Fluidigm System for Rapid, Reliable, and Cost Effective SNP Genotyping

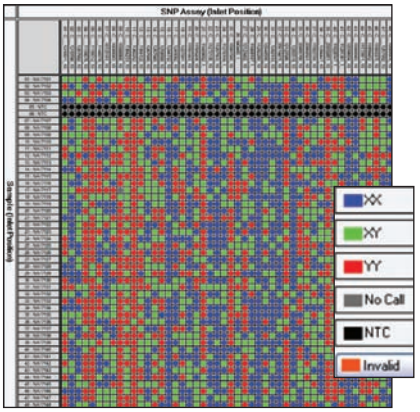
Single nucleotide polymorphisms (SNPs) are the most common form of genetic variation found in the genome of any organism. Although SNP genotypes are a powerful tool for understanding disease disposition and drug metabolism, SNP genotyping of large populations has been impractical because of the running costs and time associated with conventional microwell plate-based systems.

Comparatively, the Fluidigm system, including Fluidigm 48.48 Dynamic Arrays, is a nanofluidic system that is far more efficient while yielding equivalent call rates and reproducibility. Instead of robotic liquid-handling required to setup microwell plates, dynamic arrays have an integrated network of channels, chambers, and valves that automatically combine the reactions, conserving time, reagent, and sample amount. Following are results of experiments designed to validate call reproducibility of the Fluidigm system — and to demonstrate SNP genotyping concordance with the ABI 7900.

SNP Genotyping

Overview. Forty-eight TaqMan® SNP genotyping assays were obtained from Applied Biosystems, Foster City, CA (Table 1). Five microliters of 10x assays were loaded into the primer/probe inlets of two dynamic arrays. Forty-six different DNA samples (159–186 ng/µl) were obtained from Coriell Cell Repositories, Camden, NJ (Table 2). Each sample was mixed with 2x Universal TaqMan® PCR Master Mix (Applied Biosystems) and loaded into the sample inlets of the dynamic arrays.

Results. The results are displayed as an allele map, with each data point a pair-wise combination of a specific SNP primer/probe and sample. The results show call rates greater than 99.5% and high concordance between the duplicate dynamic array runs.



Assays Used

#	Assay Name	#	Assay Name	#	Assay Name
1	C___1210911_10	2	C___1210596_10	3	C___1207992_10
4	C___1159676_10	5	C___1156012_10	6	C___1146078_10
7	C__16125161_10	8	C__16085063_10	9	C__15988806_10
10	C___1364233_10	11	C___1382252_10	12	C___1364122_10
13	C___1267995_10	14	C___1267960_10	15	C___1258500_10
16	C___1244732_10	17	C___1243062_10	18	C___1227201_10
19	C__11561443_10	20	C__11505408_10	21	C__11469692_1_
22	C__11272136_10	23	C__11207933_10	24	C__11276169_1_
25	C__15961560_10	26	C___940460_1_	27	C__12123462_10
28	C__11668187_1_	29	C__11282289_10	30	C__12048556_10
31	C___1282193_10	32	C__25618415_10	33	C__12122586_10
34	C___1295519_10	35	C__25800766_10	36	C__25802021_10
37	C__11562154_10	38	C___1284447_10	39	C__25650851_10
40	C__11628747_1_	41	C__11281361_1_	42	C__12098654_10
43	C__15969983_10	44	C__11572811_10	45	C__11899452_10
46	C__11993375_10	47	C___1207992_10	48	C__12005976_10

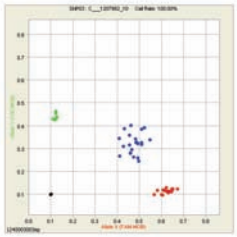
Table 1. SNP genotyping assays used in the study.

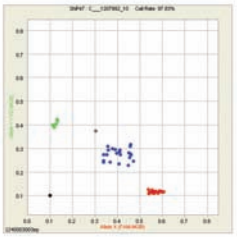
#	Sample ID	#	Sample ID	#	Sample ID
1	NA17101	2	NA17102	3	NA17103
4	NA17104	5	NA17105	6	NTC
7	NA17107	8	NA17108	9	NA17109
10	NA17110	11	NA17111	12	NA17316
13	NA17113	14	NA17114	15	NA17115
16	NA17116	17	NA17117	18	NA17118
19	NA1719	20	NA17120	21	NA17121
22	NTC	23	NA17123	24	NA17124
25	NA17125	26	NA17126	27	NA17127
28	NA17128	29	NA17129	30	NA17130
31	NA17131	32	NA17132	33	NA17133
34	NA17134	35	NA17135	36	NA17136
37	NA17137	38	NA17138	39	NA17139
40	NA17140	41	NA17141	42	NA17142
43	NA17143	44	NA17144	45	NA17145
46	NA17146	47	NA17147	48	NA17148

Table 2. DNA samples used in the study.

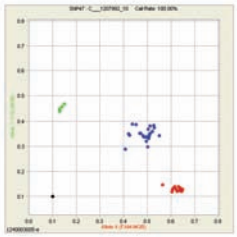
Reproducibility

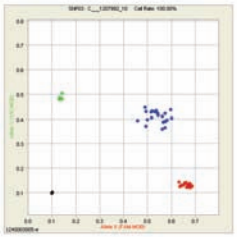
Intra- (Panel A or Panel B) and inter-chip (Panel A and B) reproducibility of assay clusters.





Panel A.

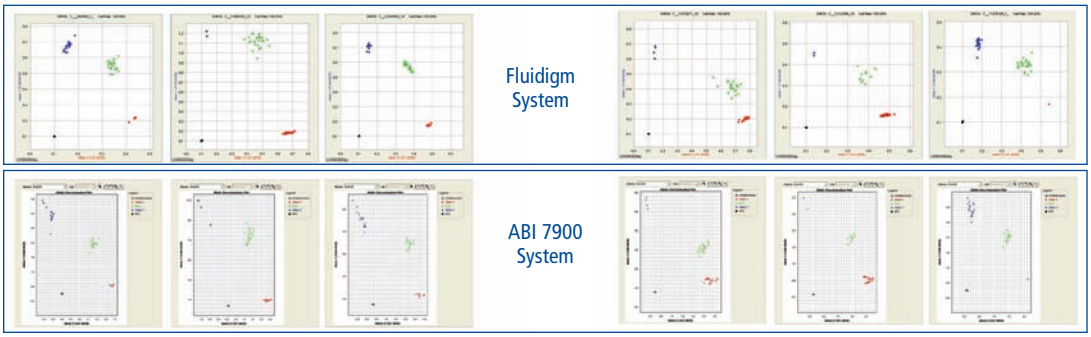




Panel B.

Concordance with Conventional Platforms

The same forty six samples were run against the same 12 primer/probe sets on both the Fluidigm system and a 7900HT Sequence Detection System (Applied Biosystems). Side-by-side comparison shows complete concordance, with a 99.5 percent call rate.



Benefits

Flexibility.
User choice of any 48 samples and any 48 SNP genotyping assays.

Set up and Run time.
Takes less than three hours.

Automation Compatibility.
Standard 384 well format.

Throughput.
Each dynamic array delivers the throughput of six 384-well plates.

SNP Genotyping Analysis Software.

- Auto-calling of SNP genotypes from the clusters.
- Visual overlay of clusters from multiple dynamic arrays.
- Flexible annotation of sample and assay information from the source plate.
- Pipetting maps of samples and primer/probes from the 96-well plates.
- Summary reports, containing the number of clusters, call rates, and confidence for each assay.

Reaction Volumes

Dynamic arrays require much smaller volumes of reaction components, which substantially reduces running costs.

	48.48 DYNAMIC ARRAY	SIX 384 WELL PLATE*	FOLD REDUCTION
10X ASSAY	192 µl	300 µl	1.6X
DNA AMOUNT	150 ng	480 ng	5.2X
TAQMAN UNIVERSAL PCR MASTER MIX	150 µl	9200 µl	61X

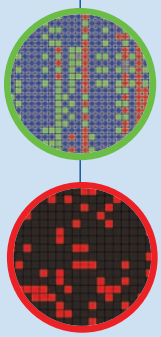
* Assumes reaction volume of 8 µl

Conclusion

The Fluidigm system is a highly efficient alternative to microwell plate-based systems for SNP genotyping studies requiring 16 or more genes to be screened against 1,000s of samples. The advantages include lower running costs, exponentially higher throughput per run, and simplified setup of reactions. The Fluidigm system provides these advantages while yielding excellent call rates and concordance with widely used microwell plate-based systems.

WORK FLOW

1. **Prime**
   Prime the IFC to prepare for samples and assays.
2. **Transfer**
   Transfer samples and assays into separate inlets on the chip.
3. **Load**
   Place the IFC on the IFC controller to automatically setup reaction chambers.
4. **Thermal Cycle**
   Place the IFC onto the Stand-Alone Thermal Cycler and start the PCR protocol.
5. **Read**
   Place the IFC on the EP1 Reader for fluorescence detection.



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