Exome capture of ThruPLEX libraries with Illumina Nextera® Rapid Capture

Introduction

Enrichment is a sample preparation strategy used to isolate and sequence only those genes of interest (in this case the entire exome), reducing cost and improving informatics efficiency. Our enrichment protocols are compatible with all ThruPLEX DNA-Seq, ThruPLEX Plasma-Seq, and ThruPLEX Tag-seq kits.

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)
- Illumina capture reagents (one of the following required):
  - Nextera Rapid Capture Exome Enrichment Kit (FC-140-1000; FC-140-1001; FC-140-1002; FC-140-1003; FC-140-1083; FC-140-1086; FC-140-1089)
  - Nextera Rapid Capture Expanded Exome Enrichment Kit (FC-140-1004; FC-140-1005; FC-140-1006)

Other consumables and equipment

Refer to Appendix A "Consumables and Equipment" (page 44–46) in the Nextera Rapid Capture Enrichment Reference Guide, Cat. # FC-140-9001DOC, Part # 15037436 Rev. J.

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 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. Combine 500 ng of each uniquely indexed ThruPLEX library.

NOTE: The number of libraries that may be pooled is determined by the kit configuration of the Nextera Rapid Capture Exome Enrichment kit purchased (1-plex to 12-plex).

2. If necessary, adjust the volume of the pooled libraries to 38 µl.

- If the total volume of libraries is >38 µl, use a vacuum concentrator without heating to reduce volume to 38 µl.
- If the total volume is <38 µl, increase the volume to 38 µl with nuclease-free water.

3. In a 0.2-ml PCR tube combine:
    - 38 µl DNA library or sample pool
    - 1 µl xGen Universal Blocking Oligo - TS HT-i5
    - 1 µl xGen Universal Blocking Oligo - TS HT-i7
    - 50 µl buffer EHB (from Nextera kit)
    - 10 µl capture oligos CEX, EEX, or RCO (from Nextera kit)
4. Continue with Procedure Step 2, page 18 of the probe hybridization per the Nextera Rapid Capture Enrichment Reference Guide, Catalog # FC-140-9001DOC, Part #15037436 Rev. J, "Shake at 1200 rpm...."
5. For Procedure Step 1 of the Second Hybridization (page 22) add the following to each well containing 25 µl of product from the first capture:
    - 13 µl buffer RSB (from Nextera kit)
    - 1 µl xGen Universal Blocking Oligo - TS HT-i5
    - 1 µl xGen Universal Blocking Oligo - TS HT-i7
    - 50 µl buffer EHB (from Nextera kit)
    - 10 µl capture oligos CEX, EEX, or RCO (from Nextera kit)
6. Continue with Procedure Step 2, page 22 of the probe hybridization per the Nextera Rapid Capture Enrichment Reference Guide, Catalog # FC-140-9001DOC, Part # 15037436 Rev. J, "Shake at 1200 rpm…"

NOTE: This protocol was developed using the Nextera Rapid Capture Exome Enrichment Kit (FC-140-1083).

Related Products

Cat. #	Product	Size	License	Quantity	Details
R400586	ThruPLEX® Tag-seq 96D Kit	96 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 96 dual 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 96 reactions with 96 dual-index primer sets.

Documents	Components	Image Data

R400585	ThruPLEX® Tag-seq 48S Kit	48 Rxns		*	
R400584	ThruPLEX® Tag-seq 6S (12) Kit	12 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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User-generated protocols

User-generated protocols are based on internal proof-of-concept experiments, customer collaborations, and published literature. In some cases, relevant results are discussed in our research news BioView blog articles. While we expect these protocols to be successful in your hands, they may not be fully reviewed or optimized. We encourage you to contact us or refer to the published literature for more information about these user-generated and -reported protocols.

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.

Questions? Protocols of your own that you would like to share?

Contact technical support | Give feedback

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