



## Careful Design, Controlled Manufacturing of Reagents and Rigorous Assay Protocols Yield High Quality *StaRT-PCR*<sup>™</sup> Multi-Gene Transcript Abundance Measurements

### Abstract

Gene Express, Inc. is proud of our client proven *StaRT-PCR*<sup>™</sup> multi-gene transcript abundance measurement technology and the GLP services provided by our Standardized Expression Measurement<sup>™</sup> (SEM) Center<sup>™</sup> or with our *StaRT-PCR*<sup>™</sup> Kits for client's own use in their laboratory. In the SEM Center<sup>™</sup> all of our *StaRT-PCR*<sup>™</sup> assays are performed under strictly controlled GLP (Good Laboratory Practice) conditions employing our proprietary Standardized Mixtures of Internal Standards<sup>™</sup> (SMIS<sup>™</sup>) and gene-specific primers. Transcript abundance measurements of the highest quality produced for clients on a fee-for-service basis at the SEM Center<sup>™</sup>. This high quality is the result of rigorous manufacturing practices for the component reagents and strict adherence to Standard Operating Procedures (SOPs) in our production facility and the SEM Center<sup>™</sup>. All of this requires the employment of stringent quality control (QC) and quality assurance (QA) measures at a number of steps in the overall process. This technical note is intended to describe the processes involved in the design and manufacture of *StaRT-PCR*<sup>™</sup> assays and reagents.

### Introduction

*StaRT-PCR*<sup>™</sup> is a patented, standardized, quantitative method with integrated quality control for measuring multi-gene expression in a tissue, blood or cell culture sample of any living organism (Apostolakos *et al.* 1993, Crawford *et al.* 2002, Pagliarulo *et al.* 2004, Willey *et al.* 1998, Willey 2004, Willey *et al.* 2004a, Willey *et al.* 2004b). The platform technique employs competitive templates incorporated into Standardized Mixtures of Internal Standards (SMIS<sup>™</sup>). Multi-gene transcript abundance measurement by *StaRT-PCR*<sup>™</sup><sup>1</sup> is unique and is differentiated from all other gene expression measurement technologies by attributes listed in Table 1 (also see the Gene Express, Inc. Technical Notes listed in the Reference section). The component reagents to conduct *StaRT-PCR*<sup>™</sup> Assays are gene specific forward and reverse primers (*StaRT-PCR*<sup>™</sup> primers) and competitive template internal standards that are formulated into Standardized Mixtures of Internal Standards<sup>™</sup> (SMIS<sup>™</sup>). This White Paper covers the stringent quality control (QC) and quality assurance (QA) in the manufacturing of these *StaRT-PCR*<sup>™</sup> reagents and the quality assurance (QA) tests conducted on new SMIS<sup>™</sup> in the Standardized Expression Measurement (SEM) Center<sup>™</sup>.

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<sup>1</sup> "*StaRT-PCR*<sup>™</sup>" – Standardized (Sta), Reverse Transcriptase (RT), Polymerase Chain Reaction (PCR) – PCR is an enzymatic process to increase the number of copies of DNA for easier detection.

<b>Table 1. <i>StaRT-PCR</i><sup>TM</sup> displays many attributes desirable for transcript abundance quantification</b>	
<i>StaRT-PCR</i> <sup>TM</sup> Meets FDA Draft Guidance for Pharmacogenomics Data Submission for Multi-Gene Assay Methods ( <a href="http://www.fda.gov/OHRMS/DOCKETS/98fr/2003d-0497-gdl0002.pdf">http://www.fda.gov/OHRMS/DOCKETS/98fr/2003d-0497-gdl0002.pdf</a> )	
<b>Methodological Analytical Performance Characteristics</b>	<b><i>StaRT-PCR</i><sup>TM</sup> Performance*</b> <ul style="list-style-type: none"> <li>• Validated in multiple independent studies</li> <li>• Published in peer-review literature</li> </ul>
Precision (Reproducibility)	Intra sample reproducibility CV 5-10% Day-to-day Reproducibility Monitoring <15%
Lower Detection Threshold	< 10 molecules (and/or as low as one transcript in 30,000 cells)
Quality Control	SMIS <sup>TM</sup> in each measurement controls for interference and all other known sources of variation
Assay Specificity	Primers validated to ensure specificity of transcript and internal standard and native template amplimer sizes verified to ensure specificity
Effective Assay Range	Dynamic range more than seven log <sub>10</sub> . Covers entire range of gene expression in cells (<10 <sup>1</sup> to >10 <sup>7</sup> molecules/10 <sup>6</sup> Reference gene molecules)
Ability to identify small differences	As little as 20% differences reliably detected due to high sensitivity and reproducibility
Credibility	Validated in multiple independent studies Published in peer-review literature
* <i>StaRT-PCR</i> <sup>TM</sup> Meets FDA Draft Guidance for Pharmacogenomics Data Submission for Multi-Gene Assay Methods (( <a href="http://www.fda.gov/OHRMS/DOCKETS/98fr/2003d-0497-gdl0002.pdf">http://www.fda.gov/OHRMS/DOCKETS/98fr/2003d-0497-gdl0002.pdf</a> ))	

The highest quality of transcript abundance data provided by *StaRT-PCR*<sup>TM</sup> is due to a number of factors including the inclusion of an internal standard and a gene-specific primer pair in every assay for each gene measured. It should be noted that none of the individual components or procedures discussed herein is solely responsible for *StaRT-PCR*<sup>TM</sup> data integrity. Rather, one should view *StaRT-PCR*<sup>TM</sup> as a complete analytical system composed of high quality standardized reagents and protocols integrated into a platform based on the concept of employing Standardized Mixtures of Internal Standards<sup>TM</sup> (SMIS<sup>TM</sup>).

Because of the high quality and standardization of the data produced, *StaRT-PCR*<sup>TM</sup> is the ideal choice for measuring transcript abundance. *StaRT-PCR*<sup>TM</sup> data remain alive, enabling easy and direct comparison across experiments, studies and laboratories, as well as over time. These attributes make *StaRT-PCR*<sup>TM</sup> desirable when gene expression data is needed to define biomarkers, assess drug affects, segment populations for drug resistance or efficacy, and to develop molecular diagnostics. Pharmaceutical and Biotech companies are developing theranostics products that will be introduced in conjunction with new drugs to determine if patients will be a responder or non-responder to the new drug.

Before making a choice regarding any technology for such purposes, it is important to understand the issues surrounding the technology. These issues include the

development of the transcript abundance assays, the manufacture and quality control of the necessary reagents, and the determination of the performance and quality assurance of each assay. The critical unique reagents in *StaRT-PCR*<sup>TM</sup> are the transcript specific primers, the transcript specific competitive template internal standards and the SMIS<sup>TM</sup> composed of these internal standards. The manufacture of these reagents occurs at Gene Express within a discretely defined and highly organized production process. These reagents are then incorporated into a highly controlled analytical system in our SEM Center<sup>TM2</sup> to produce high quality transcript abundance measurement for clients. These clients then use the data for a variety of purposes including drug target identification and validation, drug development and clinical validation, molecular diagnostic development, and for the development of biomarkers potentially for use in patient segmentation.

## Considerations for the design, manufacture and QC/QA of gene specific *StaRT-PCR*<sup>TM</sup> primers

**General Considerations:** *StaRT-PCR*<sup>TM</sup> is a state of the art method for obtaining standardized transcript abundance values. *StaRT-PCR*<sup>TM</sup> is based on quantitative competitive PCR (Gilliland, et al. 1990), an underlying technology that has a relatively long-documented, successful history for the measurement of nucleic acids (Orlando et al. 1998). As a significant and proprietary improvement, *StaRT-PCR*<sup>TM</sup> incorporates Standardized Mixtures of Internal Standards (SMIS<sup>TM</sup>) into each assay (Willey, 2004). This means that a transcript-specific internal standard is included in every assay (in the form of SMIS<sup>TM</sup>) to quantitatively determine the amount of transcript in the sample of interest. The SMIS<sup>TM</sup> provide a common standard for all transcript abundance measurements by providing an internal standard for each transcript measured. Thus, integrated quality control is provided to compensate for aberrations that may occur during the PCR process due to inhibitors in the sample or some other undefined or uncontrollable variable. Also, since the SMIS<sup>TM</sup> are prepared in large batches *StaRT-PCR*<sup>TM</sup> data are defined relative to the same standard reagents rather than to some other sample or external standard curve. These properties yield “live” data that are readily comparable across experiments, laboratories and time.

Because there is a long history of quantitative PCR to measure transcript abundance and many reviews are available that discuss the general issues regarding the subject (Bustin 2000, Bustin 2002, Bustin 2005, Orlando 1998), this note will focus on those issues most relevant to *StaRT-PCR*<sup>TM</sup> and the strategies used at Gene Express to assure the highest quality reagents and data.

One of the fundamental concepts of *StaRT-PCR*<sup>TM</sup> is that a transcript-specific internal standard is included in each assay in the form of a SMIS<sup>TM</sup>. Each **Internal Standard is a Competitive Template (IS)** that shares the primer sequences with the **Native Template (NT)** the sequence of the natural transcript that is amplified during the PCR process) as well as the majority of sequence that is flanked by the primers. The expected sizes of internal standard and NT amplicons are known and this constitutes

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<sup>2</sup> Gene Express, Inc. provides standardized and quantitative gene expression analysis at its automated Standardized Expression Measurement<sup>TM</sup> (SEM) Service or Testing Center to researchers worldwide. The SEM Center<sup>TM</sup> strictly adheres to Standard Operating Procedures (SOPs) and under strictly controlled GLP (Good Laboratory Practice) conditions. The SEM Center<sup>TM</sup> employs *StaRT-PCR*<sup>TM</sup> to provide rapid, reproducible, quantitative and standardized, as well as cost effective measurement of gene activity on high-throughput automated equipment. The entire process is controlled and operated by proprietary software.

one level of QC/QA for the specificity of each assay. As indicated previously, the internal standards are added to the PCR reactions as SMIS™ in which the concentration of each internal standard is precisely determined. During PCR amplification, the internal standard and native template for any one gene are amplified with similar kinetics such that, at the end of the reaction, the concentrations of the native template and internal standard amplimers are directly proportional to the respective initial concentrations (Apostolakos 1993, Willey 1998). Since the initial concentration of the internal standard is known, the initial concentration of the native template can be calculated.

In practice, this is done in the SEM Center™ by analyzing the products of the PCR reactions on a Caliper Life Sciences AMS-90 and an HT DNA 5000 SE30 LabChip (Caliper Life Sciences, Hopkinton, MA) analyzer that uses microfluidic electrophoresis to separate the PCR products and laser optics to quantify each amplimer. The electropherograms produced are automatically analyzed through proprietary software to identify the appropriate size amplimers and calculate the initial native template concentrations using the ratio of the native template amplimer peak area to the internal standard amplimer peak area and the known amount of internal standard initially added to the reaction. One level of specificity of each assay occurs at this step and requires that native template and internal standard amplimers of the expected sizes were produced and only those products are analyzed.

**Choice of Target Sequence:** Once the decision is made to manufacture an assay for a particular gene, an RNA/cDNA segment must be carefully chosen for which *StaRT-PCR*™ primers must be designed. All steps for primer design and manufacture are precisely defined by SOPs. In general, the attempt is made to target a specific segment that uniquely represents the target gene using proprietary methods. This maximizes the specificity of each *StaRT-PCR*™ primer pair.

**Primer Design:** All primers are designed to have a melting temperature of 58°C  $\pm$  1°C. Once primers are designed that will theoretically amplify the target sequence, they are analyzed *in silico* using the BLAST sequence utility (<http://www.ncbi.nlm.nih.gov/BLAST/>) set to screen the nr (non-redundant) database and limiting the search to *Homo sapiens*.

Additionally, all primers used for *StaRT-PCR*™ assays are evaluated for known single nucleotide polymorphisms (SNPs) in the target sequence regions that are homologous to the primers. No primers contain SNPs that are known at the time of primer design are used to develop *StaRT-PCR*™ assays. This assures that all SNP variants of any target gene sequence are measured.

Primers are accepted at this step only if they show 100% homology to the desired target and contain no known SNPs. If so, they are further evaluated for use in internal standard manufacture and *StaRT-PCR*™ assay development.

**Dealing with Alternative Transcripts and Gene Families:** There are two categories of target sequences for which the basic primer design strategy must be modified. The first of these includes those genes for which there are known alternate transcripts or transcript variants and the second includes genes that are of members of a gene family and generally have a high sequence homology (80% or more) to each other.

There are a number of genes where the primary transcript is modified after transcription. This usually occurs through the cutting and splicing of the primary transcript producing anywhere from two to over 15 alternate transcripts, each of which is different with respect to the specific splice that occurs. Different alternate transcripts are found in different concentrations in different cell types. Because alternate transcripts for any one gene are derived from the same primary transcript sequence, they all have identical homology except for the region flanked by the splice site(s). With respect to primer design, we follow one of two strategies to detect alternate transcripts. In some cases, the number of alternate transcripts is small (from 2 to 5) and, if primers are designed to flank the splice site, the different alternate transcripts can be identified by the size of the each amplicon. In these situations, we design primers that flank the splice site and create a single competitive template as an internal standard for all of the alternate transcripts for that gene. The basic strategy for primer design as described above is followed except the area for the target sequence is dictated by location of the splice site.

For some genes, there are many alternate transcripts and transcript-specific sets of primers are designed for each of the targeted variants. Usually, this is accomplished by designing one of the primers such that it flanks the sequence of the splice site, giving specificity for that particular transcript. The other primer is then designed to be common to all the known transcript variants. This strategy also is used when it is desired to have transcript-specific assays even when only a few alternative transcripts exist.

Members of a gene family also share significant sequence homology (sometimes greater than 80%). Such genes apparently evolved through the duplication of a parent gene. Because of the high degree of homology, it is particularly difficult to design transcript-specific primers for this category of genes. To maximize specificity, we employ a number of proprietary strategies to design target specific primers.

**Handling and Storage:** Once designed and synthesized, working solutions of primers are carefully prepared. This work is performed in a certified Class II Biological Safety cabinet to prevent contamination of primers with either Internal Standard Competitive Template (IS) or Native Template target (NT) sequences. After preparation, the primers solutions are certified as described below and then aliquotted into appropriate volumes and stored at  $-20^{\circ}\text{C}$ . By performing accelerated shelf life tests, the shelf life of primers stored under these conditions has been estimated to be at least five years (unpublished observations).

**Quality Control and Quality Assurance of the Performance of Primers:** Every *StaRT-PCR*<sup>TM</sup> primer pair and stock solution manufactured must pass a series of rigorous quality control analyses to generate a certificate of analyses (COAs) before any assay is used in the SEM Center<sup>TM</sup>. The COA for each primer pair includes documentation verifying the lack of native template (NT) and IS contamination in the primer working stock solutions, the assay lower detection limit, sequence homology to target priming sites, and production of correct internal standard base-pair size amplicon in its corresponding SMIS.

Each stock solution of primers is verified to be free of native template or internal standard contamination by performing *StaRT-PCR*<sup>TM</sup> reactions with sterile water as either the putative sample or IS component. Only those stock solutions that are free of native template and Internal Standard contamination (as determined by a lack of either native template or internal standard amplicon) pass this quality control step.

Each primer pair that is designed is tested for amplification of the target using an appropriate cDNA preparation. Only primer pairs that produce native template amplimers of the expected size(s) with a clean electropherogram pass this step and are used for IS construction and assay development.

It is important that each *StaRT-PCR*<sup>™</sup> assay is as sensitive as possible (i.e. has a low limit of detection). To verify this, each primer pair is used in a series of PCR reactions containing known amounts of internal standard, ranging in concentration from 6,000 to 6 molecules of internal standard template. A negative control containing no template is also tested to verify that there is no contamination of the primers. Only those assays that can detect at least 60 molecules are offered as *StaRT-PCR*<sup>™</sup> assays. The majority of *StaRT-PCR*<sup>™</sup> assays offered have a lower limit of detection of at least 6 molecules.

## **Considerations, procedures and QC/QA for the manufacture of the Internal Standards and SMIS<sup>™</sup>**

**General Considerations:** All *StaRT-PCR*<sup>™</sup> competitive template internal standards and the SMIS<sup>™</sup> composed of these are manufactured through a quality-controlled process defined by stringent SOPs. This process includes those steps described above for *StaRT-PCR*<sup>™</sup> primer design and manufacture as well as the other proprietary steps. The QA/QC requirements are summarized in Table 2.

The Internal Standard Competitive Templates (IS) are constructed through a modification of the method described originally by Celi (Celi 1993). This method involves creating an artificial Internal Standard Competitive Templates (IS) sequence from the native template through PCR amplification using the same forward primer but a different reverse primer (referred to as the CT primer) that is a chimera composed of the originally defined reverse primers at the 5' end of the CT primer contiguous at the 3' end with a sequence internal from the originally defined reverse primer by 10 to 25% of the length of the native template amplimer. The product of PCR amplification with the forward and CT primer is an artificial construct that is mostly the same sequence as the native template (75 - 90%) with the identical primer sequences as the native template but contains a short deletion. Because of these properties, the Internal Standard is amplified with the same primers and with the same kinetics as the native template but yields an amplimer that is readily resolved from the native template amplimer by microfluidic electrophoresis (Willey *et al.* 2004 a, Willey *et al.* 2004b, Willey, 2004).

**Definition of Target Sequence:** Since this subject influences the design of the *StaRT-PCR*<sup>™</sup> primers used, the factors influencing the strategies employed to define the target sequence for IS manufacture have already been discussed above in the primer design section. All target sequences lack a NotI endonuclease site. Thus, the purified plasmids containing the cloned Internal Standards can be linearized by digestion with this nuclease (see below).

**The IS Manufacturing Process:** All steps for IS manufacture are precisely defined by SOPs. Once the *StaRT-PCR*<sup>™</sup> primers for a transcript have been manufactured and certified, a CT primer is designed. The CT primer has the sequence of one of the primers at the 5' end and an internal sequence of the native template amplimer at the 3' end. The considerations used in native template primer design are applied to CT primer design. An additional consideration is that the internal sequence is no more than 25% or no less than

10% of the entire size of native template amplicon away from the 3' end of the NT primer sequence used for the construction of the CT primer. This rule is implemented to minimize the difference between the IS and the native template, yet still allow for complete electrophoretic resolution of the native template and Internal Standard (IS) amplicons.

Once designed, the CT primer is synthesized, a stock solution is made that is composed of the CT and forward primers, and a PCR reaction is performed using these primers and native cDNA from an appropriate tissue. The products of this reaction are electrophoresed in an agarose gel and the band representing the IS amplicon is excised from the gel and purified using a commercially available kit designed for the purification of PCR products from agarose gels.

The purified IS amplicon is then cloned into pCR<sup>®</sup>2.1 –TOPO 3.9 kb (Invitrogen, Carlsbad, CA). All cloned Internal Standards are sequenced. After the IS sequence is determined to be correct, each cloned Internal Standard is used to determine the sensitivity (lower limit of detection) of the respective *StaRT-PCR*<sup>™</sup> assay. To do this, purified plasmid preparations of cloned Internal Standards are first quantified (see next section) and are then verified to meet our lower limit of detection specifications (see next section). After each cloned IS passes all of the described QC/QA analyses, a large-scale plasmid preparation is made, resulting in at least 2.5 mg of purified plasmid clone. The purity of the plasmid preparations is assessed by determining the  $A_{260}/A_{280}$  and by evaluating each preparation by agarose gel electrophoresis. The concentration of each large-scale IS plasmid preparation is determined by fluorometry as described above. The desired Internal Standards are combined in equal concentrations to make a SMIS<sup>™</sup>.

**QC/QA of Individual IS:** Before use, putative clones are analyzed by first determining the size of cloned inserts after *EcoRI* digestion and then by sequencing clones with correct size inserts. In this fashion, not only are clones to be incorporated into SMIS<sup>™</sup> assured to correctly represent the desired target, there is also verification that there is 100% sequence homology between primer recognition sequences in the IS sequence and the primer sequences.

The DNA concentration of each preparation of competitive template to be used as an internal standard is accurately determined by Hoechst type 33258 dye binding and fluorimetry using the Dyna Quant 200<sup>®</sup> (Amersham Biosciences, Piscataway, NJ) using calf thymus DNA (Cat. # 80-6227-06, Amersham Biosciences, Piscataway, NJ) as a reference standard according to the manufacturers instructions (Dyna Quant<sup>®</sup> 200 Fluorometer User Manual, Amersham Biosciences, Piscataway, NJ). A standard curve is first generated using a reference calf thymus DNA standard solution and then triplicate determinations are made for each IS preparation. The average of these determinations is used to calculate the concentration of IS in each preparation. Following quantification of the DNA, each individual Internal Standard is checked for the lower limit of detection of each assay and for the appearance of the correct size IS amplicon by performing *StaRT-PCR*<sup>™</sup> assays without sample cDNA and primers for individual targets. PCR reactions then are performed using 6,000, 600, 60 and 6 molecules of IS as the template concentration. Only those assays that produce a clean PCR product of the expected size with 60 molecules or less of initial IS template become *bona fide* Internal Standard ready to be formulated into a SMIS<sup>™</sup> which in turn

can be used in *StaRT-PCR*<sup>TM</sup> assays. The majority of *StaRT-PCR*<sup>TM</sup> assays have a lower limit of detection of 6 molecules or less.

**Storage and Handling of Individual IS:** After the IS are manufactured and have passed QC, including sequence and sensitivity validation, they are aliquotted into appropriate volumes and stored at -20°C.

**The SMIS<sup>TM</sup> Manufacturing Process:** Each SMIS<sup>TM</sup> is actually a collection of six mixes (Mixes A through F) of the same internal standards. Each mix contains a different ratio of internal standards for target genes to the calibrating internal standards (ACTB and GAPD). This enables transcript abundance measurement over the entire 7-log range of gene expression in cells. Initially, Mix A is made with internal standards for 93 target genes at 10<sup>-11</sup> M, the internal standard for ACTB at 10<sup>-12</sup> M, and two internal standards for GAPD, one at 10<sup>-13</sup> M and one at 10<sup>-14</sup> M. This mix is digested with NotI to linearize the plasmids and digestion is verified by agarose gel electrophoresis of a small sample of the mix. Mix A is then diluted serially 10-fold with a diluent composed

<b>Table 2. QA/QC steps for <i>StaRT-PCR</i><sup>TM</sup> reagents</b>		
<b>Reagent</b>	<b>QC/QA Test</b>	<b>Acceptable Specifications</b>
<b>Individual primer pairs</b>	Correct sequence and amount	COA from manufacturer
	Specificity	BLAST search of primer sequence verified to be 100% correct. No hit with non-specific targets.
		Correct amplicon size verified for both internal standard and native template
		Primer sequences of internal standard verified to be 100% correct by sequence analysis
	Lack of SNP interference	Primer sequences devoid of known SNPs
	Discrimination of alternative transcripts	Primers designed to allow for differentiation of alternative transcripts
<b>Primer stock solutions</b>	PCR with no template	Free from Internal Standard or native template contamination
<b>Primer stock solutions/IS*</b>	PCR with IS only	Correct size IS amplicon
		No interfering products
<b>Internal Standard*</b>	Concentration of each internal standard determined by standard method	Each internal standard quantified using the Hoechst dye method with a calf thymus DNA standard
<b>Primer stock solutions/IS/SMIS<sup>TM</sup></b>	Lower limit of detection	Amplicon from 60 molecules or less of template
<b>SMIS<sup>TM</sup></b>	PCR with SMIS <sup>TM</sup> only	Correct size IS for each assay
		No native template contamination
	Functional test: <i>StaRT-PCR</i> <sup>TM</sup> with Universal Human RNA	Correct native template and Internal Standard amplicon produced, assay characteristics acceptable
Pipettes, hoods, fluorometer, thermocycler blocks all on a routine calibration/maintenance schedule		
*Internal Standard		

of internal standards for the calibration genes (ACTB and GAPD) at the concentrations indicated above. This gives solutions that all contain the ACTB internal standard at  $10^{-12}$  M, the two internal standards for GAPD at  $10^{-13}$  M and  $10^{-14}$  M and the internal standards for the other genes at  $10^{-12}$  M through  $10^{-16}$  M for Mix B through Mix F, respectively. All of the above manipulations are performed in a laminar flow hood with calibrated pipets. Individuals work in pairs to cross check and verify that each step is performed accurately and according to the SOPs.

**QC/QA of SMIS™:** After incorporation into a SMIS™, the assay for each gene is reevaluated for the lower limit of detection and for the appearance of the correct size for each internal standard amplicon by performing *StaRT-PCR*™ assays with primers for individual target genes without sample cDNA. In addition, a functional QA test for each assay is performed by conducting triplicate *StaRT-PCR*™ reactions with cDNA prepared from Stratagene Universal Human Reference RNA (Stratagene, La Jolla, CA) as the sample. During this evaluation, reproducibility is determined and other unacceptable performance characteristics of any particular assay are identified. If these occur, either the problem is resolved or the assay fails and is not offered as a *bona fide* *StaRT-PCR*™ assay.

**Storage and Handling of SMIS™:** After all QC/QA has been passed, SMIS™ are aliquotted into appropriate volumes and stored at  $-30^{\circ}\text{C}$ . According to accelerated shelf life tests conducted by Gene Express, Inc., the shelf life of SMIS™ stored under these conditions is estimated to be at least five years (unpublished observations).

## Summary

There are many practical reasons to understand the molecular basis of disease. One of the forces that drive the research to increase knowledge in this area is to apply this information clinically. For this application, it is critical that the molecular technologies used both in the underlying research and in the clinic must be precise and the data must be of the best quality possible. To meet this challenge, Gene Express, Inc. has developed *StaRT-PCR*™. *StaRT-PCR*™ uses reagents that are manufactured with stringent quality control and quality assurance. This results in the highest quality transcript abundance data currently obtainable. Such standardized data is not only critical for application to diagnostics and drug development but will also enhance our understanding of various disease processes.

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## Other Technical White Paper Notes Available on Request

How *StaRT*-PCR™ Works

Review of The FDA Pharmacogenomics Guidance for Industry: Role of *StaRT*-PCR™ in Increasing Value of Pharmacogenomic Data

Standardized Multi-Gene Expression Measurement – *StaRT*-PCR™ Technological Advantages

*StaRT*-PCR™ Aids Pharmacogenomics, Biomarkers and Molecular Diagnostics

*StaRT*-PCR™ – The Choice for Defining, Validating and Measuring Biomarkers or Interactive Gene Expression Indices™ (IGEs™)

*StaRT*-PCR™ Quality-Controlled, Multi-Gene Expression Measurement for Development of Drugs, Biomarkers and Molecular Diagnostics