

Protocol: Target Enrichment

Exome Capture of ThruPLEX[®] Libraries with Roche NimbleGen[®] SeqCap[®] EZ Library

For use with all ThruPLEX DNA-seq and ThruPLEX Plasma-seq Kits

Required Reagents

ThruPLEX[®] library preparation kit (one of following required):

- ThruPLEX DNA-seq Kit (Rubicon Genomics)
 - 12 rxns, 12 single indexes (CAT. NO. R400429)
 - 48 rxns, 12 single indexes (CAT. NO. R400428)
 - 48 rxns, 48 single indexes (CAT. NO. R400427)
 - 48 rxns, 48 dual indexes (CAT. NO. R400406)
 - 96 rxns, 96 dual indexes (CAT. NO. R400407)
- ThruPLEX Plasma-seq Kit (Rubicon Genomics)
 - 12 rxns, 12 single indexes (CAT. NO. R400490)
 - 48 rxns, 48 single indexes (CAT. NO. R400491)
 - 96 rxns, 96 dual indexes (CAT. NO. R400492)

Blocking oligos (both required):

- xGen[®] Universal Blocking Oligo – TS HT-i5 (Integrated DNA Technologies)
- xGen Universal Blocking Oligo – TS HT-i7 (Integrated DNA Technologies)

SeqCap[®] reagents:

- SeqCap EZ Accessory Kit v2 (Roche NimbleGen, CAT. NO. 07145594001 or 06776345001)
- SeqCap Hybridization and Wash Kit (Roche NimbleGen, CAT. NO. 05634261001 or 05634253001)
- SeqCap Pure Capture Bead Kit (Roche NimbleGen, CAT. NO. 06977952001)
- SeqCap EZ Library

Other consumables:

- Refer to Consumables Purchased from Other Vendors on page 10 in the SeqCap EZ Library SR User's Guide (Version 5.0)

Required Equipment

Refer to Laboratory Equipment on page 9 in the SeqCap EZ Library SR User's Guide (Version 5.0)

ThruPLEX Library Preparation

1. Prepare ThruPLEX libraries
 - Follow Section D in the ThruPLEX DNA-seq Kit or ThruPLEX Plasma-seq Kit Instruction Manual.
2. Perform Library Purification by AMPure XP beads
 - Follow Section E.V. in the ThruPLEX DNA-seq Kit or ThruPLEX Plasma-seq Kit Instruction Manual.
 - **CAUTION:** For the final elution, DNA must be eluted by resuspending the beads in 30 μ L of PCR grade water, not TE buffer.

Capture of ThruPLEX Libraries

1. Resuspend xGen Universal Blocking Oligos to 1 μ L per reaction (or 1 nmole/ μ L) in nuclease-free water.
2. Pool ThruPLEX libraries for hybridization by adding equal amounts of each library to obtain 1 μ g of DNA.
 - For example, to hybridize four ThruPLEX libraries with different indexes, 250 ng of each library would be added; or if pooling 10 uniquely indexed libraries, 100 ng of each library would be added.
3. In a 1.5 ml microcentrifuge tubes combine:
 - 5 μ L of COT Human DNA (1 mg/ml) from the SeqCap EZ Accessory Kit v2
 - 1 μ g pooled ThruPLEX libraries
 - 1 μ L xGen Universal Blocking Oligo – TS HT-i5
 - 1 μ L xGen Universal Blocking Oligo – TS HT-i7
4. Follow the SeqCap EZ Library SR User's Guide (Version 5.0) starting at Chapter 5, Step 5, #4 ("Close the tube's lid...") to the end of Chapter 7 to generate captured ThruPLEX libraries that are ready to be quantified and sequenced using Illumina NGS platforms.

Note: This protocol was developed using the SeqCap EZ Human Exome v3.0 Library.

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