| CORIELL INSTITUTE FOR MEDICAL RESEARCH | HIGH MOLECULAR WEIGHT (HMW) DNA EXTRACTION AND QUALITY CONTROL | |
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| | Form 0704-16 | Implemented: 2/12/2024<br>Revision Number: D-021224 |

**1. How is HMW DNA extracted?**

Nanobind HT CBB kit (PacBio) using Thermo Scientific KingFisher Flex Purification System.

**2. How is HMW DNA quality control performed?**

- HMW DNA concentration is determined using the Qubit dsDNA BR assay, a dye-based fluorescence method*. See #7 for specific information.
- HMW DNA A260/280 ratio is determined using a NanoDrop spectrophotometer*. See #7 for specific information.
- HMW DNA is run on pulsed field gel to measure molecular weight/fragment size. (See Figure 1)

The criteria for acceptance of the HMW DNA sample based upon all quality control procedures are as follows:

- Samples have a 260/280 ratio in the range of 1.70 - 2.00.
- DNA must be intact and of high molecular weight (50-300kb).

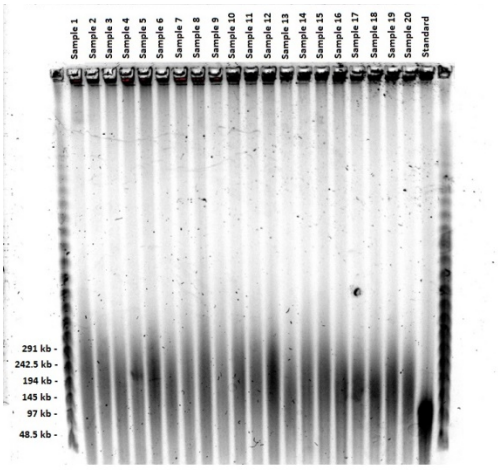


Figure 1. Pulsed field gel image showing molecular weight range of ~50-300kb

*HMW DNA is inherently difficult to work with as viscosity and inhomogeneity are common. Three measurements are recommended, sampling from top, middle, and bottom of the tube, for concentration and A260/280 readings. Concentration %CV values are usually <20%, but if the HMW DNA is very large, the %CV can exceed 30-40%.

**3. What is HMW DNA eluted in?**

HMW DNA is eluted in Buffer EB (provided in the Nanobind HT CBB kit).

**4. How much HMW DNA is shipped in the vial?**

Estimated DNA yield is 5-20µg, extracted from a starting number of $2.5x10^6$ cells.

**5. How is HMW DNA shipped?**

Shipped on cold packs at 4°C.

**6. How should HMW DNA be stored?**

Store at 4°C for up to 30 days after extraction date. Avoid freeze/thaw cycles as this can degrade HMW DNA.

**7. How should I retest the HMW DNA concentration?**

We recommend using the Qubit® dsDNA BR Assay (Q32850; ThermoFisher Scientific) to determine HMW DNA concentration. This assay uses an ultra-sensitive fluorescent nucleic acid stain for quantifying double-stranded DNA (dsDNA) in solution with a standard fluorometer and fluorescein excitation and emission wavelengths. Multiple measurements from the top, middle, and bottom of the eluate are recommended.

We recommend using a Nanodrop to determine A260/280 ratio. Multiple measurements from the top, middle, and bottom of the eluate are recommended.

8**. How are the DNA vials labeled?**

The vials will have a barcode label with the sample ID, lot number, unique Repository identifier, and the date extracted.

APPROVED

Name:_____Date:_____

Signature:_____

ISO-CONTROLLED COPY
DO NOT MAKE DUPLICATES
Copy ID:_____