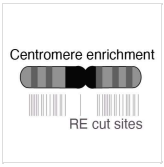

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AlphaHOR-RES: a method for enriching centromeric DNA

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ABSTRACT

Directed Methylation and Long-read sequencing (DiMeLo-seq) can be used to map protein-DNA interactions in repetitive regions, including the centromere. If you plan to use DiMeLo-seq for centromere mapping, then centromere enrichment is a valuable technique to increase the output signal and get more data. AlphaHOR-RES is a method to enrich for centromeres using MscI and AseI restriction digest.

Refer to "DiMeLo-seq: Directed Methylation with Long-read sequencing" and "pA-Hia5 Protein Expression and Purification" on protocols.io for the rest of the DiMeLo-seq protocol.

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KEYWORDS

null, centromere enrichment, centromere, DiMeLo-seq, AlphaHOR-RES, restriction digest

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MATERIALS TEXT

Monarch® High Molecular Weight DNA extraction kit (NEB T3050L)
Elution buffer/Buffer EB (QIAGEN 19086)
NEBuffer 2.1 (NEB B7202S)
MscI enzyme (NEB R0534S)
AseI enzyme (NEB R0526S)
Eppendorf DNA LoBind tubes **1.5 mL** (Fisher 022431021)
Qubit dsDNA BR Assay Kit (Fisher Q32850)
Orange DNA Loading Dye (Fisher R063)
Lonza SeaKem Gold agarose (Lonza 50512)
SYBRSafe gel stain (Fisher S33102)
Scalpel
GeneRuler High Range DNA Ladder (Fisher SM1351)
a Zymoclean Large Fragment DNA Recovery Kit (Zymo D4045)
ONT LSK-109 native library prep kit (ON SQK-LSK109, ON SQK-LSK110)
v9.4 MinION flow cell (ON FLO-MIN106D)

1 Extract genomic DNA from ~25 million cells using an NEB High Molecular Weight DNA extraction kit with 300 rpm agitation during lysis.

2 Elute DNA in 300 µL elution buffer and allow to relax at 4ºC for 2 days. DNA will remain viscous until it is digested.

3 Once fully relaxed/solubilized, add 37 µL NEBuffer 2.1, 100 units of MscI, and 100 units of AseI to reach a total volume of 370 µL in a 1.5 mL LoBind Eppendorf tube. Place the reaction on a rotator at 12 rpm and leave overnight at 37ºC.

4 Quantify DNA concentration using a Qubit Broad Range DNA kit.

5 Mix DNA with orange loading buffer and load onto a 0.3% TAE agarose gel made with Lonza SeaKem Gold agarose and 15 µL SYBRSafe gel stain per 100 mL gel. Load a GeneRuler High Range DNA Ladder in an adjacent lane. To avoid overloading, load no more than 250-300 ng of DNA per mm of lane width. Run gel at 2 V/cm for 1 hour and image over a blue light transilluminator.

6 Cut gel to remove fragments smaller than 20 kb. Keep everything larger than 20kb (up to the well itself).

7 Purify DNA from the resulting gel slice using a Zymoclean Large Fragment DNA Recovery Kit with the following modifications:

7.1 Melt the gel slice at room temperature on a rotator at 12 rpm.

7.2 Elute DNA from the column twice with elution buffer heated to 70ºC.

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8 Quantify DNA concentration with a Qubit Broad Range DNA kit.

9 Prepare DNA for sequencing using an ONT LSK-109 (multiplexing) or LSK-110 (not multiplexing) native library prep kit.
For a more detailed library prep, please refer to "DiMeLo-seq: Directed Methylation with Long-read sequencing" step 12.

10 Sequence on a v9.4 MinION flow cell.

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