FLUIDIGM®

100-6812 Rev 06

D3 Assay Design

User Guide

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Contents

About This Guide

Purpose

This guide describes how to use the D3™ assay design website to design and order custom primers for NGS library prep, genotyping, and gene expression applications.

How to Use This Guide

The guide is organized according to the typical workflow for designing and ordering customer primers.

Help with Assay Design or General Technical Support

Fluidigm has several resources to support you in your assay designs. System and chemistry user guides, quick references, and safety data sheets are available at fluidigm.com.

NOTE D3 was tested successfully for compatibility with Google Chrome™, Mozilla Firefox®, Microsoft® Edge, and Safari®. D3 is not fully compatible with Internet Explorer, which does not fully support all the JavaScript functions in use by D3 on the Assay Design website.

Call or email us if you need help with assay design, want to check the status of your orders, or if you have questions about product specifications.

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Help with the Website

Email us at webmaster@fluidigm.com.

Chapter 1: Getting Started

D3 Assay Design

Define. Design. Deliver.

Fluidigm D3™ assay design is a web-based tool to streamline assay design for next-generation sequencing (NGS) library preparation (LP), qPCR, and genotyping studies. Its intuitive user interface enables researchers to design—with only a few clicks—high-coverage assay panels for gene expression, genotyping, or targeted sequencing experiments. Backed by expert, specialized technical support from the Fluidigm Assay Design Group, D3 ensures that researchers can:

- Minimize time and resources expended on primer design and assay formulation.
- Rapidly design a single assay or many high-throughput batches of assays.
- Customize assays, leveraging the ability of D3 to control and fine-tune design parameters.
- Track hundreds of assay design panels with multiple versions per panel, making D3 a convenient, secure, referable, and reusable repository for any laboratory's designs.

Fluidigm custom-designed assays support a variety of genomes. Supported genomes are listed as shown in Step 2 in Create a New Panel from Scratch on page 14. Assay types include:

- Targeted NGS Assays: These assays are designed to the region of interest with the necessary sequence tags for fast and easy incorporation of barcodes and NGS adapters. Targeted sequencing primers offer low amplicon dropout and high map-to-target rate. Access Array™ Target-Specific Primers support the LP 48.48 integrated fluidic circuit (IFC). and up to 480 amplicons.
- Delta Gene™ assays: These are MIQE-compliant, qPCR gene expression assays designed to high specificity and sensitivity.
- SNP Type™ assays: High-throughput, cost-effective allele-specific assays enable rapid single-nucleotide polymorphism (SNP) screening by PCR. They facilitate quick, cost-effective development and implementation of top-quality SNP genotyping panels.

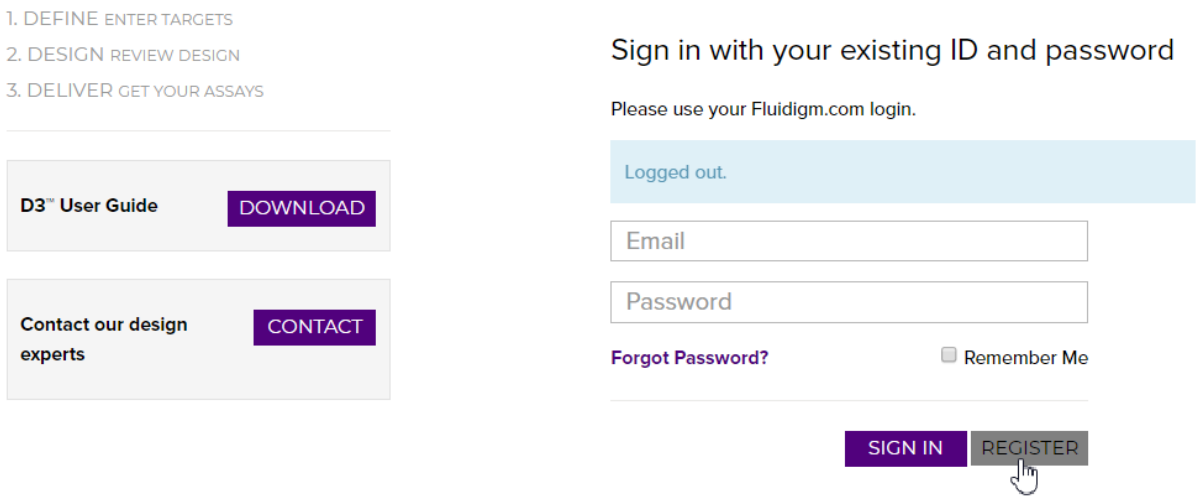
D3 Workflow

Tailored for simplicity, speed, and accuracy across a diverse set of primers, D3 is geared to span expertise levels, from broad targeting at the gene level to specific target coordinates. D3 users follow five fundamental steps:

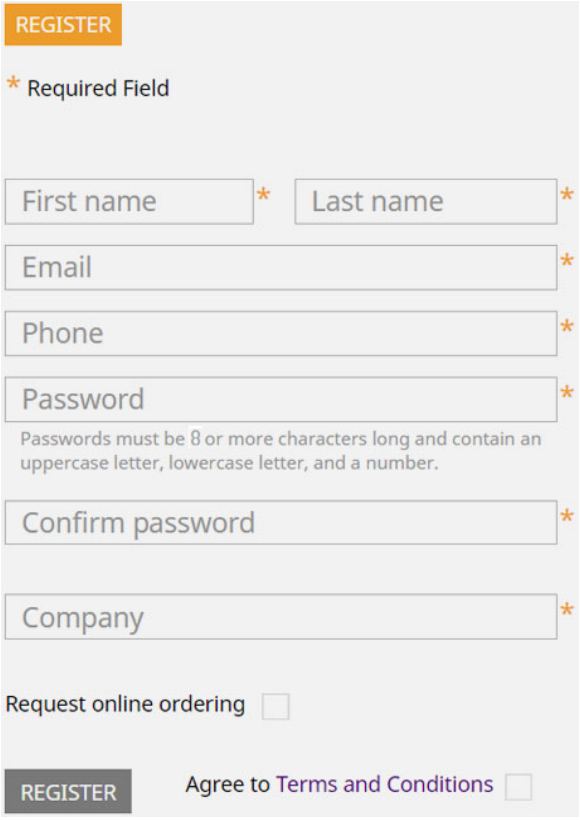
	Step	Go To
1	Create an assay panel of the desired assay type.	D3 Panel Creation Options on page 13
2	Select targets for design in D3.	Adding Targets for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays on page 22
3	Submit a design request for the assay panels.	Submitting a Target for Design on page 45
4	Receive a design review through D3 from the Fluidigm Assay Design Group. If necessary, go back to Step 2.	Preparing an Order on page 57
5	Submit a quote request.	Requesting a Quote on page 57

Registering as a D3 User

1. Open a browser window and navigate to d3.fluidigm.com.
2. In the Sign in panel, click **REGISTER**:



3 Enter your account information in the Register panel:



Your password must contain at least:

- 8 characters
- 1 uppercase character
- 1 lowercase character
- 1 number or special character (such as & or %)

There should be no spaces between characters. For example:

ILOVE D3! is not a valid password because it contains a space and has no lowercase character.

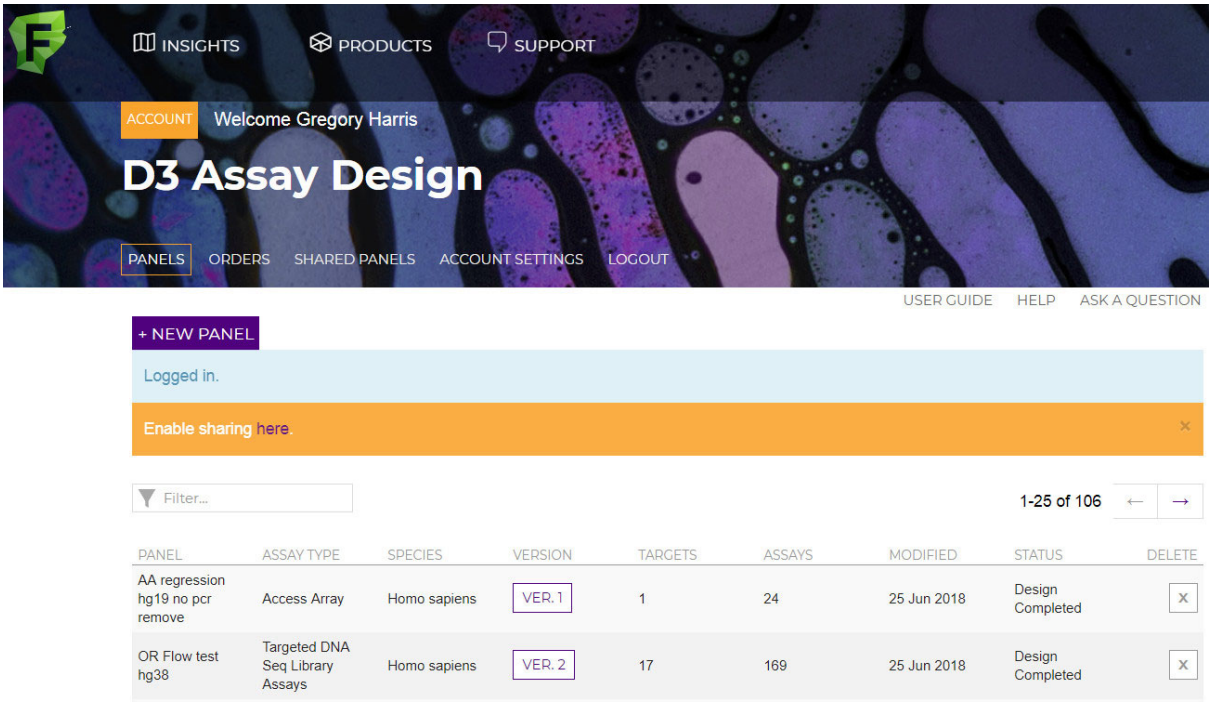
ILoveD3! is a valid password as it meets all criteria.

4 If you are in North America, check the box **Request ecommerce enablement** to be able to order online via a Salesforce account.

5 Read the user agreement, check the box **Agree to Terms and Conditions**, and click **REGISTER**. An activation email is sent to your email address.

In the unlikely event that you still have issues with password registration, email us with any error messages you receive, the browser you are using, and your contact information.

6 Click the link in your activation email and log in. When you see the Projects page, you can begin creating your study-specific assay design projects. For example:



Managing Your D3 Account

This section helps you to manage details regarding your D3 account.

- **You forgot your email or password**. Contact Technical Support for assistance and include your name and the name of your institution, including the date you registered. We will do our best to locate your account.
- **You are not receiving D3 confirmatory emails for your account**. Although we do everything we can to ensure that you receive our emails, there are many reasons why you may not receive them. Here are a few things to check.
  - Check whether D3 emails are going into your spam or junk folder. Mark them as “not spam” and redirect them to your inbox.
  - Confirm that you have received emails from @fluidigm.com in the past.
  - Validate with your IT department that D3 emails are not being blocked due to any blacklisting or spam filters at the company server level.
  - Validate that your D3 account is active and accessible.
  - If you still have issues, contact Technical Support to help resolve them.
- **You relocated**. Create a new account with a new profile and email address for your new lab or new employer.
- **You want to consolidate multiple accounts**. To maintain data integrity and confidentiality, D3 does not permit account consolidation. Maintain a single account and control experimental separation through the use of projects and versions.

- **You want to disable or delete unwanted accounts**. For each unwanted account, download or record any information you will still require. On D3, log a request to either disable or delete your unwanted accounts. Include your full name, institution, and email address. You will be notified by email when your request is completed. All projects, versions, assays, and quotes related to each deleted account are deleted.

Chapter 2: Designing on D3

D3 Assay Design Options

All assays are ready to use via Fluidigm standard protocols and are accompanied by a detailed informatics packet. Singleplex Access Array™ and Delta Gene™ assays are also offered with or without wet-lab testing.

Access Array Target-Specific Primers

When used with the Fluidigm Access Array system, Access Array Target-Specific Primers allow the fast, simple, and inexpensive preparation of a large number of amplicons. They are ideal for analysis across focused genomic regions to better understand human genetic variation. Primers are designed for quick and specific amplification of targets, incorporation of sample-specific barcodes, and addition of sequencer-specific adapters all at the same time. All Access Array Target-Specific Primers are:

- Flexible: up to 480 plex/sample
- Universal: barcodes and adapters for Illumina® sequencers
- Compatible with Thermo Fisher Scientific and Roche® sequencers
- Accurate: highly uniform, sensitive, and specific amplicon libraries

The supported sequencers for use with the Access Array 48.48 IFC. and the LP 48.48 IFC for Access Array are as follows:

Manufacturer	Supported Sequencer
Illumina	MiniSeq™, MiSeq™, NextSeq™, and HiSeq®
Thermo Fisher Scientific	Ion Torrent™ PGM
Roche	454

Access Array Target-Specific Primers are provided as follows:

- Singleplex primers are provided in nuclease-free water at a final volume of 100 µL per well in a single 96-well plate with mixed forward and reverse primers at a final concentration of 50 µM.
- Multiplex primer sets are provided in nuclease-free water at a final volume of 100 µL per well in six 96-well plates per set at a final concentration of 60 µM.

Targeted DNA Seq Library Assays

Targeted DNA Seq Library Assays for use with Juno™ and Access Array system are custom targeted sequencing solutions that offer maximum efficiency in singleplex and pooled formats. These assays are offered in two formats:

- ASY-MPX assays are provided in singleplex mixes (individual forward and reverse primer mixes), which allow more efficient troubleshooting options for panel development. These assays are normalized to a concentration of 450/150 µM in 15 µL. Assays are delivered in 96-well plates based on the D3™ pooling algorithm.
- ASY-MPX-P assays are pre-pooled assays that are provided in pooled and singleplex formats:

  Pooled: One 96-well plate contains pre-pooled mixes (50 µL) to minimize hands-on times. These assays are normalized for LP 48.48 and LP 192.24 workflows to a concentration of 2.5/0.83 µM. Contact assay_design_group@fluidigm.com to discuss alternative pooling formats to accommodate the LP 8.8.6 workflow.

  Singleplex: The singleplex retains are similar to ASY-MPX.

Standard assays are designed for the human genome for targets with an associated RefSeq ID, gene name, target sequence, or genomic coordinates. Non-standard requests for other species can be submitted on D3 or to the Assay Design Group at assay_design_group@fluidigm.com.

Delta Gene qPCR Gene Expression Assays

Delta Gene Assays enable you to take full advantage of the Biomark™ HD System. Experiment setup and validation times are minimized. Biologically related gene lists are available to assist with identifying gene targets of interest.

Delta Gene assays offer:

- Rapid turnaround time for real-time qPCR gene expression while maintaining high data quality
- MIQE compliance
- A low-cost alternative to the use of labeled probes for gene expression analysis

Delta Gene qPCR Gene Expression assays are provided as a mixture of forward and reverse primers in equal molar ratios and normalized to 100 µM in nuclease-free water at a final volume of 100 µL per well (1 target per well) of a 96-well plate.

SNP Type Genotyping Assays

SNP Type™ assays are custom, high-throughput, and low-cost SNP genotyping solutions for rapid screening. Based on allele-specific PCR SNP detection, SNP Type assays combine the advantages of minimum setup time and flexible assay choice with the reliability of Dynamic Array™ integrated fluidic circuits (IFCs). They employ tagged, allele-specific PCR primers and a common reverse primer. A universal probe set is used in every reaction, producing uniform fluorescence while significantly reducing startup and running costs. All SNP Type assays:

- Are economical and have low startup and running costs
- Produce robust locus-specific primer sequences

- Are flexibly designed to your custom target list

SNP Type assays are provided in three separate oligo plates per 96 assays, as follows (and all oligos are in nuclease-free water):

- Allele-Specific Primers (ASP1 and ASP2): Each primer is mixed in equal molar ratios to a final concentration of 100 µM (per primer).
- Preamplification Primer: Each primer is normalized to 100 µM (per primer).
- Locus-Specific Primer (LSP): Each primer is normalized to 100 µM (per primer), as shown in Table 1.

Table 1. SNP Type product options

Product Code	Volume (µL)
ASY-GT-XS	50
ASY-GT-S	100
ASY-GT-M	200
ASY-GT-L	500

D3 Panel Creation Options

Panels define top-level areas of investigation. Depending on the experiment, panel definition may be by genome, disease function, or biochemical pathway. Panels can intuitively be grouped in alignment with specific laboratory projects or areas of investigation, thereby maintaining a separation between studies.

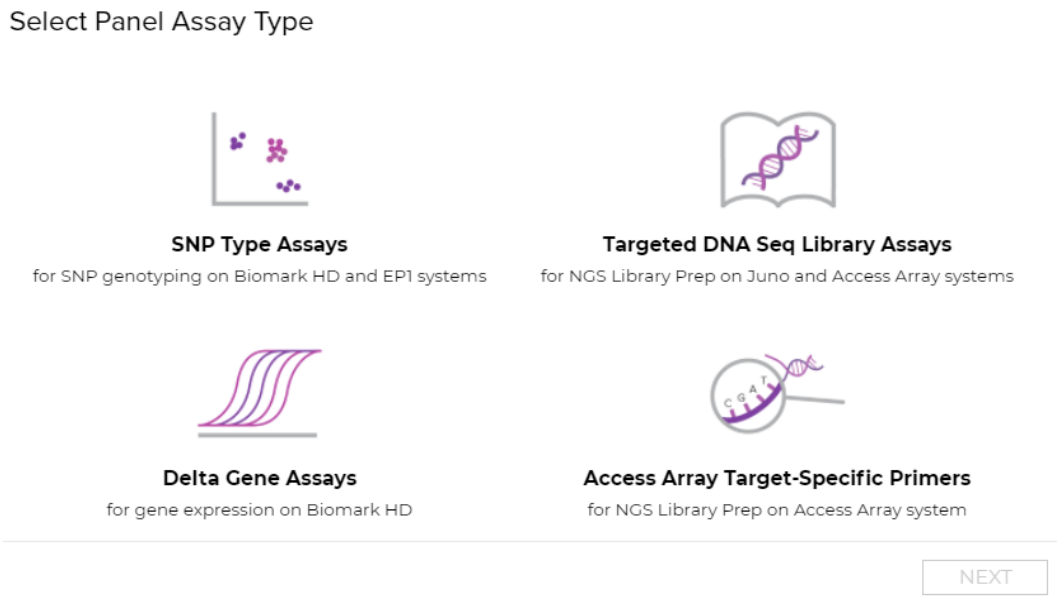
Versions help track the history of a panel. Since the assay design process is typically iterative, versions enable a researcher to preserve a snapshot of the design process, allowing tracking not only of design changes but also of order status and details.

When a new panel is created in D3, it is automatically designated as Version 1 of that panel. Its version description should be written to indicate how it is unique or different from other versions in the same panel.

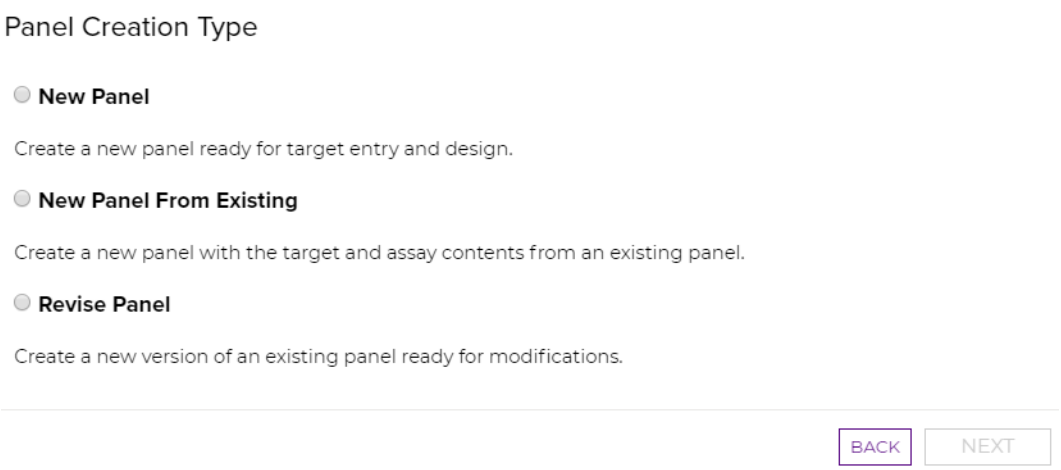
Create a new panel if you change species. Create a new version of an existing panel if you make small changes without changing the species or the majority of the targets.

Creating a New D3 Panel

1. Click **+NEW PANEL** on the Panels page to be guided through the creation of your new D3 panel.
2. Select the type of assay for your new panel on the Panel Assay Type screen and click **NEXT**. Panels, genomes, and SNP masking options are filtered to show only the options that are available for the assay type that you select.



After you click **NEXT**, you see the Panel Creation Type screen:



3 Finalize the creation of your new D3 panel through your interactions within a set of screens as described in the following instructions:

- Create a New Panel from Scratch on page 14
- Create a New Panel from a Shared Panel on page 17
- Create a New Panel by Revising an Existing Panel on page 18

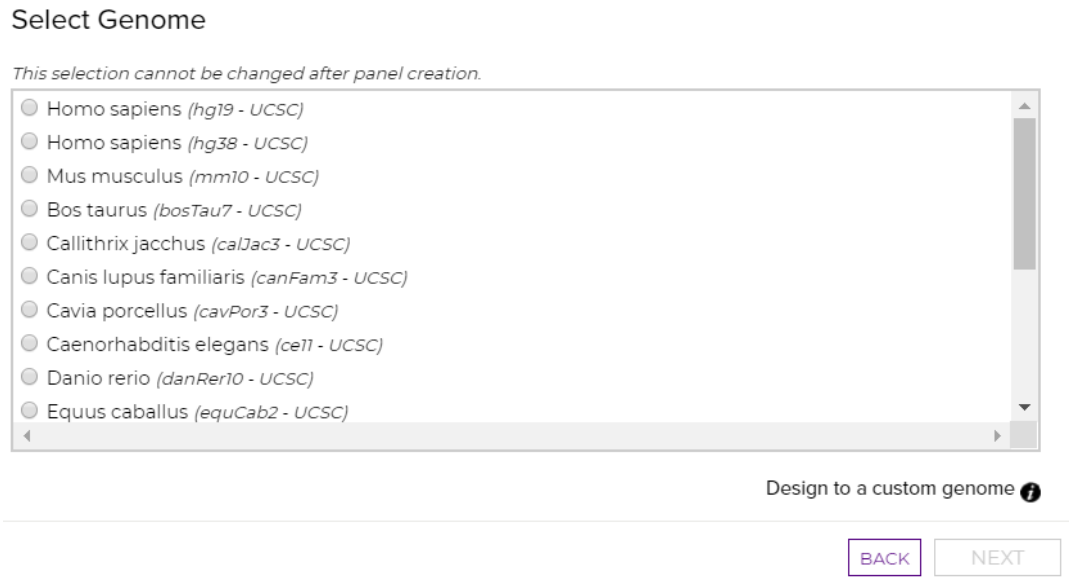
Create a New Panel from Scratch

1 Select **New Panel** on the Panel Creation Type screen and click **NEXT** to create a new empty panel designated as Version 1.

2 Select a genome from the list on the Select Genome screen and click **NEXT**. The list is filtered to show only those genomes that are available for design with the selected assay type. (Contact Technical Support to have a custom genome added to your account.)

IMPORTANT

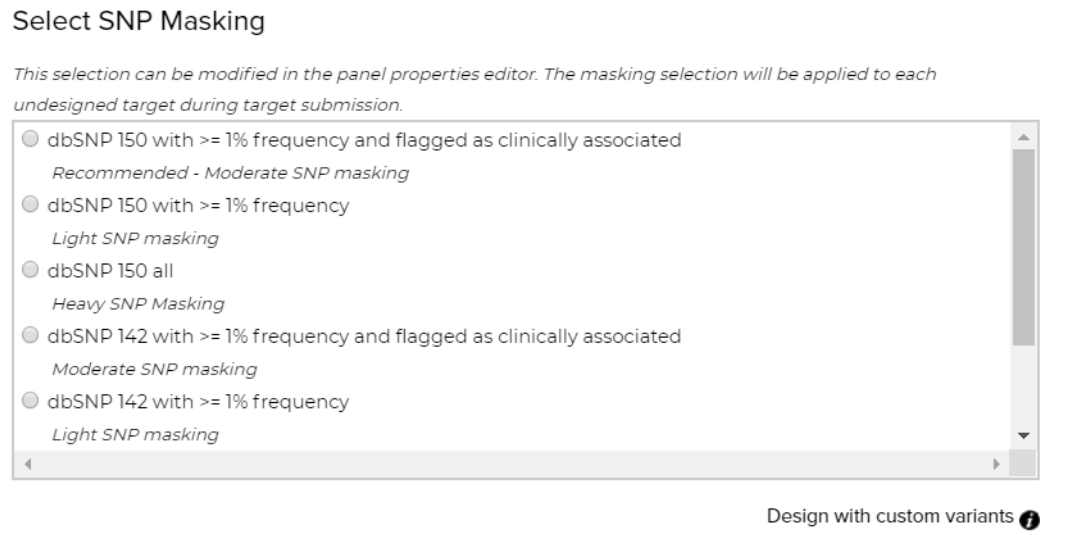
- A panel can target only one genome.
- You cannot change the genome after panel creation.



3 Select the type of SNP masking on the Select SNP Masking screen and click **NEXT**. The list contains SNP masking types for the species and assay type that you selected.

More SNP masking affects the coverage or designability of targets. Your selection applies to each undesigned target upon target submission, but you can modify your selection between target design submissions.

You can also change the panel name, description, and version comments of your selection on the Panel Properties screen in Step 5. (Contact Technical Support to have a custom variant masking available for design.)



4 Enter the sequencing platform and minimum/maximum amplicon size on the Assign Design Parameters screen and click **NEXT**. The parameters listed in Table 2 are dependent on the assay type that you choose:

- For Access Array Target-Specific Primers, D3 supports the Illumina, Roche 454™, and Ion Torrent sequencing platforms.
- For Targeted DNA Seq Library Assays, D3 supports only the Illumina sequencing platform.

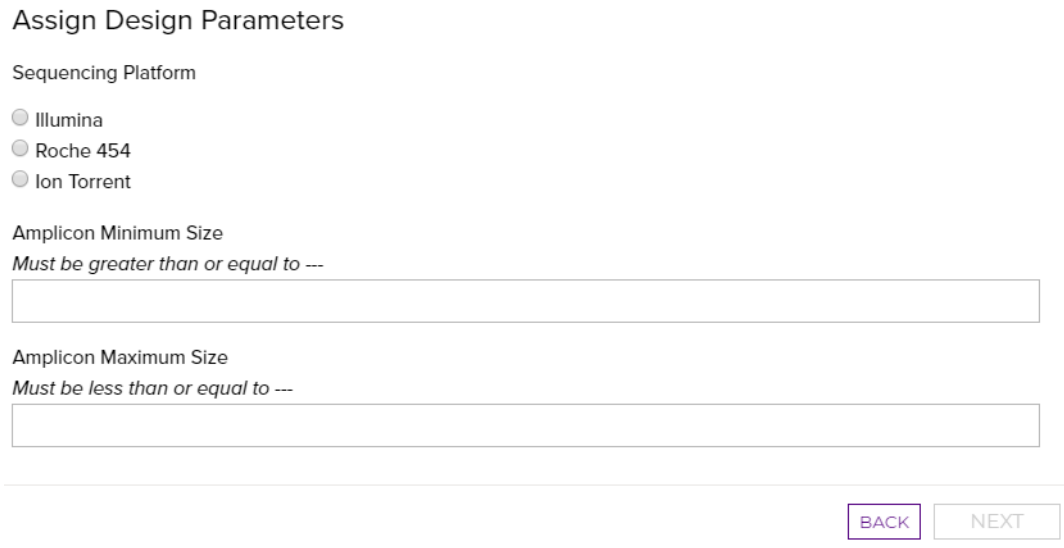


Table 2. Allowable amplicon sizes for each sequencing platform

Sequencing Platform	Amplicon Length (bp)
Illumina	150–500 (The default is minimum 150 and maximum 200.)
Roche	100–600 (The default is minimum 100 and maximum 200.)
Ion Torrent	100–400 (The default is minimum 100 and maximum 200.)

IMPORTANT

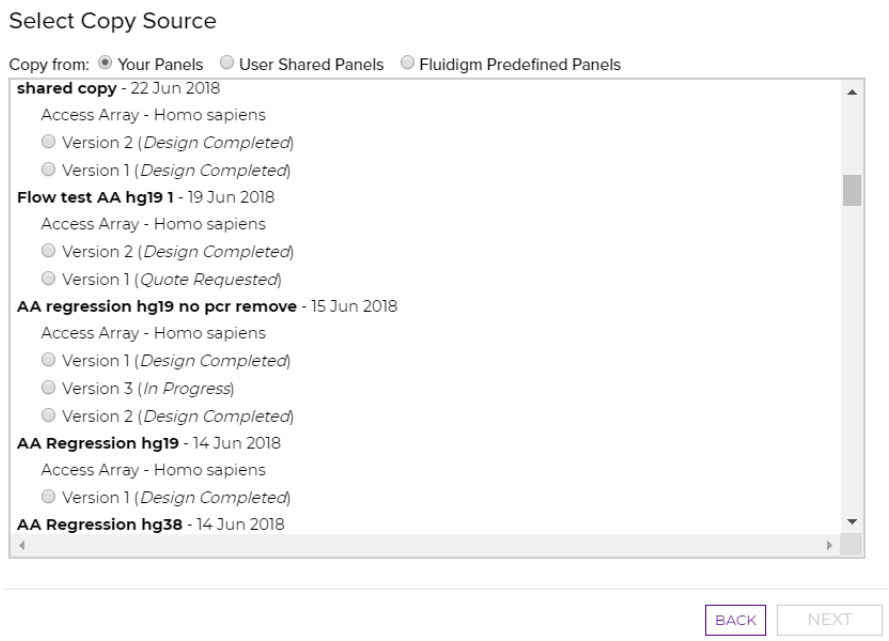
- Always specify minimum and maximum amplicon lengths for your project. For optimal designs, it is best to keep amplicon lengths within 25% of each other.
- You can change the amplicon length only when there is no design associated with any target in that version.

5 Enter a unique name for the new panel (in Panel Name) on the Panel Properties screen. You may also include a description (in Panel Description) and comments for this specific version of the panel (in Version Comments) as needed. Click **FINISH**.

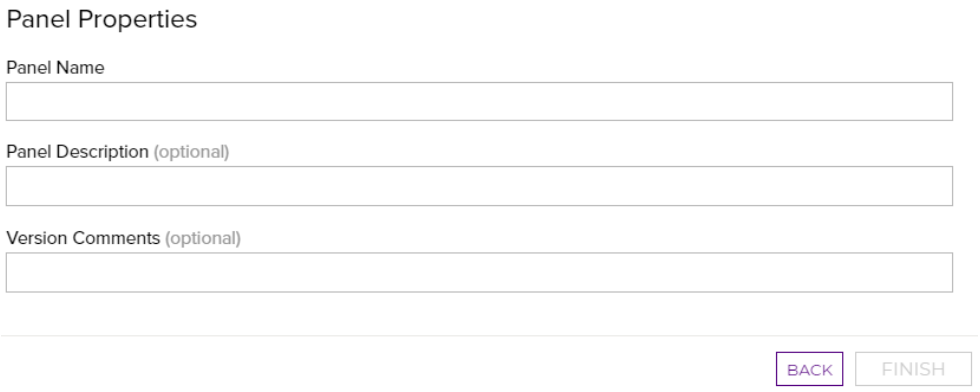
Create a New Panel from a Shared Panel

1 Select **New Panel From Existing** on the Panel Creation Type screen. Click **NEXT** to create a new panel by copying the contents of an existing panel from your account, from a shared panel with another user, or from a shared panel in a list of predefined Fluidigm panels.

2 Select a copy source and its location on the Select Copy Source screen and click **NEXT**.

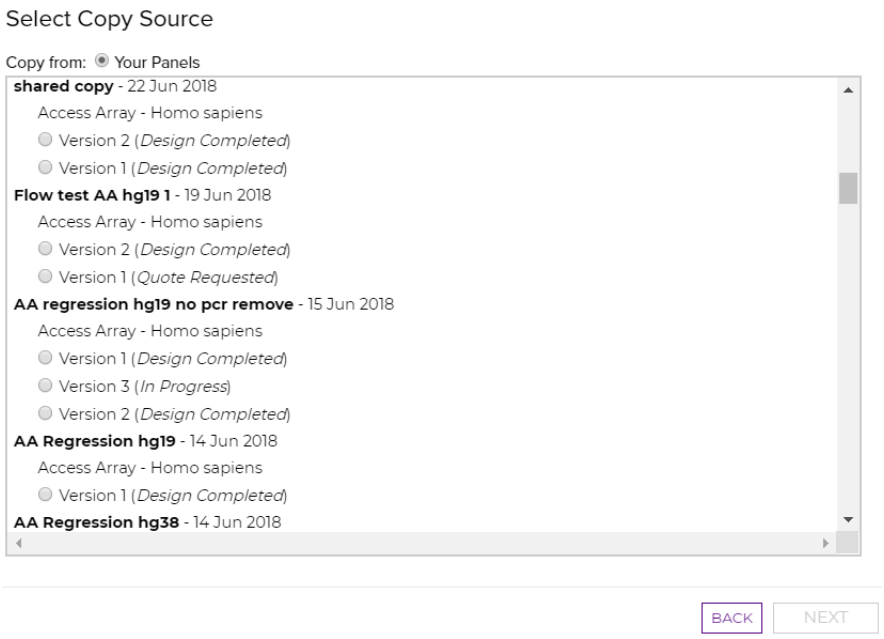


3 Enter a unique name for the new panel (in Panel Name) on the Panel Properties screen. (Optional) You may include a description (in Panel Description) and comments for this specific version of the panel (in Version Comments). Click **FINISH**.



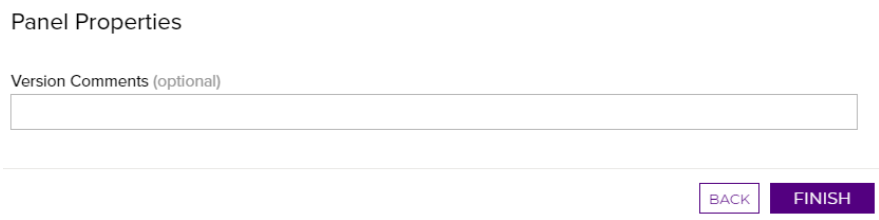
Create a New Panel by Revising an Existing Panel

1 Select **Revise Panel** on the Panel Creation Type screen and click **NEXT** to create a new version of an existing panel from your account.



2 Select a panel to copy from the Select Copy Source list and click **NEXT**.

3 (Optional) Enter comments for this specific version of the panel (in Version Comments) and click **FINISH**.



Customizing a Fluidigm Panel

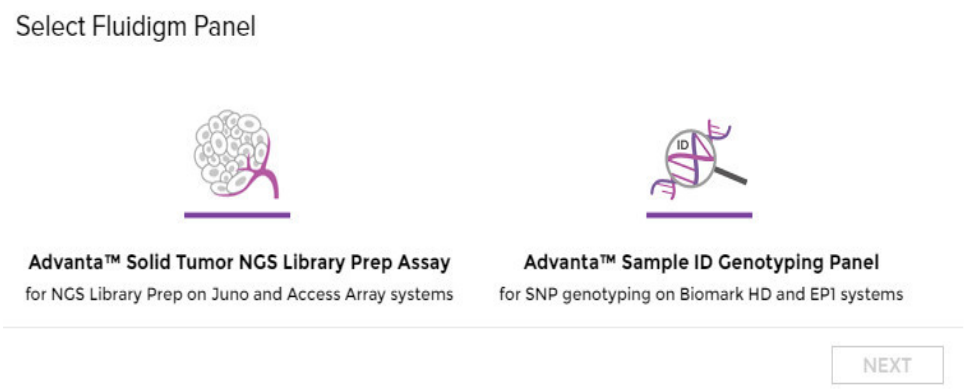
You can customize an existing panel from the Fluidigm catalog to fit the requirements of your experiment. Customization can include increased coverage of existing targets, addition of new targets, or removal of your choice of provided targets.

You can reorder the full customized panel or, for TSP panels only, you can reorder an addition to the catalog panel. See Fluidigm Panel Customization for Targeted DNA Seq Library Assays on page 59 for more information.

NOTE Fluidigm product panels are optimized through multiple iterations of experiment execution and analysis. Modifying the contents of a catalog panel by re-pooling making additions might result in decreased performance of the catalog panel. Optimization of the new panel design might be required to ensure equitable performance.

To customize a Fluidigm panel:

1. Click **+CUSTOMIZE FLUIDIGM PANEL** on the Panels page to be guided through the creation of a customized Fluidigm panel.
2. Select the type of assay for your customized panel on the Select Fluidigm Panel screen and click **NEXT**.



After you click **NEXT**, you see the Panel Properties screen:

3 Enter a unique name for the customized panel (in Panel Name) on the Panel Properties screen. You may also include a description (in Panel Description) and comments for this specific version of the panel (in Version Comments) as needed. Click **FINISH**.

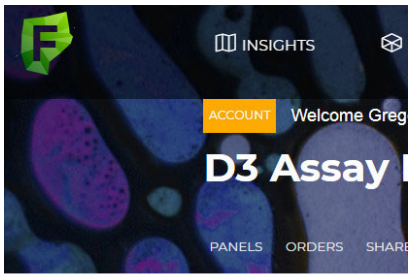
NOTE When customizing a Fluidigm panel design, the assay design parameters (for example, genome and amplicon sizes) are predefined from the selected panel.

Project and Version Control Settings

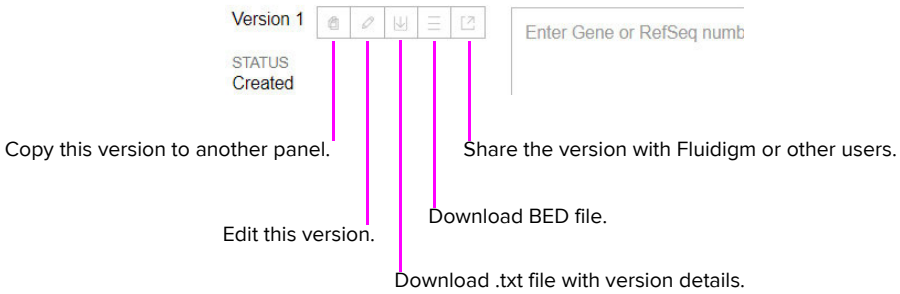
D3 projects and versions can be controlled via the left panel buttons in the project/version view.

Project-Level Controls

ACCOUNT shows you who is logged in. It is not a command. The commands on this page are beneath the title D3 Assay Design. For example, click **PANELS** to see the list of all the panels in your account. When you select a specific panel, you can edit or delete it with the icons that you see here:



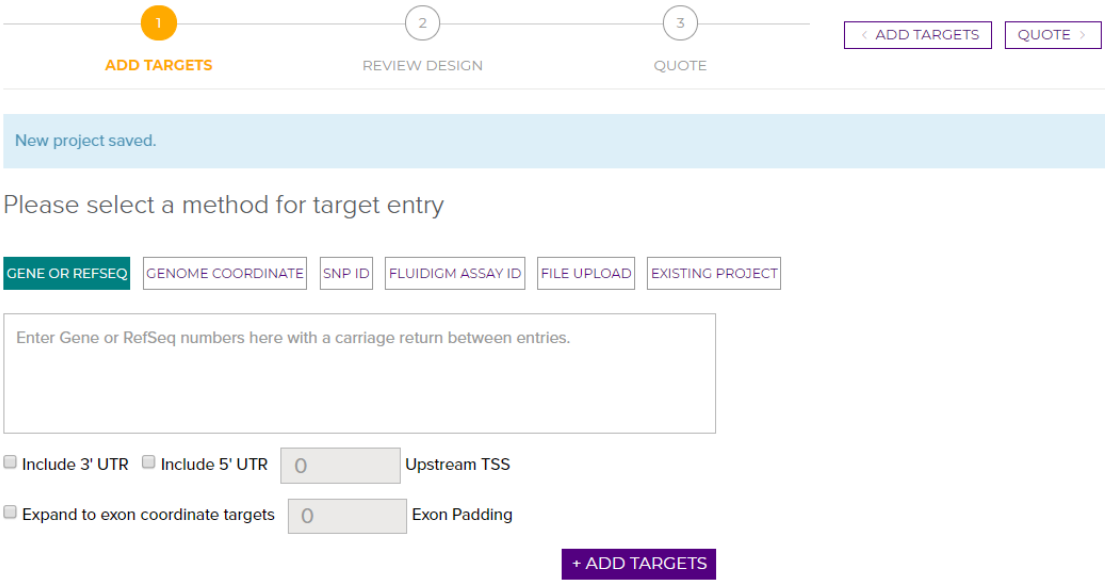
Version-Level Controls



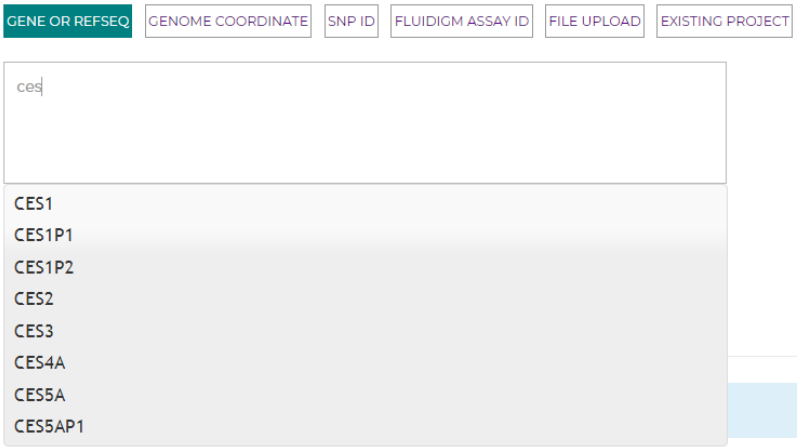
Adding Targets for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays

Depending on the assay type, you can add targets from the Add Targets screen in any of the ways described in this section.

NOTE Target entries are not case-sensitive.



GENE OR REFSEQ. Enter the gene names (such as GADPH) or the Reference Sequence Numbers (such as NM_000181). As you enter a target value, D3 provides suggestions:



GENOME COORDINATE. Enter the genome coordinate range (such as chr21:33,043,963-33,044,163).

Targets with genome coordinates must not exceed 5,000 base pairs (bp) in length. Genome coordinate regions greater than 5,000 bp can be entered as separate targets.

If you entered genome coordinates, each alias must be defined and unique. Aliases are used to name primers. For example, the primers for alias APC_ex3 are named APC_ex3_1, APC_ex3_2, and APC_ex3_3.

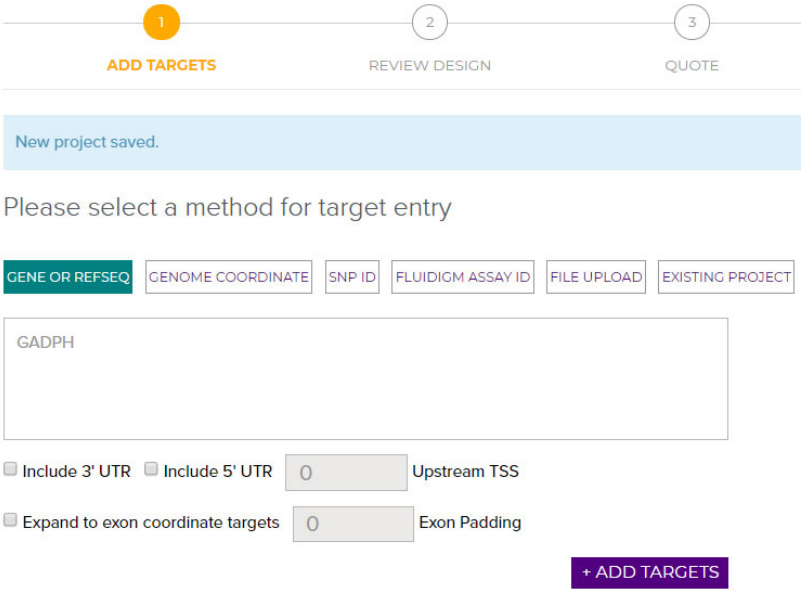
SNP ID. Enter one or more RS numbers to create coordinate targets that cover the genomic location of the SNP. Cosmic IDs are also supported for some species (hg38).

FLUIDIGM ASSAY ID. Enter one or more known Fluidigm assay identifiers to add that assay to the panel. A target is created for each assay ID entered. The targets must be submitted for design to return the assay data. The assay may contribute to coverage for other targets.

FILE UPLOAD. For example, download a template spreadsheet from the Add Targets screen, complete the required fields, and upload the spreadsheet back into the project.

EXISTING PROJECT. For example, copy targets by gene symbols from a D3 project that you created earlier or targets by gene symbols that the Fluidigm Assay Design Group shared with you.

Access Array Target-Specific Primers and Targeted DNA Seq Library Assays: Adding Targets by Gene Name or RefSeq ID



1 With **GENE OR REFSEQ** selected, enter the gene name or the **RefSeq ID**. Only NCBI NM accession IDs are accepted.

IMPORTANT

- Use the NCBI library to identify the official gene symbol. If the gene name is not accepted, attempt to enter aliases.
- Assays can be designed to a specific gene isoform by entering a NCBI RefSeq ID instead of the official gene symbol.

2 Check the boxes to determine whether the design should cover the 5′ UTR (untranslated region), the 3′ UTR, or both.

3 Enter the number of bases upstream of the transcription start site (TSS) of the gene of interest. This is only available if coverage of 5′ UTR is selected.

4 Check the box to expand the target regions to coordinate targets. (Optional) Add additional bases around each of the exons in the target.

NOTE Expanding the target to coordinate regions creates one target for each exon in the supplied gene or RefSeq target. Exons that are within 150 bases are merged into a single target. The individual target regions can be modified or removed after the target is added.

5 Targeted DNA Seq Library Assays only: Select **Design dual coverage** to request each base in the target to be covered by at least two assays.

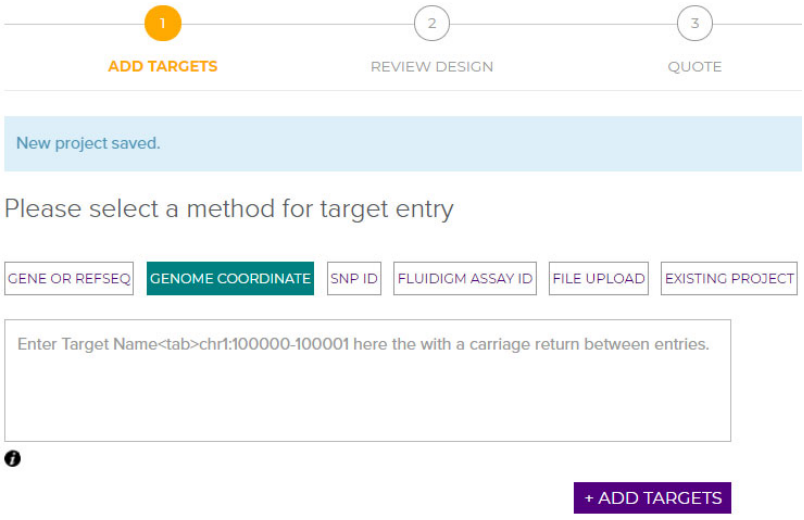
6 Targeted DNA Seq Library Assays only: Select **High quality design filter** to request only assays that have no primers in repeat regions or that amplify more than one genomic region.

7 When you are finished, click **+ADD TARGETS**.

NOTE D3 validates NM accession IDs and official gene names. Only valid targets can be added; non-NM accession IDs are not supported. Refer to the NCBI database to determine if an entry is valid.

Access Array Target-Specific Primers and Targeted DNA Seq Library Assays: Adding Targets by Genome Coordinates

NOTE Be certain to provide genome coordinates corresponding to the currently supported genome build. The supported genome build is identified in the Genome Build field, located in your project properties.

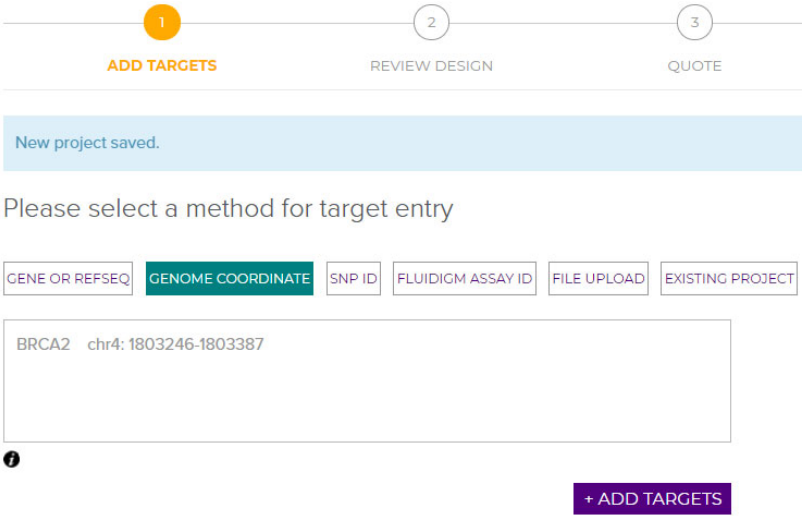


1 With **GENOME COORDINATE** selected, enter one or more genome coordinate targets using a gene name or target alias and the coordinate string separated by a tab per line.

The coordinate string is comprised of the chromosome (For example, Chr7, chr7, or 7) and the start and end coordinates separated with a dash, making sure that the end coordinate is larger than the start coordinate. A coordinate can be entered in the format

123456789 or 123,456,789 but not in the format 123.456.789. The upper limit is 5,000 per coordinate range.

For example:

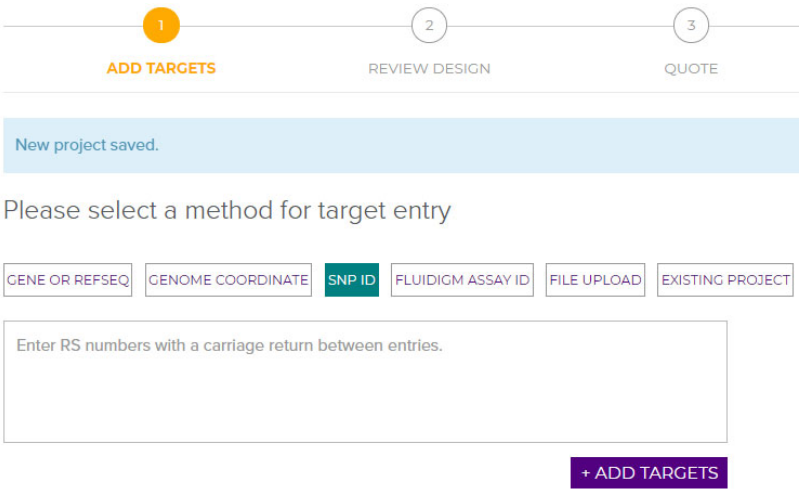


2 Targeted DNA Seq Library Assays only: Check the box **Design dual coverage** to request that each base in the target be covered by at least two assays.

3 Targeted DNA Seq Library Assays only: Select **High quality design filter** to request only assays that have no primers in repeat regions or that amplify more than one genomic region.

4 When you are finished, click **+ADD TARGETS**.

Access Array Target-Specific Primers and Targeted DNA Seq Library Assays: Adding Targets by SNP ID



1 With **SNP ID** selected, enter one or more COSMIC ID or RS numbers per line to create genome coordinate targets that cover the bases associated with the variant.

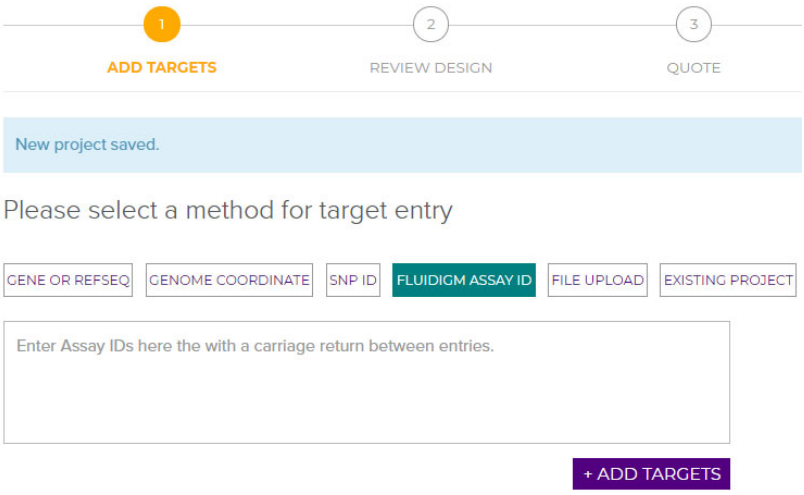
NOTE COSMIC ID and RS number submissions are not supported for all genomes.

2 Targeted DNA Seq Library Assays only: Check the box **Design dual coverage** to request that each base in the target be covered by at least two assays.

3 Targeted DNA Seq Library Assays only: Select **High quality design filter** to request only assays that have no primers in repeat regions or that amplify more than one genomic region.

4 When you are finished, click **+ADD TARGETS**.

Access Array Target-Specific Primers and Targeted DNA Seq Library Assays: Adding Targets by Fluidigm Assay ID

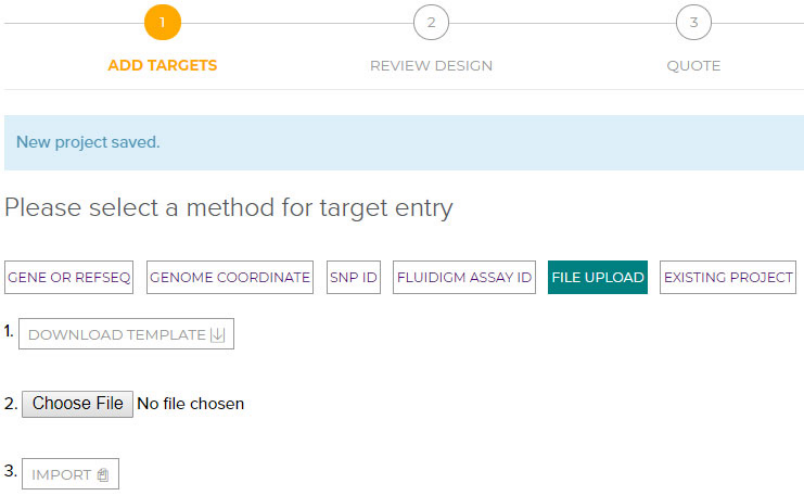


1 With **FLUIDIGM ASSAY ID** selected, enter one or more Fluidigm Assay IDs per row to import the specific assay primers into the panel.

NOTE Each valid Assay ID adds a target to the panel, which retrieves the assay data when submitted for design.

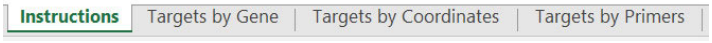
2 When you are finished, click **+ADD TARGETS**.

Access Array Target-Specific Primers and Targeted DNA Seq Library Assays: Adding Targets by Uploading a File



1 With **FILE UPLOAD** selected, click **DOWNLOAD TEMPLATE** to access the Excel template spreadsheet file.

2 When the Excel file displays at the bottom of your screen, open it and view the 4 tabs:



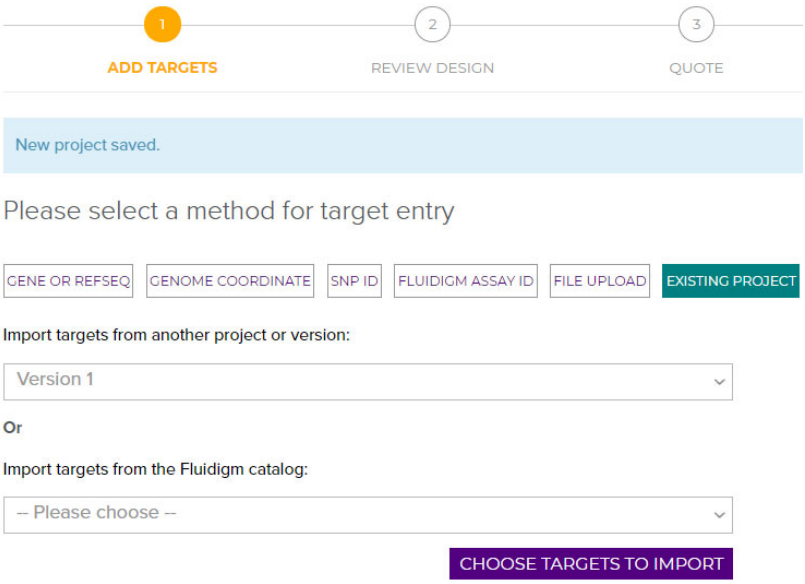
3 In Excel, read the instructions, enter your targets in the appropriate tab, and save your work as a tab-delimited Excel file with a *.txt filename extension.

You can only save one Excel sheet (one tab) of targets at a time. This is the active sheet.

4 In D3, click **Choose File,** navigate to the file that you saved in Step 3 and click **Open**. The file containing the active sheet with your specified targets is now ready to be imported.

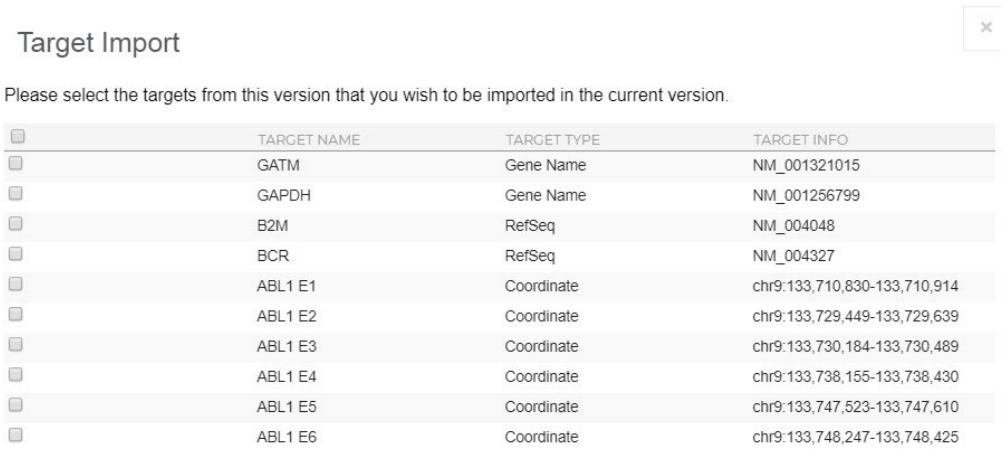
5 Click **IMPORT**.

Access Array Target-Specific Primers and Targeted DNA Seq Library Assays: Adding Targets from Existing Projects



With **EXISTING PROJECT** selected, you can view the current targets at the bottom of the screen.

1. Use a drop-down list to import targets from another project or version that you created on D3 (**Import targets from another project or version**) or from the catalog where applicable (**Import targets from the Fluidigm catalog**).
2. Click **CHOOSE TARGETS TO IMPORT**. A list of targets displays. For example:



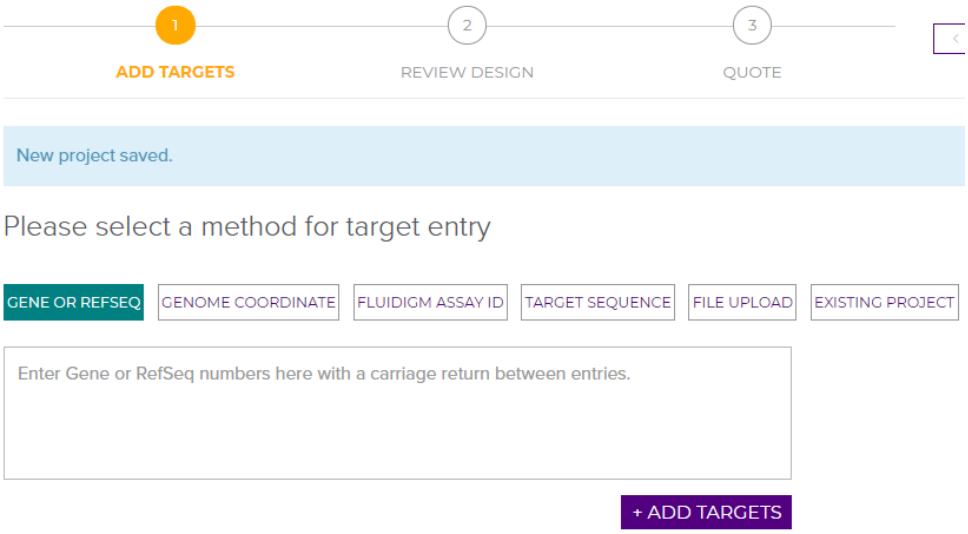
	TARGET NAME	TARGET TYPE	TARGET INFO
☐	GATM	Gene Name	NM_001321015
☐	GAPDH	Gene Name	NM_001256799
☐	B2M	RefSeq	NM_004048
☐	BCR	RefSeq	NM_004327
☐	ABL1 E1	Coordinate	chr9:133,710,830-133,710,914
☐	ABL1 E2	Coordinate	chr9:133,729,449-133,729,639
☐	ABL1 E3	Coordinate	chr9:133,730,184-133,730,489
☐	ABL1 E4	Coordinate	chr9:133,738,155-133,738,430
☐	ABL1 E5	Coordinate	chr9:133,747,523-133,747,610
☐	ABL1 E6	Coordinate	chr9:133,748,247-133,748,425

3. Check the boxes of the targets to be imported into the current version.
4. Click **IMPORT TARGETS**.

Adding Targets for Delta Gene Assays

- If the species is in the supported list, you can add targets by RefSeq ID, gene symbol, genome coordinate, target sequence, or Fluidigm Assay ID. Otherwise, only target sequence or Fluidigm Assay ID are valid entry methods.
- If genome coordinates or sequences are entered, each alias must be defined and be unique.
- If Assay IDs are entered, they are automatically populated when the design query is complete, assuming there is a design for the target.

Delta Gene Assays: Adding Targets by Gene Name or RefSeq ID



1 With **GENE OR REFSEQ** selected, enter one or more gene names or RefSeq IDs per line. NCBI nomenclatures are acceptable.

IMPORTANT

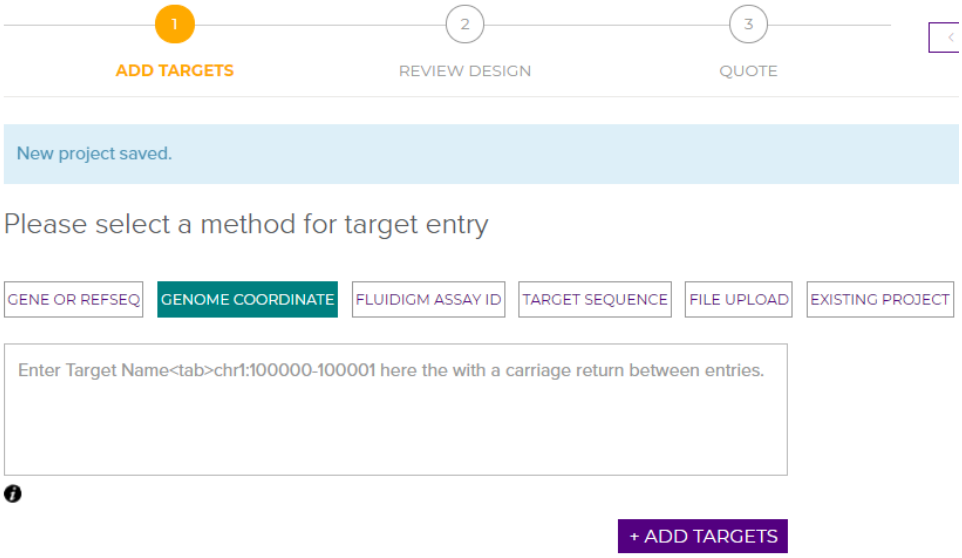
- Use the NCBI library to identify the official gene symbol. If the gene name is not accepted, try to enter aliases. Always use NCBI RefSeq IDs.
- Delta Gene designs are designed to the gene level whenever possible. If designs do not detect all known isoforms, a design comment is included during design review.
- Assays can be designed to ensure coverage of a specific gene isoform by entering an NCBI RefSeq ID instead of the official gene symbol.

2 When you are finished, click **+ADD TARGETS**.

NOTE If a gene cannot be located, it might be known by a different alias on the support list.

Delta Gene Assays: Adding Targets by Genome Coordinates

NOTE Be certain to provide genome coordinates corresponding to the currently supported genome build. The supported genome build is identified in the Genome Build field located in your project properties.



1 With **GENOME COORDINATE** selected, enter one or more genome coordinate targets using a gene name or a target alias and the coordinate string separated by a tab per line.

   Each coordinate string contains at least 1 chromosome (For example, Chr7, chr7, or7) with a starting and ending coordinate range (separated by a hyphen). For example:

   `chr4:1803246-1803387`

   The lowest value in a coordinate range is the start coordinate and the highest value is the end coordinate. The format of each coordinate string can be 123456789 or 123,456,789 but not 123.456.789. The upper limit of the ending coordinate value is 5,000.

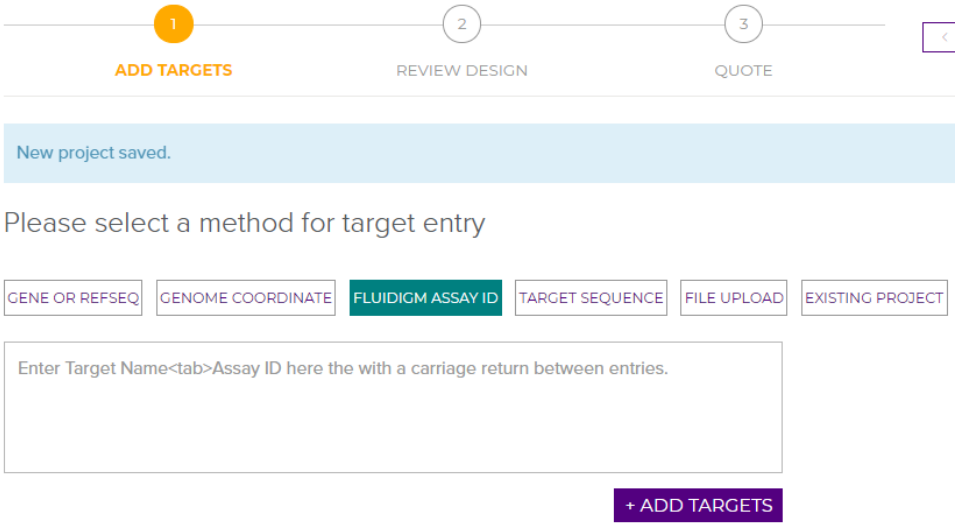
   (Optional) Place square brackets [ ] around the regions of interest for primer design. Assays are designed to flank the bracketed area, with a primer on either side of the bracket. For example:

   `chr4:1803246-1803387[]chr4:1803388-1803435`

2 Click **+ADD TARGETS**.

Delta Gene Assays: Adding Targets by Fluidigm Assay ID

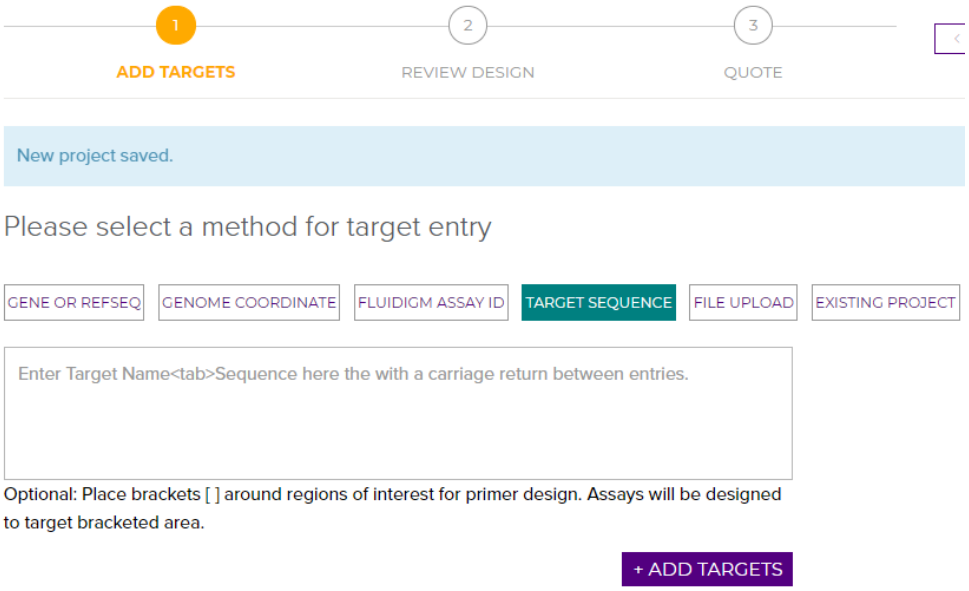
An easy way to reorder assays from a previous order is to enter the associated Fluidigm Assay ID. Assay IDs are available in the informatics packet that is supplied with your assays.



1 With **FLUIDIGM ASSAY ID** selected and referring to your previously ordered assay, copy and paste the gene names and the Fluidigm Assay IDs.

2 When you are finished, click **+ADD TARGETS**.

Delta Gene Assays: Adding Targets by Target Sequence

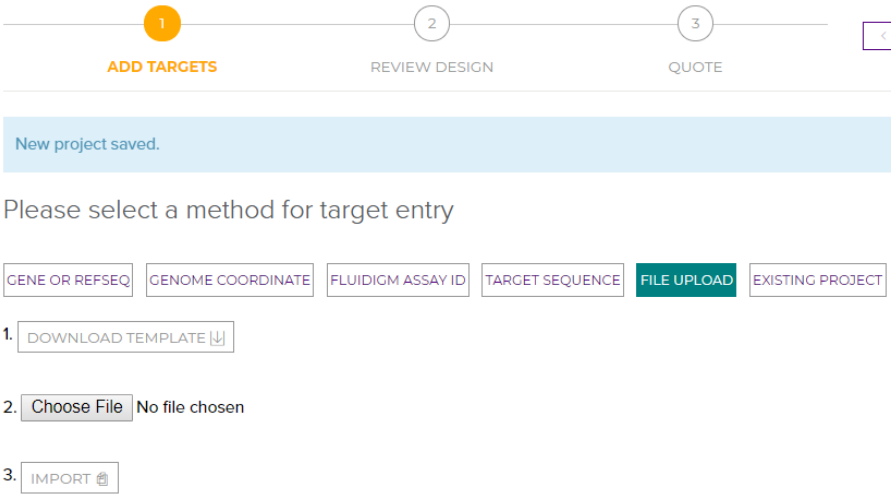


1 With **TARGET SEQUENCE** selected, enter one or more target names and associated target sequences per line. Each target name and a sequence (up to 1,000 bp) must be separated by a tab.

(Optional) Place square brackets [ ] around the regions of interest in each sequence for primer design. Assays are designed to flank the bracketed area, with a primer on either side of the bracket.

2 When you are finished, click **+ADD TARGETS**.

Delta Gene Assays: Adding Targets by Uploading a File



1 With **FILE UPLOAD** selected, click **DOWNLOAD TEMPLATE** to access the Excel template spreadsheet file.

2 When the Excel file displays at the bottom of your screen, open it and view the 6 tabs:

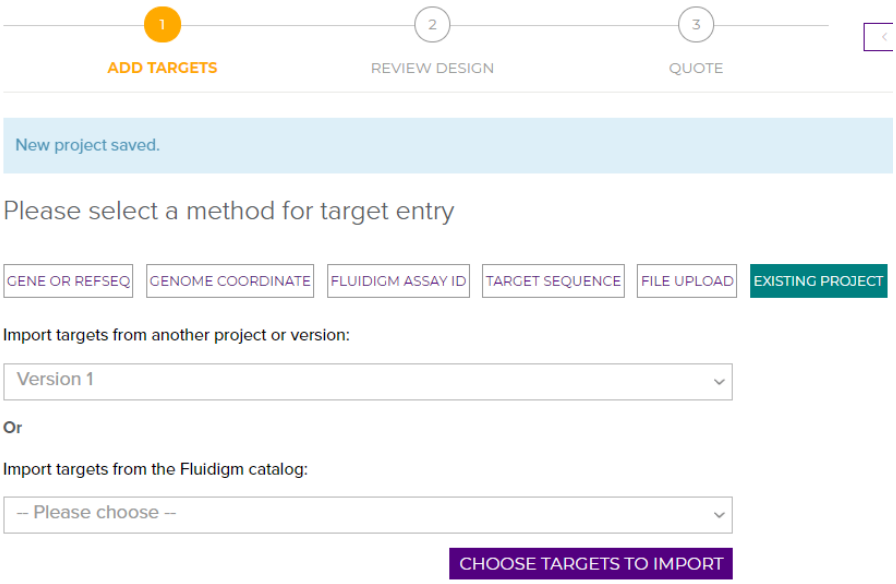
3 In Excel, read the instructions, enter your targets in the appropriate tab, and save your work as a tab-delimited Excel file with a *.txt filename extension.

You can only save one Excel sheet (one tab) of targets at a time. This is the active sheet.

4 In D3, click **Choose File,** navigate to the file that you saved in Step 3 and click **Open**. The file containing the active sheet with your specified targets is now ready to be imported.

5 Click **IMPORT**.

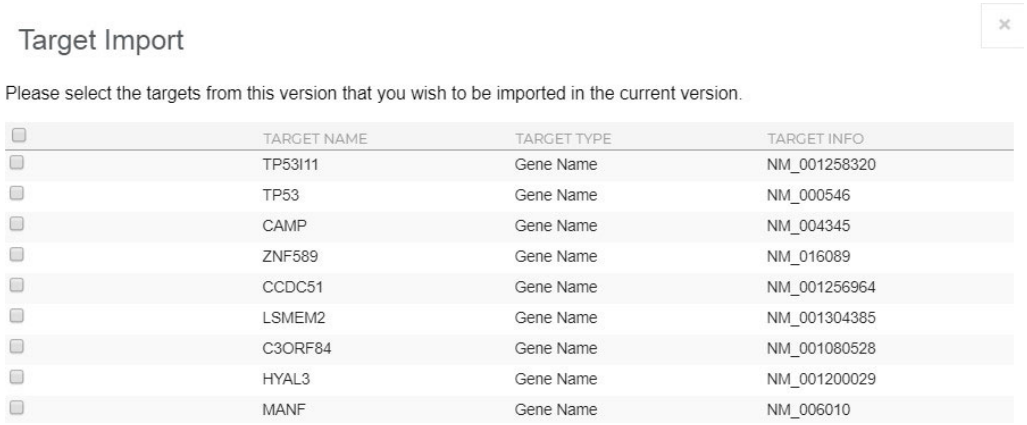
Delta Gene Assays: Adding Targets from Existing Projects



1 With **EXISTING PROJECT** selected, you can view the current targets at the bottom of the screen.

2 Use a drop-down list to import targets from another project or version that you created on D3 (Import targets from another project or version) or from the catalog where applicable (Import targets from the Fluidigm catalog).

3 Click **CHOOSE TARGETS TO IMPORT**. A list of targets displays. For example:



	TARGET NAME	TARGET TYPE	TARGET INFO
☐	TP53I11	Gene Name	NM_001258320
☐	TP53	Gene Name	NM_000546
☐	CAMP	Gene Name	NM_004345
☐	ZNF589	Gene Name	NM_016089
☐	CCDC51	Gene Name	NM_001256964
☐	LSMEM2	Gene Name	NM_001304385
☐	C3ORF84	Gene Name	NM_001080528
☐	HYAL3	Gene Name	NM_001200029
☐	MANF	Gene Name	NM_006010

4 Check the boxes of the targets to be imported into the current version.

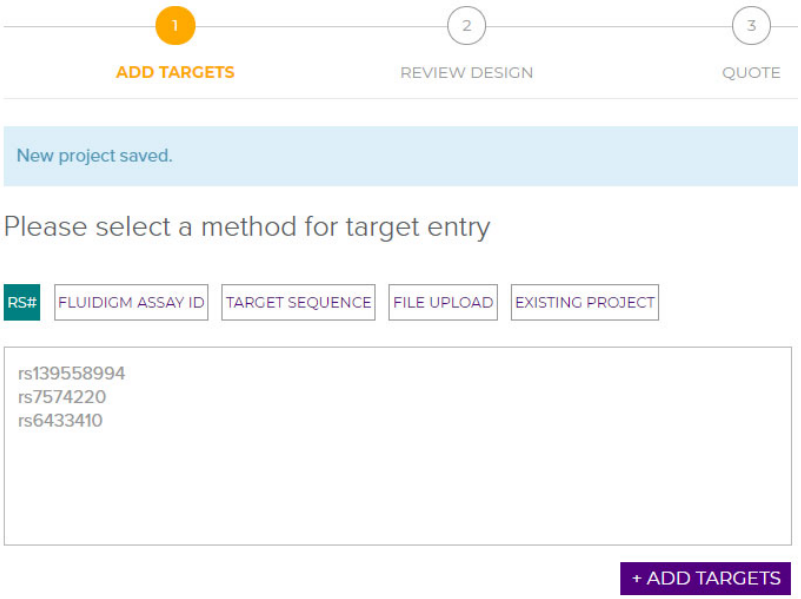
5 Click **IMPORT TARGETS**.

Adding Targets for SNP Type Assays

For SNP Type Assays, the following parameters apply:

- If the species is on the supported list, you can add targets by RefSNP (RS) number, target sequence, or Fluidigm Assay ID.
- If the species is not on the supported list, only the target sequence or Fluidigm Assay ID are valid entry methods.
- If either the target sequence or Fluidigm Assay ID is entered, the SNP name must be defined.
- If the Fluidigm Assay ID is not entered, it is automatically populated when the design query is complete, assuming that there is a design for the target.

SNP Type Assays: Adding Targets by RS Number



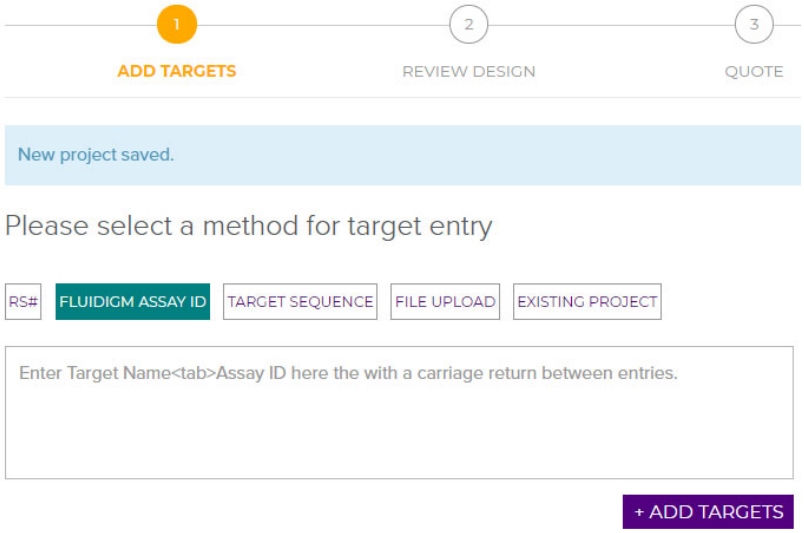
1 With **RS#** selected, enter one RS number per line.

NOTE The designable sequence for each valid RS number are queried and masked with the selected SNP masking option during target design.

2 When you are finished, click **+ADD TARGETS**.

SNP Type Assays: Adding Targets by Fluidigm Assay ID

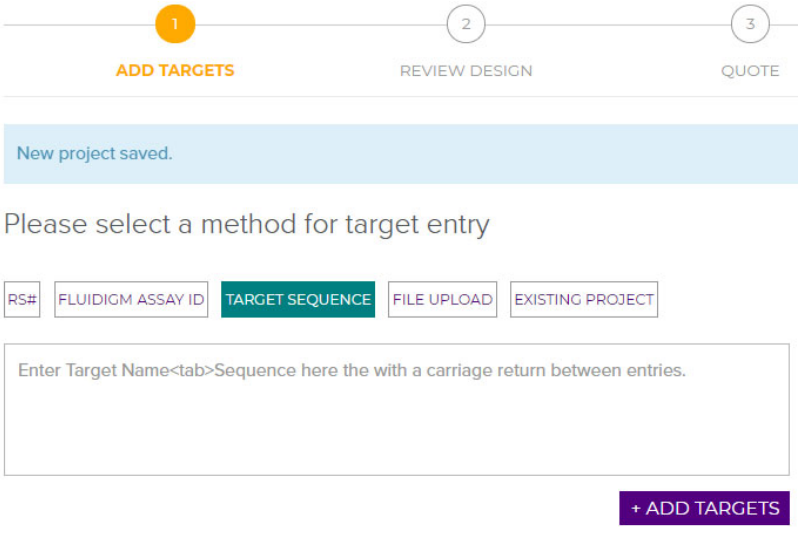
An easy way to reorder assays from a previous order is to enter the associated Fluidigm Assay ID. Assay IDs are available in the informatics packet that is supplied with your assays.



1 With **FLUIDIGM ASSAY ID** selected and referring to your previously ordered assay, copy and paste one or more lines containing the RS number or target name and the Fluidigm Assay IDs, separated by a tab.

2 When you are finished, click **+ADD TARGETS**.

SNP Type Assays: Adding Targets by Target Sequence



1 With **TARGET SEQUENCE** selected, enter one or more target names and associated target sequences per line. Each target name and a sequence (up to 1,000 bp) must be separated by a tab.

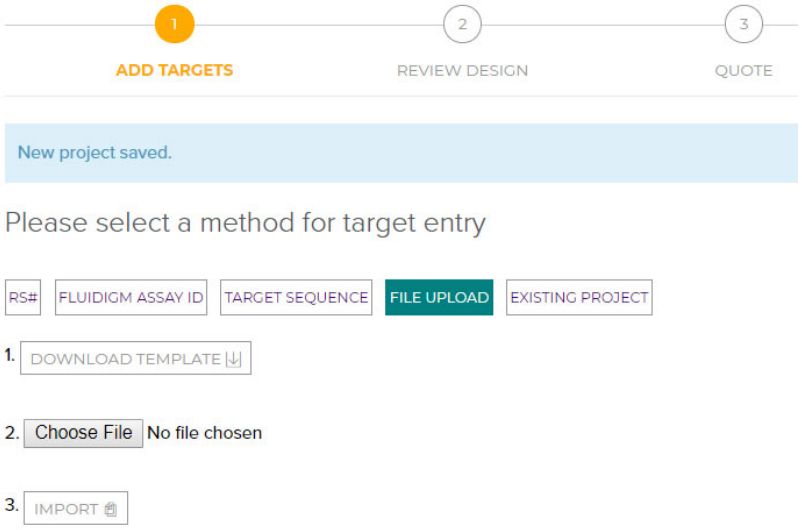
Place square brackets [ ] around the targeted biallelic SNP for primer design. Assays are designed to target the bracketed area. For example:

Biallelic SNP	...GTGTGAGT[C/A]TGACAGC...
Deletion	...GGTAATGAC[G/-]GTACGSG...
Insertion	...GTGACAG[-/G]TGAAGAGA...

NOTE Non-biallelic target SNP sequences are not accepted.

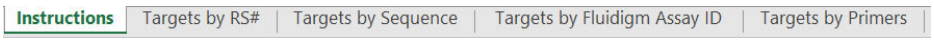
2 When you are finished, click **+ADD TARGETS**.

SNP Type Assays: Adding Targets by Uploading a File



1 With **FILE UPLOAD** selected, click **DOWNLOAD TEMPLATE** to access the Excel template spreadsheet file.

2 When the Excel file displays at the bottom of your screen, open it and view the 6 tabs:



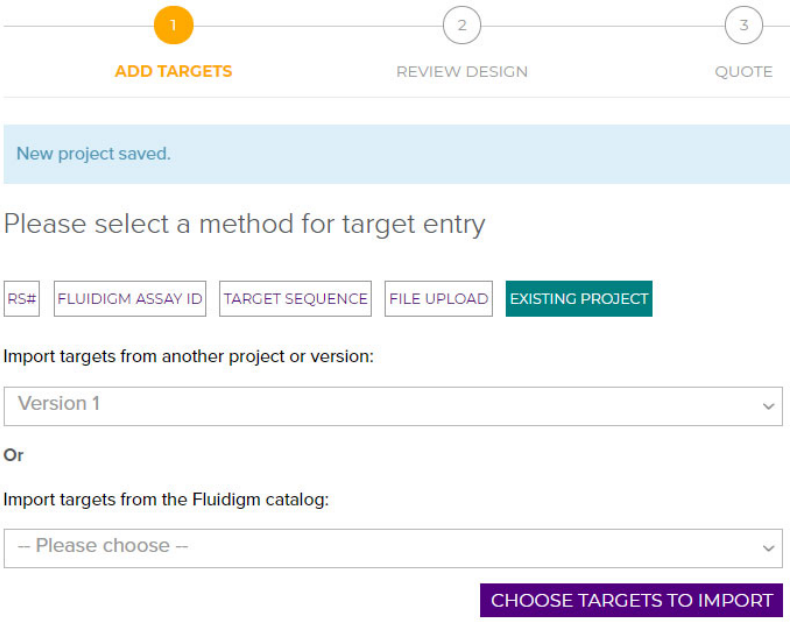
3 In Excel, read the instructions, enter your targets in the appropriate tab, and save your work as a tab-delimited Excel file with a *.txt filename extension.

You can only save one Excel sheet (one tab) of targets at a time. This is the active sheet.

4 In D3, click **Choose File,** navigate to the file that you saved in Step 3 and click **Open**. The file containing the active sheet with your specified targets is now ready to be imported.

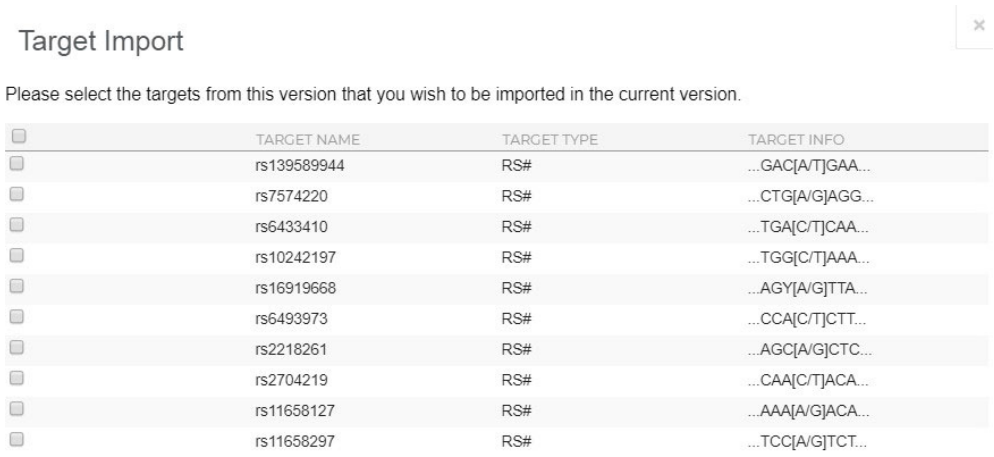
5 Click **IMPORT**.

SNP Type Assays: Adding Targets from Existing Projects



1 Use a drop-down list to import targets from another project or version that you created on D3 (**Import targets from another project or version**) or from the catalog where applicable (**Import targets from the Fluidigm catalog**).

2 Click **CHOOSE TARGETS TO IMPORT**. A list of targets displays. For example:



3 Check the boxes of the targets to be imported into the current version.

4 Click **IMPORT TARGETS**.

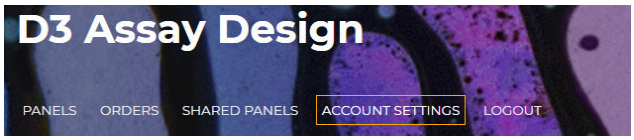
Sharing Your Design with Colleagues

Shared users receive read-only access to the shared version of the panel on their own dashboard. The read-only version is be updated if the original owner makes changes. Shared

users may make their own copies of the shared version to rename and edit. Modifications to copies do not affect the original owner's panel.

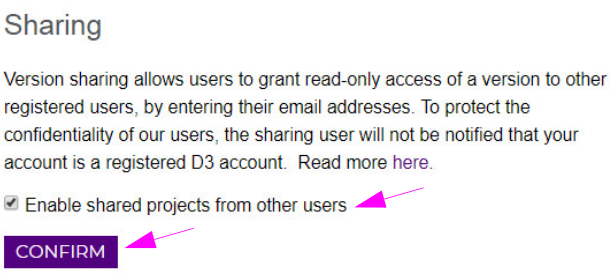
1 Be certain that you and your colleagues have sharing enabled.

   a Click **ACCOUNT SETTINGS**:



   b Check the box **Enable shared projects from other users**.

   c Click **CONFIRM** to enable sharing.



2 Navigate to the project or version.

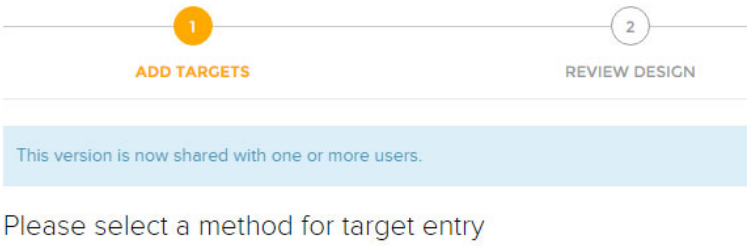
3 Click the **Options** icon for that version, and then click the **Share** icon:

4 Read and agree to the conditions of the Fluidigm sharing agreement.

5 Enter email addresses for users you want to share with.

6 Click **CONFIRM**.

   Your project or version is now shared with the users you designated. A sample shared version is shown here:

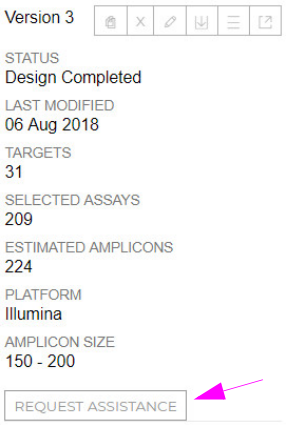


To stop sharing a version, delete the shared email addresses and confirm the change.

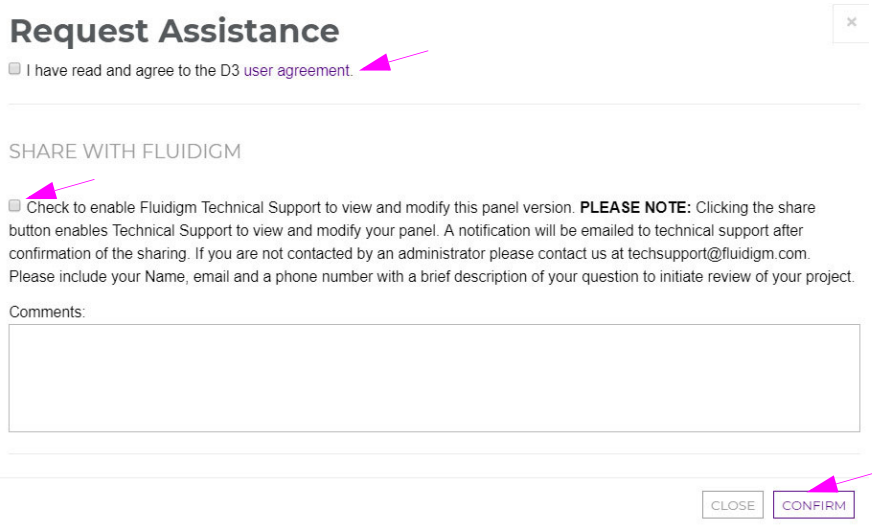
Requesting Assistance for Panel Design

You can request assistance in creating, designing, or ordering your D3 panel. Sharing a panel with Technical Support enables our D3 support staff at Fluidigm to have full control over one version of the panel. It is recommended that you create a new version of the panel prior to requesting our assistance.

1 Click **REQUEST ASSISTANCE**:



You see the Request Assistance screen:



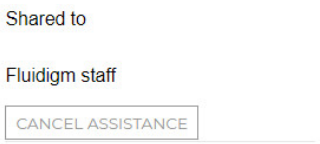
2 Read the user agreement and check the box I **have read and agree to the D3 user agreement**.

3 Read the paragraph in the SHARE WITH FLUIDIGM section and check the box that enables Fluidigm Technical Support to access this version of the panel.

4 Enter your contact information into the Comments box with a brief description of the reason for your request.

5 Click **CONFIRM**. You will receive notification by email from Fluidigm Technical Support. If you are not contacted by an administrator, email us at techsupport@fluidigm.com.

Your panel version is now shared with the Fluidigm staff:



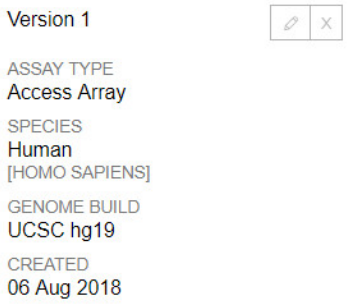
To stop sharing a version, click **CANCEL ASSISTANCE**, uncheck the sharing checkbox, and click **CONFIRM**.

D3 Best Practices

Adherence to best practices optimizes the D3 user experience. This section provides design recommendations, with pointers to increase assay coverage.

Design Recommendations

Be certain to provide genome coordinates corresponding to the currently supported genome build. The supported genome build is identified in the Genome Build field located in your project properties, below ASSAY TYPE and SPECIES fields. For example:



For Access Array Target-Specific Primers and Targeted DNA Seq Library Assays

- To list targets by genomic coordinates, the format should be chromosome: start coordinates-end coordinates. For example, `chr4:1803246-1803387`. This can be used for genomic region resequencing, SNP calling, or selecting specific exons.
- When genomic coordinates are provided, aliases must be added. An alias should not exceed 15 characters. Aliases are used to name primers. For example, the primers for alias APC_ex3 are named APC_ex3_1, APC_ex3_2, and APC_ex3_3.
- To obtain uniform coverage across amplicons, the optimal size for the target range of an assay is ≥150 bp. However, amplicon lengths should be within 25% of the average in a given library.

For Delta Gene Assays

- You must ensure that Assay IDs, genome coordinates, and sequence submissions refer to the intended target species. D3 cannot check whether all targets entered belong to the same species.
- Assays are designed to target all known isoforms of the gene. If an assay does not target all known isoforms, a design comment is displayed next to the assay along with a list of detectable isoforms.

For SNP Type Assays

- When targets are entered by sequence, you must indicate the targeted SNP using square brackets separated by a forward slash. For example, [A/G].
- A minimum of 60 bp is recommended both upstream and downstream of the targeted SNP, with a maximum of 250 bp to increase the probability of receiving a design.
- Indicate any relevant additional non-targeted SNPs as International Union of Pure and Applied Chemistry (IUPAC) codes, which are tabulated in Appendix B.
- You must specify RefSNP numbers. A sequence is retrieved using the most recent build on human NCBI dbSNP and any adjacent SNP, with a reported allele frequency ≥1% (excluding SNPs with no frequency data).
- If both sequence and RS number are provided, the assay is designed to the sequence. No additional sequence is retrieved from dbSNP.
- Only one SNP can be identified per target number.
- Insertions or deletions >10 bp should not be included.
- Non-biallelic SNPs should not be included.
- Targets with adjacent SNPs within 30 bp on both sides of the target SNP will not be designed. Fluidigm recommends removal of an adjacent SNP if it is not pertinent within the study population.
- Sequences are filtered based on % GC content. Sequences that bear greater than 65% GC are assigned a nonstandard design rank automatically.

Design Ranks (Standard vs. Nonstandard)

The design review process ranks assays as either standard or nonstandard, depending on the designability of the assay. The rank is not an indicator of the quality of your assay design. However, it affects the level of support provided. Although every D3 assay type has its supported species list, you can enter unsupported species if the selected assay type allows sequence submission.

- Standard assays are designed to product specifications and do not include a design comment.

- Nonstandard assays fall outside typical design criteria. They are offered with limited support and might not meet product specifications. They include a design comment providing a rationale for the ranking.

  A nonstandard rank is always assigned to assays that are either designed with relaxed algorithm parameters (such as relaxed requirements for GC filter, amplicon length, SNP filter, or repeat filter) or that are designed to customer-provided sequences. Such assays are provided with limited support and might not meet product specifications.

  Assays that are designed with relaxed design parameters typically have a lower success rate than standard Fluidigm assays, yet they offer a viable alternative for studying difficult targets.

Design comments are specific to each assay type and are listed in the following sections.

Design Comments

For Access Array Target-Specific Primers and Targeted DNA Seq Library Assays

1 Nonstandard Assay: GC content is outside of the product specification.

2 Nonstandard Assay: One primer is designed within a repeat region.

3 Nonstandard Assay: Both primers are designed within a repeat region.

4 Nonstandard Assay: Designed without SNP and repeat annotation.

5 Nonstandard Assay: *In silico* check shows assays may hybridize to multiple loci.

6 Nonstandard Assay: Designed with amplicon lengths outside of specified lengths.

7 Nonstandard Assay: Designed outside of specifications.

For Delta Gene Assays

1 Nonstandard Assay: Designed to customer-provided sequence or genome coordinate.

2 Nonstandard Assay: Designed to non-NM_accession ID.

3 Nonstandard Assay: Not designed to gene level.

4 Nonstandard Assay: Designed outside of specifications.

For SNP Type Assays

1 Nonstandard Assay: GC content is outside of the product specification.

2 Nonstandard Assay: Target is an insertion or deletion or not a SNP.

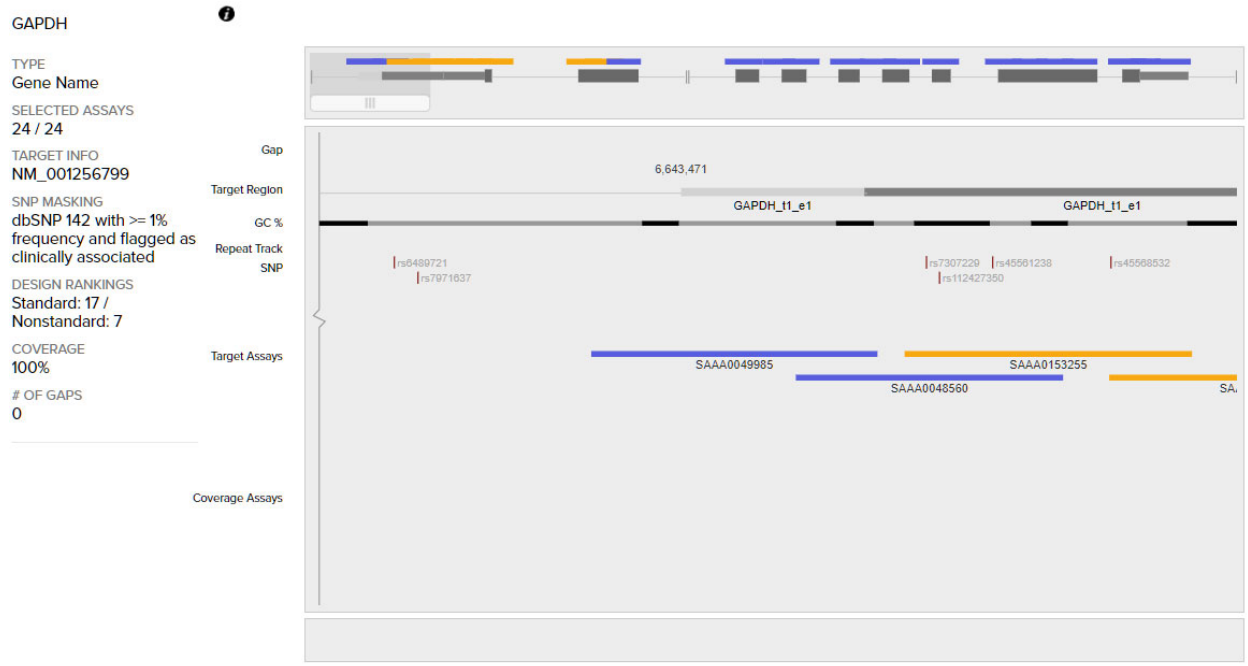
3 Nonstandard Assay: Designed outside of specifications.

Assay Design Coverage

The D3 coverage report calculates coverage based on the inner amplicon (or true amplification sequence) between the locus-specific forward primer and the locus-specific reverse primer. It excludes regions covered by synthetic oligonucleotides.

Primers. Primer specificity is tested *in silico* against the latest genome build available at the time of release for the selected species. Occasionally two, three, or more primers may be required to cover the specific region. In such cases, coverage is accomplished by tiling inner amplicons across the region.

Amplicon length. You can see the length beneath the AMPLICON LENGTH column at the right of the ASSAY ID in the assays table:



INCLUDE ASSAY(S) EXCLUDE ASSAY(S) Filter... 1-24 of 24

	SELECTED	Assay Name	ID	Amplicon Length	Unique Coverage	GC%	Design Rank	Comments	User Annotation
	True	GAPDH_1	SAAA0049985	193	40%	63%	Standard		
	True	GAPDH_2	SAAA0048560	187	9.7%	65%	Standard		
	True	GAPDH_3	SAAA0153255	193	15.4%	66%	Nonstandard	1 - Nonstandard Assay: GC content is outside product specifications.	
	True	GAPDH_4	SAAA0153253	197	21.6%	71%	Nonstandard	1 - Nonstandard Assay: GC content is outside product specifications.	

Unique coverage. D3 designs reference the plus strand of the human genome reference and do not imply gene transcription directionality. The unique coverage value is a representation of the genomic coverage provided by an assay, not including overlapping assays. This value is important for tiling designs where particular regions may be covered by two or more assays.

The coverage wheel changes dynamically as targets are added or removed from a project and as assays are included or removed from the assays list.

For example:

Targeted DNA Seq CNV Design Recommendations

Target definition for copy number variant (CNV) panels in D3 with Targeted DNA Seq Library assays varies based upon the method of sequencing analysis.

The following recommendations that assist in defining the targets for a CNV panel also take into consideration downstream analysis:

- Include a reference gene target in the panel (for example, RPPH1). Check the box to select the **High quality design filter** option for the reference target to ensure that no nonstandard, off-target assays are returned.
- Target definition is dependent on the similarities between the regions of interest:

  Highly similar regions. Identify the unique bases between the genomic sequences and define coordinate targets that cover only the unique bases. Primers will be designed to flank the coordinates provided.

  Partially similar regions. Create coordinate targets for the unique region with the High quality design filter option, and coordinate targets for the common regions without the High quality design filter option.

Singleplex or Multiplex Access Array Target-Specific Primers

Your choice of singleplex or multiplex Access Array Target-Specific Primers depends on the needs of your experiment. These assays are performed on the Access Array system on either the Access Array 48.48 or LP 48.48 IFC. Individual or singleplex Access Array reactions contain one forward and one reverse primer in each microfluidic reaction chamber on the IFC and are provided with wet-test data. Multiplex reactions contain up to 12 sets of forward and reverse primers in each reaction chamber. Assays for multiplex are singleplex assays arrayed in a format for easy pooling into your primer pools. No wet-test data is provided with this option.

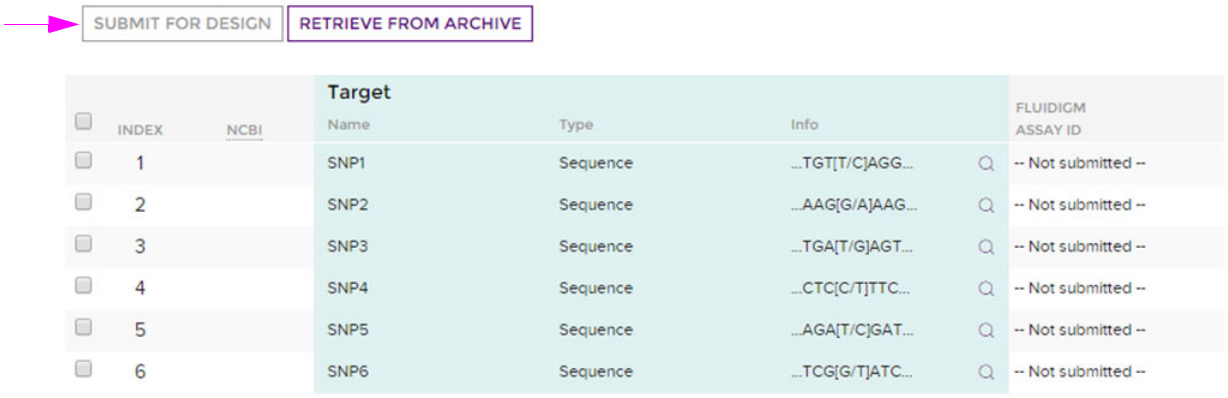
Chapter 3: Design Review and Ordering

D3™ is designed to automatically perform an *in silico* verification after you have added all targets. If a design passes verification, you can submit it for design. Our design experts generate your custom assay and notify you by email when the design is ready for review. Expect a design review response in 48–72 hours.

Submitting a Target for Design

1 Check the list of targets to make sure you have added all targets you want to include in this version of your design. You can add more later.

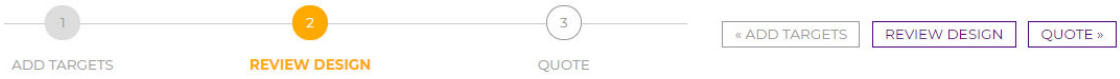
2 Click **SUBMIT FOR DESIGN**:



3 Review the confirmatory email that you receive from the system.

Target Coverage for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays

1 Click **REVIEW DESIGN** in the panel navigation links to display the target design coverage:



2 In the panel summary on display, review the following items related to the overall panel coverage statistics:

Selected Assays: The number of assays associated with non-archived targets currently included for ordering in the panel.

Designed Targets: The number of targets in the panel with assay designs.

Bases Covered: The count of target bases in the entire panel covered by at least 1 amplicon.

Bases Dual Covered: The count of target bases in the panel covered by at least 2 amplicons.

Design Rankings: The count of nonstandard- and standard-ranking assays in the panel selected for ordering.

The percentage of coverage displayed in the circle at the right is the percentage of bases across all non-archived targets that are covered by at least 1 assay. This is the overall coverage.

NOTE For Access Array™ Target-Specific Primers and Targeted DNA Seq Library Assays, each target's coverage is calculated by counting the total bases for all design regions in the target that are covered by one or more assay inner amplicons. In addition, target coverage is calculated using all assays in the panel rather than only those assays associated with the target.

3 Click **TARGET COVERAGE** in the review navigation tabs to review the following items related to the overall target coverage:

Assays Designed: The number of assays specifically associated with this target by the design process. These assays are removed from the panel if the target is archived.

Assays Selected: The number of assays associated with this target that are marked as included for ordering.

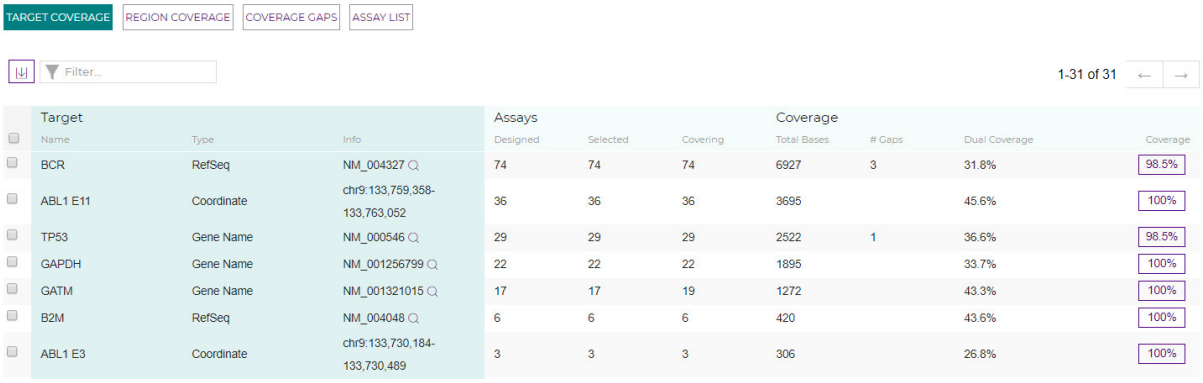
Assays Covering: The number of assays across the entire panel that cover the design regions of this target.

Coverage Total Bases: The count of bases for all design regions associated with this target.

Coverage Gaps: The number of gaps across all design regions associated with this target.

Coverage Dual Coverage: The percentage of bases of all design regions associated with this target that are covered by more than one assay inner amplicon.

Coverage: The percentage of bases of all design regions associated with this target that are covered by at least 1 assay inner-amplicon.



Target			Assays			Coverage			
Name	Type	Info	Designed	Selected	Covering	Total Bases	# Gaps	Dual Coverage	Coverage
BCR	RefSeq	NM_004327	74	74	74	6927	3	31.8%	98.5%
ABL1 E11	Coordinate	chr9:133,759,358-133,763,052	36	36	36	3695		45.6%	100%
TP53	Gene Name	NM_000546	29	29	29	2522	1	36.6%	98.5%
GAPDH	Gene Name	NM_001256799	22	22	22	1895		33.7%	100%
GATM	Gene Name	NM_001321015	17	17	19	1272		43.3%	100%
B2M	RefSeq	NM_004048	6	6	6	420		43.6%	100%
ABL1 E3	Coordinate	chr9:133,730,184-133,730,489	3	3	3	306		26.8%	100%

4 Click **REGION COVERAGE** in the review navigation tabs to review the following items related to the individual target region coverage:

Region Name: The title of the region for this coordinate range. For gene and RefSeq designs, it is the type of the region (UTR, Upstream).

Region Coordinates: The positions of the starting and ending bases for this designable region.

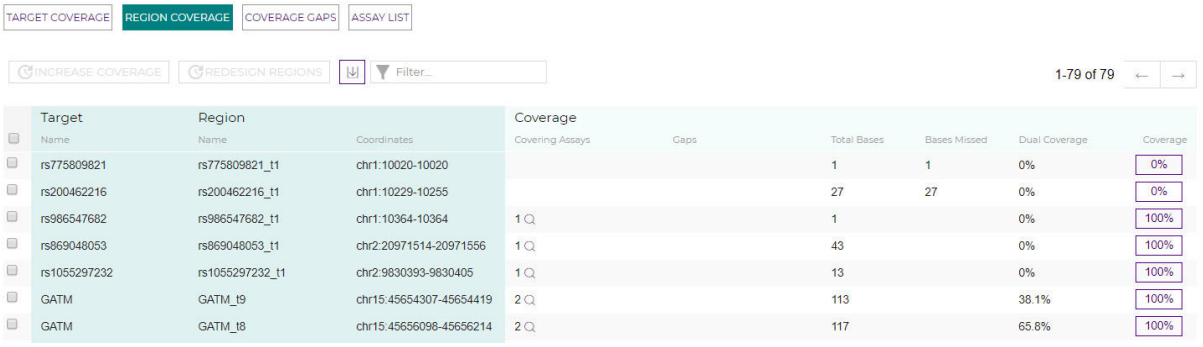
Covering Assays: The count and list of assay IDs that cover this specific region.

Coverage Gaps: The count and list of gaps in the coverage of the region.

Total Bases: The length of this design region.

Bases Missed: The number of bases in this region that are without coverage.

Dual Coverage and Coverage: The percentage of coverage of this region by ≥1 assay inner-amplicon.



Target	Region		Coverage					
Name	Name	Coordinates	Covering Assays	Gaps	Total Bases	Bases Missed	Dual Coverage	Coverage
rs775809821	rs775809821_t1	chr1:10020-10020			1	1	0%	0%
rs200462216	rs200462216_t1	chr1:10229-10255			27	27	0%	0%
rs986547682	rs986547682_t1	chr1:10364-10364	1		1		0%	100%
rs869048053	rs869048053_t1	chr2:20971514-20971556	1		43		0%	100%
rs1055297232	rs1055297232_t1	chr2:9830393-9830405	1		13		0%	100%
GATM	GATM_t9	chr15:45654307-45654419	2		113		38.1%	100%
GATM	GATM_t8	chr15:45656098-45656214	2		117		65.8%	100%

NOTE For Access Array Target-Specific Primers and Targeted DNA Seq Library Assays, a designed target has 1 or more target regions depending on the target input type. A gene or RefSeq target has multiple design regions per target, whereas all other target types have only 1 region.

5 Click **COVERAGE GAPS** in the review navigation tabs to review the following items related to the gaps in coverage:

Region Name: The title of the region that contains this gap.

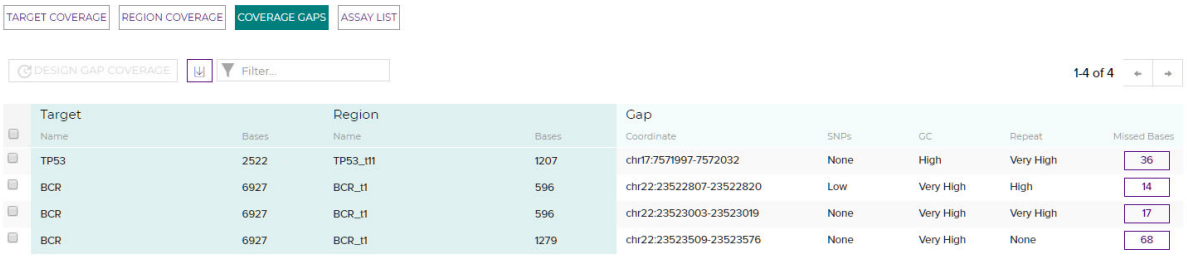
Gap Coordinates: The positions of the starting and ending bases for this gap in coverage.

SNPs: The relative percentage of bases in and around the gap region that are masked for SNPs.

GC: The relative percentage of bases in and around the gap region that have high or low amounts of GC.

Repeat: The relative percentage of bases in and around the gap region that are masked as a repeat.

Missed Bases: The number of bases that comprise the gap.

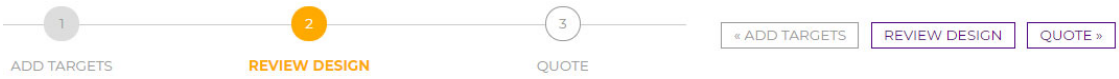


Target		Region		Gap				
Name	Bases	Name	Bases	Coordinate	SNPs	GC	Repeat	Missed Bases
TP53	2522	TP53_t11	1207	chr17:7571997-7572032	None	High	Very High	36
BCR	6927	BCR_t1	596	chr22:23522807-23522820	Low	Very High	High	14
BCR	6927	BCR_t1	596	chr22:23523003-23523019	None	Very High	Very High	17
BCR	6927	BCR_t1	1279	chr22:23523509-23523576	None	Very High	None	68

NOTE For Access Array Target-Specific Primers and Targeted DNA Seq Library Assays, a gap in coverage might result from a number of factors, including the GC% of the region or bases masked for variants or repeats. The gap coverage table provides information that may explain why the gap region is difficult to cover.

Assay List for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays

1 Click **REVIEW DESIGN** in the panel navigation links to display the target design coverage:



2 Click **ASSAY LIST** in the review navigation tabs to review the following items related to all assays in the panel:

Target Name: The title of the target with which this assay is associated.

Assay Name: The title of the assay provided by the design process. The title is subject to change.

Assay ID: The identification number of the assay assigned by processing. The ID cannot be changed and might be the same across multiple targets.

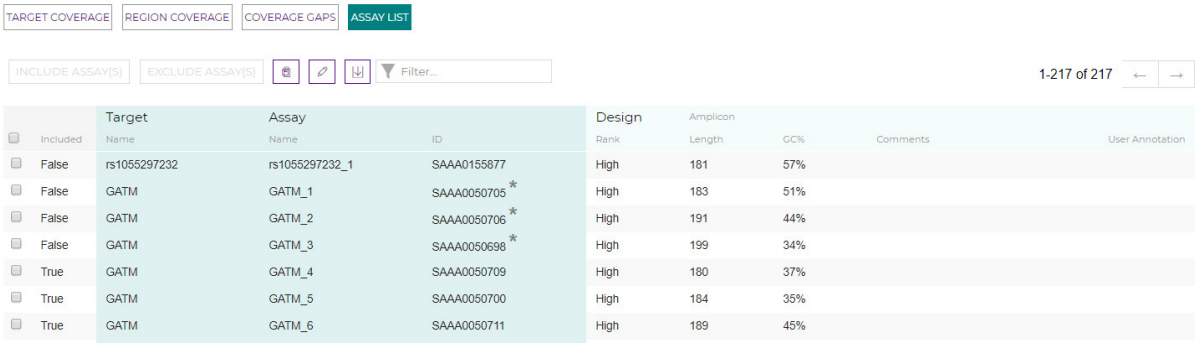
Design Rank: The support level for this assay, based on design parameters.

Amplicon Length: The total length of the amplicon, including primers.

Amplicon GC: The total GC% of the amplicon, including primers.

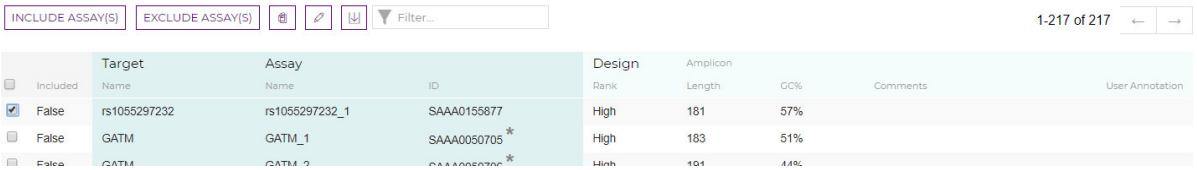
Comments: Information regarding why an assay is ranked nonstandard.

User Annotation: Specific panel information supplied by the user to the assay.

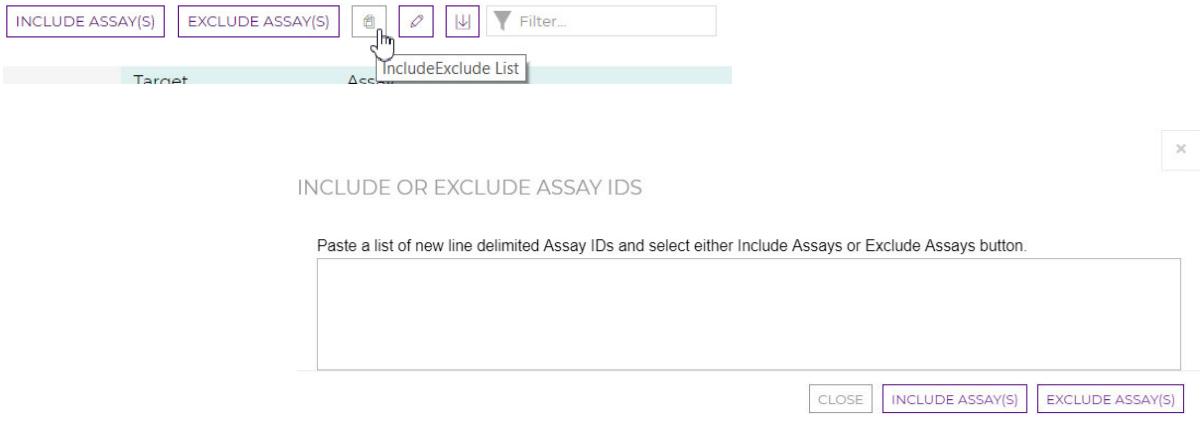


3 Check the boxes of those assays that you want to be:

- Included in the panel and click **INCLUDE ASSAY(S)**.
- Excluded from the panel and click **EXCLUDE ASSAY(S)**.

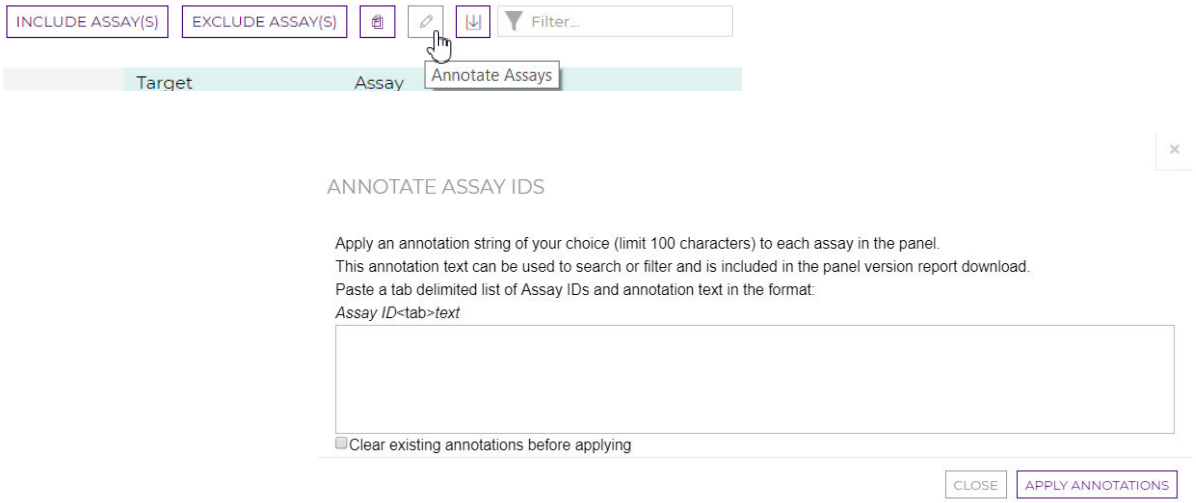


Alternatively, you can click **IncludeExclude List**, copy and paste a list of Assay IDs into the box on the INCLUDE OR EXCLUDE ASSAY IDS screen, and click either **INCLUDE ASSAY(S)** or **EXCLUDE ASSAY(S)** to include or exclude assays in batch.



NOTE For Access Array Target-Specific Primers and Targeted DNA Seq Library Assays, excluding an assay removes it from the panel order and from coverage calculation.

4 Click **Annotate Assays**, copy and paste a tab-delimited list of Assay IDs with associated user-defined comments into the box on the ANNOTATE ASSAY IDS screen, and click **APPLY ANNOTATIONS** to annotate assays in batch.

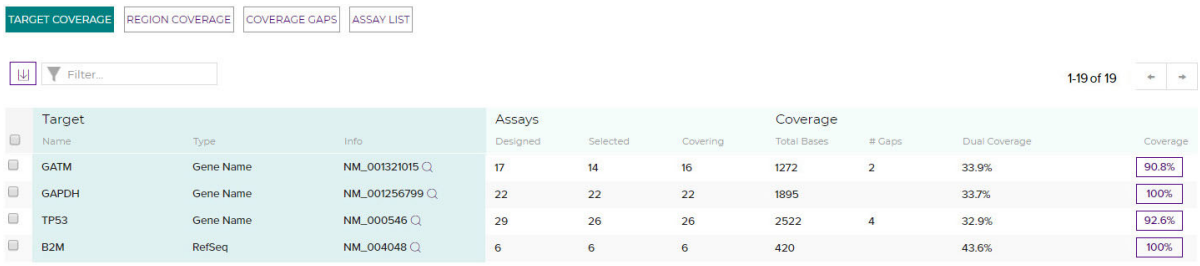


The Target Coverage Display for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays

For Access Array™ Target-Specific primers and Targeted DNA Seq Library Assays, D3 graphically displays the coverage results for each target and each design region within the target. This includes an alignment of the genomic region with all relevant information (SNPs, GC content, and repeat regions) for each assay design. A complete target region and a zoom window enable you to examine coverage at the assay level.

To access the coverage display:

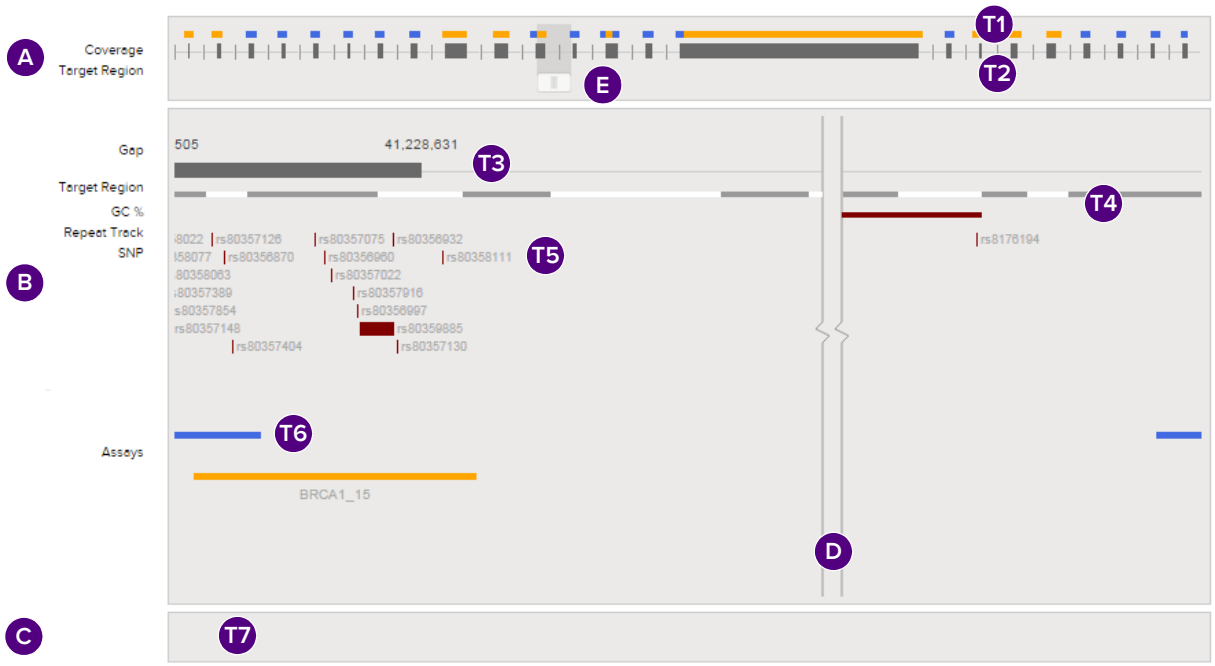
1 Navigate to the Design Review screen of your Access Array Target-Specific Primers or Targeted DNA Seq Library Assays design:



Target			Assays			Coverage			
Name	Type	Info	Designed	Selected	Covering	Total Bases	# Gaps	Dual Coverage	Coverage
GATM	Gene Name	NM_001321015	17	14	16	1272	2	33.9%	90.8%
GAPDH	Gene Name	NM_001256799	22	22	22	1895		33.7%	100%
TP53	Gene Name	NM_000546	29	26	26	2522	4	32.9%	92.6%
B2M	RefSeq	NM_004048	6	6	6	420		43.6%	100%

2 In the Coverage column, click any coverage percentage (at the right) to view coverage results for that target. A multipanel track displays with tracks and panels. For more information, see Coverage Results Track Definitions for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays on page 51.

Coverage Results Track Definitions for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays

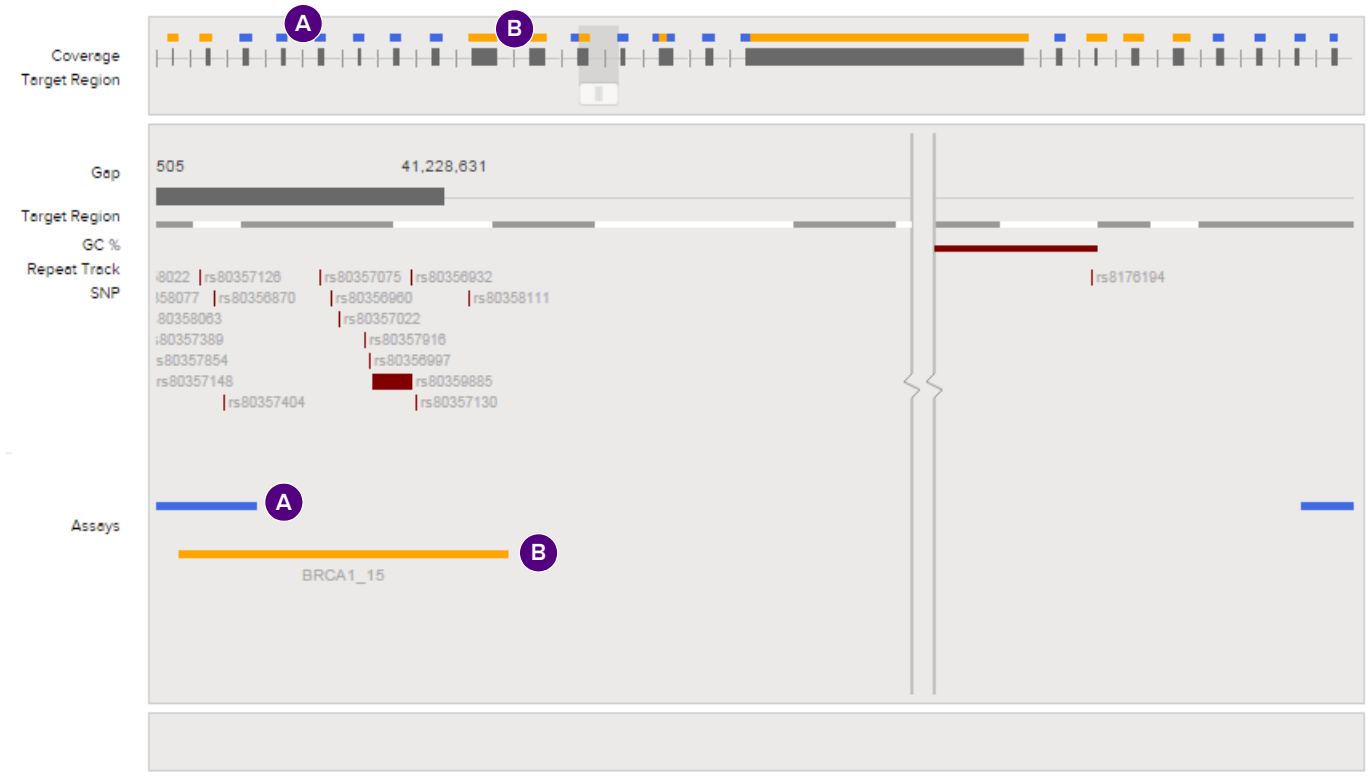


Display tracks in the coverage results:

Label	Definition	Content
A	—	The panel displays target and overall coverage for all selected assays.
B	—	The panel displays zoomed area. By default it covers about three assay lengths.
C	—	The panel lists gaps in zoomed area, with genome coordinates that can be copied and pasted into .txt file.
D	—	Ellipsis punctuations denote the omission of the sequence for the current design.
E	—	The slider bar sizes and aligns to zoomed area.
T1	Overall coverage track	A list of lines is dynamically calculated by the coverage script as the user selects and deselects assays.
T2	Target definition	Regions of targets are defined.
T3	Zoomed target definition	The regions of defined targets are determined by the zoomed coordinates.
T4	GC content	The GC portion is extracted from the full definition by the zoomed coordinates.
T5	SNPs	Relative locations of SNPs are displayed with associated dBSNP IDs.

Label	Definition	Content
T6	Inner amplicon	The inner amplicon (minus primers) of selected assays is displayed within the zoomed coordinates. Inner amplicons displayed in the Target Assays band are assays that are associated with the displayed target and listed in the assay table. Inner amplicons displayed in the Coverage Assays band are amplicons that cover the target but are associated with another target and not displayed in the assay table.
T7	Gap track with gap coordinates	The coordinates and size of a gap are displayed for a region not covered by designs.

Coverage Color Coding Definitions for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays



Label	Definition
A	Standard-ranked assays (blue)
B	Nonstandard-ranked assays (yellow)
—	Not shown: not selected for ordering (light gray)
—	Not shown: gaps in coverage (red)

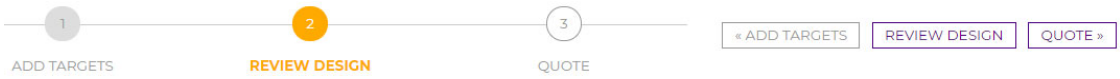
Increase Coverage for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays

Coverage for Access Array Target-Specific Primers and Targeted DNA Seq Library Assays can be increased by resubmitting a design region or gap region to Fluidigm for reprocessing using alternate design parameters.

Increasing Coverage in Design Regions

You can design additional assays to cover new targets in the target design region. The assays designed through this action are be different from the assays already in the panel.

1 Click **REVIEW DESIGN** in the panel navigation links to display the target design coverage:

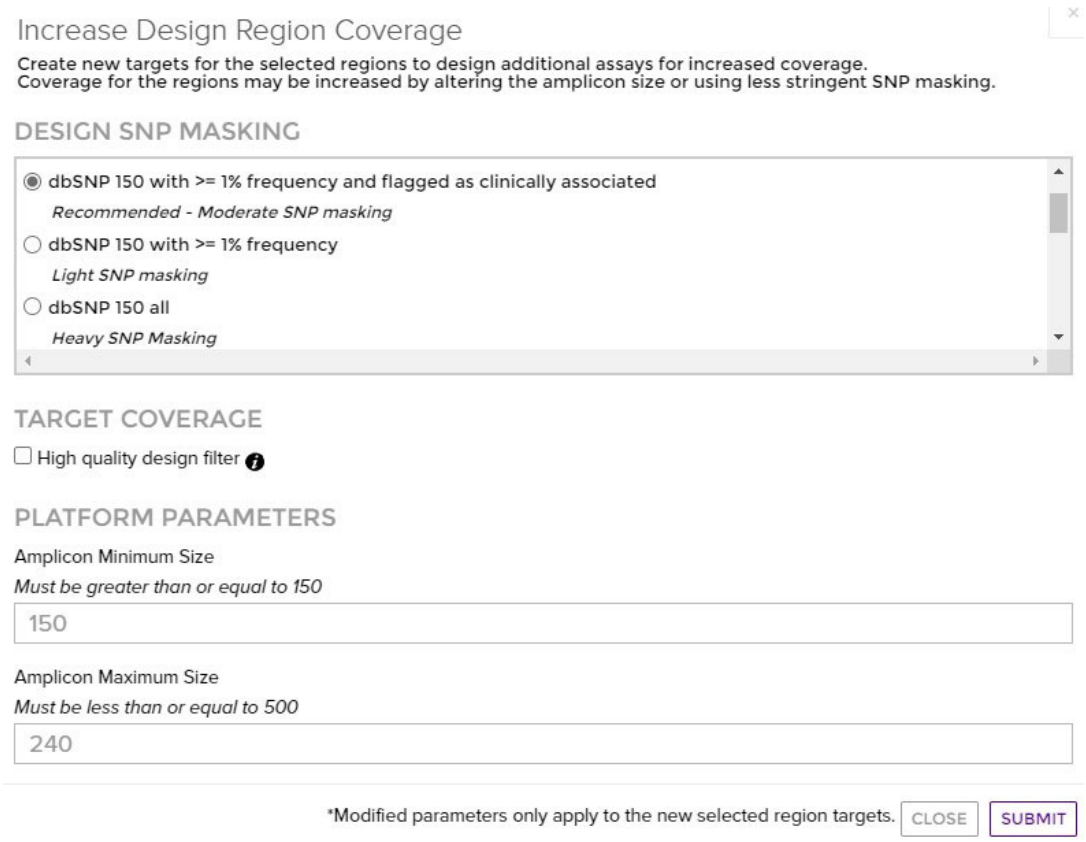


2 Click **REGION COVERAGE** in the review navigation tabs.

3 Check the boxes of those design regions that you want to be included and click **INCREASE COVERAGE**.

4 In the DESIGN SNP MASKING section of the Increase Design Region Coverage screen, select an alternate SNP masking option or adjust the minimum and maximum amplicon sizes.

5 For Targeted DNA Seq Library Assays only: In the TARGET COVERAGE section of the Increase Design Region Coverage screen, select **High quality design filter** to request only assays that have no primers in repeat regions or that amplify more than one genomic region.



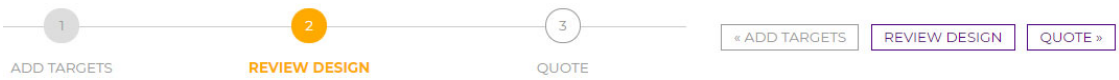
NOTE Changing the parameters increases the likelihood that alternate assays can be designed.

6 Click **SUBMIT** to create the new coordinate targets with the alternate design parameters. The new targets are submitted automatically for design to Fluidigm.

Redesigning Coverage of Design Regions

You can redesign coverage if the current target design region is not acceptable. A new coordinate target is created and submitted for design using your alternate design parameters. Some of the assays designed through this action might match assays that are in the panel already.

1 Click **REVIEW DESIGN** in the panel navigation links to display the target design coverage:

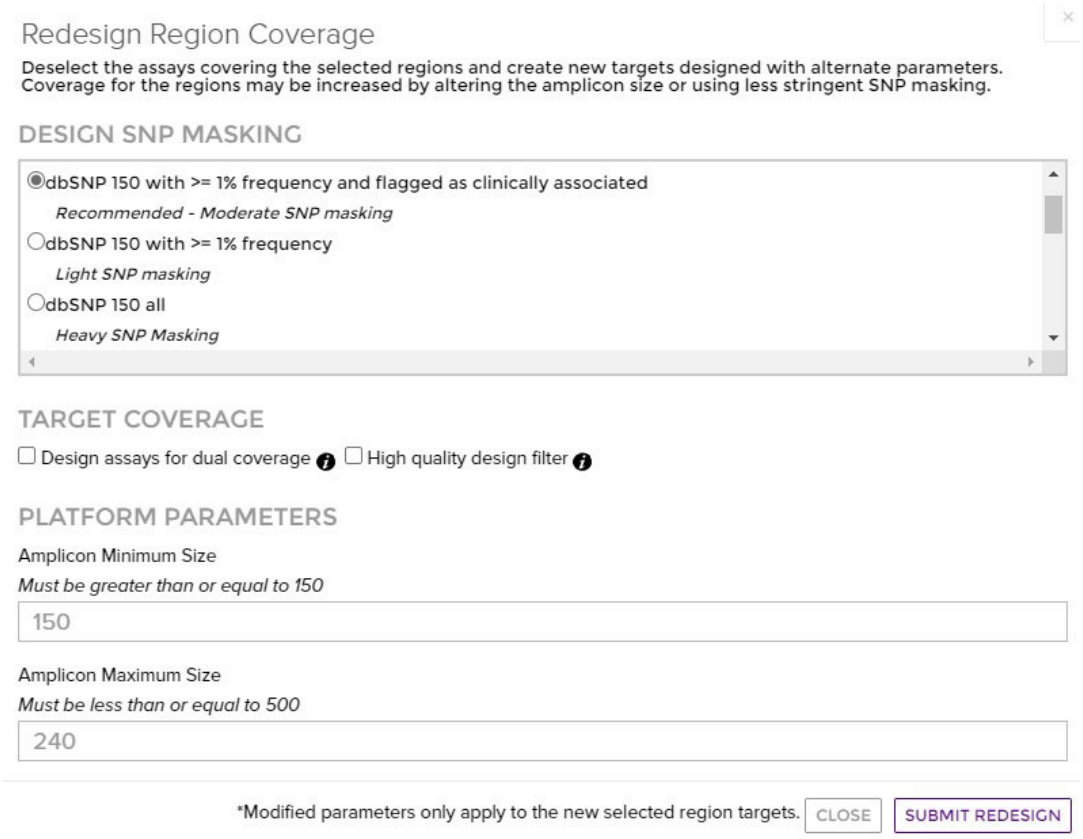


2 Click **REGION COVERAGE** in the review navigation tabs.

3 Check the boxes of those design regions that you want to be redesigned and click **REDESIGN REGIONS**.

4 In the DESIGN SNP MASKING section of the Redesign Region Coverage screen, select an alternate SNP masking option or adjust the minimum and maximum amplicon sizes.

5 For Targeted DNA Seq Library Assays only: In the TARGET COVERAGE section of the Redesign Region Coverage screen, select **Design dual coverage** to request each base in the target to be covered by more than one assay. Select **High quality design filter** to request only assays that have no primers in repeat regions or that amplify more than one genomic region.



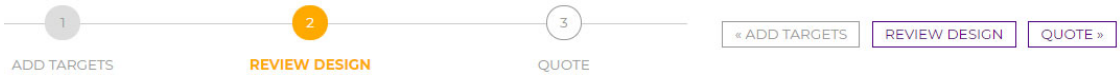
NOTE Changing the parameters increases the likelihood that alternate assays can be designed.

6 Click **SUBMIT** to create the new coordinate targets with the alternate design parameters. The new targets are submitted automatically for design to Fluidigm.

Designing Coverage for Gaps

Using alternate parameters, you can create and submit for design a new coordinate target that covers part or all of a gap region. The existing assays in the panel are avoided so that only new assays are returned if they pass.

1 Click **REVIEW DESIGN** in the panel navigation links to display the target design coverage:

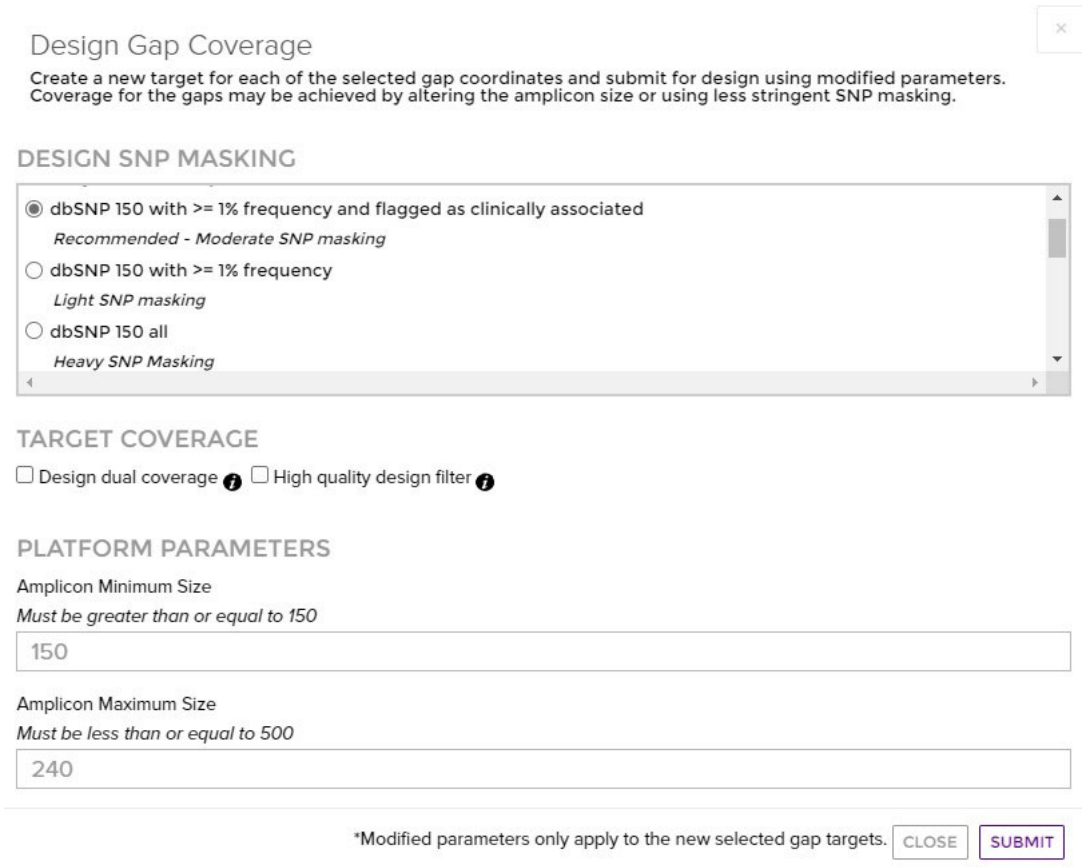


2 Click **COVERAGE GAPS** in the review navigation tabs.

3 Check the boxes of those design regions that you want to be included and click **DESIGN GAP COVERAGE**.

4 In the DESIGN SNP MASKING section of the Design Gap Coverage screen, select an alternate SNP masking option or adjust the minimum and maximum amplicon sizes.

5 For Targeted DNA Seq Library Assays only: In the TARGET COVERAGE section of the Design Gap Coverage screen, select **Design dual coverage** to request each base in the target to be covered by more than one assay. Select **High quality design filter** to request only assays that have no primers in repeat regions or that amplify more than one genomic region.



NOTE Changing the parameters increases the likelihood that alternate assays can be designed.

6 Click **SUBMIT** to create the new coordinate targets with the alternate design parameters. The new targets are submitted automatically for design to Fluidigm.

Optimizing Your Design

Our aim is to help you to optimize all aspects of your designs, so we invite you to request assistance with them. Click **REQUEST ASSISTANCE** at the left of the screen and read D3 Best Practices on page 40.

Preparing an Order

D3 offers the convenience of arraying assays using either default parameters or your customized layout in standard 96-well plates. Plated assays allow the use of multichannel pipettes to reduce pipetting steps, significantly reducing your effort and minimizing the possibility of pipetting errors.

Requesting a Quote

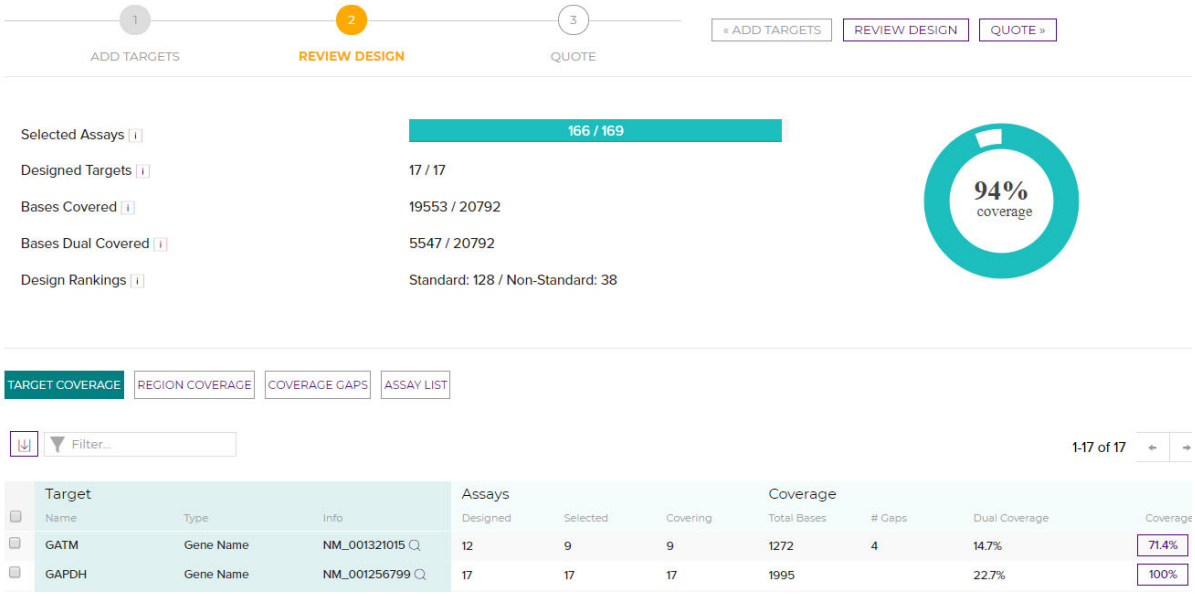
When the design review step is complete, you are notified by email. You can now either:

- Request a quote. Your local Fluidigm sales representative or distributor will provide you with a quote and handle all the ordering logistics. When you submit a purchase order (PO), the manufacture of your assays commences.
- If you have established a blanket PO with Fluidigm, you can request that your order be processed without a quote. Your local Fluidigm sales representative or distributor will handle all the ordering logistics.

To request a quote:

1 Follow the link in the notification email to access the project/version that is ready for your review.

2 If you are satisfied with the assays, click **QUOTE** at top-right of your screen:



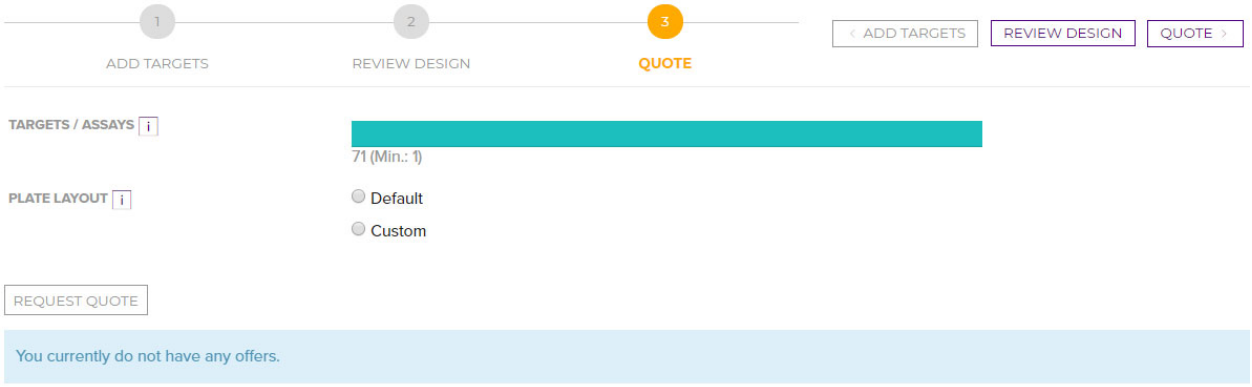
NOTE Click **QUOTE** to begin ordering. The first step in the order process is to request a price quote. Assays are not manufactured until a P.O. is received.

3 Select the plate layout.

Plate Layouts

The Default Plate Layout for SNP Type or Delta Gene

The default (standard) layout places your assays onto plates column by column, sorted alphabetically by target name.



1 To accept the default layout, click **Default**.

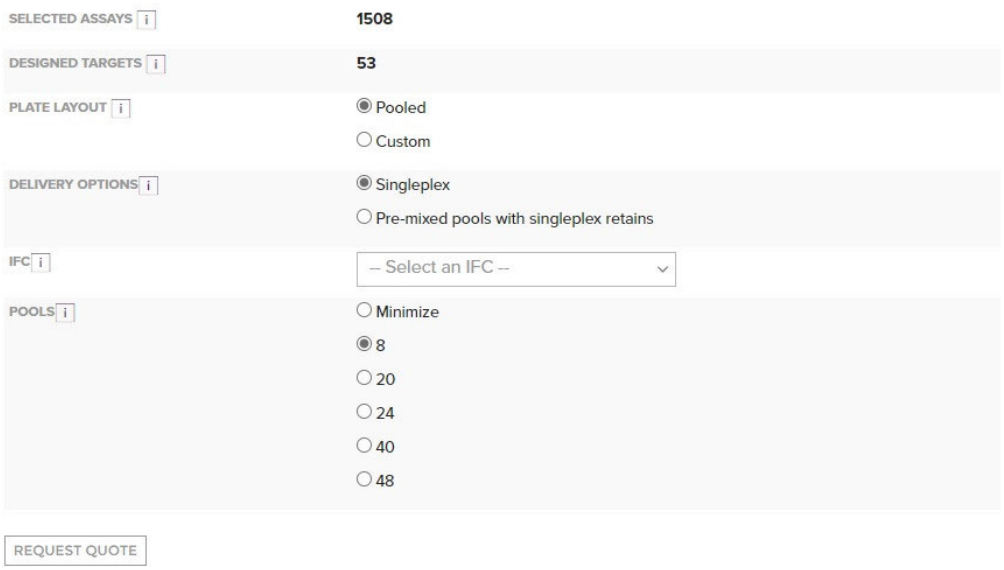
2 Click **REQUEST QUOTE**.

The Pooled Layout for Targeted DNA Seq Library Assays

The pooled layout option orients the selected assays into pools for each pooling algorithm that Fluidigm uses. For projects that are ≤2,400 amplicons, 20 or 24 pools can be provided. For projects that are >2,400 amplicons, 40 or 48 pools are mixed. Pooled assays can be delivered pre-mixed in tubes or in well plates.

NOTE

- There is an additional cost for pre-pooling the assays in tubes.
- The Plate Layouts received from informatics packets that contain empty wells (without TSP assays) are shown as buffer. The empty wells contain buffer for secondary QC purposes.



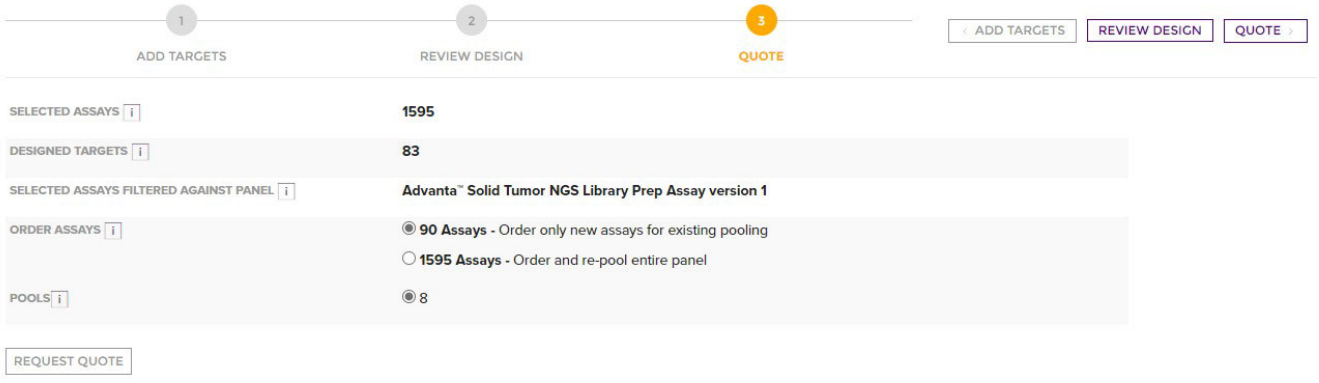
1. To accept the pooled plate layout, click **Pooled**.
2. Click the delivery option **Singleplex** or **Pre-mixed pools with singleplex retains**.
3. (Optional) Select an IFC to filter the pools options so that only selections that are compatible with the target IFC are included.
4. Select the desired number of pools into which the selected assays are distributed. (The Minimize option pools the assays into the smallest number of pools that can be achieved.)
5. Click **REQUEST QUOTE**.

Fluidigm Panel Customization for Targeted DNA Seq Library Assays

Customizations to a Fluidigm Targeted DNA Seq Library Assays panel can be ordered as additions to the product pools of an existing panel or ordered as an entirely new (and complete) panel. Generation of a quotation for a customized panel requires the selection of the assay order type:

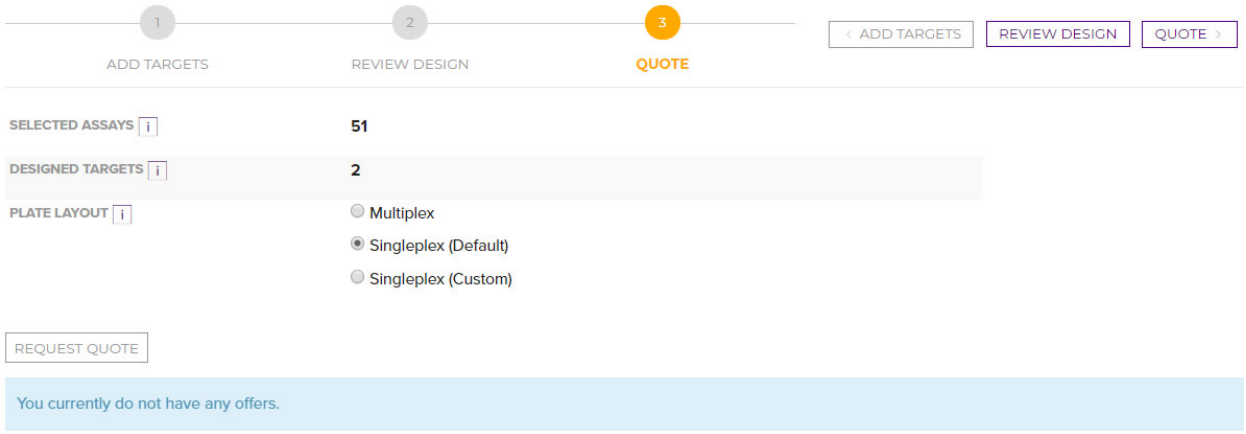
- **Ordering new assays for existing product pools.** Product pooling is used to determine the optimal pool into which each assay should be added. The order includes only assays that are not included in the catalog product. The assays are delivered in singleplex plates that are laid out for easy pipetting into the appropriate pools.
- **Ordering a new panel, complete with all product assays.** This option should be used if any assays or targets have been removed from the product panel or if the number of desired pools is different from the number listed in the catalog. All assays in the panel will be re-pooled and delivered based upon the layout selections.

  NOTE Fluidigm Advanta™ panels are optimized through multiple iterations of sequencing analysis, including the increase in concentration of certain lower-performing assays. These optimizations are not considered when re-pooling, and panel optimization might need to be performed. For example:



The Multiplex Layout for Access Array Target-Specific Primers

The default layout option for Access Array Target-Specific Primers is multiplexed into pools. The internal algorithm by Fluidigm groups the selected assays in pools. The pools are distributed row by row across one or more well plates.



1 To accept the multiplexed layout, click **Multiplex**.

2 Click **REQUEST QUOTE**.

The Singleplex Layout for Access Array Target-Specific Primers

The singleplex layout places your assays onto plates column by column, sorted alphabetically by target name.

1 To accept the Singleplex layout, click **Singleplex (Default)**.

2 Click **REQUEST QUOTE**.

The Custom Plate Layout

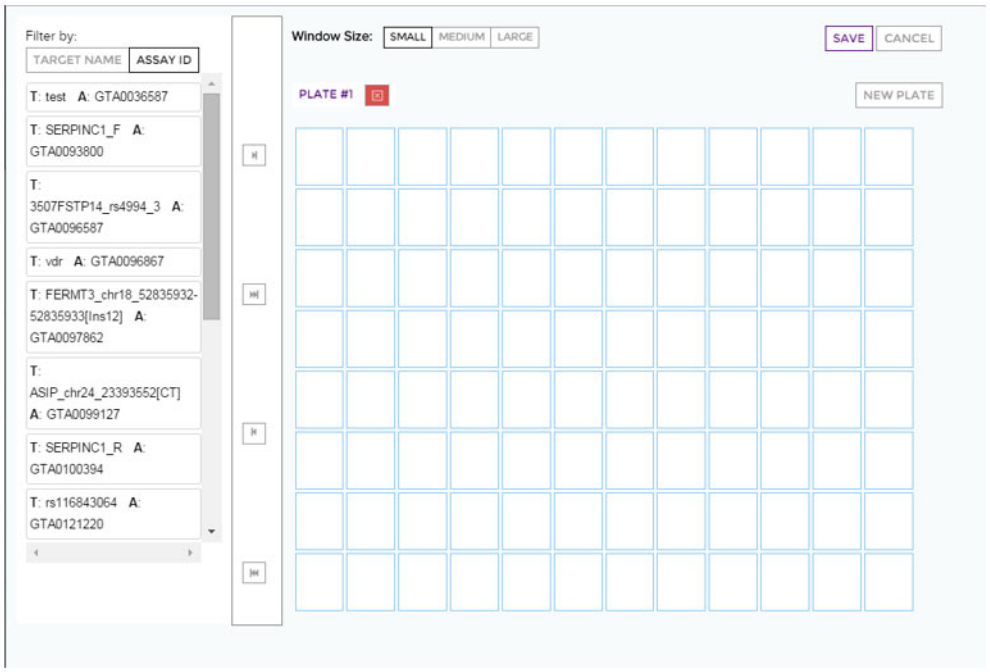
The Custom layout allows you to assign your assays to specific wells. When you order multiplexed Access Array Target-Specific Primers, Fluidigm determines the optimal array of your assays for your multiplex PCR.

IMPORTANT

- Before you can request a quote, you must assign all assays to well positions.
- For Targeted DNA Seq Library Assays, the plates are renamed pools.
- To conform to the configuration of Fluidigm IFCs, you must plate by columns, not by rows.

1 To create a custom layout, select **Singleplex (Custom).**

2 Click **EDIT** to display a 96-well plate template:



3 (Optional) Click **TARGET NAME** or **ASSAY ID** (the default) in the left pane to filter the set of targets or assays.

4 (Optional) Click a window size (**SMALL**, **MEDIUM**, or **LARGE**) to change the size of the window. The default is SMALL.

5 (Optional) Click **NEW PLATE** add more plates. The default is PLATE #1. For more information, see Multiple Plates on page 62.

6 Assign targets (assays) to well locations vertically (by column) in the following order:

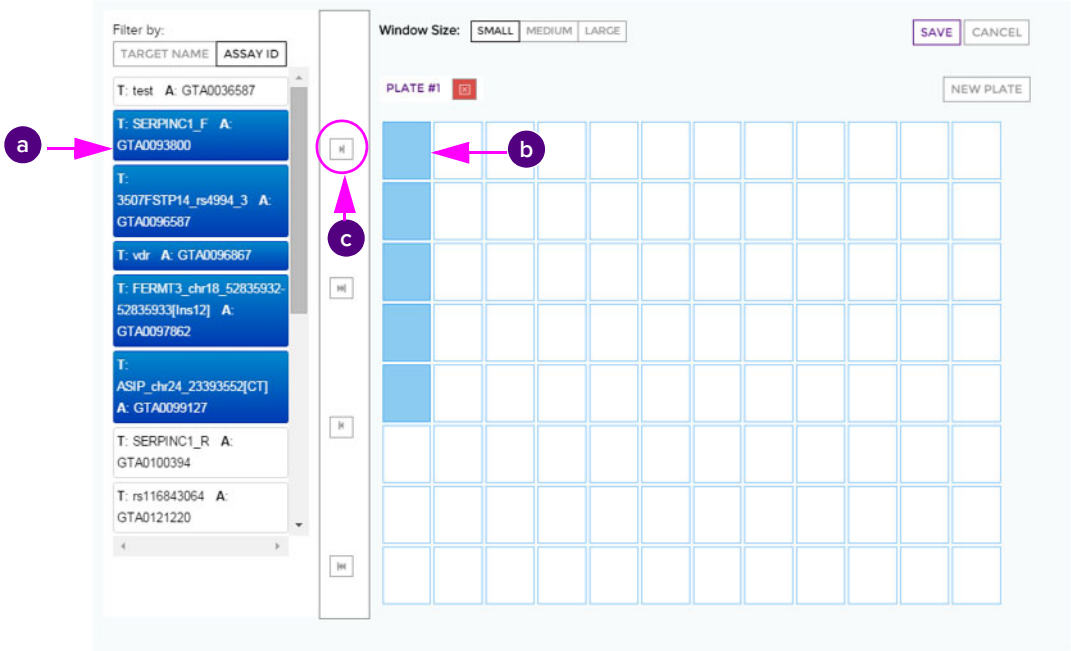
a. Drag-select or Ctrl-select multiple targets.

b. Drag-select or Ctrl-select well locations vertically (by column).

c. Click one of the following command tools that assign assays to wells:

- Click ⏭ to assign selected assays to specific well locations. The first selected assay is assigned to the well you specified, and all other selected assays are assigned vertically (by column) after the first selected well location.
- Click ⏭ to automatically assign assays to unselected well locations starting at the first available well.

You can click ⏮ to remove one or more selected assays or click ⏮ to remove all assays without selecting them first.



7 When all assays are assigned to wells, click **SAVE**. (Delete all empty plates before saving.)

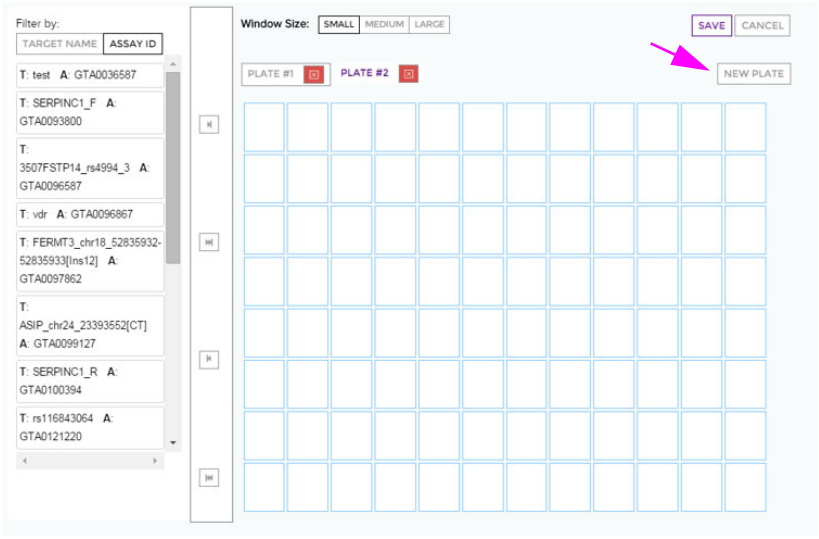
8 Click **REQUEST QUOTE**.

IMPORTANT The version is now locked and cannot be edited. To edit targets, assays, or the layout, you can only copy the version (click **Copy Version**) and edit the copy.

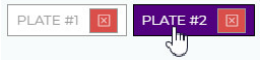
Multiple Plates

You can add assays to as many plates as you require:

1 Click **NEW PLATE** to add another plate:



Click the plate to display. For example, to see the contents of plate 2, click **Plate #2**:



2 Repeat Step 1 at any time to add as many plates as you want.

3 On each plate, assign targets (assays) to well locations vertically (by column), as described in Step 6 of The Custom Plate Layout on page 60. When you complete plate layout, click **SAVE**. (You are now ready to place your order by requesting a quote. See Placing Your Order on page 65.)

Panel Redesign

Creating a new version of an ordered panel enables you to redesign poor performing assays or to add design regions to the panel. Panel redesign begins by creating a new version of the panel or a new panel from a copy.

Creating a New Panel from an Existing Panel

1 Click **+NEW PANEL** from the panels list.

2 Select the assay type and click **NEXT**.

3 Select from the available redesign options:

- Click **New Panel From Existing** to create a new panel by copying the contents of an existing panel.
- Click **Revise Panel** to create a new version of the panel.

4 (Optional) If the selected panel version was ordered already, you can accept to filter the source panel assays when ordering. See Filtering the Assays in Your Order for a Resigned Panel on page 63.

5 If you are creating a new panel, enter a panel name and click **FINISH**.

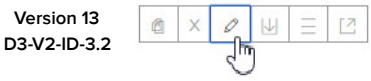
The new panel version displays and is ready for target changes (addition, removal, or redesign).

Filtering the Assays in Your Order for a Resigned Panel

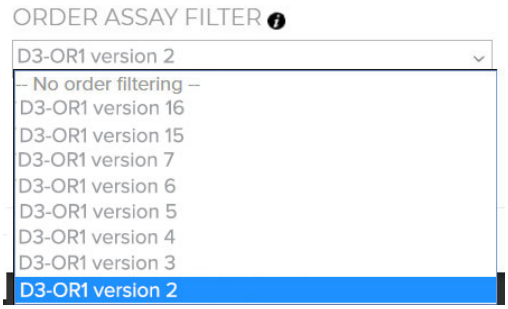
When redesigning a previously ordered panel, you can remove existing assays from the ORDER ASSAY FILTER screen. These assays are included in the panel during the design phase but are not added to the quotation when ordering.

1 Open a version of a previously ordered panel.

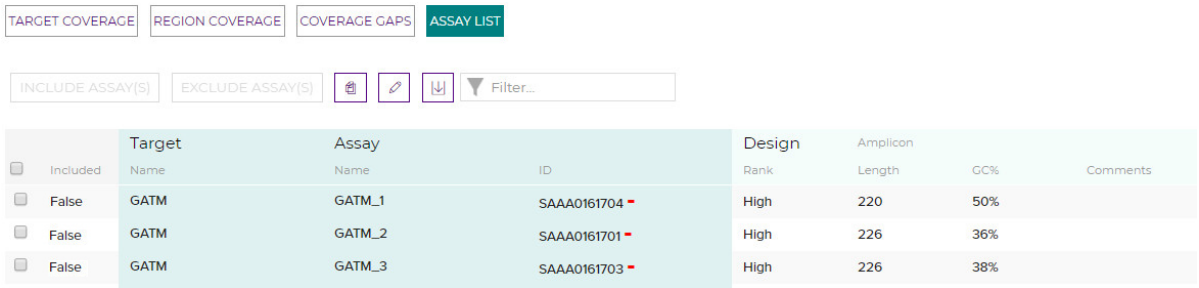
2 Click the version icon (**Edit Panel Version**). For example:



3 Select the source panel version to be used to filter the assays. For example:



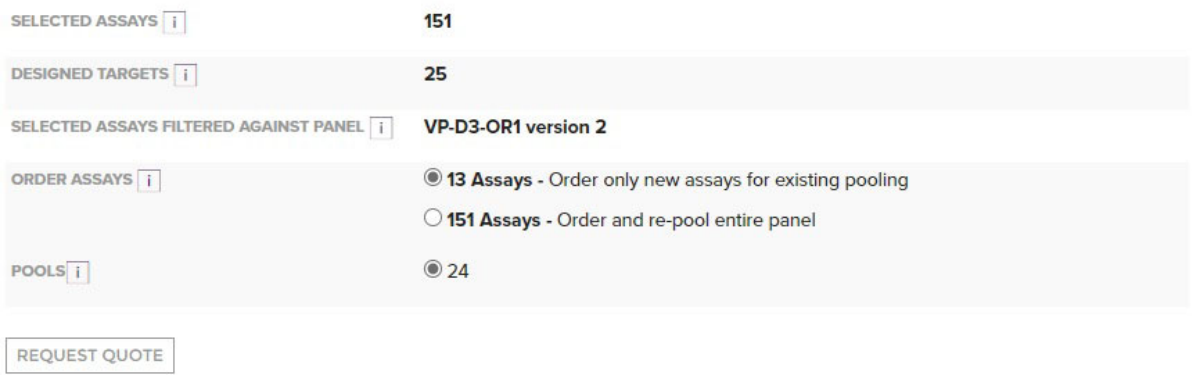
4 Click **DESIGN REVIEW** and click **ASSAY LIST** to review the filtered assays. Assays that are marked with the red mark ▬ after the ID will not be ordered. For example:



5 Redesign or add new targets to the version. (The count of new assays that do not exist in the filtered panel are displayed in the Panel Design summary.)

6 Click **QUOTE** at the upper-right. The quotation can be registered for (a) only the new assays for the existing pool or for (b) the entire panel with a new pooling scheme.

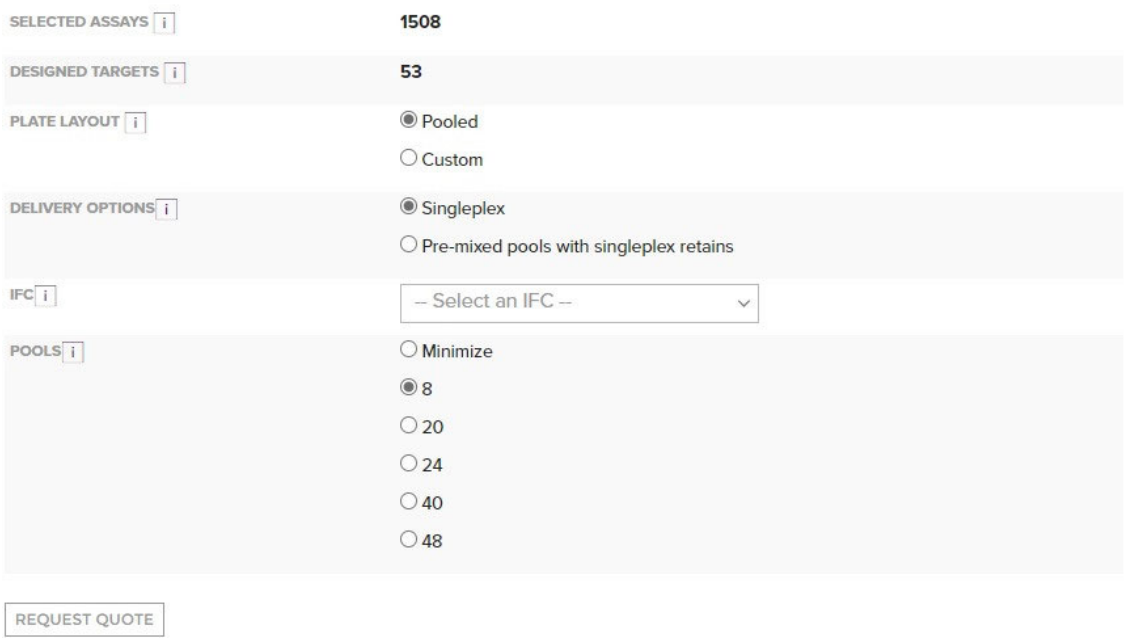
For example:



Placing Your Order

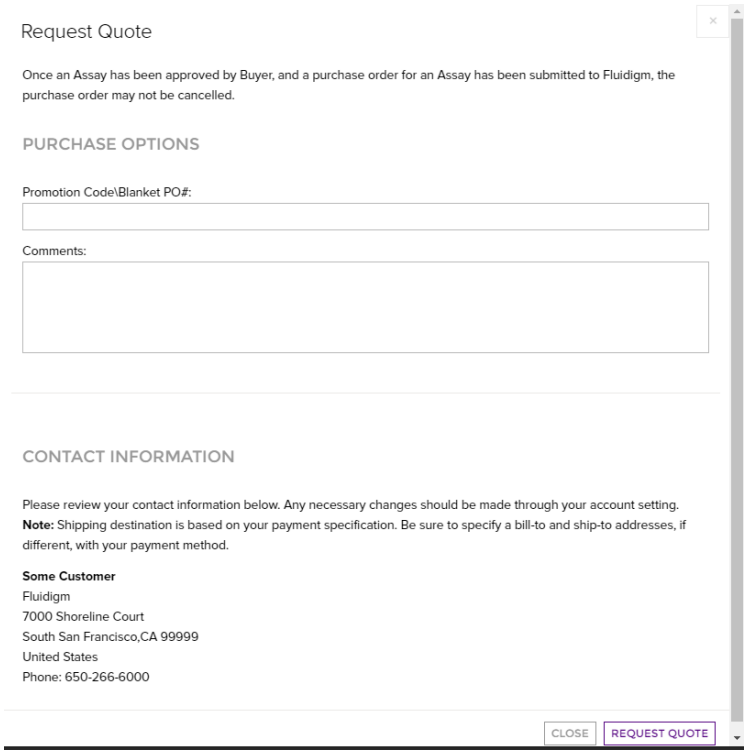
An order can be placed only after design review of your assay is complete. After you select your targets and send a design review request, you will be notified within 48–72 hours that review is complete. At that point you can iteratively add or modify targets with requested reviews if you want to improve coverage. Your design is ready to order when the targets meet your satisfaction, failed targets are removed, and minimum requirements are met.

To begin the order placement process, click **REQUEST QUOTE**:



NOTE You can include notes and promotional codes and verify your contact information before you request a quote for your project.

On the screen that appears, several purchase options are available:



- Promotion Code\Blanket PO#: If you have a valid promotional code or a blanket P.O. number, enter it here. To expedite processing, submit only valid promotional codes or established blanket P.O. numbers with Fluidigm.
- Comments: You can include notes for your order. For example, you can enter the following note: “Please include a quote for 10 units of part number AA-M-48.48.”
- Volume (SNP Type™): Specify x-small (50 µL), small (100 µL), medium (200 µL), or large delivery (400 µL). You can only see this option with SNP Type assays.
- Wet-Tested (Access Array/DELTAgene): Fluidigm offers an optional wet-lab test for Access Array and Delta Gene™ assays. This option results in an additional charge and two additional weeks of turnaround time.
- Contact information: Review your contact information.

IMPORTANT To avoid delays with processing and quote generation, include only valid Fluidigm promotional codes or valid blanket P.O. numbers.

Changing Your Order

When you click **ORDER** to submit a request for a quote, the version that is associated with your order becomes locked and cannot be edited. If you decide not to purchase the assays as presented in the locked version and you want to change your order:

1 Create a new version or the panel from the locked version using the Revise Panel option in the procedure Creating a New D3 Panel on page 13.

2 Edit the new version or panel.

3 Click **ORDER** to submit a new request for a quote.

Tracking Your Status

The status of your design or order is updated in D3 automatically. Update notices include:

Status	Notification
DESIGN	Created: An empty project has been created without targets.
	In Progress: Targets have been added and/or targets have been submitted for design.
	Completed: Designs are completed and ready for your review.
ORDER	Quote Request Submitted
	Quote Request Received
	P.O. Processed
	Shipped

Contact sales.admin@fluidigm.com for an update on your order.

When to Expect Delivery

Typically, assays that are not wet-tested are delivered within 4 weeks; wet-tested assays are delivered within 6 weeks.

An informatics packet is emailed at the time of product shipment. It includes primer sequences, plate layout, and instructions on where to download supporting documents, such as user guides and quick references. Informatics packets are also available online within your D3 orders.

Timely updates are provided through the D3 website while assays are being manufactured. An update is generated immediately upon the P.O. being processed and subsequently when assays are shipped.

Reordering an Assay

You can reorder a previously purchased assay:

1 Select the specific version of the assay in your order history tab.

2 Copy the version to a new version or product.

3 Request a quote for the new version or product.

Appendix A: Glossary

For an exhaustive glossary of genomics terms, visit genome.gov.

Term or Acronym	Definition
Base pair (bp)	Two chemical bases bonded to one another forming a rung of the DNA ladder
CDS	Coding sequence
Ensembl	Genome browser, online at ensembl.org
Exon	Portion of a gene that codes for amino acids
Gene expression	Process by which the information encoded in a gene is used to direct the assembly of a protein molecule. The cell reads the sequence of the gene in groups of three bases. Each group of three bases (codon) corresponds to one of 20 different amino acids used to build the protein.
Inner amplicon	The region of DNA amplified between two synthetic primers (does not include the primers)
Intron	Non-coding portion of a gene
Marker	DNA sequence with a known physical location on a chromosome
Multiplexing	Simultaneous analysis of more than one target in the same reaction. Specific quantification of multiple targets that are amplified within a reaction can be performed using a differentially labeled primer or probes. Amplicon or probe melting curve analysis allows multiplexing in allelic discrimination if a dsDNA-binding dye is used as the detection chemistry.
NCBI	The National Center for Biotechnology Information, online at ncbi.nlm.nih.gov.
Phenotype	An observable characteristic displayed by an organism
Primer	A short, single-stranded DNA sequence used in PCR. In the PCR method, a pair of primers is used to hybridize with the sample DNA and define the region of the DNA to be amplified.
RefSeq	NCBI Reference Sequence Database, a collection providing a comprehensive, integrated, non-redundant, well-annotated set of sequences, including genomic DNA, transcripts, and proteins
SNP	Single-nucleotide polymorphism, a single base difference found when the same DNA sequence from two different individuals is compared
Total amplicon	The PCR product resulting from the amplification of DNA by two synthetic primers (includes primer)
TSS	Transcription start site
UTR	Untranslated region
Wild type	The non-mutant form of a gene encoding the normal genetic function. Generally, but not always, a dominant allele.

Appendix B: IUPAC Codes

The following table lists the IUPAC standard representation of DNA bases by single characters that specify either a single base (for example, G for guanine, A for adenine) or a set of bases (for example, R for either G or A).

Symbol	Meaning
G	G
A	A
T	T
C	C
R	G or A
Y	T or C
M	A or C
K	G or T
S	G or C
W	A or T
H	A or C or T
B	G or T or C
V	G or C or A
D	G or A or T
N	G or A or T or C

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For technical support visit
fluidigm.com/support.