

Technical Validation of RNA-Based Biomarkers for Drug Development and Companion Diagnostics



TACT	JUN	CTH
IL10	IL15	IL15
TGFB	PTGSF13	IL10
NOS2A	VEGF	IL10
HSPA1A	IL18N	IL10
JUN	OD14	IL10
MMP9	TLR4	IL10
PTGS2	STAT3	IL10

Source MDx Technical Validation of RNA-Based Biomarkers for Drug Development and Companion Diagnostics

Background

Source MDx has completed the technical validation process described below for hundreds of inflammation and immune response genes now available as part of the molecular biomarker services offered to the pharmaceutical and diagnostics industries. The Company is currently applying this validation process to technically qualify more than a thousand genes in therapeutic areas of interest to our clients. This document outlines the technical validation process that yields precision and calibration across multiple gene assays that form the basis of the Source MDx Precision Profiles™. The Precision Profile assay is a multiplexed Polymerase Chain Reaction (PCR) assay optimized by Source MDx to measure the gene expression of a biological condition or agent in untreated and treated biological samples derived from body fluids (such as whole blood), tissues (such as skin) or culture medium (such as keratinocytes and THP-1 cells). Total RNA is extracted from each sample and a first strand synthesis is performed using a reverse transcriptase (RT) to generate cDNA. The cDNA serves as a template for use in a Precision Profile assay where individual gene amplification is quantified by real-time Quantitative Polymerase Chain Reaction (QPCR) using the ABI Prism® 7900HT Sequence Detection System.

Key to the Precision Profile Assay is the concept of precision and calibration (Source MDx U.S. patent 6,692,916B2) achieved through proprietary reagents (target gene primer-probe sets), high performance plates (Precision Profile plates) and strict adherence to narrow permissible levels of experimental variables. By rigorously controlling all assay variables, the gene expression levels in a sample of a given biological condition can be measured and clear comparisons can be made between gene loci, biological conditions and individuals. At Source MDx, precision is monitored at the: A. individual target gene level, B. reagent level and C. plate production level as described below.

Technical Validation Process Summary

Source MDx target gene primer-probe sets are optimized through multiple version design and testing at the small scale. Specific design criteria are proprietary to Source MDx. Primer probe design candidates undergo quality control testing to ensure that specificity and efficiency are within required specifications. Having met all acceptance criteria, the optimal primer-probe set is ordered in a production scale lot and undergoes further quality control testing to ensure that the production scale lot is performing according to the specifications

established at the small scale. Future production scale lots are also quality control tested and compared to previous production scale lots, ensuring uniform reagent lot performance over time. Precision Profile plates are manufactured in a 384-well format according to strict Standard Operating procedures. Plate production quality control tracks template matched plate lot performance. A quarantine and release process provides the highest quality reagents and plates for use in the Precision Profile assay.

A. Small Scale Primer-Probe Quality Control

1. *The Primer-Probe Amplification Test* measures transcript-specific amplification by QPCR using Source MDx's standard template. The primer-probe amplification test evaluates the following:

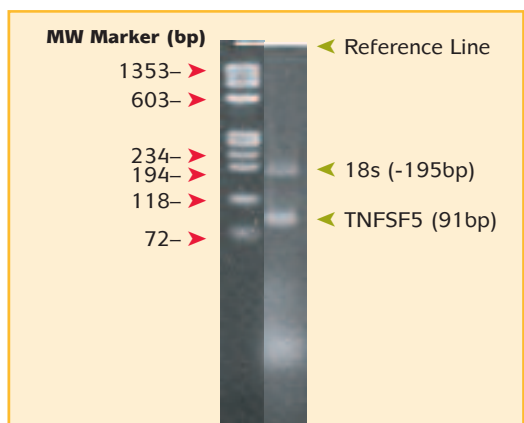
- RT+ (in quadruplicate, multiplexed with 18S, using Source MDx standard QC template)
- RT+ (in duplicate, without 18S, using Source MDx standard QC template)
- RT- (in duplicate, using PAX RNA)
- gDNA (in duplicate, using genomic DNA)
- NTC (in duplicate, using DEPC water)

In each case, appropriate levels of expression or lack of expression must be met in order to proceed to the gel test.

2. *The Primer-Probe Gel Test* is performed to ensure specificity of the primer-probe candidate. The primer-probe gel test evaluates the following:

- RT+ (multiplexed with 18S)
- RT+ (without 18S)
- RT-
- gDNA
- NTC

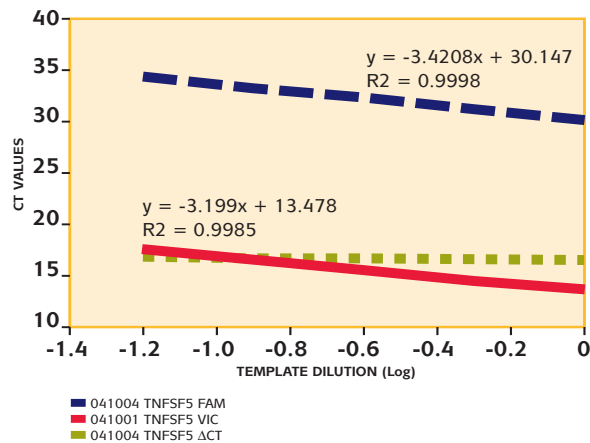
TNFSF5 Primer-Probe Gel Test (RT+, multiplexed with 18S)



Note: Source MDx will be incorporating the Agilent Bioanalyzer System in 2006.

A single product band of expected size with lack of genomic DNA amplification and competing primer dimer formation must be met in order to proceed to the next test. An example of TNFSF5 primer-probe gel test (RT+ multiplexed with 18S only) is shown below.

TNFSF5 Dilutional Linearity – FAM, VIC and Delta CT



3. *The Primer-Probe Efficiency Test* is performed to demonstrate that the primer-probe set has an optimal amplification efficiency within specified parameters. Linearity of response is measured across a series of 5-fold template dilutions. The primer-probe efficiency test evaluates the following:

- Dilutional linearity of FAM labeled primer-probe set of interest
- Dilutional linearity of VIC labeled 18S endogenous control
- Dilutional linearity of primer-probe set of interest normalized to 18S endogenous control (delta CT)

A plot of the FAM, VIC and delta CT values versus the log of template dilution provides a linearity of response measure. The slope translates to an amplification efficiency measure for the FAM labeled

Gene Sequence Summary						
Gene	Amplicon	% Identity	Mismatches ("N's")	Query Start	Query End	Reference
TNFSF5	Forward	100	0	7	68	NM_000074.1
	Reverse	100	0	4	68	

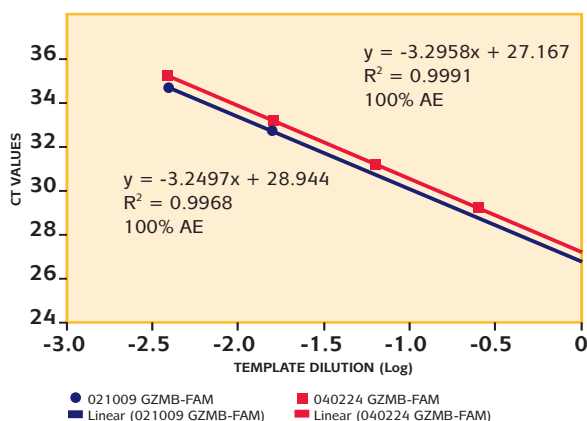
primer-probe of interest and the VIC labeled 18S endogenous control. Substantially similar efficiencies across target gene primer-probes and the 18S endogenous control primer-probe are required. An example of TNFSF5 primer-probe dilutional linearity is shown on the previous page.

4. The Sequence Verification Test ensures that the amplification of the primer-probe of interest matches the intended targeted sequence. The sequence verification of TNFSF5 shown above, results in 100% identity to the reference sequence used for the original primer-probe design.

B. Production Scale Primer-Probe Quality Control

Production scale reagent lot uniformity is tested using the same methods for amplification and specificity as described above. New lots are quarantined and compared with previous lots of reagents. New lots must demonstrate specificity and amplification efficiency comparable to previous lots. As an example, linearity of response across a series of 5-fold template dilutions is demonstrated below for a previous lot and new lot of production scale GZMB.

GZMB Reagent Lot Uniformity - FAM CT (previous vs. new)



C. Precision Profile™ Plate Production and Quality Control

Precision Profile plate production runs are prepared in batches of up to 20 plates in a 384-well format. Each plate production run is prepared identically using pre-qualified reagents and the Biomek® FX liquid handling automation. The primer-probe reagents targeting each gene of interest reside in triplicate wells with 18S specific primer-probe reagent multiplexing in order to monitor reagent delivery and PCR inhibition. Each well of a Precision Profile plate contains the following:

- Source MDx designed, production scale, 20X primer-probe stock
- TaqMan Universal PCR Master Mix, (ABI #4304437)
- Human 18S rRNA, 20X primer-probe stock, (ABI #4310893E)

Plate batch quality control testing involves PCR analysis of the selected test plates by the ABI Prism 7900®HT Sequence Detector, using the appropriate QC cDNA template(s). Representative plates from each batch are quality control tested for reagent performance and delivery. Comparability to previously established baseline data ensures production consistency across replicates and plates. Production batches meeting established criteria are released from quarantine for project specific use and only template addition is required.



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