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MiSeqDx™ Universal Kit 1.0 Manifest File Instructions

The Manifest file specifies the amplicon and primer sequences of the genomic regions being targeted by this custom assay. Each sample listed in the sample sheet must be associated with one and only one manifest file. Different manifest files may be specified for different samples in the sample sheet. Once a manifest file has been generated, it should be saved to the Manifest Repository (a folder specified in the MiSeq Operating Software).

File Structure

The manifest file must be saved as a tab-delimited.txt file. The Manifest Template can be opened with Excel for ease of use; 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, it should be located 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. Please see the Manifest Template for the layout.

[Probes] Column Descriptions

- **Target ID**–A unique identifier used as the display name of the amplicon, consisting of numbers and letters, must not contain any spaces/symbols.
- **Chromosome**–The chromosome of the amplicon (ex. chr1, chr2, chrX), this must match the reference genome.
- **Start Position**–Genomic coordinate of the amplicon start position, including sequence matching the DLSO.
- **End Position** – Genomic coordinate of the amplicon end position, including sequence matching the ULSO.
- **ULSO Sequence**–Sequence of the upstream primer used to generate the amplicon (also called 'Custom Probe 1' in Package Insert).
- **DLSO Sequence**–Sequence of the downstream primer used to generate the amplicon (also called 'Custom Probe 2' in Package Insert).

[Targets] Column Descriptions

- **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 will be the same.
- **Target Number**–The number 1; the target region for a probe pair has index 1 and will be labeled TargetID.1 in the data. Any off-target sequences have indexes of 2, 3, etc. (labeled TargetID.2, TargetID.3).
- **Chromosome** –The chromosome of the amplicon (ex. chr1, chr2, chrX), this should match the reference genome.
- **Start Position**–Genomic coordinate of the amplicon start position, including sequence matching the DLSO.
- **End Position**–Genomic coordinate of the amplicon end position, including sequence matching the ULSO.
- **Probe Strand**–Should be + or – to indicate the strand of the amplicon.
- **Sequence**–Sequence of the region between the ULSO/ DLSO, should come from forward strand if Probe Strand is +, should come from reverse strand if Probe Strand is –.

Storing Manifest Files

Once a Manifest file has been generated, it should be saved as a tab-delimited.txt file to a network location or USB drive. When generating the Sample Sheet, the name of the Manifest (without .txt) should be referenced.

Before starting the sequencing run, the Manifest should be saved to the Manifest Repository directory and the Sample Sheet should be saved in the Sample Sheet Repository specified in the MiSeq Operating Software (MOS). In MOS, Manifests and Sample Sheets can be moved, deleted, renamed, or uploaded from a USB drive through the 'Manage Files' option. For more details, please see the MiSeqDx Instrument Reference Guide.

Diagnosing an Issue with your Manifest

It is important to make sure 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. Before using your manifest and starting a sequencing run, compare the total number of amplicons in your oligo pool to the number specified in the manifest to make sure none are missing.

The final results of MSR analysis will be in .vcf file and .bam file format. To do a quick check of your data, coverge information bam files can be visualized using the Integrative Genome Viewer (IGV, located: www.broadinstitute.org/igv, from the Broad Institute).This Viewer utilizes the .bam files from your data, which can be found in the run folder of your sequencing run. The data from the run is saved to the network location you specified in

MOS (MiSeq Operating Software); and the bam files will be located in the Alignment folder, in Data >> Intensities >> Basecalls. Through the data visualization, you will be able to determine if there are: (1) regions with low coverage or (2) regions with unexpectedly high coverage. These two phenotypes could indicate an issue with either the manifest file (incorrect coordinates entered) or the probe pool (homologous genes, cross-binding, etc.).