

Targeted capture of ThruPLEX libraries with Roche NimbleGen SeqCap EZ

Introduction ↑

Enrichment is a sample preparation strategy used to isolate and sequence only those genes of interest, 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, or 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)
- SeqCap reagents:
 - SeqCap EZ Accessory Kit V2 (Roche NimbleGen; Cat. # 07145594001 or 06776345001)
 - SeqCap EZ Hybridization and Wash Kit (Roche NimbleGen, Cat. # 05634261001)
 - SeqCap Pure Capture Bead Kit (Roche NimbleGen; Cat. # 06977952001)
 - SeqCap EZ Library
- Other consumables as specified in the the SeqCap EZ Library SR User's Guide (version 5.0) under "Consumables Purchased from Other Vendors"

Equipment

As specified in the "Laboratory Equipment" section of the SeqCap EZ Library SR User's Guide (version 5.0)

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 μ l of PCR grade water, *not* TE buffer.

ThruPLEX library capture
























1. Resuspend xGen Universal Blocking Oligos to 1 μ l per reaction (or 1 nmol/ μ l) in nuclease-free water.
2. Pool ThruPLEX libraries for hybridization by adding equal amounts of each library to obtain 1 μ g of DNA.
For example, to hybridize four ThruPLEX libraries with different indexes, 250 ng of each library would be added; or if pooling 10 uniquely indexed libraries, 100 ng of each library would be added.
3. In a 1.5-ml microcentrifuge tube combine:

- 5 µl of COT Human DNA (1 mg/ml) from the SeqCap EZ Accessory Kit v2
- 1 µg pooled ThruPLEX libraries
- 1 µl xGen Universal Blocking Oligo - TS HT-i5
- 1 µl xGen Universal Blocking Oligo - TS HT-i7

4. Follow the SeqCap EZ Library SR User's Guide (version 5.0) starting at Chapter 5, Step 5, #4 ("Close the tube's lid...") and continue to the end of Chapter 7 to generate captured ThruPLEX libraries that are ready to be quantified and sequenced using Illumina NGS platforms.

NOTE: This protocol was developed using the SeqCap EZ Human Exome v3.0 Library.

Related Products

Cat. #	Product	Size	License	Quantity	Details			
R400584	ThruPLEX® Tag-seq 6S (12) Kit	12 Rxns		*				
<p>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.</p> <p style="text-align: center;"></p> <table border="1" style="width: 100%; text-align: center;"> <tr> <td>Documents</td> <td>Components</td> <td>Image Data</td> </tr> </table>						Documents	Components	Image Data
Documents	Components	Image Data						
R400586	ThruPLEX® Tag-seq 96D Kit	96 Rxns		*				
R400585	ThruPLEX® Tag-seq 48S Kit	48 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

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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](#).

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

Contact technical support

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