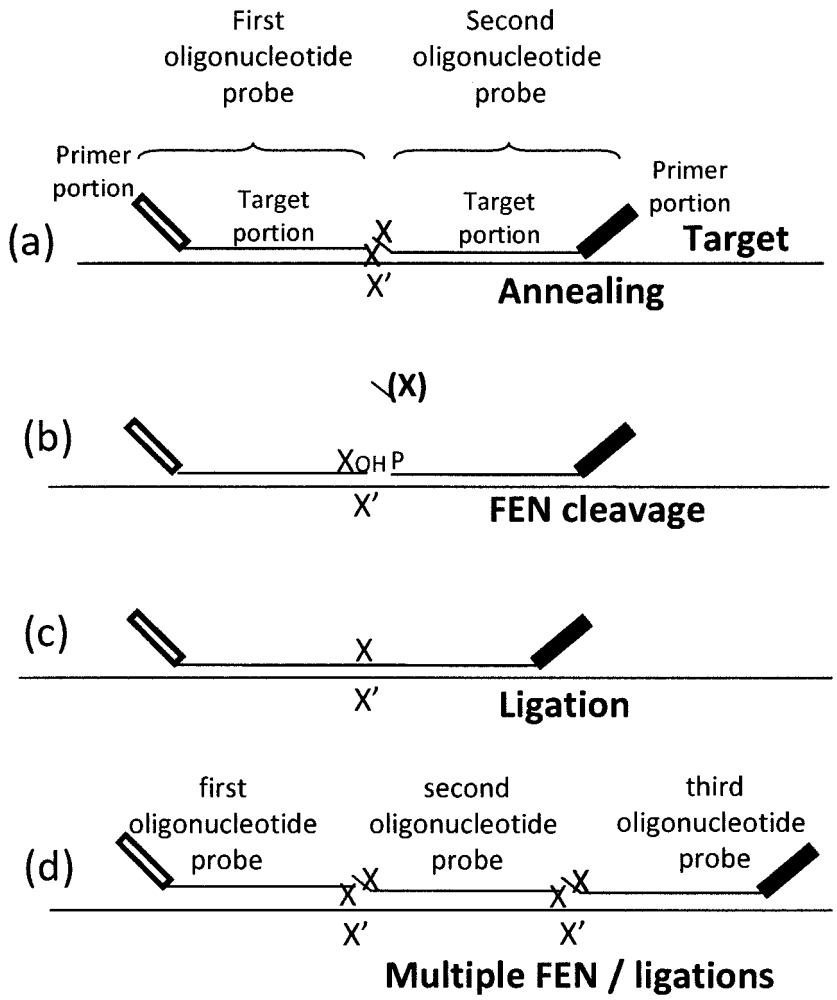

First oligonucleotide probe
Second oligonucleotide probe
Primer portion
Target portion
Target portion
Primer portion
(a)
Target
X
X'
Annealing
(X)
(b)
XOH P
X'
FEN cleavage
(c)
X
X'
Ligation
first oligonucleotide probe
second oligonucleotide probe
third oligonucleotide probe
(d)
X'
X'
Multiple FEN / ligations

Figure 1

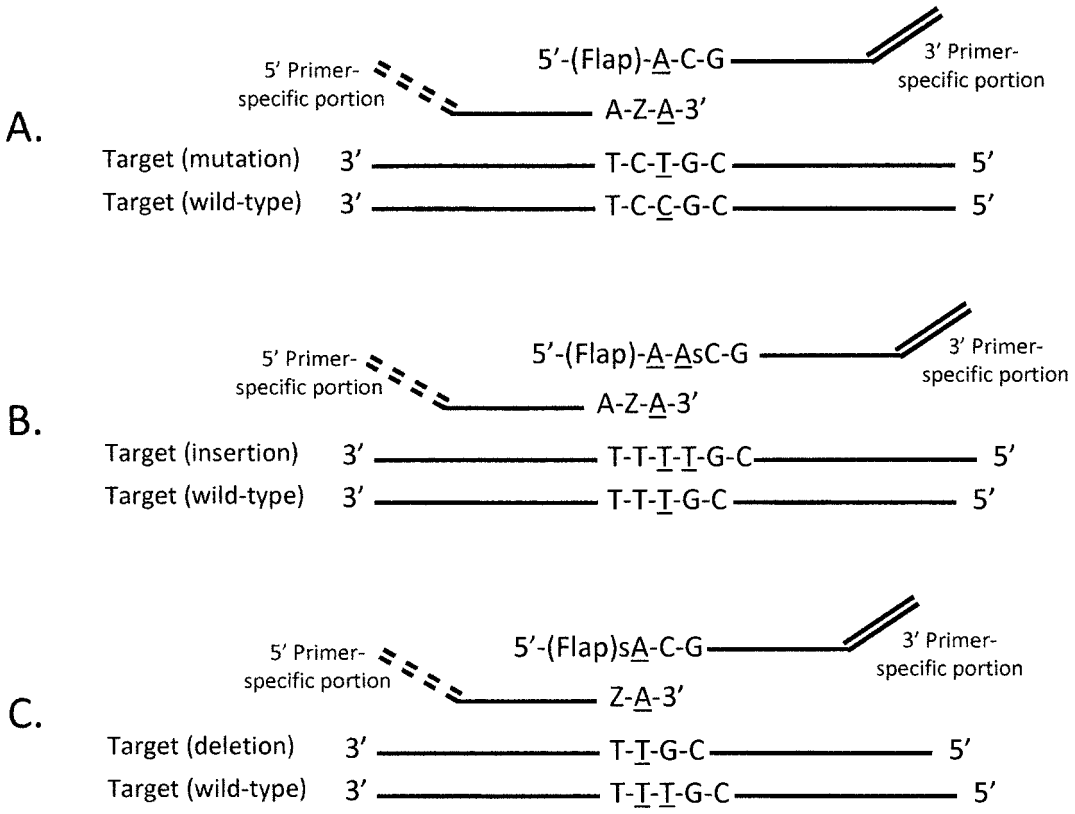

A.
5′ Primer-specific portion
5′-(Flap)-A-C-G
3′ Primer-specific portion
A-Z-A-3′
Target (mutation) 3′ T-C-T-G-C 5′
Target (wild-type) 3′ T-C-C-G-C 5′
B.
5′ Primer-specific portion
5′-(Flap)-A-AsC-G
3′ Primer-specific portion
A-Z-A-3′
Target (insertion) 3′ T-T-T-T-G-C 5′
Target (wild-type) 3′ T-T-T-G-C 5′
C.
5′ Primer-specific portion
5′-(Flap)sA-C-G
3′ Primer-specific portion
Z-A-3′
Target (deletion) 3′ T-T-G-C 5′
Target (wild-type) 3′ T-T-T-G-C 5′

Figure 2

1. Nuclease-ligation reaction to detect G→A, T, C mutation in K-ras gene includes 4 5'-N first oligonucleotide probes also containing unique zip-code portions, and four N-3' second oligonucleotide probes containg a detectable label.

2. 5'-nuclease activity cleaves off only matching 5'-overlapping base, e.g., 5'-C in case of wild type and 5'-D (D=A,G or T) in case of a mutation (5'-A shown) leaving ligation-competent 5'-phospate.

3. Ligation covalently seals the two oligo probes together (only mutation case is shown).

4. Detection of labeled ligation products on array of capture oligonucleotides

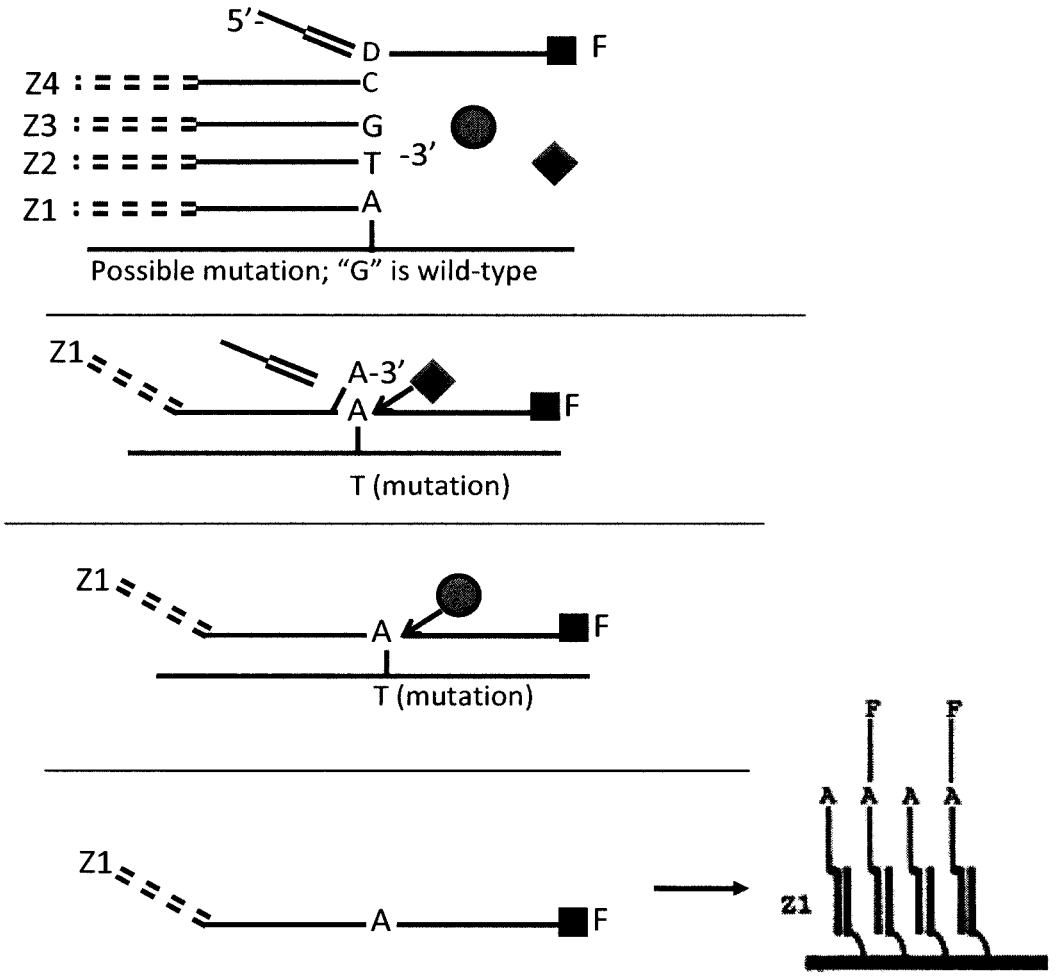
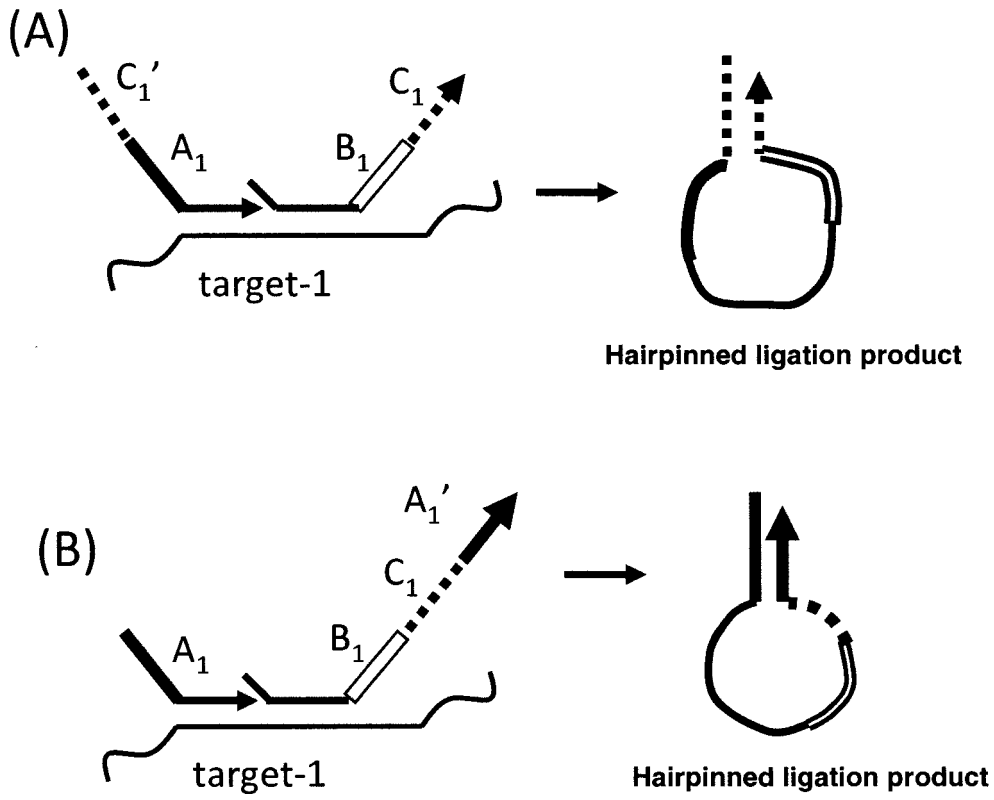


Figure 3



Figures 4A-4B

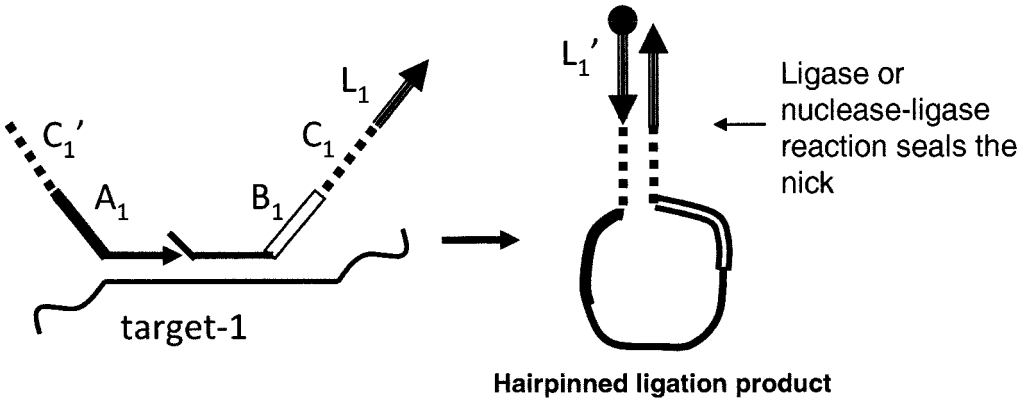

C1'
A1
B1
C1
L1
target-1
L1'
Ligase or nuclease-ligase reaction seals the nick
Hairpinned ligation product

Figure 4C

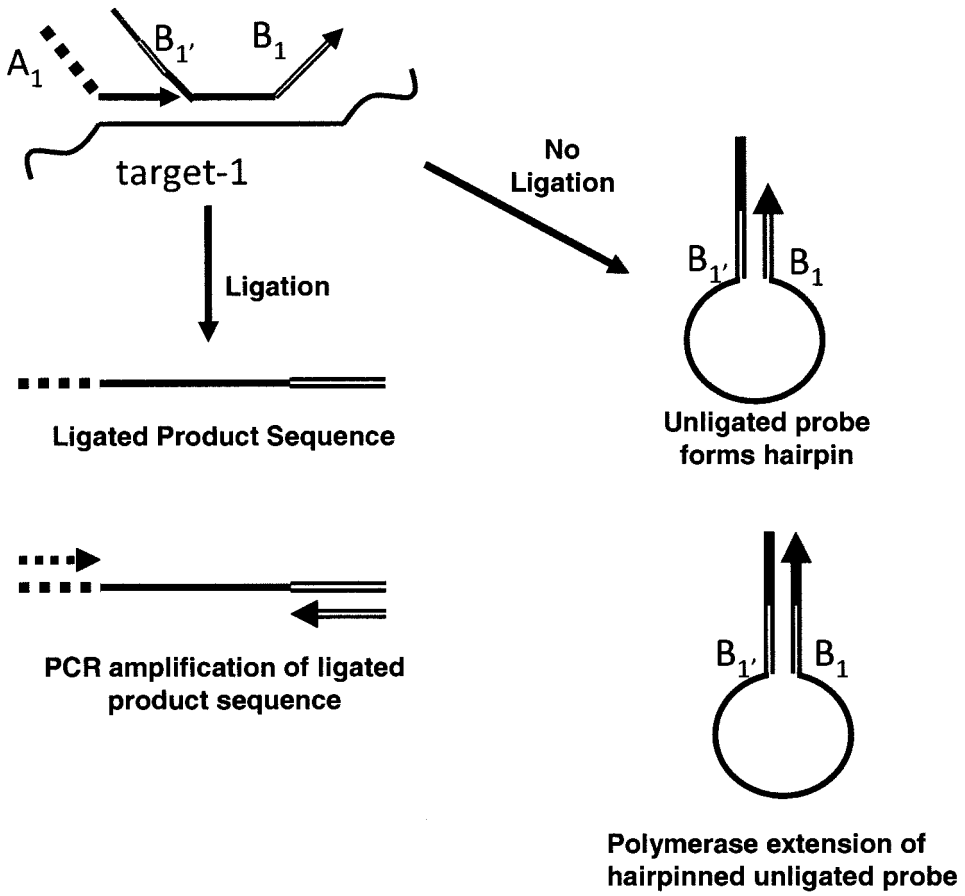

A1
B1'
B1
target-1
Ligation
No Ligation
Ligated Product Sequence
Unligated probe forms hairpin
PCR amplification of ligated product sequence
Polymerase extension of hairpinned unligated probe

Figure 5

1. Nuclease-ligation reaction to detect G→A, T, C mutation in K-ras gene includes 4 5′-N second oligonucleotide probes, some containing additional 5′ flaps, and four N-3′ first oligonucleotide probes. Mutation-specific probes contain primer-specific portions for subsequent amplification, but wild-type probes contain short sequences that will not amplify.

2. 5′-nuclease activity of polymerase cleaves off only matching 5′-overlapping base and additional flap, e.g., 5′-C in case of wild type and 5′-D (D=A,G or T) in case of a mutation (5′-A shown) leaving ligation-competent 5′-phospate.

3. Ligation covalently seals the two probes together (only mutation case is shown). Unligated probes form a hairpin due to complementary regions of the 5′flap and 3′ primer-specific portion

4. PCR amplifies only ligation products for the mutant, but not wild-type alleles. Unligated hairpinned second oligonucleotide probes are extended by polymerase to occlude binding of, and subsequent extension or amplification by, the secondary primer.

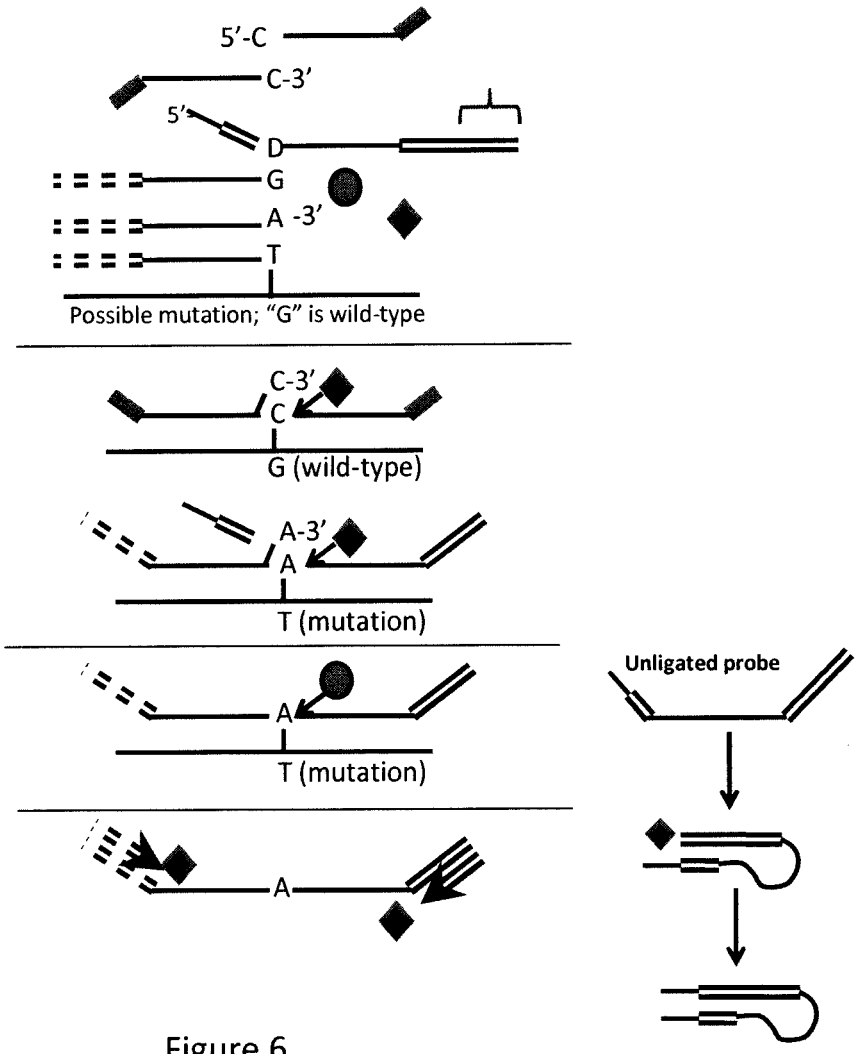


Figure 6

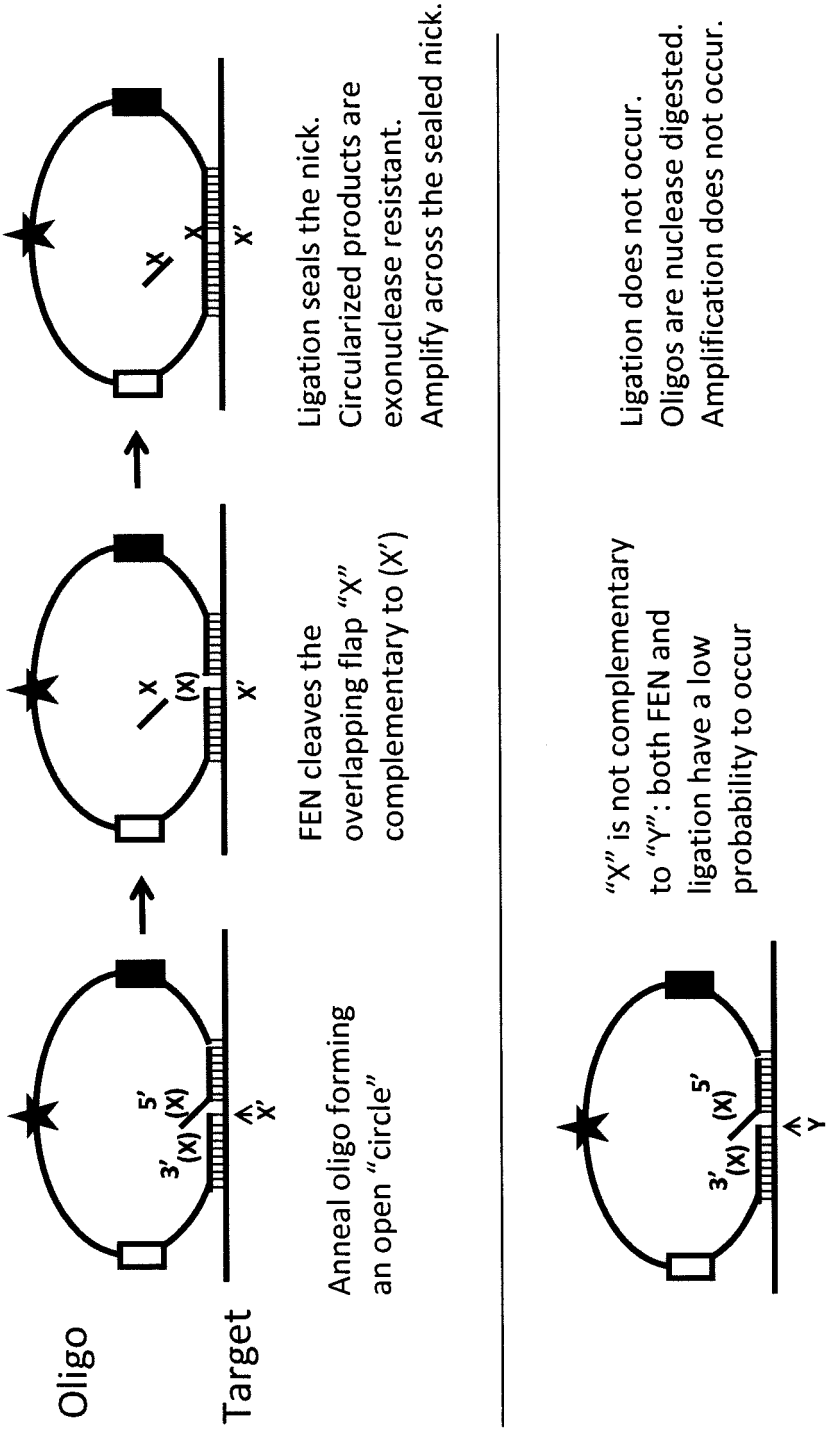

Oligo
Target
Anneal oligo forming an open "circle"
FEN cleaves the overlapping flap "X" complementary to (X')
Ligation seals the nick. Circularized products are exonuclease resistant. Amplify across the sealed nick.
"X" is not complementary to "Y": both FEN and ligation have a low probability to occur
Ligation does not occur. Oligos are nuclease digested. Amplification does not occur.

Figure 7

A. Nuclease-ligation reaction to detect G→A, T, C mutation in K-ras gene includes three coupled oligonucleotide probes with the mutation-specific base on the 3' end of the upstream probe and on or near the 5' end of the downstream probe (only "A" - specific probe is shown).

B. 5'-nuclease cleavage of only matching 5'-overlapping base, leaving ligation-competent 5'-phospate.

C. Ligation covalently seals the two free ends of the coupled probes to create covalently closed ligation products. Unligated probes form hairpins due to hybridization between complementary (Kr) and (Kr') regions.

D. PCR amplifies only ligation products for the mutant, but not wild-type alleles. Unligated hairpinned probes are extended by polymerase to occlude binding of, and subsequent extension or amplification by, the secondary primer.

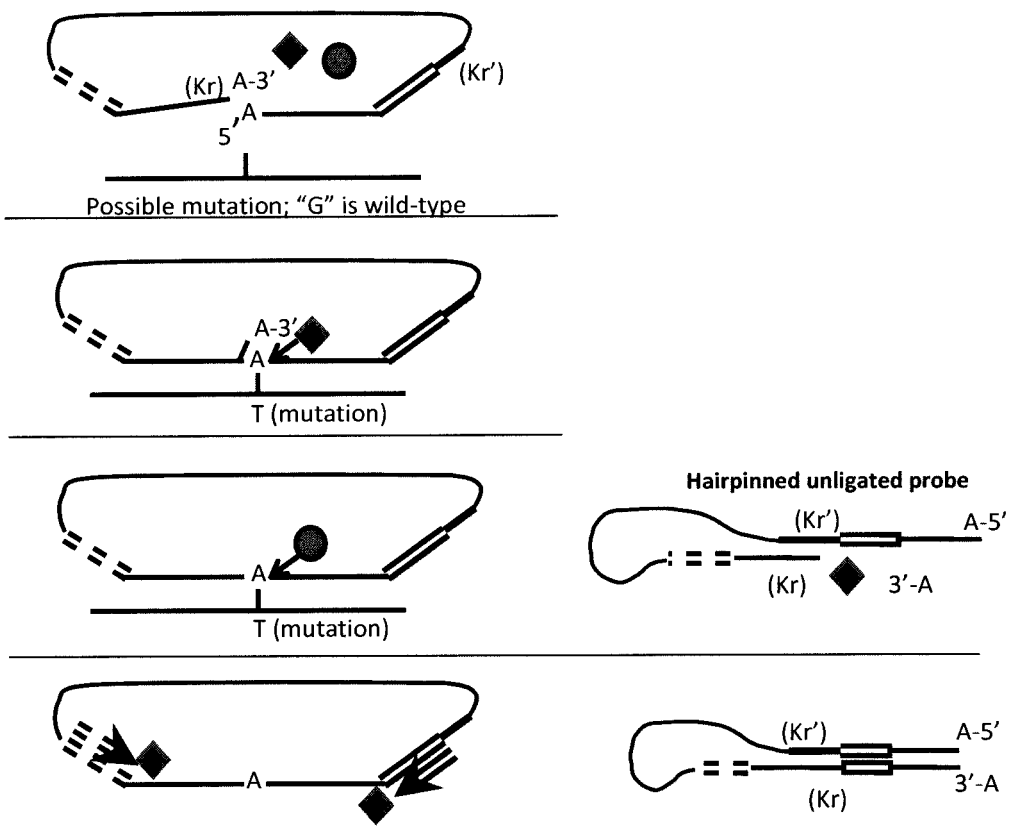
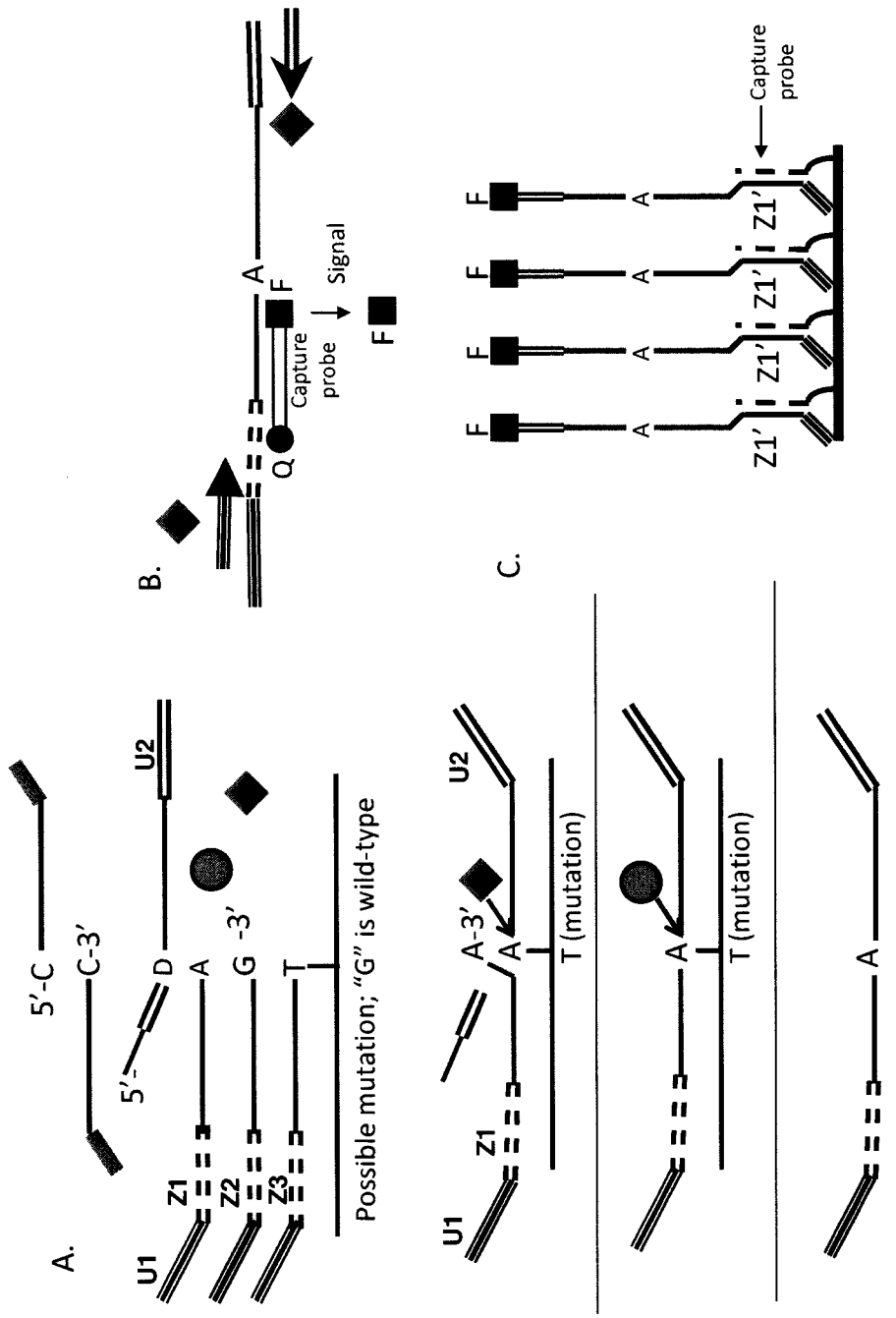
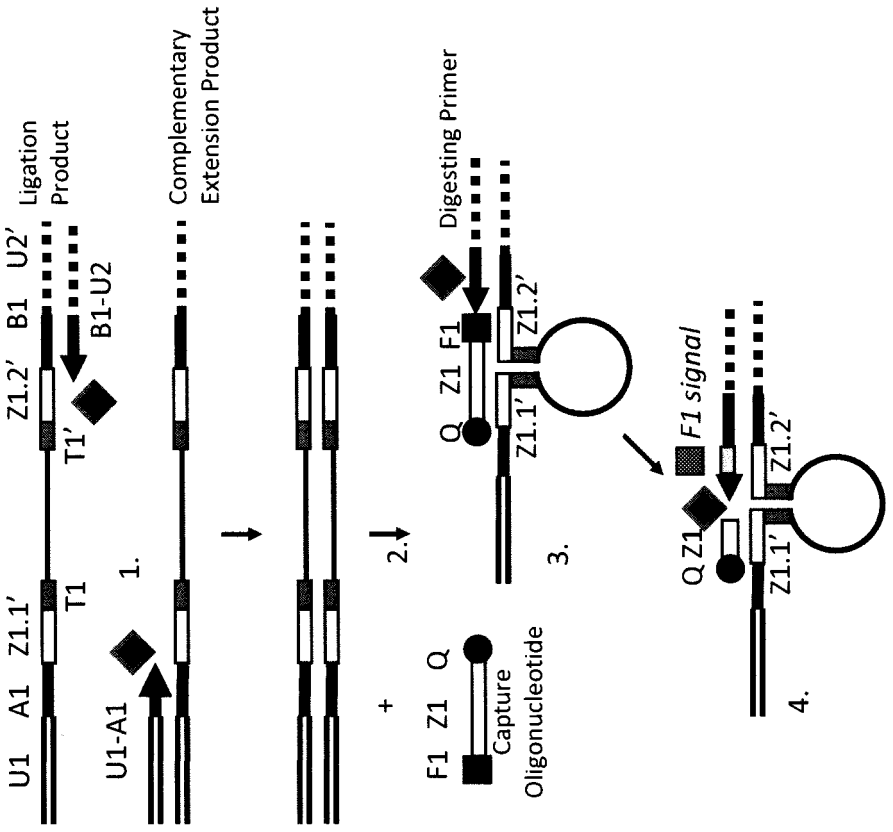


Figure 8

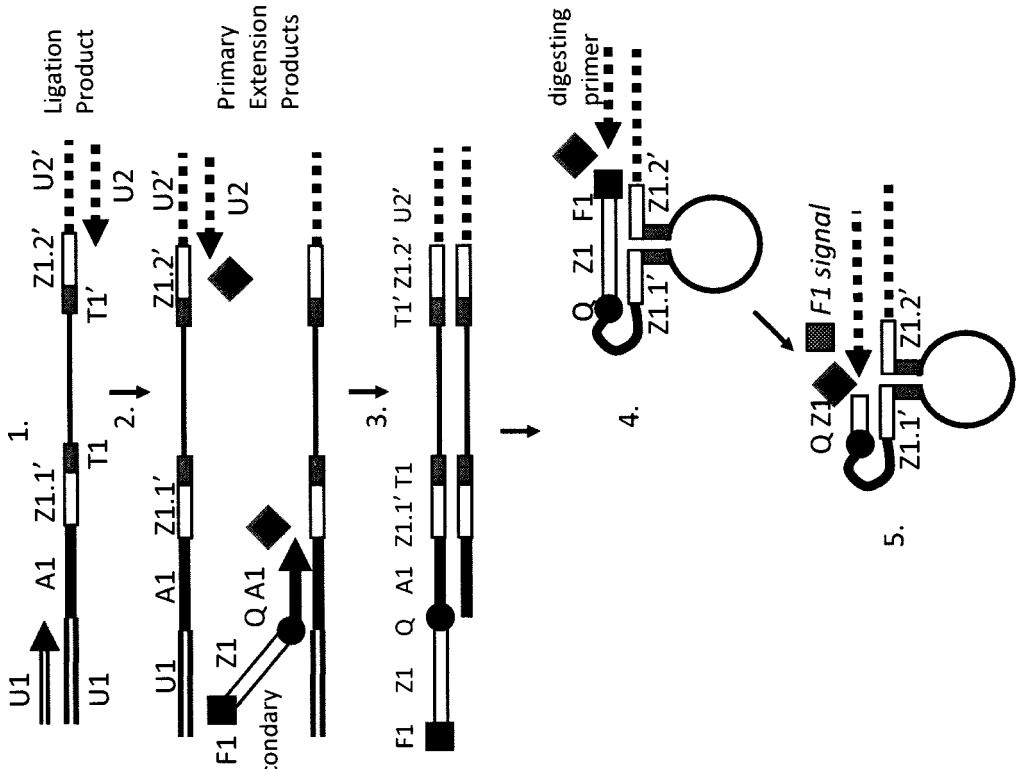


Figures 9A-9C



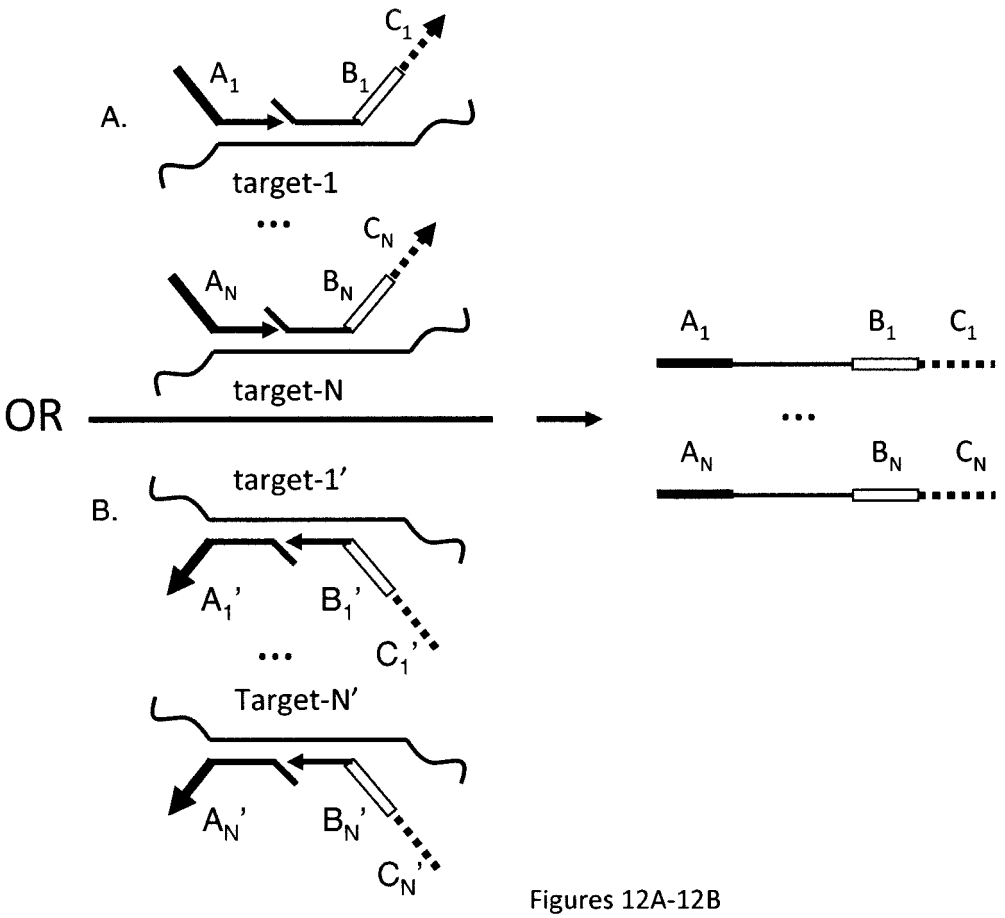
1. Priming on the tag-encoded ligation product and complementary extension product thereof.
2. dsDNA products of PCR.
3. Hairpin formation: after dsDNA melts and the temperature decreases hairpin forms with the stem between Z1.1' + Z1.2'.
4. Signal is generated by 5' nuclease activity of polymerase when it extends primer, the "digesting primer". As soon as polymerase has traversed Z1.2', the short stem between Z1.2' and Z1.1' falls apart and polymerase continues extending to create the dsDNA product.

Figure 10

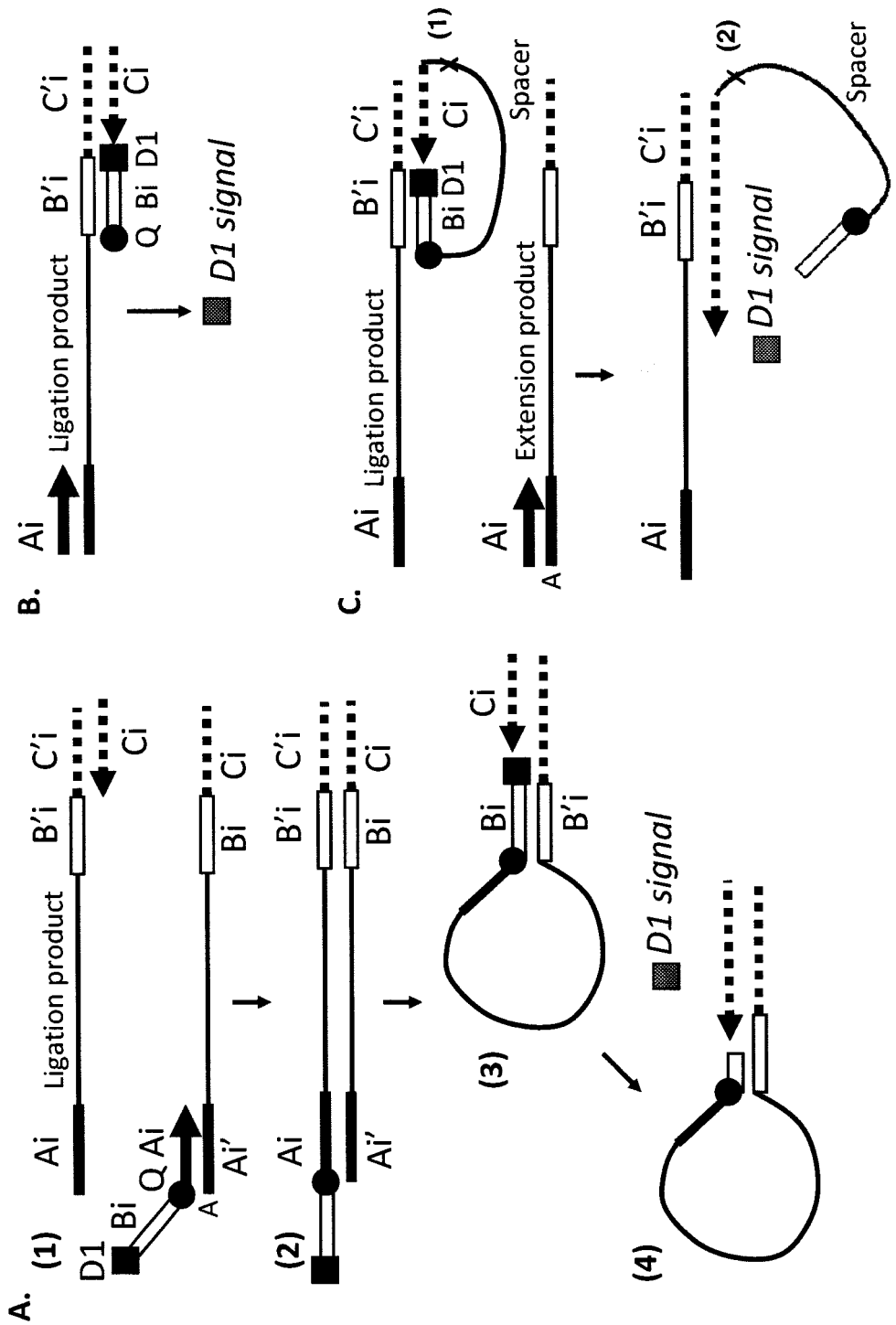


1. Universal PCR amplification of ligation product
2. Priming on both strands of primary extension products of the ligation products.
3. The dsDNA products of PCR; the polymerase blocker stops extension of the bottom strand.
4. Dual Hairpin formation: after dsDNA melts and the temperature decreases two hairpins form with the stems between Z1 and Z1.1' + Z1.2'.
5. Signal is generated by 5' nuclease activity of polymerase when it extends the "digesting primer". As soon as polymerase has traversed Z1.2', the short stem between Z1.2' and Z1.1' falls apart and polymerase continues extending until it comes to the polymerase blocker to create a dsDNA product.

Figure 11



Figures 12A-12B



Figures 13A-13C

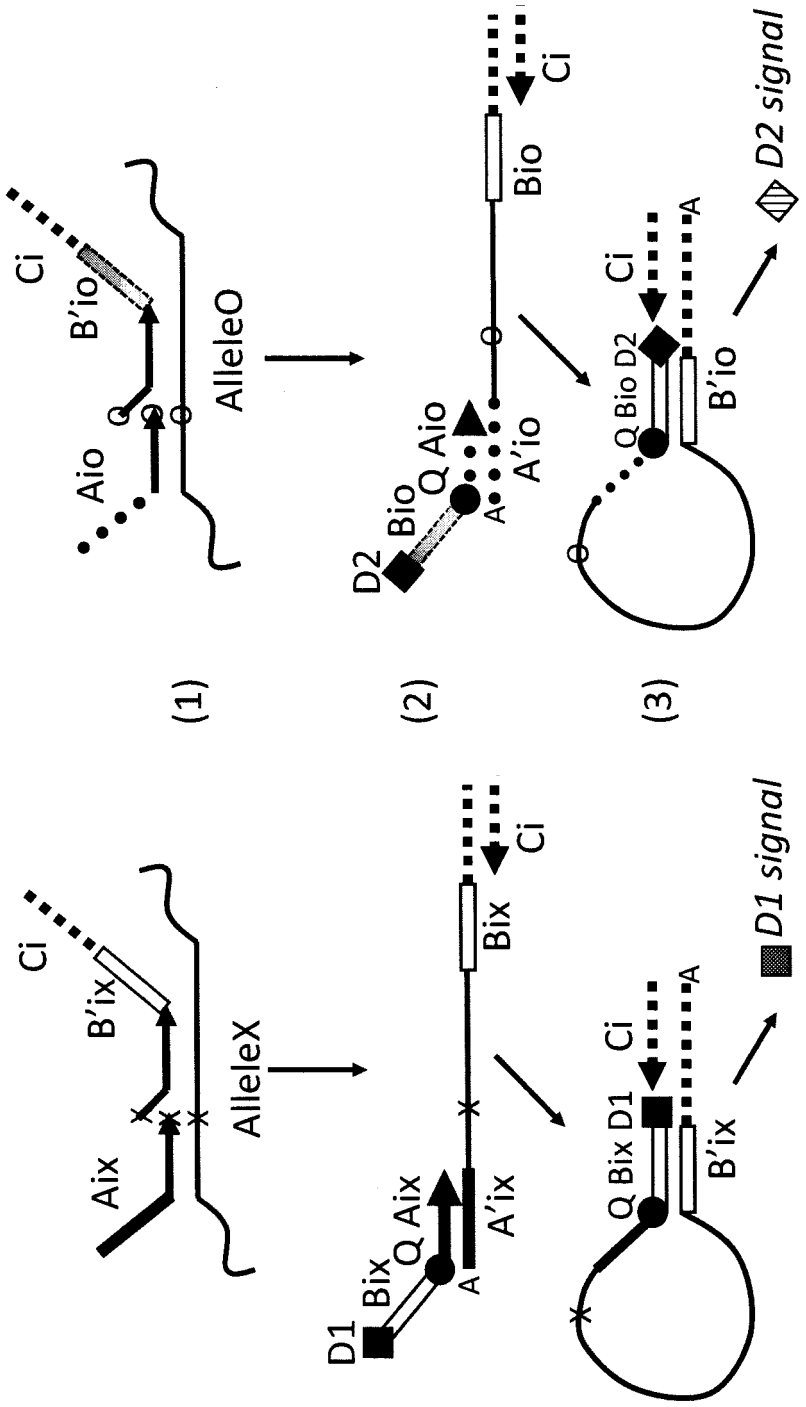

Ci
B'io
Aio
AlleleO
(1)
Bio
Ci
Q Aio
A'io
D2
Bio
(2)
Ci
A
Q Bio D2
B'io
D2 signal
(3)
Ci
B'ix
Aix
AlleleX
Bix
Ci
Q Aix
A'ix
D1
Bix
Ci
A
Q Bix D1
B'ix
D1 signal

Figure 14

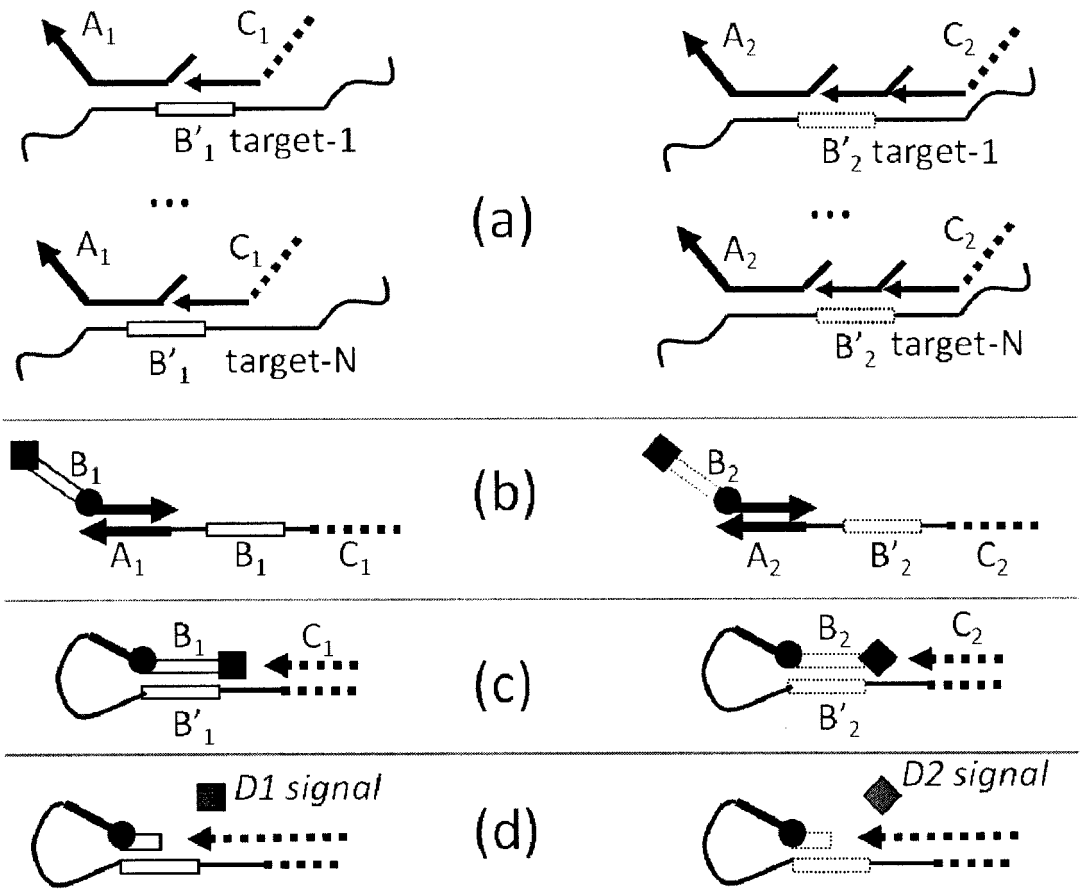

A1
C1
B'1 target-1
A2
C2
B'2 target-1
(a)
A1
C1
B'1 target-N
A2
C2
B'2 target-N
B1
A1
B1
C1
(b)
B2
A2
B'2
C2
B1
C1
B'1
(c)
B2
C2
B'2
D1 signal
(d)
D2 signal

Figure 15

1. The first and second oligonucleotide probes anneal to the template DNA. The first probe is extended towards the second probe, strand-displacing an internal matched base, which is 5' to two internal thiophosphate bonds.

2. 5-nuclease activity cleaves at that internal base, but further nuclease activity is halted by internal thiophosphate bonds, which are not cleavable.

3. Ligase ligates the extended first probe to the second probe. Unligated second probes (that were not cleaved) form hairpins that are extended by polymerase to occlude binding of, and subsequent extension or amplification by, the secondary primer.

4. The target DNA is suitable for direct sequencing, or alternatively PCR amplification and sequencing.

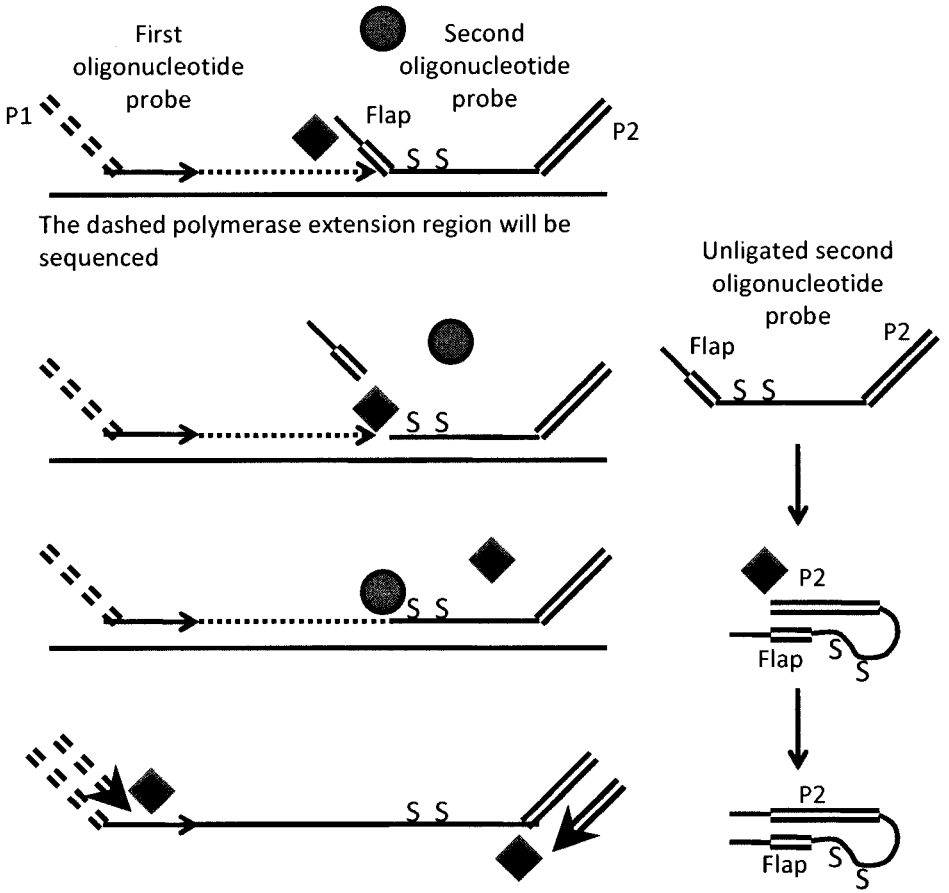


Figure 16