Nov 19, 2024 Version 1

TEnCATS - Transposable Element nanopore Cas9-Targeted Sequencing V.1

DOI
https://dx.doi.org/10.17504/protocols.io.kqdg3q66ev25/v1

Torrin McDonald[1], Camille Mumm[1], Jessica Switzenberg[1], Alan Boyle[2]
[1]University of Michigan; [2]University of Michigan - Ann Arbor

SMaHT

Jessica Switzenberg
University of Michigan - Ann Arbor

DOI: https://dx.doi.org/10.17504/protocols.io.kqdg3q66ev25/v1

Protocol Citation: Torrin McDonald, Camille Mumm, Jessica Switzenberg, Alan Boyle 2024. TEnCATS - Transposable Element nanopore Cas9-Targeted Sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg3q66ev25/v1Version created by **Jessica Switzenberg**

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

Created: November 18, 2024

Last Modified: November 22, 2024

Protocol Integer ID: 112392

Keywords: Cas9, nCATS, transposable elements, TE, R10, ONT, SMaHT, transposable elements within genomic dna, using oxford nanopore technology, oxford nanopore technology, using cas9, r10 flow cell, genomic dna, cas9, dna, boyle lab, transposable element, sequencing

Abstract

This is the Boyle lab's protocol for using Cas9 to target transposable elements within genomic DNA using Oxford Nanopore Technologies (ONT) R10 flow cells.

Materials

Monarch HMW DNA Extraction Kit for Tissue (T3060S, NEB)
Proteinase K (3115879001, Roche)
Thermomixer
EnGen sgRNA Synthesis kit (E3322S, NEB)
Molecular biology grade water
Trizol
Chloroform
Ethanol
3M sodium acetate
Refrigerated centrifuge
RNase-free water
Qubit with BR RNA assay kit, dsDNA BR assay kit, dsDNA HS assay kit
Alt-R S.p. Cas9 HiFi Nuclease V3 (IDT)
QuickCIP (M0525S, NEB)
Taq DNA polymerase (M0273L, NEB)
10mM dATP
T4 DNA ligase (M0202M, NEB)
1X TE buffer
SQK-LSK114 kit (ONT)
MinION R10 flow cells (ONT)
MinION sequencer (ONT)
Tube rotator
1.5mL microcentrifuge tubes
PCR tubes

High Molecular Weight (HMW) genomic DNA (gDNA) extraction from tissue

45m

1 Aliquot the desired amount of tissue for gDNA extraction

2 Follow the manufacturer's guidelines from the Monarch HMW DNA Extraction Kit for Tissues (T3060S, NEB) with following changes to the lysis step:

2.1 Add 40 µL 10 mg/mL Proteinase K to 580 µL Tissue Lysis Buffer

2.2 Incubate tissue sample in Tissue Lysis Buffer 56 °C 00:15:00 in Thermomixer at 2000rpm 15m

2.3 Incubate the tissue sample an additional 00:30:00 at 56 °C without agitation 30m

2.4 Continue with Monarch protocol

in vitro transcription of guide RNAs (sgRNAs)

1h 15m

3 Lyophilized custom DNA oligos of the desired guide RNAs were resuspended in molecular biology grade water to a final concentration of 100uM. A 1:10 dilution of the oligos was used as a working stock for *in vitro* transcription.

4 For each guide, set up the below mix in a PCR tube using the EnGen sgRNA Synthesis Kit (E3322S, NEB):

- 10 µL 2x sgRNA Reaction Mix
- 0.5 µL DTT
- 2.5 µL custom DNA oligo
- 2 µL EnGen Enzyme Mix
- 5 µL molecular biology grade water

5 Incubate the reaction 37 °C 01:00:00 in a thermocycler 1h

6 Add 30 µL molecular biology grade water then 2 µL DNase I to the reaction to remove the DNA oligos

7 Incubate the reaction 37 °C 00:15:00 15m

Purification of sgRNAs 55m

8 Move the sgRNAs produced from the *in vitro* transcription reactions into a new 1.5mL microcentrifuge tube

9 Add 200 µL Trizol and 50 µL Chloroform to the samples and vortex to mix thoroughly

10 Centrifuge 20000 x g, Room temperature, 00:05:00 5m

11 Pipet out the aqueous layer (should be clear) carefully to avoid the pink layer and place into a new 1.5mL microcentrifuge tube

12 Add 50 µL Chloroform and vortex to mix thorougly

13 Centrifuge 20000 x g, Room temperature, 00:05:00 5m

14 Pipet out the top layer and place into a new 1.5mL microcentrifuge tube

15 Add 2 volumes of 70% ethanol and 3M sodium acetate solution to a final concentration of 0.3M and invert to mix 10 times

16 Centrifuge 20000 x g, 4°C, 00:30:00 30m

17 Remove supernant being careful not to disturb the white RNA pellet at the bottom

18 Wash the sgRNA by adding 250 µL 70% ethanol and centrifuging 20000 x g, 4°C, 00:10:00 then removing the supernant. Repeat this wash for a total of 2 washes. 10m

19 Remove as much of the residual ethanol as possible and air dry on bench for 00:05:00 5m

20 Resuspend the sgRNA in 11 µL RNase-free water

21 Quantify the RNA concentration using your specific method, we used the Qubit

Cas9-targeting of transposable elements in HMW gDNA 1h 31m

22 Aliquot out 10ug of extracted gDNA using a wide-bore tip into a PCR tube, try to get this to below 30uL. If not, you can scale up the following reaction if needed.

23 Add the following to the gDNA sample:
4 µL 10X Cutsmart Buffer
6 µL QuickCIP
X µL molecular biology grade water to a total of 40uL

24 Incubate the sample to dephosphorylate the gDNA using the below protocol in a thermocylcer: 50m
37 °C 00:30:00
80 °C 00:20:00

24.1 Make a 1:5 dilution of the Cas9 nuclease as follows:
2.5 µL 1X Cutsmart Buffer
0.5 µL Alt-R S.p Cas9 HiFi nuclease

24.2 While the gDNA is dephosphorylating, make the Cas9:sgRNA (RNP) by mixing the following in a PCR tube:
850 ng sgRNA

1 µL 1:5 dilution of Cas9 nuclease
1.5 µL 10X Cutsmart Buffer
X µL RNase-free water to a total of 15uL

24.3 Mix by flicking and incubate Room temperature 00:20:00

25 Cool the dephosphorylated gDNA on ice for 00:01:00 1m

26 Add the following to the gDNA sample for Cas9 cleavage and a-tailing of open ends:
15 µL RNP
2 µL Taq DNA polymerase
1.5 µL 10mM dATP
Mix by inversion

27 Incubate the sample using the protocol below in a thermocycler: 40m
37 °C 00:30:00
72 °C 00:10:00

ONT library preparation for R10 MinION flow cells 56m

28 Cool the Cas9-treated sample on ice for 00:01:00 1m

29 Transfer the sample to a new 1.5mL microcentrifuge tube using a wide-bore tip

30 Add the following to the sample from the ONT SQK-LSK114 kit:
25 µL LNB
5 µL LA
then add 5 µL T4 DNA ligase (M0202M, NEB) and
X µL molecular biology grade to 100uL total and mix by flicking

31 Incubate Room temperature 00:30:00 with rotation 30m

32 Add 1 volume of 1X TE buffer and invert to mix

33 Add 40 µL Axygen magnetic beads to the sample and invert to mix

34 Incubate Room temperature 00:10:00 with rotation 10m

35 Incubate Room temperature 00:05:00 without rotation 5m

36 Place tube on a magnetic rack and pellet the beads

37 Remove supernant

38 Wash beads twice by adding 200 µL LFB , resuspending the beads by inversion, pellet beads on magnetic rack and removing the supernant

39 Remove as much residual LFB as you can

40 Add 13 µL EB to the sample and resuspend the beads in the buffer

41 Incubate 37 °C 00:10:00 10m

42 Place tube on magnetic rack and pellet the beads

43 Pipet out the eluate and place in new 1.5mL microcentrifuge tube

44 Quantify the library using your method of choice, we use the Qubit

Loading onto the R10 MinION flow cell

45 For sequencing, we load as much library as we can onto the flow cell thus the entire 12uL of library is used. Add the following to the library:

- 37.5 µL SQB
- 25.5 µL LB

46 Prime the MinION following ONT's instructions

47 Mix the library to resuspend the LB and load onto the flow cell following ONT's instructions