**UW Medicine - Pathology**

400-11-01-15

**DNA Sample Requirement, Preparation & Quality Control**

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| Adopted Date: Oct. 12, 2009  Review Date: Oct. 21, 2009  Revision Date: Nov. 2009, Oct. 21, 2012  Under Revision: |

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

### Material and Equipment

1. Spectrophotometer NanoDrop 2000c
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).
   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. Lower the arm and when the pop-up window appears, click **Ok** on the screen.
   5. Go to File then choose New workbook and click save.
   6. 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.
   7. Put 1.5 μL of gDNA per sample at a time and 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.
   8. NanoDrop does not have to be blanked again unless the next sample to be measured was resuspended in a different reagent.
   9. Wipe the surfaces of the pedestal and arm with kimwipe after every sample.
   10. Once all samples are measured, clean the surfaces with pure water on a kimwipe.
   11. Click **Print** **Report** for the record.
2. To determine the quality of the sample, run 100-300 ng of gDNA on a 0.8or 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.*



1. **REFERENCES**
2. The NanoDrop user's manual.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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**UW Medicine - Pathology**

**Cytogenetics and Genomics**

**SIGNATURE PAGE FOR POLICIES AND PROCEDURES**

Procedure / Policy Title: **DNA Sample Requirement, Preparation & Quality Control**

Procedure / Policy Number: 400-11-01-15

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