Protocol: Target Enrichment

Targeted Capture of ThruPLEX® Libraries with xGen® Lockdown® Probes

For use with all ThruPLEX DNA-seq Kits 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)

Enrichment panel:

- xGen Lockdown® Panel

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)

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 µL of PCR grade water, not TE buffer.

Capture of ThruPLEX DNA-seq Libraries

1. Resuspend xGen Lockdown Panel to 4.5 µL per hybridization in nuclease-free water and aliquot for single-use into 0.2 mL tubes.
2. Resuspend xGen Universal Blocking Oligos to 1 µL per reaction (or 1 nmole/ µL) in nuclease-free water.
3. Pool ThruPLEX libraries for hybridization by adding equal amounts of each library to obtain 1 µg 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.
4. In a 1.5 ml microcentrifuge tubes combine:
   - 5 µl of COT Human DNA (1 mg/ml) from the SeqCap EZ Accessory Kit v2
   - 1 µg pooled ThruPLEX libraries
   - 1 µl xGen Universal Blocking Oligo – TS HT-i5
   - 1 µl xGen Universal Blocking Oligo – TS HT-i7
5. 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 with the following modification:
   Chapter 5, Step 5, #12:
   - Transfer the cocktail to the 4.5 µL aliquot of xGen Lockdown Panel in a 0.2 mL tube prepared above.

Note: This protocol was developed using the Roche NimbleGen SeqCap EZ System and the xGen® Acute Myeloid Leukemia (AML) Cancer Panel v1.0.