STANDARD OPERATING PROCEDURE	**SOP No. 005** **QC of DNA**
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Electronic Filename: PCBN.SOP05.v2.0 QC of DNA	

1. PURPOSE

To describe the procedure for the QC of DNA extracted from frozen tissue by real-time PCR. NOTE: This SOP does not cover detailed procedures for handling Human Biological Materials or hazardous chemicals and it is recommended that personnel following this SOP refer to institutional safety guidelines.

The following SOP for QC of DNA is followed by the JHU, NYU, MSKCC and UW Network Sites of the PCBN.

2. RESPONSIBILITIES

Authorized personnel performing QC of DNA extracted from frozen prostate tissue must ensure that:

- all procedures are followed correctly
- all samples are adequately coded during processing
- all documentation is completed, and accurate records maintained on all samples

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS FOR JHU & NYU NETWORK SITES

Equipment	Materials
iCycler real time Thermocycler Nanodrop	96-well plates Primers as described SYBR Green PCR Master Mix (Invitrogen Catalog # 4309155)

5. EQUIPMENT AND MATERIALS FOR UW & MSKCC NETWORK SITES

Equipment	Materials
Gel electrophoresis system Gel Doc Nanodrop	Agarose TAE Buffer 6X Sample Loading Buffer DNA Ladder Standard

6. PROCEDURES FOR JHU AND NYU NETWORK SITES ONLY

- Quantitate DNA samples extracted using **PCBN SOP004 DNA Extraction from Frozen Tissue** by Nanodrop. Refer to the Nanodrop user guide for instructions of use.
- If DNA concentration is greater than 100 ng/ul, dilute DNA sample to 100 ng/ul.
- QC of DNA samples extracted using **PCBN SOP004 DNA Extraction from Frozen Tissue** by real-time PCR.

    - Rxn mix (20 µl): µl/rxn

        gDNA 2
        SYBR Green 10
        F-primer 1
        R-primer 1
        dH2O 6

        - A. β-globin primers (100 bp product)

        Forward: 5'-GTGCACCTGACTCCTGAGGAGA-3'
        Reverse: 5'-CCTTGATACCAACCTGCCCAG-3'

    - B. 18S primers (110 bp product)

        Forward: 5'-GATGGTAGTCGCCGTGCC-3'
        Reverse: 5'-GCCTGCTGCCTTCCTTGG-3'

    - Cycling parameters

        Cycle 1: (1X)
        Step 1: 95.0ºC for 03:00
        Cycle 2: (35X)
        Step 1: 95.0ºC for 00:30
        Step 2: 60.0ºC for 00:30
        Step 3: 72.0ºC for 00:45

7. PROCEDURES FOR UW AND MSKCC NETWORK SITES ONLY

- Quantitate DNA samples extracted using **PCBN SOP004 DNA Extraction from Frozen Tissue** by Nanodrop. Refer to the Nanodrop user guide for instructions of use.
- Measure the A_{260}/A_{280} ratio for an estimate of DNA purity with respect to contaminants that absorb UV.
- DNA length can be determined using agarose gel electrophoresis.
- DNA samples with sharp 10kb band are considered as high-quality DNA.