Primer Express® Software v3.0

Simple Primer and Probe Design for Real-Time PCR Applications

- Enables individual oligo design for real-time PCR applications
- Supports assays based on TaqMan® and SYBR® Green I dye chemistries
- Provides design flexibility, ease-of-use, and requires minimal optimization
- Provides robust assay performance when used in accordance with Rapid Assay Development Guidelines (RADG)

Figure 1. Graphic results of one primer and probe set are displayed and annotated, using various colors, on the Sequence tab. Placing the mouse over each oligo displays its start/stop positions, Tm and %GC.

Introduction

Applied Biosystems new Primer Express® Software v3.0 allows you to design your own primers and probes using TaqMan® and SYBR® Green I dye chemistries for gene quantitation and allelic discrimination (SNP) real-time PCR applications. Developed specifically for use with the Applied Biosystems 7500 Fast and 7900HT Fast Real-Time PCR Systems, and the 7300 and 7500 Real-Time PCR Systems, Primer Express® Software provides customized application-specific documents for absolute/relative quantitation and allelic discrimination. This latest software version includes an improved workflow for allelic discrimination assay designs and supports the Windows XP® operating system*.

Easy-to-Learn Software

Primer Express® Software v3.0 is flexible, easy to learn and to use, and requires minimal optimization. It includes an algorithm optimized specifically for use with TaqMan®-based reagents, recommended primer/probe concentrations, and default thermal cycling conditions. Primer Express® Software also supports assays based on the SYBR Green I dye. This minimizes the need for assay optimization and streamlines the design process.

The software also allows customized designs if the probe and/or one or more primers are known. Default and adjustable parameters (Figure 2) provide the flexibility required for all users, regard-
less of experience level. When used in accordance with Applied Biosystems Rapid Assay Development Guidelines (RADG), which are used with our real-time PCR instruments and reagents, Primer Express® Software provides robust assay performance.

**Simple to Use**

Primer Express® Software v3.0 is the ideal solution for researchers with varying levels of experience and expertise. Simplicity and flexibility are the hallmarks of Primer Express® Software v3.0, which is fully compatible with all 7300, 7500, 7500 Fast, and 7900HT Fast Real-Time PCR Systems. Whether your research includes gene quantitation or allelic discrimination studies, Primer Express® Software belongs in your lab.

### Automated and Manual Primer/Probe Design

**Flexible Primer Express® Software v3.0** will design your primers and probes automatically, or you can design them manually.

For automated design, simply choose either TaqMan® or TaqMan® MGB assays. If you know the sequence, you need only to import (or copy and paste) the sequence and quickly view your results.

Alternatively, if you prefer to design the primers and probes manually, the procedure is equally simple and straightforward.

1. Choose either TaqMan® or TaqMan® MGB assays.
2. Import or enter the sequence.
3. Annotate the probe of your choice in the target sequence (or, alternatively, annotate the forward and/or reverse primer(s) in the target sequence).
4. Let the software automatically locate the correct primer pairs.

### Additional Features and Benefits

- **Batch process tool**: Automates primer and probe design from multiple sequence files for quantitation assays.
- **Primer/probe test tool**: Interactively investigates primer and probe sequences to determine Tm, percent of GC, length, and secondary structure.
- **Online ordering of primers and probes**: Provides access to the Applied Biosystems Web site from within the application for online ordering (Figure 4).

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**Figure 2.** Primer Express® Software contains default parameters for primer and probe design, in accordance with Applied Biosystems rapid assay development guidelines (RADG).

**Figure 3.** Tabular results for candidate primer/probe sets along with any secondary structure information are displayed in the Primer/Probe tab. Oligo properties include oligo sequences, start/stop positions, Tm, %GC, length and any secondary structure information. Amplicon properties include Tm, GC and length.
• **Exporting and printing:** Exports sequences, parameters, candidate primers/probes and ordering information to tab-delimited text. You can also print the information that you need. Columns in the candidate primer/probe list can be organized per your preference, and the custom format can be printed.

• In addition, Primer Express® Software also provides robust assays that use universal thermal cycling conditions and easy-to-use annotation tools.

**Primer Express® Software and SYBR® Green-Based Assays**

If you wish to design primers and probes using the SYBR® Green I chemistry assays, it is important to use the documents and parameters that are appropriate for gene quantitation assays. Simply use the primer pair sequences for your assay. You may choose to save the probe sequence for future use in a TaqMan®-based assay.

**Rapid Assay Development Guidelines**

Primer Express® Software v3.0 was designed with Applied Biosystems Rapid Assay Development Guidelines (RADG). The RADG are a simple yet comprehensive set of guidelines for use with our real-time PCR instruments and reagents. Many traditional variables, including magnesium chloride concentration and the thermal cycling protocol itself, have been standardized, which substantially reduces assay development time.

**Conclusion**

Researchers whose study focus is gene quantitation and allelic discrimination now have an easy, yet accurate method of primer/probe design. Assay optimization is minimal, and the process is streamlined and efficient. Primer Express® Software v3.0, in conjunction with Applied Biosystems suite of real-time PCR instruments, allows you to customize documents for absolute/relative quantitation and allelic discrimination applications.

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**Figure 4.** Oligo sequences can be ordered directly from the Applied Biosystems Store.
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Applied Biosystems has developed a comprehensive set of guidelines to ensure success when using sequence detection systems (SDS) reagents and instrumentation. These guidelines are simple and easy to follow. Many traditional variables, such as magnesium chloride concentration and the thermal cycling protocol itself, have been standardized, greatly reducing assay development time. All SDS reagent kits contain a passive internal reference to normalize non-PCR related fluorescence fluctuations. Normalizing with a passive internal reference minimizes well-to-well variability that can result from a variety of causes, such as pipetting errors and sample evaporation. Real-time quantitative PCR was first performed using the 5’ fluorogenic nuclease assay, which uses a TaqMan® probe during the PCR amplification to provide added specificity. An additional chemistry is also available, using SYBR® Green I dye, that can provide real-time quantitative PCR information. Assays using the SYBR® Green I double-stranded DNA binding dye do not require a TaqMan® probe and provide additional experimental flexibility.

Purchase of this product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process for research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler.

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ORDERING INFORMATION

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*Note: While Primer Express® Software v3.0 is designed for the Windows® XP operating system, it is not supported on Windows® NT, Windows® 2000, or Macintosh® operating systems.

Get more information at http://www.appliedbiosystems.com