Validation Study of FUJIFILM QuickGene System for Affymetrix GeneChip®

Reproducibility of Extraction of Genomic DNA from Whole Blood samples in EDTA using FUJIFILM membrane technology on the QuickGene-810 extraction platform.
Validation study was performed at Affymetrix Clinical Services Laboratory.

Introduction:

The purpose of this study was to demonstrate that genomic DNA can be reliably extracted from whole blood samples using **FUJIFILM DNA Whole Blood kit S (DB-S)** on the **QuickGene-810 system**,(both available from **AutoGen***) with minimal variations. Being able to produce consistent results validates the FUJIFILM membrane technology and chemistry as a reliable solution to DNA extraction in a clinical environment. Extractions were performed on 25 samples of 200µl whole blood in anticoagulated EDTA tubes according to manufacturer's instructions. All samples were analyzed on a NanoDrop ND-1000 spectrophotometer for purity, yield, concentration and on a 1% agarose gel for integrity. Purity was monitored by the A260/A280 ratios and the A260/A230* (*monitored but not a recorded metric).
The accepted average sizing of the product was $\geq$ 10 kilobases (kb), concentration $\geq$ 50ng/ul and yield > 2 µg, as defined by Affymetrix for analysis on whole genome amplification. After the samples were determined to be within the defined metrics, they were run on the **Affymetrix GenomeWide 5.0 arrays**, scanned and analyzed. The results demonstrated that the chemistry and the extraction procedure are consistent in terms of A260/A280, A260/A230, concentration, yield and sizing. All samples successfully hybridized to the arrays and produced call rates within the metrics.

Method:

The blood samples were supplied by ProMedDx in purple top EDTA tubes and stored at 4° for 7 days prior to extraction. The QuickGene-810 system can extract DNA from 8 samples at a time, so extractions of the 25 samples were performed in batches of 8. The samples (200 uls of whole blood) were initially mixed with a protease and a lysis buffer and then incubated at 56°C for 3 minutes. 100% ethanol was added to each sample before pipetting the entire sample (750µl) onto the individual membrane cartridges.

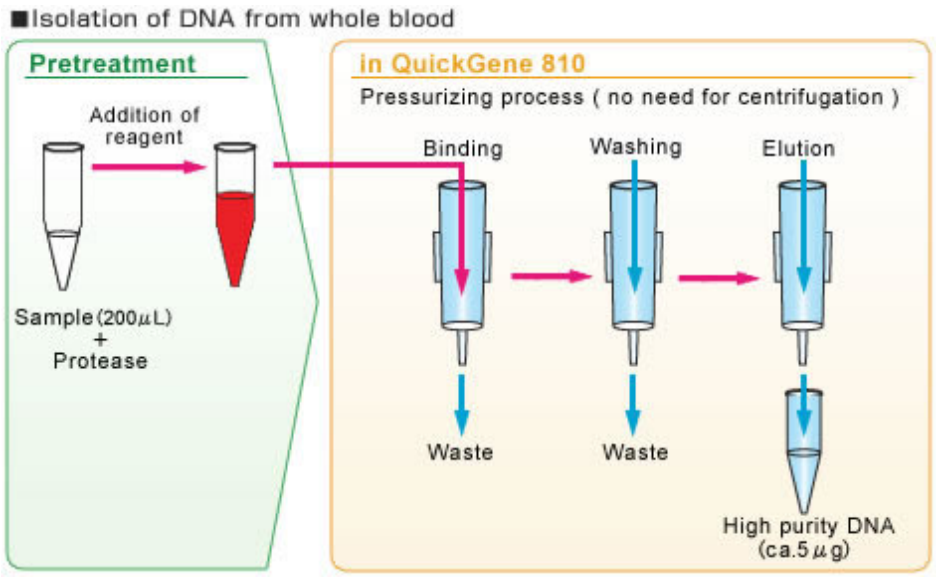

Figure 1. Overview of extraction methods for QuickGene-810 from whole blood.

The kit reagents were distributed into the appropriate reservoirs according to the manufacturer instructions and placed in the defined location within the QuickGene-810. Waste collection containers as well as elution tubes for each sample are placed on the cartridge tray within the unit. The isolation method (whole blood) was selected and the program initiated.

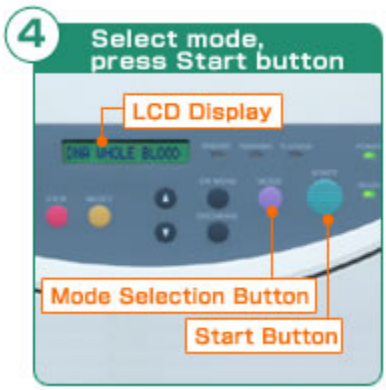

Figure 2. Method selection and start of QuickGene-810

Once the program is initiated, the machine will automatically step through three pressurized processes: binding, washing and elution. The entire automated process takes about 6 minutes to complete. The sample was eluted in AutoGen WDB buffer at 100ul. Once the program has been finished, the samples are removed from the cartridge tray and either immediately analyzed or stored at 4°C. The waste collected into the individual vials are appropriately disposed of in a hazardous waste collection carboy. The collection containers and membranes are disposed of in laboratory debris.

Each sample was individually analyzed on a NanoDrop ND-1000 and 1% agarose gel electrophoresis. Concentration (ng/μL), A260/A280, A260/A230 ratios and yield (μg) were all determined by pipetting 1.5μl of sample onto the

NanoDrop pedestal. The samples were analyzed on an agarose gel by adding 5µl of sample to 10ul of a loading buffer mixture. Each sample and loading buffer mixture were pipetted into a 1% agarose gel well (up to 48 samples) and run for approximately 25 minutes. The gel image was photographed and analyzed by a Kodak Gel Logic 2000 imaging system. Size was estimated according to a standard DNA ladder run alongside the samples on each gel.

All 25 samples were then prepared and run on the GenomeWide 5.0 arrays (GW5.0). GeneChip microarrays consist of small DNA fragments (or probes), that are synthesized to specific locations on a coated array quartz surface. Millions of probes can be contained on one array. By extracting and labeling nucleic acids from experimental samples, and then hybridizing those prepared samples to the array, the amount of label can be monitored at each probe location, enabling a wide range of applications on a whole-genome scale. These include, but are not limited to, gene- and exon-level expression analysis, novel transcript discovery, genotyping, and resequencing. The GW5.0 is a single microarray featuring 500,000 single nucleotide polymorphisms (SNPs), as well as 420,000 additional non-polymorphic probes that can measure other genetic differences, such as copy number variation (CNV). Probes (SNPs and CNVs) on the array are present on 200 to 1,100 base pair (bp) Nsp I or Sty I digested fragments in the human genome, and are amplified using the fifth generation of the Whole-genome Sampling Assay (WGSA).

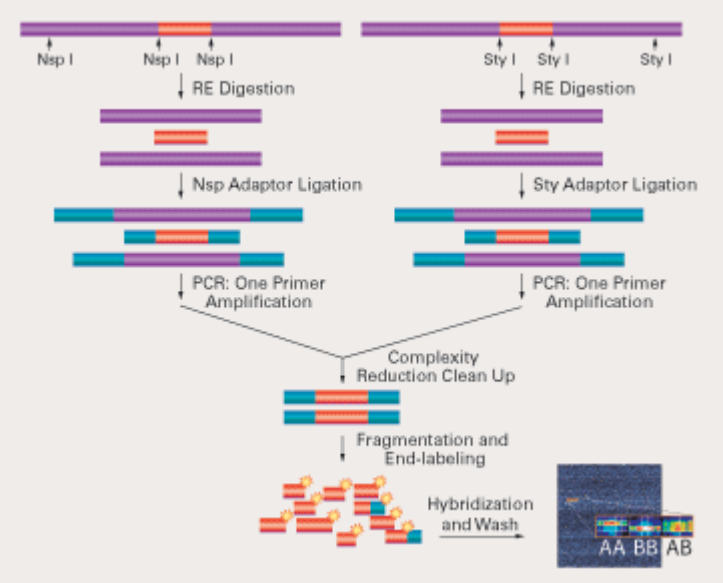

Figure 3. Overview of GW5.0 microarray process

All the samples are scanned and analyzed on Affymetrix platforms using the BRLMM-P algorithm. BRLMM-P (Bayesian Robust Linear Model with Mahalanobis distance classifier perfect-match only; pronounced “B-realm") is an evolution of the RLMM genotype calling algorithm [Rabbee and Speed, 2006]. BRLMM and BRLMM-P perform a multiple-chip analysis fitting probe effects to increase precision on signal estimates for the two alleles of each SNP, followed

by a Bayesian classification approach to make genotype calls. BRLMM seeds the classifier with calls made using the MM probes, while BRLMM-P classifies based on cluster properties alone

Results:

Overall the QuickGene-810 was a very easy instrument to use. The extraction procedure preparation was minimal and very straightforward. The instrument interface is also very user-friendly. Protocol selection and any parameter changes can be completed with minimal effort. The extracted DNA is eluted directly into a 0.5mL tube that can either be stored at 4°C or taken directly into metric analysis. The automated portion of the extraction is extremely efficient at 6 minutes or less to process all 8 samples.

In analyzing the microarray data, all the blood samples had a passing BRLMM call rate with a 0% failure rate. The quality of the DNA extracted from the whole blood by the QuickGene-810 can be demonstrated by the success of the GenomeWide5.0 assay and the high call rate percentages.

File Name	gender	brlmm-p_call_rate	AB_%	AA_%	BB_%	A260/A280	A260/A230	ng/ul	Yield
Autogen_B_Ref103	male	99.56	27.22	36.88	35.46				
Autogen_B_6971	female	99.43	29.66	35.32	34.45	1.93	1.77	78.89	7.89
Autogen_B_6972	female	99.42	29.79	35.28	34.35	1.88	1.56	67.12	6.71
Autogen_B_6973	male	99.52	28.82	35.78	34.92	1.89	1.30	61.46	6.15
Autogen_B_6974	male	99.32	28.57	35.84	34.91	1.89	1.32	36.88	3.69
Autogen_B_6975	male	99.46	29.32	35.43	34.71	1.85	1.05	38.28	3.83
Autogen_B_6976	male	99.56	29.29	35.55	34.72	1.92	1.32	101.55	10.16
Autogen_B_6977	female	99.51	30.36	35.04	34.11	1.99	1.68	122.24	12.22
Autogen_B_6978	male	99.57	20.2	40.12	39.25	1.88	1.26	54.31	5.43
Autogen_B_6979	male	99.36	29.67	35.28	34.4	1.89	1.27	32.32	3.23
Autogen_B_6980	female	99.50	29.62	35.28	34.59	1.89	1.31	72.37	7.24
Autogen_B_6981	male	99.48	28.98	35.74	34.77	1.88	1.38	49.05	4.91
Autogen_B_6982	male	99.47	28.65	35.82	35	1.94	1.61	45.78	4.58
Autogen_B_6983	male	99.33	29.13	35.51	34.69	1.93	1.68	54.63	5.46
Autogen_B_6984	male	99.48	29.13	35.62	34.73	1.94	1.64	46.21	4.62
Autogen_B_6985	male	99.64	29.63	35.45	34.56	1.92	1.76	37.57	3.76
Autogen_B_6986	male	99.63	28.72	35.81	35.1	1.85	1.73	61.75	6.18
Autogen_B_6987	male	99.59	29.65	35.4	34.54	1.82	1.39	32.90	3.29
Autogen_B_6988	female	99.55	30.16	35.12	34.27	1.90	1.83	78.95	7.90
Autogen_B_6989	female	99.54	29.66	35.32	34.57	2.14	1.35	45.88	4.59
Autogen_B_6990	female	99.54	30.15	35.15	34.23	1.97	1.88	85.89	8.59
Autogen_B_6991	female	99.54	30.1	35.17	34.26	1.94	1.87	88.10	8.81
Autogen_B_6992	male	99.52	29.49	35.47	34.55	1.89	1.81	74.59	7.46
Autogen_B_6993	male	99.62	26.92	37.07	35.64	1.94	1.90	106.99	10.70
Autogen_B_6994	male	99.49	28.96	35.67	34.86	1.89	1.53	58.68	5.87
Autogen_B_6995	female	99.55	29.6	35.38	34.56	1.92	1.88	95.61	9.56

	MEAN	99.5	28.97	35.70	34.83	1.92	1.56	65.12	6.51
	MEDIAN	99.52	29.49	35.45	34.59	1.90	1.61	61.46	6.15
	StDev	0.08	1.96	1.00	0.98	0.06	0.25	24.84	2.48
	SEM	0.02	0.39	0.20	0.20	0.01	0.05	4.97	0.50

All samples run on 1% agarose gel electrophoresis were of similar size and showed only a single band, meaning that the DNA was intact The differing intensities of the bands are indicative of concentration variation seen from sample to sample.

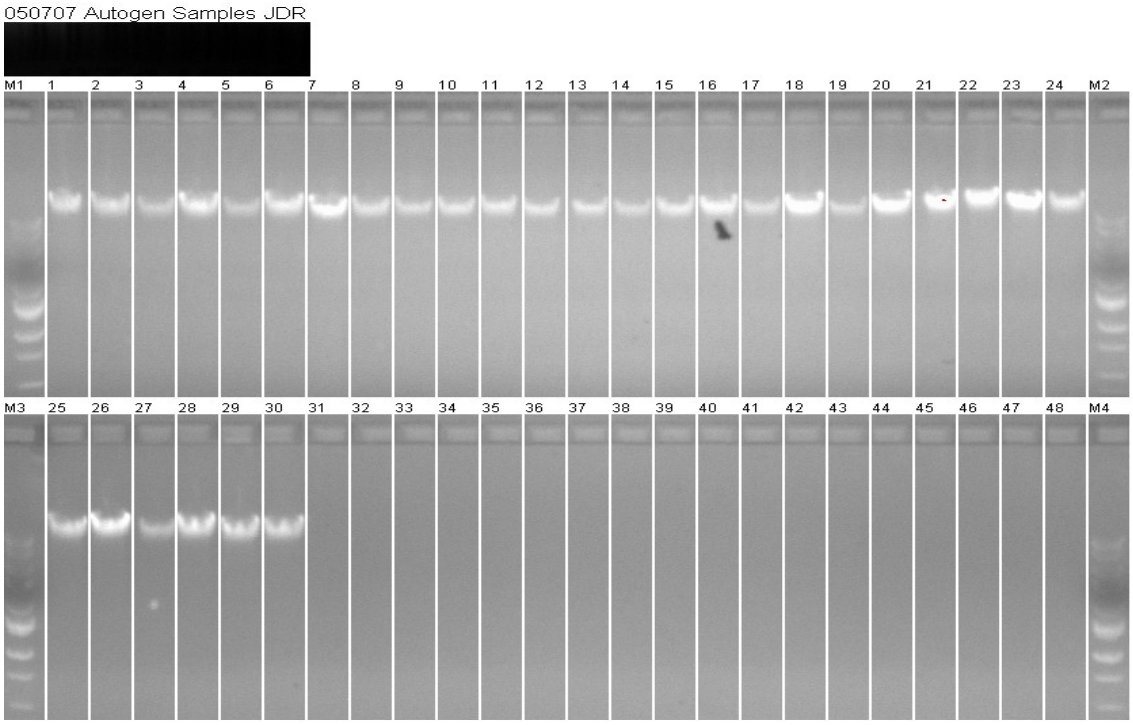

Figure 4. 1% Agarose gel image of AutoGen whole blood sample extractions

Summary:

In conclusion, Affymetrix Clinical Services Laboratory using the FUJIFILM DNA Whole Blood kit S on the QuickGene-810 platform can extract high quality DNA from EDTA-stabilized whole blood. This demonstrates that FUJIFILM chemistry on the QuickGene-810 can be used to reliably extract good quality DNA from whole blood for GeneChip® Applications.

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North American Distributor
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Tel: 508-429-5965
info@autogen.com
www.autogen.com