

Designing an Assay

Start a Project

1. 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)
 - 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.

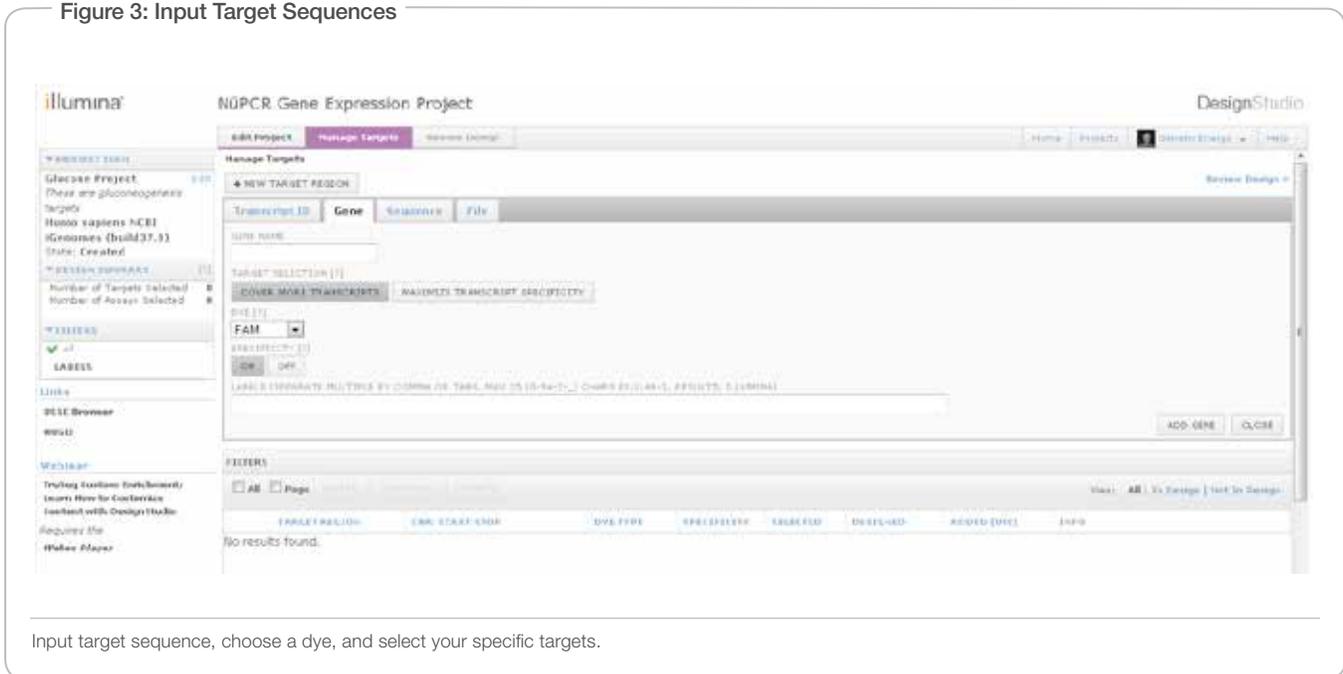
Duplex Design

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.

Figure 3: Input Target Sequences



Input target sequence, choose a dye, and select your specific targets.

