PANEL INFORMATION

TwinStrand Duplex Sequencing™ AML-29 Panel (Human), v1.0

Tube Label:	PAN AML-29 Panel (Human), v1.0	**Number of Genes:**	29
Part Number:	06-1002-XX	**Number of Target Intervals:**	150
Pipeline ID:	aml29-panel-v1.0	**Target Type:**	mutation hotspots or full coding regions
Total Panel Size:	58.6 kb	**Genome Reference:**	hs38DH (Human)

This acute myeloid leukemia (AML) panel is designed to detect ultra low-frequency somatic mutations in 29 genes recurrently mutated in AML patients. 90%–95% of adult AML patients carry a mutation in at least one panel gene. Many of these genes are also recurrently mutated in clonal hematopoiesis of indeterminate potential (CHIP) and myelodysplastic syndrome (MDS).

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AML Panel Genes					
ASXL1	*FAM5C (BRINP3)*	*IDH2*	*NPM1*	*RAD21*	*TET2*
CBL	*FLT3*	*KIT*	*NRAS*	*RUNX1*	*TP53*
CEBPA	*GATA2*	*KRAS*	*PHF6*	*SMC1A*	*U2AF1*
DNMT3A	*HNRNPK*	*MLL-X (KMT2A-X)*	*PTEN*	*SMC3*	*WT1*
EZH2	*IDH1*	*MYH11-CBFB*	*PTPN11*	*STAG2*	

Full Target List		
Gene	Accession #	Target Region
ASXL1	NM_015338	a.a. 363-1542
CBL	NM_005188	a.a. 366-477
CEBPA	NM_001287424	full coding region
DNMT3A	NM_022552	a.a. 286-913
EZH2	NM_004456	a.a. 87-208, 244-302, 503-752
FAM5C (BRINP3)	NM_199051	a.a. 80-142, 396-767
FLT3	NM_004119	a.a. 569-647, 807-847
GATA2	NM_032638	a.a. 77-481
HNRNPK	NM_002140	a.a. 21-85, 173-215, 319-336, 371-453
IDH1	NM_005896	a.a. 106-138
IDH2	NM_002168	a.a. 126-178
KIT	NM_000222	a.a. 412-448, 788-828
KRAS	NM_004985	a.a. 1-96

Full Target List - Continued		
Gene	Accession #	Target Region
MLL-X (KMT2A-X)	NM_005933 *(KMT2A)*	*MLL* intron 9
MYH11-CBFB	NM_022844 *(MYH11)*	*MYH11* intron 30, exon 31
NPM1	NM_002520	a.a. 258-282, 283-295
NRAS	NM_002524	a.a. 1-96
PHF6	NM_032458	full coding region
PTEN	NM_001304717	a.a. 258-337, 385-440
PTPN11	NM_002834	a.a. 47-110, 484-533
RAD21	NM_006265	full coding region
RUNX1	NM_001754	full coding region
SMC1A	NM_006306	a.a. 38-99, 447-515, 578-637, 687-732, 772-902, 1096-1145
SMC3	NM_005445	a.a. 184-268, 365-435, 656-705, 882-1035, 1100-1158
STAG2	NM_001282418	a.a. 42-128, 155-297, 436-472, 513-546, 578-675, 787-844, 892-1155
TET2	NM_001127208	a.a. 1-2003
TP53	NM_000546	full coding region
U2AF1	NM_006758	a.a. 16-44
WT1	NM_024426	a.a. 372-523

a.a. = amino acids

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Panel-Specific Protocol Recommendations

Cot-1 DNA Recommended:	Human: 03-2020-XX	**Number of Final PCR Cycles:**	14 (PCR-2), 6 (PCR-3)
Number of Captures Required:	1	**Conditioning Reagent:**	Recommended
Typical On-Target Percentage:	80%-90% (1 capture), 90%-95% (2 captures)		

Panel-Specific Protocol Notes

DNA Conditioning: The Library Conditioning step is recommended for this panel. Refer to the TwinStrand Duplex Sequencing Kit Manual for a full explanation of the benefits vs. costs of performing Library Conditioning.

1 vs. 2 Captures: With panels of this size, it is possible to perform only a single round of hybrid capture. However, some AML-29 panel targets have pseudogenes or nearby repetitive elements that can affect efficiency of capture and sequencing. A single round of hybrid capture is generally sufficient to obtain 80% or greater on-target bases with this panel. We recommend starting with 2 captures the first time through the protocol to generate data that can be compared against. An increase in off-target reads means that raw sequencing must be increased proportionally to generate the same amount of informative data, so a trade-off exists between time saved with a single capture vs. potential additional sequencing cost required. The user should consider the pros and cons in the context of their specific situation.

Final PCR Cycles: The number of final PCR cycles varies with probe panel, number of captures, input DNA mass, input DNA quality, thermocycler calibration and other factors. If 2 rounds of hybrid capture are performed then PCR-3 is the final PCR, and with 1 round of capture PCR-2 is the final PCR. The number of final PCR cycles listed in the table for 1 vs. 2 captures should be considered a starting point, assuming 500 ng of high quality genomic DNA input at the beginning of library preparation.

Statistical Power: To detect ultra-low frequency mutations, sufficient Duplex Sequencing Depth is required. Duplex Depth is the denominator when calculating mutation frequency. For example, to observe a mutation frequency of 1/5,000 a library must be sequenced to at least 5,000x Duplex Depth across the specific genomic locus of interest. Additionally, since DNA molecules are being randomly sampled from a pool, to have 95% power to detect a mutation at a frequency of 1/X, a library should be sequenced to at least 3X depth at the locus of interest. For example, to achieve 95% power for detecting a mutation with a true frequency of 1/5,000, a depth of 15,000x is required at that locus. Although TwinStrand™ probe panels are designed for maximum capture and sequencing depth uniformity, coverage cannot ever be fully uniform due to natural sequence context variation. Some sites of interest may achieve below average depths and this should be accounted for in power calculations.

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****
250	75	150	$216	5,500x	8,000x
500	150	300	$434	11,000x	16,000x
1,000	300	600	$864	22,000x	32,000x

* 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.

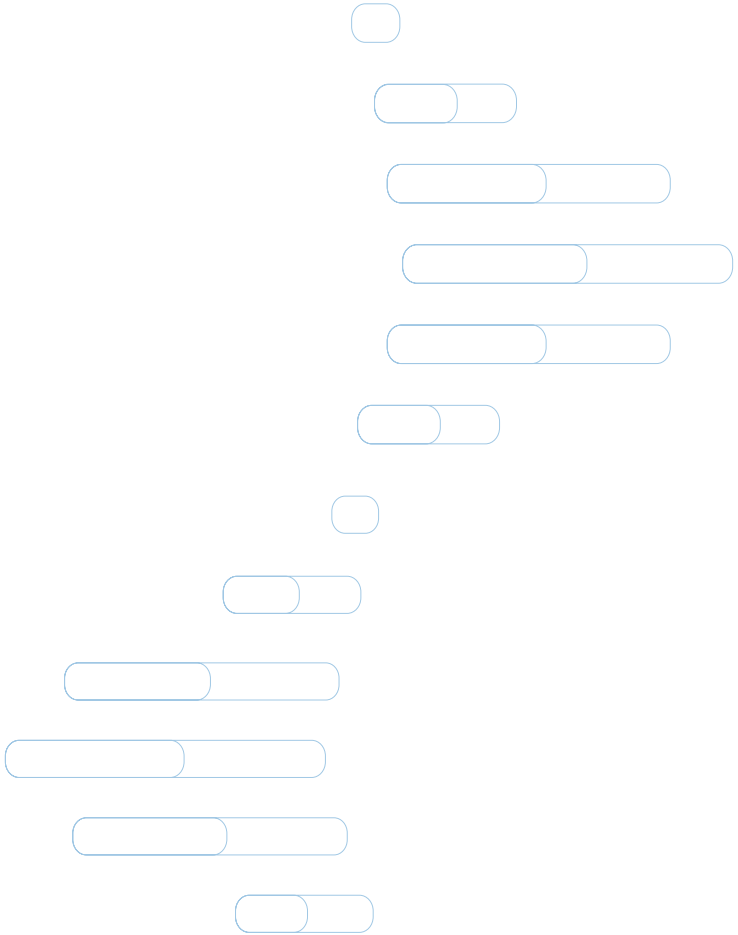
** Approximate sequencing required to achieve a peak tag family size ~10.

*** 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.

Notes



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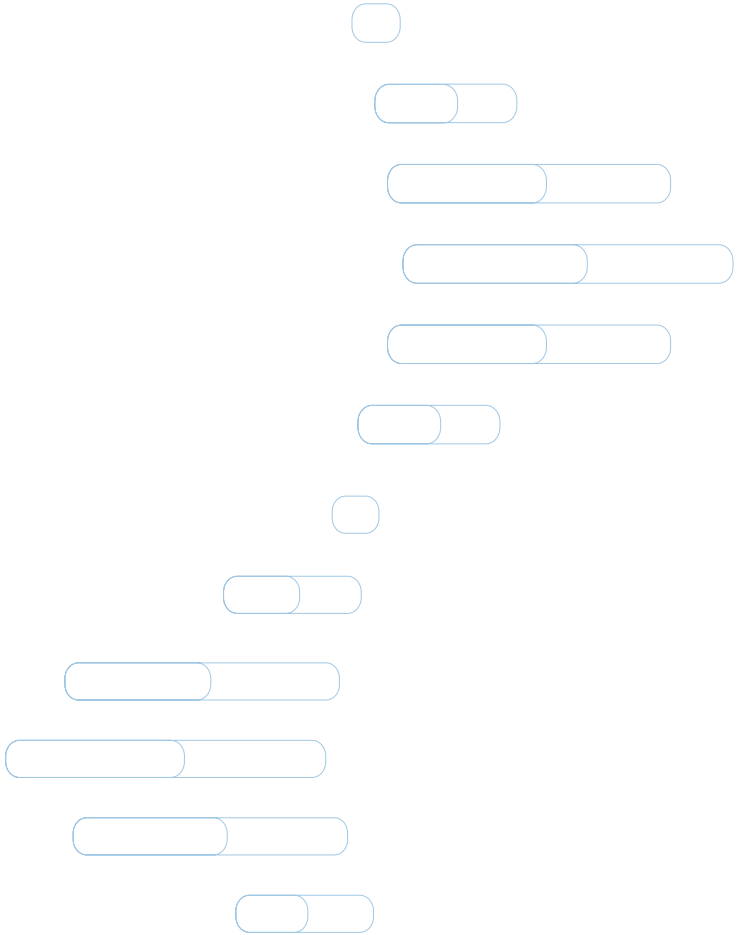

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