

Protocol: Target Enrichment

Exome Capture of ThruPLEX® Libraries with Agilent SureSelect®XT2 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)

SureSelect®XT2 reagents:

- Refer to Required Reagents on page 14 in the Agilent SureSelectXT2 Protocol (Version E.0, January 2015)
- SureSelectXT2 Capture Library (e.g. SureSelectXT2 Human All Exon V5, 16; Agilent Technologies, CAT. NO. 5190-6208)

Required Equipment

Refer to Required Equipment on page 16 in the Agilent SureSelectXT2 Protocol (Version E.0, January 2015)

Important Notes

When integrating ThruPLEX with the Agilent SureSelectXT2 Target Enrichment System, all components of the SureSelectXT2 Reagent Kit are used except the following:

- SureSelect End Repair Enzyme Mix
- SureSelect End Repair Oligo Mix
- SureSelect dA-Tailing Master Mix
- SureSelect Ligation Master Mix
- SureSelectXT2 Pre-Capture Indexes

Contact Agilent to order a SureSelectXT2 Reagent Kit without the SureSelectXT2 Library Prep Kit ILM.

CAUTION: This custom kit may require the following items for the post-capture amplification step:

- Herculase II Fusion DNA Polymerase with dNTPs (Agilent Technologies, CAT. NO. 600677 or 600679)
- Illumina® P5 Primer: AATGATACGGCGACCACCGA
- Illumina P7 Primer: CAAGCAGAAGACGGCATACGA

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. Pool ThruPLEX libraries for hybridization by adding equal amounts of each library to obtain 1.5 µg of DNA.
 - Depending on capture library size, equal amounts of 8 or 16 libraries are pooled.
 - For example, the SureSelectXT2 protocol recommends pooling of 8 libraries with different indexes (187.5 ng of each) when using the Human All Exon v5 Capture Library.
3. Combine the following in a 1.5 ml microcentrifuge tube:
 - 1.5 µg pooled ThruPLEX libraries
 - 1 µL xGen Universal Blocking Oligo – TS HT-i5
 - 1 µL xGen Universal Blocking Oligo – TS HT-i7

4. Concentrate the ThruPLEX libraries/xGen Universal Blocking Oligo mixture using a vacuum concentrator held at $\leq 45^{\circ}\text{C}$ to reduce the volume in the tube to $< 7 \mu\text{L}$. Do not completely dry the mixture.
5. Bring the volume to $7 \mu\text{L}$ with nuclease-free water.
6. Vortex the tube vigorously for 30 seconds and centrifuge to bring contents of the tube to the bottom.
7. Add $9 \mu\text{L}$ SureSelectXT2 Blocking Mix; pipette up and down to mix.
8. Transfer the contents to a 0.2 mL PCR tube or to one well of a 96-well plate.
9. Proceed with the SureSelectXT2 Protocol (Version E.0, January 2015) starting at Chapter 4, Step 2, #2 (“Cap the wells,...”) to the end of Chapter 5, Step 3.

Note: This protocol was developed using the SureSelectXT2 Human All Exon v5 Capture Library and the ClearSeq DNA Kinome Panel.

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ThruPLEX DNA-seq and ThruPLEX Plasma-seq are protected by U.S. Patents 7,803,550; 8,071,312; 8,399,199; 8,728,737 and corresponding foreign patents. Additional patents pending.

