Quick Guide to Using
Beacon Designer Software

To Design Primers, Molecular Beacons, and TaqMan Probes
for Real-Time PCR Assays on the iCycler iQ™ Detection System

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Creating a Project

File your sequences and results in a folder by opening a **Project**. This enables multiple users to clearly organize projects and sequences in the program.

- Under **File**, select **New>Project** to name your Project. Click **Create** to save
- To open a previously saved project, select **Open>Project**

Opening a Sequence

You have the option to paste a sequence directly into the program or to open a sequence from a local file, Entrez, or dbSNP database.

- To paste in your sequence, select **File>New Sequence**
    1. Name the sequence in **Sequence Definition** box
    2. Paste your sequence into the box and click **Add**
- To open a sequence from Entrez or from dbSNP:
    1. Select **File>Open Sequence>From Entrez** or **>From dbSNP**
    2. Type in accession number(s) or assay ID(s)
- To open a sequence from an existing file:
    1. Copy your sequence into another program to create a text file in FASTA format
    2. On first line before the first base, type “>” symbol followed by a name, and save as a text file; example: >beta actin AAACCCTTTGGG. . .
    3. Select **File>Open Sequence>From File**, and select file created above
- Select **View>Sequence Details** to view your sequence, which is renumbered starting with 1. This sequence is opened in your Web browser and may be saved as a text file
- Create an SNP sequence by selecting **Tools>SNP** to add or delete a SNP for your sequence. Details can be viewed under **SNP Information** tab

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Designing Primers for Molecular Beacons

- Under **Options**, choose **Beacon Design**
- Select sequence, then **Analyze>Primer Search**
- Click **Search**; results are displayed under the **Primer Properties** tab. Click **Alternate Primers** to view other primer results
- Primer search parameters may be changed; example: if a primer set is not found, change the default value of **Target Tm** range to **+/-10°C** (instead of 5°C)
- You may also import primers by selecting **Analyze>Add Primers**
- Select **Tools>Reaction Conditions** to change the default reaction conditions for which primers are designed

Designing Molecular Beacons

- After designing primers for sequence, select **Analyze>Beacon Search**
- Click **Search**. Results are displayed under the **Beacon Properties** tab
- **Beacon Search Parameters** may be changed; example: if a molecular beacon is not found, change the **TaOpt** range to **+/-10°C**
- You may also import a molecular beacon. Select **Analyze>Add Beacon**, which gives you the option to design optimal primers for that molecular beacon
- Click **Alternate Beacons** to view other results
- Select **Tools>Export>Beacon Results** to export results to a spreadsheet or right-click directly on the sequences to copy and paste into another program

Designing TaqMan Probes and Primers

- Under **Options**, choose **TaqMan® Design**
- Select **Analyze>TaqMan® Search**
- Click **Search**; TaqMan results are displayed under the **TaqMan® Properties** tab and primer results are displayed under **Primer Properties** tab
- Click **Alternate Primers** and **Alternate TaqMans®** to view other results
- TaqMan search parameters may be changed; example: if a TaqMan probe is not found, change parameters, such as the **Target Tm** range to **5–15°C**. Another option is to choose between **Design Sense TaqMan®** or **Design Anti-sense TaqMan®**
- Select **Tools>Export>TaqMan® Results** to export results to a spreadsheet or right-click directly on the sequences to copy and paste into another program

BLAST Searches

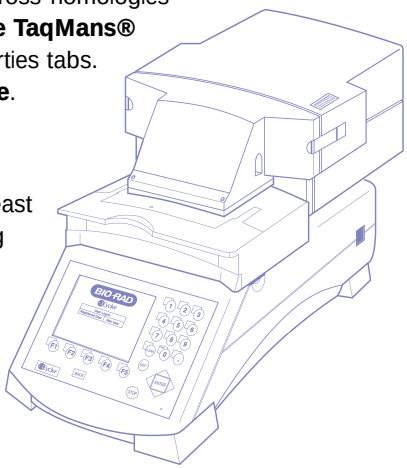
The cross homologies of the PCR products can be searched with the integrated BLAST search.

- Select **Analyze>BLAST Search** to launch **Search Parameters** window
- Select **Human Genome BLAST** or **Standard BLAST** and database type
- Results are displayed under **BLAST Properties** tab. Click on **BLAST Details** to go directly to NCBI BLAST results to further analyze

Designing for Multiplex Reactions

The software supports design for multiplex reactions by automatically checking for cross-dimers on probes and primers designed in a single search. You may also use the multiplex options to check for cross-dimers among probes and primers designed in separate searches and to verify the multiplex reaction.

- Design for the multiplex reaction by selecting sequences for the multiplex, and design probes and primers as described above
- To use the multiplex options, highlight the sequences for the multiplex reaction and select **Analyze>Multiplex Beacon** or **Analyze>Multiplex TaqMans®** to view cross-dimers among the probes and primers. Use **Analyze>Multiplex Primers** for primers only
- **Multiplexing Results** displays the cross-dimers formed from most stable to least stable
- Use this information to select TaqMan probe and primers with the least cross homology for the multiplex reaction
- You can choose another primer set, TaqMan probe, or molecular beacon probe for the multiplex to compare cross-homologies by selecting **Alternate Primers** or **Alternate TaqMans®** or **Alternate Beacons**, located in the properties tabs. Select the alternate choice and click **Replace**. Then repeat the multiplex function to check for cross-dimers
- The optimal multiplex reaction will have the least amount and least stable cross-dimers among the probes and primers



Designing for Allelic Discrimination — Recommended Parameters

Allelic discrimination assays require shorter probes to maximize specificity to the target sequence to effectively discriminate. Parameters below are suggested as a starting point for optimizing the assay.

- **TaqMan® Search** — start with **Primer Target Tm**: **54°C** and **TaqMan® Target Tm**: **Primer Tm + 5°C**. This will enable a design of a shorter TaqMan probe with a T_m of 55–58°C and primer set T_m around 53°C
- **Beacon Search** — start with **Primer Target Tm**: **51°C**, and change **Target Beacon Tm** to **TaOpt + 11**. Aim for an assay with a primer set of T_m = 53–54°C and a beacon of T_m = 58°C

Rating System

The primers and probes designed are displayed under **Primer Properties, Beacon Properties**, and **TaqMan® Properties.** The designed primers and probes receive a numerical rating, which are also described at the bottom right corner as **Poor 0-50, Good 50-75,** and **Best 75-100**. The **Search Status** tab next to **Sequence Information** also displays the Poor, Good, and Best rating for the probes and primers designed. A **Best** rating does not guarantee a successful probe and primer set, but indicates that this set has characteristics that typically generate acceptable results.