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TruSeq Custom Amplicon Kit Dx

Manifest File Instructions

FOR IN VITRO DIAGNOSTIC USE

The TruSeq Custom Amplicon™ Kit Dx enables a custom oligonucleotide pool to target specific, pre-defined regions of the genome for sequencing. The manifest is used during analysis to guide alignment sof the sequence reads to targeted regions, rather than to the entire genome.

The specific targeted regions for a particular custom oligonucleotide pool are summarized in the Manifest file. The manifest specifies the amplicon and primer sequences of the genomic regions being targeted by the custom oligonucleotide pool.

Although each prepared library (one library equates to one reaction) must be associated with a single manifest, different libraries sequenced in a single run can use different manifests.

For the Germline workflow, each sample requires one custom oligonucleotide pool for complete coverage of targeted regions; therefore, one library is created per sample, which corresponds to one manifest.

For the Somatic workflow, each sample requires two different custom oligonucleotide pools for complete coverage: one oligonucleotide pool "A" designed with amplicons for one strand (ex: plus strand or forward pool), and a complementary custom oligonucleotide pool "B" designed to the other strand (ex: minus strand or reverse pool). Therefore, two libraries are created per sample, each with a unique manifest.

Create Manifest File

A manifest file can be created manually using the Manifest Template download. The Manifest Template can be opened with Excel; however, it should then be saved as a tab-delimited *.txt file.

The Manifest has three sections: [Header], [Probes], and [Targets]. The Header section is optional and can contain information such as the name of the technician running the assay, the name of the experiment, etc. If a Header section is used, locate it before the Probes section. The Probes and Targets sections are mandatory and are used during data analysis.

The following are the column descriptions for the mandatory sections. See the Manifest Template for the layout.

[Probes] Column Descriptions

Probes Column	Description
Target ID	A unique identifier used as the display name of the amplicon, consisting of numbers and letters. Must not contain any spaces or symbols.
Chromosome	The chromosome of the amplicon, for example chr1, chr2, or chrX. Must match the hg19 reference genome.
Start Position	Genomic coordinate of the amplicon start position, excluding the sequence matching the probes.
End Position	Genomic coordinate of the amplicon end position, excluding the sequence matching the probes.
ULSO Sequence	Sequence of the upstream primer used to generate the amplicon, also called 'Custom Probe 1' in the TruSeq Custom Amplicon Kit Dx package insert.
DLSO Sequence	Sequence of the downstream primer used to generate the amplicon, also called 'Custom Probe 2' in the TruSeq Custom Amplicon Kit Dx package insert.

[Targets] Column Descriptions

Targets Column	Description
Target A	Same text as the Target ID for the amplicon listed in [Probes].
Target B	Same text as the Target ID for the amplicon listed in [Probes], Target A and Target B are the same.
Target Number	The number 1; the target region for a probe pair has index 1 and is labeled TargetID.1 in the data. Any off-target sequences have indexes of 2, 3, etc., and are labeled as TargetID.2, and TargetID.3.

Targets Column	Description
Chromosome	The chromosome of the amplicon, for example chr1, chr2, or chrX. Must match the reference genome.
Start Position	Genomic coordinate of the amplicon start position, including the sequence matching the probes.
End Position	Genomic coordinate of the amplicon end position, including the sequence matching the probes.
Probe Strand	Enter + or - to indicate the strand of the amplicon.
Sequence	Sequence of the region between the ULSO/DLSO. If the probe strand is +, the sequencing region is from the forward strand. If the probe strand is -, the sequencing region is from the reverse strand.

Check Manifest and Oligonucleotide Pool Design

Before using your manifest and starting a sequencing run, compare the total number of amplicons in your oligonucleotide pool to the number specified in the manifest. Make sure no amplicons are missing.

Final results of secondary analysis are in *.vcf files and other analysis files in the run folder of your sequencing run. The data from the run is saved to the network location you specified in the operating software of the sequencing instrument. Analysis reports and output files are located in the Alignment folder. See Germline Variant or Somatic Variant module guides for more details on these files.

When optimizing your assay, refer to output coverage files to perform a quick check of your data. Regions with low or zero coverage can indicate an issue with the manifest file. For example, coordinates in the manifest file might not reflect the genomic regions targeted by the oligonucleotide pool. Alternatively, regions with unexpectedly high coverage can indicate a different issue with the oligonucleotide pool, such as homologous genes or cross-binding, which can then guide further pool redesign and optimization.

Make sure that amplicons on the same strand do not overlap. Overlapping amplicons, such as those used to tile a region, must be on different strands to prevent unintentional amplification events.

Finally, make sure that the manifest file is a tab-delimited *.txt file.

Store and Access Manifest File

The manifest file is required to set up a run with Local Run Manager software. After a manifest file is created, save the tab-delimited *.txt file to a location available for upload.

Before starting the sequencing run, the manifest can be saved to module settings in Local Run Manager Germline Variant or Somatic Variant analysis modules. During run creation, saved manifests are accessible from the Manifest drop-down list. Manifests can also be imported for a specific run when entering run information. For more information, please see the instrument reference guide for your high-throughput DNA sequence analyzer.

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