Targeted capture of ThruPLEX libraries with Agilent SureSelectQXT

Introduction

Enrichment of ThruPLEX libraries with Agilent SureSelect platforms is easily performed. The chart below details the reagents necessary for this SureSelect^{QXT} protocol. The module marked in red is not required when integrating with ThruPLEX kits. This target enrichment protocol is compatible with all ThruPLEX DNA-Seq, ThruPLEX Plasma-Seq, and ThruPLEX Tag-seq kits.

Integration of SureSelect^{QXT} with ThruPLEX kits			
Additional reagents	Primers	Required	Illumina P5 and P7 primers
	Blocking oligos	Required	IDT xGen Universal Blocking Oligos (TS HT-i5 and TS HT-i7)
	Agilent Herculase II Fusion DNA Polymerase	Required	Agilent Cat. # 600677, 600679 (with dNTPs)
Agilent SureSelect^{QXT} Reagent Kit	SureSelect^{QXT} Library Prep Kit, ILM, Box #2	Not used	Replace with a ThruPLEX kit.
	SureSelect^{QXT} Target Enrichment Kit, ILM (Hyb module, Box #1)	Required	The following reagent is *not* used: SureSelect^{QXT} Stop Solution
	SureSelect^{QXT} Target Enrichment Kit, ILM (Hyb module, Box # 2)	Required	The following reagent is *not* used: SureSelect^{QXT} Primer Mix

Materials required

Reagents

- A ThruPLEX library preparation kit (choose from the ThruPLEX DNA-Seq kits, ThruPLEX Plasma-Seq kits, and ThruPLEX Tag-seq kits listed in the Related Products section at the bottom of this page)
- Two blocking oligos (both required):
  - xGen Universal Blocking Oligo - TS HT-i5 (Integrated DNA Technologies; IDT)
  - xGen Universal Blocking Oligo - TS HT-i7 (IDT)
- Primers (both required):
  - Illumina P5 Primer: AATGATACGGCGACCACCGA
  - Illumina P7 Primer: CAAGCAGAAGACGGCATACGA
- SureSelect^{QXT} reagents: Refer to the "Required Reagents" section of the Agilent SureSelect^{QXT} protocol.

NOTE: The following item may be required for the post-capture amplification step: Herculase II Fusion DNA Polymerase with dNTPs (Agilent Technologies, Cat. # 600677 or 600679)

Equipment

- As specified in the "Required Equipment" section of the Agilent SureSelect^{QXT} protocol.

NOTE: When integrating ThruPLEX kits with the SureSelect^{QXT} library capture system, all components of the SureSelect^{QXT} Reagent Kit are used *except* the following:

- SureSelect^{QXT} Buffer
- SureSelect^{QXT} Enzyme Mix ILM

- DMSO
- SureSelect^{QXT} Read Primer 1
- SureSelect^{QXT} Read Primer 2
- SureSelect^{QXT} Index Read Primer
- SureSelect^{QXT} P7 dual indexing primers
- SureSelect^{QXT} P5 dual indexing primers
- SureSelect^{QXT} Stop Solution
- SureSelect^{QXT} Primer Mix

Contact Agilent to order a SureSelect^{QXT} Reagent Kit *without* the SureSelect^{QXT} Library Prep Kit ILM, Box 2.

Protocol

ThruPLEX library preparation

1. Prepare ThruPLEX libraries according to the ThruPLEX DNA-Seq, Plasma-Seq, or Tag-seq kit user manual.
2. Perform library purification using AMPure XP beads as described in the appropriate ThruPLEX user manual.

CAUTION: For the final elution, DNA must be eluted by resuspending the beads in 30 µl of PCR grade water, *not* TE buffer.

ThruPLEX library capture

1. Resuspend xGen Universal Blocking Oligos to 1 µl per reaction (or 1 nmol/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 xGen Universal Blocking Oligo - TS HT-i5
    - 1 µl xGen Universal Blocking Oligo - TS HT-i7
6. Follow procedures in the Agilent SureSelect^{QXT} Protocol starting at Chapter 3, Step 2 through the end of Chapter 4, Step 5 with the following modification:
   At Chapter 4, Step 1. Amplify the Captured Libraries, modify the Post-Capture PCR Reaction Mix to the following:

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

The ThruPLEX libraries are already indexed, so do *not* use the SureSelect^{QXT} indexing primers.

NOTE: This protocol was developed using the SureSelect^{XT} Human All Exon v5 Capture Library.

Related Products

Cat. #	Product	Size	License	Quantity	Details
R400584	ThruPLEX® Tag-seq 6S (12) Kit	12 Rxns		*	

The ThruPLEX Tag-seq Kit includes all necessary reagents for generating and multiplexing DNA-seq libraries with the incorporation of Unique Molecular Indexes (UMIs), and includes 6 unique single index PCR primer sets. Once purified and quantified, the resulting library is ready for Illumina NGS instruments using standard Illumina sequencing reagents and protocols. Only 50 pg to 50 ng of fragmented double-stranded DNA is required for library preparation. The entire three-step workflow takes place in a single tube or well in about two hours. No intermediate purification steps or sample transfers are necessary, preventing handling errors and loss of valuable samples. This kit includes reagents sufficient for 12 reactions with 6 single-index primer sets.

Documents | Components | Image Data

R400585	ThruPLEX® Tag-seq 48S Kit	48 Rxns		*	
R400586	ThruPLEX® Tag-seq 96D Kit	96 Rxns		*	
R400674	ThruPLEX® DNA-Seq Kit	24 Rxns		*	
R400675	ThruPLEX® DNA-Seq Kit	48 Rxns		*	
R400676	ThruPLEX® DNA-Seq Kit	96 Rxns		*	
R400677	ThruPLEX® DNA-Seq Kit	480 Rxns		*	
R400679	ThruPLEX® Plasma-Seq Kit	24 Rxns		*	
R400680	ThruPLEX® Plasma-Seq Kit	48 Rxns		*	
R400681	ThruPLEX® Plasma-Seq Kit	96 Rxns		*	
R400682	ThruPLEX® Plasma-Seq Kit	480 Rxns		*	

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If you are looking for a product-specific, fully optimized User Manual or Protocol-At-A-Glance, please visit the product's product page, open the item's product details row in the price table, and click Documents. More detailed instructions for locating documents are available on our website FAQs page.

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