**END-seq (variant)**

Reagents and consumables required are detailed at the end of the protocol

*Input material*

Start with agarose plugs containing 1-4x10^7 yeast cells that were ethanol fixed at harvest or 1-2x10^6 mammalian cells[[1]](#footnote-1) (see protocol for Agarose Embedding), you need ½ of a plug per sample.

*Stopping points*

This protocol is very long but can be done in bits, and we have stopped at many points without problems. To our knowledge all the following stopping points are fine:

You can pause indefinitely at any point where plugs are in tris buffer, store at 4°

You can pause indefinitely when DNA is in solution in TE, store at 4°.

You can pause indefinitely when DNA solutions have ethanol added for precipitation, is a pellet in the 70% ethanol, or the pellet is re-suspending in TE. Store at 4°.

You can pause indefinitely when DNA is bound to magnetic beads once beads are in bead wash or TE, store at 4°. Vortex well to re-suspend when you re-start.

Once you start the library preps using the NEBNext Ultra II kit, you must continue until the beads are in the wash solution, but then you can store at 4°.

After eluting the DNA from the magnetic beads with USER, store the libraries in the freezer until ready to amplify. Similarly, amplified libraries before or after purification are stored in the freezer.

*Protocol*

Transfer ½ plug 2ml tube

Equilibrate plugs 1 hr in 100µl 1x NEBuffer 2 + 0.1 mM dNTPs

Replace with fresh 100µl NEBuffer 2 + 0.1mM dNTPs containing 1µl Klenow

 1 µl Exonuclease VII could be included in this step to remove bound proteins

Incubate over night at 37°

Rinse twice with 1ml tris buffer (10mM Tris pH8.0), transfer to 15ml tubes and wash 3x15min with 10ml tris buffer on rocker

Transfer plugs back to 2ml tubes (I normally keep the 15ml tubes for further washes)

Equilibrate plugs with 100µl 1xCutSmart + 5mm DTT[[2]](#footnote-2) + 1mm dATP

 Incubate 1hr at RT

Exchange for 100µl same with 1µl Klenow exo- and 1µl T4 PNK

 2h r at 37°

Rinse 2x 1ml tris buffer then wash 15min with 10ml tris buffer

Equilibrate 1 hr with 100 µl 1x Quick ligase buffer containing 2.7µl END-seq adaptor 1

Replace with 100µl 1x Quick ligase buffer containing 2.7µl END-seq adaptor 1, 1 µl high conc. T4 DNA ligase

Incubate over night to over weekend at 25°

Rinse 2x 1ml, transfer to 15ml tubes then wash 4x15 min with 10ml tris buffer

Wash over night with 10ml tris buffer

Preheat heating blocks to 65° and 42°

Transfer plugs to 1.5ml tubes

Equilibrate 30 min with 1ml agarase buffer

Remove buffer and melt agarose 20min at 65°

Cool to 42° (transfer quickly!), add 1µl β-agarase and flick to mix (don't let it cool down!)

Incubate 1 hour at 42°

Meanwhile, pre-chill Covaris to 11°

Add 12.5µl 10M NH4OAc, 1µl Glycoblue and 125µl of ethanol, chill on ice for 5 min

Spin 15min 4° top speed

Wash with 70% ethanol, dry 5 min, resuspend in 132µl 1xTE 15min at 65°

 Set aside 2µl diluted to 100ul for qPCR validation

From here, move to the TrAEL-seq protocol and follow that starting from the transfer to Covaris tubes. The only difference is to use END-seq adaptor 2c for the ligation although END-seq adaptor 2 should work as well.

**Reagents**

*Adaptors*

END-seq adaptor 1:

[Phos]GATCGGAAGAGCGTCGTGTAGGGAAAGAGTGUU[BtndT]U[BtndT]UUACACTCTTTCCCTACACGACGCTCTTCCGATC∗T

Annealing:

20µl 100uM ENDseq Adaptor 2c in 200ul 1x T4 DNA ligase buffer

Incubate in heating block at 95°C 5 min then remove block from the heat and leave to cool to room temperature

END-seq adaptor 2c:

[Phos]GATCGGAAGAGCTATTATTTAAATTTTAATTUGACTGGAGTTCAGACGTGTGCTCTTCCGATC\*T

 Purchase PAGE purified from Sigma-Genosys or other supplier

Annealing:

20µl 100uM ENDseq Adaptor 2c in 200ul 1x T4 DNA ligase buffer

Incubate in heating block at 95°C 5 min then remove block from the heat and leave to cool to room temperature

*Reagents and consumables*

10x NEBuffer 2 (NEB)

Klenow fragment of DNA polymerase I (NEB M0210S)

10x CutSmart (NEB)

1M DTT

100mM dATP

Klenow fragment of DNA polymerase I, 3’-5’ exo- (NEB M0212S)

T4 Polynucleotide Kinase (T4 PNK - NEB M0201S)

5x Quick Ligation buffer (NEB B6058S)

High concentration T4 DNA Ligase (NEB M0202M)

β-agarase (NEB M0392S)

GlycoBlue (Thermo AM9515)

Ethanol

AFA microTUBEs (Covaris 520045)

Dynabeads MyOne streptavidin C1 beads (Thermo, 65001)

NEBNext Ultra II DNA kit (NEB E7645S), additional Q5 mix may be required which can be purchased separately from NEB

1ng/µl sonicated salmon sperm DNA or other carrier

NEBNext Multiplex Oligos set (e.g. NEB E7335S), additional USER mix may be required which can be purchased separately from NEB

AMPure XP beads (Beckman A63881)

Bioanalyser high sensitivity DNA chip (Agilent 5067-4626)

KAPA qPCR (Roche KK4835) or equivalent library quantification kit

*Solutions*

These solutions are made with milliQ water

Tris buffer 10mM Tris pH8.0

Agarase buffer 10 mM Bis-Tris-HCl, 1 mM EDTA pH 6.5

These solutions are made with certified DNase/RNase free water

10 M NH4OAc

1x TE and 0.1x TE Diluted from 100x stock

2xTN 10 mM Tris pH 8, 2 M NaCl

Wash buffer 5 mM Tris pH 8, 0.5 mM EDTA, 1 M NaCl

Bead prep buffer 5mM Tris pH8, 0.5mM EDTA, 1M NaCl, 0.05% Tween

*Equipment*

Magnetic rack for 1.5 ml tubes

Magnetic rack for 0.2 ml tubes (optional)

Covaris E220 sonicator

 Other sonicators may be suitable, protocol adjustments will be required

PCR machine

Agilent Bioanalyzer or equivalent gel system

Wheel

Rocker

2 heating blocks

LoBind 1.5ml tubes

LoBind tips for P20

1. Ethanol fixing of mammalian cells yet to be tested [↑](#footnote-ref-1)
2. DTT is from single use DTT aliquots [↑](#footnote-ref-2)