Simultaneous detection of two SNPs from crude extracts using TaqMan® Multiplex PCR

Jordan Lang
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Life Technologies Real-time PCR Portfolio

Formats

Strategies

Sample Prep Reagents

Instruments

Flexibility & Value

Life Technologies™ Proprietary and Confidential

QuantStudio™ Families

<table>
<thead>
<tr>
<th></th>
<th>QuantStudio™ 6 Flex</th>
<th>QuantStudio™ 7 Flex</th>
<th>QuantStudio™ 12K Flex</th>
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</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>3 interchangeable blocks — 96, 56, 384</td>
<td>4 interchangeable blocks — 96, 56, 192, TaqMan® Array Card</td>
<td>5 interchangeable blocks — 96, 56, 288, TaqMan® Array Card + OpenArray® plates</td>
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<tr>
<td>Detection channels</td>
<td>5-color</td>
<td>6-color, 21 filter</td>
<td>6-color, 21 filter</td>
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<tr>
<td>Excitation Source</td>
<td>Halogen Lamp</td>
<td>Halogen Lamp</td>
<td>White LED</td>
</tr>
<tr>
<td>Automation</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

TaqMan® Reagents for SNP Genotyping Workflow

Direct Lysis

SNP Genotyping

Learn more at www.lifetechnologies.com/taqman

TaqMan® Reagents Empower Your Plant Research

What type of experiment are you conducting?
- Gene Expression
- SNP Genotyping
- Copy Number
- miRNA
- NGS

Life Technologies™ Proprietary and Confidential
Direct Lysis:
DNA Extract All Reagents Kit

- Lowers cost per sample
- Compatible with various sample types
- Reduces bottleneck of sample prep
  - Simple 5 minute protocol
  - No purification
  - Lysate added directly to PCR reaction

DNA Extract All Reagents Kit Workflow

- Simple 3 step protocol
- Sample preparation completed in 5 minutes or less
- Scalable volume according to needs with a 1:1 ratio of Lysis and Stabilizing Solution

Plant Sample
95°C for 3 min
75 uL of Lysis Solution
75 uL of Stabilizing Solution

TaqMan® 5’ Nuclease Chemistry Basics

- Still the gold standard for SNP allele discrimination
- Robust assay design pipeline successfully used for millions of assay designs

Custom TaqMan® SNP Assay Design

- FAM® and VIC® labeled assays designed and ordered using the online Custom TaqMan® Assay Design Tool (CADT)
  - Any SNP in any organism
  - Enter target sequences into tool and submit for assay design
  - Complete service including
    - Secure and confidential ordering
    - Assay design and manufacturing
    - Quality-control testing for synthesis accuracy and formulation completeness
  - Available at www.lifetechnologies.com/snpcadt

Multiplex qPCR for Plant Genetic Analysis

Upcoming Products
- New ABI® and JUN® reporter dyes
- New probes using DQY7 quencher

Application
- Gene expression
- Zygosity & copy number
- Multiple SNP detection

Benefit
- Increase throughput
- Decrease reagent usage
- Decrease labor time

Information
- Product launch spring 2014
- Early access available—please inquire with your local sales rep

New Reporter Dyes:
ABI® and JUN®

Traditional NED and PET Labels:
- Optimized Spectral Separation to match Detection Windows

New ABI®/JUN® Labels:
- Optimized Spectral Separation to match Detection Windows
Multiplex PCR Dye Selection

New ABY®/JUN® Labels: Optimized Spectral Separation to match Detection Windows

<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, VIC, ABY, JUN</td>
<td>530 ± 10 nm</td>
<td>570 ± 10 nm</td>
</tr>
<tr>
<td>2</td>
<td>FAM, VIC, ABY, JUN</td>
<td>580 ± 10 nm</td>
<td>620 ± 10 nm</td>
</tr>
<tr>
<td>3</td>
<td>FAM, VIC, ABY, JUN</td>
<td>600 ± 10 nm</td>
<td>650 ± 10 nm</td>
</tr>
<tr>
<td>4</td>
<td>FAM, VIC, ABY, JUN</td>
<td>550 ± 10 nm</td>
<td>590 ± 10 nm</td>
</tr>
<tr>
<td>5</td>
<td>FAM, VIC, ABY, JUN</td>
<td>550 ± 10 nm</td>
<td>590 ± 10 nm</td>
</tr>
<tr>
<td>6</td>
<td>FAM, VIC, ABY, JUN</td>
<td>560 ± 10 nm</td>
<td>600 ± 10 nm</td>
</tr>
</tbody>
</table>

New Probes with QSY7 Quenchers

- QSY7 quenchers are compatible with all four reporter dyes
- QSY7 probes labeled with FAM, VIC, ABY® and JUN® will be available for online ordering in April 2014

Early access available - please inquire with your local sales rep

TaqMan® Probe Quenchers

1) TAMRA quencher (1994-1998)
2) MGB-NFQ quencher (1999-)
3) NFQ quencher (2006-)
4) QSY7 quencher (2009-)

- QSY7 probe quenchers are better than NFQ at reducing background reporter dye signal

NFQ Quencher vs. QSY7 Quencher - Background Signal and ΔRn - Rn vs Cycle

Crude Lysate Prep for Dual-SNP Genotyping Experiment

- Direct Lysis with DNA Extract All Reagents Kit
  - 3 mm seed chips from 23 maize and 22 soy samples
  - Crude lysates added directly to PCR reaction
  - Normalization not required
- Quantification with Qubit® 2.0 Fluorometer

Dual-SNP Genotyping Experiment with 4 Reporter Dyes

- TaqMan® Assays
  - 6 markers for Maize (4 VIC®/FAM™ & 2 ABY®/JUN®)
  - 2 markers for Soy (1 VIC®/FAM™ & 1 ABY®/JUN®)
- Dual-SNP Genotyping
  - R&D Multiplex Master Mix (contains MP)
    - MP = Mustang Purple Passive Reference Dye
  - Single reaction containing 2 SNP Assays (VIC®/FAM™, ABY®/JUN®)
  - 10uL PCR reactions in 384-well plate format
  - ViiA™ 7 with Genotyping Protocol
**Dual-SNP Genotyping PCR-Protocol (~70 min)**

1. 95.0°C
2. 1.9°C/s → 60.0°C
3. 1.9°C/s
4. 1.6°C/s
5. 95.0°C
6. 60.0°C
7. 00:30
8. 00:30
9. 00:30
10. 05:00

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**Real-Time Genotyping Analysis**

- ViiA™ 7 and QuantStudio™ systems have Reveal Traces slide bar

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**Screening of Probe Designs**

- **ABY®/JUN®-QSY7** probes have different quenchers than VIC®/FAM™-MGB probes
- Non-MGB designs for ABY®/JUN® probes have a few more bases than FAM/VIC®-MGB counterparts
- Verify performance of ABY®/JUN® assay in single-SNP reaction before moving to duplex SNP

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**Single-SNP/reaction**

**Maize: Marker #3 (csu1171.2)**

- VIC®/FAM™
- ABY®/JUN®

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**Maize: Marker #13 (PZA03240.2)**

- VIC®/FAM™
- ABY®/JUN®

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**Soy: Marker #2 (rs3727453)**

- VIC®/FAM™
- ABY®/JUN®
Dual-SNP Genotyping Experiment
SNP Assay Pairs

<table>
<thead>
<tr>
<th>ABY®/JUN® Markers</th>
<th>Maize 3</th>
<th>Maize 10</th>
<th>Maize 12</th>
<th>Maize 14</th>
<th>Soy 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>A/G</td>
<td>A/T</td>
<td>C/T</td>
<td>C/G</td>
<td>C/T</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VIC®/FAM® Markers</th>
<th>Maize 1</th>
<th>Maize 10</th>
<th>Maize 12</th>
<th>Maize 14</th>
<th>Soy 10</th>
</tr>
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</tr>
</tbody>
</table>

Dual-SNP: Assay Pair 1
Maize SNP 1 (ae1.7) Maize SNP 3 (csu1171.2)

Dual-SNP: Assay Pair 2
Maize SNP 10 (PZA03713.1) Maize SNP 3 (csu1171.2)

Dual-SNP: Assay Pair 3
Maize SNP 12 (PZA03639.1) Maize SNP 3 (csu1171.2)

Dual-SNP: Assay Pair 4
Maize SNP 14 (PZA02750.3) Maize SNP 3 (csu1171.2)

Dual-SNP: Assay Pair 5
Maize SNP 1 (ae1.7) Maize SNP 13 (PZA03249.2)

Maize SNP 3 Single-SNP
Dual-SNP: Assay Pair 6
Maize SNP 10 (PZA03713.1) Maize SNP 13 (PZA03240.2)

Dual-SNP: Assay Pair 7
Maize SNP 12 (PZA03639.1) Maize SNP 13 (PZA03240.2)

Dual-SNP: Assay Pair 8
Maize SNP 14 (PZA02750.3) Maize SNP 13 (PZA03240.2)

Dual-SNP: Assay Pair 9
Soy SNP 10 (rs3727378) Soy SNP 2 (rs3727453)

Dual-SNP Genotyping Summary
- DNA Extract All Kit compatible with TaqMan® dual-SNP multiplex
- QSY7 probes performance similar to MGB probes
- Real-time traces allow for troubleshooting of ambiguous data and PCR cycle optimization for assays

TaqMan® Multiplex Reagents for Your Plant Research
- Product launch in spring 2014
  - Probes with new reporters/quencher
  - Calibration plates
  - Controls
- Assays previously designed with MGB can be designed to QSY7 through our custom assay design service
- Multiplex Products
  - Increases throughput
  - Lowers reagents and labor time

For Research Use Only. Not for use in diagnostic procedures.
Thank you!

Questions?