Protocol: Target Enrichment

Exome Capture of ThruPLEX® Libraries with Illumina Nextera® Rapid Capture Exome Enrichment Kit

Required Reagents

ThruPLEX library preparation kit (one of the following required):

- ThruPLEX DNA-seq Kit (Rubicon Genomics)
  - 12 rxns, 6 single indexes (CAT. NO. R400523)
  - 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)

Illumina Capture Reagents (One of the following required):

- Nextera Rapid Capture Exome Enrichment Kit (FC-140-1000; FC-140-1001; FC-140-1002; FC-140-1003; FC-140-1083*; FC-140-1086; FC-140-1089).

  *This configuration was used for protocol development
- Nextera Rapid Capture Expanded Exome Enrichment Kit

Other Consumables and Equipment

Refer to Appendix A "Consumables and Equipment" (page 44-46) in the Nextera Rapid Capture Enrichment Reference Guide, Catalog # FC-140-9001DOC, Part # 15037436 Rev. J

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 re-suspending the beads in 30µL of PCR grade water, not TE Buffer

Capture of ThruPLEX Libraries

1. Combine 500 ng of each uniquely indexed ThruPLEX library
   - NOTE: The number of libraries that may be pooled is determined by the kit configuration of the Nextera Rapid Capture Exome Enrichment kit purchased (1-plex to 12-plex)
2. If necessary, adjust the volume of the pooled libraries to 38 µl
   - If the total volume of libraries is >38 µl, use a vacuum concentrator without heating to reduce volume to 38 µl
   - If the total volume is <38 µl, increase the volume to 38 µl with nuclease free water
3. Combine the following in a 0.2 ml PCR tube:
   - 38 µl DNA Library or Sample Pool
   - 1 µl xGen Universal Blocking Oligo – TS HT-i5
   - 1 µl xGen Universal Blocking Oligo – TS HT-i7
   - 50 µl buffer EHB (from Nextera kit)
   - 10 µl capture oligos CEX, EEX, or RCO (from Nextera kit)
4. Continue with Procedure step 2, page 18 of the probe hybridization per the Nextera Rapid Capture Enrichment Reference Guide, Catalog # FC-140-9001DOC, Part # 15037436 Rev. J, "Shake at 1200 rpm…."
5. For Procedure step 1 of the Second Hybridization (page 22) add the following to each well containing 25 µl of product from the first capture:
   - 13 µl buffer RSB (From Nextera kit)
   - 1 µl xGen Universal Blocking Oligo – TS HT-i5
   - 1 µl xGen Universal Blocking Oligo – TS HT-i7
   - 50 µl buffer EHB (from Nextera kit)
   - 10 µl capture oligos CEX, EEX, or RCO (from Nextera kit)
6. Continue with Procedure step 2, page 22 of the probe hybridization per the Nextera Rapid Capture Enrichment Reference Guide, Catalog # FC-140-9001DOC, Part # 15037436 Rev. J, "Shake at 1200 rpm…."

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