TWIST CUSTOM PANEL DESIGN SUITE

At Twist Bioscience, we combine precise oligonucleotide synthesis with a scalable silicon-based manufacturing platform to generate high-performing probe panels for NGS target enrichment. We complement this powerful technology with proprietary design algorithms and a rapid iteration pipeline to enable the quick custom design, synthesis, and optimization of panels. Twist Custom Panels can be designed and built to cover a wide range of panel sizes, target regions, and complex design requirements — all with exceptional and consistent performance.

Whether you design your own panel from scratch or add targets to enhance the content of our Human Core Exome, you can use your Twist Custom Panel with Twist's modular library preparation kits or seamlessly integrate them into your existing workflow.

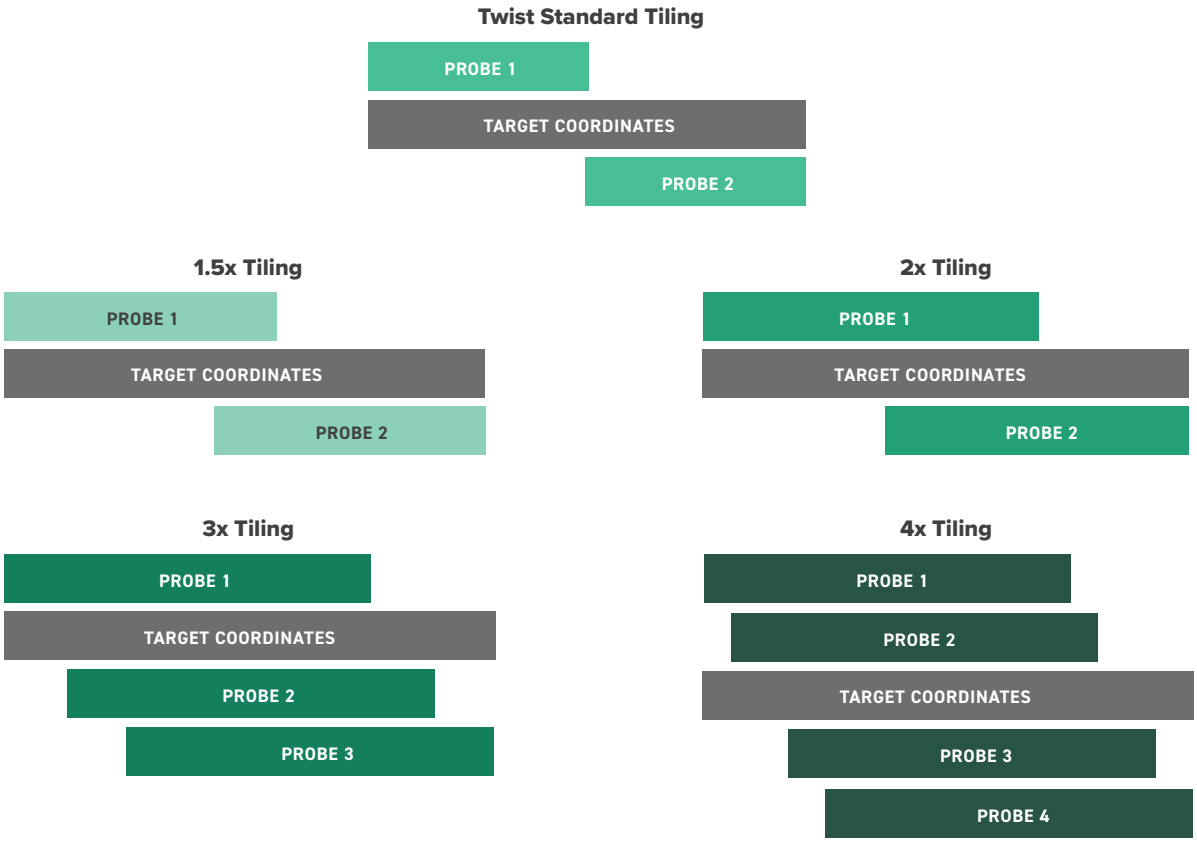
TABLE OF CONTENTS

TWIST CUSTOM PANEL DESIGN SUITE

Twist Custom Panel design options provide a comprehensive approach to designing custom probe panels that satisfy an array of NGS target enrichment approaches.

You can design your Twist Custom Panel(s) using target coordinates, gene names, or FASTA files. In addition, you can request panels that add content to our core/fixed panels ("spike-in" target coordinates added to our Twist Human / Mouse Core Exomes) or that increase coverage of specific target coordinates (Nx-based tiling).

Though Twist Custom Panels are optimized for efficient enrichment of desired targets using 120-bp oligonucleotide probes, we have also had success with other oligo lengths for unique experimental needs. Contact your local Field Application Scientist or Account Manager for more details.



Twist standard and Nx-tiling approaches to probe design. Nx tiling may improve coverage of difficult to sequence regions, repetitive regions, single-nucleotide polymorphisms (SNPs), among other unique genomic elements.

Target Coordinate-Based Custom Panel

Purpose: Design a Twist Custom Panel from a target coordinates file(s).

Reference genomes: Twist currently supports target coordinates from the following genome assemblies:

- Human (hg19 and hg38)
- Mouse (mm10)
- Rat (rnor6)
- Canine (canFam3)

Input: Provide .xls/.txt/.bed files with target coordinates in the following format:

Chrom	Start	Stop
chr1	8412873	8412997
chr1	29646065	29646189
chr1	76116000	76116124
chr1	161178622	161178746
chr1	196642171	196642295

Genes-Based Custom Panel

Purpose: Design a Twist Custom Panel from a file(s) with gene names.

Reference genomes: Twist currently supports gene names from the following genome assemblies:

- Human (hg19 and hg38)

Input: Provide .xls/.txt/.bed files with gene names in the following format:

Gene_Name
C7A
C4B
IGLL13
IKBKG
NCF1

Additional Parameters: We can strategically design your gene-based target coordinates using the following databases:

- Reference Sequence Database (RefSeq)
- GENCODE
- Consensus CDS (CCDS)

In addition, 3’ and 5’ UTRs can be included in the design.

FASTA File-Based Custom Panel

Purpose: Design a Twist Custom Panel from FASTA sequence file(s).

Input: Provide .txt/.fa file(s) with FASTA sequences. For expedited processing, begin each unique FASTA sequence with “>” followed by a multi-word description using “_” to separate words.

```
>Descriptive_name_for_Sequence
GTTGTAAAACGACGGCCAGTGAATTCGAGC
GGGCGGCCGCCTGCAGACCAGGTCTNNNNN
GATCTGGATCCCTCGAGTCTAGAGTCGACC
```

Human Core Exome with Spike-in Custom Panel

Purpose: Design a Twist Custom Panel from gene names or target coordinate file(s) that will be spiked-in to the Twist Human Core Exome.

Reference genomes: Twist currently supports spike-in target coordinates / gene names from the following genome assemblies:

- Human: hg19 and hg38

Input: Provide .xls/.txt/.bed files with gene names or target coordinates in the following format:

Target Coordinate File

Chrom	Start	Stop
chr1	8412873	8412997
chr1	29646065	29646189
chr1	76116000	76116124
chr1	161178622	161178746
chr1	196642171	196642295

Gene Name File

Gene_Name
C7A
C4B
IGLL13
IKBKG
NCF1

Nx-Tiled Custom Panel

Purpose: Design a Twist Custom Panel with Nx tiling from target coordinates / gene names / FASTA file(s). Nx tiling may improve coverage of difficult to sequence regions, repetitive regions, single-nucleotide polymorphisms (SNPs), among other unique genomic elements.

Input: PProvide .xls/.bed/.txt/.fa files with target coordinates / gene names/FASTA file(s) in the format detailed above.

EVALUATING TWIST CUSTOM PANEL DESIGNS

How to Evaluate Custom Panel Design Using the UCSC Genome Browser

When your Twist Custom Panel design is complete, you will receive a folder containing panel design files. This section describes the steps for viewing and evaluating those files using the UCSC Genome Browser.

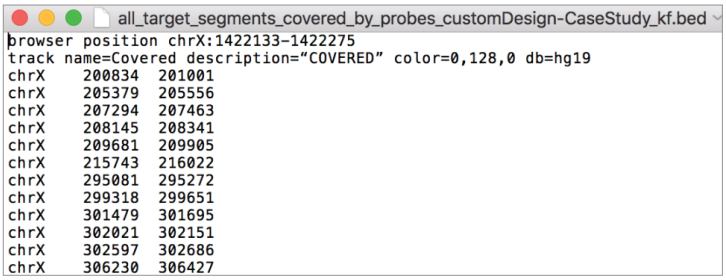
Panel Design Coverage Report and Files

When the design of your panel is complete, you will receive the following:

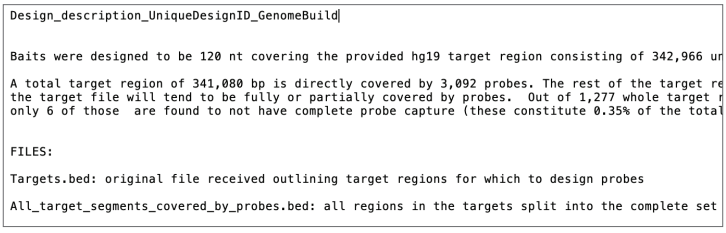
1. Three .bed files:
   - A .bed file of the regions you targeted (targeted regions)
   - A .bed file of the regions covered by your custom panel
   - A .bed file of the regions not covered

   Each .bed file contains a header with the information required for upload to the UCSC Genome Browser.
2. A .txt file with the probe count and coverage information (probe coverage report).
3. A screenshot from the UCSC Genome Browser of a selected region of your panel created during the quality control process.

The UCSC browser header information in each .bed file allows you to easily upload the .bed files to the UCSC Genome Browser for visualization.



An example of one of the .bed files. This example summarizes the regions covered by a Twist custom panel. The header information provides details the UCSC Genome browser needs to upload these annotation tracks.

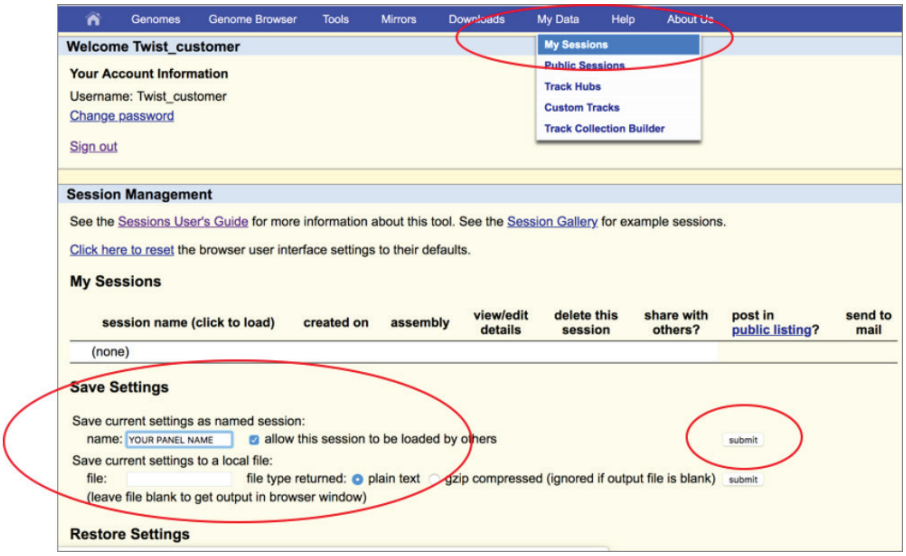


An example of a probe coverage report.

USING THE UCSC GENOME BROWSER

Use the UCSC Genome Browser to evaluate your panel design. A basic introduction to features of the Genome Browser is provided here. For more information about the UCSC Genome Browser, refer to the Genome Browser User Guide.

1. Access the UCSC Genome Browser and navigate to **My Data > My Sessions**.
2. Create an account and login in. Use the options under My Sessions to save or restore your browser settings (to display specific track combinations, including custom tracks from Twist).

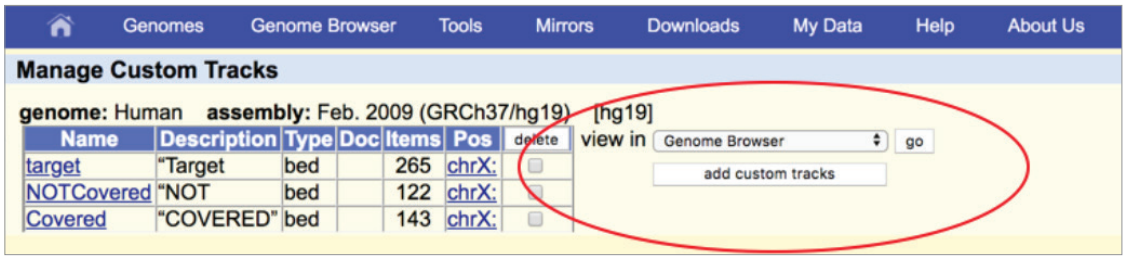


Use the options under My Data > My Sessions to save your custom browser configuration.

3. To upload the panel design .bed files, select **My Data > Custom Tracks**. In the Add Custom Tracks page:
   - Select the following in the pull-downs:
     - Clade: Mammal
     - Genome: Human
     - Assembly: (ensure the appropriate assembly is selected)
   - Click **Choose File** and select a .bed file to review: Covered Regions, Not Covered Regions, or your Target Regions file
   - Click **Submit**
4. The Manage Custom Tracks window opens with the .bed file and following listed:
   - **Name:** links to the update page where you can edit the track data
   - **Description:** description from the track line
   - **Type:** track type, based on the data format (.bed files in this case)
   - **Items:** number of data items in the custom track file
   - **Pos:** default chromosomal position (only the chromosome number is shown)

5. To add the other files, click **Add Custom Tracks** and repeat the process until you have a complete listing. You can use the viewer options to display or hide any of the tracks you upload.

6. Select **View In > Genome Browser** and click go to view the files in the Genome Browser.

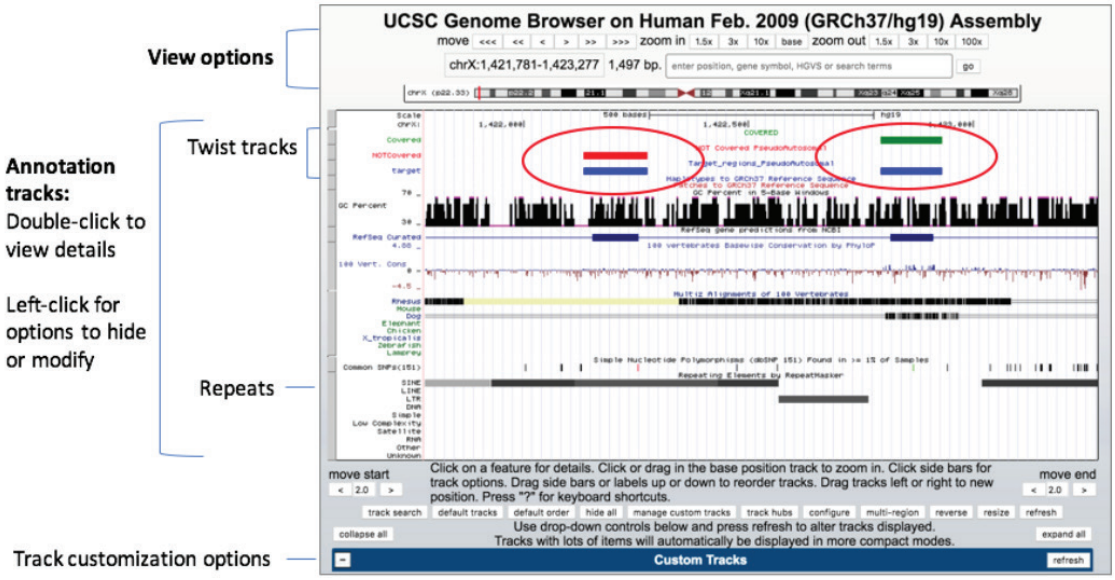


Uploading .bed files. Click add custom tracks to add each file. Once all files are added, click go to view the data in the Genome Browser.

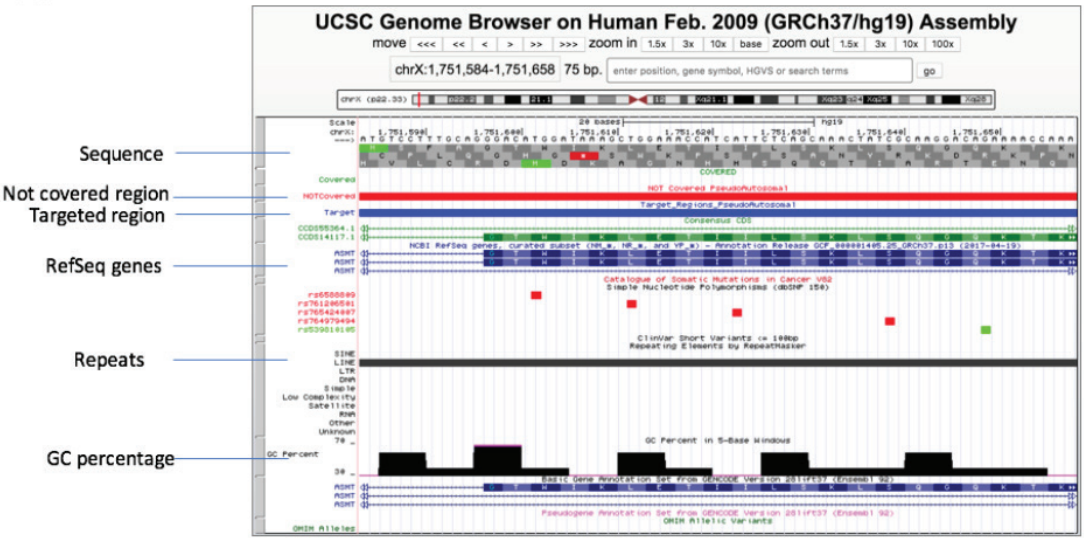
The Genome Browser opens to display the region of the assembly.

- Covered regions appear in green
- Regions not covered appear in red
- Target regions appear in blue

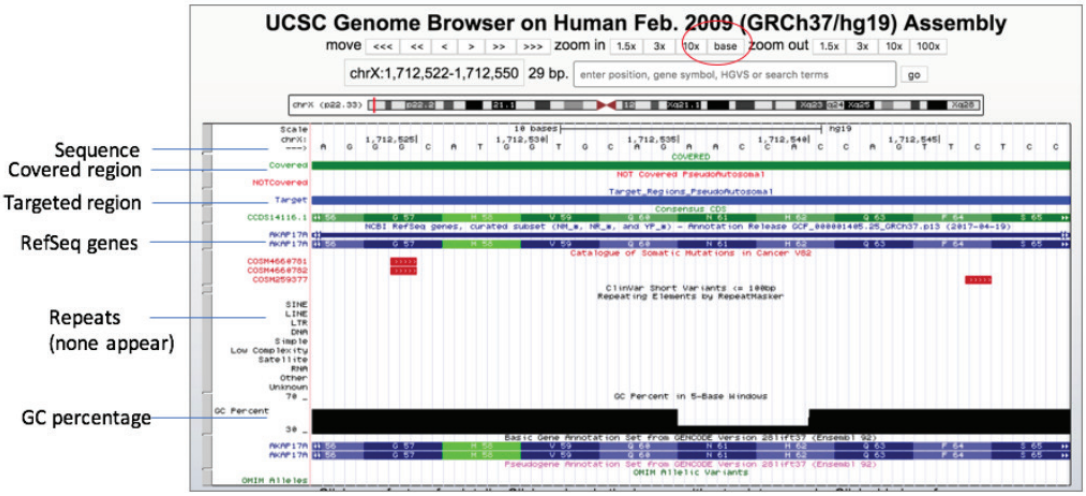
7. The metadata headers contain a default browser position. Use the controls within the Genome Browser to adjust the view:
   - Use the **Move** arrows to navigate along the genome
   - Use the **Zoom In / Zoom Out** buttons above the viewer for a more / less detailed view
   - Click **Base** to zoom in to the nucleotide level
   - Highlight a region in the browser or in the chromosome image to specify the region for view
   - Use the browser position search box to enter positions and interrogate a region of interest (for example, chrX: 1421781-1423277)



The genome annotation tracks appear in the Genome Browser viewer. Use the view options to navigate through the genome. This example shows two targeted regions (blue): one that is not covered by a probe (red), and another that is covered (green).



A covered region expanded to the base level. The top annotation track (Base Position) displays the nucleotide sequence. Note the region is covered by the panel design, and it shows no repeats and no regions of extreme GC content. Other annotation tracks include NCBI RefSeq, CCDS, GenCode Genes, COSMIC Regions, and OMIM Alleles.



A region that is covered expanded to the base level. The display of the top annotation track (Base Position) has been expanded to "Full" display to show the nucleotide sequence as well as the predicted translation in all three reading frames. Note the region is not covered in the panel design, and it shows regions of repeats and extreme GC content. Other annotation tracks include NCBI RefSeq, CCDS, GenCode Genes, COSMIC Regions, and OMIM Alleles.

Why did I not get 100% coverage?

If you see target regions that were not covered, check the **Repeatmasker** annotations. Twist filters out highly repetitive regions that may result in off-target capture and lower performance of the panel. Covering or forcing probes over repeat regions can result in non-specific capture and alignment issues at sequencing.

If targets essential to your panel are filtered out because they occur in repeat regions, Twist can reintroduce probes. Doing so, however, will increase off-target rates and lower the performance of your panel. In addition, these regions may be challenging to uniquely map in downstream analysis and may result in poor coverage.

FREQUENTLY ASKED QUESTIONS (FAQS)

Are Twist probes RNA or DNA?

Our probes for target enrichment are double-stranded DNA. They can, however, be used to enrich targets from cDNA libraries made from RNA.

How do I request a Custom Panel from Twist?

To submit a request for a Twist Custom Panel, please provide the relevant files and information detailed in this manual for your specific design request.

For panel designs for unique genomes and collaborations for development, contact your regional or local Sales and Support Account Manager.

How long will it take to design my Twist Custom Panel?

On average, the process of custom panel design takes two business days, but this time depends in part on the complexity of the request.

Can I create other probe lengths if I am using a different library preparation protocol?

Yes. Please consult with your local regional Twist Bioscience Account Manager in Sales and Support to design a panel using 60–120 bp probe length.

What is the turnaround time from when I place my order until I receive the probes?

Twist turn-around time averages three weeks.

What applications are compatible with Twist Custom Panels?

Twist Custom Panels can be used in various applications, including exome, custom exome, cfDNA, viral sample genotyping and detection, comprehensive cancer and cancer-specific panels, and methylation sequencing.

What is the minimum number of reactions for Twist Custom Panels?

12 sample reactions.

What are the requirements for fusion panel designs?

Please contact Twist NGS Technical Support at NGStechnicalsupport@twistbioscience.com for help with design strategies to analyze gene fusions.

How large of a panel can Twist make?

There are no size limitations on custom panel designs. We tailor our capabilities to your needs.

Please contact Twist NGS Technical Support at NGStechnicalsupport@twistbioscience.com or your Account Manager for help with any other questions you may have.

Twist Custom Panels is a component of the Twist portfolio of products for NGS Target Enrichment.

LEARN MORE

twistbioscience.com/ngs
sales@twistbioscience.com