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HEK3/LINC01509 Library Preparation

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Library preparation for HEK3 recording site (LINC01509) using PCR and digestion.

Materials

- NGS primer F ("CTGGCCTGGGTCAATCCTTG")
- NGS primer R ("CCCAGCCAAACTTGTCAACC")
- Q5 HiFi polymerase 2X master mix (NEB)
- BccI (NEB)
- LMW agarose
- SYBR Safe gel stain
- Generuler 1kb PLUS
- SPRI beads (Nucleomag)

Troubleshooting

HEK293T Cell Lysis

1 Make up **1:50** Thermolabile Proteinase K (NEB) in Viagen (cell).

2 Resuspend cells in 25 µL of Viagen/Proteinase K.

Quickly transfer cells into a PCR strip to avoid viscosity.

3 Perform lysis in a PCR machine: 1h 30m

37 °C 01:00:00
55 °C 00:30:00
On ice

Store lysates at -20 °C in the pre-PCR freezer.

PCR Amplification of Genomic DNA

4 Make up a 100 µL PCR reaction per sample (Q5) using 10 µL of cell lysate.

4.1 98 °C 00:00:30 30s

4.2 98 °C 00:00:05
68 °C 00:00:10
72 °C 00:00:20 35s

4.3 go to step #4.2 24 X

4.4 72 °C 00:02:00 2m

SPRI Bead Concentration

5 Purify amplicons by **1X SPRI** bead cleanup, eluting in 10 µL of water.

Digestion of Zero-Length Recordings

6 Digest zero-length recordings with 5 µL BccI in **50 uL** volume: 1h 20m

37 °C 01:00:00
65 °C 00:20:00
On ice

Gel Separation and Extraction of Recordings

7 Perform gel electrophoresis in **4% LMP agarose** at **3 V/cm** for 01:00:00 . 1h

8 Extract the region from **300 bp to 500 bp** and elute in 12 µL of water.

Store gel products at -20 °C in the post-PCR freezer.

Indexing PCR of Recombinant DNA

9 Using 1 µL of gel product, perform indexing PCR in 12 µL volume with **Nextera primers**.

9.1 98 °C 00:00:30 30s

9.2 98 °C 00:00:05 35s
67 °C 00:00:10
72 °C 00:00:20

9.3 go to step #9.2 9 X

9.4 72 °C 00:02:00 2m

SPRI Bead Purification

10 Purify amplicons by **1X SPRI** bead cleanup, eluting in 10 µL of water.

D1000 Tapescreen

11 Take 1 µL of each indexed amplicon to D1000 Tapescreen.

Expected result

Recordings should be above 158 + 67 (adaptors) + 69 (indexes) = **294 bp**.

12 Pool indexed amplicons according to desired relative molarities for sequencing.