

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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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

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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.

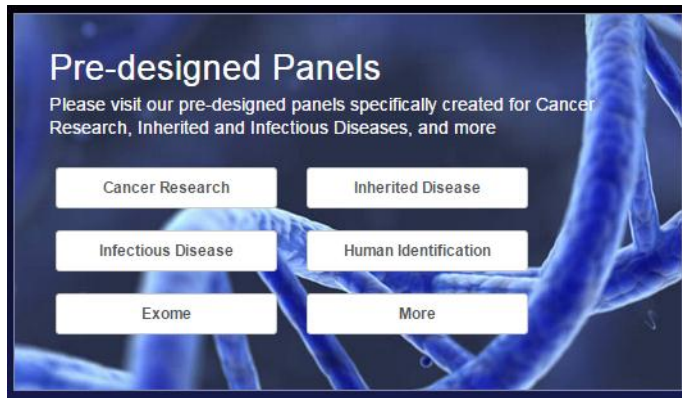
The screenshot shows the Ion AmpliSeq Designer website. At the top, the Ion Torrent logo is on the left, and navigation links for 'Browse Your Designs', 'Browse Our Ion Research Panels', 'Browse Your Custom References', 'Browse Pre-Loaded Genomes', and 'Help' are on the right. The main content area is divided into two columns. The left column, labeled 'a', is titled 'Welcome to Ion AmpliSeq Designer' and contains a 'Sign In' button and a 'Register new account' button. The right column, labeled 'b', is titled 'Pre-designed Panels' and lists categories: 'Cancer Research', 'Inherited Disease', 'Infectious Disease', 'Human Identification', 'Exome', and 'More'. Below these columns is a section titled 'What our Customers are saying' with a quote from Dr. Ian Cree. To the right of the quote is a red box labeled 'c'. Below this section is a row of three boxes. The first box, labeled 'e', is titled 'Ion AmpliSeq Designer Video' and includes a 'Need help? Watch our "My First Design" tutorial video.' link. The second box is titled 'Custom Gene Fusion panels' and mentions 'New functionality to create Custom Gene Fusion panels from over 1,000 fusion targets now available.' The third box is titled 'Showcase your breakthrough with Ion Torrent Technology' and features the 'ASHG2015' logo. Below these boxes are two sections: 'What's New in AmpliSeq' and 'Why Use AmpliSeq'. The 'What's New in AmpliSeq' section includes 'Support for RNA Gene Fusion designs', 'Special pricing for Custom Gene Fusion panels', and 'Panel search user interface revamp'. The 'Why Use AmpliSeq' section lists five benefits: 'Easy to use software for the design of highly specific primers', 'Low input amount: 10ng for DNA, 5ng for RNA', 'Superior quality, specificity and coverage', 'Supports short (140bp), medium (275) and long (375) sized Amplicons', and 'Design new or leverage our existin validated panels'. At the bottom is a 'Have Questions?' section with a red box labeled 'e' and a list of options for getting help: 'Send an email to ampliseq-designs@lifetech.com', 'Post a question on the Ion Community', 'Contact Technical & Order Support', and 'Get premium help from our Ion AmpliSeq Assist Service'.

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.

Ion Research Panels: Cancer Research

Search for keyword, gene name or symbol:

Search... Go

[Clear all filters](#)

Research Area

- ☐ Inherited Disease (16)
- ☒ Cancer Research (9)
- ☐ Infectious Disease (3)
- ☐ Exome (2)
- ☐ Pharmacogenomics (1)
- ☐ Human Identification (1)
- ☐ Dementia Research (1)
- ☐ Developmental Disorders (1)

DNA Type

- ☐ Standard (25)
- ☐ FFPE (5)

Genome

- ☐ HG19 (9)
- ☐ TB_H37RV
- ☐ Human Papillomavirus (HPV16)
- ☐ EBOLA_KM034562V1

Pipeline

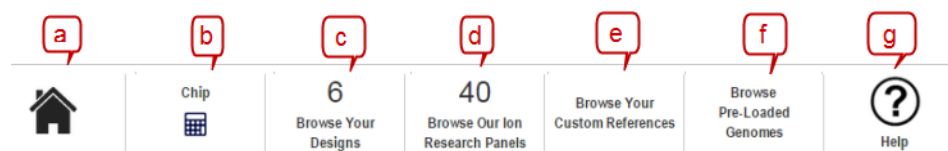
- ☐ DNA (6)

Panel Name	Research Area	Genome	Number of amplicons per pool (# of tubes)	Tags	Actions
Ion AmpliSeq™ Cancer Hotspot Panel v2 4475346	Cancer Research	Human (hg19)	Pool1: 207 amplicons (1 tube)	DNA FFPE	Review panel Sign in to order
More Summary:					
Ion AmpliSeq BRCA1 and BRCA2 Panel	Cancer Research	Human (hg19)	Pool1: 55 amplicons Pool2: 56 amplicons Pool3: 56 amplicons (3 tubes)	DNA Standard	Review panel Sign in to order
More Summary:					
Ion AmpliSeq Comprehensive Cancer Panel 4477685	Cancer Research	Human (hg19)	Pool1: 3996 amplicons Pool2: 4008 amplicons Pool3: 3991 amplicons Pool4: 3997 amplicons (4 tubes)	DNA FFPE	Review panel Sign in to order
More					

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

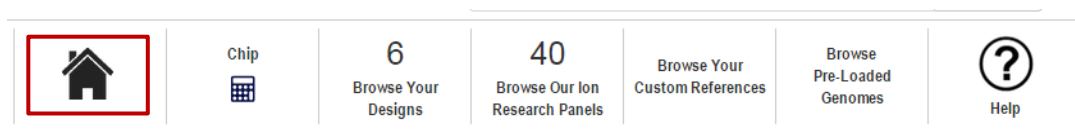
Ion Chip Type:	520	530	540
Approximate # Library Samples / Chip:	>384	>384	>384

[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.

The screenshot shows the 'Start a new design' page. At the top are four tabs: 'Start a New Design...' (active), 'Review Draft Designs (0)', 'Review Completed Designs & Order (1)', and 'Review Ordered Designs (0)'. The main content area has three steps. Step 1, 'Name and details', has a 'Design Name' field with 'Lab x 414' and a 'Details' field with 'Human test'. Step 2, 'Application type', shows four options: 'DNA Gene designs (multi-pool)' (selected with a blue checkmark), 'DNA Hotspot designs (single-pool)', 'RNA Gene Expression designs (single-pool)', and 'RNA Gene Fusion designs (multi-pool)'. Step 3, 'Select genome to use', shows a grid of genome options. 'Human (hg19)' is selected with a blue checkmark. Other options include Mouse (mm10), Cow (bosTau7), Chicken (galGal4), Dog (canfam3), Chinese hamster (cricr1), Pig (susScr3), Sheep (oviAri3), Maize (AGPv3), Rice (IRGSP-1.0), Soybean (Glyma1.1), and Tomato (SL2.40). A 'Custom Reference' option is also available. At the bottom left is a 'Next: Add Targets' button. At the bottom right is a 'Get Started Video' link.

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**.

The screenshot shows the 'Review Draft Designs (1)' tab in the Ion AmpliSeq Designer. A modal window titled 'Add Gene/Region' is open, showing options for 'Type' (Gene (CDS Only), Gene (CDS + UTR), Region) and a 'Gene Symbol' input field. Below the modal, a green banner indicates 'Target saved successfully'. The main interface shows a table of targets with columns: Type, Name, Symbol, Chrom, Start, and End. Two targets are listed: EGFR-AS1 and ALK-5. The bottom of the interface shows pagination controls: '<< First < Previous Showing 2 of 2 results Next > Last >>' and a dropdown for '15 Per page'.

Type	Name	Symbol	Chrom	Start	End
Gene (CDS + UTR)	EGFR-AS1	EGFR-AS1			
Gene (CDS + UTR)	ALK-5	TGFB1			

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.

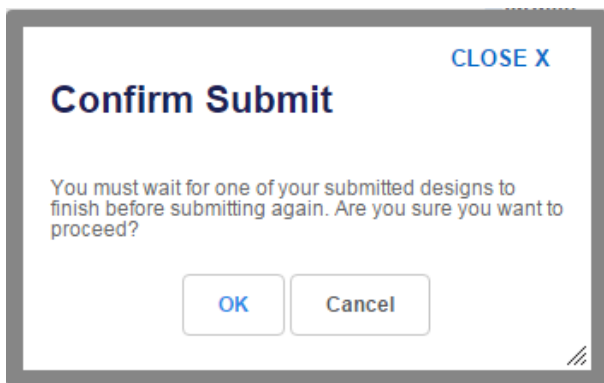
- **Upload File** allows you to upload genomic coordinates of several targets at once, via a CSV or BED file (choose from the “Type” dropdown):

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

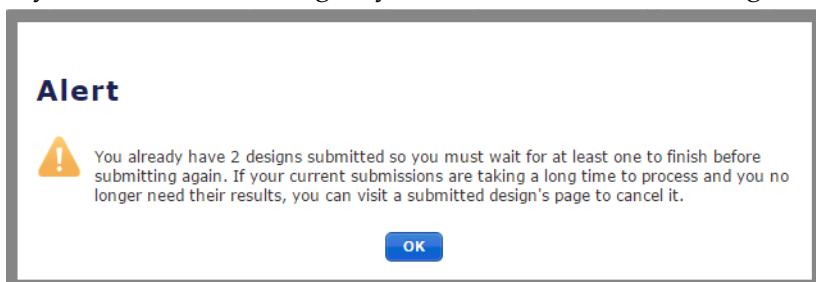
Ion AmpliSeq™ Designer uploads the targets, checks them, and verifies regions.

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:



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

Start a New Design...
Review Draft Designs (1)
Review Completed Designs & Order (1)
Review Ordered Designs (0)

Lab x 414
Switch design: Lab x 414
Edit
Copy Targets
Add design

IAD301356 - Submitted
Export targets
Cancel Submission

✓ Targets have been successfully submitted. You will be notified by email when the results are ready for review.

DNA Type	375 bp	Exon padding	5 bp	Targets size	~9.3 kb	Amplicons	~29	Genome reference	Human (hg19)
Type	Name	Symbol	Chrom	Start	End				
Gene (CDS + UTR)	EGFR-AS1	EGFR-AS1							
Gene (CDS + UTR)	ALK-5	TGFBP1							

<< First
< Previous
Showing 2 of 2 results
Next >
Last >>
15
Per page

- 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).

Start a New Design... Review Draft Designs (2) **Review Completed Designs & Order (4)** Review Ordered Designs (0)

Lab x 414 a Switch design: Lab x 414 b Edit c Copy Targets d Add design

IAD301356 - Results ready Hide -

Solution ID	Solution Type	DNA Type	Amplicon Range	Instrument & Application	Pools (Input DNA)	Amplicons	Missed (bp)	Coverage (%)
IAD301356_152	High Specificity	cDNA	125 - 140 bp	• S5/S5 XL (520,530,540) • PGM • Proton germline and somatic germline and somatic	2 (20 ng)	116	1,744	81.46
IAD301356_167	High Specificity	FFPE DNA	125 - 175 bp	• S5/S5 XL (520,530,540) • PGM • Proton germline and somatic germline and somatic	2 (20 ng)	98	635	93.25
IAD301356_182	High Specificity	Standard DNA	125 - 275 bp	• S5/S5 XL (520,530) • PGM germline germline	2 (20 ng)	56	204	97.83
IAD301356_197	High Specificity	Standard DNA	125 - 375 bp	• S5/S5 XL (520,530) • PGM germline germline	2 (20 ng)	41	0	100

Recommended for: S5/S5 XL™ (520,530)

Also supported with: PGM™ Hi-Q™

100.00% Coverage ? See how to [increase coverage](#) up to 100%

Chip Calculator

2 (20 ng) ?
Pools (Input DNA) ?
Pool1: 21 amplicons | Pool2: 20 amplicons

125 - 375 bp ?
Amplicon Range ?

11.13 kb ?
Panel Size ?

e Add to Cart f Download results a Sharing h Export targets i Copy amplicons j View Cart

- Switch design**—Click the drop down to change the design in view.
- Edit**—Allows you to edit the Design Name and Details only.
- Copy Targets**—Allows you to copy your design if you want to make modifications to it:

CLOSE X

Copy Design

Name *

Details


(Copied from cluck_demo on Wed Sep 10 17:00:23 UTC 2014)

1944 characters remaining (2000 maximum)

Save
Cancel

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

 This design does not meet the minimum requirement that each pool have at least 12 amplicons; this prerequisite is dictated by our manufacturing capabilities. The pool has only 4 amplicons. Please add at least 8 additional targets to meet the design requirement.

Note: This design has fewer than 48 amplicons and is subject to our minimum order quantity policy of 48 amplicons (96 oligos). Your order will be priced accordingly.

Note: Our 375bp solutions have been optimized to be used with our Hi-Q™ sequencing kit. Please use this kit during your sequencing efforts when using 125-375bp amplicon solutions.

This design has been created using externally provided Custom Reference contigs (sequences). Though our algorithms make every effort to achieve a high level of specificity and performance, results cannot be guaranteed when a full reference genome assembly is not available. Incomplete genomes (fragments) may lead to potential false positives, off-target hits and other undesired mapping events. Please refer to our documentation for strategies on how to improve your designs when using Custom References.

- 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.

CLOSE X

Sharing

Share with your Collaborators

This feature provides you with a link where you can share your (read-only) designs with collaborators.

Share

Share with the Ion Community

You can also propose this panel to Ion Torrent to be shared as a Community Panel under the "Panels" tab in the Ion AmpliSeq™ Designer. Simply click the button below, after telling us why you think this panel is suitable for sharing to the wider Ion Community.

Tell us why you want to share this panel:

Why this panel is suitable for sharing...

2000 characters remaining (2000 maximum)

Propose as community panel

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.

CLOSE X

Sharing

Share with your Collaborators

This feature provides you with a link where you can share your (read-only) designs with collaborators.

Unshare

Link to share with collaborators (registered users only):

<https://test.ampliseq.com/protected/shared/viewSharedDesign.action?key=9fc2b7976a552241100ea8391ad33f92aae021e0fdcd1aaec4afec0efad421>

Share with the Ion Community

You can also propose this panel to Ion Torrent to be shared as a Community Panel under the "Panels" tab in the Ion AmpliSeq™ Designer. Simply click the button below, after telling us why you think this panel is suitable for sharing to the wider Ion Community.

Tell us why you want to share this panel:

Why this panel is suitable for sharing...

2000 characters remaining (2000 maximum)

Propose as community panel

- 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.

CLOSE X

Copy Amplicons

18 of 18 targets (with their amplicons) will be copied.

Select targets from your design to copy only specified amplicons to another design.

If you need to edit the amplicons at a detailed level, use the "Download amplicon list" button to download a CSV format file. You can remove unnecessary amplicons from the file and then re-upload it to one of your custom designs.

Cancel

Download amplicon list

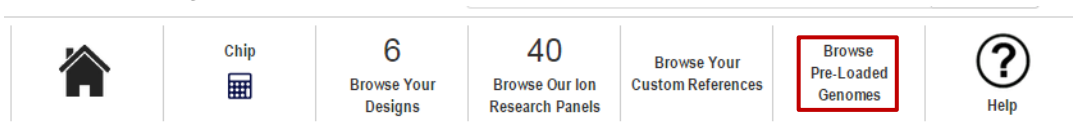
Copy amplicons to another design

- 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.

Design References								Add reference
Public Genomes		Custom References						
Organism Name	Scientific Name	Assembly Version	Assembly Release Date	Number of Contigs	Size	Gene Annotation Sources	Variant Sources	Reference Downloads
Human	<i>Homo sapiens</i>	hg19	Feb. 2009	24 chromosomes	3.10 Gb	UCSC	dbSNP v138, COSMIC v68	sequence (FASTA)
Mouse	<i>Mus musculus</i>	mm10	Dec. 2011	21 chromosomes	2.72 Gb	UCSC	dbSNP v138	sequence (FASTA)
Chicken	<i>Gallus gallus</i>	galGal4	Nov. 2011	31 chromosomes, 1 scaffold	1.00 Gb		dbSNP v138	sequence (FASTA)
Cow	<i>Bos taurus</i>	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:

Need help? Read our "Working with Custom References" guide. ?

Add a custom reference

Custom references are composed of a reference sequences (FASTA) file and an optional polymorphic regions (BED) file. These consist of a required FASTA format file containing the context sequence, and an optional known polymorphism BED file that contains regions of high variation over which to avoid placing primers for designs against the custom reference. See our [Basic & Biological Filtering](#) help page for more information about how the pipeline avoids variant regions during the design process.

Reference name *

 ?

Associated organism for primer specificity check *

 ?

Reference source (Recommended)

Reference description

1972 characters remaining (2000 maximum)

Reference sequences (FASTA) file. Maximum upload size is 2.0 GB. Uploaded ZIP files must decompress to 2.0 GB or less. [Enter FASTA data in a text area instead.](#) *

If your reference sequence file exceeds 2.0 GB or you're having trouble with your upload, please [contact us](#) for help.

 ?

Genome short name for Torrent Server *

 ?

Known polymorphism (BED) file. Maximum size is 2.0 GB.

 ?

* indicates the field is required

- 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.

Associated organism for primer specificity check *

Chicken (galGal4) ▼ ?

Select Associated Organism

None

Human (hg19)

Mouse (mm10)

Cow (bosTau7)

Chicken (galGal4)

Pig (susScr3)

Sheep (oviAri3)

Maize (AGPv3)

Rice (IRGSP-1.0)

Soybean (Glyma1.1)

Tomato (SL2.40)

- 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):

Reference sequences (FASTA) file. Maximum upload size is 2.0 GB. Uploaded ZIP files must decompress to 2.0 GB or less. [Enter FASTA data in a text area instead.](#) *

If your reference sequence file exceeds 2.0 GB or you're having trouble with your upload, please [contact us](#) for help.

chicken.fasta ?



[illegible]

- 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.

chicken-polymorphisms.bed   

- 18

Design References

Add reference

Uploaded reference: GMO_chicken

Public Genomes

Custom References

Name	# Contigs	Size	Source	Description	Uploaded ▲
Edit GMO_chicken	2	2.6 kb	UCSC	Genetically modified chicken	May 7, 2015

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

Custom Reference

GMO_chicken

Download reference file + BED file

Edit

Add design

Upload date: May 7, 2015 Size: 2.6 kb Associated organism for primer specificity check: Chicken (galGal4) FASTA File: reference.fasta
Variant Regions File: chicken-polymorphisms.bed Genome short name for Torrent Server: gmo_chicken_123

Contigs

ID ▲	Length
contig_1	1382
contig_2	1174

<< First < Previous Showing 2 of 2 results Next > Last >> 10 ▼ Per page

Known polymorphisms

Name ▲	Contig	Start	End
snp_1	contig_1	18	19
snp_10	contig_2	68	69
snp_11	contig_2	389	390
snp_12	contig_2	472	473
snp_13	contig_2	665	666
snp_14	contig_2	695	696
snp_15	contig_2	802	803
snp_16	contig_2	807	808
snp_17	contig_2	940	941
snp_18	contig_2	945	946

<< First < Previous Showing 1–10 of 20 results Next > Last >> 10 ▼ Per page

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

- Reference name
- Reference source
- Reference description

Need help? Read our "Working with Custom References" guide. ?

Edit custom reference

Reference name *

GMO_chicken



Reference source (Recommended)

UCSC

Reference description

1972 characters remaining (2000 maximum)

Genetically modified chicken

Genome short name for Torrent Server *

gmo_chicken_123



Save

Cancel

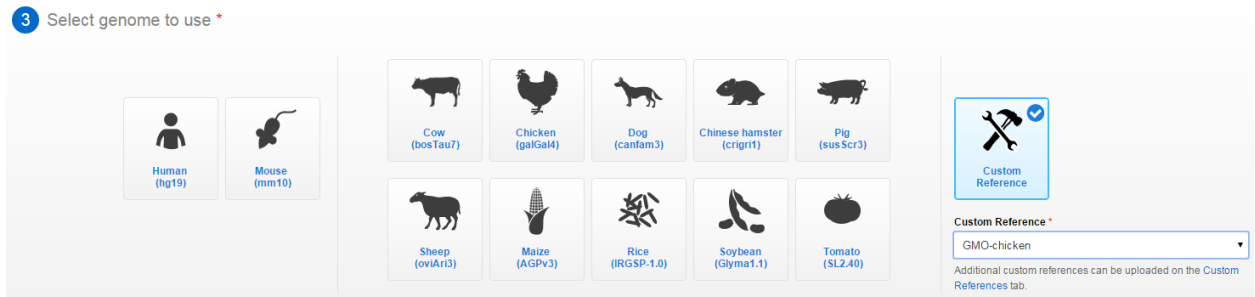
Delete

* indicates the field is required

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.

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

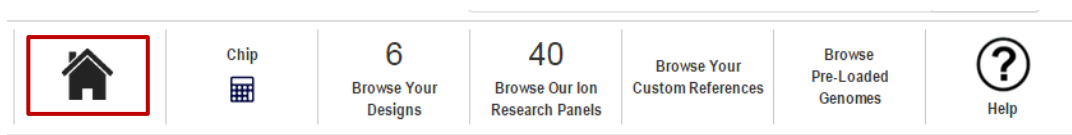


Start an RNA Gene Fusions design

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:



- 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.

Start a new design

1 Name and details **Design Name *** RNA Gene Fusions 234

Details Lung disease panel
1982 characters remaining (2000 maximum)

2 Application type *

RNA Fusion designs support gene fusion detection through AmpliSeq assays targeting known fusion transcripts

DNA Gene designs (multi-pool) DNA Hotspot designs (single-pool) RNA Gene Expression designs (single-pool) **RNA Gene Fusion designs (multi-pool)**

3 Select genome to use *

Human (RefSeq/Ensembl) This pipeline is only compatible with Human (RefSeq/Ensembl).

Next: Add Targets * indicates the field is required

Get Started Video

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

Start a New Design... **Review Draft Designs (2)** Review Completed Designs & Order (1) Review Ordered Designs (0)

RNA Gene Fusions 234 Switch design: RNA Gene Fusions 234 Edit Copy Targets Add design

IAD301358 - Draft

Add Fusion Upload File

Symbol Pair

Input Specifications

Submit targets Delete Delete all

Each gene fusion design must contain at least 12 targets per panel that are expected to be expressed in your test samples. We have pre-populated your design with 12 gene expression assays to get you started. You may accept the pre-populated targets or substitute with genes of interest to meet your custom panel needs.

Fusions Genes

ID	Donor Gene	Donor Transcript Id	Donor Breakpoint Exon	Acceptor Gene	Acceptor Transcript Id	Acceptor Breakpoint Exon	Cosmic Fusion Id	Publications
No data available in table								

Genome reference Human (Ensembl)

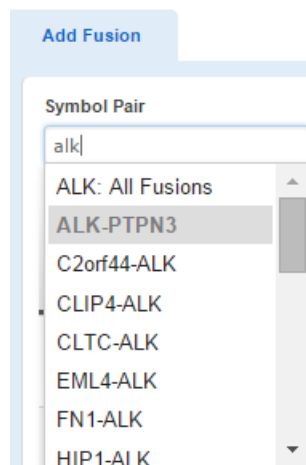
<< First < Previous Showing 0 of 0 results Next > Last >> 15 Per page

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.

Add Fusion

Symbol Pair
ALK-PTPN3

[Input Specifications](#)

ID	Donor Gene	Donor Transcript Id	Donor Breakpoint Exon	Acceptor Gene	Acceptor Transcript Id	Acceptor Breakpoint Exon	Cosmic Fusion Id	Publications
<input type="checkbox"/> ALK-PTPN3.A11P3	ALK	ENST00000389048	11	PTPN3	ENST00000374541	3		View
<input type="checkbox"/> ALK-PTPN3.A11P3.1	ALK	ENST00000389048	11	PTPN3	ENST00000412145	3		

[Add fusion\(s\)](#)

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

Add Fusion

Symbol Pair

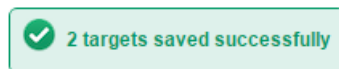
Input Specifications

<input type="checkbox"/>	ID ▲	Donor Gene	Donor Transcript Id	Donor Breakpoint Exon	Acceptor Gene	Acceptor Transcript Id	Acceptor Breakpoint Exon	Cosmic Fusion Id	Publications
<input checked="" type="checkbox"/>	ALK-PTPN3.A11P3	ALK	ENST00000389048	11	PTPN3	ENST00000374541	3		
<input checked="" type="checkbox"/>	ALK-PTPN3.A11P3.1	ALK	ENST00000389048	11	PTPN3	ENST00000412145	3		

Add fusion(s)

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.

The screenshot shows the 'RNA Gene Fusions 234' interface. At the top, there are four tabs: 'Start a New Design...', 'Review Draft Designs (2)', 'Review Completed Designs & Order (1)', and 'Review Ordered Designs (0)'. The 'Review Draft Designs (2)' tab is active. Below the tabs, there's a 'Switch design:' dropdown set to 'RNA Gene Fusions 234', and buttons for 'Edit', 'Copy Targets', and 'Add design'. The main section is titled 'IAD301358 - Draft'. It contains a form to 'Add Gene/Transcript' with an 'Upload File' button and a text input for 'Enter gene symbol or transcript accession (RefSeq)'. Below the input are 'Add target' and 'Restore pre-populated genes' buttons. A green message bar states '2 targets saved successfully'. An orange warning bar says 'Each gene fusion design must contain at least 12 targets per panel that are expected to be expressed in your test samples. We have pre-populated your design with 12 gene expression assays to get you started. You may accept the pre-populated targets or substitute with genes of interest to meet your custom panel needs.' Below this, there are two tabs: 'Fusions' and 'Genes'. The 'Genes' tab is selected, showing a table of 12 pre-selected gene targets. The table has columns for 'Type', 'Name', and 'Symbol'. The 'Name' column contains text input fields with the gene symbols. The 'Symbol' column contains the corresponding gene symbols. At the bottom, there's a 'Genome reference' dropdown set to 'Human (RefSeq)' and a pagination bar showing 'Showing 1-12 of 12 targets'.

Type	Name	Symbol
Gene	<input type="text" value="APOB"/>	APOB
Gene	<input type="text" value="AFM"/>	AFM
Gene	<input type="text" value="MRPL13"/>	MRPL13
Gene	<input type="text" value="LRP1"/>	LRP1
Gene	<input type="text" value="ITGB7"/>	ITGB7
Gene	<input type="text" value="JUN"/>	JUN
Gene	<input type="text" value="HMBG"/>	HMBG
Gene	<input type="text" value="LMNA"/>	LMNA
Gene	<input type="text" value="TBP"/>	TBP
Gene	<input type="text" value="CFHR5"/>	CFHR5
Gene	<input type="text" value="MYC"/>	MYC
Gene	<input type="text" value="MTTP"/>	MTTP

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**.

The screenshot shows a web interface for adding genes. At the top, there are two tabs: 'Add Gene/Transcript' (active) and 'Upload File'. Below the tabs is a search bar labeled 'Enter gene symbol or transcript accession (RefSeq)'. The search bar contains the text 'TP53'. To the right of the search bar are two buttons: 'Add target' and 'Restore pre-populated genes'. Below the search bar is a dropdown menu showing a list of gene symbols: 'TP53', 'TP53AIP1', 'TP53AP1', and 'TP53BP1'.

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.

The screenshot shows a design management interface. At the top, there is a 'Switch design:' label followed by a dropdown menu currently showing 'test_expressions_1'. To the right of the dropdown are three buttons: 'Edit', 'Copy Targets', and 'Add design'. Below these buttons are three more buttons: 'Submit targets', 'Delete', and 'Delete all'.

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

The screenshot shows a 'Confirm Submit' dialog box. The title bar says 'CLOSE X'. The main text reads 'Confirm Submit'. Below that, it says 'You will be able to submit 1 more job(s) at this time. Are you sure you want to proceed?'. At the bottom, there are two buttons: 'OK' and 'Cancel'.

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

✓ Targets have been successfully submitted. You will be notified by email when the results are ready for review.

- 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.

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

The screenshot shows the Ion AmpliSeq Designer web interface. At the top, there's a header with the ThermoFisher Scientific logo and 'Ion AmpliSeq Designer'. A search bar is on the right. Below the header, there's a navigation bar with icons for Home, Chip, and a '6 Browse Your Designs' button. To the right of this are buttons for '40 Browse Our Ion Research Panels', 'Browse Your Custom References', 'Browse Pre-Loaded Genomes', and a Help icon. Below the navigation bar, there's a progress bar with four tabs: 'Start a New Design...', 'Review Draft Designs (2)', 'Review Completed Designs & Order (4)' (which is highlighted in blue), and 'Review Ordered Designs (0)'. Below the progress bar, there's a 'My Designs' section with an 'Add design' button. The 'My Designs' section has tabs for 'DNA' and 'RNA'. Below these tabs, there's a status filter: 'Status: In Cart, Results ready, Design error, Undesignable' with a 'See All' link. A table lists two designs:

Design	Genome	ID	Status	DNA Type	Targets	Targets Size	Amplicons	Pools	Coverage (%)	Modified	Pipeline Version
Lab x 414	Human (hg19)	IAD301356	Results ready	Standard DNA	2	11.13 kb	41	2	100	Oct 1, 2015	4.4.8
GMO_chicken	GMO-chicken	IAD301326	Results ready	Standard DNA	1	853 bp	3	2	100	Sep 25, 2015	4.4.8

- 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.

RNA Gene Fusions 234

Switch design: RNA Gene Fusions 234 | Edit | Copy Targets | Add design

IAD301358 - Results ready

Recommended for: SS™/SS XL™ (520,530,540) | Also supported with: PGM™, Proton™

14 of 14 Designed | Chip Calculator | 1 Pool | 150 bp Amplicon Size

Add to Cart | Download results | Export targets | Copy amplicons | View Cart

Not happy with this design? Let us help.

Fusions | Genes

Fusion Pair | **Transcripts**

▼ ALK-PTPN3 | 2 designed

ID ▲	Donor Gene	Donor Transcript Id	Donor Breakpoint Exon	Acceptor Gene	Acceptor Transcript Id	Acceptor Breakpoint Exon	Cosmic Fusion Ids	Publications
ALK-PTPN3.A11P3	ALK	ENST00000389048	11	PTPN3	ENST00000374541	3		📄
ALK-PTPN3.A11P3.1	ALK	ENST00000389048	11	PTPN3	ENST00000412145	3		

- 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.

[Start a New Design...](#) [Review Draft Designs \(2\)](#) [Review Completed Designs & Order \(4\)](#) [Review Ordered Designs \(0\)](#)

RNA Gene Fusions 234 Switch design: RNA Gene Fusions 234 [Edit](#) [Copy Targets](#) [Add design](#)

IAD301358 - Results ready

Recommended for:

S5™/S5 XL™ (520,530,540)

Also supported with:

PGM™
Proton™

14 of 14
Designed [?](#)

Chip Calculator

1
Pool [?](#)

150 bp
Amplicon Size [?](#)

[Add to Cart](#) [Download results](#) [Export targets](#) [Copy amplicons](#) [View Cart](#)

Not happy with this design? Let us help.

[Fusions](#) [Genes](#)

Type	Name ▲	Transcripts
▼ Gene	AFM Amplicon ID: AMPL1744499 Show accessions: <div>All</div> <div>Transcript accessionCompatible</div> <div>NM_001133 ?</div>	1 total, 1 designed
► Gene	APOB	1 total, 1 designed

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:

- **chromStart** may be a value between 0 and length of the sequence specified by **chrom** minus 1.
- **chromEnd** must be greater than **chromStart**.
- **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
TGGGATTATCAAGACAACAAGCAGTAGGACTtcaaaaagttttttttt
```

Sample variants of interest (highlighted in blue):

<u>chrom</u>	<u>chromStart</u>	<u>chromEnd</u>
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

The information in this guide is subject to change without notice.

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