De-multiplexing Cell3™Target Data – Illumina Sequencing

This document outlines an example procedure for sample de-multiplexing of pooled Cell3™Target libraries following Illumina sequencing. The i7 indexing read contains a 9bp molecular tag in addition to the unique 8bp sample index. The i5 index contains 8bp unique sample index only. The final read structure is Y*, I8Y9,I8,Y*.

Input requirements

1. Illumina bcl2fastq software – a Base Call (BCL) Files to Fastq conversion software http://emea.support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software.html
2. Basecalls directory
3. Samplesheet.csv – ensure there are no N's present in the sample sheet (examples provided on request)
4. Check RunInfo.xml to ensure the correct number of cycles have been performed for the indexes
    a. i7 = 17 cycles
    b. i5 = 8 cycles

Expected file outputs per sample

1. Read 1 fastq file (R1)
2. Read 2 fastq file (R3)
3. fastq file containing information on a per fragment molecular tag (R2)
4. Index 1 and 2 fastq files (these are not required)

Procedural steps

1. Open a command terminal
2. Move into the basecalls directory of the data to be de-multiplexed
3. Run the following bcl2fastq command

    bcl2fastq --create-fastq-for-index-reads --mask-short-adapter-reads 0 --use-bases-mask Y*,I8Y9,I8,Y* --no-lane-splitting (optional)

    * replace with the number of cycles performed for read 1 and 2