**UW Medicine -1 Pathology**

400-11-01-05

DNA Sampling Requirements, Preparation and Quality Control Procedure

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| Adopted Date: 10/12/09Review Date: 02/14/10Revision Date: 04/10/2013 |

PURPOSE

To determining the concentration and the quality of genomic DNA (gDNA) samples to ensure they show no signs of RNA contamination or degradation.

PROCEDURE

### Equipment

1. Spectrophotometer NanoDrop ND-1000
2. Agarose Gel Electrophoresis

### Procedures

1. Use NanoDrop Spectrophotometer to QC and quantify the DNA samples.
	1. Genomic DNA should be prepared in nuclease-free water or 1X TE buffer (10mM Tris-HCl and 0.1mM EDTA, pH 7.5 - 8.0). For the procedure of DNA isolation, please see page136.
	2. Clean the surfaces of the pedestal and arm of the NanoDrop with pure water on a kimwipe.
	3. Click on NanoDrop software icon and choose **Nucleic Acid** module.
	4. Once the program has been **initialized**, put 1.5 μL of the reagent that was used to resuspend the gDNA pellet at the end of the DNA extraction procedure. Click **Blank** on the upper left hand side of the program window.
	5. Put 1.5 μL of gDNA one sample at a time, then click **Measure** on the upper left hand side of the program window to measure the concentration of the sample, A260/A280 ratio, and the A260/A230 ratio. If the concentration is too low (<5-10 ng/μL) or too high (>3000 ng/μL), the concentration might not be accurate. In this case, the sample must be concentrated or diluted, respectively. A260/A280 and A260/A230 ratios show the purity of the sample, and they should be ≥ 1.8 for optimal labeling yields.
	6. NanoDrop does not have to be blanked again unless the next sample to be measured was resuspended in a different reagent.
	7. Wipe the surfaces of the pedestal and arm with kimwipe after every sample.
	8. Once all samples are measured, clean the surfaces with pure water on a kimwipe.
	9. Click **Print** report for the record.
2. To determine the quality of the sample, run 200-300 ng of gDNA on a 1-1.2 % agarose gel to ensure it shows no sign of RNA contamination or degradation as long as its molecular weight. Take a photo of the gel image.

 ***Important:*** *genomic DNA should appear as a single prominent band greater than 12kb. If the sample appears as more than one band or as a smear, the DNA may be degraded or have a contaminant that could affect the labeling procedure. RNA contamination will result in a smear less than 200bp.* *Genomic DNA exhibiting significant degradation (all bands < 500bp) is unsuitable for CGH analysis.*

REFERENCES

1. The NanoDrop user's manual.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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 Cytogenetic Supervisor

**UW Medicine - Pathology**

 **Cytogenetics - UWMC**

**SIGNATURE PAGE FOR POLICIES AND PROCEDURES**

Procedure / Policy Title: DNA Sampling Requirements, Preparation and Quality Control Procedure

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