Target capture of ThruPLEX libraries with Agilent SureSelectXT

Introduction

Enrichment of ThruPLEX libraries with Agilent SureSelect platforms is easily performed. The chart below details the reagents necessary for this SureSelect^{XT} 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^{XT} with ThruPLEX kits			
Additional reagents	Primers	Required	Illumina P5 and P7 primers
	Blocking oligos	Requred	xGen Universal Blocking Oligos (TS HT-i5 and TS HT-i7)
	Agilent Herculase II Fusion DNA Polymerase	Required	
Agilent SureSelect^{XT} Reagent Kit	SureSelect^{XT} Library Prep Kit ILM	Not used	Replace with a ThruPLEX kit. Contact Agilent to purchase a Reagent Kit without this component.
	SureSelect Target Enrichment Box #1	Required	The following components are *not* used: • SureSelect Elution Buffer • SureSelect Neutralization Buffer
	SureSelect Target Enrichment Kit ILM Indexing Hyb Module Box #2	Required	The following components are *not* used: • SureSelect ILM Indexing Pre Capture Reverse PCR Primer • SureSelect ILM Indexing Post Capture Forward PCR Primer

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
  - Ilumina P7 Primer: CAAGCAGAAGACGGCATACGA
- SureSelect^{XT} reagents: Refer to the "Required Reagents" section of the Agilent SureSelect^{XT} Protocol

Equipment

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

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

- SureSelect^{XT} Library Prep Kit ILM*
- SureSelect ILM Indexing Pre Capture Reverse PCR Primer
- SureSelect ILM Indexing Post Capture Forward PCR Primer

*Contact Agilent to order a SureSelect^{XT} Reagent Kit *without* the SureSelect^{XT} 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. Follow the Agilent SureSelect^{XT} Protocol starting at the beginning of Chapter 4 and continuing to 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 SureSelect^{XT} 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 µl of xGen Universal Blocking Oligo - TS HT-i5
    - 1 µl of xGen Universal Blocking Oligo - TS HT-i7

  NOTE: This results in a volume of 7.6 µl per reaction instead of 5.6 µl as stated in the SureSelect^{XT} 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 per 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 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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