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Barcoding Next Gen Experiment 2 Description

Basics:

1. 1 Lane of a MiSeq Illumina machine (Biopolymer Facility Pooled Library ID LIB023222: JO 8\_16\_16)
2. 2 libraries were pooled and run on that single lane
   1. Each library contains a TruSeq Index
      1. JO Library 1 (GEN00067700) TruSeq Index 7 (CAGATC)
      2. JO Library 2 (GEN00067701) TruSeq INDEX 8 (ACTTGA)

Vector-135 bp

Barcode-24 bp

Vector-23 bp

Index-8bp

Forward read: 162 bp

Reverse read: at least 161 bp

Library Preparation

**Library 1**  - JO Library 1 (GEN00067700) TruSeq Index 7 (CAGATC)

1. gDNA was extracted from barcoded cell lines or tumor samples.
2. PCR was performed on genomic DNA extracted in step 1, amplifying the barcode and flanking vector regions and adding the first index (8 bp)
3. PCR products were pooled in equal molar concentrations and submitted to the BPF for library preparation. During library prep, TruSeq Index 7 was added to the pooled library sample.

This library contains 30 samples with a total of 20 indexes. 20/30 samples (indexes 1-10) contained a single barcode (single cell clone cell lines). The remaining 10 samples (indexes 11-20) had gDNA extracted from mixtures of barcode cells and may contain up to 33 unique barcodes each.

**Library 2 -** JO Library 2 (GEN00067701) TruSeq INDEX 8 (ACTTGA)

1. gDNA was extracted from barcoded cell lines
2. PCR was performed on genomic DNA extracted in step 1, amplifying the barcode and flanking vector regions and adding the first index (8 bp)
3. PCR products were pooled in equal molar concentrations and submitted to the BPF for library preparation. During library prep, TruSeq Index 8 was added to the pooled library sample.

This library contains 20 samples with a total of 20 indexes. 17/20 samples had gDNA extracted from mixtures of barcoded cells and may contain up to 33 unique barcodes each. 1 sample will contain only 1 barcode. The final 2 samples were prepped from non-barcoded cell lines as a negative control.

**Desired analysis:** Please identify barcode (24 bp)/index (8 bp) pairings for each read from each library. I will need to know which library each read came from and the barcodes that are associated with each index within that library.