



# **StaRT-PCR™ Platform Data Generation Plan AND Standard Operating Procedures for MAQC Study**

## **Test Site**

Test site: Gene Express, Inc.  
Contact Person: James C. Willey, M.D.  
Address: 975 Research Drive  
Toledo, OH 43614-2788  
Phone: (419) 383-3541  
E-mail: [jwilley@umedohio.edu](mailto:jwilley@umedohio.edu)

Contact Person: Elizabeth Herness Peters, Ph.D.  
Address: 975 Research Drive  
Toledo, OH 43614-2788  
Phone: (419) 380-9930  
E-mail: [ehpeters@geneexpressinc.com](mailto:ehpeters@geneexpressinc.com)

## **Introduction**

The purpose of this document is to describe the procedures that will be performed using the *StaRT-PCR*™ platform for the MAQC study.

## **Materials**

### **Gene Express, Inc. Products**

1. Standardized Mixtures of Internal Standards™ (SMIS™)
2. Gene-Specific Primers (forward + reverse at a concentration of 0.05 µg/ µl each)
3. HT S-GEM Suite™ software program

### **RNA Samples**

1. Stratagene Universal Human Reference RNA (UHRR) – Catalog No. 740000
2. Ambion Human Brain Reference RNA (AB) – Catalog No. TBA
3. RNA mixture of 25% AB vs. 75% UHRR
4. RNA mixture of 75% AB vs. 25% UHRR

## Disposables

- |  |
|--|
| 1. Disposables are listed in the SOP manual and include items such as clean Kim wipes, microcentrifuge tubes, pipette tips, 96 well plates, buffer troughs, plastic adhesive seals, etc. |
|--|

## User Manual

- |   |
|---|
| 1. Standardized Expression Measurement Center (SEM™) SOP manual |
|---|

## Reagents

ITEM	SOURCE
First Strand cDNA buffer	Invitrogen Cat. No. Y00146
dNTPs, 10 mM	Promega Cat. No. C1141
Oligo dT Primer, 500 µg/µl	Promega Cat. No. 15199905
RNasin, 40 units/µl	Promega Cat. No. 15140508
MMLV Reverse Transcriptase, 200 units/ml	Invitrogen Cat. No. 28025-013
Sterile RNase/DNase free water	Invitrogen Cat. No. 10977015
Tris-EDTA Buffer, pH 8.0 and pH 7.4	Fisher Cat. No. BP1338-1
10X PCR Buffer without Mg <sup>2++</sup>	Invitrogen Cat. No. Y02028
MgCl <sub>2</sub> , 50 mM	Invitrogen Cat. No. Y02016
Platinum Taq polymerase, 5U//µl	Invitrogen Cat. No. 10966-026
DNA Buffer Solution	Invitrogen Cat. No. Y02028
DNA Ladder	Caliper Life Sciences Cat. No. P/N 760124
DNA Gel-Dye Mixture	Caliper Life Sciences Cat. No. P/N 760124
HT DNA 5000SE 30 Marker	Caliper Life Sciences Cat. No. P/N 760124

## Equipment

ITEM	SOURCE
Block Thermocycler	MWG
Agilent 2100 Bioanalyzer	Agilent Technologies
MultiPROBE II HT EX Liquid Handler	Perkin Elmer
Caliper AMS90 SE	Caliper
AMS-90 Sipper Chip	Caliper
Centrifuge	Beckman
Laminar Flow Hoods	Baker and Hemco
Pipettes	Rainin and Pipetman
Freezer, -80°C	Sanyo
Freezer, -20°C	LRP
Refrigerator, 4°C	Kenmore

## Experimental Design

The RefSeq numbers of a total of 90 to 100 genes will be matched to the MAQC 1,042 gene list. The remaining genes, up to a total of 200 genes, will be selected from the list of available *StaRT-PCR*<sup>™</sup> gene assays, located at the following web address <http://www.geneexpressinc.com/products2.asp>, and approved by the MAQC.

Three replicate measurements for each gene will be obtained for each of the four mRNA samples listed above. The loading control genes will be ACTB and GAPD.

## StaRT-PCR™ Gene Assay Workflow

Day 1	Day 2	Day 3	Day 4	Day 5
Receive Sample	RNA Evaluation	E screen	C Screen	Replicate TA Measurements
Login Sample	Reverse Transcription			
Store Sample	Calibration			

Day 6	Day 7	Day 8	Day 9	Day 10
Replicate TA Measurements				

Day 11	Day 12	Day 13		
Replicate TA Measurements	Replicate TA Measurements	Prepare Final Report		

### 1. Sample Receipt, Login, and Initial Storage of Samples

All personnel will follow SOP0001 for receipt, login, and initial storage for the four (4) mRNA samples. Samples will be stored at -80°C.

1.1 New Order Function will be used to begin an order by following SOP0035.

1.2 A SOURCEPLATE.csv file will be prepared by following SOP0017.

1.3 Prepare SOURCEPLATE(s).

### 2. RNA Preparation and Analysis

Four (4) mRNA samples, designated as Stratagene Universal Human Reference RNA, Ambion Brain Reference RNA, and two titration mixtures will be received from Ambion at a concentration 1 mg/ml per tube in a total volume of 50 µl.

2.1 RNA quantity assessment will be performed on each sample using the Agilent Bioanalyzer and recording the 28S/18S ratio and RIN, if available.

All personnel will follow SOP0002 for RNA quantity assessment.

2.2 RNA quality assessment will be performed using 200 ng of each sample and assessed with the Agilent Bioanalyzer. All personnel will follow SOP0002 for RNA quality assessment.

### 3. Reverse-Transcription of RNA to cDNA

All personnel will follow SOP0004 for reverse transcription of mRNA samples to cDNA using oligo dT priming and MMLV reverse transcriptase.

### 4. Quantitative Calibration

Each of the cDNA samples will be Quantitatively Calibrated according to the reference gene (ACTB) such that 1 µl of cDNA sample when included into the PCR reaction with 600,000 β-actin molecules yields both native and internal standard PCR products that are approximately equal in peak height and area under the curve according to the SOPs of Gene Express, Inc. and the HT S-GEM Suite™ software program.

4.1 Each cDNA sample will be serially 10-fold diluted using the MultiPROBE II HT EX Liquid Handler according to SOP0052.

4.2 cDNA quantitative calibration reactions will be prepared in microtiter plates by the MultiPROBE II HT EX Liquid Handler using SOP0015 and completed using SOP0005.

4.3 PCR reactions will be prepared in microtiter plates by the MultiPROBE II HT EX Liquid Handler following SOP0018, SOP0023, SOP0024 and SOP0025.

4.4 PCR amplification of samples prepared in 4.3 will be performed using a MWG Block Thermocycler and following SOP0013.

- 4.5 After amplification is complete, a LabChip 90 sipper chip will be prepared using SOP0006.
- 4.6 The PCR reaction products from 4.4 will be electrophoretically separated and quantified with the Caliper AMS90 SE by using SOP0028.
- 4.5 Data from the Caliper AMS90 SE will be exported and a report file will be generated using SOP0009.

## 5. E Screen

Each gene will be assessed with E level SMIS™ containing 600 molecules of internal standards. The E screen results determine which level of SMIS™ is required during High Throughput PCR.

- 5.1 PCR reactions will be prepared using 1 µl of calibrated cDNA and 1 µl of E SMIS™ following SOP0018, SOP0023, SOP0024 and SOP0025.
- 5.2 PCR reactions will be performed using a MWG Block Thermocycler and following SOP0013.
- 5.3 After amplification is complete, a LabChip 90 sipper chip will be prepared using SOP0006.
- 5.4 The PCR reaction products from 5.2 will be electrophoretically separated and quantified with the Caliper AMS90 SE by using SOP0028.
- 5.5 Data from the Caliper AMS90 SE will be exported and a report file will be generated using SOP0009.

## 6. Transcript Abundance (TA) Measurements

*StaRT-PCR*™ will be performed using an Internal Standard Competitive Template (CT) within the PCR reaction for transcript abundance measurement of each gene. *StaRT-PCR*™ does this through inclusion of a Standardized Mixture of Internal Standards™ (SMIS™) and gene specific forward and reverse primers in transcript abundance measurement of each gene. Based on results from Quantitative Calibration and E screen, 1 µl of calibrated cDNA sample, and 1 µl of the appropriate SMIS™ (A, B, C, D, E, or F) will be PCR amplified. Proper selection of SMIS™ A thru F enables measurement of target gene expressed across a more than 7 log<sub>10</sub> range. For target genes in balance with 1 µl of A, B, C, or D SMIS™, 10-fold dilution of both cDNA and SMIS™ will be used. Thus, cDNA and SMIS™ containing approximately 60,000 molecules of b-actin transcript and internal standard, respectively, will be in each PCR reaction.

- 6.1 PCR reactions will be prepared following SOP0018, SOP0023, SOP0024 and SOP0025.
- 6.2 PCR reactions will be performed using a MWG Block Thermocycler and following SOP0013.
- 6.3 After amplification is complete, a LabChip 90 sipper chip will be prepared using SOP0006.
- 6.4 The PCR reaction products from 6.2 will be electrophoretically separated and quantified with the plate with the Caliper AMS90 SE by using SOP0028.
- 6.5 Export and generate a report file using SOP0009.

## 7. Data Acquisition

*StaRT-PCR*™ transcript abundance measurements will be obtained using the Caliper AMS90™ data by analyzing the area under the curve. Data will be registered and automatically analyzed using the HT S-GEM Suite™ software program. The data will be exported into a text file.

## 8. Data Management and Quality Control Specifications

*StaRT-PCR*™ Data are automatically processed and analyzed as described in SOP0009. Quality control specifications include appropriate negative and positive

control wells on each 96 well plate. Peaks must meet a minimum peak height and peak area requirement. Both Native and Internal Standard peak base pair sizes must lie within a maximum designated range of  $\pm 35$  base pairs.

**9. Final Report**

The final data report will contain the average of the three replicate TA measurements, the standard deviation and the coefficient of variation. The final report will be sent directly to the MAQC via electronic file.