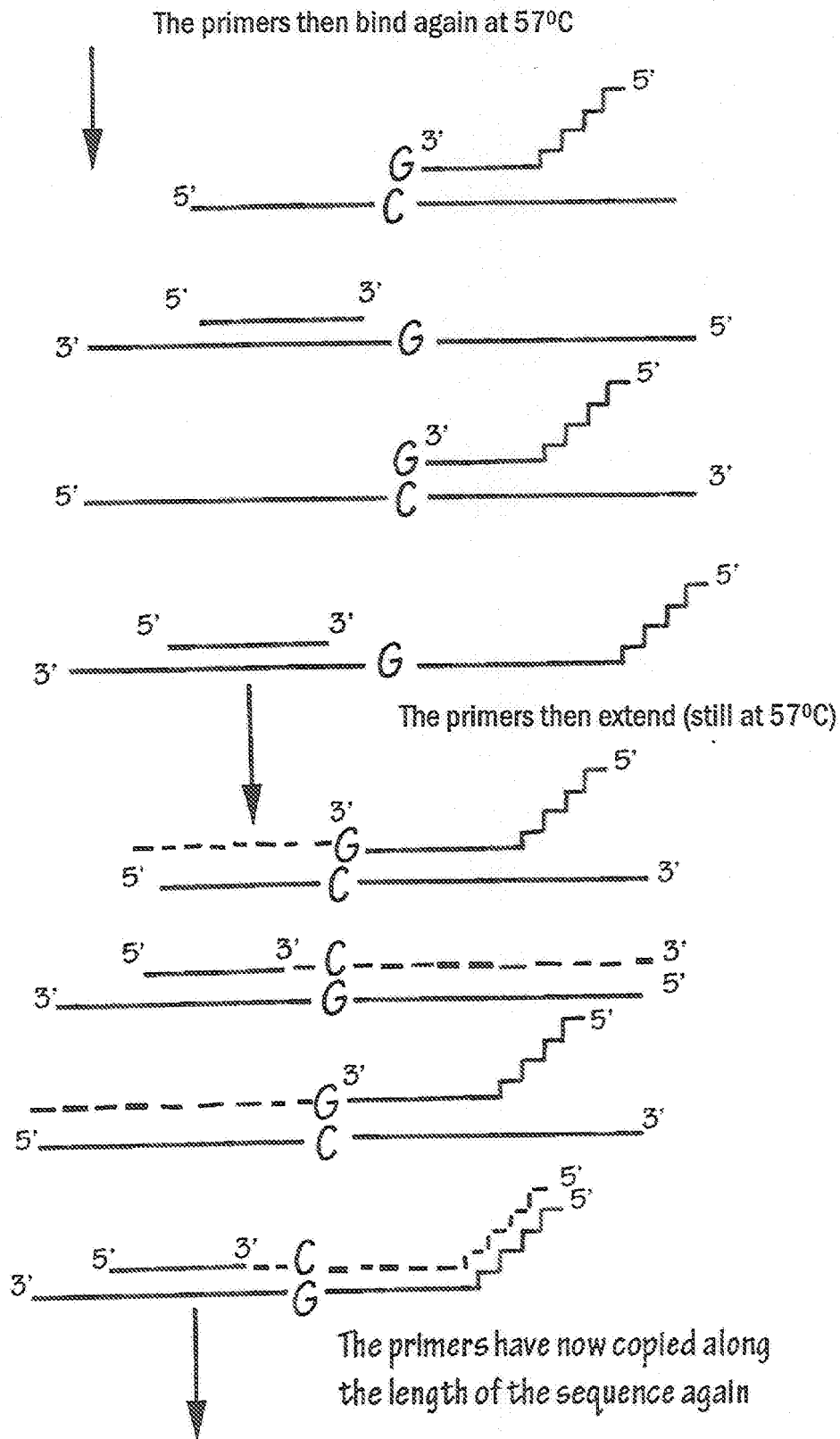
2a

Allele Specific primer 2

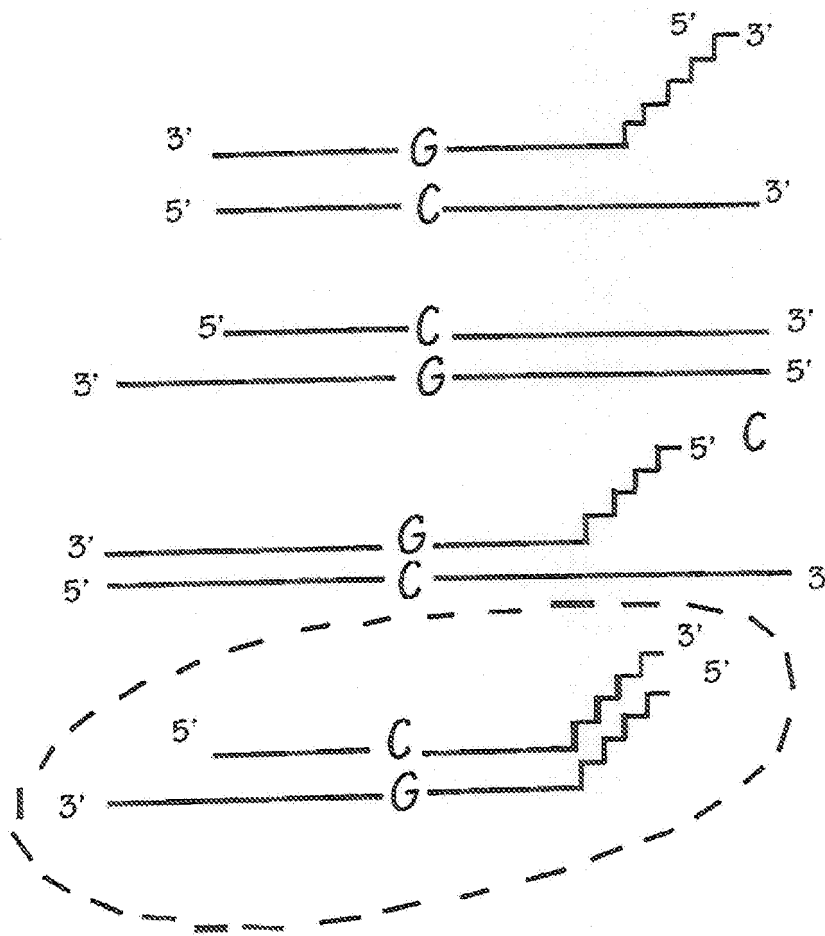


2b.





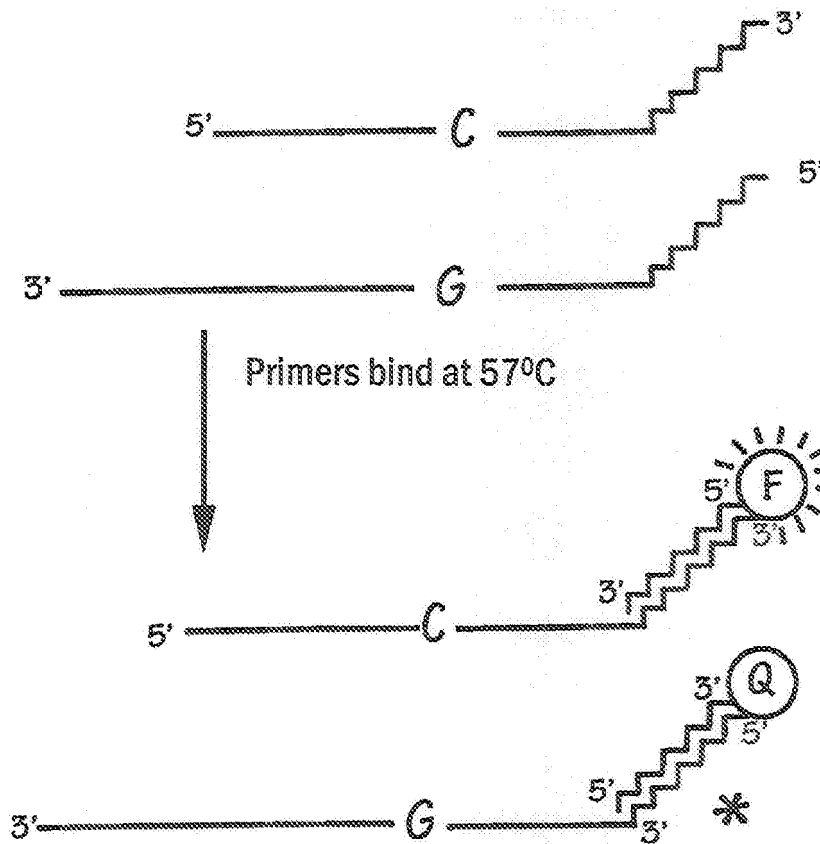
2d



At this point the most important part
is the extension of the common primer
along the allele specific primer to create
a reverse compliment of the tail sequence

Although in the next stage all denature at 94°C it is this
last scenario with the reverse compliment of the tail
sequence that is illustrated below

2e



The compliment fluorescent primer is then able to bind leaving it free from the quenching effect of the quencher and able to fluoresce when excited.

* N.B. Although the quencher can bind
It is unable to extend in a 3' to 5' direction

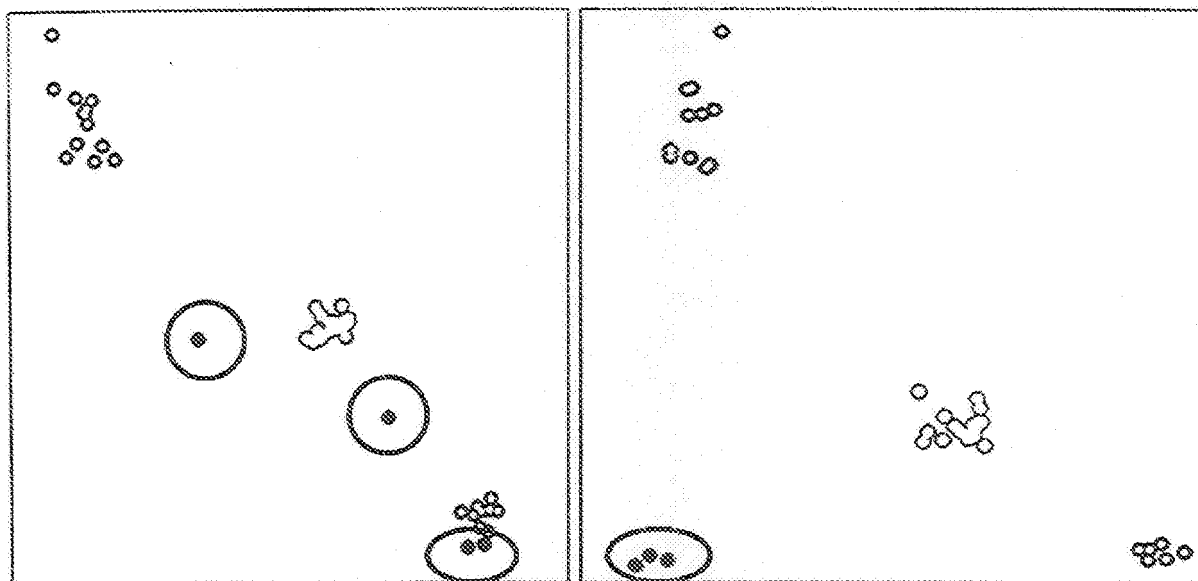


Figure 2:

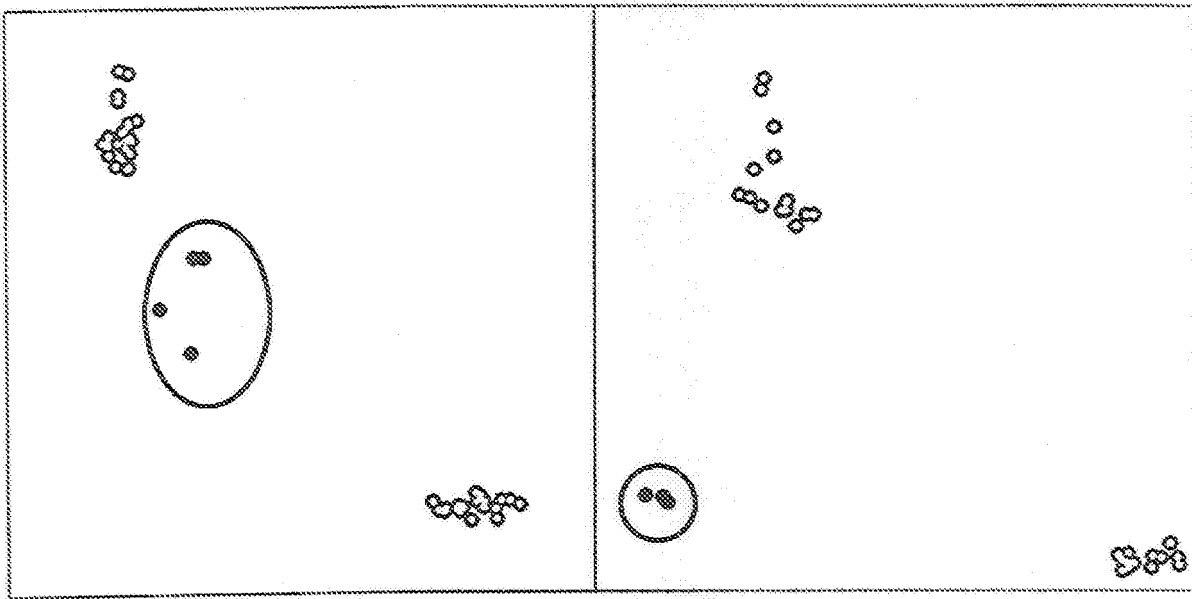


Figure 3:

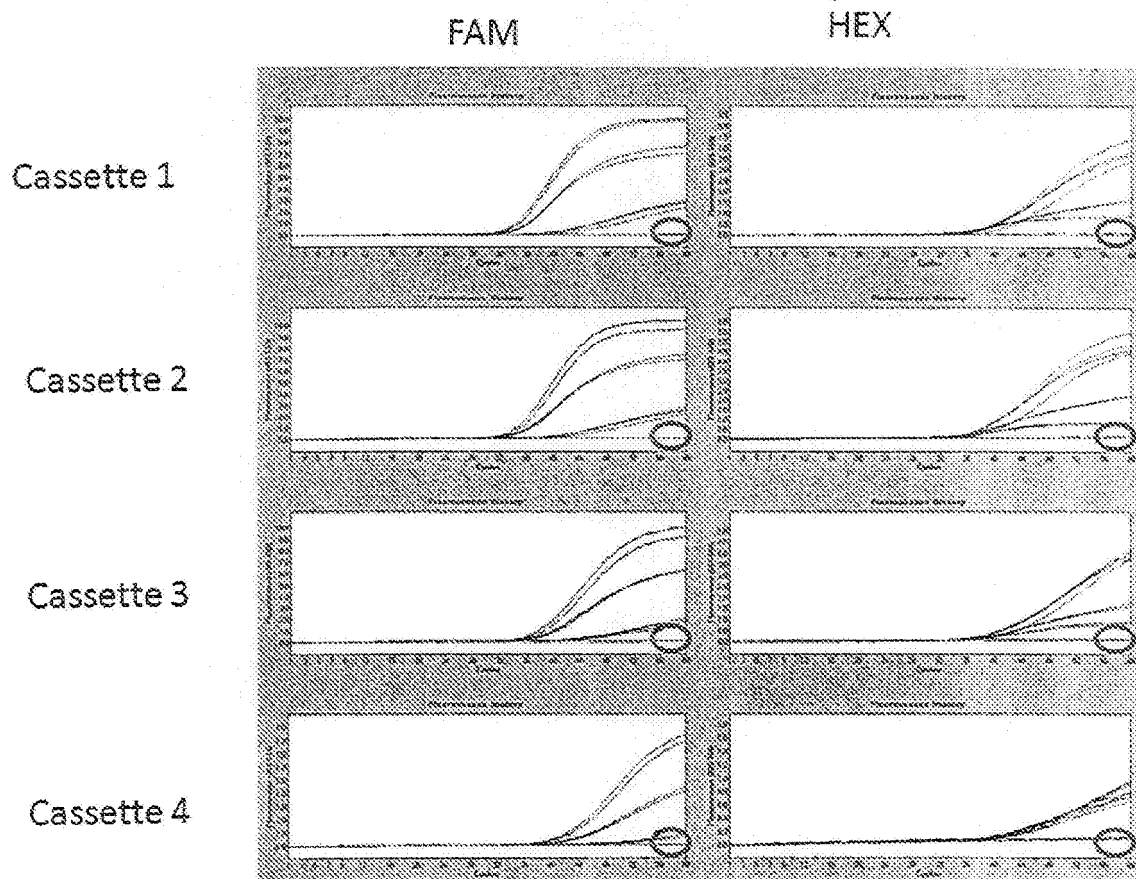


Figure 4:

SNP ————— [A/G/T/C] —————

There are four
possible outcomes
Per Allele for this SNP

————— A —————
or
————— G —————

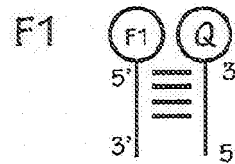
————— T —————
or
————— C —————

The primers involved in this reaction are as follows:

Allele specific primer 1



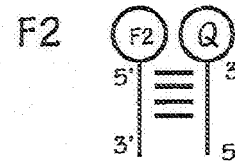
Respective Fluor/Quencher



Allele specific Primer 2



Respective Fluor/Quencher

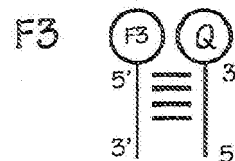


Common Primer 1 5' ————— 3'

Allele specific primer 3



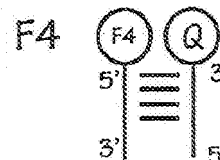
Respective Fluor/Quencher



Allele specific Primer 4



Respective Fluor/Quencher



Common Primer 2 5' ————— 3'

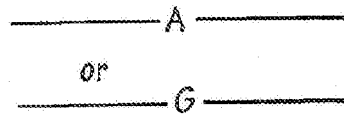
Figure 5

SNP 1 ——— [A/G] ———

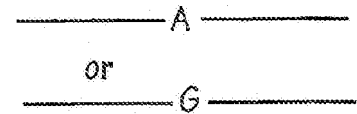
SNP 2 ——— [A/G] ———

1

There are two
possible outcomes
Per Allele for this SNP



There are two
possible outcomes
Per Allele for this SNP

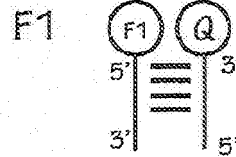


The primers involves in this reaction are as follows:

Allele specific primer 1



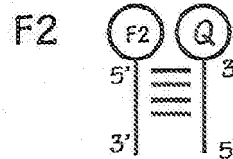
Respective Fluor/Quencher



Allele specific Primer 2



Respective Fluor/Quencher

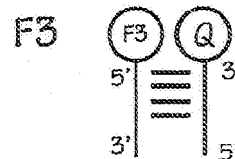


Common Primer 1 5' ————— 3'

Allele specific primer 3



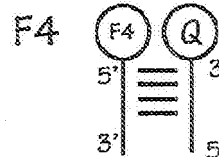
Respective Fluor/Quencher



Allele specific Primer 4



Respective Fluor/Quencher



Common Primer 2 5' ————— 3'