USER GUIDE

ion torrent
by Thermo Fisher Scientific

Ion AmpliSeq™ Designer: Getting Started

Uploading custom references and creating new research assays and fusion designs for use with v5.0 Software

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Revision D

October 5, 2015

For Research Use Only. Not for use in diagnostic procedures.

About This Guide

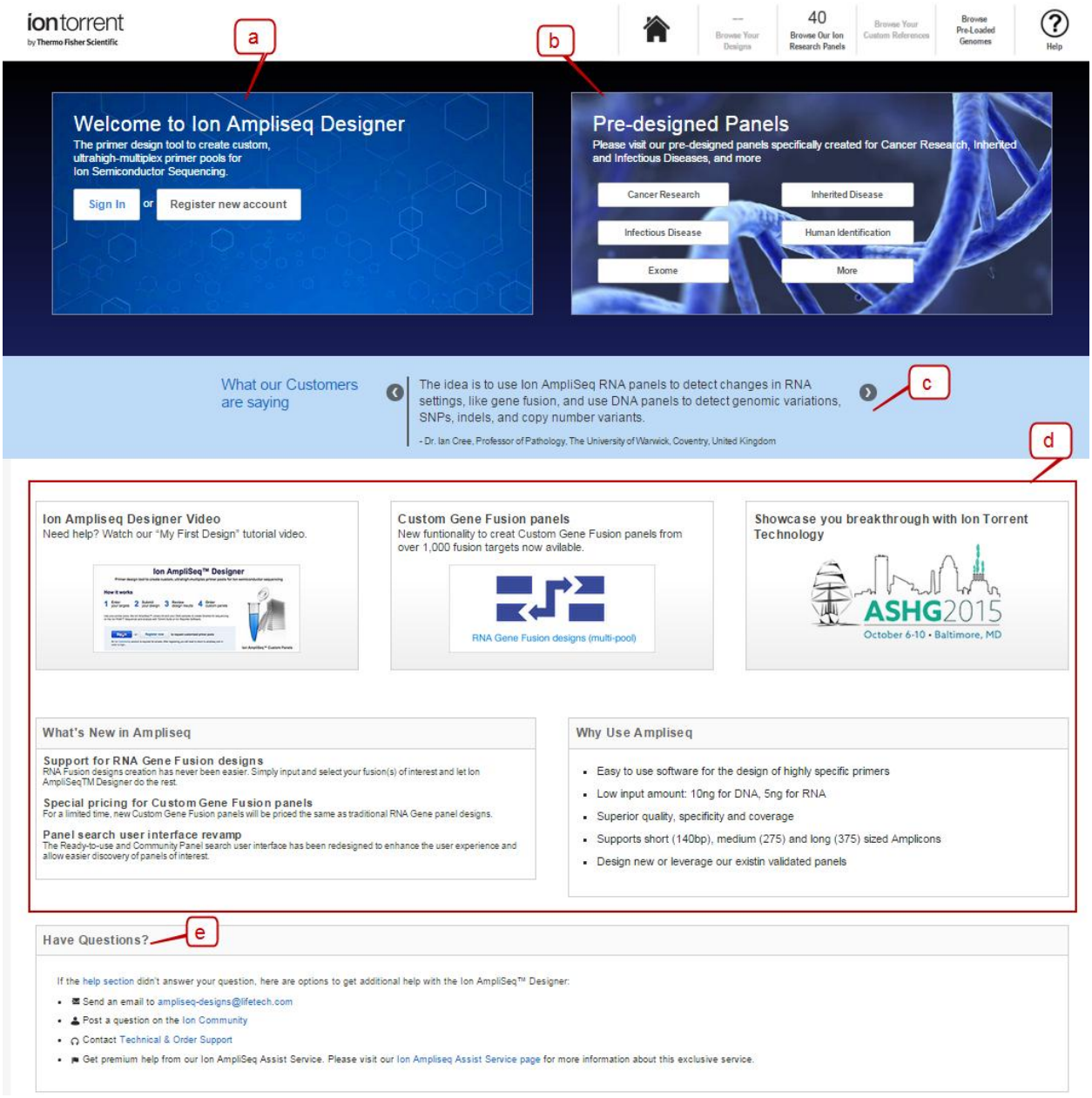
Changes from previous version

Revision	Date	Description
A.0	September 12, 2014	Original
B.0	May 27, 2015	RNA Gene Fusions added
C.0	June 23, 2015	Note added about required primers for RNA Gene Fusion designs
D.0	October 5, 2015	User interface changes implemented to ease customer use, added chip coverage calculator

Contents

Custom or Pre-designed Panels

The ampliseq.com website has been redesigned to improve your panel design experience. If just browsing for panel design information or the latest news, there is no need to sign in. From the landing page, you will see two main options for beginning a panel design: Custom and Pre-designed. Below are news updates and a help section.



a. Sign in to begin custom designs.

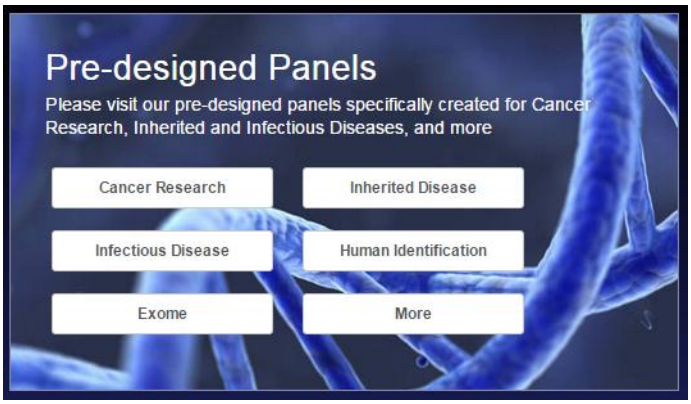
b. Browse to see pre-designed panel options (formerly known as Ready-to-Use and Community panels).

c. What customers are saying about AmpliSeq™ Designer.

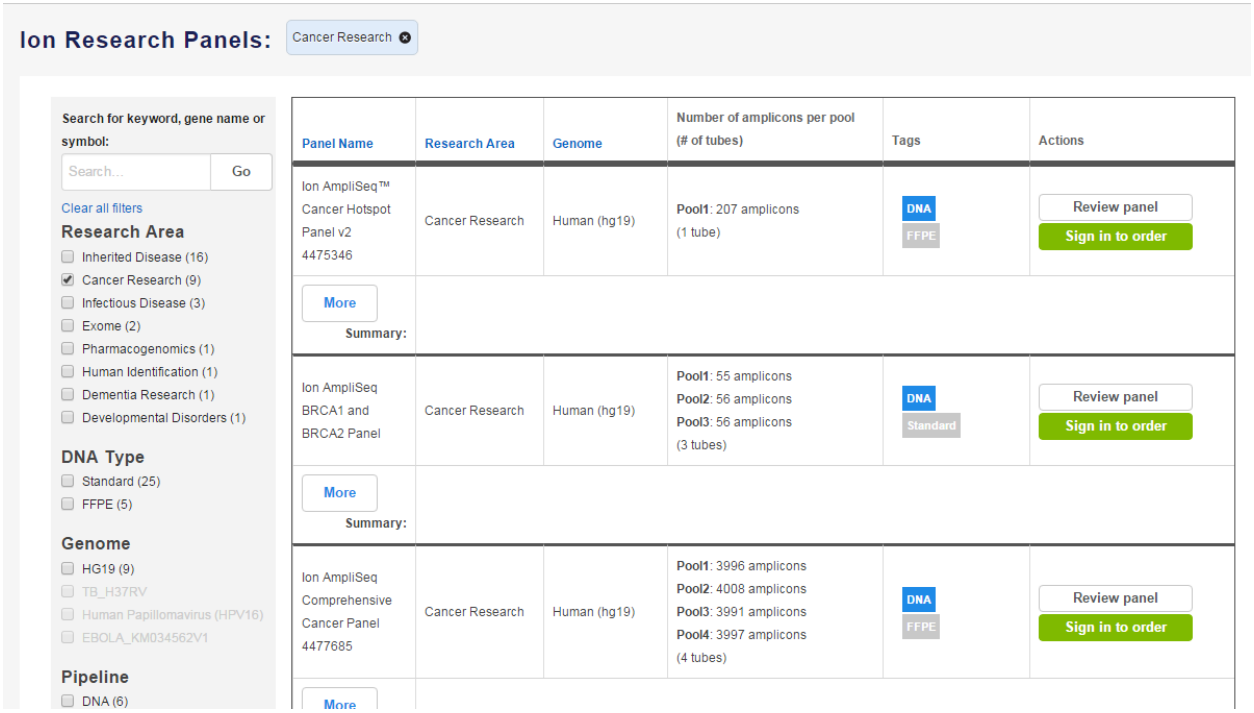
d. News, training and events.

e. Access to Help section, frequently asked questions and customer support.

To browse Pre-designed Panels, click your area of interest, such as Cancer Research.



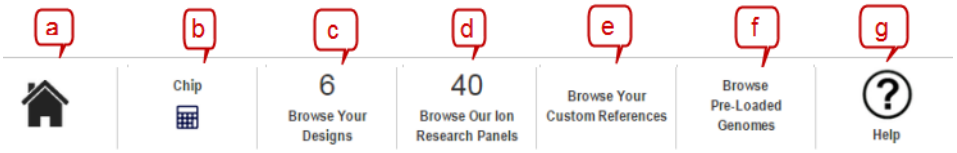
You will now see a table displaying cancer research panels and their descriptions.



To see other categories, check the other research areas to expand your list.

Navigation Bar

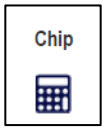
Once you log into AmpliSeq™ Designer, notice there is a new navigation bar.



a. Home - Takes you to starting point for creating a custom panel design.
b. Chip Calculator – Allows you to roughly calculate amplicon coverage for a variety of applications.
c. Browse Your Designs – Allows you to see the designs you have already created.
d. Browse Our Ion Research Panels – Takes you to page listing all available Ion and Community panels and their descriptions.
e. Browse Your Custom References – Provides access to all custom references you have uploaded to your account.
f. Browse Pre-Loaded Genomes – Takes you to list of Public Genomes available through AmpliSeq™ Designer and any custom references you have uploaded.
g. Help – Takes you to the customer help page.

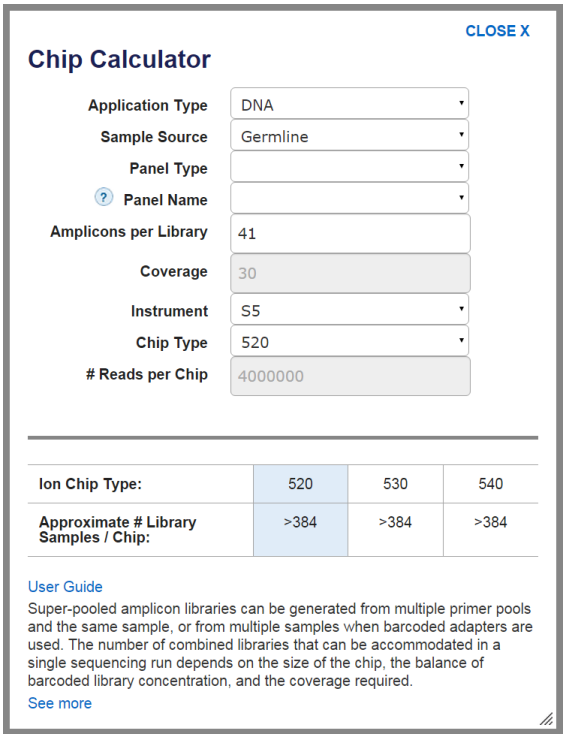
Ion AmpliSeq™ Chip Calculator

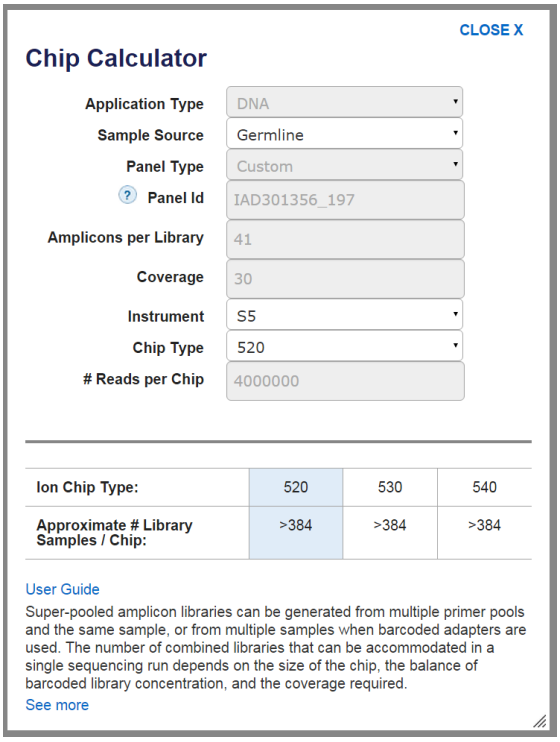
New in Ion AmpliSeq™ v5.0, you can use a chip coverage calculator to aid your design process. You can access this calculator either by the navigation bar at the top of the screen, or on individual panel pages.



The navigation bar version of the calculator is good for obtaining rough estimates of amplicon coverage for a variety of application and chip types. As you proceed down the information list, the drop-down options become application-specific. (See calculator example on the left image below.) Also note, instrument selection changes the Chip options in the lower table.

The panel-specific chip calculator (right image below) contains pre-populated fields pertaining to that panel.

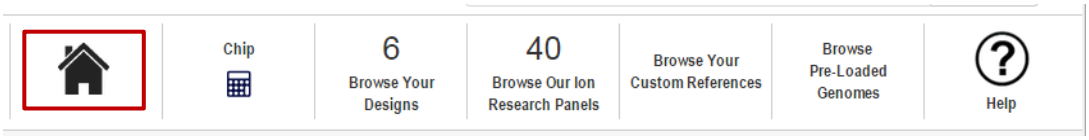

CLOSE X
Chip Calculator
Application Type
DNA
Sample Source
Germline
Panel Type
Panel Name
Amplicons per Library
41
Coverage
30
Instrument
S5
Chip Type
520
Reads per Chip
4000000
Ion Chip Type:
520
530
540
Approximate # Library Samples / Chip:
>384
>384
>384
User Guide
Super-pooled amplicon libraries can be generated from multiple primer pools and the same sample, or from multiple samples when barcoded adapters are used. The number of combined libraries that can be accommodated in a single sequencing run depends on the size of the chip, the balance of barcoded library concentration, and the coverage required.
See more


CLOSE X
Chip Calculator
Application Type
DNA
Sample Source
Germline
Panel Type
Custom
Panel Id
IAD301356_197
Amplicons per Library
41
Coverage
30
Instrument
S5
Chip Type
520
Reads per Chip
4000000
Ion Chip Type:
520
530
540
Approximate # Library Samples / Chip:
>384
>384
>384
User Guide
Super-pooled amplicon libraries can be generated from multiple primer pools and the same sample, or from multiple samples when barcoded adapters are used. The number of combined libraries that can be accommodated in a single sequencing run depends on the size of the chip, the balance of barcoded library concentration, and the coverage required.
See more

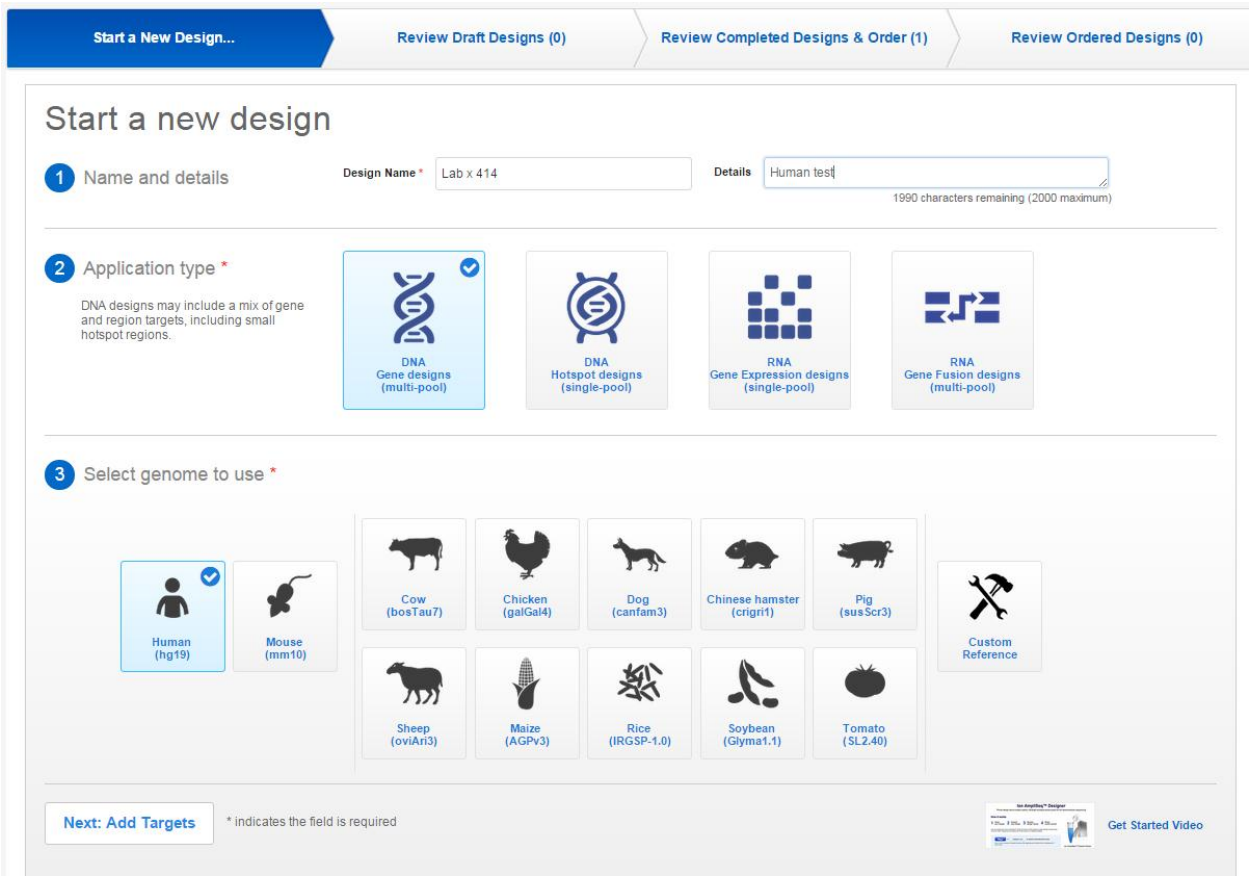
Start a new design

If you are using one of the AmpliSeq™ standard genome references, starting a new design is a relatively simple process. The redesigned website has eliminated some screens to speed the process.

If you are not on the home page, click the "home" icon in the navigation bar to return to the main page.



1. Enter a Design Name and, optionally, details. The *Start a new design* page now expands to add additional elements for creating a new panel.

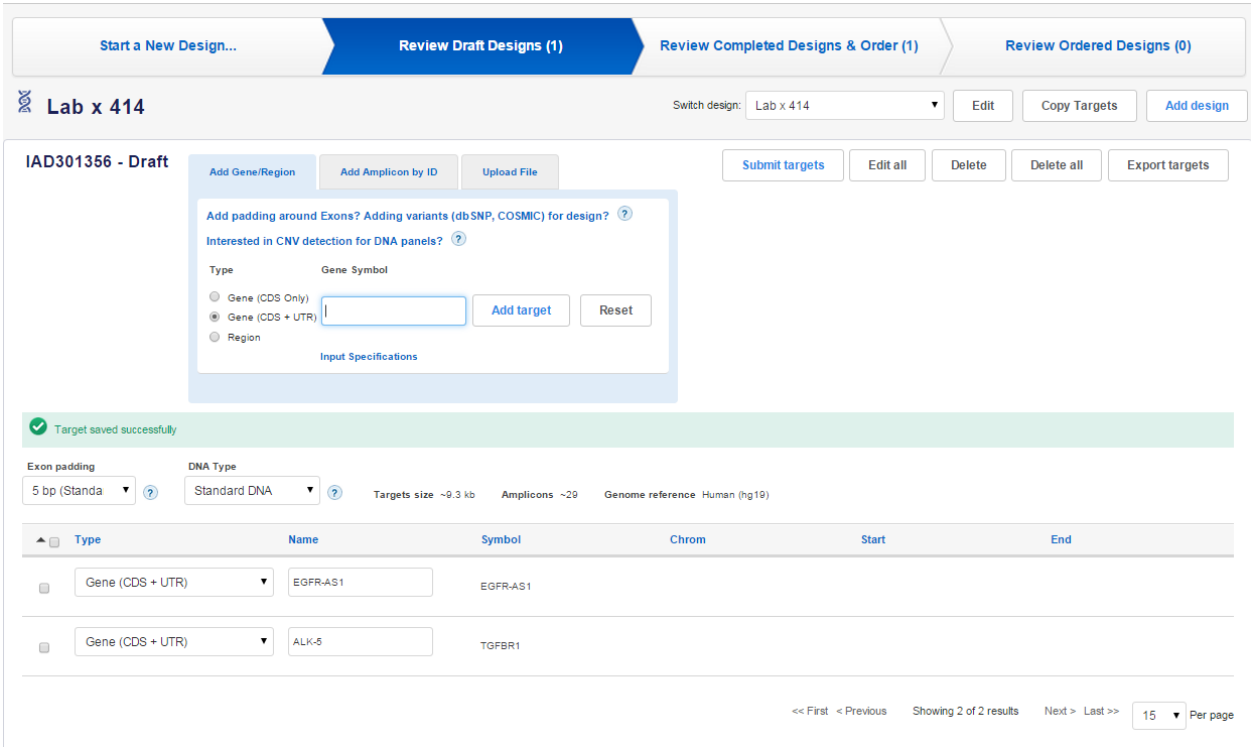


2. Select your Application type. Choose **DNA Gene designs** (multi-pool), **DNA Hotspot designs** (single-pool), **RNA Gene Expression designs** (single-pool), or **RNA Gene Fusion designs** (multi-pool). Clicking on the application type filters the compatible genomes below.

Note: Custom reference genomes are currently only compatible with DNA designs. RNA Gene designs are only compatible with the human genome.

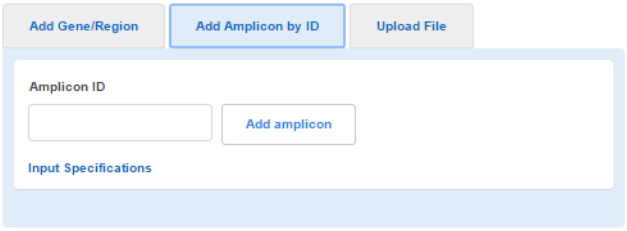
3. Select a **Reference Genome.** For custom references, click **Custom Reference**.
4. Click **Next: Add Targets** to proceed.
5. You have three options for adding targets: Add Gene/Region manually, Add Amplicons by ID, or Upload File.

    - **Add a Gene/Region** allows a variety of manual options:
        a. Choose type: Gene (CDS only), Gene (CDS +UTR), or Region.
        b. Begin typing the gene symbol or region.
        c. Click **Add target** after each entry. A green or red text box appears after each to let you know if the target was added successfully.
        d. When finished, click **Submit targets**.

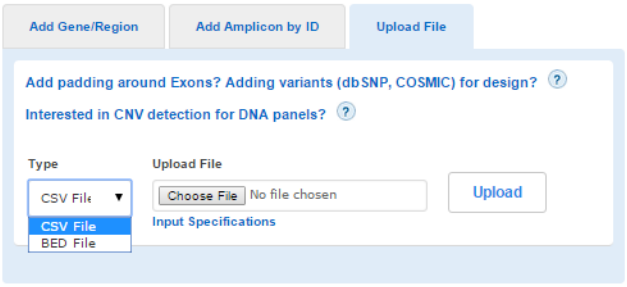


Alternatively, use **Add Amplicon by ID** or **Upload File** tabs.

- **Add Amplicon by ID** Allows you to enter amplicon IDs assigned to specific genomic coordinates.



- **Upload File** allows you to upload genomic coordinates of several targets at once, via a CSV or BED file (choose from the "Type" dropdown):



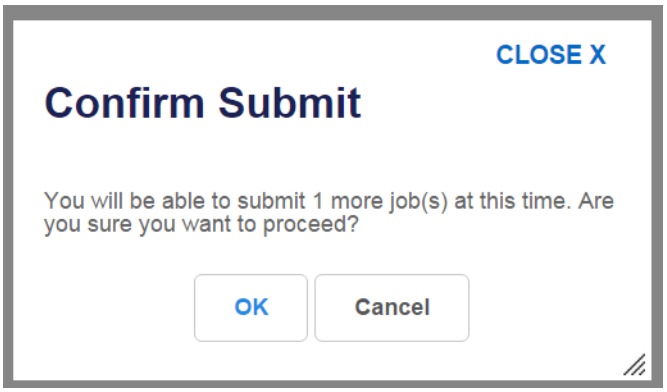
  For guidelines regarding file specifications and restrictions, click the "Input Specifications" link. After browsing and choosing your file, click **Upload** to proceed.

Ion AmpliSeq™ Designer uploads the targets, checks them, and verifies regions.

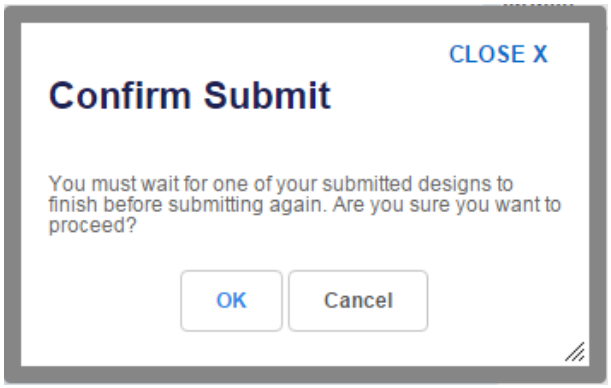
6. For any highlighted erroneous target(s), either correct the coordinates inside the table or remove them by checking their checkbox(es) and clicking the **Delete** button.

7. (Optional) Click **Export targets** to download your targets into a CSV file.

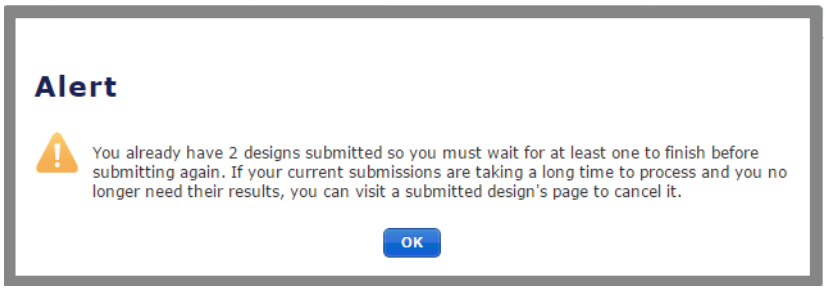
8. To submit your panel, click **Submit targets.** New in AmpliSeq™ v5.0, two designs can be submitted at a time.
9. Confirm your submission.



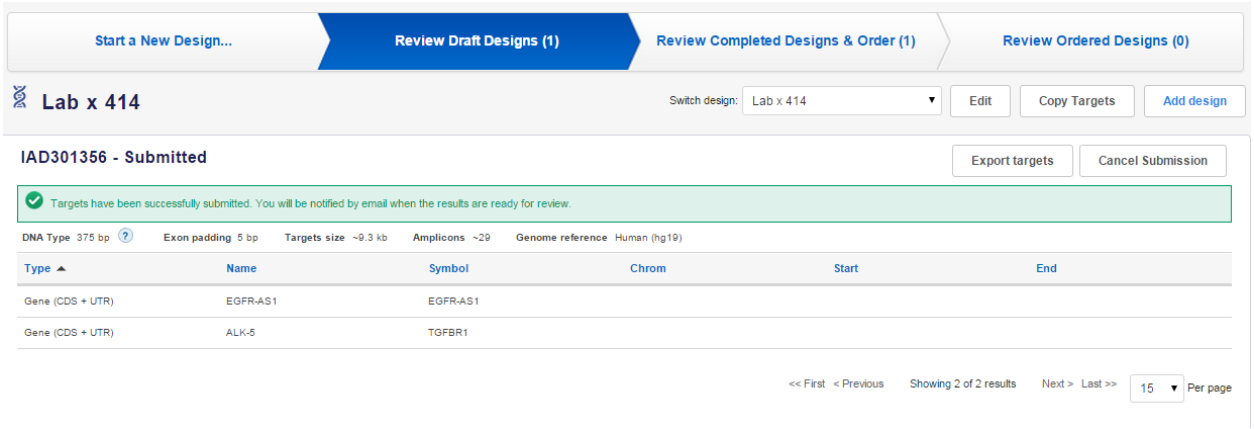
Note: If you submit two designs, you will see this Confirmation pop-up:



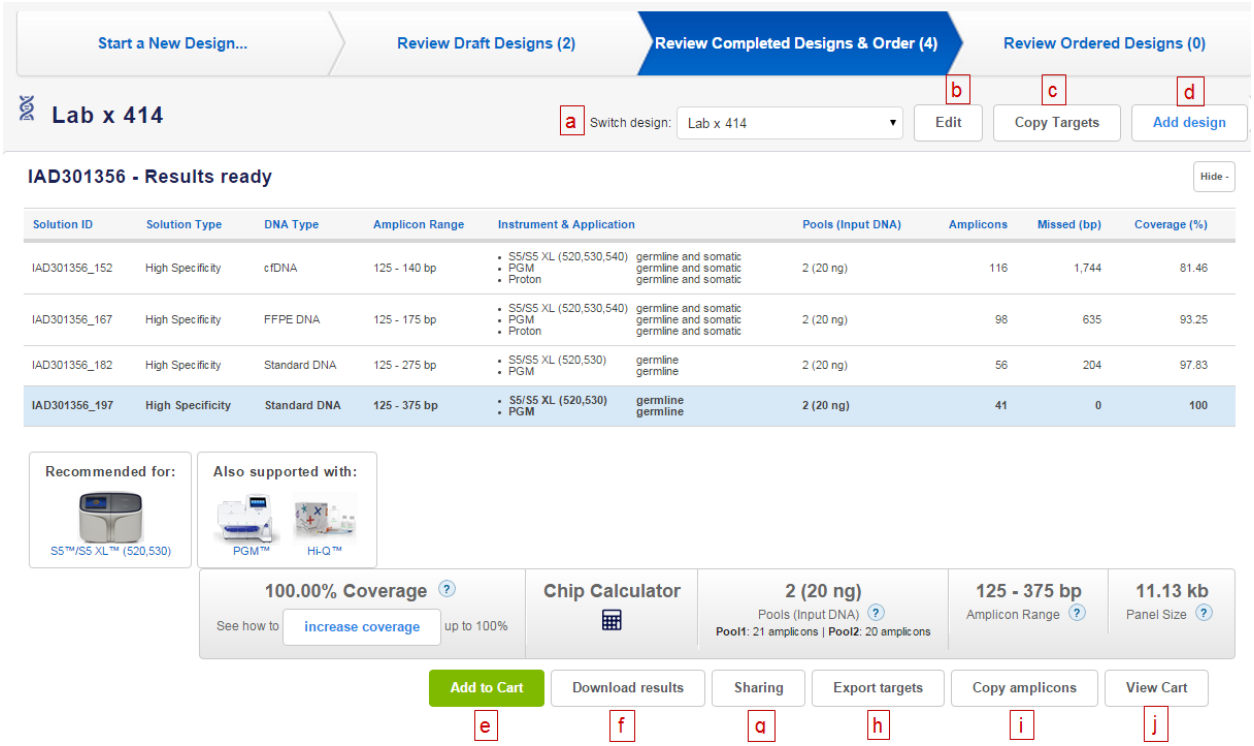
If you submit three designs, you will see this Alert message:



10. Once confirmed you will see the following confirmation and also receive an email confirming the design submission.



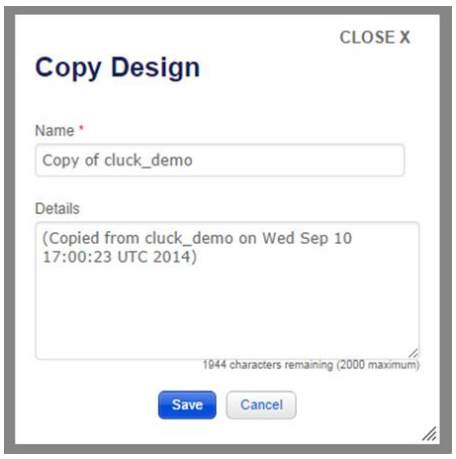
11. When the Assay Design results are ready, you will receive an email instructing you to review the results in Ion AmpliSeq™ Designer. Click the **View results** provided in the email to be directed to the results page (or navigate to http://www.ampliseq.com and click the notification or navigate to the completed design using the **My Designs** tab).



a. **Switch design**—Click the drop down to change the design in view.

b. **Edit**—Allows you to edit the Design Name and Details only.

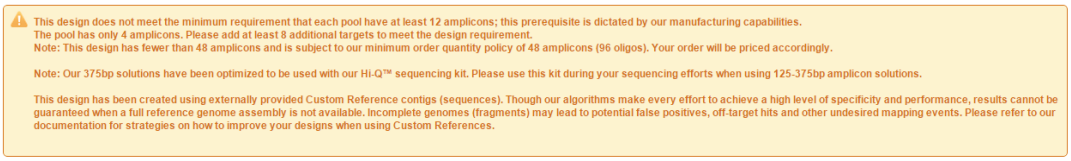
c. **Copy Targets**—Allows you to copy your design if you want to make modifications to it:



d. **Add design:** Starts a new design.

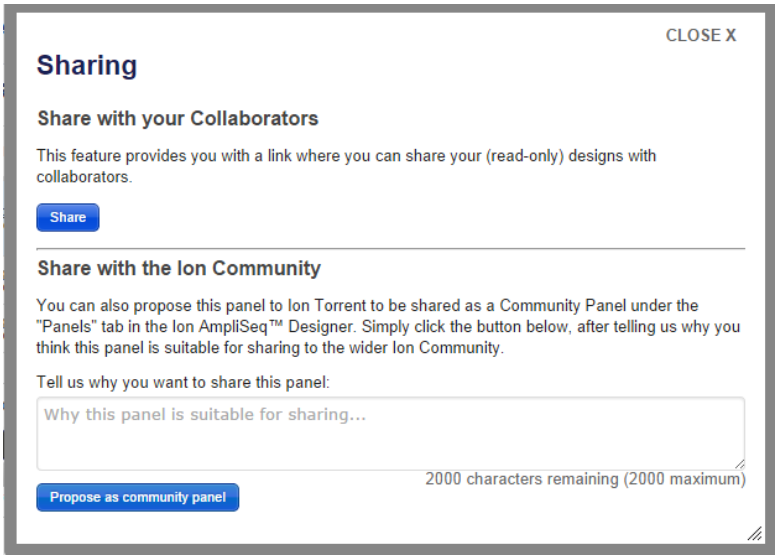
e. **Add to cart and request quote**: When this button is green you may click it to add the highlighted design to your cart.

Note: If there are not enough amplicons (at least 12) the button is greyed out and the following message appears:

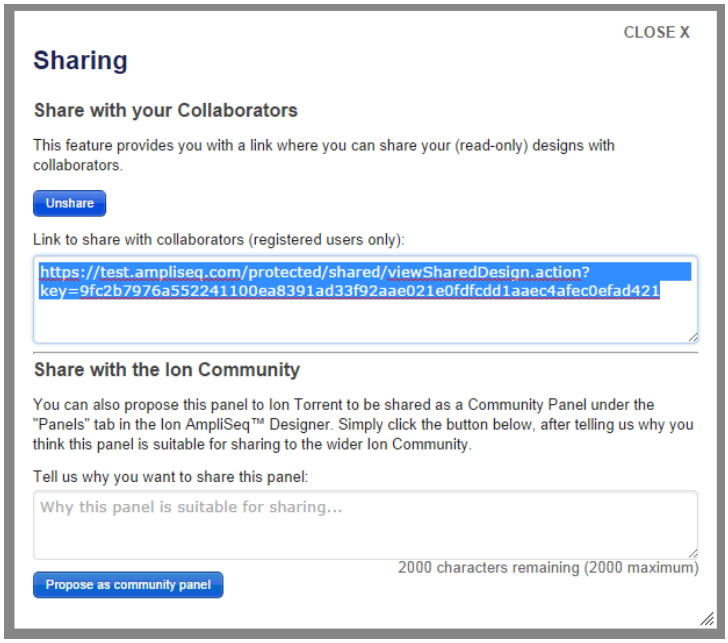


f. **Download results:** Design data results are available for download once your assay design is complete. A compressed folder will download containing a number of results files.

g. **Sharing:** Creates a link to your designs that you can email to another Ion AmpliSeq™ Designer account holder.

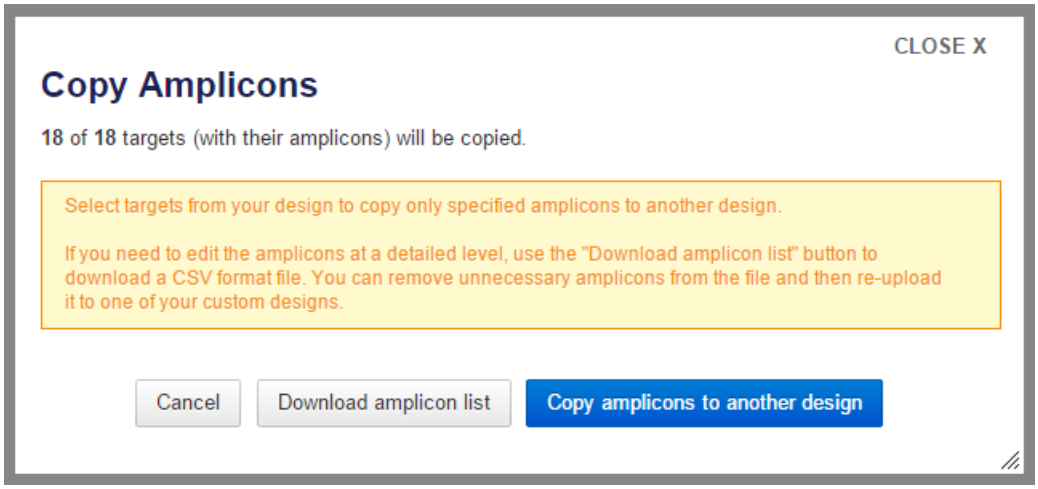


Note: Sharing your design also makes your custom reference available for review and downloading by anyone to whom you provide the link to the design.



h. **Export targets**: Downloads your targets into a CSV file.

i. **Copy Amplicons**: Allows you to copy your amplicons and download an amplicon list or copy your amplicons to another design.

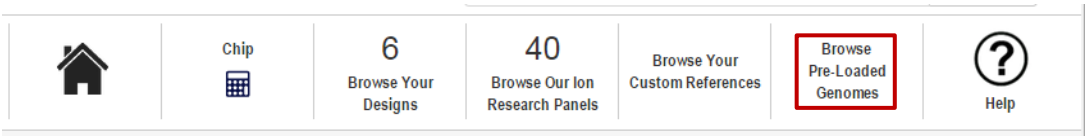


j. **View Cart**: Once you add your designs to your cart you may view the cart and request a quote.

Create and manage Reference Genomes

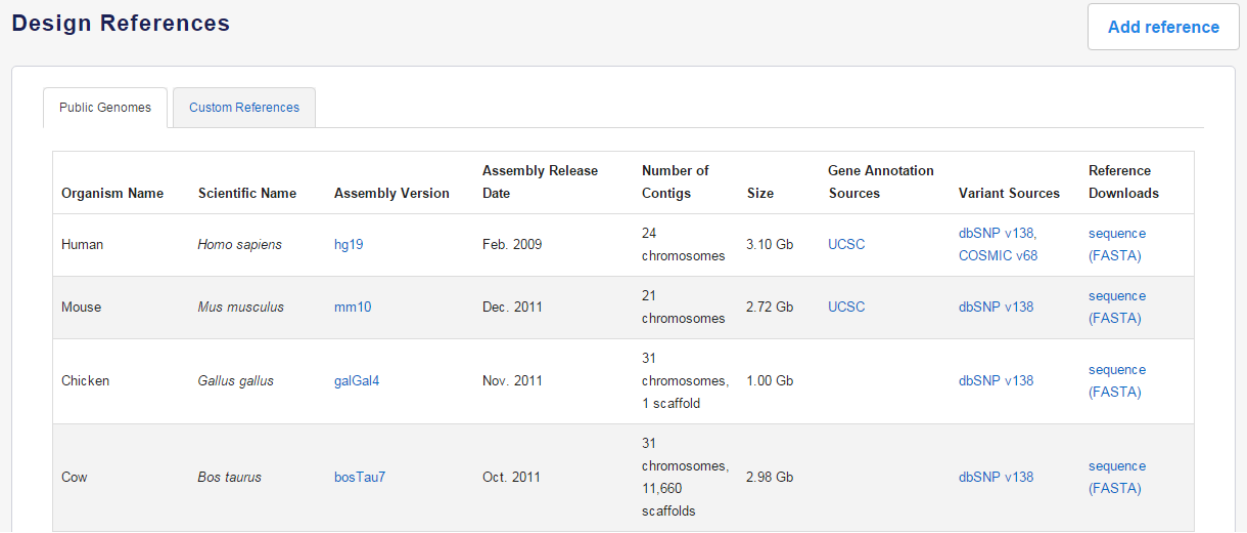
In AmpliSeq™ Designer, you can use a variety of human, animal, and plant reference genomes to build your panels. You can also upload your own. The steps below describe uploading your own.

1. From the navigation bar, select **Browse Pre-loaded Genomes**.



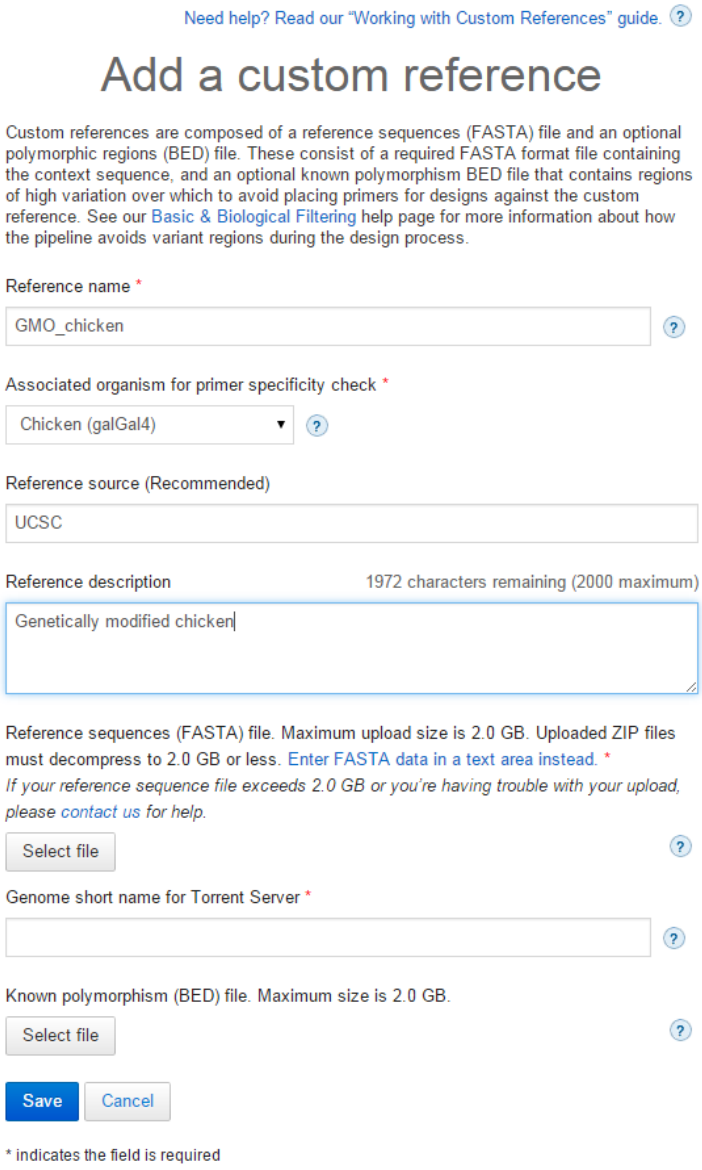
The Design Reference screen appears and Public Genomes are displayed. If you have previously uploaded custom references, click on the Custom References tab to view them.

Note: You cannot choose a public genome at this point; these are the available choices when you start a new design. Here, the list of public genomes is for informational purposes only.



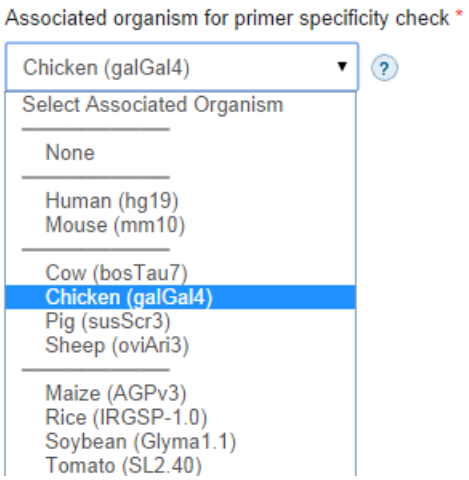
Organism Name	Scientific Name	Assembly Version	Assembly Release Date	Number of Contigs	Size	Gene Annotation Sources	Variant Sources	Reference Downloads
Human	*Homo sapiens*	hg19	Feb. 2009	24 chromosomes	3.10 Gb	UCSC	dbSNP v138, COSMIC v68	sequence (FASTA)
Mouse	*Mus musculus*	mm10	Dec. 2011	21 chromosomes	2.72 Gb	UCSC	dbSNP v138	sequence (FASTA)
Chicken	*Gallus gallus*	galGal4	Nov. 2011	31 chromosomes, 1 scaffold	1.00 Gb		dbSNP v138	sequence (FASTA)
Cow	*Bos taurus*	bosTau7	Oct. 2011	31 chromosomes, 11,660 scaffolds	2.98 Gb		dbSNP v138	sequence (FASTA)

2. To upload a new custom reference, click the **Add reference** button.
3. Fill in the required information on the following screen:



a. **Reference name**—Must be composed of US-ASCII letters, numbers, and spaces, between 3 and 32 characters in length.

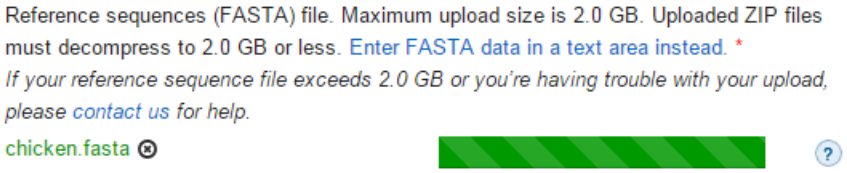
b. **Associated organism for primer specificity check**—Click to view the dropdown menu containing list of organisms. If your data are associated with one of our supported organisms, providing this information may improve primer specificity to your custom reference by favoring primers with few optimal binding sites in the consensus sequence. Primer specificity check refers to the process of identifying potential primer mispriming events. Primers with high number of potential mispriming events are avoided in our designs.



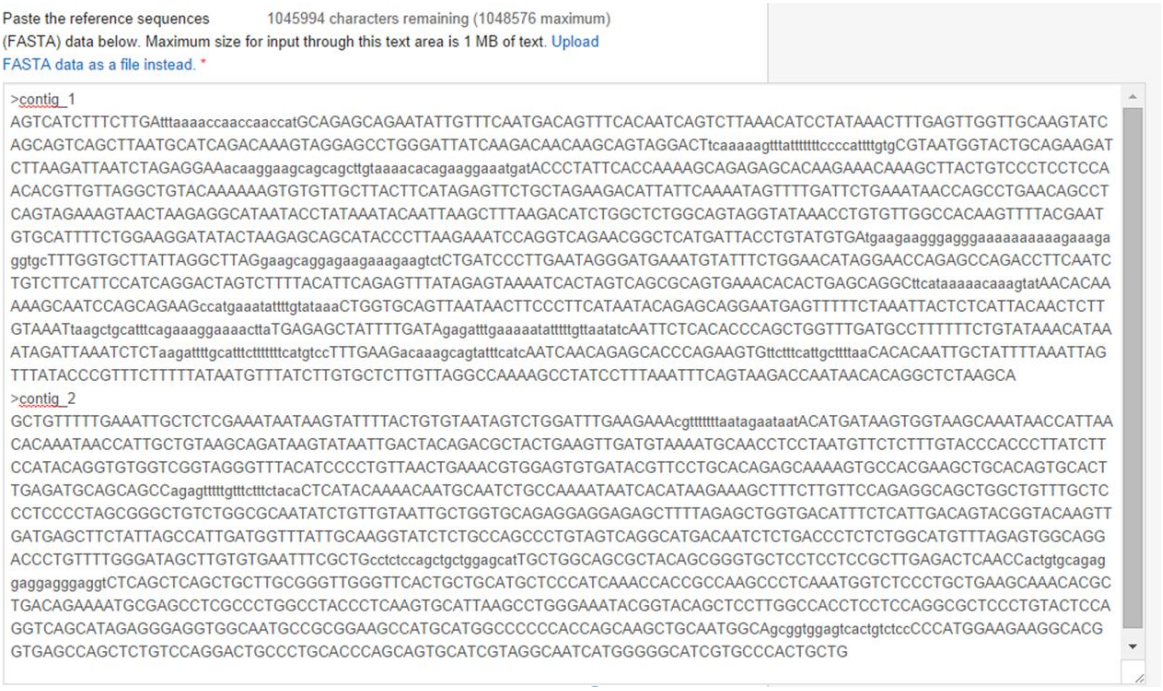
c. **Reference source (Recommended)**—Name the database/source of the DNA sequence.

d. **Reference description**—Add any notes about the custom reference sequence.

e. **Reference sequences**—You may either upload a FASTA file (Default size is 2.0 GB; however, upon request the limit can be extended to 4.0 GB):

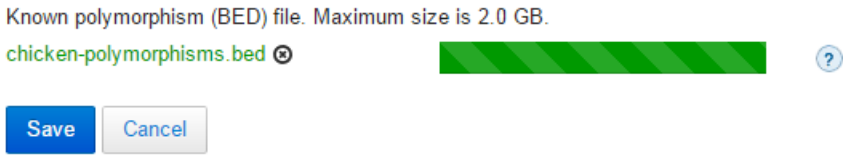


or copy and paste the reference sequence:

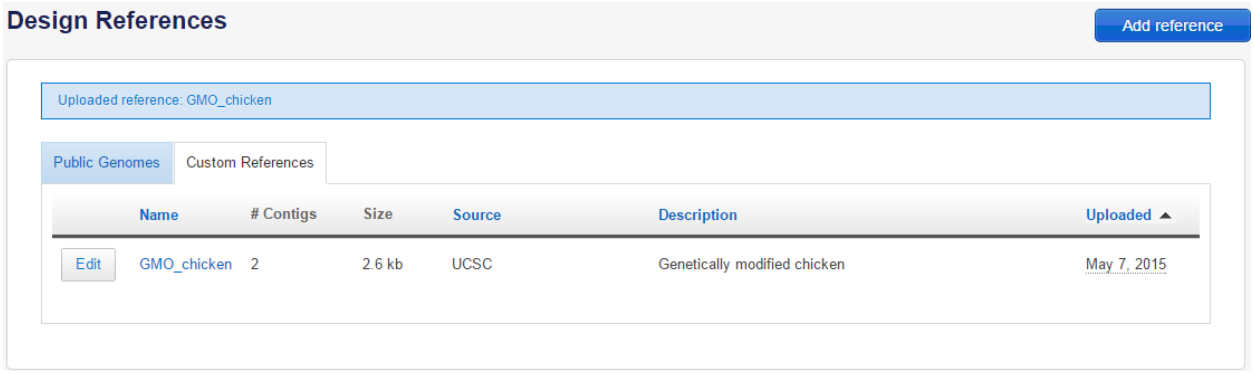


f. **Genome short name for Torrent Server** —Should be composed of lowercase US-ASCII letters, numbers, and underscores, between 1 and 30 characters in length.

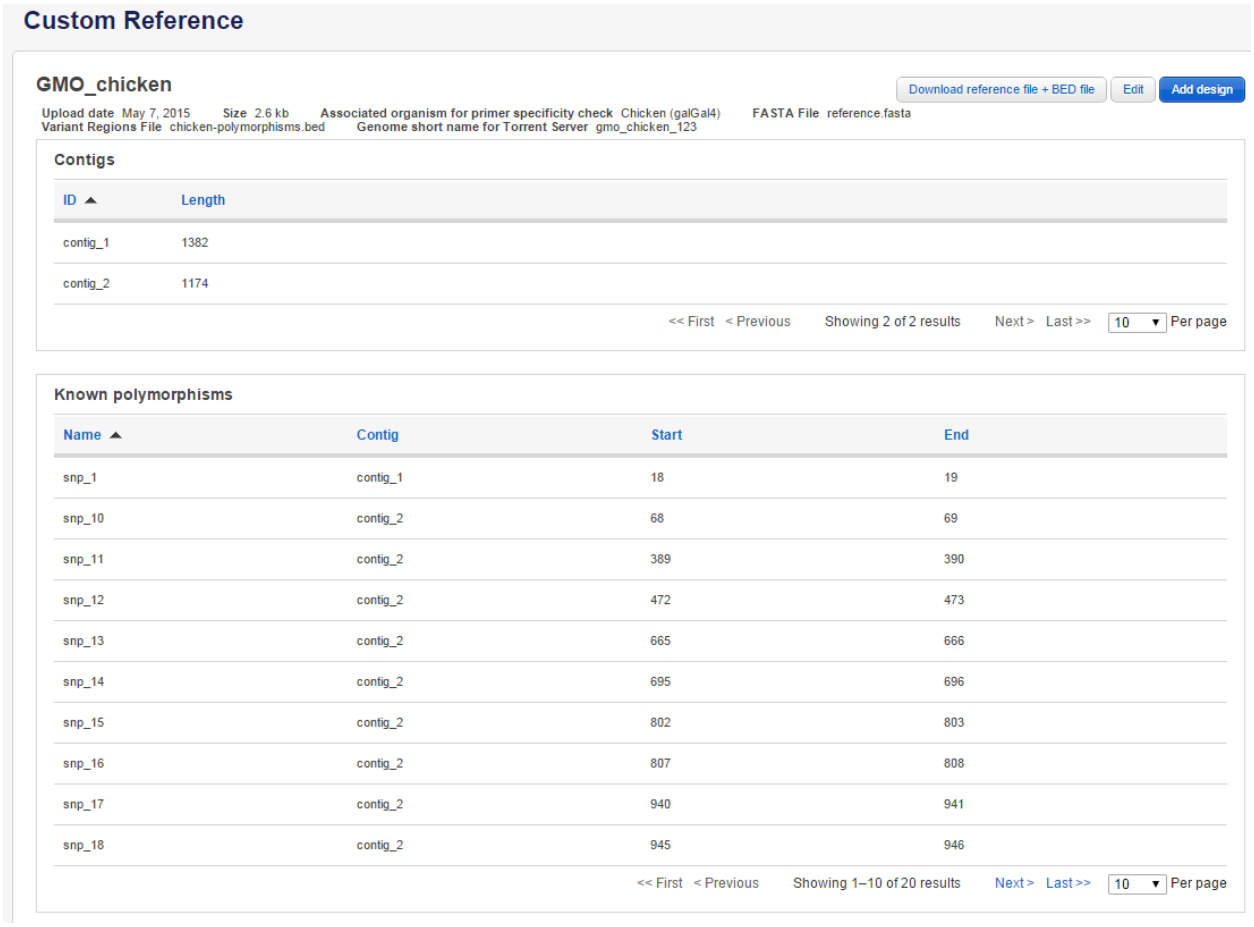
g. **Known polymorphism (BED) file**—Indicates regions of the sequences in the custom reference FASTA file with high polymorphism (i.e., SNPs, indels, or other variation). Ion AmpliSeq™ Designer minimizes primer overlap with these regions. This file is optional. See ***Appendix*** for specifications on creating and formatting BED files for uploading.



4. Save your custom reference by clicking the blue **Save** button and click on the **Custom References** tab to view your uploaded reference:

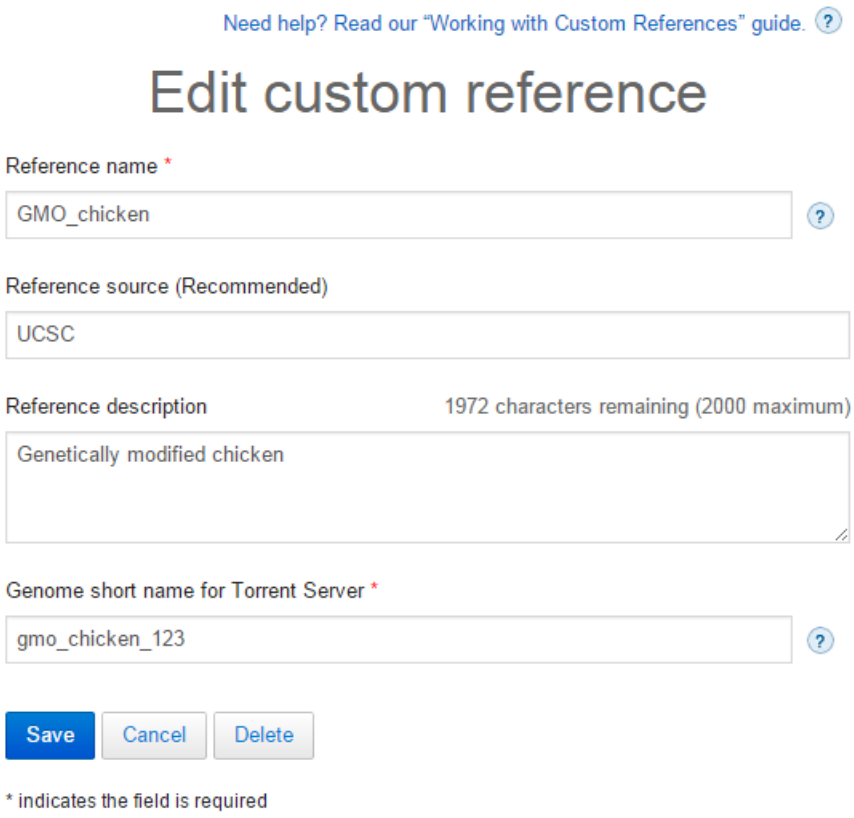


5. Click on the custom reference name to show more information:



6. Click on the blue **Edit** button in the upper right of the window to edit the following:

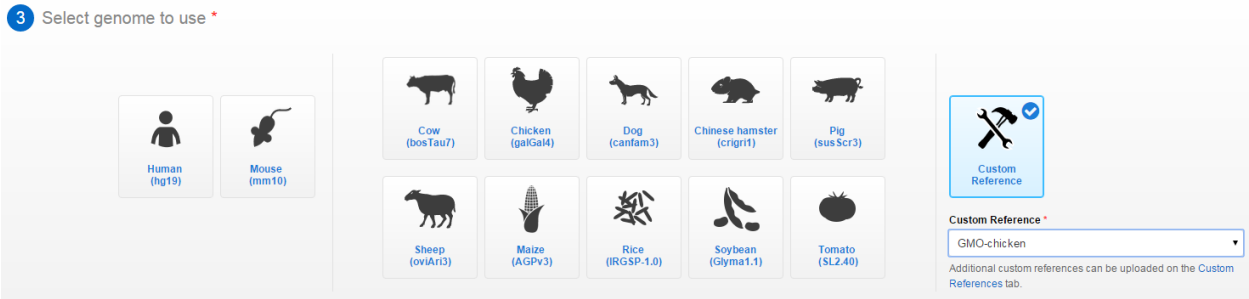
- Reference name
- Reference source
- Reference description



Note: Updates to these textual identifiers are made throughout the entire site.

Note: You cannot make changes to the uploaded files (genomic data) as they are permanently associated with this assigned custom reference genome. If you want to make changes, you will need to delete and re-upload your edited files using the **Add reference** button. Click **Delete** to remove the reference from the list of active custom references. This will not affect existing designs; associated custom references will still be downloadable.

7. When building your custom panel, click **Custom Reference** and select your custom reference from the drop-down box.

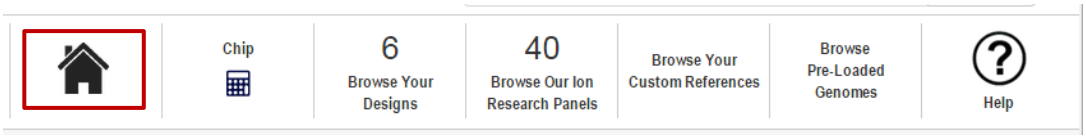


Start an RNA Gene Fusions design

To make RNA Gene Fusions panels with a combination of Gene Fusion and Gene Expression Assays (GEX), follow the steps below.

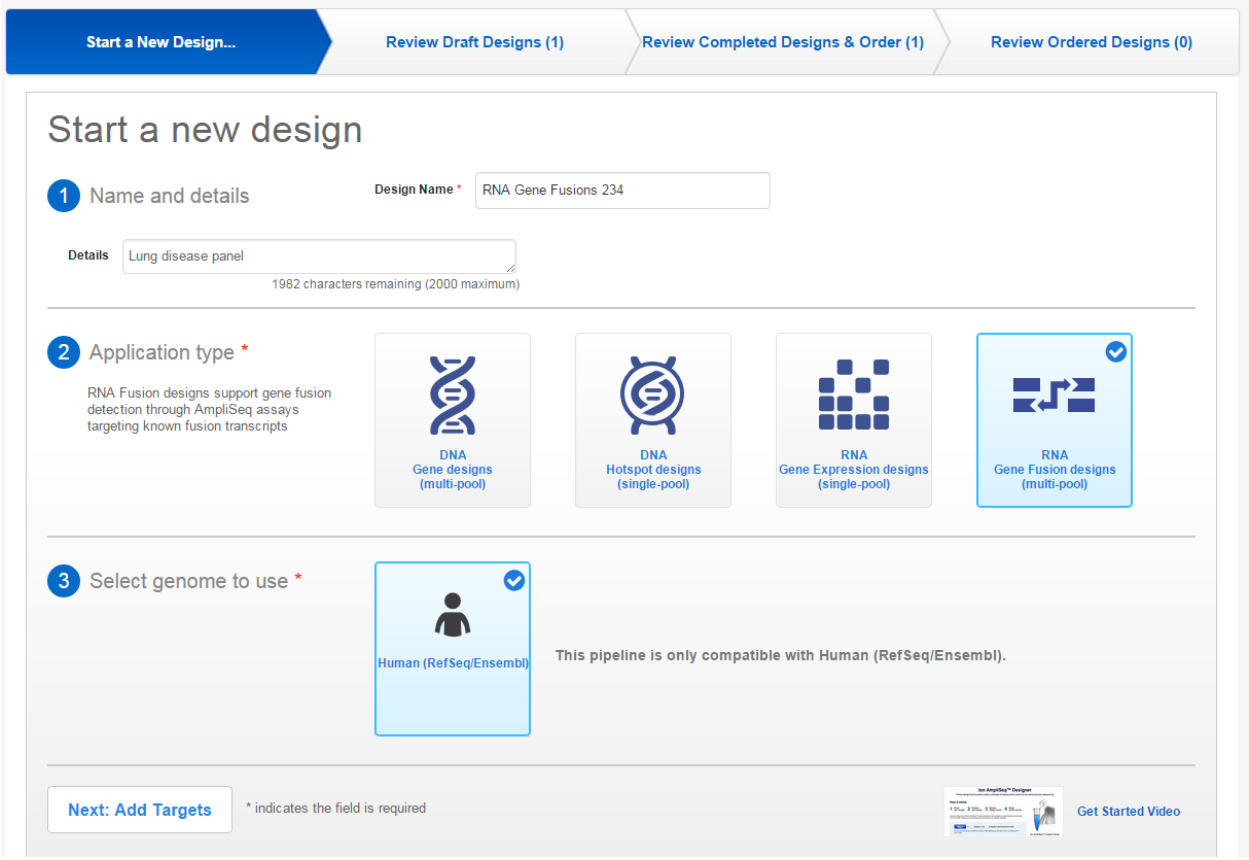
Important:	For successful panels, you must have at least 12 GEX assays per panel. We provide 12 default GEX assays for each panel that you may accept or replace with targets of your choice. For panels requiring two pools, the GEX assays will be split between the two pools.

If you are already on Ampliseq.com working in another application, click the "home" icon in the navigation bar to return to the main page:

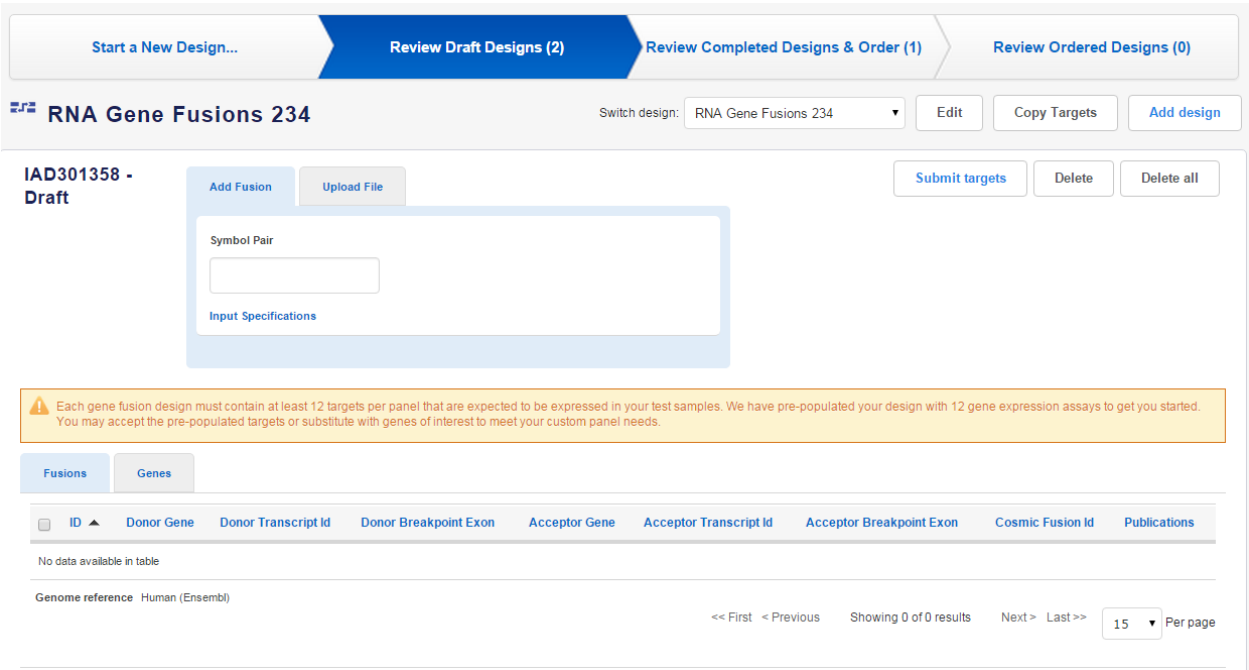


1. Enter a Design Name and, optionally, details. The *Start a new design* page now expands to add additional elements for creating a new panel.

2. Select **RNA Gene Fusion designs (multi-pool)** button, in application type.
   Note: Human (RefSeq/Ensembl) is the only genome permitted with this panel type.



3. Click **Next: Add Targets** button.

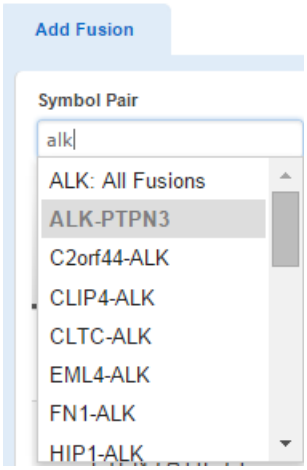


At the top of this screen, you can Add Fusions by Symbol Pair or upload them from a file.

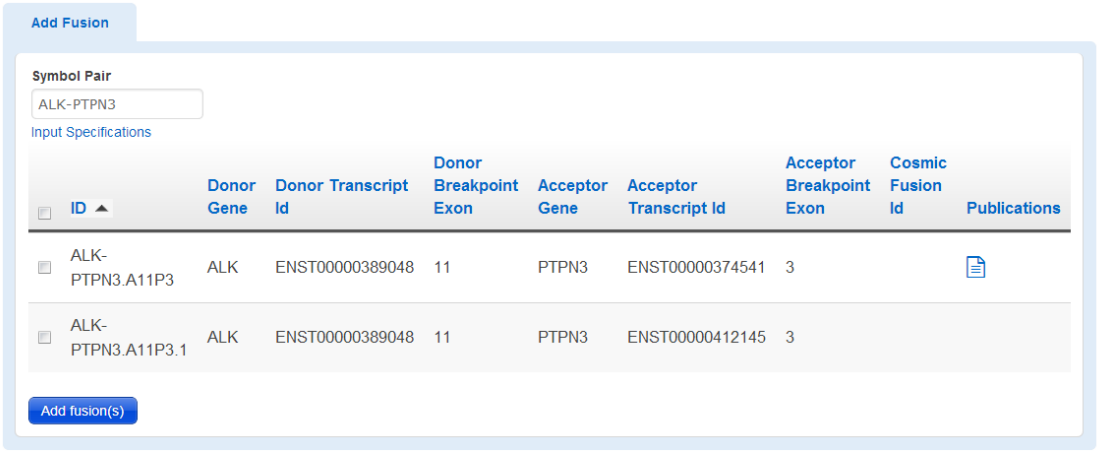
Click “Input Specifications” link for details. At the bottom of the screen you see two tabs, one to add gene fusion targets and the other to add gene expression targets. These tabs represent the two stages mentioned in the introduction for this section. By default the Fusion tab is selected.

4. On the Fusions tab, add your targets.

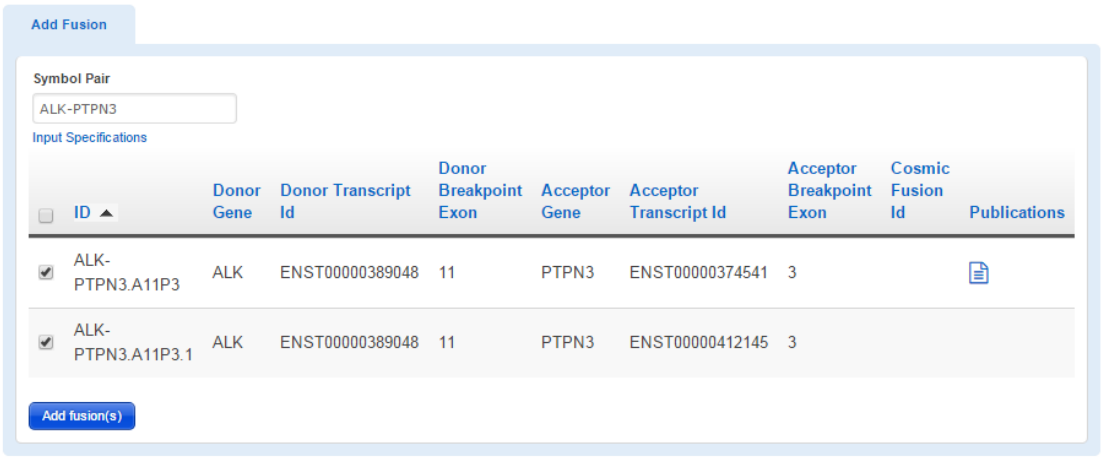
    a. Start typing the gene symbol that you would like to add the targets for, and the available fusions partners are displayed.



    b. Select the desired gene pair. The available fusions for that gene pair are displayed.

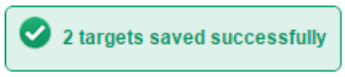


c. Add the fusions by checking each checkbox on the left of each target, and then click the **Add fusion(s)** button.



5. Repeat step 4 a-c to add additional targets.

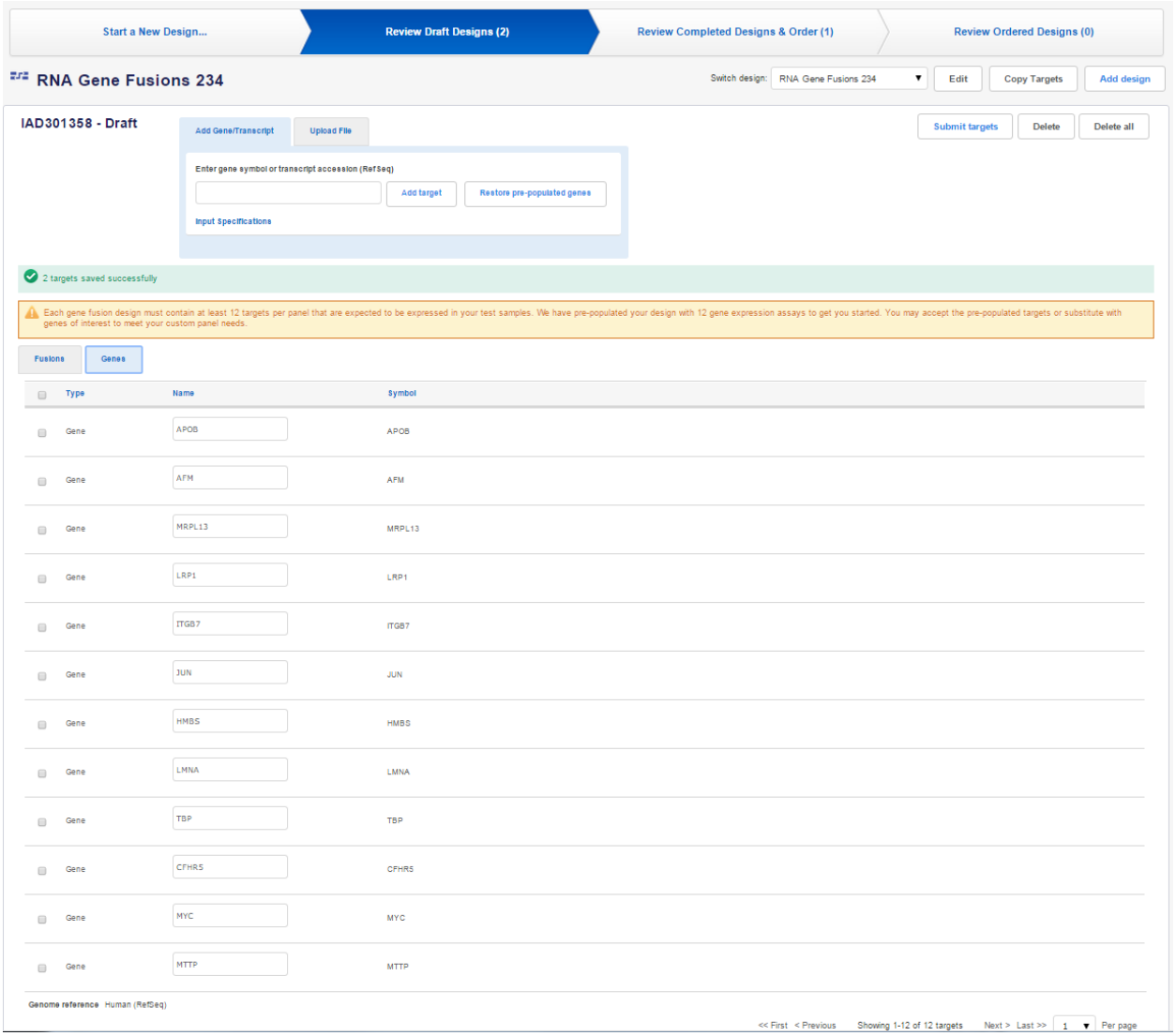
Ion AmpliSeq™ Designer uploads the targets and checks them. Once complete, a status message appears at the top of the screen: either "Target saved successfully" in green text, or "# duplicate fusions ignored" in red text.



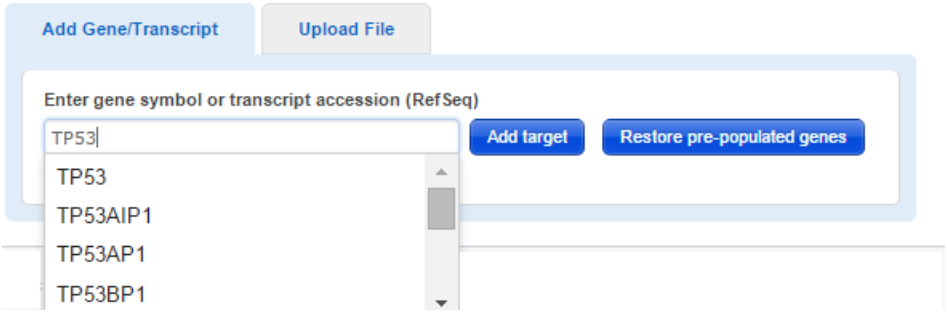
Gene Expression Assays

Now add your gene expression assays. Every pool is required to have 12 gene expression assays, and we have pre-selected them to get you started. If you want to pick your own gene expression assays, delete the proposed ones and add your own gene expression targets by gene symbol or RefSeq transcript accession.

1. Click on the **Genes** tab.



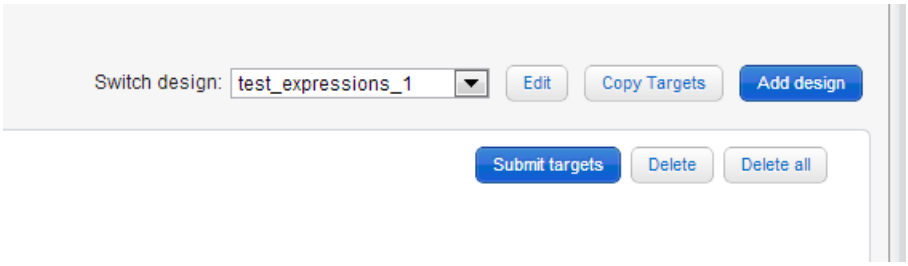
2. At the top of the screen, enter valid gene symbols (preferably an HGNC-approved symbol) or valid RNA RefSeq accession numbers as in the example below and click **Add target**.



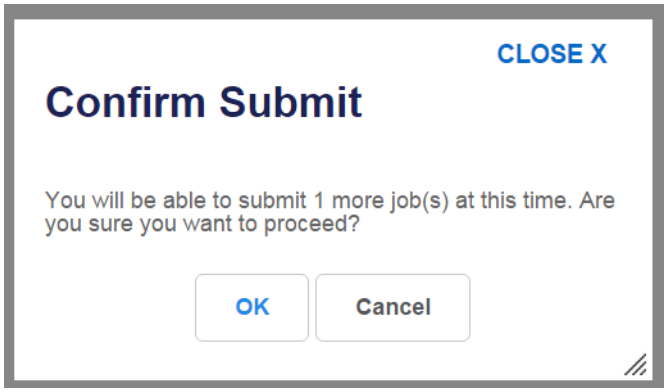
Alternatively, you can upload your own gene expression assays. Please click Input Specification link in the software user interface for more details and a CSV template for creating your own list of genes or RefSeq Accession numbers.

Note: If you would like to restore the pre-populated gene expression assays, click the Restore pre-populated genes button and they will be restored.

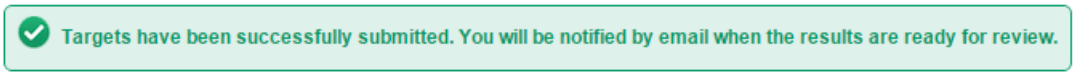
3. Repeat step 2 to add additional genes or RNA RefSeq accession numbers.
4. To submit your design once you have selected your gene fusion targets and gene expression assays, click **Submit targets** button. New in AmpliSeq™ v5.0, two designs can be submitted at a time.



5. Confirm your submission. **Note**: You can submit a second submission if required.



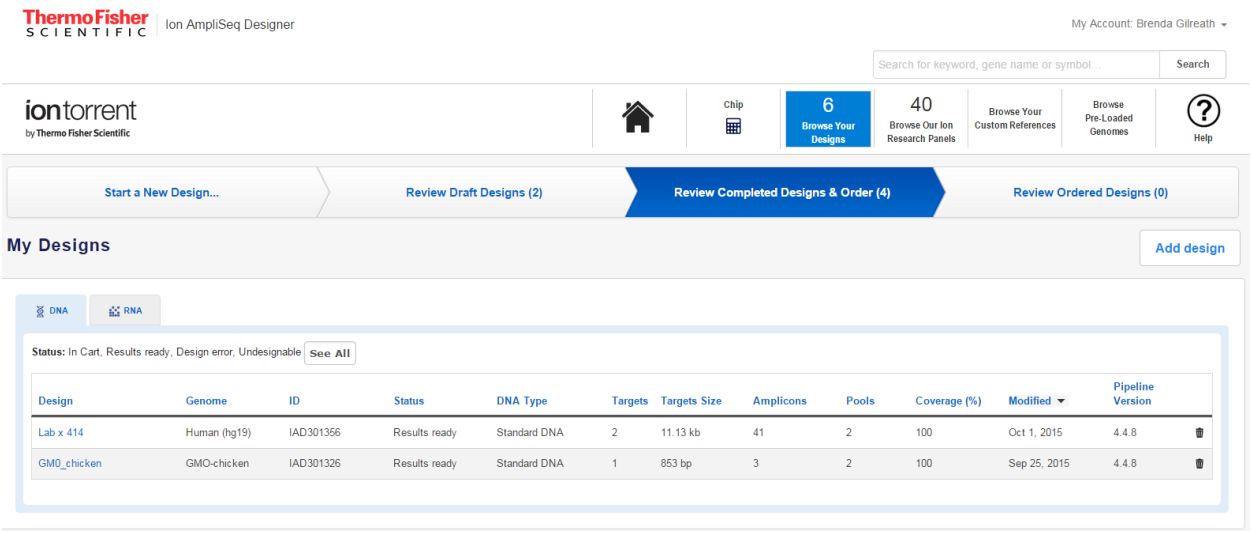
Acknowledgement of the submission appears at the top of the screen and is also sent to you via email.



6. When your fusion results are ready, you will receive an email instructing you to review the results in Ion AmpliSeq™ Designer.

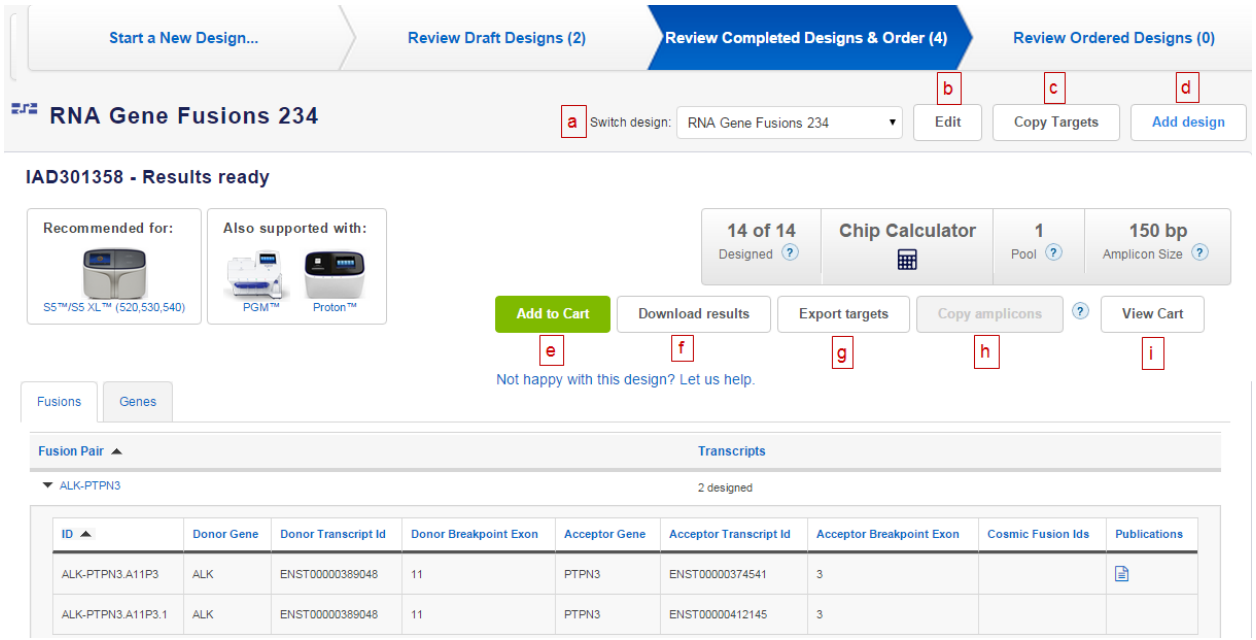
   Note: You may need to check your Spam folder and move the email to your Inbox and therefore enable its links.

7. Click the link provided in the email, or go to website and navigate to the **Review Completed Designs & Order** tab.



8. Click on the Design.

9. Review results on the **Fusions** tab and make changes if necessary by clicking the **Copy Targets** button and submitting a new design with your changes.



a. **Switch design**—Click the drop-down menu to change the design in view.

b. **Edit**—Allows you to edit the Design Name and Details only.

c. **Copy Targets**—Allows you to copy your design if you want to make modifications to it.

d. **Add design**—Starts a new design. Once you have reviewed your designs, you can go ahead and place the order.

e. **Add to cart**—When this button is green you may click it to add the highlighted design to your cart.

f. **Download results**—Design data results are available for download once your assay design is complete. A compressed folder will download containing a number of results files.

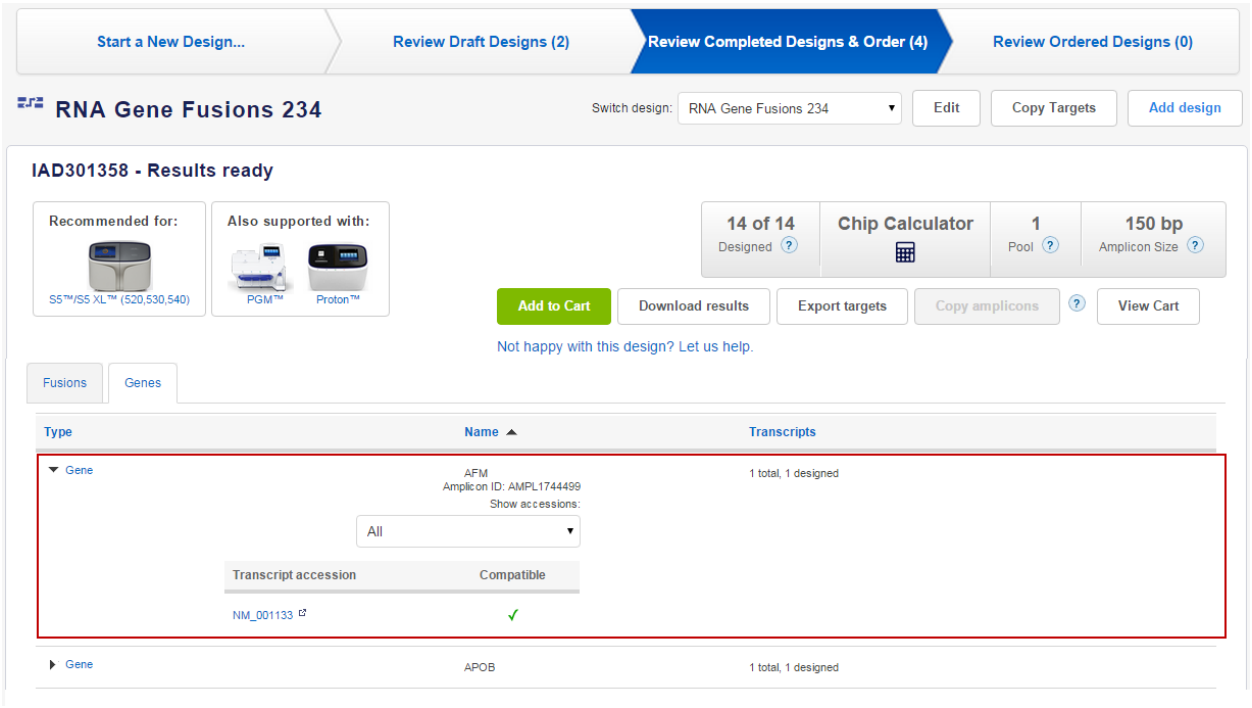
g. **Export targets**—Downloads your targets as a CSV file.

h. **Copy Amplicons**—Not applicable for RNA designs.

i. **View Cart**—Once you add your designs to your cart you may view the cart and request a quote.

10. Review gene assay results on the **Genes** tab.

11. Click on the "Gene" link to view its compatible transcripts.



Appendix

Reference FASTA sequence

Uploading sequences

One or more reference sequences in FASTA format can be uploaded by:

a. Selecting a "plain text" or compressed file (in either ZIP or GZIP formats) containing the sequence(s). The maximum file size allowed for upload is 1 GB after decompression.
b. Copying and pasting the sequences in the text area available after clicking on the link "Enter FASTA data in a text area instead."

FASTA format

A sequence in FASTA format is expressed in 2 or more lines of text: The first line is an identifying "header", the rest of the lines (one or more) represent the sequence itself.

- **The header:** The header line starts with a "greater-than" symbol (">") followed by at most 64 ASCII characters. Allowed characters are A-Z, a-z, 0-9, "_" and "-", with NO SPACES between them. Since the header is used to identify the sequence, it is required to be unique for each sequence in the reference.

- **The sequence:** The only characters accepted for representing a sequence are "A," "C," "G," "T," and "N" (lower case versions are also allowed for representing low complexity regions). Although a sequence can be just one or multiple lines of different size after the header, it is traditional to use separate lines of 50 or 60 characters in length.

- **Sequence size:** The minimum length of a sequence is 160 bp: allowing 60 bp for minimum insert size, plus 50 bp upstream and 50 bp downstream to serve as a design buffer for primer positioning during amplicon design; however, the recommended upstream and downstream context buffer sequence for optimal designs is 1,000 bp.

Known polymorphism BED file

The known polymorphism BED file indicates regions of the sequences in the custom reference FASTA file with high polymorphism (i.e., SNPs, indels, or other variation). AmpliSeq™ Designer will minimize primer overlap with these regions. This file is optional. You may upload it at the time of creating a new custom reference.

The BED format is a tab-delimited file, with one line per region. Required fields are **chrom**, **chromStart**, and **chromEnd** in the first three columns of the BED file format. Additional fields will be ignored. The **chrom** field must match one contig ID in the accompanying FASTA file. **chromStart** and **chromEnd** fields are the zero-based, half-open coordinates indicating the region to target in the sequence identified by the ID in the **chrom** field. **chromStart** and **chromEnd** are relative to the sequence of the FASTA record corresponding to the given ID.

They must meet the following criteria:

- **chromStar**t may be a value between 0 and length of the sequence specified by **chrom** minus 1.
- **chromEnd** must be greater than **chromEnd**.
- **chromEnd** may have a maximum value of the length of the sequence specified by **chrom**.
- No region should overlap any other region in the file. Overlapping regions should be merged by the customer into a single contiguous region.
- The variant coordinates BED file must have no header (i.e., no "track" lines).

Example FASTA file (*50 bases per line*):

```
>contig_1
AGTCATCTTTCTTGAtttaaaaccaaccaaccatGCAGAGCAGAATATTG
TTTCAATGACAGTTTCACAATCAGTCTTAAACATCCTATAAACTTTGAGT
TGGTTGCAAGTATCAGCAGTCAGCTTAATGCATCAGACAAAGTAGGAGCC
TGGGATTATCAAGACAACAAGCAGTAGGACTtcaaaaagtttatttttt
```

Sample variants of interest (highlighted in blue):

chrom	chromStart	chromEnd
contig_1	0	1
contig_1	95	96
contig_1	105	106
contig_1	199	200

Sample formatted BED file:

contig_1	0	1
contig_1	95	96
contig_1	105	106
contig_1	199	200

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