

GoldenGate® Assay Workflow

Illumina's GoldenGate Assay protocol provides high-quality, high-multiplex genotyping results with a streamlined workflow.

INTRODUCTION

The GoldenGate Assay (Figure 1) allows for a high degree of loci multiplexing (1536-plex) during the extension and amplification steps, minimizing time, reagent volumes, and material requirements of the process.

ASSAY OVERVIEW

The DNA sample used in this assay is activated for binding to paramagnetic particles **1**. This activation step is a highly robust process that requires a minimum input of DNA (250ng at 50ng/μl). Depending upon the multiplex level, this equates to only 160pg

of DNA per SNP genotype call. Assay oligonucleotides, hybridization buffer, and paramagnetic particles are then combined with the activated DNA in the hybridization step **2**. Three oligonucleotides are designed for each SNP locus. Two oligos are specific to each allele of the SNP site,

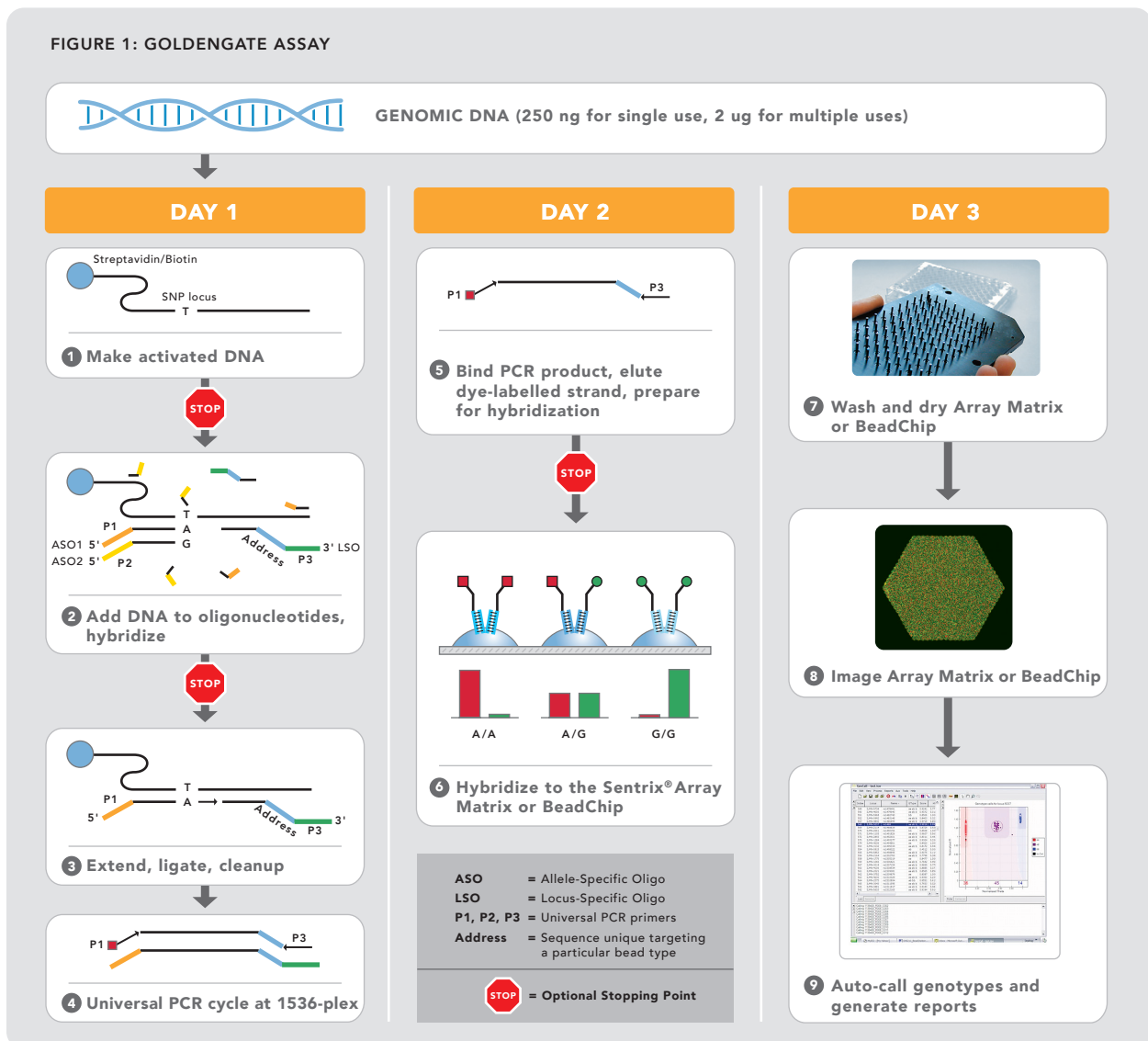


FIGURE 2: PROTOCOL TIME REQUIREMENTS

	PROTOCOL STEP	ONE PLATE		TWO PLATES		THREE PLATES ¹		FOUR PLATES ¹	
		Hands-on	Total time	Hands-on	Total time	Hands-on	Total time	Hands-on	Total time
DAY 1	1 Make activated DNA	2:00	2:00	2:30	2:30	3:00	3:00	3:30	3:30
	2 Add DNA and hybridize to oligonucleotides	0:30	3:00	0:30	3:00	0:45	3:15	1:00	3:30
	3 Extend, ligate, and clean-up	1:30	2:00	2:00	2:30	2:45	3:15	3:30	4:00
	4 PCR cycle	–	3:00	–	3:30	–	3:00	–	3:00
DAY 1 TOTALS		4:00	10:00	5:00	11:00	6:30	12:30	8:00	14:00
DAY 2	5 Bind PCR product, elute dye-labelled strand, prepare for hybridization	1:30	2:30	2:15	3:15	3:00	4:00	3:45	4:45
	6 Hybridize to Array Matrix ²	0:30	0:30	0:30	0:30	0:30	0:30	0:45	0:45
	DAY 2 TOTALS	2:00	3:00	2:45	3:45	3:30	4:30	4:30	5:30
DAY 3	7 Wash and dry Array Matrix	0:10	0:30	0:10	0:30	0:15	0:45	0:15	0:45
	8 Image Array Matrix	–	2:00	–	4:00	–	6:00	–	8:00
	DAY 3 TOTALS	0:10	2:30	0:10	4:30	0:15	6:45	0:15	8:45
TOTAL GENOTYPES (1536-PLEX)		147,456		294,912		442,368		589,824	

¹ Requires two technicians

² Times listed are for processing on the Sentrix Array Matrix; analysis can also be carried out on the Sentrix BeadChip format.

called the Allele-Specific Oligos (ASOs). A third oligo that hybridizes several bases downstream from the SNP site is the Locus-Specific Oligo (LSO). All three oligonucleotide sequences contain regions of genomic complementarity and universal PCR primer sites; the LSO also contains a unique address sequence that targets a particular bead type. Up to 1,536 SNPs may be interrogated simultaneously in this manner using GoldenGate technology. During the primer hybridization process, the assay oligonucleotides hybridize to the genomic DNA sample bound to paramagnetic particles. Because hybridization occurs prior to any amplification steps, no amplification bias can be introduced into the assay.

Following hybridization, several wash steps are performed, reducing noise by removing excess and mis-hybridized oligonucleotides. Extension of the appropriate ASO

and ligation of the extended product to the LSO joins information about the genotype present at the SNP site to the address sequence on the LSO 3. These joined, full-length products provide a template for PCR using universal PCR primers P1, P2, and P3 4.

Universal PCR primers P1 and P2 are Cy3- and Cy5-labeled. After downstream-processing 5 the single-stranded, dye-labeled DNAs are hybridized to their complement bead type through their unique address sequences 6. Hybridization of the GoldenGate Assay products onto the Array Matrix or BeadChip allows for the separation of the assay products in solution, onto a solid surface for individual SNP genotype readout 7. After hybridization, the BeadArray Reader is used to analyze fluorescence signal on the Sentrix Array Matrix or BeadChip 8, which is in turn analyzed using software for

automated genotype clustering and calling 9.

Figure 2 illustrates both hands-on and total technician time required for manually completing the GoldenGate Assay on the BeadStation system.

FOR ADDITIONAL INFORMATION

The power of the GoldenGate Assay can be accessed with an Illumina system or through Illumina Genotyping Services. To find out more, visit us at our website at www.illumina.com.

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