

Infinium® II Assay Workflow

Illumina's Infinium II Assay provides unlimited multiplexing for whole-genome genotyping applications with manual or automated workflow

INTRODUCTION

The Infinium II Whole-Genome Genotyping Assay (Figure 1) is designed to interrogate a large number of SNPs at unlimited levels of loci multiplexing. Using a single bead type and dual color channel approach, the Infinium II Assay scales genotyping from 10,000 to hundreds of thousands of SNPs per sample. Illumina's optional Laboratory Information Management System (LIMS) and automation ensure positive sample

tracking while reducing time required and labor costs.

ASSAY PROTOCOL

The DNA sample used for this assay is isothermally amplified in an overnight step (Figure 1; Step 1 and Step 2). This amplification has no appreciable allelic partiality. Additionally, a relatively low DNA sample requirement of 750 ng is sufficient to assay over 500,000 SNP loci. The amplified product is then fragmented by a controlled enzy-

matic process that does not require gel electrophoresis (Step 3). After alcohol precipitation and resuspension of the DNA (Step 4), the BeadChip is prepared for hybridization in the capillary flow-through chamber (Step 5); samples are applied to BeadChips and incubated overnight. The amplified and fragmented DNA samples anneal to locus-specific 50-mers (covalently linked to one of over 500,000 bead-types) during the hybridization step (Step 6). One bead type corresponds

FIGURE 1: INFINIUM II ASSAY PROTOCOL

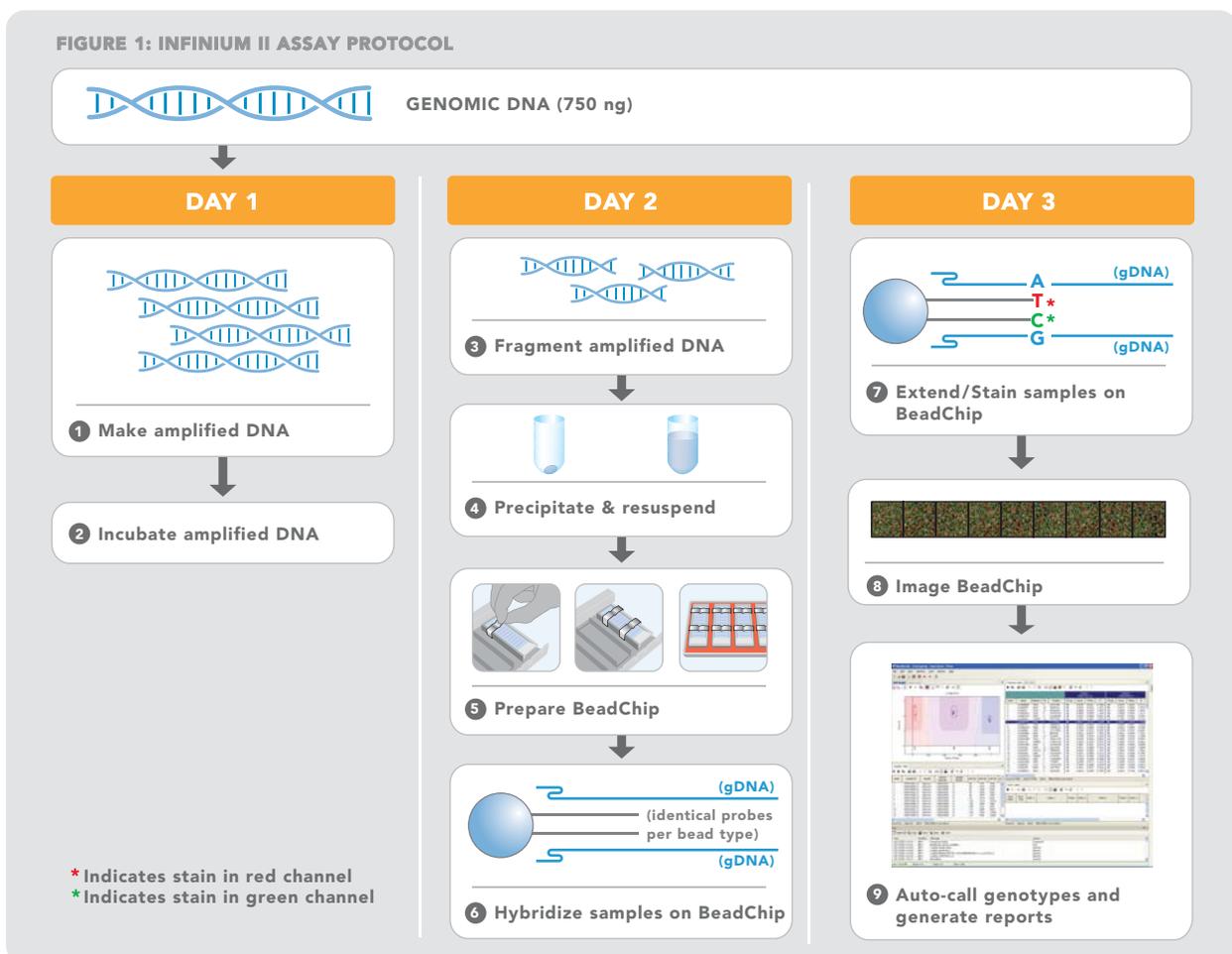


FIGURE 2: INFINIUM II ASSAY WORKFLOW

AUTOMATED

DAY	PROTOCOL STEP	8 BEADCHIPS		16 BEADCHIPS		24 BEADCHIPS		Incubation*
		Hands-on	Total	Hands-on	Total	Hands-on	Total	
DAY ONE	① Set up DNA amplification	5min	25min	5min	35min	5min	40min	20h (o/n)
DAY TWO	② Fragment amplified DNA	5min	1h 15min	5min	1h 20min	5min	1h 25min	1h
	③ Precipitate amplified DNA	5min	2h 15min	5min	2h 25min	5min	2h 30min	1h 50min
	④ Resuspend amplified DNA	5min	1h 10min	5min	1h 15min	5min	1h 20min	1h
	⑤ Prepare BeadChip	30min	30min	40min	40min	50min	50min	-
	⑥ Hybridize sample to BeadChip	5min	5min	5min	10min	5min	15min	20m + 16h (o/n)
DAY 2 TOTALS		50min	5h 15min	1h	5h 50min	1h 10min	6h 10min	
DAY THREE	⑦ Extend and stain BeadChip	5min	2h 25min	5min	4h 50min	5min	7h 15min	-
	⑧ Scan BeadChip (1 scanner)	5min	6h	5min x 2	12h	5min x 3	18h	-
TOTAL GENOTYPES (HUMANHAP550v1.0)		> 4x10⁶		> 8.5x10⁶		> 13x10⁶		

* These are total incubations, but several can be nested within each other

MANUAL

DAY	PROTOCOL STEP	8 BEADCHIPS		16 BEADCHIPS		24 BEADCHIPS		Incubation
		Hands-on	Total	Hands-on	Total	Hands-on	Total	
DAY 1	① Set up DNA amplification	5min	15min	5min	15min	5min	15min	10m + 20h (o/n)
DAY 2	② Fragment amplified DNA	10min	1h 10min	10min	1h 10min	10min	1h 10min	1h
	③ Precipitate amplified DNA	5min	1h 55min	5min	1h 55min	5min	1h 55min	1h 50min
	④ Resuspend amplified DNA	10min	1h 10min	10min	1h 10min	10min	1h 10min	1h
	⑤ Prepare BeadChip	30min	30min	40min	40min	50min	50min	-
	⑥ Hybridize sample to BeadChip	30min	1h	40min	1h	40min	1h 5min	20m + 16h (o/n)
DAY 2 TOTALS		1h 25min	5h 45min	1h 45min	5h 55min	1h 45min	6h 10min	
DAY 3	⑦ Extend and stain BeadChip	35min	2h 25min	1h 10min	4h 50min	1h 45min	7h 15min	1h 50min
	⑧ Scan BeadChip (1 scanner)	5min	6h	5min x 2	12h	5min x 3	18h	-
TOTAL GENOTYPES (HUMANHAP550v1.0)		> 4x10⁶		> 8.5x10⁶		> 13x10⁶		

to each allele per SNP locus. After hybridization, allelic specificity is conferred by enzymatic base extension. Products are subsequently fluorescently stained (Step 7). The intensities of the beads' fluorescence are detected by the Illumina BeadArray Reader (Step 8), and are in turn analyzed using Illumina's software for automated genotype calling (Step 9).

Figure 2 shows the estimated hands-on time required for completing the Infinium II Assay using the Illumina BeadStation 500 system. With Illumina's optional Laboratory Information Management System (LIMS) to ensure positive sample tracking, the Infinium II Assay is a robust protocol with a straight forward workflow that can be automated or processed manually.

ADDITIONAL INFORMATION

For more information about Infinium II or other products and services from Illumina, visit our website or contact technical support at the address below.

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