Sep 27, 2024

Preparation of Linked-Read Sequencing Libraries using Haplotagging beads

DOI
dx.doi.org/10.17504/protocols.io.5jyl827m8l2w/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5jyl827m8l2w/v1

Protocol Citation: marek.kucka, Yingguang Frank Chan 2024. Preparation of Linked-Read Sequencing Libraries using Haplotagging beads. **protocols.io https://dx.doi.org/10.17504/protocols.io.5jyl827m8l2w/v1**

Protocol status: Working
We use this protocol and it's working

Created: September 25, 2024

Last Modified: September 27, 2024

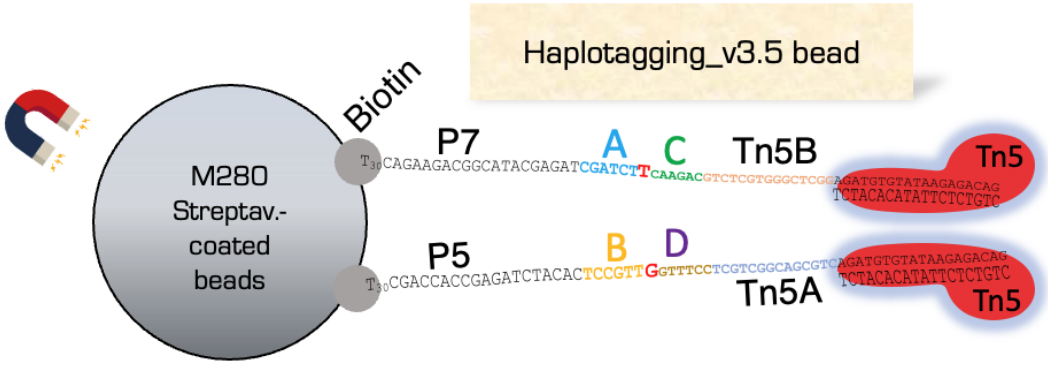
Protocol Integer ID: 108352

Funders Acknowledgement:
European Research Council
Grant ID: 101069216
"HAPLOTAGGING"

Abstract

Description:
Haplotagging beads are prepared in form of a 96 well plate where each well contains M280-Streptavidin beads linked with complete and barcoded i5 and i7 Tn5-sequencing adapters. Each well contains 884736 well-specific and barcoded Tn5-adapters assembled with Tn5 transposase. Whole plate then has almost 85 million barcodes (96 x 884736 = 84934656 barcodes), with each bead carrying many copies of a single segmented barcode.

The haplotagging beads are used to prepare short-read linked-read sequencing libraries with both i7 and i5 index being 13bp long. High molecule DNA is tagmented with a single bead and all the reads of that DNA molecule will carry the same barcode combination (one of 85 million).



Haplotagging bead v3.5 design.
The 4 plates of haplotagging beads can be distinguished based on different combination of ligation overhang connecting two 6bp segments of i7 or i5 barcode (overhang being the red highlighted base, here shown design of Plate2 with **T**-overhang in i7 barcode and **G**-overhang in i5 barcode).
A and C represent 6bp segments of i7-barcode; B and D represent 6bp segments of i5-barcode.

1bp overhangs in i7/i5 barcode identifying Haplotagging bead plate:
Plate1: A/C
Plate2: T/G
Plate3: C/T
Plate4: G/A

Materials

Haplotagging beads

Qubit™ dsDNA Quantification Assay Kits (Thermo-Fisher, Q32854)

0.6% SDS in H2O
1M Tris, pH=8
5M NaCl
Triton X-100

Exonuclease I (*E. coli*) (NEB, M0293)

Q5® High-Fidelity 2X Master Mix (NEB, M0492)
NEBNext® High-Fidelity 2X PCR Master Mix (NEB, M0541)

P7 primer
AATGATACGGCGACCACCGAGATCTACAC

P5 primer
CAAGCAGAAGACGGCATACGAGAT

Ampure or Home-made magnetic beads for DNA extraction/library size selection

Linked-read library preparation from High molecular gDNA

50m 50s

1 **Aliquot 1 U of Haplotagging beads per DNA sample.**
1 U of Haplotagging beads corresponds to 1 ul of original M280 Streptavidin beads.
Use max of 0.3 ng gDNA per 1 U of Haplotagging beads.

2 **Dilute gDNA** of each sample to 0.15 ng DNA/ul in 10mM Tris, pH=8
Check with HS Qubit concentration of your diluted gDNA using 10 (1.5ng) or 20 (3ng) ul of diluted 0.15ng/ul gDNA

3 **WASH buffer**
10 mM Tris, pH=8
30 mM NaCl
0.1% Triton X-100

4 **TAGMENTATION**: mix in same order: 10m

1 U of Haplotagging beads from one well of Haplotagging bead plate
16 ul WASH buffer
2 ul 0.15ng/ul gDNA
5 ul 5xTagmentation buffer

mix by inverting to re-suspend the gDNA with beads
incubate at 55 °C for 00:10:00

5 **STOP TAGMENTATION and WASH the beads** 3m
add 12 ul of 0.6% SDS in each sample
mix by inverting
Room temperature 00:02:00
Pulse spin down and place on magnetic stand.
Remove supernatant.
Add 150 ul WASH buffer to the beads, mix by inverting for 00:01:00
Place on magnet and repeat the bead washing step one more time.

6 **Pool or Subsample all samples' beads into one tube.**

Pool all or only proportion of beads from each sample depending on the sequencing coverage needed.
Note: 1 ng of DNA needs 30 Gb of sequencing (or 200 million reads) to achieve high enough linked-read information per DNA molecule.

Example: If we tagmentated 10 DNA samples and we will sequenced with 30Gb of sequencing output, then total input amount of DNA from all these 10 samples has to be 1ng. Each sample thus brings in 0.1 ng DNA.
This protocol did tagmentation using 0.3ng DNA, which is why we only pool 1/3 of the beads from each of the 10 samples into one single pooled sample for further preparation, to achieve 1 ng of gDNA input. Keep the rest of the beads in 4 °C as backup.

7 **Exonuclease I treatment of beads to remove un-integrated Tn5-transposomes** 24m

On magnet remove WASH buffer from the beads
Add 60 ul 1x Exonuclease I buffer and 2 ul Exonuclease I enzyme
Mix well and incubate:
48 °C 00:24:00
Mix beads by inverting 5 time every 5 minutes during the incubation

8 WASH the beads twice at RT with WASH buffer to remove Exonuclease I

9 **Amplification of the libraries (libraries bound to bead surface)** 13m 50s

Prepare 50 ul PCR mix for every 5 U of beads:

- 25 ul of NEBNext® High-Fidelity 2X PCR Master Mix / Q5® High-Fidelity 2X Master Mix
- 22.5 ul H2O
- 2.5 ul 20 uM P5+P7-primer mix

P7 primer AATGATACGGCGACCACCGAGATCTACAC
P5 primer CAAGCAGAAGACGGCATACGAGAT

72 °C 00:10:00
98 °C 00:00:20
11 cycles of:
98 °C 00:00:15
62 °C 00:00:30
72 °C 00:00:45
final elongation:
72 °C 00:02:00

10 run 5 ul of PCR on 2% agarose gel

11 perform Ampure/Home-made magnetic beads size selection on the leftover library to remove library fragments shorter than 300bp and longer than 800bp