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Protocol TE Display sequencing (TED-Seq)

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We use this protocol and it's working

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Abstract

Background: Mobilization of transposable elements (TEs) can generate large effect mutations. However, because new TE insertions are challenging to detect and transposition is typically rare, the actual rate and landscape of new insertions remains unexplored for most TEs.

Results: Here, we introduce a TE display sequencing approach that leverages target amplification of TE extremities to detect non-reference TE insertions with high sensitivity and specificity. By implementing this approach on serial dilutions of genomic DNA from A. thaliana lines carrying different repertoires of new TE insertions, we show that the method can detect TE insertions that are present at frequencies as low as 1:250 000 within a DNA sample. In addition, TE display sequencing can be multiplexed to simultaneously detect insertions for distinct TE families, including both retrotransposons and DNA transposons, increasing its versatility and cost-effectiveness to investigate complex “mobilomes”. Importantly, when combined with nanopore sequencing, this approach enables the identification of insertions using long-reads and achieves a turn around time from DNA extraction to insertion identification of less than 24h, significantly reducing the time-to-answer. Analysis of TE insertions in large populations of A. thaliana plants undergoing a transposition burst demonstrate the power of the multiplex TE display sequencing to assess the rates and allele frequencies of heritable insertions, enabling its implementation to study large-scale ‘evolve and resequence’ experiments. Furthermore, we show that ~6% of de novo TE insertions show recurrent allele frequency changes consistent with either positive or negative selection.

Conclusions: TE display sequencing is an ultra-sensitive, specific, quick, and cost-effective approach to investigate the rate and landscape of new insertions for multiple TEs in large scale population experiments. We provide a step-by-step experimental protocol as well as ready-to-use bioinformatic pipelines, ensuring straightforward implementation of the method.

Materials

- NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs E7645, E7103)

- NEBNext Ultra II Q5 Master Mix (New England Biolabs M0544S)

- AMPure XP beads (Beckman Coulter A63880)

Adapter Primer

The following primers are designed for ATCOPI93 detection, the underlined primer sequence should be specific for your TE of interest

FORKED ADAPTER

5’ GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*t 3’
| || | ||| | || |||
3’**CTACGAGAAGGCTAGp 5’

P7_adapter_up
5’ GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T 3’

P7_adapter_bottom
5’ pGATCGGAAGAGCATC** 3’

Modifications: p 5’phosphate
* phosphorothioate bond
** dideoxy-C

1st PCR primer

TEDseq_outer_for : (complementary to the P7_adapter_up)

5’ GTGACTGGAGTTCAGACGTG 3’ (FIXED)

Reverse Primer (complementary to the target TE copy and downstream of the P5_linker_TE primer, in this particular case it corresponds to ATCOPIA93)

TE_outer_rev:

5’ GTGAGTCCTCTTCAACGGCT 3’ (should be changed accordingly to the specific targeted TE)

nested PCR primer

P7_primer_index4
5’ CAAGCAGAAGACGGCATACGAGAT**GCCAAT**GTGACTGGAGTTCAGACGTG 3’
P7_primer_index6
5’ CAAGCAGAAGACGGCATACGAGAT**CTTGTA**GTGACTGGAGTTCAGACGTG 3’
P7_primer_index1
5’ CAAGCAGAAGACGGCATACGAGAT**CGATGT**GTGACTGGAGTTCAGACGTG 3’
P7_primer_index3
5’ CAAGCAGAAGACGGCATACGAGAT**ACAGTG**GTGACTGGAGTTCAGACGTG 3’
So on so for….

Bold sequences match barcode

NEXTFLEX® PCR-Free Barcodes:
Barcode Adapter 1 CGATGT
Barcode Adapter 2 TGACCA
Barcode Adapter 3 ACAGTG
Barcode Adapter 4 GCCAAT
Barcode Adapter 5 CAGATC
So on so for…

TE Specific primer. The underlined sequence should correspond to the **(-) strand of the specific TE sequence of interest if we amplify the 5' extremity.**

Bold nucleotides are introduced to increase sequence diversity during illumina sequencing of pooled libraries generated by TED-seq.

When sequencing together multiple TED-Seq libraries targeting different TEs, only the P5_TE_primer without linker should be used.

Example ATCOPIA93, underlined sequence (For each TE-specific family, change the TE sequence):

P5_linker_TE primer :

5’ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCCACTCTCTTGTAGTACATATC 3’
5’ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**T**GCCCACTCTCTTGTAGTACATATC 3’
5’ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**AT**GCCCACTCTCTTGTAGTACATATC 3’
5’
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**CATA**GCCCACTCTCTTGTAGTACATATC 3’
5’
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**GAATT**GCCCACTCTCTTGTAGTACATATC 3’

Amplicon sequences

Amplicon after the first PCR: (the TE sequence is underlined)
GTGACTGGAGTTCAGACGTG(neighboring_genomic_sequence+TSD)TGCTCTTCCGATCTATTGATCAAGACTCAAATAAGAAAGGCCTAGTATTGGATATGTACTACAAGAGAGTGGGCCGAACATATGAGAAGTCTATGAAGAGCTTCTAGAAGAGGTGAAGGCACACAAATATCTCTTGTAGCCGTTGAAGAGGACTCAC

Amplicon after the nested PCR (using Index 4):

CAAGCAGAAGACGGCATACGAGATGCCAATGTGACTGGAGTTCAGACGTG(neighboring_genomic_sequence+TSD)TGCTCTTCCGATCTATTGATCAAGACTCAAATAAGAAAGGCCTAGTATTGGATATGTACTACAAGAGAGTGGGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT

ie P7adapter+(neighboring_genomic_sequence+TSD)**+**COPIA93 **+** P5adapter

Before Starting

1 Before starting, be sure to have:

P7_adapter_up
P7_adapter_bottom
P7 primer (P7_Primer_index#)
Primer for the 1st PCR (outer Primers)
Primer for the nested PCR (P7_Primer_index#, P5_linker_TE primer)

NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs E7645, E7103)
NEBNext Ultra II Q5 Master Mix (New England Biolabs M0544S)
AMPure XP beads (Beckman Coulter A63880)

To sequence with Oxford Nanopore Technology:

Ligation Sequencing Kit V14 (ONT SQK-LSK114)
R10.4.1 MinION Flow Cells (ONT FLO-MIN114) (if sequencing with MinION)
R10.4.1 PromethION Flow Cells (ONT FLO-PRO114M) (if sequencing with PromethION)

PREPARING CUSTOM ADAPTER

2 **Annealing adapters (final concentration 30uM):**
In a 0.2ml tube, mix:
90µl of 100µM P7_adapter_up (in 10mM Tris-HCl pH8)
90µl of 100µM P7_adapter_bottom (in 10 mM Tris-HCl pH8)
30µl T4 DNA Ligase Reaction Buffer (NEB)
90µl H_2O
In a thermocycle,heat to 95 °C and maintain the temperature for 2 min.
Cool to 25 °C over 45 min. (0.026C per seg)
Cool to 4 °C for temporary storage.
Centrifuge the PCR tube briefly to draw all moisture away from the lid.
Note: We recommend preparing aliquots of 50ul (40 reactions) of the custom adapter to avoid repeated freeze–thawing
Long term storage at -20°C

[C] P7_Primer_index = 10µM in 10 mM Tris-HCl pH8 (stock in 100uM)
[C] P5_TE_primer = 10µM in 10mM Tris-HCl pH8 (stock in 100uM)

3 Select the best protocol based on the technology used to sequence your library

STEP CASE

Standard Libraries 45 steps

Protocol for use with Standard Libraries (~400 bp)

Sonication

4 Sonicate 50µl of 20ng/µl DNA (ie 1µg)
Covaris program: Peak incident power 75W, Duty factor (18.6%), 200 Cycles per burst, for 175 seconds (Could be fragmented by any sonication system or enzymatic fragmentation. For short-reads you should aim fragments between 400-700bp).
Load in Agarose gel 1% 100 ng to control the fragment size
Only **500ng** will be used in the following protocol

End repair and A tailing

5 Add the following components to a sterile nuclease-free tube:

(green) NEBNext Ultra II End Prep Enzyme Mix	1.5 µl
(green) NEBNext Ultra II End Prep Reaction Buffer	3.5 µl
Fragmented DNA (500ng)	25 µl
Total Volume	30 µl

Note: For End Prep and Ligation step, NEBNext® Ultra™ II DNA Library Prep Kit's volume have been adjusted to reduce the price of each library. 48 samples can be processed with one kit (E7546S) instead of 24.

6 Set a 100 µl or 200 µl pipette to 25 µl and then gently pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.
Note: It is important to mix well. The presence of a small amount of bubbles will not interfere with performance.

7 Place in a thermocycler, with the heated lid set to ≥ 75°C, and run the following program:
30 minutes @20°C
30 minutes @ 65°C
Hold at 4°C

If necessary, samples can be stored at -20°C; however, a slight loss in yield (~20%) may be observed. We recommend continuing with adapter ligation before stopping.

Adapter Ligation

8 Add the following components to a sterile nuclease-free tube:

End Prep Reaction Mixture (Step 7 in End repair and A tailing Section)	30 µl
(red) NEBNext Ultra II Ligation Master Mix*	15 µl
(red) NEBNext Ligation Enhancer	0.5 µl
Custom Adapter (30µM)	1.25 µl
Total volume	46.75 µl

* Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.
Note: The Ligation Master Mix and Ligation Enhancer can be mixed ahead of time and is stable for at least 8 hours @ 4°C. We do not recommend adding adapter to a premix in the Adapter Ligation Step.

9 Set a 100 µl or 200 µl pipette to 40 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

10 Incubate at 20°C for 15 minutes in a thermocycler with the heated lid off.

Size Selection of Adapter-ligated DNA

11 Vortex AMPure XP Beads to resuspend.

12 Add 7.5 µl (~ 0.16X) of resuspended beads to the 46.75 µl ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

13 Incubate samples on bench top for at least 5 minutes at room temperature.

14 Place the tube on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing on the magnetic stand.

15 After 5 minutes (or when the solution is clear), carefully transfer the supernatant containing your DNA to a new tube (**Caution**: do not discard the supernatant). Discard the beads that contain the unwanted large fragments.

16 Add 7.5 µl (~0.16X) of resuspended Beads to the supernatant and mix at least 10 times. Be careful to expel all of the liquid from the tip during the last mix. Then incubate samples on the

bench top for at least 5 minutes at room temperature.

17 Place the tube on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing on the magnetic stand.

18 After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA targets (**Caution**: do not discard beads).

19 Add 200 µl of 80% freshly prepared ethanol to the tube while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

20 Repeat Step 19 once. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

21 Air dry the beads for up to 5 minutes while the tube is on the magnetic stand with the lid open. Caution: Do not overdry the beads.

22 Remove the tube from the magnetic stand. Elute the DNA target from the beads into 8µl of 10 mM Tris-HCl.

23 Mix well on a vortex mixer or by pipetting up and down 10 times. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing back on the magnetic stand.

24 Place the tube on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 7.5 µl to a new PCR tube.
Samples can be stored at -20°C.

1st PCR: Enrichment of Adapter-ligated DNA

25 Add the following components to a sterile strip tube:

Adapter Ligated DNA Fragments (Step 24)	7.5 µl
(blue) NEBNext Ultra II Q5 Master Mix	12.5 µl
(blue) TEDseq_outer_for (10µM)	2.5 µl
(blue) TE_outer_rev (10µM)	2.5 µl
Total volume	25 µl

Set a 100 µl or 200 µl pipette to 20 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

Place the tube on a thermocycler and perform PCR amplification using the following PCR cycling conditions:
1. 98°C 30”
2. 98°C 10”
3. 61°C 75”
4. GO TO .2 20x
5. 61°C 5’
6. Hold at 4°C

Cleanup of PCR Reaction

26 Vortex AMPure XP to resuspend.

27 Add 22.5 µl (**0.9X**) resuspended beads to the PCR reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

28 Incubate samples on bench top for at least 5 minutes at room temperature.

29 Place the tube on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing on the magnetic stand.

30 After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (Caution: do not discard the beads).

31 Add 100 µl of 80% freshly prepared ethanol to the tube while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

32 Repeat Step 31 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

33 Air dry the beads for up to 5 minutes while the tube is on the magnetic stand with the lid open. Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

34 Remove the tube from the magnetic stand. Elute the DNA target from the beads by adding 17µl of 10 mM Tris-HCl.

35 Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing back on the magnetic stand.

36 Place the tube on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 17 µl to a new PCR tube for and store at –20°C.

Samples can be stored at -20°C.

Nested PCR: Adding P5 and P7 sequences

37 Add the following components to a sterile strip tube:

Adapter Ligated DNA Fragments (Step 36)	7.5 µl
(blue) NEBNext Ultra II Q5 Master Mix	12.5 µl
(blue) P7_Primer_index# (10µM)	2.5 µl
(blue) P5_linker_TE_primer (10µM)	2.5 µl
Total volume	25 µl

Set a 100 µl or 200 µl pipette to 20 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

Place the tube on a thermocycler and perform PCR amplification using the following PCR cycling conditions:

1. 98°C 30”
2. 98°C 10”
3. 61°C 75”
4. GO TO .2 6X
5. 98°C 10”
6. 72°C 75”
7. GO TO .5 10X
8. 72°C 5’
9. Hold at 4°C

Cleanup of PCR Reaction

38 Vortex AMPure XP to resuspend.

39 Add 22.5 µl (**0.9X**) resuspended beads to the PCR reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing

for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

40 Incubate samples on bench top for at least 5 minutes at room temperature.

41 Place the tube on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube wells before placing on the magnetic stand.

42 After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (Caution: do not discard the beads).

43 Add 100 µl of 80% freshly prepared ethanol to the tube while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

44 Repeat Step 43 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

45 Air dry the beads for up to 5 minutes while the tube is on the magnetic stand with the lid open. Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

46 Remove the tube from the magnetic stand. Elute the DNA target from the beads by adding 33 µl of 10 mM Tris-HCl.

47 Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube wells before placing back on the magnetic stand.

48 Place the tube on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 30 µl to a new PCR tube for and store at –20°C.

Library can be stored at -20°C.