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Twist Bioscience's quality management system governing the design and manufacture of NGS Target Enrichment Panels is ISO 13485:2016 certified (San Francisco, CA).

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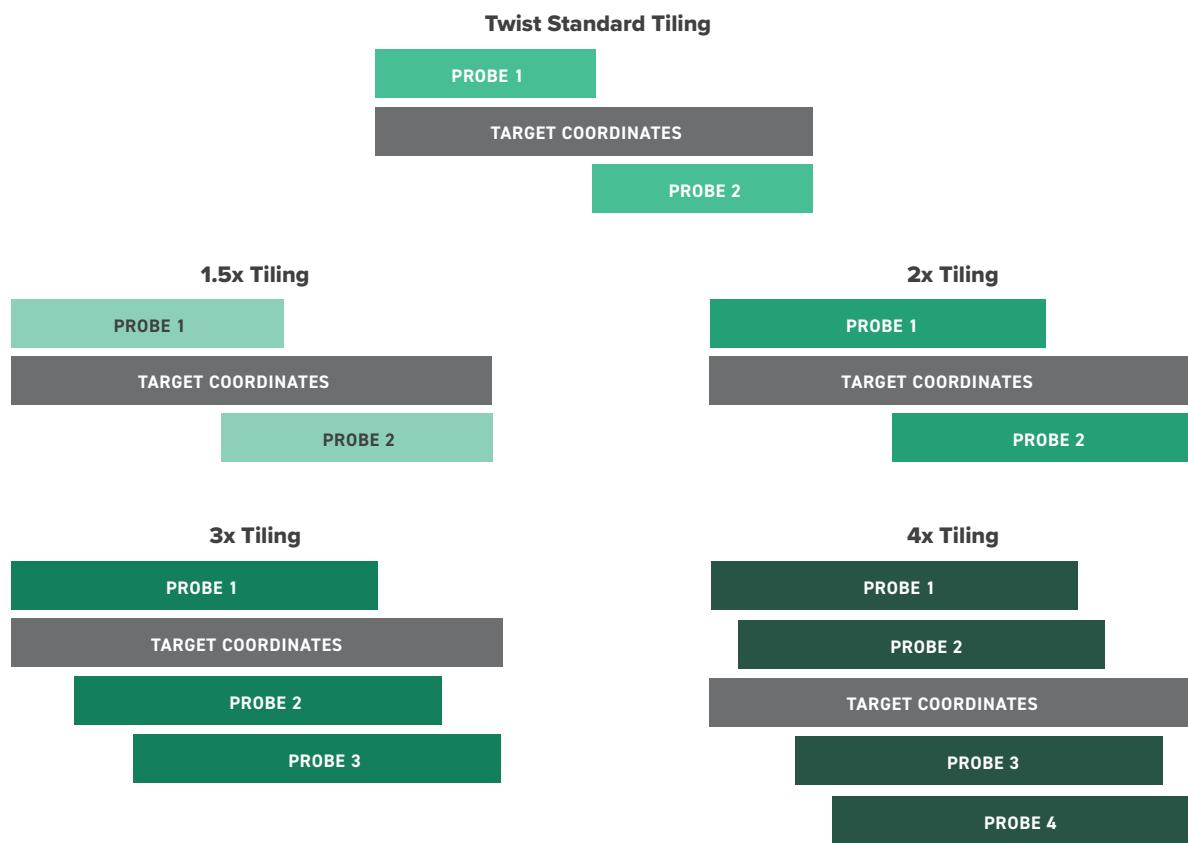
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TWIST CUSTOM PANEL DESIGN SUITE

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
GTTGTAAAACGACGCCAGTGAATTGAGC
GGGCAGGCCCTGCAGACCAGGTCTNNNNN
GATCTGGATCCCTGAGTCTAGAGTCGACC
```

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 | | | Gene Name File |
|------------------------|-----------|-----------|----------------|
| Chrom | Start | Stop | Gene_Name |
| chr1 | 8412873 | 8412997 | C7A |
| chr1 | 29646065 | 29646189 | C4B |
| chr1 | 76116000 | 76116124 | IGLL13 |
| chr1 | 161178622 | 161178746 | IKBKG |
| chr1 | 196642171 | 196642295 | 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: Provide .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.

```
all_target_segments_covered_by_probes_customDesign-CaseStudy_kf.bed
browser position chrX:1422133-1422275
track name=Covered description="COVERED" color=0,128,0 db=hg19
chrX 200834 201001
chrX 205379 205556
chrX 207294 207463
chrX 208145 208341
chrX 209681 209905
chrX 215743 216022
chrX 295081 295272
chrX 299318 299651
chrX 301479 301695
chrX 302021 302151
chrX 302597 302686
chrX 306230 306427
```

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.

```
Design_description_UniqueDesignID_GenomeBuild
Baits were designed to be 120 nt covering the provided hg19 target region consisting of 342,966 un
A total target region of 341,080 bp is directly covered by 3,092 probes. The rest of the target re
the target file will tend to be fully or partially covered by probes. Out of 1,277 whole target r
only 6 of those are found to not have complete probe capture (these constitute 0.35% of the total
FILES:
Targets.bed: original file received outlining target regions for which to design probes
All_target_segments_covered_by_probes.bed: all regions in the targets split into the complete set
```

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).

The screenshot shows the 'My Sessions' page of the UCSC Genome Browser. At the top, there is a navigation bar with links for Genomes, Genome Browser, Tools, Mirrors, Downloads, My Data (which is highlighted with a red circle), Help, and About Us. The 'My Data' menu has a sub-menu with options: My Sessions (highlighted with a red circle), Public Sessions, Track Hubs, Custom Tracks, and Track Collection Builder. Below the navigation bar, there is a 'Welcome Twist_customer' section with account information (Username: Twist_customer, Change password, Sign out). Under 'Session Management', there is a note about the Sessions User's Guide and a link to reset browser settings. The 'My Sessions' section contains a table with a single row: '(none)'. Below this is a 'Save Settings' section with fields for 'name:' (set to 'YOUR PANEL NAME') and 'allow this session to be loaded by others' (checkbox checked). There are also fields for 'file:' (set to 'plain text') and 'file type returned:' (radio buttons for 'plain text' and 'gzip compressed (ignored if output file is blank)'). A 'submit' button is located to the right of these fields. At the bottom of the page is a 'Restore Settings' link.

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.

Manage Custom Tracks

genome: Human assembly: Feb. 2009 (GRCh37/hg19) [hg19]

| Name | Description | Type | Doc | Items | Pos | delete |
|------------|-------------|------|-----|-------|--------------------------|--------|
| target | "Target" | bed | | 265 | chrX: 1421,781-1,423,277 | |
| NOTCovered | "NOT" | bed | | 122 | chrX: 1421,781-1,423,277 | |
| Covered | "COVERED" | bed | | 143 | chrX: 1421,781-1,423,277 | |

view in **Genome Browser** go
add custom tracks

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)

View options

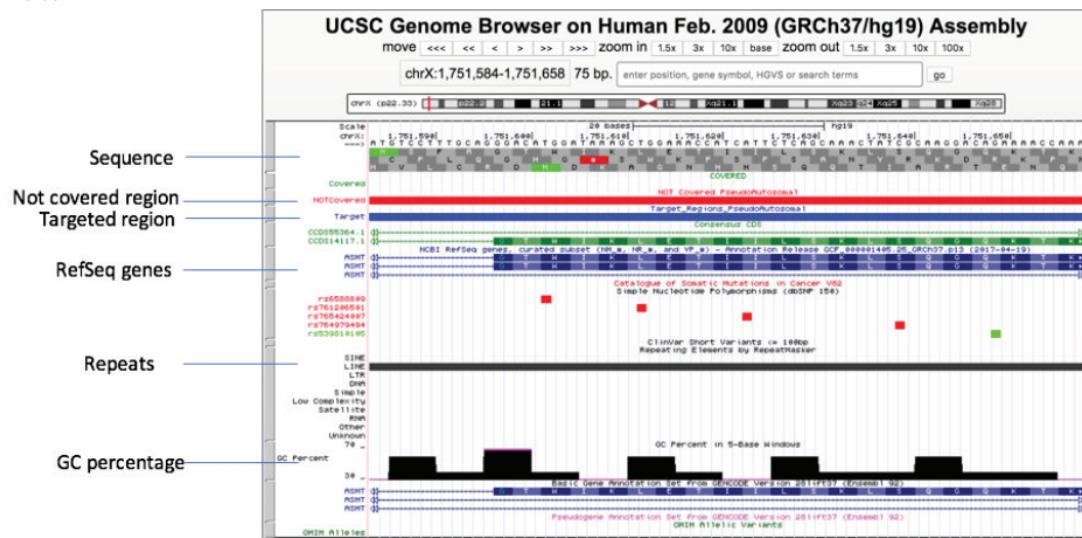
Annotation tracks:
Double-click to view details

Left-click for options to hide or modify

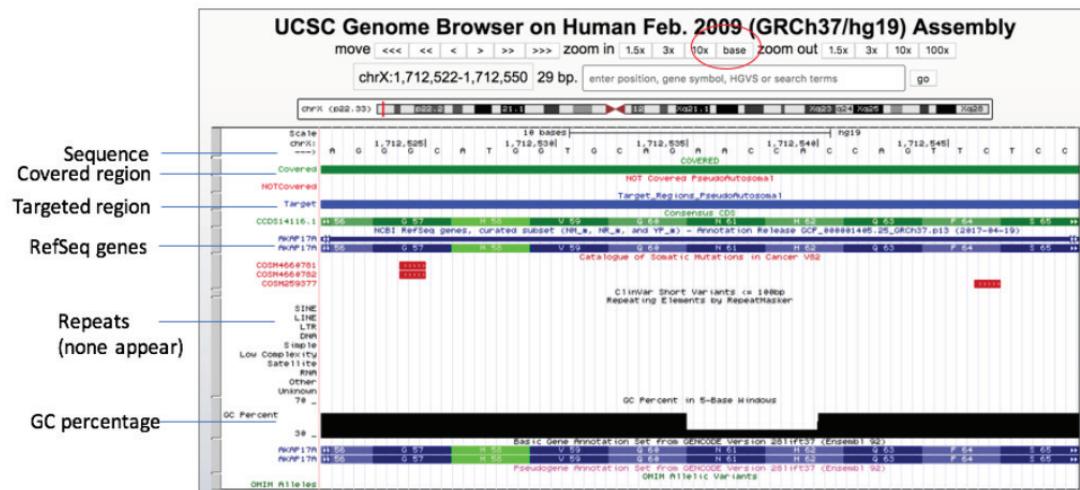
Repeats

Track customization options

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

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