

SNPlex™ Genotyping System

Turn your capillary electrophoresis (CE) instrument* into a SNP genotyping workhorse.

- 12–48 multiplex SNP genotyping capability
- Insertion/deletion (up to 6 bases) and SNP detection in the same assay
- Manual protocol to minimize startup costs
- Resources available to support full automation for high-throughput genotyping
- Cost-effective for projects with a range of SNPs and samples



Figure 1. The Applied Biosystems 3130x/ Genetic Analyzer supports the SNPlex™ System and a broad range of applications on one instrument.

EXAMPLE USES OF THE SNPlex SYSTEM

Diseases Researched:	Species Studied:
Autism	<i>Arabidopsis</i>
Breast cancer	Cattle
Bulimia	Chicken
Crohn's disease	Chimpanzee
Hypertension	Corn
Obsessive-compulsive disorder	Dog
Prostate cancer	Mouse
Schizophrenia	Pig
Sleep disorder	Salmon
	Soybean

Introduction

The SNPlex™ Genotyping System is a comprehensive solution for medium to large-scale genotyping studies, such as candidate gene or region association studies. It uses CE to separate selectively amplified gene regions, reports easy-to-survey cluster plots, and manages large numbers of data sets.

Ideal for Fine Mapping and Association Studies

The SNPlex Genotyping System is well suited to perform a range of studies such as:

- Linkage mapping
- Fine mapping
- Disease association
- Population stratification
- Microarray replication
- Quantitative trait loci analysis
- Assisted breeding

*The SNPlex system is supported on the Applied Biosystems 3130x/ Genetic Analyzer and the 3730/3730x/ DNA Analyzers

An Integrated Research Solution

- Platform flexibility to meet your throughput needs—choose the 3130xI, the 3730, or the 3730xI Analyzer
- Application flexibility on a single platform—perform genotyping, sequencing, resequencing, and fragment analysis
- SNPLex system core reagents—universal conditions and reagents eliminate time-consuming optimization
- Custom and fixed content—choose only the SNPs you need, or use our validated SNPLex™ System Human Linkage Mapping Set
- Convenient online assay-design and ordering system
- Integrated software suite for data collection, analysis, and quality control
- Fully supported protocols and user documents

The SNPLex System Assay

The SNPLex system assay uses an oligonucleotide ligation assay (OLA) followed by PCR, and can be divided into 3 basic steps:

1. Encoding—Multiplex OLA to generate genotype-specific products
2. Amplification—Multiplex PCR using a universal primer pair
3. Decoding
 - a. Hybridization of universal reporter probes to amplicons
 - b. CE to rapidly identify eluted probes

The same set of universal reporter probes is used with every SNPLex probe pool, which ensures extremely reproducible detection and identification using GeneMapper® Software v4.0

Performance

The SNPLex system and the automated design pipeline have undergone extensive testing, with key metrics summarized in Table 1.



Figure 2. The SNPLex system offers an integrated solution including CE detection, data collection, and automated genotype calling.

TABLE 1. SNPLex GENOTYPING SYSTEM PERFORMANCE[§]

Characteristic	Definition	Value
Concordance	The ability to call the correct genotype. Defined by comparison to TaqMan® assays or sequencing reactions	≥ 99.8%
Precision	The ability to call the same genotype across repeated runs of the same SNP and sample	≥ 99.8%
Call rate	The fraction of potential genotypes called (only passing SNPs counted)	≥ 99.32%
Conversion rate [°]	Percentage of the initial submission that produces passing SNPs	≥ 80–90%

[°] The conversion rate combines two components: (1) the in silico design rate; and (2) the number of SNPs that pass. This rate is highly dependent on the DNA quality, state of SNP validation, and presence of genomic repeats.

[§] Two representative experiments (Tobler et al.) contributing to these metrics are described below:

1) 48 SNPs with minor allele frequencies ≥ 0.1 in 4 populations were tested against 44 Caucasian gDNA samples, each occurring 8 times per 384-well plate. This experiment was replicated on three separate instruments. Genotype results were compared to known genotypes generated by TaqMan® probe based assays.

2) 521 SNPs, selected from the first 10 Mb of chromosome 21, were tested against 92 triplicate gDNA samples.

Time to Results

With up to a 48-plex assay format and multicapillary detection, the SNPlex system generates several hundred to > 500,000 genotypes per week. A 3730 DNA Analyzer is capable of processing up to 400,000 genotypes per day.

Using a multichannel pipettor and an inexpensive manual plate washer, the manual protocol is time-effective and easy to implement. Documentation and scripts are also available to support common liquid-handling robotics.

To balance speed and cost, use a combination of manual assay setup and postamplification automation. Table 2 estimates how much time you'll spend on each step for different numbers of 384-well plates.

Assay Design and Ordering

Our convenient online assay-design pipeline designs allele-specific probes and optimizes multiplexed assays using your SNPs of interest in three easy steps:

1. Enter sequence or SNP IDs
2. Check for formatting errors
3. Select organism and submit design

Our probe design prevents self-complementarity and dimerization, and annealing efficiencies are optimized for ligation. Furthermore, we determine the optimal combination of SNPs to produce the highest yield per multiplex reaction. Typically, in less than an hour, you'll receive a design report containing multiplex assays that can then be ordered from Applied Biosystems.

To optimize human SNP selection, download free SNPbrowser™ Software and choose from over 5 million prescreened SNPlex-compatible SNPs, including 2.8 million HapMap SNPs.

Web Resource

We invite you to learn more about the SNPlex Genotyping System available at <http://www.appliedbiosystems.com>

TABLE 2. HOURS REQUIRED TO GENERATE DATA FROM N 384-WELL PLATES*

Day	Protocol Step	1 Plate†		2 Plates‡		3 Plates‡		4 Plates‡	
		Hands-On Time	Total Time	Hands-On Time	Total Time	Hands-On Time	Total Time	Hands-On Time	Total Time
1	Encode genotype information	1:00	7:00	2:00	8:00	2:00	8:00	2:00	8:00
	Amplify	0:30	1:45	1:00	2:15	1:00	2:15	1:00	2:15
Day 1 totals		1:30	8:45	3:00	10:15	3:00	10:15	3:00	10:15
2	Decode	1:30	4:00	3:00	5:30	3:00	5:30	3:00	5:30
	Load and run sample plates on CE instrument	0:15	1:15	0:45	1:45	0:45	1:45	0:45	1:45
Day 2 totals		1:45	5:15	3:45	7:15	3:45	7:15	3:45	7:15
Total genotypes (48 plex)		17,856		35,712		53,568		71,424	

* It is often more convenient to prepare gDNA in advance of running the assay

† Manual protocol

‡ Automated protocol

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The Applied Biosystems 3130/3130x/ Genetic Analyzers and 3730/3730x/ DNA Analyzers include patented technology licensed from Hitachi, Ltd., as part of a strategic partnership between Applied Biosystems and Hitachi, Ltd., as well as patented technology of Applied Biosystems.

NOTICE TO PURCHASER: SNPlex™ System. PLEASE REFER TO THE SNPlex™ SYSTEM USER'S GUIDE, PRODUCT INSERT, OR APPLIED BIOSYSTEMS PRODUCT PAGE FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION

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Printed in the USA. 06/2006 Publication 132PB02-01



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