Quantitation of Gene Expression and Protein Expression on a Single Analytical Platform

Dr. Amy Lam
Field Application Scientist
TaqMan® Protein Assays
Protein Detection from 10-500 cells or 1-1000 ng protein from tissues

- Combines protein detection using Antibodies with robust, sensitive real-time PCR
- Relative quantification of proteins in cell and tissue lysates
  - no purification of proteins required!
    - Just lyse and dilute....
- Applications:
  - Small sample protein analysis
  - Correlation of RNA & Protein
    - miRNA:protein
    - mRNA:protein
  - Validation of siRNA induced silencing
  - Validation of Gene Transfection/Transduction
  - Sample analysis from FFPE & Frozen Tumor tissues
  - Analysis of in vitro protein:protein interactions
TaqMan® Protein Assays Utilize Proximity Ligation Assay Technology

- Homogeneous assay based on two antibodies, each conjugated to a different oligonucleotide (one 3’ oligo, one 5’ oligo)
- When the two conjugated antibodies bind and are in close proximity, the oligonucleotides can be ligated, serving as the template for real-time PCR amplification and quantification

**TaqMan® Protein Assay Overview**

**Prepare Sample**
- Lyse cells and dilute into plate
- Only 2 uL sample required!

**Binding**
- Bind Assay Probes (2 uL) to protein in crude lysate

**Ligation**
- Ligate Oligos after binding (100 uL)

**TaqMan® Fast Real-Time PCR**
- Run Fast Real-Time PCR (20 uL)

**Data Analysis**
- Analyze Data for Relative Quant

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<th>Total Time</th>
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<tr>
<td>Hands on Time</td>
<td>~1.5 hrs</td>
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<tr>
<td>Time to Results</td>
<td>~3.5 hrs</td>
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</tbody>
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Compatible with StepOnePlus™, 7500, 7500 Fast, 7900 HT, & ViiA™ 7 Real-Time PCR Systems
Example Plate Setups for RQ experiments

- In general, dilution curves are required (e.g., 5 dilutions x 3 process replicates).

- **Example plate layouts:**

<table>
<thead>
<tr>
<th>Reference Cell Lysate</th>
<th>Test Cell Lysate 1</th>
<th>Test Cell Lysate 2</th>
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<tr>
<td><strong>NPCs</strong></td>
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<tr>
<td>Reference &amp; 2 Test samples</td>
<td>7 dilutions/quadruplicates</td>
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<tr>
<td>Reference &amp; 3 Test Samples</td>
<td>7 dilutions/triplicates</td>
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<td>Reference – 11 dilutions</td>
<td>4 Test Samples</td>
<td>4 dilutions/triplicates</td>
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</table>

- Data normalized based on cell count, total protein (or gDNA / RNAaseP assay).

- Relative quant between reference lysate and test lysates
  - No endogeneous control assays.
TaqMan® Protein Assay Data Characteristics

NPC = No Protein Control
(i.e. buffer only, no cell lysate)
Cystatin B Assay in Raji Cell Lysate

CT plots converted to ΔCT plots

Avg. Ct

ΔCt

Linear dynamic range

Cell Input per reaction

NPC

"hook"

ΔCt vs cell input

ΔCT vs cell input
Pre-Designed Assays Address Stem Cell Pluripotency & Differentiation

Pluripotent cells: Self renewal → Loss of Pluripotency: Differentiation

Embryonic Stem Cells

- Neural cells (neurons)
- Mesenchymal cells (connective tissue)
- Hematopoietic cells (blood)

Key Markers of Pluripotency

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<tr>
<td>NANOG</td>
<td>OCT3/4</td>
</tr>
<tr>
<td>SOX2</td>
<td>LIN28</td>
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</table>

CSTB and ICAM1 – positive control protein expression markers
New! TaqMan® Protein Assays Open Kit
Enables you to make an Assay to your target of choice!

Open Kit Components
- Oligo Probe Kit
- Buffer Kit
  - Lysate Dilution
  - Antibody Dilution
  - Assay Probe Dilution
  - Assay Probe Storage

Enough to make 40 x 96-well plates of Assay Probes
- plus extra for QC of biotinylated-antibodies
Assay Workflow with Open Kit – 1 added step

**Build the Assay**

1. **Prox-Oligo A**
2. **Prox-Oligo B**
3. **Assay Probe A**
4. **Assay Probe B**

Customer-supplied biotinylated antibodies

**Prepare Sample**

1. Lyse cells and dilute into plate (2 μL sample)

**Binding**

1. **Target**

**Ligation & Inactivation**

1. **Target**

**TaqMan® Fast Real-Time PCR**

1. **Cycle number**

**Data Analysis**

1. **Fold Change**
Assay probes are made from biotinylated-antibodies & streptavidin-linked oligonucleotides

- Single tube of biotinylated polyclonal antibodies
- polyclonal + monoclonal
- matched monoclonal (ELISA) pair

Combine for Binding in Assay

Split in half

Streptavidin-oligo
3' SA

Assay probe A

Streptavidin-oligo
5'

Assay probe B

Connector oligo
Taking your research to the next level utilizing protein assays and the strengths of real-time PCR

- Validate siRNA knockdown at the protein level
- Correlate mRNA and protein expression
- Correlate non-coding RNA and protein expression
- Quickly make protein assays with a single polyclonal antibody or matched antibody pair to targets of interest
- Measure *in vitro* protein-protein interactions
Follow on to siRNA knockdown
Expression changes after transfection with a Lin28 siRNA

IHC

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<th>Protein</th>
<th>Parental</th>
<th>siRNA LIN28</th>
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TCAM2 Seminoma cell line

TaqMan® Protein Assays provide a higher sensitivity, more quantitative readout
Application: mRNA / protein Comparative Expression

Starting Sample

Crude cell lysate

Protein Expression Sample Prep kit

TaqMan® Protein Expression Assay

Real-time PCR System

Crude cell lysate

PARIS™ RNA kit

TURBO DNA-free™ DNAse treatment

TaqMan Gene Signature plate/ Gene Expression assay

Similar amount of starting sample

Similar workflow and time to results

Same readout platform

mRNA vs Protein
TaqMan® Array Gene Signature Plates

- Preconfigured with most appropriate gene assays that represent a specific biological process, pathway, biomarker set or disease state
- Contain high quality TaqMan Gene Expression assays in a new convenient, dried-down, 96-well format.
- There are 90 TaqMan Array Gene Signature Plates
  - Some were based on existing TaqMan array Gene Signature MFC
  - Some are based on customer collaborations
TaqMan® Array Gene Signature Plates

Plate Guide

Which TaqMan® Array Gene Signature 96-Well Plate is right for you? Simply select a species and disease or pathway below.

### Find Plates:

**By Species**
- All Species
- Human
- Mouse
- Rat

**By Disease/Pathway**
- All Diseases/Pathways
- Apoptosis
- Biomarkers Related Pathway
- Cancer
- Cell Cycle Proliferation and Regulation
- Development and Stem Cells
- ECM Matrix and Adhesion
- Endogenous Controls
- Immune System and Inflammation
- Neurology
- Reproduction
- Signal Transduction
- Toxicology and Drug Metabolism

### Stem Cell Pluripotency Plate, Human

**Diseases/Pathways:** Biomarkers Related Pathway, Cancer, Cell Cycle Proliferation and Regulation, Development and Stem Cells, Neurology, Reproduction

**Plate Description**
The TaqMan® Array Human Stem Cell Pluripotency 96-well Plate contains 92 assays to stem cell associated genes and 4 assays to candidate endogenous control genes.

The panel of assays in this plate was selected in cooperation with the International Stem Cell Initiative (2007 Nature Biotechnology, 25:803-816). The assays target genes which are validated as markers for the characterization of human embryonic stem cell identity, and which assess variations between embryonic stem cell isolates. Genes are included based on the following criteria:

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Pathway Study (I): TaqMan® Array Gene Signature 96-Well Plates


Find Fast and standard plates by species and disease or pathway. View a list of genes and assay IDs for each plate.

Find Plates:
By Species
- All Species
- Human
- Mouse
- Rat

By Disease/Pathway
- All Diseases/Pathways
- Apoptosis
- Biomarkers Related Pathway
- Cancer
- Cell Cycle Proliferation and Regulation

14-3-3 Induced Intracellular Signaling Plate, Human
ABC Transporters Plate, Human
ABC Transporters Plate, Mouse
Activation of cAMP-Dependent PKA Plate, Human
AHR Pathway Plate, Human
Alzheimer’s Disease Plate, Human
Alzheimer’s Disease Plate, Mouse
Androgens Plate, Human
Angiogenesis Plate, Human
Antigen Processing and Presentation By MHCs Plate, Human

Angiogenesis Plate, Human

Diseases/Pathways: Apoptosis, Cancer, Cell Cycle Proliferation and Regulation, Development and Stem Cells, ECM Matrix and Adhesion, Immune System and Inflammation, Signal Transduction

Plate Description: The TaqMan® Array Human Angiogenesis 96-well Plate contains 92 assays to angiogenesis and lymphangiogenesis associated genes and 4 assays to candidate endogenous control genes.

The panel of assays included in this plate targets known angiogenesis growth factors such as VEGF as well as matrix derived inhibitors such as endostatin. Additionally, the panel contains markers and targets for angiogenesis and lymphangiogenesis.

Overview of Angiogenesis:
Higher eukaryotes have two vascular systems: blood and lymphatic. The blood vasculature transports oxygen, carbon dioxide, nutrients, metabolic products, cells of the immune system, hormones and other factors. The lymphatic vessels collect and transport proteins and lymph with a fluid through lymph nodes and lymphatics. Angiogenesis and lymphangiogenesis are the developmental processes.

<table>
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17
Correlation of p53 mRNA & Protein Expression Changes
Same samples, same real-time system

• p53 protein much higher in irradiated NTERA2 cells, mRNA unaffected
• ↑p53 protein leads to apoptosis at 24 hrs post-irradiation

*PPIA endogenous reference for mRNA
Correlate non-coding RNA, mRNA and protein expression on the same samples, same system

<table>
<thead>
<tr>
<th>TaqMan® MicroArray Card A</th>
<th>377 miRNAs plus 3 endogenous references (U6, RNU44 and RNU48)</th>
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</thead>
<tbody>
<tr>
<td>TaqMan® Protein Assays</td>
<td>10 protein targets</td>
</tr>
<tr>
<td>TaqMan® Gene Expression Assays</td>
<td>10 mRNA targets plus ACTB</td>
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<td>10 protein-mRNA targets</td>
<td>ALCAM   NANOG   CSTB</td>
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<td>p53       ICAM-1   SOX2   LIN28</td>
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Cell fate and apoptosis control: miR-125b and p53

Down-regulation of p53 ensures reduced proliferation and an anti-apoptotic effect in neurogenesis

Ensure cell lineage programming in sync with cell cycle cues

Cell survival

Cell fate and apoptosis control: miR-125b and p53

miR-125b shows significant translational repression of p53 mRNA

### mir125b/p53 Dynamics

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<th>Day 4</th>
<th>Day 10</th>
<th>Day 14</th>
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<td>p53 protein</td>
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Quickly make assays to Targets of Interest with a polyclonal antibody or matched antibody pair

Example protein assay sensitivity in buffer with proximity probes made utilizing commercially available antibodies on the TaqMan Protein Assays Open Kit

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<th>Protein</th>
<th>VENDOR</th>
<th>Ab type</th>
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<th>Total/well (2uL)</th>
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<tr>
<td>SCF</td>
<td>RnD</td>
<td>polyclonal</td>
<td>27</td>
<td>54 fg</td>
<td>0.3 ng/well**</td>
</tr>
<tr>
<td>CD117</td>
<td>RnD</td>
<td>polyclonal</td>
<td>14</td>
<td>28 fg</td>
<td>N/A</td>
</tr>
<tr>
<td>p53</td>
<td>RnD</td>
<td>polyclonal</td>
<td>130</td>
<td>260 fg</td>
<td>N/A</td>
</tr>
<tr>
<td>Pro-CASP3</td>
<td>RnD</td>
<td>polyclonal</td>
<td>400</td>
<td>800 fg</td>
<td>N/A</td>
</tr>
<tr>
<td>CASP8</td>
<td>RnD</td>
<td>polyclonal</td>
<td>1400</td>
<td>2.8 pg</td>
<td>N/A</td>
</tr>
<tr>
<td>CDH1/E-cadherin</td>
<td>RnD</td>
<td>polyclonal</td>
<td>4</td>
<td>8.0 fg</td>
<td>1.0 ng/well**</td>
</tr>
</tbody>
</table>

*Typical limit of detection (LOD) reported by vendor: calculated as 3 SDs above background (NPC) on recombinant protein standard curves

**Typical limit of detection (LOD) reported by vendor: calculated as 3 SDs above background (NPC) utilizing direct ELISA method
Example Protein:Protein Interaction Application
PhosphoTyrosine interaction – EGF stimulation

Data & figures courtesy of Kazuya Machida, University of Connecticut, US
ProteinAssist™ Software:
Free Software for multi-plate (study) analysis - downloadable from www.appliedbiosystems.com/taqman4antibodies

Plate setup screen (96 or 384 well plates)
Import *.eds files or exported text files

Supports 7900, 7500, StepOnePlus™ and ViiA™ 7 Real-Time PCR Systems
ProteinAssist™ Software:
Fold Change Results – with grouping
TaqMan Protein® Assays - Summary

● Pre-designed assays
  – Stem cell pluripotency- hOCT3/4, hSOX2, hNANOG, hLIN28
  – Common proteins – hICAM-1, hCystatin B

● NEW! Open Kit system to build your own assays

● Enables many applications for cell biology research are including:
  – Small sample protein analysis
  – siRNA knockdown validation
  – Validation of gene transfection/transduction
  – mRNA/miRNA : protein correlation
  – Tumor characterization
  – Fixed tissue analysis
  – Protein-protein interactions

www.appliedbiosystems.com/proteinassays
www.appliedbiosystems.com/taqman4antibodies
A New Era in High-Productivity qPCR
Applied Biosystems® ViiA™ 7 Real-Time PCR System

- Responsive touch-screen interface
- Rapid block change
- Intuitive software with ReadiApp™ Application and QuickStart functions
- Integrated TaqMan® Array block
- OptiFlex™ System
Instrument
Touch Screen

- Name the instrument and create an Avatar
- Create shortcuts to common protocols

*Ideal for Core Labs and multi-user environments*
Block Change

- Block change typically in less than 1 min
  - Heated Cover (96 vs. 384)
  - Plate Adaptor (96 vs. 384 vs. TAC)

- 384-well and TAC Blocks

<table>
<thead>
<tr>
<th></th>
<th>Validated</th>
<th>Programmable</th>
</tr>
</thead>
<tbody>
<tr>
<td>384</td>
<td>5-20uL</td>
<td>1-30uL</td>
</tr>
<tr>
<td>TAC</td>
<td>1uL</td>
<td>1uL</td>
</tr>
</tbody>
</table>

- 96-well and Fast 96-well coming soon!

<table>
<thead>
<tr>
<th></th>
<th>Validated</th>
<th>Programmable</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>10-100uL</td>
<td>1-200uL</td>
</tr>
<tr>
<td>Fast 96</td>
<td>5-30uL</td>
<td>1-100uL</td>
</tr>
</tbody>
</table>
The Optics: OptiFlex™ System

- Decoupled Excitation and Emission Filters
- Select up to 21 color combinations

<table>
<thead>
<tr>
<th>Channel</th>
<th>Dye Examples</th>
<th>Excitation Filter</th>
<th>Emission Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FAM™, SYBR®, SYTO®9 (MeltDoctor™), Fluorescein, SYPRO® Orange</td>
<td>470 ± 15nm</td>
<td>520 ± 15nm</td>
</tr>
<tr>
<td>2</td>
<td>VIC®, JOE™, TET™, HEX™</td>
<td>520 ± 10nm</td>
<td>558 ± 12nm</td>
</tr>
<tr>
<td>3</td>
<td>TAMRA™, NED™, BODIPY® TMR-X</td>
<td>550 ± 10nm</td>
<td>586 ± 10nm</td>
</tr>
<tr>
<td>4</td>
<td>ROX™, Texas Red®</td>
<td>580 ± 10nm</td>
<td>623 ± 14nm</td>
</tr>
<tr>
<td>5</td>
<td>LIZ™</td>
<td>640 ± 10nm</td>
<td>682 ± 14nm</td>
</tr>
<tr>
<td>6</td>
<td>Alexa Fluor®, Joda-4</td>
<td>662 ± 10nm</td>
<td>711 ± 12nm</td>
</tr>
<tr>
<td>Performance Specs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Well-to-well variability</strong></td>
<td>+/- 0.25°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Max Block Ramp Rate</strong></td>
<td>3.0°C/sec (384-well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Run time</strong></td>
<td>~35 min for 384-well, using Fast Master Mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Demonstrated Sensitivity</strong></td>
<td>Down to 1 copy</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dynamic Range</strong></td>
<td>Up to 9 logs</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Resolution</strong></td>
<td>Down to 1.5 fold change for single plex reaction</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Performance
ViiA™ 7 System: 1.5 Fold Sensitivity

RNaseP copies:
- 10,000
- 6,667
- 4,500
- 3,000
- 1,500
- 1,000

TaqMan Gene Expression MMx, 5ul Rxn, 384-well format, Standard Run conditions
Dynamic Range, Fast Run (384-well)

Amplification of plasmid DNA (7-7E10 total copies/rxn)

9 logs of linear dynamic range in 35 minutes.

TaqMan Fast Universal MMx, 5ul Rxn, 384-well format
Expanded Multiplex Capabilities

Multiplex on cDNA with 5 probes + passive reference. Proprietary dyes and master mix in development.
Detection Limit: Down to 1 Copy

Digital PCR run showing single molecule detection: Raji gDNA diluted to 1.05 copies per well. 71% (260/368) wells positively amplified.

**POISSON DISTRIBUTION:**
1.05 copy per well will result in ~70% positive reactions. Single molecule detection is possible following Poisson distribution.

384-well plate format (368 replicates +16 NTC). Fast conditions, 5ul Rxn, Fast Universal MMx).
C<sub>t</sub> Uniformity of 384-well

Amplification of the RNase P Gene showing 144 replicates of 5,000 and 10,000 copy populations on a 384-well block using fast run conditions.
Ct Uniformity on TAC

Ct Standard Deviation of 0.092
Amplification of the TGF-Beta Gene on a TaqMan Array Card with a Full-Plate of replicates.

Gene Expression Master Mix, standard run conditions
Thermal Uniformity

- Minimal variability across plate
Multiple Instruments, Multiple Blocks

Highly Reproducible Results

<table>
<thead>
<tr>
<th></th>
<th>Block</th>
<th>TGF-Beta FAM</th>
<th>18s VIC</th>
<th>Ave Ct</th>
<th>TGF-Beta FAM</th>
<th>18s VIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>384w</td>
<td>0.052</td>
<td>0.027</td>
<td>27.17</td>
<td>12.38</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>384w</td>
<td>0.057</td>
<td>0.037</td>
<td>27.13</td>
<td>12.38</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>384w</td>
<td>0.054</td>
<td>0.028</td>
<td>27.16</td>
<td>12.37</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>384w</td>
<td>0.061</td>
<td>0.032</td>
<td>27.25</td>
<td>12.24</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>384w</td>
<td>0.053</td>
<td>0.028</td>
<td>27.24</td>
<td>12.24</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>384w</td>
<td>0.053</td>
<td>0.028</td>
<td>27.24</td>
<td>12.23</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>TAC</td>
<td>0.088</td>
<td>0.137</td>
<td>27.11</td>
<td>12.39</td>
<td></td>
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<tr>
<td>A</td>
<td>TAC</td>
<td>0.084</td>
<td>0.130</td>
<td>27.15</td>
<td>12.42</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>TAC</td>
<td>0.080</td>
<td>0.140</td>
<td>27.14</td>
<td>12.41</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>TAC</td>
<td>0.087</td>
<td>0.084</td>
<td>27.24</td>
<td>12.30</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>TAC</td>
<td>0.095</td>
<td>0.090</td>
<td>27.22</td>
<td>12.27</td>
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<tr>
<td>B</td>
<td>TAC</td>
<td>0.091</td>
<td>0.098</td>
<td>27.21</td>
<td>12.28</td>
<td></td>
</tr>
</tbody>
</table>
Software
Start a Run in Less than 3 Clicks!

Select an instrument and an experiment template file to run a new experiment. Load the reaction plate into the instrument, then click **Start Run**.

**Select Instrument**

- FOSSANTAREFL01 READY
- FOSSANTAREFL01 READY
- FOSSANTAREFL02 READY

**Enter Experiment Name and Location**

- **Experiment Name:** 2010-03-28 164413
- **Barcode (Optional):**
- **User Name (Optional):**
- **Location:** /ViA7 Software v1.0\experiments\2010-03-28 164413 eds

**Select Experiment Template**

- **Experiment Template:** D:\Applied Biosystems\ViA7 Software v1.0\config\templates\ViA7_TaqMan_Array_Std_Curve_Template.adl

**Samples**

- **Sample 1**

You may import a plate setup file or a sample definition text file. Alternatively, you may directly edit the sample names in the table to the left, or copy and paste sample names from a spreadsheet.
Quickly Set Up Experiments

- Copy/Paste Sample Information within ViiA™ 7 Software to and from Microsoft Excel®
Expanded Import Capability

- Compatible with 7900 Set Up Files
- Directly import sample name files from Excel
- Ability to import extra sample information

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample Name</th>
<th>ID</th>
<th>Age</th>
<th>Sex</th>
<th>Weight</th>
<th>HairColor</th>
<th>Smoker</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample 1</td>
<td>1</td>
<td>22</td>
<td>Female</td>
<td>25</td>
<td>black</td>
<td>Yes</td>
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<tr>
<td>2</td>
<td>Sample 2</td>
<td>2</td>
<td>25</td>
<td>Male</td>
<td>26</td>
<td>brown</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Sample 3</td>
<td>3</td>
<td>45</td>
<td>Female</td>
<td>50</td>
<td>blonde</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Sample 4</td>
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<td>31</td>
<td>Male</td>
<td>33</td>
<td>red</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Sample 5</td>
<td>5</td>
<td>29</td>
<td>Female</td>
<td>46</td>
<td>grey</td>
<td>No</td>
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</tbody>
</table>
# File Compatibility Summary

<table>
<thead>
<tr>
<th>ViiA™ 7 Software Importing Capability</th>
<th>Can Import From:</th>
<th>Can Not Import From:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment Set up Files (.txt)</td>
<td>7900 SDS v 2.3</td>
<td>7000, 7300</td>
</tr>
<tr>
<td></td>
<td>7500 v2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7500 Fast v2.1</td>
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<tr>
<td></td>
<td>StepOnePlus™ v2.1</td>
<td></td>
</tr>
<tr>
<td>Template and Experiment Files (.sds or .eds)</td>
<td>7900 SDS v 2.3.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7500 v2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>StepOnePlus™ v2.1</td>
<td></td>
</tr>
<tr>
<td>Sample Data Files (.txt or .csv)</td>
<td>Excel or Text</td>
<td></td>
</tr>
<tr>
<td>Run Data Files (Ct values)</td>
<td>All Platforms</td>
<td></td>
</tr>
</tbody>
</table>
Recover Data from Instrument

- Store up to 100 runs on the instrument
- Start run using USB
- Save completed run file onto USB
- Completed run file saved onto network
- Auto-delete downloaded experiments if there is not enough room on instrument
Maximize your Plates: Run TaqMan® and SYBR® Assays on One Plate

- De-coupled filter selection lets you run different chemistries
- Explore custom dyes and enhance your research

ViiA™ 7 Software Filter Selection Features

<table>
<thead>
<tr>
<th>Run Method</th>
<th>TAM</th>
<th>VIC</th>
<th>DyelO</th>
<th>ROX</th>
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</thead>
<tbody>
<tr>
<td>Emission Per</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAM</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DyelO</td>
<td></td>
<td>✔</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROX</td>
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</table>

<table>
<thead>
<tr>
<th>Emission Filter</th>
<th>HAM</th>
<th>VIC</th>
<th>DyelO</th>
<th>ROX</th>
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</thead>
<tbody>
<tr>
<td>Load</td>
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</tr>
<tr>
<td>Save</td>
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<td></td>
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</tr>
<tr>
<td>Reset to Default</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TaqMan Assay w/ SYBR Assay Run

Amplification Plot

Derivative Melt Curve
Different Ways to Obtain $C_T$

- Flexibility to analyze data from any temperature hold step

**Data Step Selection**

Select the step and stage to use for $C_T$ analysis. Only stage/step combinations for whic...
Endogenous Control Selection

- Helps you determine the best housekeeping gene
- Assess overall $C_T$ distribution of your genes across samples
Quickly Assess $C_T$ Distribution

- Use Box-Whisker plots to assess $C_T$ distribution of your samples for each target
- Determine which sample may be an outlier
- Mouse over each box plot to view descriptive statistics
Quality Control Plots

- Assess $C_T$ correlation ($r$) between samples using Pearson’s Correlation
- Quickly determine non-ideal samples in your analysis
Import Standard Curves

- Import a standard curve from another experiment
  - Save wells on the plate for more reactions
Amplification of the TGF-beta Gene (FAM dye- Red) and 18S Gene (VIC dye - Green) showing 384 replicates.

5ul rxn, Gene Expression Master Mix, Standard conditions.
ViiA™ 7 High Resolution Melting Module

- Embedded within the ViiA™ 7 Software!
- 10 User Licenses per purchase
- New HRM Features:
  - Specify number of data points per degree
  - Analysis settings: user can define number of variant calls
Genotyping
Genotyping Optimization Tool

- Reveal traces to determine ideal cycle for clustering
- Use the scroll bar to determine the best cycle for optimal clustering
- Benefit: optimize protocols
ViiA™ 7 Security Features

- Authenticating user’s login info
- User login time-out for inactivity
- Event log for unauthorized login attempts
- Pre-defined functions based on user’s setting
- Detects changes to data
ViiA™ 7 Data Auditing Features

- Record date/time users create, delete, or update data.
  - Allow users to review the audit trails.
- Track creation and changes to experiments
- Track system configuration changes
Legal Acknowledgements

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