

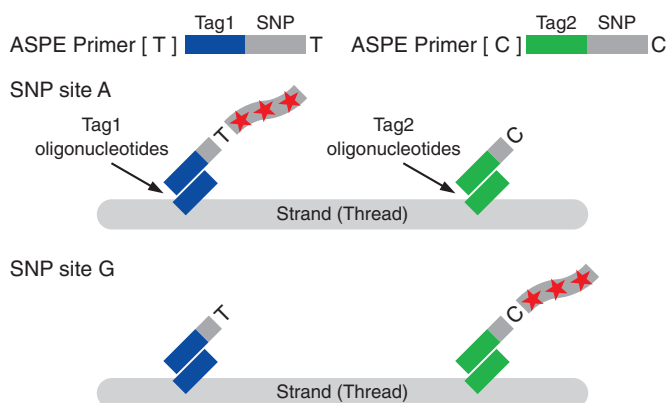
Handy Bio-Strand® System for Simultaneous multi SNPs analysis (Format II)



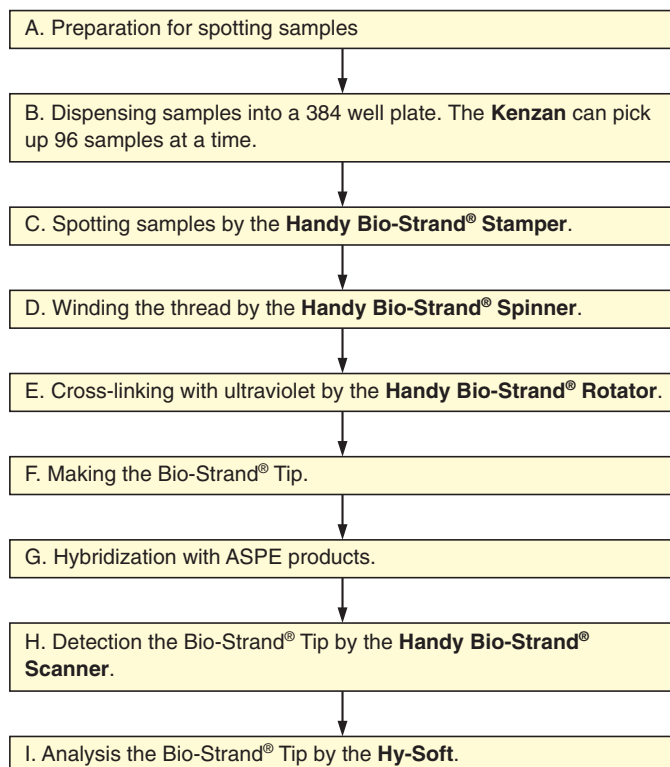
Introduction

Multi-SNPs analysis using Handy Bio-Strand® enables detection of 168 SNPs utilizing a Tag oligonucleotide simultaneously. By fixing Tag oligonucleotides on a strand and hybridizing with ASPE (Allele Specific Primer Extension) products using primers complementary to Tag oligonucleotides.

The results were completely consisted with the sequence of the SNP site.



Experimental Procedure



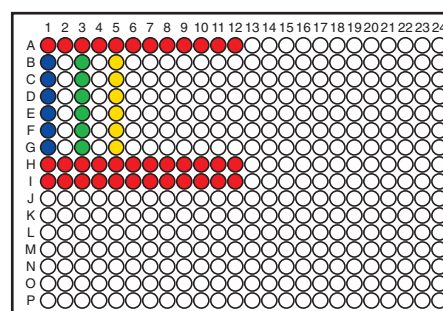
Method

A. Spotting samples preparation

Table 1. Sequence of Spotting samples

Probe oligonucleotide (5'-3')	
S Cy5 R4a (=Marker)	Cy5-ATCTTTTCCAGATATTCACCC
Biotin C12 Tag1	Biotin (spacer C12) - CCTGGTGGTTGACTGATCACCATAA
Biotin C12 Tag2	Biotin (spacer C12) - GCTAGATGAAGAGCAAGCGCATGGA
Biotin C12 Tag6	Biotin (spacer C12) - TTGAAGTTCGAGAATCGTATGTGT

B. Sample spot



- 150nM Cy5 labeled oligonucleotide marker (S Cy5 R4a)
- 10uM Biotin labeled oligonucleotide (Tag 1)
- 10uM Biotin labeled oligonucleotide (Tag 2)
- 10uM Biotin labeled oligonucleotide (Tag 6)

Figure 1. Spot Map

E. Cross-linking

Table 2. Intensity of UV irradiation for probe immobilization

UV energy	100 mJoules/cm ²
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G. Hybridization

Table 3. Primer sequence for ASPE products

PCR Primer (5'-3')	
2D6 Fw	CAGTCAACACAGCAGGTTCA
2D6 Rv2	CCGAAACCCAGGATCTGGGT
ASP Primer (5'-3')	
2D6T1T	TTATGGTGATCAGTCAACCACCAGGAACGCTG GGCTGCACGCTACT
2D6T6C	ACACATACGATTCTGCGAACTTCAAACGCTG GGCTGCACGCTACC