Using the SNPlex™ System Dried gDNA Plates Kit

The SNPlex™ System Dried gDNA Plates Kit (PN 4362637) contains two 384-well plates of ready-to-use, dried down genomic DNA that can be used for performance evaluation and training purposes. When used with the SNPlex™ System Control Pool (P/N 4362635), genotype accuracy can be evaluated using the genotypes table provided on the included CD. Because the gDNA plates contain a number of replicate samples (details below), within plate and/or within run genotyping precision can also be measured.

Genotyping experiments using the SNPlex System Dried gDNA plate can be analyzed with either the Applied Biosystems 3130*xl*, 3730 or 3730*xl* DNA Analyzers (16-capillary, 48-capillary or 96-capillary arrays, respectively). SNPlex System users preferring the 96-well assay protocol can transfer samples from the 384-well gDNA plate to a 96-well plate for performance evaluation and training purposes

The SNPlex System Dried gDNA plates can be used with custom SNPlex probe pools in order to evaluate pool performance with DNA samples of consistent quality.

Plate Layout

The layout of the SNPlex System Dried gDNA plate is shown in Figure 1, below. This layout results in each plate quadrant containing 40 DNAs in duplicate, 3 DNAs in triplicate, and 1 DNA is repeated 5 times. For details on plate quadrants, please consult the SNPlex™ System User Guide.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	5a	5a	12a	12a	19a	19a	26a	26a	33a	33a	40a	40a	7b	7b	14b	14b	21b	21b	28b	28b	35b	35b	41b	41b
B	5a	5a	12a	12a	19a	19a	26a	26a	33a	33a	40a	40a	7b	7b	14b	14b	21b	21b	28b	28b	35b	35b	41b	41b
C	6a	6a	13a	13a	20a	20a	27a	27a	34a	34a	41a	41a	8b	8b	15b	15b	22b	22b	29b	29b	36b	36b	42b	42b
D	6a	6a	13a	13a	20a	20a	27a	27a	34a	34a	41a	41a	8b	8b	15b	15b	22b	22b	29b	29b	36b	36b	42b	42b
E	7a	7a	14a	14a	21a	21a	28a	28a	35a	35a	42a	42a	9b	9b	16b	16b	23b	23b	30b	30b	37b	37b	43b	43b
F	7a	7a	14a	14a	21a	21a	28a	28a	35a	35a	42a	42a	9b	9b	16b	16b	23b	23b	30b	30b	37b	37b	43b	43b
G	8a	8a	15a	15a	22a	22a	29a	29a	36a	36a	43a	43a	10b	10b	17b	17b	24b	24b	31b	31b	38b	38b	44b	44b
H	8a	8a	15a	15a	22a	22a	29a	29a	36a	36a	43a	43a	10b	10b	17b	17b	24b	24b	31b	31b	38b	38b	44b	44b
I	9a	9a	16a	16a	23a	23a	30a	30a	37a	37a	44a	44a	11b	11b	18b	18b	25b	25b	32b	32b	39b	39b	2d	2d
J	9a	9a	16a	16a	23a	23a	30a	30a	37a	37a	44a	44a	11b	11b	18b	18b	25b	25b	32b	32b	39b	39b	2d	2d
K	10a	10a	17a	17a	24a	24a	31a	31a	38a	38a	5b	5b	12b	12b	19b	19b	26b	26b	33b	33b	40b	40b		
L	10a	10a	17a	17a	24a	24a	31a	31a	38a	38a	5b	5b	12b	12b	19b	19b	26b	26b	33b	33b	40b	40b		
M	11a	11a	18a	18a	25a	25a	32a	32a	39a	39a	6b	6b	13b	13b	20b	20b	27b	27b	34b	34b	3c	3c	2e	2e
N	11a	11a	18a	18a	25a	25a	32a	32a	39a	39a	6b	6b	13b	13b	20b	20b	27b	27b	34b	34b	3c	3c	2e	2e
O	1a	1a	1b	1b	2a	2a	2b	2b	3a	3a	3b	3b	4a	4a	4b	4b	1c	1c	2c	2c	4c	4c		
P	1a	1a	1b	1b	2a	2a	2b	2b	3a	3a	3b	3b	4a	4a	4b	4b	1c	1c	2c	2c	4c	4c		

Figure 1: Sample layout of the gDNA plate. Empty wells are reserved for allelic ladders (AL) and no-template control (NTC) samples. The numbers 1-44 indicate the 44 unique samples included on the plate. The suffixes a, b, c, d and e indicate within-quadrant replicates of specific DNA samples. The suffixes are excluded from the template samples sheets (included on the CD) in order to simplify analysis.

Protocols

Laboratories using the 384-well assay protocol can use the SNPlex System Dried gDNA plate as supplied. Note that the DNA included on the gDNA plate has been previously fragmented, and can be used directly in the OLA reaction, according to the procedure for dried down DNA.

Laboratories using the 96-well protocol will first need to resuspend the dried down DNA in the gDNA plate and transfer it to a 96-well plate as follows:

1) Taking care to avoid cross contamination, add 2.3 uL of nuclease free water to the 96-wells defining a single quadrant of the gDNA plate. For details on plate quadrants, please consult the SNPlex System User Guide.

2) Cover and vortex the plate thoroughly.

3) Spin down the plate and allow it to equilibrate for 10 min.

4) After the 10 min. equilibration, vortex and spin down the plate once more.

5) Using a fresh tip each time, transfer 2 uL from each hydrated well of the 384-well gDNA plate to the corresponding well of a 96-well reaction plate.

After transferring to the 96-well reaction plate, the DNA can be used directly in the OLA reaction, according to the procedure for wet gDNA as described in the SNPlex System User Guide.

Preparing Samples for Electrophoresis

In preparation for running the SNPlex System hybridization products on the 3130*xl*, 3730 or 3730*xl* DNA Analyzer, allelic ladder is added to specific wells of the reaction plate immediately before capillary electrophoresis. Depending on which capillary array is being used (16, 48 or 96 capillaries), allelic ladder will be added to wells that may contain assay products from DNA samples. Assay results from wells to which allelic ladder is added must be excluded from allele calling by changing the sample type indicated in the sample sheet to “Allelic Ladder”. Template plate records are included on the CD which have the instrument-specific sample type correctly identified (details below).

Figures 2 through 7 show the location of NTCs and Allelic Ladders when used with the indicated plate and instrument combinations. The shaded areas indicate locations where allelic ladders are added to wells that contain assay results from DNA samples, after completion of the SNPlex System assay.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																							NTC	NTC
L																							NTC	NTC
M																							AL	AL
N																							AL	AL
O																							AL	AL
P																							AL	AL

Figure 2: 384-well plate, 96-capillary array

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																							NTC	NTC
L																							NTC	NTC
M																					AL	AL	AL	AL
N																					AL	AL	AL	AL
O																					AL	AL	AL	AL
P																					AL	AL	AL	AL

Figure 3: 384-well plate, 48-capillary array

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																							NTC	NTC
L																							NTC	NTC
M																								
N																								
O			AL	AL			AL	AL			AL	AL			AL	AL			AL	AL			AL	AL
P			AL	AL			AL	AL			AL	AL			AL	AL			AL	AL			AL	AL

Figure 4: 384-well plate, 16-capillary array

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												NTC
G												AL
H												AL

Figure 5: 96-well plate, 96-capillary array

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												NTC
G											AL	AL
H											AL	AL

Figure 6: 96-well plate, 48-capillary array

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												NTC
G												
H		AL		AL		AL		AL		AL		AL

Figure 7: 96-well plate, 16-capillary array

Template Plate Records

Four template plate records (sample sheets) have been included on the CD for use with the SNPlex System Dried gDNA plates. These include:

Template_384-well_96cap.txt (3730*xl*)
Template_96-well_96cap.txt (3730*xl*)
Template_384-well_48cap.txt (3730)
Template_96-well_48cap.txt (3730)
Template_384-well_16cap.txt (3130*xl*)
Template_96-well_16cap.txt (3130*xl*)

The template plate records include sample names and sample types that are specific to the plate-array combination specified. Before importing the plate records into the Data Collection software, certain fields must be changed to match user-specific content. The templates are tab-delimited text files and can be viewed and edited in a number of text editing programs. Table 1 specifies the changes required prior to importing the various template plate records.

Table 1: Required Template Plate Record Changes

Field Content in Template	Purpose	Change To	Number of Changes
"Change_ContName"	Plate Name	Your Plate Name	1
"Change_PlateID" *	Plate ID	Your Plate ID	1 (0 for 3130*xl*)
"OwnName"	Plate Owner	Plate Owner's Name	1
"OpName"	Inst Operator	Operator's Name	1
"Change_ResultsGroup"	Results Group	Your Results Group	96 or 384
"Change_Protocol"	Instrument Protocol	Your Inst Protocol	96 or 384

*Plate ID field is not applicable to the 3130*xl*

After editing and saving the appropriate template plate record, it can be imported into the Data Collection software. If necessary, the plate seal type can be changed from septa (default) to heat-seal after importing the plate record. Additionally, the SNP set can be added after importing the plate record. The SNP set for the SNPlex System Control Pool is included on the CD in a folder called "AIF _Data". For more information on plate records, please consult the SNPlex System User Guide.

Evaluating Performance

When used with the SNPlex System Control Pool, the SNPlex System Dried gDNA Plate typically results in the following performance:

Three genotype clusters should be observed for 45 of 48 SNPs. Exceptions are hcv7505765, hcv7571632, and hcv2962785, which show only two clusters.

The genotype precision between identical gDNA samples should be 99.7% or better.

The genotype accuracy, when compared to the provided expected genotypes, should be 99.5% or better.

The call rate should be 95% or better.

For more detailed information on the SNPlex System Control Pool, please consult the SNPlex System User Guide.

Sample Data

Sample files from a single quadrant of a SNPlex System Dried gDNA Plate using the SNPlex System Control Pool, collected using both a 3730*xl* and a 3130*xl*, are included in the folder called Sample Data. These data can be loaded into GeneMapper® Software for comparison and/or training purposes. Please consult the GeneMapper Software User Guide and the SNPlex System User Guide for details on analyzing SNPlex Data.

Genomic DNA Sources

The SNPlex System Dried gDNA Kit contains certain internal control DNA samples supplied by the European Collection of Cell Cultures. Purchase of the SNPlex System Dried gDNA Kit conveys the right to use such DNA samples as internal controls in connection with use of the SNPlex System Dried gDNA Kit but does not convey any right to replicate or redistribute such DNA samples.

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