MJ Health Research Foundation

MJ Health Resource Center

Technical Report

Quality Control

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I. Objective

MJ Biobank stores 3 types of biological samples: serum, plasma and buffy coat. Among these samples, buffy coats are mainly used to study association between genes and diseases through experiments such as whole genome sequencing, SNP genotyping etc. MJ Biobank's biological samples have been stored in the freezers for many years, and quality and purity of the sample might change as a result of long term storage. Therefore, quality control measures must be implemented to ensure good sample quality. From a researcher's perspective, a buffy coat sample can be accepted only if the genomic DNA extracted from it is good for the downstream experiments. Therefore the quality control for buffy coat is focused on ensuring the high quality and quantity of genomic DNA extracted from the buffy coat samples.

II. Preliminary Sampling

1. Experimental group: experimental group consisted of samples donated between 2002 and 2008 by donors who have donated only once and have no record of cancers. Samples were chosen based on year of donation (note) and were to be destroyed after completing the quality control experiment. A total of 16 buffy coat samples were chosen. Note: 2 tubes from each year between 2002 and 2008 were to be chosen from different freezers. But 2002 buffy coat samples in freezer #1 have already been converted to DNA, as a result both 2002 samples were chosen from freezer #2 instead.

2. Control group: Controls consisted of buffy coat samples taken and processed within the shortest period of time possible, stored temporarily in 4°C, then entered the quality control process directly, without storing in -80°C. A total of 2 positive control buffy coat samples were prepared.

3. Sampling steps:
    1) Export the list of donors who have declined to re-consent to their previous sample donations between 2002 and 2008 as of 2015/9/23(total of 917 individuals)
    2) Remove those who donated more than once or whose records include cancer (642 individuals left)

3) Find the storage locations for the samples donated by these 642 donors.
4) For each year try to select at least 2 buffy coat tubes from different freezers (table 1, total of 16 tubes)

Table 1: Quality Control Sample Selection List

Sample Number	Freezer Number	Donate Year	Sample Number	Freezer Number	Donate Year
1	1	2003	9	9	2005
2	2	2002	10	10	2005
3	2	2002	11	11	2006
4	3	2003	12	12	2006
5	3	2004	13	17	2006
6	4	2004	14	18	2006
7	5	2008	15	19	2007
8	6	2008	16	20	2007

III. Experiment Procedure

A kit was used to extract genomic DNA from the selected buffy coat samples. The extracted genomic DNA samples were analyzed using 1% Agarose gel electrophoresis and optical density, specifically A260/A280 and A260/A230 ratios. The quality of genomic DNA was assessed based on guidelines as followed:

1. Genomic DNA Purity and Quantitation by UV/Vis Spectrophotometry: if 1.8 <= A260/A280 <= 2.0 and A260/A230 > 2.0, then the sample is considered having passed purity test (1). Final DNA quantity will be computed based on optical density as well.

2. 1% Agarose Gel Electrophoresis: if electrophoresis result only shows high molecular weight genomic DNA bands and no other bands or smear, then it means the DNA sample has not been degraded due to freeze-thaw cycles or damaged during extraction process.

IV. Contractor for Quality Control Experiments

Genomics BioSci & Tech. Ltd

V. Equipment and Methods:

1. Genomic DNA Extraction
   DNA Extraction Kit: QIAGEN Gentra Puregene Blood Kit 158389

2. Optical Density
   Spectrophotometer: NanoDrop 2000 UV-Vis Spectrophotometer by Thermo Scientific

3. Gel Electrophoresis
   1) Gel Sample Concentration: 100 ng/uL
   2) gDNA Volume per well: 2uL
   3) Marker: λDNA/Hind III
   4) Voltage: 100V
   5) Experiment time: 45mins
   6) Buffer type: TAE
   7) Dye: Genomics/6x loading dye
   8) Agarose gel percentage: 1%

VI. Result Analysis:

1. Optical Density Analysis
   Purity for the buffy coat samples at MJ Biobank was measured by UV/Vis Spectrophotometry based on the following guidelines:
   1) 1.8 <= A260/A280 <= 2.0
   2) A260/A230 > 2.0

   Low A260/A280 ratio indicates leftover proteins, phenol carryover due to the nucleic acid extraction process, or insufficient DNA quantity for the genomic DNA extracted from the buffy coat. On the other hand, low A260/A230 may indicate carbohydrate carryover (usually with plant-derived samples), phenol, guanidine or glycogen carryover due to the extraction process (2).

   Table 2 shows the raw data for the optical density values provided by the contractor Genomics. All 18 samples had A260/A230 ratios greater than 2.0, but 6 out of 18 samples showed suboptimal A260/A280 ratios (<1.8), meaning samples might have residual protein or phenol remained after genomic DNA extraction.

Table 2: Sample Optical Density Analysis Result

Group	Sample Number	Donate Year	Conc. (ng/μl)	A260	A280	A260/A280	A260/A230	Factor	Total Volume (μl)	Quantity (μg)	Freezer Num.	Result
Experiment	1	2003	345.5	6.909	3.822	1.81	2.32	50	300	103.65	1	○
	2	2002	293.7	5.874	3.239	1.81	2.26	50	300	88.11	2	○
	3	2002	284.1	5.683	3.106	1.83	2.20	50	300	85.23	2	○
	4	2003	248.7	4.974	2.761	1.80	2.34	50	200	49.74	3	○
	5	2004	309.3	6.186	3.521	1.76	2.17	50	300	92.79	3	Failed
	6	2004	311.9	6.239	3.516	1.77	2.10	50	200	62.38	4	Failed
	7	2008	351.7	7.035	3.845	1.83	2.32	50	100	35.17	5	○
	8	2008	311.3	6.226	3.427	1.82	2.34	50	300	93.39	6	○
	9	2005	197.2	3.943	2.153	1.83	2.84	50	200	39.44	9	○
	10	2005	462.6	9.252	5.151	1.80	2.09	50	200	92.52	10	○
	11	2006	350.9	7.019	3.903	1.80	2.25	50	300	105.27	11	○
	12	2006	335.9	6.717	3.737	1.80	2.35	50	200	67.18	12	○
	13	2006	340.4	6.808	3.758	1.81	2.34	50	200	68.08	17	○
	14	2006	346.7	6.935	3.826	1.81	2.36	50	200	69.34	18	○
	15	2007	243.6	4.873	2.723	1.79	2.36	50	300	73.08	19	Failed
	16	2007	218.1	4.363	2.432	1.79	2.36	50	200	43.62	20	Failed
Control	17	2015	252.3	5.047	2.840	1.78	2.34	50	300	75.69	No	Failed
	18	2015	179.1	3.583	2.038	1.76	2.59	50	200	35.82	-80°C	Failed
Average			299.06	5.981	3.322	1.80	2.33		238.89	71.14		

Figure 1 plots the distribution for genomic DNA concentrations and quantities. Results indicate that all 18 buffy coat samples have produced at least 30ug of genomic DNA. With regard to the concentrations, except for sample #9 and #18 (control), all other samples have produced greater than 200ng/uL of genomic DNA.

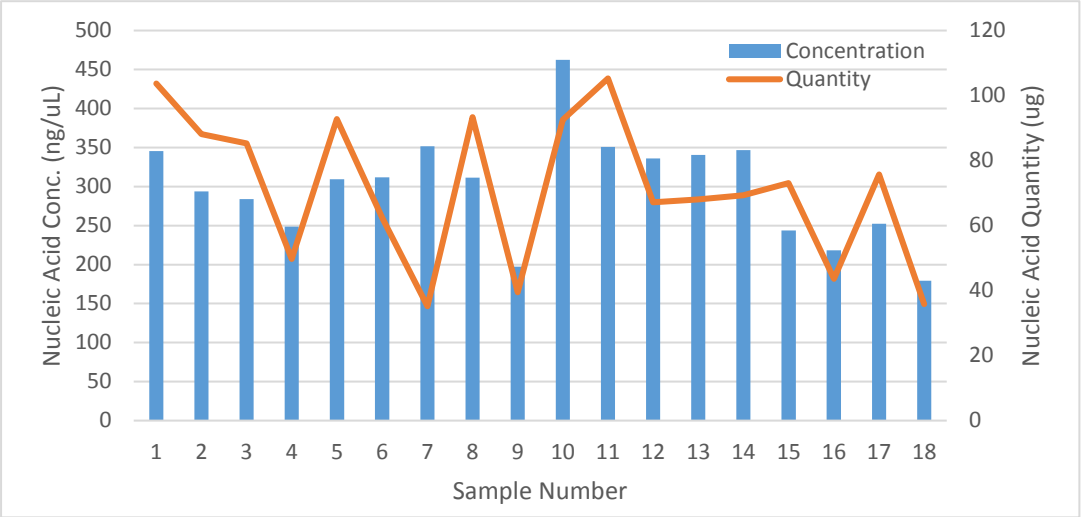

Figure 1. Distribution of Buffy Coat Genomic DNA Concentration and Quantity

2. Gel Electrophoresis

Optical density only shows the quantity and concentration of genomic DNA in the samples, it does not indicate whether DNA has been degraded. DNA structure must be analyzed using gel electrophoresis. The result of gel electrophoresis is shown in figure 2, where well M represents λDNA/Hind III Marker, the other wells represent 18 genomic DNA from buffy coats being tested for quality. According to the gel image, DNA quality is generally acceptable with correct band size, but light smear indicates the presence of degradation. Genomics, the quality control contractor believed it is normal to have smears above the major bands, the absence of smear below major bands, especially at 4000bp, indicates genomic DNA quality is good and can be used for downstream experiments such as SNP genotyping or sequencing.

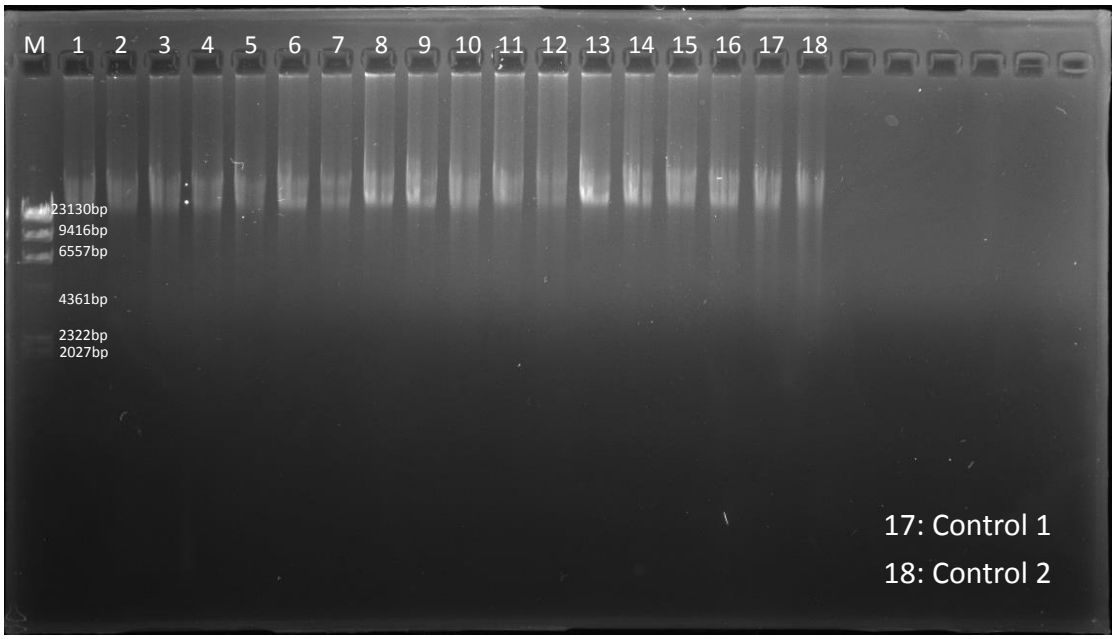

Figure 2. Genomic DNA Gel Electrophoresis

VII. Conclusion

Quality control was performed by using electrophoresis and UV/Vis spectrophotometry to assess the quality and quantity of genomic DNA extracted from buffy coat samples selected based on years of donation and storage freezers. The result indicates that after years of storage in the -80°C freezer, structural integrity and quantity of genomic DNA remains good and can be used for subsequent experiments such as NGS sequencing. High quality DNA also reflects a generally stable storage environment.

References:

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