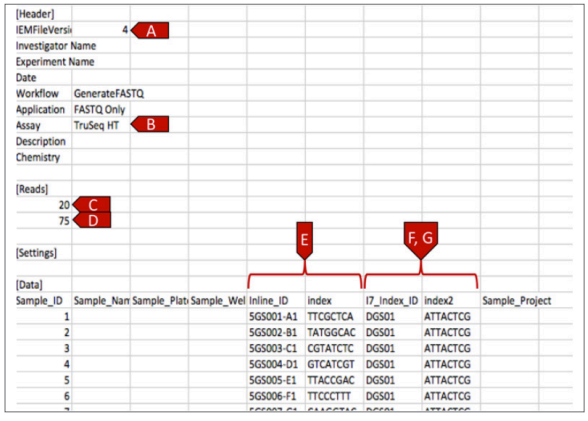
Guidelines for Setting Up a Sample Sheet for Illumina® Sequencing

NEBNEXT DIRECT® GENOTYPING SOLUTION PANELS (NEB #E9500B/#E9530B)

1. Download an NEBNext Direct GS sample sheet containing all 768 barcode combinations from the product page for the NEBNext Direct GS Target Enrichment Kit (NEB #E9530).
2. Update the [Data] fields with the barcodes used in your experiment.
3. Add optional, user specific information in the remaining (Data) fields.
4. Save the sample sheet as a csv file (.csv) and transfer the sample sheet to an Illumina sequencer.

Example Sample Sheet for Illumina Sequencing:



A. For use with Illumina Experiment Manager v5, change this value to 5.

B. For use with Illumina Experiment Manager v5, change the Assay field to "TruSeq Nano DNA".

C. 20 cycles of Read1 sequences the 12 nt unique molecular identifier (UMI) followed by the 8 nt inline sample index.

D. 75 cycles of Read2 sequences the targeted regions containing loci of interest.

E. The Inline_ID/Index0 fields indicate the sample barcodes that are added during 5´ Adaptor ligation. This column will not be recognized by the sequencer and is for documentation purposes only.

F. The I7_Index_ID/index fields instruct the sequencer to read 8 cycles of Index1 to sequence the pool index. The pool index is added to each pool of samples that is processed through the NEBNext Direct GS Target Enrichment Kit as a single reaction using an indexed PCR primer mix during the Library Amplification step of the protocol.

G. If a single target enrichment library pool is sequenced alone (only one i7 barcode is used), these fields should be left blank in order to instruct the sequencer to omit the Index1 read.

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