PANEL INFORMATION

TwinStrand Duplex Sequencing™ Control Panel (Human), v1.0

Tube Label: CTP — Control Panel (Human), v1.0

Part Number: 03-2038-XX

Pipeline ID: ctp-human-v1.0

Total Panel Size:	2.4 kb
Number of Target Intervals:	1
Target Type:	arbitrary genomic region
Genome Reference:	hs38DH (Human)

This human probe panel has been empirically validated to perform well using the TwinStrand Duplex Sequencing Kit Manual. It is intended to be used as a positive control in situations such as: (1) together with Human Control DNA (03-2023-XX) to generate a technical control library if using a Universal kit without an application-specific panel, (2) as a side-by-side comparator when testing the performance of new, user-supplied human probe panels with a Universal kit and (3) as a small, sequencing cost-effective probe set to use when testing the performance of new sources of human DNA with either Universal or application-specific kits.

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Target Regions in Panel			
Chromosome	Start	End	Genomic context
chr6	155,239,014	155,241,414	Coding & Non-coding

Coordinates in the BED format (0 start, half-open)

Panel-Specific Protocol Recommendations

Cot-1 DNA Recommended: Human: 03-2020-XX
Number of Captures Required: 2
Typical On-Target Percentage: 95%-99% (2 captures)
Number of Final PCR Cycles: 8 (PCR-3)
Conditioning Reagent: Recommended

Panel-Specific Protocol Notes

1 vs. 2 Captures: For smaller panels such as this one, 2 sequential rounds of hybrid capture are strongly recommended. The reduction in on-target % with only 1 capture, and thus the additional raw sequencing required, will likely outweigh any benefit from time saved.

Final PCR Cycles: The number of final PCR cycles (PCR-3) listed in the table above assumes a starting library preparation input (pre-shearing) of 500 ng of high quality genomic DNA. The required number of final PCR cycles may vary with DNA input, DNA quality, thermocycler calibration and other factors. The value listed should be considered a starting point and adjustments made as described in the TwinStrand Duplex Sequencing Manual.

DNA Input vs. Projected Duplex Sequencing Data						
DNA Input (ng)*	Number of Clusters (Million)**	Number of Paired-End Reads (Million)**	Illumina Sequencing Cost (NovaSeq, S4, List Price)***	Mean Duplex Depth****	Max Duplex Depth****	Informative Duplex Bases (Million)****
250	3	6	$9	6,000x	7,500x	18
500	6	12	$17	12,000x	15,000x	36
1,000	12	24	$35	24,000x	30,000x	72

* Assumes high molecular weight genomic DNA (quantified pre-fragmentation). DNA requirements to achieve a given Duplex Depth when using cell-free DNA or heavily damaged DNA such as that from FFPE will be lower and higher, respectively.

** Approximate sequencing required to achieve a peak tag family size ~10.

*** S4 list price = $7,200/lane (2.5 billion clusters/lane) as of December 2019.

**** Data estimates assume 150 bp paired-end reads

TwinStrand Duplex Sequencing data yields are dependent on accurate quantification, and the quality of input DNA. We recommend Qiagen silica column-based kits or “salting out” methods for DNA extraction. Phenol-chloroform extraction can damage DNA and reduce data yields. DNA should be quantified using a double-stranded DNA-binding fluorescent dye (e.g. Qubit fluorometer, Thermo Fisher Scientific) rather than UV spectrometry which can overestimate concentration if contaminants are present, which will reduce data yields. If possible, check genomic DNA quality using a 0.5% agarose gel, or a fragment analyzer system (e.g. Agilent TapeStation system). DNA integrity number (DIN) >7 on a fragment analyzer or a single high molecular weight band on a gel generally indicates good quality DNA, although oxidation or other forms of non-fragmentation chemical damage may still be present and not appreciable.

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