Part II: Pooled Exome Enrichment

In this protocol, genomic libraries representing multiple individuals are pooled together and enriched as a single exome capture. The libraries are pooled evenly based on molarity (from Bioanalyzer assay). A combined total of **3 pMol** prepared library is placed into the enrichment workflow.

To pool 3 individuals into a single exome capture, 1 pMol of each library are pooled and lyophilized in a vacuum concentrator. A novel indexed blocking scheme (see below) is used to prevent non-specific pull down. The Indexed Blocking Reagent (IBR) replaces SureSelect Block #3 during hybridization.

Indexed Blocking Oligos

P5_b1_f

5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTT CCGATCT 3'

P5_b1_r

5' AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT 3'

P7_b1_f 5' CAAGCAGAAGACGGCATACGAG 3'

P7_b1_r 5' CTCGTATGCCGTCTTCTGCTTG 3'

P7_b2_f 5' GTGACTGGAGTTCAGACGTGTGCTCTTCCGAT/3ddC/ 3'

P7_b2_r 5' AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC 3'

Order oligos HPLC purified. /3ddC/ corresponds to 3' dideoxyCytosine. This notation is consistent with the format for IDT.

Indexed Blocking Reagent (IBR)

Reconstitute oligos to 300uM with PCR Grade water. Combine equal volumes of each oligo to make 50uM indexed blocking reagent. Store at -20ºC

Hybridize the Pooled Library

Three components of hybridization reaction:

Hybridization Buffer
SureSelect Block Mix
Exome Capture (plus RNAse dilultion)