Targeted capture of ThruPLEX libraries with Agilent SureSelectXT2

Introduction

Enrichment of ThruPLEX libraries with Agilent SureSelect platforms is easily performed. The chart below details the reagents necessary for this SureSelect^{XT2} protocol. The modules marked in red are 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^{XT2} with ThruPLEX kits			
Additional reagents	Primers	Required	Illumina P5 and P7 primers
	Blocking oligos	Required	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^{XT2} Reagent Kit	SureSelect^{XT2} Library Prep Kit, ILM	Not used	Replace with a ThruPLEX kit. Contact Agilent to purchase a Reagent Kit without this component.
	SureSelect^{XT2} Pre-capture Indexes, ILM	Not used	Indexes are included in the ThruPLEX kit
	SureSelect^{XT2} Pre-capture Box #1	Required	
	SureSelect^{XT2} Pre-capture Box #2	Required	

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)
- SureSelect^{XT2} reagents:
  - Refer to the "Required Reagents" section of the Agilent SureSelect^{XT2} Protocol
  - A SureSelect^{XT2} Capture Library (e.g. SureSelect^{XT2} Human All Exon V5, 16; Agilent Technologies, Cat. # 5190-6208)

NOTE: This following items may be required for the post-capture amplification step:

- Herculase II Fusion DNA Polymerase with dNTPs (Agilent Technologies, Cat. # 600677 or 600679)
- Illumina P5 Primer: AATGATACGGCGACCACCGA
- Illumina P7 Primer: CAAGCAGAAGACGGCATACGA

Equipment

- As specified in the "Required Equipment" section of the Agilent SureSelect^{XT2} Protocol.

NOTE: When integrating ThruPLEX kits with the SureSelect^{XT2} library capture system, all components of the SureSelect^{XT2} 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
- SureSelect^{XT2} Pre-Capture Indexes

Contact Agilent to order a SureSelect^{XT2} Reagent Kit *without* the SureSelect^{XT2} Library Prep Kit ILM.

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. 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 SureSelect^{XT2} protocol recommends pooling of 8 libraries with different indexes (187.5 ng of each) when using the Human All Exon v5 Capture Library.
3. In a 1.5 ml microcentrifuge tube combine:
    - 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 ≤45°C to reduce the volume in the tube to <7 µl. Do not completely dry the mixture.
5. Bring the volume to 7 µl with nuclease-free water.
6. Vortex the tube vigorously for 30 sec and centrifuge to bring contents to the bottom of the tube.
7. Add 9 µl SureSelect^{XT2} 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 SureSelect^{XT2} Protocol 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 SureSelect^{XT2} Human All Exon v5 Capture Library and the ClearSeq DNA Kinome Panel.

Related Products

Cat. #	Product	Size		License	Quantity	Details
R400584	ThruPLEX® Tag-seq 6S (12) Kit	12 Rxns	USD $850.00			

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	USD $2833.00			
R400586	ThruPLEX® Tag-seq 96D Kit	96 Rxns	USD $4987.00			
R400674	ThruPLEX® DNA-Seq Kit	24 Rxns	USD $740.00			
R400675	ThruPLEX® DNA-Seq Kit	48 Rxns	USD $1428.00			
R400676	ThruPLEX® DNA-Seq Kit	96 Rxns	USD $2617.00			
R400677	ThruPLEX® DNA-Seq Kit	480 Rxns	USD $11592.00			
R400679	ThruPLEX® Plasma-Seq Kit	24 Rxns	USD $757.00			
R400680	ThruPLEX® Plasma-Seq Kit	48 Rxns	USD $1432.00			
R400681	ThruPLEX® Plasma-Seq Kit	96 Rxns	USD $2484.00			
R400682	ThruPLEX® Plasma-Seq Kit	480 Rxns	USD $12220.00			

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User-generated protocols

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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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