# illumina®

## Designing Custom Probes for NūPCR<sup>™</sup> Assays

Guidelines for efficiently creating and ordering custom NuPCR Assays using the DesignStudio<sup>™</sup> web-based design tool.

### Introduction

NuPCR assays provide researchers with a specific, sensitive, and cost-effective real-time PCR method for analyzing gene expression results. Probes can be designed with FAM, HEX, ROX, or Q670 dyes and can be used on any PCR instrument.

The DesignStudio web-based tool (www.illumina.com/designstudio) provides researchers with an easy and convenient method for creating probes and primers optimized for target-specific detection. The design process begins with the creation of a project and uploading of sequence information. The DesignStudio software evaluates the sequence and provides suggested bioinformatically optimized probes and primers that are specific to that target sequences and have predicted success.

This technical note describes the process of designing and ordering NuPCR assays (Figure 1).

### Accessing DesignStudio

Figure 2: Starting Your Project

DesignStudio is available through Mylllumina. To access, go to https://my.illumina.com and supply your login information. If you do not have a Mylllumina account, you will be asked to register for one.

Once logged in to Mylllumina, click on the Custom Products link on the left and then choose NuPCR DesignStudio.



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## Designing an Assay

#### Start a Project

- Choose the type of qPCR project you would like to create. There are currently three options available:
  - NuPCR Gene Expression Assays
  - qPCR Binding Dye Assays
  - qPCR High Resolution Melting Assays
- 2. Input the following information (Figure 2):
  - Project Name
  - Project Description (optional)
  - Species (Human, Mouse, Rat)
  - Genome Source (NCBI or UCSC)
  - Genome Build
- 3. Click <NEXT>.

#### **Input Target Sequences**

You should now be on the Manage Targets screen and ready to enter target sequence information (Figure 3).

- 1. Input your target sequence by:
  - Transcript ID-RefSeq IDs
  - Gene-Gene symbols
  - Sequence-Direct sequence input (A, C, G, T)

Figure 3: Input Target Sequences

- File—Upload a .csv file (templates provided for Gene, Transcript, or Sequence)
- 2. Choose a dye:
  - FAM
  - HEX
  - ROX
  - Quasar 670 (Q670)

- 3. Choose a Specificity check.
  - ON: only assays targeting a unique location in the genome will be designed
  - OFF: assays will not be checked for "uniqueness", useful if uploading a sequence that is not human, rat, or mouse
- 4. Add target labels that can be used in searching and sorting targets (optional).
- 5. Select targets by clicking on the ADD GENE button on the middle right side of the page. These targets are added to the Filter at the bottom half of the page.

DesignStudio will begin designing optimal probe and primer sequences for the chosen targets.

#### **Duplex Design**

Once multiple targets are added to the filter, users can choose two for use in designing duplex assays (Figure 4).

- 1. Click the box to the left of the target to select it for multiplexing.
- 2. Click MULTIPLEX (2 TARGETS).
- 3. Assign a Multiplex name.
- 4. Assign each target a different dye.
- 5. Click CREATE MULTIPLEX.

The DesignStudio tool will generate assays for each target that can be combined into a single qPCR experiment.

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Choose assays from the graphical display or table.

#### Assay Selection and Purchase

Once design is complete, clicking on the TARGET REGION or DESIGNED tabs will take you to the assay selection page. In the top panel, transcripts for the selected targets are displayed in graphical representation. The table in the lower panel displays the assays you can choose from.

Select assays by clicking on their graphical representation in the top panel, or the assay name in the lower panel (Figure 5).

Once assays are selected, you can choose the assays you wish to purchase.

- Click ADD TO DESIGN to choose the assay(s) you wish to purchase. A green check mark will appear next to the chosen assay(s).
- 2. Click the REVIEW DESIGN tab to review your order.
- 3. Click the REQUEST QUOTE button to complete your project.

Note that finalized projects cannot be edited.

#### Order an Assay

Once your assay design is finalized, you will be taken to the qPCR calculator to select the size and quantity of each assay (Figure 6). The calculator will determine the amount of master mix required for the total number of reactions in the project. These are guidelines and can be edited. When done, click REQUEST A QUOTE. This will send an email to your distributor with the project details. You will receive a confirmation email and the distributor will contact you with pricing and delivery information.

#### Learn More

NuPCR reagents provide the Nu gold standard in real-time PCR specificity, sensitivity, and simplicity. Amplify your story. Get the details at www.illumina.com/nupcr.

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