Protocol: Target Enrichment

# Exome Capture of ThruPLEX® Libraries with Agilent SureSelect®QXT 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®QXT reagents:

- Refer to Required Reagents on page 13 in the Agilent SureSelectQXT Protocol (Version B.2, October 2014)

## Required Equipment

Refer to Required Equipment on page 15 in the Agilent SureSelectQXT Protocol (Version B.2, October 2014)

## Important Notes

When integrating ThruPLEX with the Agilent SureSelectQXT2 library capture system, all components of the SureSelectQXT Reagent Kit are used except the following:

- SureSelectQXT Buffer
- SureSelectQXT Enzyme Mix ILM
- DMSO
- SureSelectQXT Read Primer 1
- SureSelectQXT Read Primer 2
- SureSelectQXT Index Read Primer
- SureSelectQXT P7 dual indexing primers
- SureSelectQXT P5 dual indexing primers
- SureSelectQXT Stop Solution
- SureSelectQXT Primer Mix

**Contact Agilent to order a SureSelectQXT Reagent Kit without the SureSelectQXT Library Prep Kit, ILM, Box 2.**

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

- Herculase II Fusion DNA Polymerase with dNTPs (Agilent Technologies, CAT. NO. 600677 or 600679)

## 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. Using a narrow gauge needle, poke hole(s) in the lid of each tube containing a ThruPLEX library to be used for capture.
3. Concentrate the ThruPLEX library using a vacuum concentrator held at ≤45°C to reduce the volume in the tube to <10 µL. Do not completely dry the mixture.
4. Bring the volume to 10 µL with nuclease-free water.

5. To each resuspended library, add:
    - 1 µL of xGen Universal Blocking Oligo – TS HT-i5
    - 1 µL of xGen Universal Blocking Oligo – TS HT-i7
6. Follow procedures in the Agilent SureSelectQXT Protocol (Version B.2, October 2014) starting at Chapter 3, Step 2 through the end of Chapter 4, Step 5 with the following modification:

Chapter 4, Step 1. Amplify the Captured Libraries

- Modify the Post-Capture PCR Reaction Mix (Table 18, page 47) to the following:

| **Reagent** | **Volume for 1 rxn** |
|---|---|
| Nuclease-free water* | 10.5 µL |
| 5x Herculase Rxn Buffer | 10.0 µL |
| 100 mM dNTP Mix | 0.5 µL |
| Herculase II Fusion DNA Polymerase | 1.0 µL |
| 10 µM Illumina P5 Primer | 2.5 µL |
| 10 µM Illumina P7 Primer | 2.5 µL |
| **Total** | **27.0 µL** |

- ThruPLEX libraries are already indexed, so do **not** use the SureSelectQXT indexing primers.

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