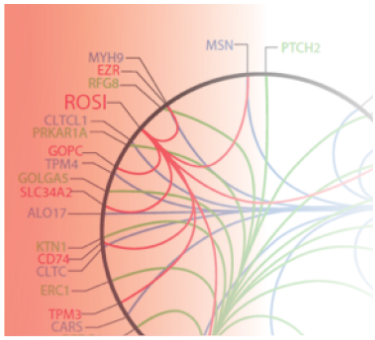

Instructions for Use

ARCHER™

Archer™ Assay Designer

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Introduction

Product Description

The Archer FusionPlex™ and VariantPlex™ target-enrichment assays are used to create libraries for next generation sequencing. Libraries are created by using the FusionPlex assay in conjunction with the Archer Universal RNA Reagent Kits V2 (AK0040-8, AK0042-8), or VariantPlex assay in conjunction with the Archer Universal DNA Reagent Kit V2 for Illumina® (AK0037-8) and Archer™ MBC Adapters for Illumina or Ion Torrent™. Please note that DNA custom design is not supported on the Ion Torrent platform at this time. **These products should never be used independently** (see Associated Products).

Once sequenced, Archer FusionPlex or VariantPlex libraries may be analyzed via Archer Analysis (http://archer.archerdx.com/) to detect and identify SNPs, InDels and fusion partners of designated genes. Copy number variation is not supported through Assay Designer at this time. **For Research Use Only.** Not for use in diagnostic procedures.

While Archer has a large and growing family of FusionPlex and VariantPlex assay panels, these panels cannot possibly provide detection for the vast combination of gene fusions and mutations possible in the human genome. For this reason, Archer has designed an online tool to facilitate panel customization. **The Archer Assay Designer** enables the user to pick and choose desired genes and gene exons. In the following pages, you will find detailed instructions for using and navigating this powerful tool.

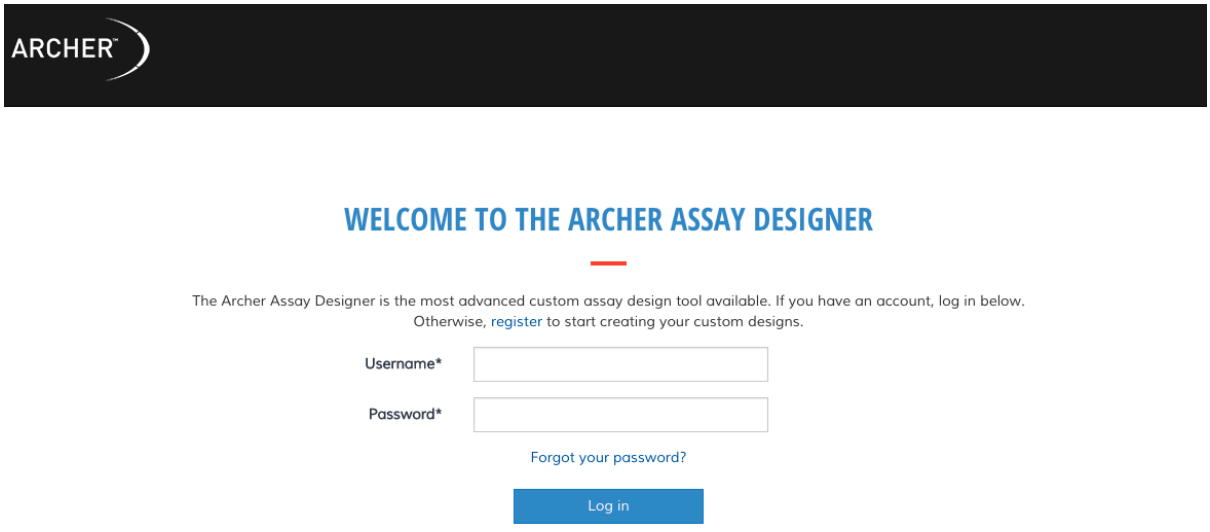
Assay Type

Currently, the Archer Assay Designer offers RNA fusion detection, RNA full exon detection and DNA full exon detection. If you require Copy Number Variation detection, or DNA fusion detection, please contact tech@archerdx.com.

Overview

1. Register / login
2. Create a new project
3. Add gene targets
4. Specify target exons, fusion or tiling direction, and optional inclusion of UTRs
5. Submit design
6. Review completed design
7. Order assay

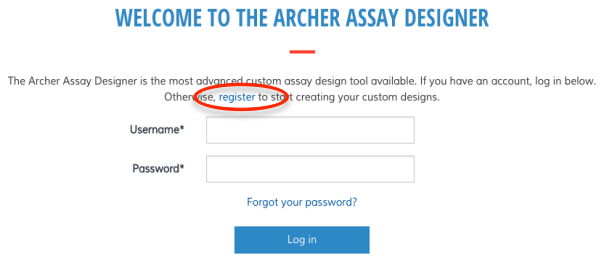
Getting Access

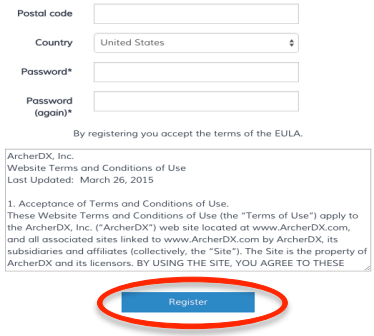


http://assay.archerdx.com/

Registration

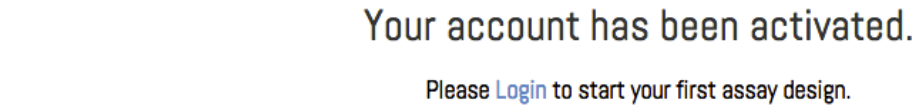
- First-time users must register before gaining access to the Archer Assay Designer. Follow the link circled to the left to go to the Registration Page.
- Enter your username, email address, institution, and password on the registration page, read the Terms and Conditions of Use, and click Register at the bottom.



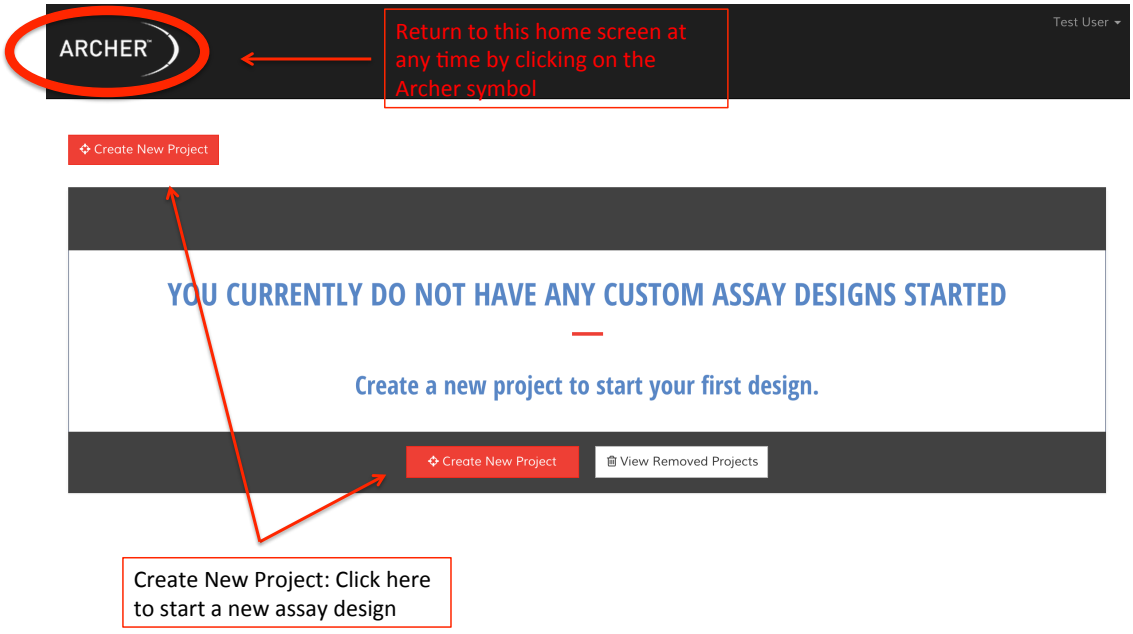


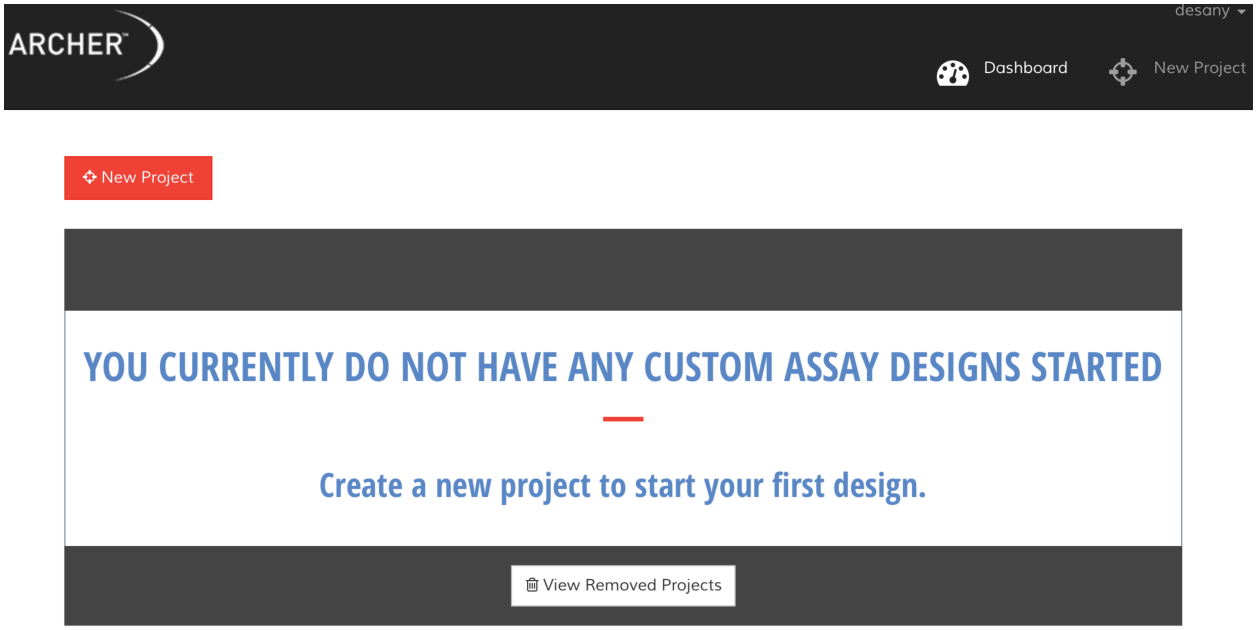
Confirmation

- Upon successful registration you will receive an activation e-mail at the address provided. To activate your account simply follow the link in the e-mail. Once your account has been successfully activated, you will see the following message.



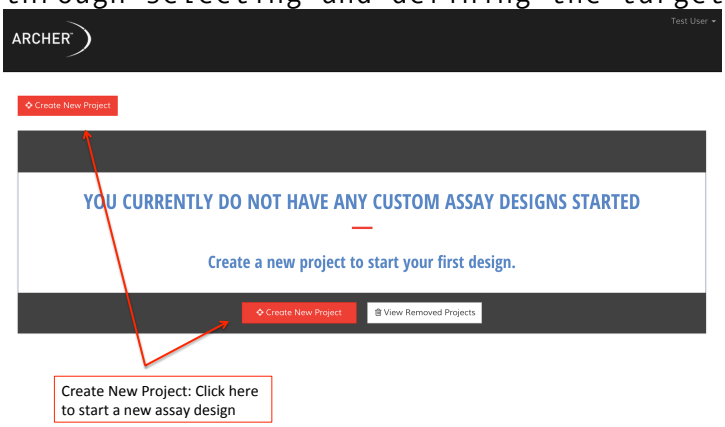
Website at a Glance





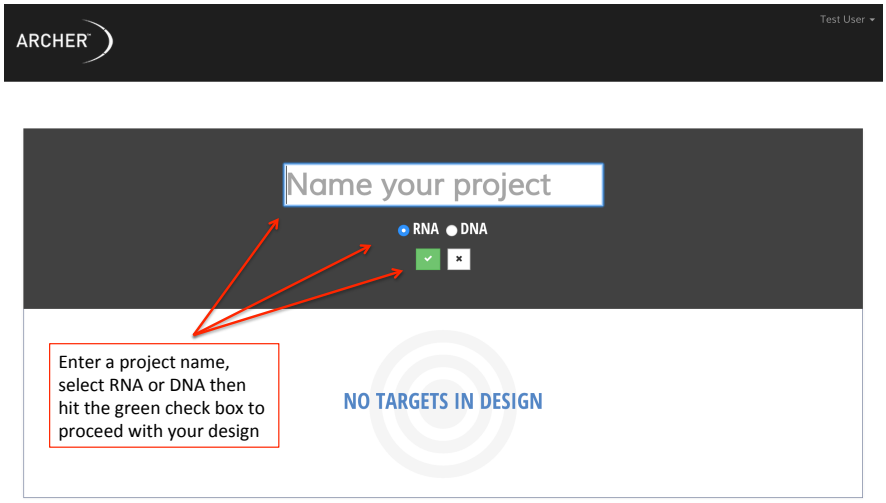
New Project

- Click the "Create New Project" button to create a new project.
- After selecting Create New Project, Assay Designer will walk you through selecting and defining the target exons of interest.



Design Name and Type

1. Enter a name for your project.
2. Select RNA or DNA
   - If you'd prefer to analyze DNA for fusion or CNV contact: tech@archerdx.com.
3. Click the green checkbox to start customizing your design.



- After clicking on the green box above you will be prompted to add the target regions of interest.

Things to Consider

Before adding targets to the design, you may want to consider a few important features.

Target Gene Variants

- When choosing a gene target, please note that some genes have multiple transcript variants.
- It is important to consider transcript variants because exon numbers may vary between them.
- It is critical to make sure you are specifying exons numbers relative to the transcript you have selected

Fusion Exon

- Genes fusions may form at several different exon junctions.
- For this reason it is necessary to know the exact exon(s) by which this occurs.

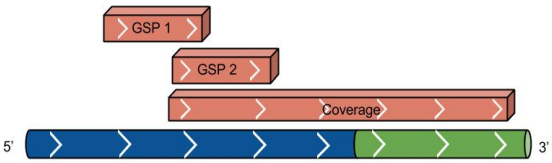
Fusion Direction

- Fusion partners may bind at the 5’ end, 3’ end, or in some cases both ends of a gene exon. You will be given the option to designate which direction you expect the fusion partner to be located.

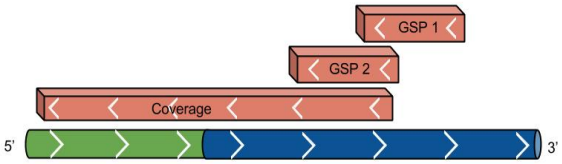
Target Gene

Fusion

3’ Directional Fusion Detection



5’ Directional Fusion Detection

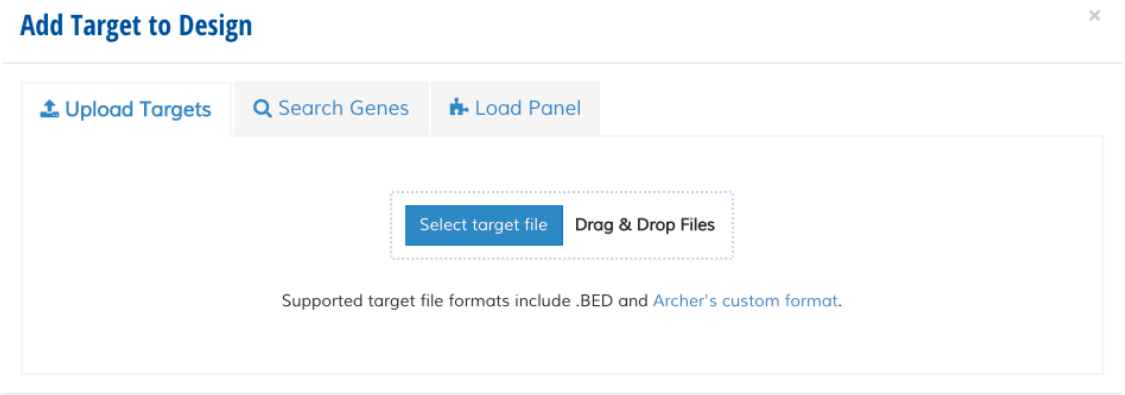


References

- If you are uncertain about any of the previously mentioned features, please visit the following sources for clarification:
  - Quiver - http://archerdx.com/software/quiver
    - This curated database designed by Archer™ contains entries from the sources listed below as well as several others, including manual entries from our in-house fusion discoveries.
  - COSMIC - http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/
  - ChimerDB - http://biome.ewha.ac.kr:8080/FusionGene/index.jsp
  - TICdb - http://www.unav.es/genetica/TICdb/

Add New Targets

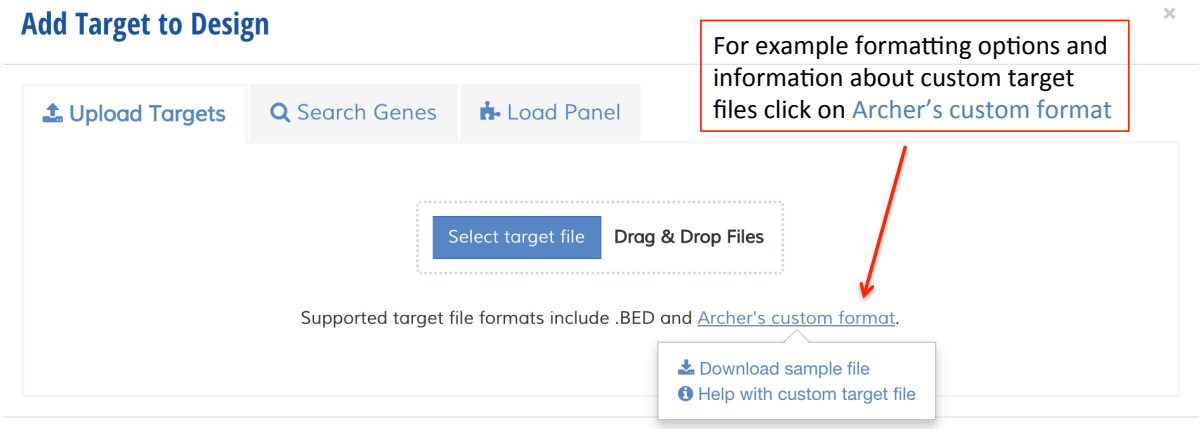
- After entering in your project name, molecule type, and clicking on the green box you will be prompted to add the target regions of interest.



- There are three methods for adding gene targets:
- 1. Upload a primer design file,
- 2. Upload all the targets from one of our commercially available panels, then edit
- 3. Search for the target regions of interest by gene name

1. Adding Targets by Uploading a Target File

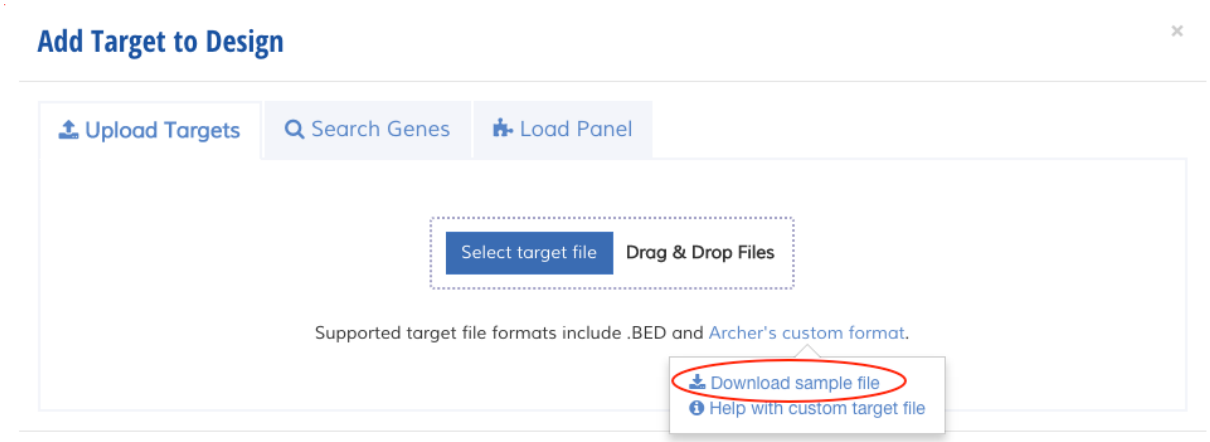
- Click the indicated link to download a template file for adding targets.



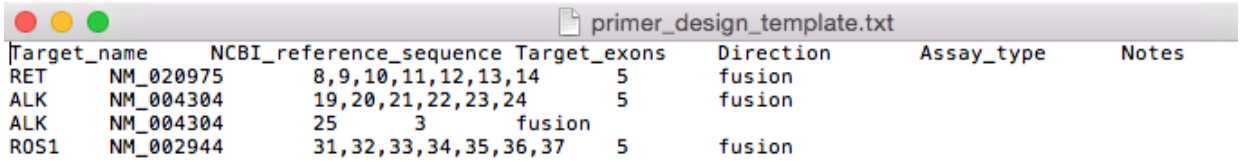
Clicking on the “Help with custom target file” will display the following:

Adding Targets to Custom Target File

INSTRUCTIONS FOR GENERATING AND UPLOADING A CUSTOM TARGET FILE



1. Click on the "Download sample file" link to download a template file showing sample targets.

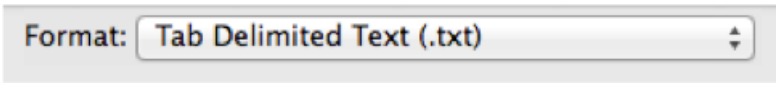


2. By default, this template file is downloaded as a "txt" file, which may be difficult to add information to. Copy the template into an Excel spreadsheet, or similar application, to easily add gene targets and other necessary information. Below is an example of a correctly formatted custom target file.

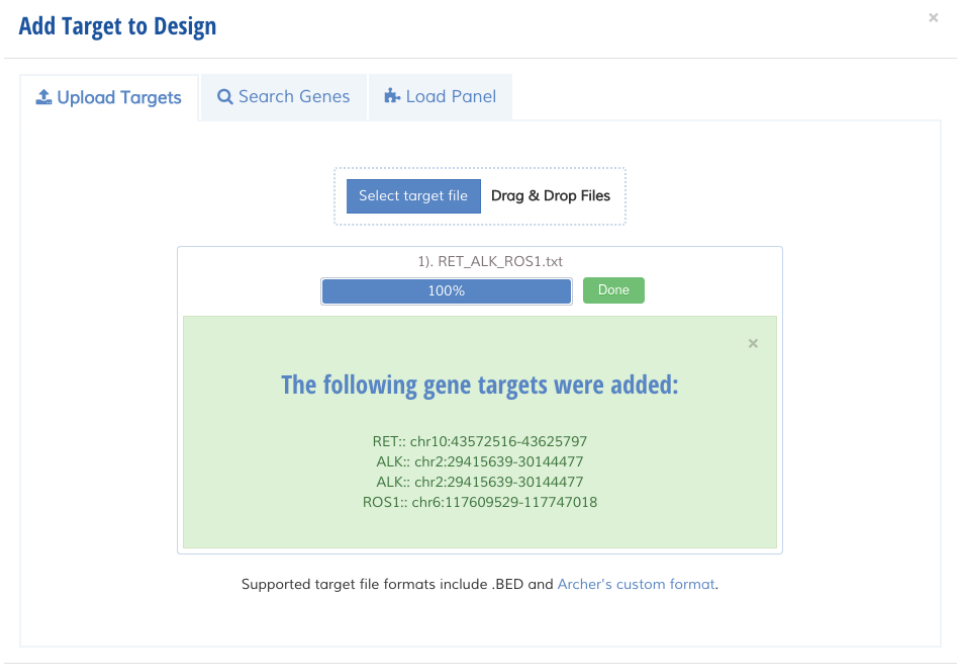
	A	B	C	D	E	F
1	Target_name	NCBI_reference_sequence	Target_exons	Direction	Assay_type	Notes
2	RET	NM_020975	8,9,10,11,12,13,14	5	fusion	
3	ALK	NM_004304	19,20,21,22,23,24	5	fusion	
4	ALK	NM_004304	25	3	fusion	
5	ROS1	NM_002944	31,32,33,34,35,36,37	5	fusion	
6						

Each column name signifies the following:

- Target Name: Canonical gene symbol for target gene.
- NCBI_reference_sequence: The reference sequence associated with the target gene. Reference sequences must start with "NM".
- Target Exon: The exons within the target gene, which will be amplified for gene fusions. Exons must be comma delimited.
- Direction: The direction of primer amplification, designated by either the "5" or "3" prime end of the gene. If you would like to detect fusions in both directions you may do this by adding the target gene and gene exons twice but selecting opposing directions (see NTRK3 entries in example).
- Assay_type: Currently supported assay types include "Fusion" and "Tile".
- Notes: This section will not be considered by the assay designer. It is for your convenience to keep note of ideas while building your custom file.



3. When you have finished adding targets to your custom file, be sure to save your spreadsheet file as a Tab Delimited Text (.txt) file.

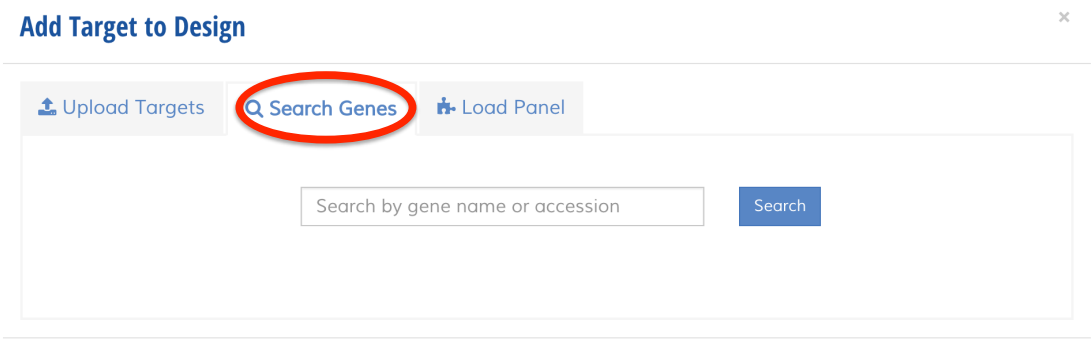


4. Upload your file by dragging and dropping it in to the designated

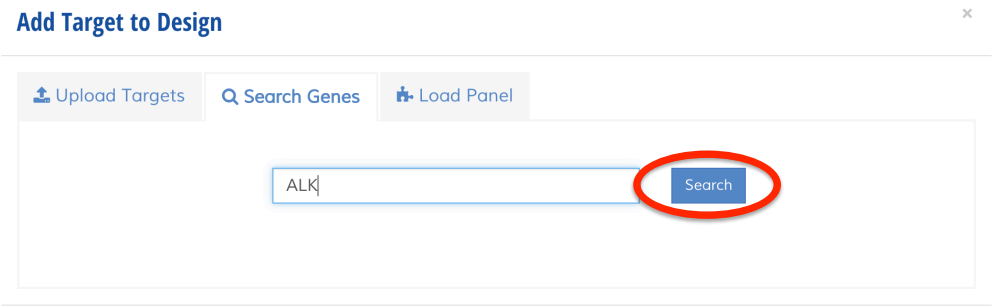
area. Alternatively, you may select the file to be uploaded by navigating to its location on your computer.

2. Adding Targets by a Gene Name Search

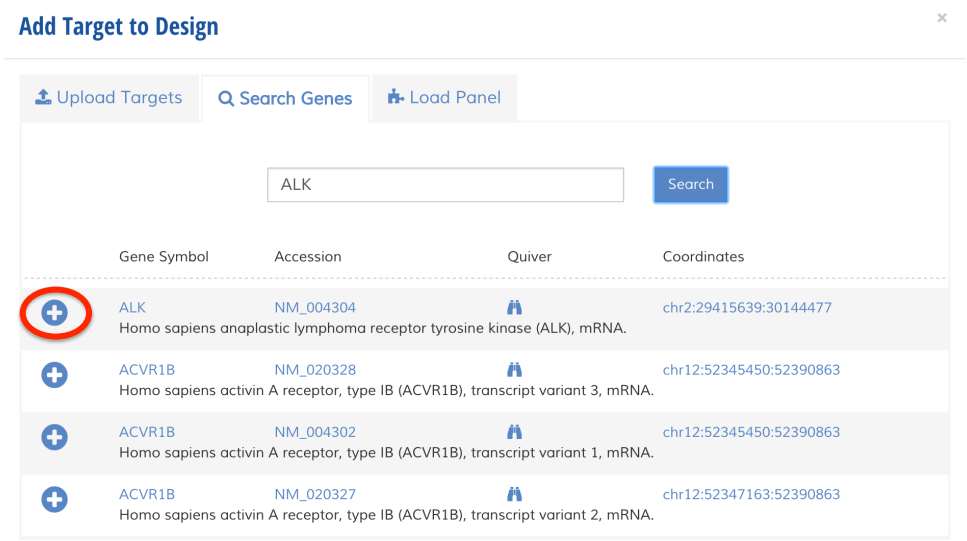
- Select the Search Genes tab circled below.



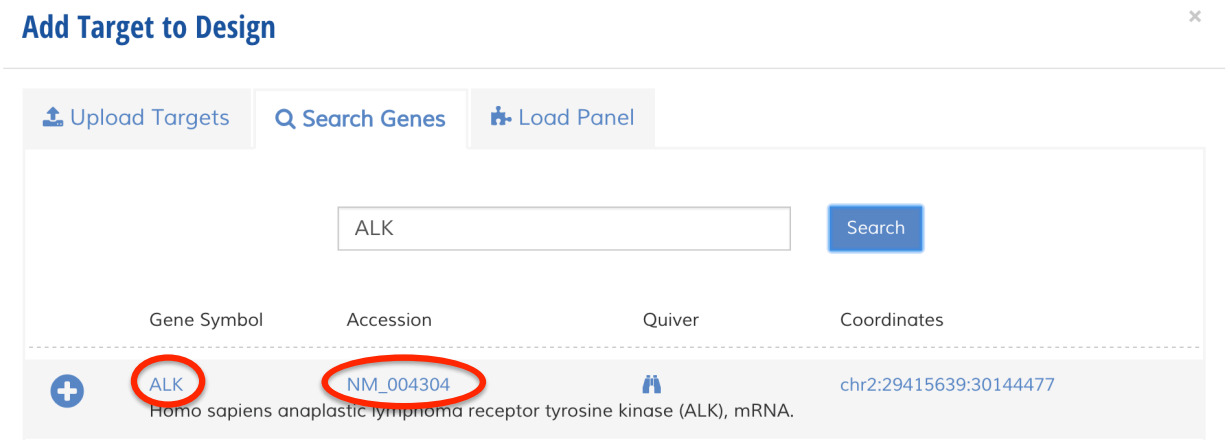
- Enter either the full or partial gene symbol and then click search.



- Select the correct transcript variant from the resulting list by clicking the corresponding ⊕ (see Things to Consider to find out more about transcript variants).



- Verify you have the correct gene transcript by clicking the gene symbol link to view your selected target in the UCSC Genome Browser or click the accession number link to read about your selection at the NCBI GenBank.



Target Options

The software will walk you through the options for each target after you have selected it including the following:

Direction

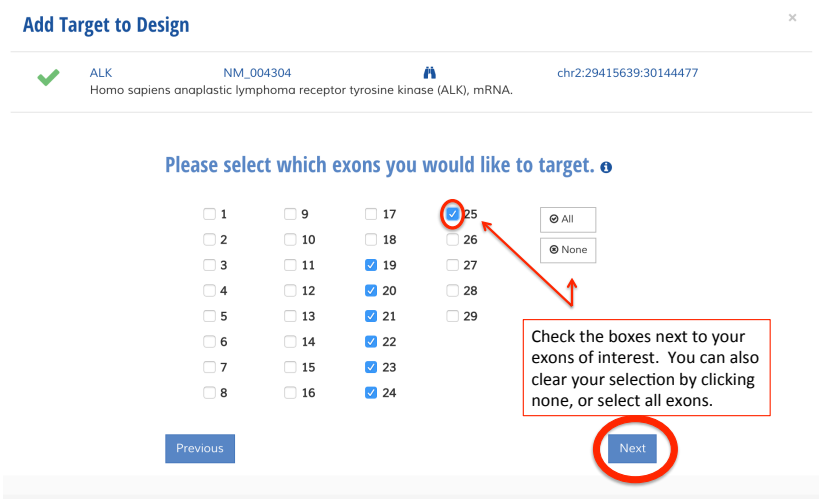
- The direction of primer amplification is relative to the gene of interest and is based on the location or potential location of the fusion partner (see Things to Consider).
- DON'T FORGET: if you want fusion detection at both ends of a target exon, you must add that gene and select that target exon twice: once in the 3' direction and once in the 5' direction
- Click Next after making your selection

Untranslated Regions

- Specify whether or not to include untranslated regions in the design.

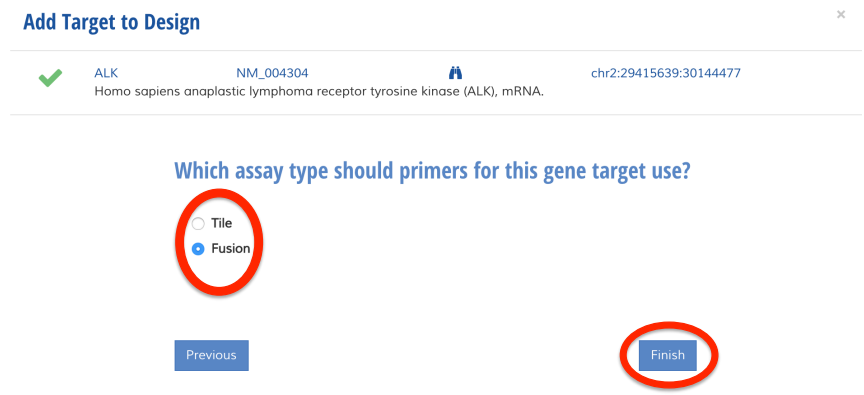
Exon Selection

- The assay designer provides the flexibility to choose exactly which exons in the target gene you want to sequence
- If you intend to detect fusion across all exons click Check All, otherwise, click the box next to the desired target exon(s).
- When the appropriate exons have been selected, exit by clicking Next.



Exon Tiling or Fusion Detection

- Choose either exon tiling or fusion detection for the exons chosen for the target.

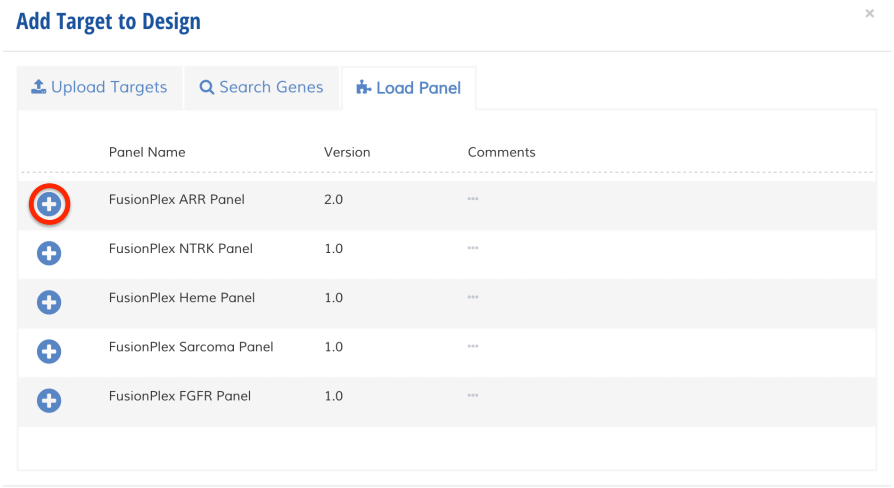


- After selecting finish, you will be directed back to the main Add Targets to Design screen. Choose more targets or proceed to submit your design by closing the Add Targets to Design window.

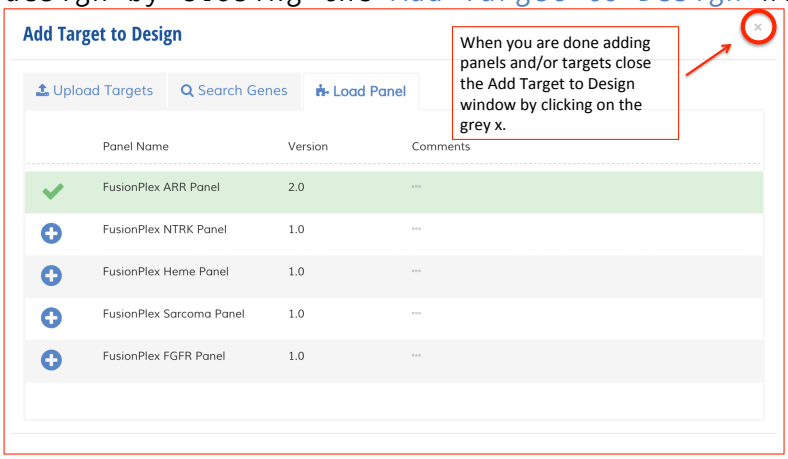
3. Adding Targets by Loading a Panel

Select from one of our commercially available panels

- This tab will give you the option of utilizing one of our commercially available panels. You may add targets, remove targets or combine panels.
- Select the desired panel(s) by clicking the corresponding

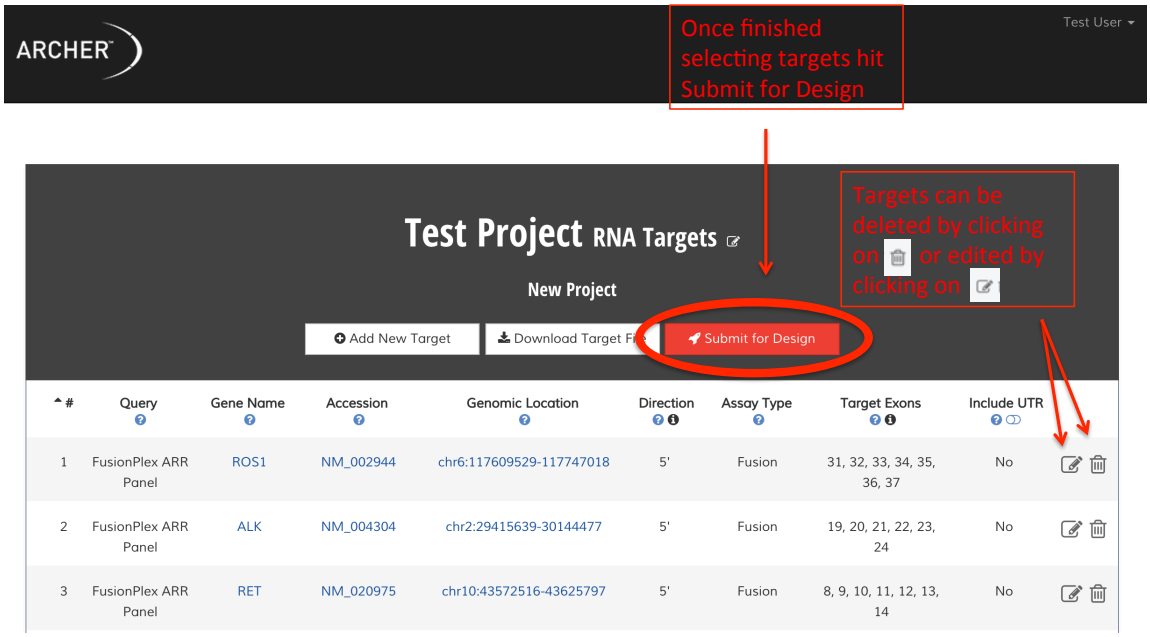


- After selecting a panel you may still add more targets or remove unwanted targets. Choose more targets, or proceed to submit for design by closing the Add Target to Design window.

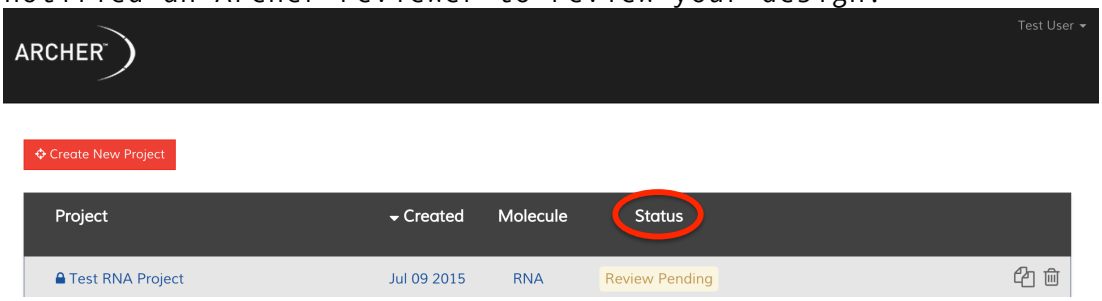


Submit for Design

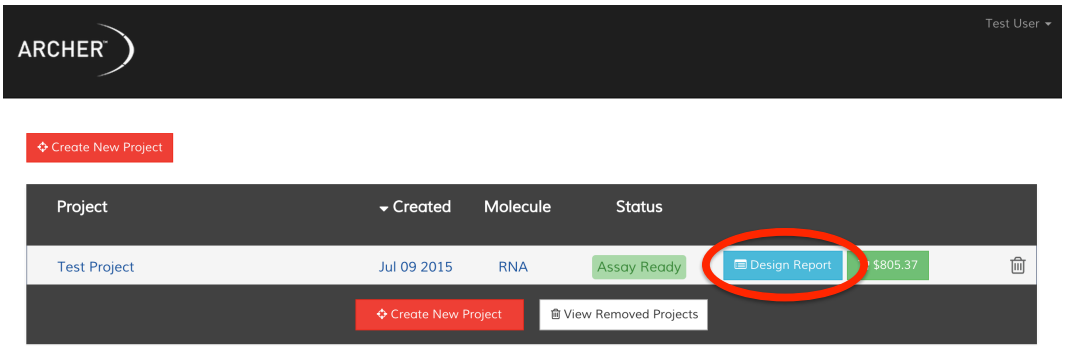
- Once you have uploaded all genes of interest and selected the correct directions and exons, click Submit for Design to initiate the design process.
- While the most recent project is being designed, another project may be created in the interim. However, only one project may be submitted for design at a time.



- Once the design has been submitted you will be able to see the status of the design. A status of Review Pending indicates that Archer Assay Designer has completed designing your primers and notified an Archer reviewer to review your design.

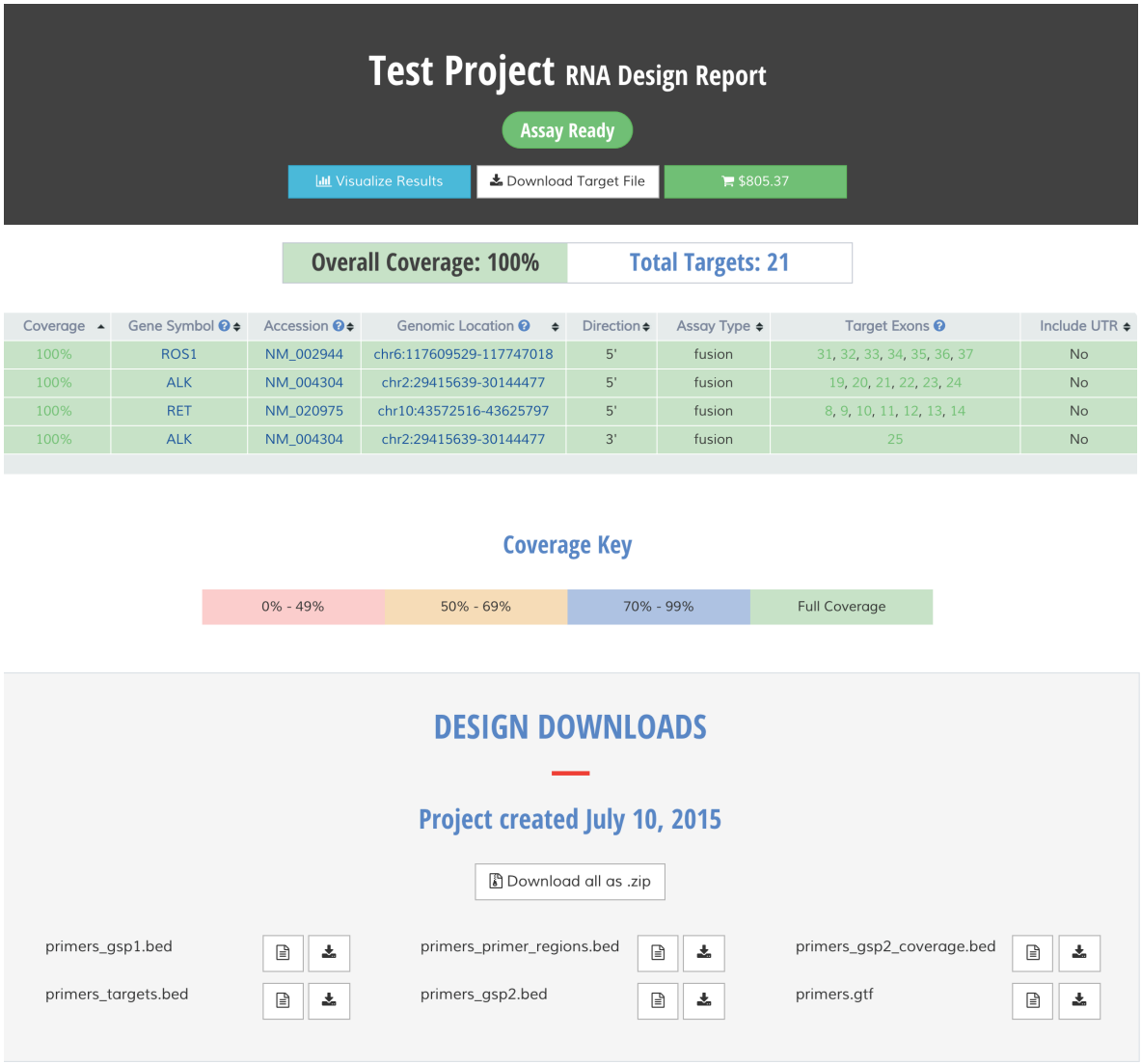


- After the design is reviewed, you will receive an e-mail with a quote, and a sales representative will contact you.
- Once the Design has been approved, the Dashboard status of the project will change to say "Assay Ready" and will provide you with new options. You will receive an additional email from the reviewer with any notes for your design.



Review Order

- You may review your order by either following the link in the e-mail or by clicking Design Report on the Dashboard.



Coverage	Gene Symbol	Accession	Genomic Location	Direction	Assay Type	Target Exons	Include UTR
100%	ROS1	NM_002944	chr6:117609529-117747018	5'	fusion	31, 32, 33, 34, 35, 36, 37	No
100%	ALK	NM_004304	chr2:29415639-30144477	5'	fusion	19, 20, 21, 22, 23, 24	No
100%	RET	NM_020975	chr10:43572516-43625797	5'	fusion	8, 9, 10, 11, 12, 13, 14	No
100%	ALK	NM_004304	chr2:29415639-30144477	3'	fusion	25	No

Check Coverage

- The coverage rating is an indication of how many of the designated targets will be amplified by the design.
- Unfortunately, the Assay Designer may not always produce an assay with 100% coverage due to complications with the target of interest.
  - Non-unique regions (contains gene homologues)
  - A-T rich regions
  - Low complexity regions (polynucleotide repeats)
- Overall coverage can quickly be assessed by comparing the coverage score to the color-coded coverage key below the target list.

- Each gene target, as well as the individual exons within that target is assigned a coverage rating. Clicking on the exon number will bring up a report. The Target Exon Report displays the percent coverage for each exon. If there was less than 100% coverage, the reason for design failure is specified. The cause of incomplete coverage will also be discussed in the email from the reviewer.

Coverage	Gene Symbol	Accession	Genomic Location	Direction	Assay Type	Target Exons	Include UTR
100%	ROS1	NM_002944	chr6:117609529-117747018	5'	fusion	31, 32, 33, 34, 35, 36, 37	No
100%	ALK	NM_004304	chr2:29415639-30144477	5'	fusion	19, 20, 21, 22, 23, 24	No
100%	RET	NM_020975	chr10:43572516-43625797	5'	fusion	8, 9, 10, 11, 12, 13, 14	No
100%	ALK	NM_004304	chr2:29415639-30144477	3'	fusion	25	No

Click on the target exon to bring up the Target Exon Report

Target Exon Report

Target	Genomic Location	Coverage
31	chr6:117658334-117658503	100%
32	chr6:117650491-117650609	100%
33	chr6:117647386-117647577	100%
34	chr6:117645494-117645578	100%
35	chr6:117642421-117642557	100%
36	chr6:117641030-117641193	100%
37	chr6:117639350-117639415	100%

View Coverage in Genome Browsers – IGV

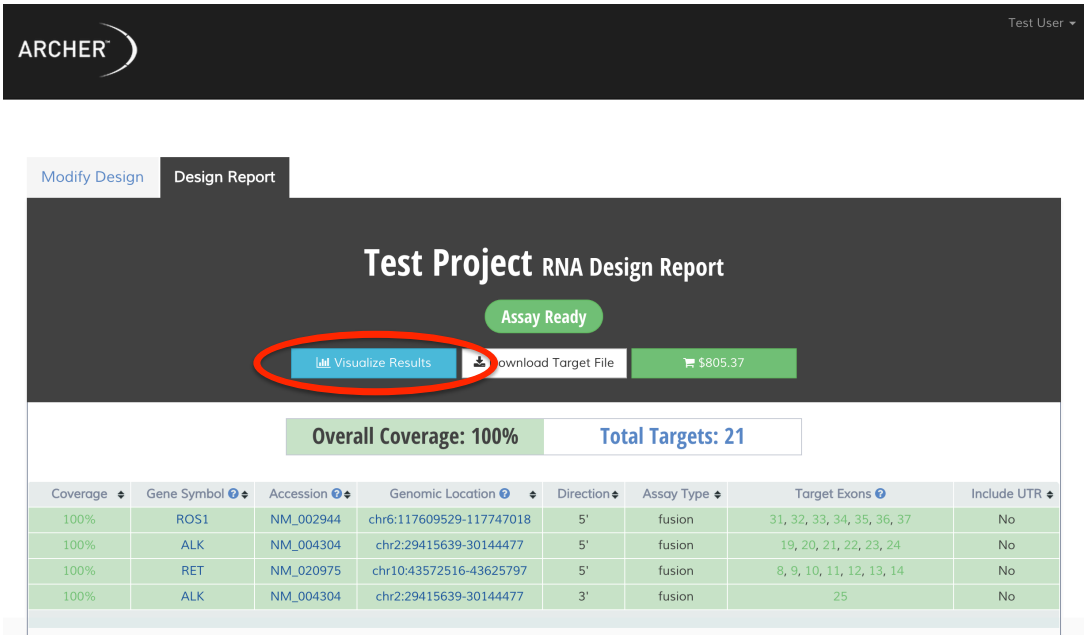
- If you don't already have IGV on your computer, download and install the program.
- IGV: http://www.broadinstitute.org/software/igv/download
- IGV User guide: http://www.broadinstitute.org/igv/UserGuide
- IGV compatible .bed files pertaining to your design may be downloaded by clicking any of the .bed files present at the bottom of the design report.

- Upload .bed files in IGV to view coverage

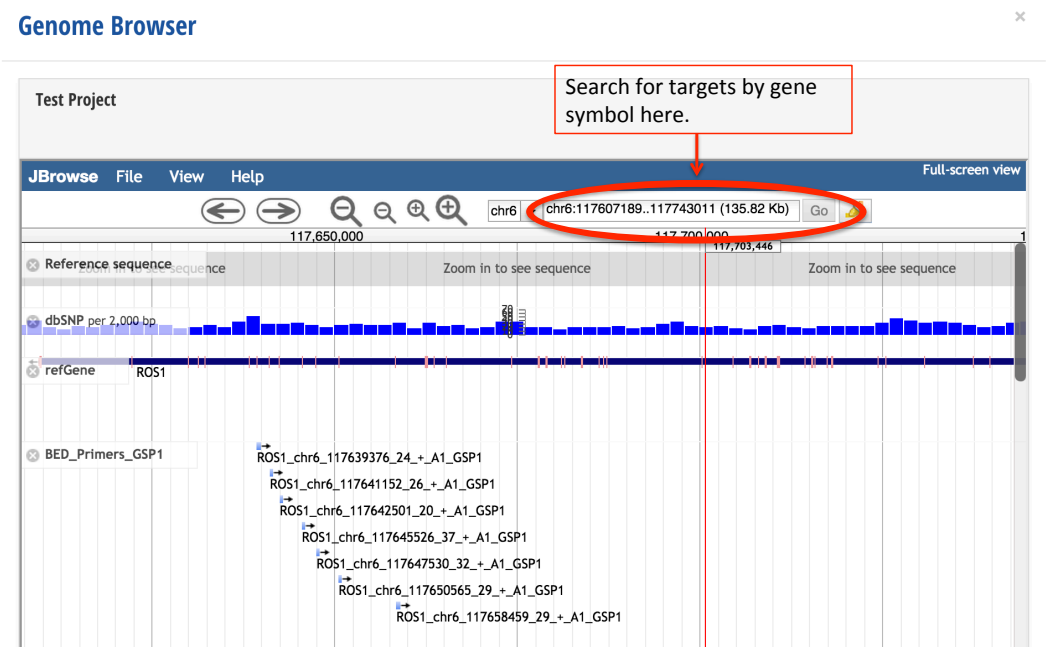
File name	Description
primers_targets.bed	Position of the chosen target exons.
primers_gsp1.bed	Position of the first PCR gene specific

	primer.
primers_gsp2.bed	Position of the second PCR gene specific primer. Also, the Starting position of the amplicon
primers_gsp2_coverage.bed	The projected coverage resulting from the gene specific primer pair amplification.
Additional Files	
primers.gtf	A GTF formatted file, containing information necessary to analyze sample results with the Archer™ Analysis Pipeline.

- Alternatively you can open our Genome Browser containing the .bed files by clicking on Visualize Results.



This link will bring up a searchable window containing ther .bed files

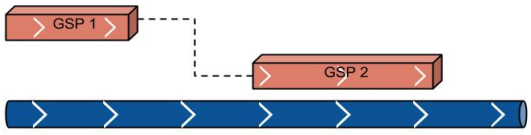


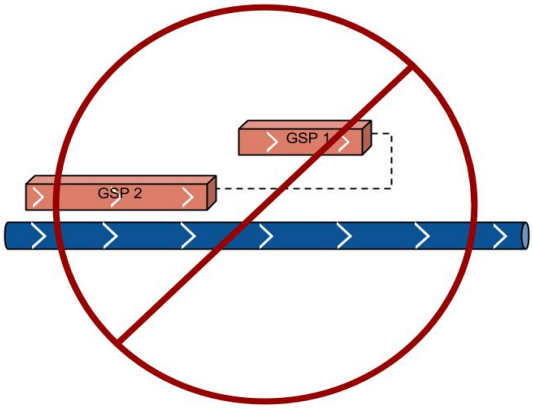
View Coverage – What to Look For

- NOTE: This section is relevant to RNA fusion designs ONLY

Nested Primers:

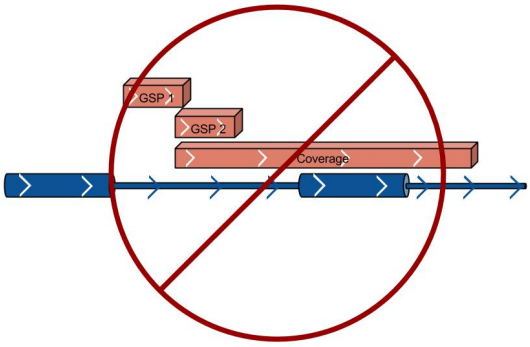
- The GSP2 should always be located (nested) inside of the GSP1





Exons Only:

- Primers should be positioned only on exons

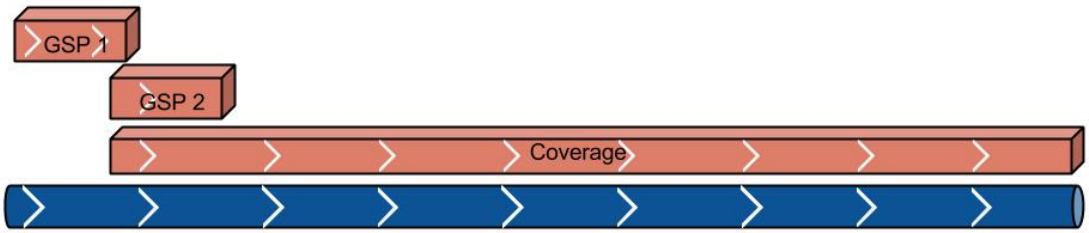


Correct Directionality:

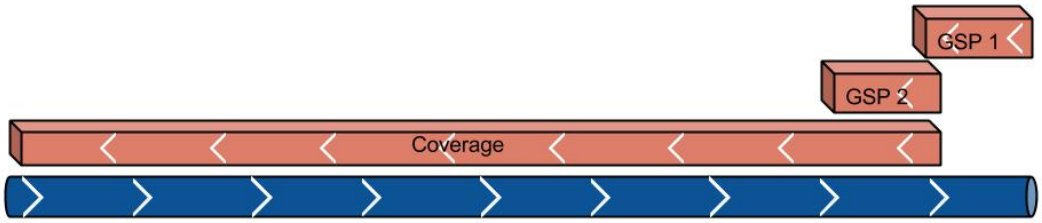
- Ensure that GSP1, GSP2, and

the Coverage direction all match the direction specified in the design.

3’ Directionality:

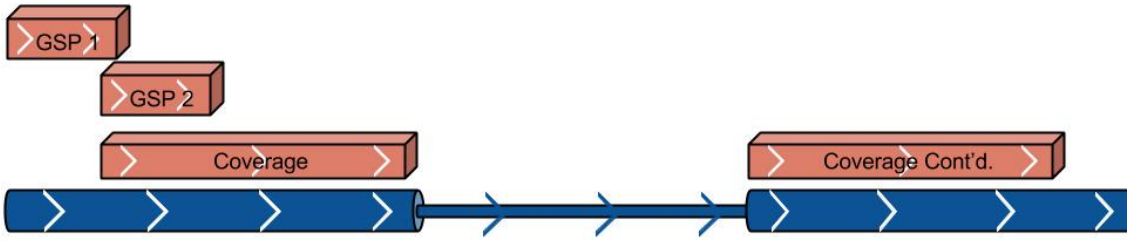


5’ Directionality:



Junction Coverage

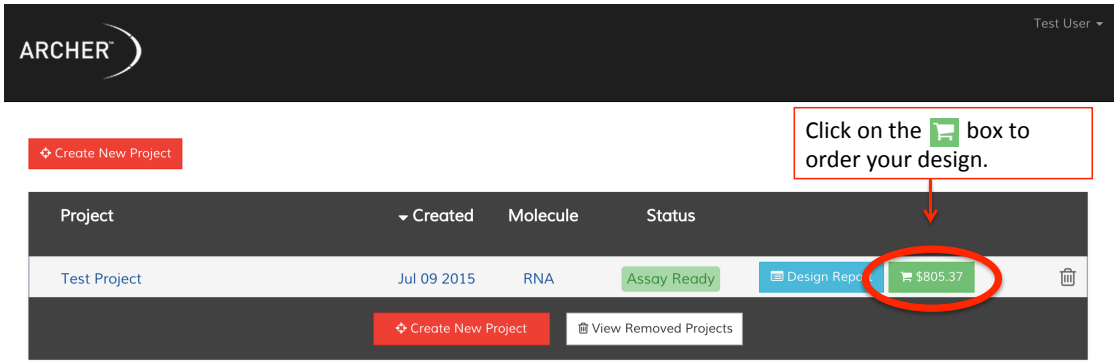
- Ensure that primer coverage extends through exon-exon junctions of all exons specified in design.



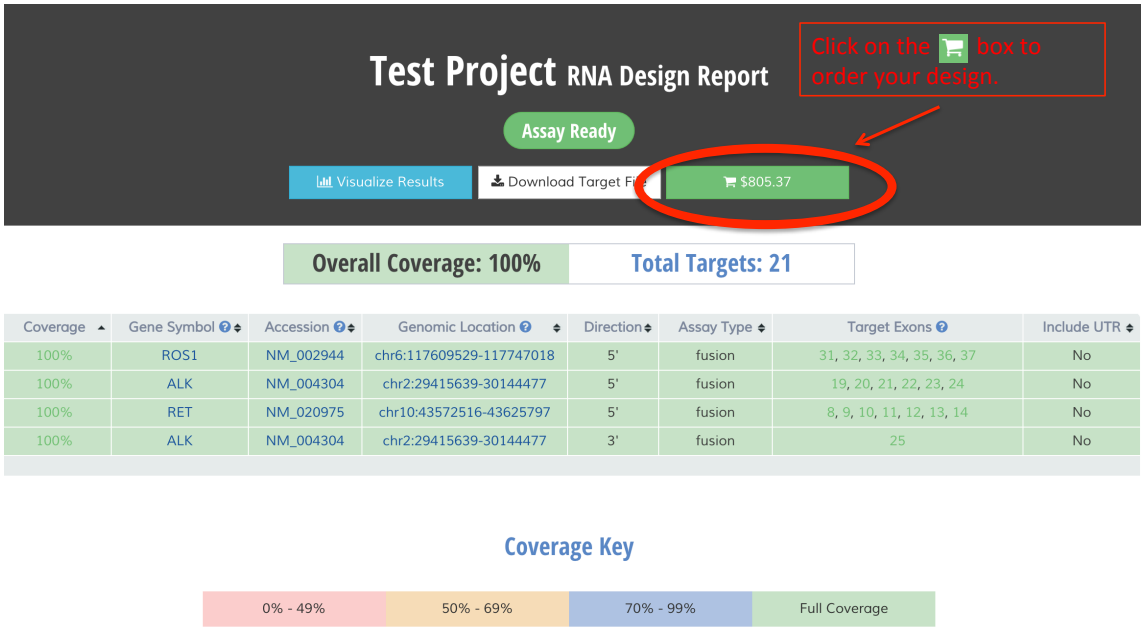
Ordering Your Assay

- “Order Assay” is accessible at two locations in the Assay Designer.

1. From the Assay Design Dashboard



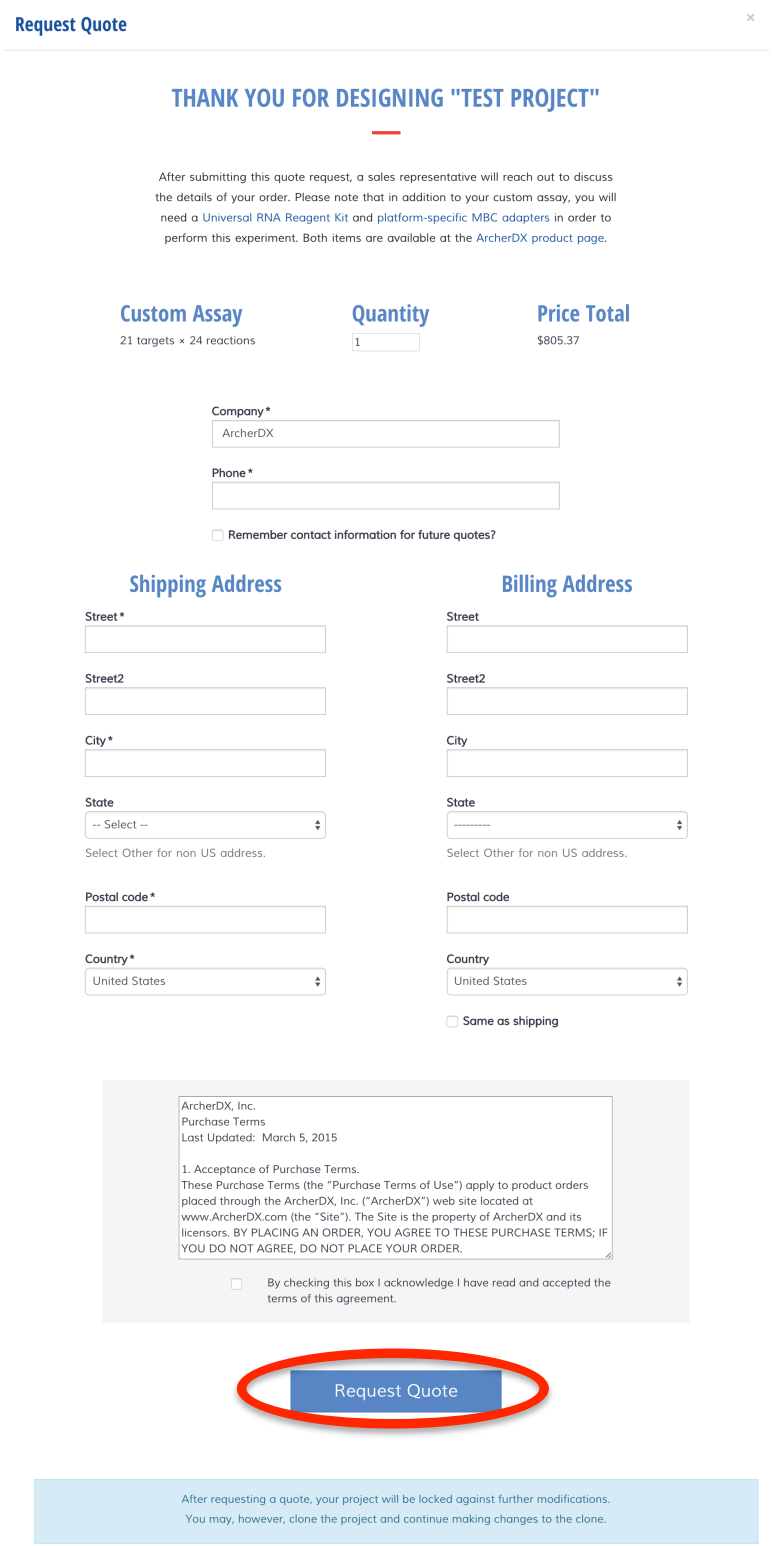
2. From the Design Report



Coverage	Gene Symbol	Accession	Genomic Location	Direction	Assay Type	Target Exons	Include UTR
100%	ROS1	NM_002944	chr6:117609529-117747018	5'	fusion	31, 32, 33, 34, 35, 36, 37	No
100%	ALK	NM_004304	chr2:29415639-30144477	5'	fusion	19, 20, 21, 22, 23, 24	No
100%	RET	NM_020975	chr10:43572516-43625797	5'	fusion	8, 9, 10, 11, 12, 13, 14	No
100%	ALK	NM_004304	chr2:29415639-30144477	3'	fusion	25	No

Complete Order Information

- Fill out the following fields and click Request Quote when done.



- A confirmation message is displayed if the ordering information was entered correctly.

Thank you!

We have received your quote request for Project #640 – "Test Project".

A sales representative will be in touch shortly. Please note that in addition to the assay you've designed, you will also need a Universal RNA Reagent Kit and platform-specific MBC adapters in order to perform this experiment. Both items are available at the ArcherDX product page.

- **NOTE**: Unless you have already been given an order number, you must contact orders@archerdx.com and provide payment information before the order is processed.

FAQs

URL: http://archerdx.com/faqs

Associated Products

P/N	Product Description
AK0040-8	Archer™ Universal RNA Reagent Kit for Illumina®
AK0042-8	Archer™ Universal RNA Reagent Kit for Ion Torrent™ AK0037-8 Archer™ Universal DNA Reagent Kit for Illumina®
N/A	Archer™ Analysis Pipeline - http://analysis.archerdx.com

URL: http://archerdx.com/

Limitations of Use

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

This product was developed, manufactured, and sold for in vitro use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.

For more information please visit http://www.archerdx.com

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