STANDARD OPERATING PROCEDURE	**SOP No. 007** **QC of RNA**
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1. PURPOSE

To describe the procedure for the QC of RNA 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 RNA is followed by the JHU, NYU and UW Network Sites of the PCBN.

2. RESPONSIBILITIES

Authorized personnel performing QC of RNA 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

Equipment	Materials
Agilent Bioanalyzer iCycler real time Thermocycler Nanodrop	96-well plates DNase I (Ambion, Catalog # AM2222) Primers as described SuperScript First Strand Synthesis System for RT-PCR (Invitrogen, Cat # 11904-018) SYBR Green PCR Master Mix (Invitrogen Catalog # 4309155)

5. PROCEDURES

- Quantitate RNA samples extracted using **PCBN SOP006 RNA Extraction from Frozen Tissue** by Nanodrop. Refer to the Nanodrop user guide for instructions of use.
- Obtain RIN number for samples extracted using **PCBN SOP006 RNA Extraction from Frozen Tissue** by Agilent Bioanalyzer.

Procedures for JHU and NYU Network Sites Only:

- DNase I Treatment (Ambion, Cat # AM2222)
  - Treat 50 µl of RNA (<200 µg/ml) with 1 µL DNase I.
    Refer to standard protocol for 'Removal of Contaminating Genomic DNA from RNA' (37°C for 30 min).
  - Heat inactivate at 75°C for 10 min following addition of EDTA to a final concentration 5 mM.
- cDNA synthesized using SuperScript First Strand Synthesis System for RT-PCR (Invitrogen, Cat # 11904-018) following standard protocol for 'First-Strand Synthesis Using Random Primers'.
- QC RNA samples extracted using **PCBN SOP006 RNA Extraction from Frozen Tissue** by real-time PCR.
  - Rxn mix (20 µl): µl/rxn

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

  - GAPDH primers (81 bp product)

    Forward: 5'-CGCTCTCTGCTCCTCCTGTT-3'
    Reverse: 5'-CCATGGTGTCTGAGCGATGT-3'

  - 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