Protocol: Target Enrichment

Targeted Capture of ThruPLEX® Libraries with Agilent SureSelect®XT Target Enrichment System

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)

Primers (both required):

- Illumina® P5 Primer: AATGATACGGCGACCACCGA
- Illumina P7 Primer: CAAGCAGAAGACGGCATACGA

SureSelect®XT reagents:

- Refer to Required Reagents on page 16 in the Agilent SureSelectXT Protocol (Version B.3, June 2015)

Required Equipment

Refer to Required Equipment on page 19 in the Agilent SureSelectXT Protocol (Version B.2, April 2015)

Important Notes

When integrating ThruPLEX with the Agilent SureSelectXT library capture system, all components of SureSelectXT Reagent Kit are used **except** the following:

- SureSelectXT Library Prep Kit ILM
- SureSelect ILM Indexing Pre Capture PCR Reverse Primer
- SureSelect ILM Indexing Post Capture Forward PCR Primer

Contact Agilent to order a SureSelectXT Reagent Kit without the SureSelectXT Library Prep Kit ILM.

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 Libraries

1. Resuspend xGen Universal Blocking Oligos to 1 µL per reaction (or 1 nmole/ µL) in nuclease-free water.
2. Follow the Agilent SureSelectXT Protocol (Version B.3, June 2015) starting at the beginning of Chapter 4 until the end of Chapter 5 with the following modifications: Chapter 4, Step 1. Hybridize DNA Samples to the Capture Library:

- Depending on sample type, quality, fragment size, and thermal cycler used, ThruPLEX library preparation may not yield 750 ng as called for in the SureSelectXT Protocol. If this is the case, use the entire volume of library for concentration.
- In addition to the reagents included in the SureSelect Block Mix in Table 32 on page 66, add:
  - 1 uL of xGen® Universal Blocking Oligo – TS HT-i5
  - 1 uL of xGen® Universal Blocking Oligo – TS HT-i7

  CAUTION: This results in a volume of 7.6 µL per reaction instead of 5.6 µL as stated in the SureSelectXT Protocol.

Chapter 5, step 1A. Amplify the Capture Libraries with Indexing Primers:

- Modify the Post-Capture PCR Reaction Mix (Table 38, page 71) to the following:

Reagent	Volume for 1 rxn
Nuclease-free water**	19.5 µL
5x Herculase Rxn Buffer (clear cap)*	10.0 µL
Herculase II Fusion DNA Polymerase (red cap)*	1.0 µL
100 mM dNTP Mix (green cap)*	0.5 µL
10 µM Illumina P5 Primer	2.5 µL
10 µM Illumina P7 Primer	2.5 µL
Total	**36.0 µL**

Note: This protocol was developed using the SureSelectXT Human All Exon v5 Capture Library.

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