Twist MRD Rapid 500 Panel Ordering and Design Output Reference Guide

For Research Use Only (RUO). Not for use in diagnostic procedures.

Twist MRD Rapid 500 Panels leverage Twist's best in class target enrichment technology to deliver fully customized NGS tests for liquid biopsy samples, in as little as six days. Each MRD Rapid 500 panel provides sufficient material for 12 tests in a single matrix tube for ease of use and tracking over multiple timepoints.

Twist MRD Rapid 500 Panels utilizes a streamlined order intake, design, and manufacturing process to ensure the fastest possible turnaround time. Order intake can be completed within a single business day, facilitated by close partnership with Twist account managers and straightforward criteria for target submission. The ordering process begins with submission of a browser extensible data (BED) file, containing all genomic coordinates for variants of interest. Each BED file can contain targets for up to 150 panels at once. Capture probes are then designed by Twist's proprietary algorithms based on the submitted BED file and selected filtering metrics.

Please review the following details to guide BED file creation and submission. If you have any questions, please contact your account manager.

ORDER PROCESS OVERVIEW

STEP 1: SET UP BLANKET PURCHASE ORDER (BPO)

Once you have discussed the scope of the project with your account manager and would like to proceed with placing an order, Twist will receive a blanket purchase order that can be applied to current and future Twist MRD Rapid 500 Panel orders.

STEP 2: CREATE AND SUBMIT BED FILE

Each order starts with a BED file containing variant genomic coordinates for each panel. Once you have completed the order BED file per the instructions below, the file is provided to your account manager for processing. In the event that a probe cannot be designed to a selected target, or the file cannot be uploaded to the designer, Twist Customer Service will contact you with an error description and recommendations for correction.

STEP 3: REVIEW AUTOMATED PANEL DESIGN OUTPUT

Twist's automated design platform uses the submitted BED file to output a set of custom probes for the selected panel targets. Once design is complete, you will receive instructions from Twist's Customer Service team on how to download and review the design output files for each panel.

STEP 4: TRACK YOUR ORDER

Successfully designed MRD panels are moved into Twist's production environment. The 120 base pair biotinylated probes are synthesized, quality controlled, and vialed in barcoded matrix tubes. You will receive emailed status updates to inform you of how your order is progressing, all the way to shipment.

STEP 5: BEGIN YOUR RESEARCH!

FORMATTING YOUR BED FILE FOR SUBMISSION

Proper formatting of the submitted BED file is essential for error-free order intake. Please read the submission details carefully to ensure a seamless process.

Each Twist MRD Rapid 500 panel can accommodate a minimum of 50 targets and a maximum of 500 targets. Up to 150 panels can be submitted in a single BED file.

All BED files must contain variant genomic coordinates; probe files are not accepted. Incorrectly formatted BED files will be incompatible with Twist's panel designer, and will be returned for correction.

1. CONFIGURE MRD TARGET BED FILE

Each line of the tab delimited BED file should contain 4 fields, with no header line included. These fields outline the variant genomic coordinates as well as the panel the target is associated with

- **Column 1:** The chromosome the variant is located on including the "chr" nomenclature used by BED files (i.e. chr7).
- **Column 2:** The genomic start coordinate location within the reference build the variant is sourced from.
- **Column 3:** The genomic end coordinate location within the reference build the variant is sourced from. The end coordinate must be at least 1 base pair greater than the start coordinate.
- **Column 4:** The name of the panel the target is associated with.
  NOTE: Target size is limited to 120 base pairs.

2. SELECT PROBE DESIGN FILTER

Twist MRD Rapid 500 probes are designed to be centered over each genomic target. At order submission, you may choose to employ Twist's proprietary repeat filter, which assesses regions of the reference genome that are highly repetitive or contain low complexity sequences.

Enabling this filter will remove probes from a submitted panel if more than half of the probe sequence overlaps the repeat track. While the filter can mitigate potential off target effects incurred by probes over repetitive regions, removal of these probes may result in a lack of probe coverage over your submitted targets.

If you choose not to employ the repeat filter, all probes will be automatically designed and included in your panel. You will not be able to modify the probe content upon receipt of the design output files. Intergenic and intronic regions are less conserved than exons; variants in these regions are more likely to intersect with the repeat filter.

NOTE: All Twist MRD Rapid 500 Panels support alignment to either human genome reference hg19 or hg38.

3. NAME BED FILE FOR SUBMISSION

To ensure Twist processes your order using the desired parameters, the file name should follow the below structure, to include your selected order name, filtered or unfiltered, and the genome build used (hg19 or hg38).

- **Naming template:** {Order_Name}_{Filtered/Unfiltered}_{Genome_Build}.bed

UNDERSTANDING YOUR DESIGN OUTPUT FILES

Each Twist MRD Rapid 500 Panel will include six design output files. These files provide detailed information about probe coverage over the submitted targets, which can be used for downstream analysis.

- **Probes Merged BED File:** Contains the genomic coordinates for the probe footprint of the panel.
- **Target Bases Covered BED File:** Contains the genomic coordinates for each of the submitted targets for which a probe covers.
  NOTE: The target bases covered BED file will contain every submitted target if the repeat filter was not applied.
- **Target Bases Not Covered BED File:** Contains the genomic coordinates for each of the submitted targets for which a probe could not be placed because of screening from the repeat filter.
  NOTE: The target bases not covered BED file will be empty if the repeat filter was not applied.
- **Target Regions with Zero Probes BED File:** Contains the genomic coordinates for each of the submitted targets for which zero probe coverage exists. This file should mimic the target bases not covered file.
- **All Tracks File:** an aggregate of the previous BED files that has been formatted for upload into the UCSC genome browser as a custom track. This provides a visual representation of the BED file coordinates as a convenience to our customers.
- **Report File:** provides summary of the design information, coverage summary, and design files.

FREQUENTLY ASKED QUESTIONS

AT WHAT TIME POINTS WILL TWIST GIVE ME UPDATES ON MY ORDER?

Once your target BED file has been uploaded into Twist's processing system, you will receive email notifications when:

- The panels have been designed and pushed to production
- The panel design output files are available for download
- The panels are ready for shipment
- The panels are delivered to the customer

Twist's customer support team may also contact you if there are any unexpected errors with your file upload or design.

WHAT HAPPENS IF THERE IS AN ERROR UPLOADING MY TARGET BED FILE?

Twist's customer support will contact you with a descriptive summary of BED file validation errors for you to review and correct. Orders will then be resubmitted through your account manager for redesign.

NOTE: Twist MRD Rapid 500 Panel 1-day order intake turnaround time will begin upon receipt of **an error-free**, corrected target BED file.

CAN PROBES BE MODIFIED AFTER THEY HAVE BEEN DESIGNED?

After target BED files have been processed, Twist's proprietary design algorithms create capture probes which are automatically sent for synthesis. At this stage, probe sequences and design coverage cannot be modified. If you would like to alter probe coverage, another order for a new Twist MRD Rapid 500 Panel will need to be submitted.

WHAT DOCUMENTATION WILL TWIST PROVIDE WHEN MY PANELS ARE SHIPPED?

Twist will provide an E-manifest for each order with panel-specific CoAs.

CAN I ORDER A TWIST MRD RAPID 500 PANEL WITH MORE THAN 500 PROBES?

The Twist MRD Rapid 500 panels are available for research where 500 or less probes are required. If you would like to design panels with more than 500 probes, Twist can support you through the standard custom panel workflow.