

Infinium® Assay Workflow

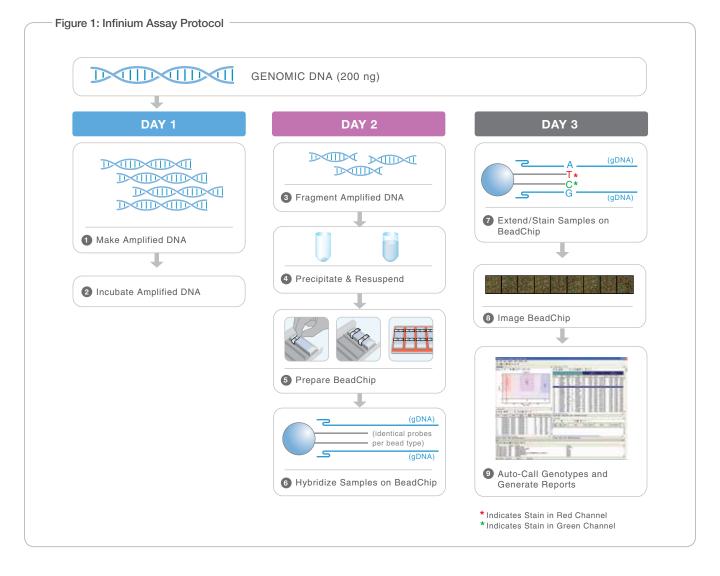
The Illumina Infinium assay provides unlimited multiplexing for whole-genome genotyping applications with a manual or automated workflow.

Introduction

The Infinium whole-genome genotyping assay (Figure 1) is designed to interrogate a large number of SNPs at unlimited levels of loci multiplexing. Using a single beadtype and dual-color channel approach, the Infinium assay scales genotyping from 10,000 to millions of SNPs per sample. The optional Illumina Laboratory Information Management System (LIMS) and automation with a Tecan liquid-handling robot ensure positive sample tracking while reducing hands-on time and labor costs.

Assay Protocol

The Infinium assay protocol is depicted in Figure 1. It begins with an overnight amplification of the DNA sample (Steps 1 and 2). This amplification has no appreciable allelic partiality. Additionally, a relatively low DNA sample requirement of 200 ng is sufficient to assay over 300,000 SNP loci. The amplified product is then fragmented by a controlled enzymatic process that does not require gel electrophoresis (Step 3). After alcohol precipitation and DNA resuspension (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 DNA samples are then annealed to locus-specific 50-mers covalently linked to one of over 300,000 beadtypes in an



overnight hybridization step (Step 6). One beadtype corresponds 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 iScan system (Step 8), and are in turn analyzed using Illumina software for automated genotype calling (Step 9). Table 1 shows the estimated processing time required for completing the Infinium assay, for both automated and manual workflows, using the Infinium CytoSNP-12 BeadChip and the iScan system.

Additional Information

For more information about Infinium products, visit www.illumina.com.

Table 1: Infinium Assay Workflow Using the CytoSNP-12 BeadChip*

Day	Step	Automated Workflow (Robot time)	Manual Workflow (Hands-on time)	Incubation/Dry Time
1	Set up DNA amplification	1 hr (hands-on)	1 hr	20–24 hr
	Fragment amplified DNA	5 min	15 min	1 hr
0	Precipitate amplified DNA	10 min	15 min	1 hr 50 min
2	Resuspend amplified DNA	10 min	15 min	1 hr
	Hybridize sample to BeadChip	15 min 40 min (hands-on)	1 hr 15 min	17–24 hr
	Wash BeadChip	30 min (hands-on)	30 min	25 min
3	Stain BeadChip	3 hr	3 hr	1 hr
	Image BeadChip	2 hr 24 min (scan time)	2 hr 24 min (scan time)	_

^{*} Time indicated for processing 4 BeadChips, 48 samples using the iScan system.

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