

Transposon mapping using flanking sequence exponential anchored (FLEA) PCR

Anton Henssen (✉ henssena@mskcc.org)

Kentsis Research Group

Julianne Carson

Kentsis Research Group

Alex Kentsis

Kentsis Research Group

Method Article

Keywords: Transposition, genomic integration, PCR, flanking sequence PCR

Posted Date: July 22nd, 2015

DOI: <https://doi.org/10.1038/protex.2015.071>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

This protocol describes a modified version of flanking sequence exponential anchored (FLEA) PCR. The original FLEA-PCR method was developed to amplify integration sites of retroviruses or retro-transposons. Such transposons contain two long terminal repeats (LTR), which have the same orientation. In contrast, DNA transposons have inverted terminal repeats (ITR), which are inverted in their orientation. Here, we adapt FLEA-PCR for mapping genomic DNA transposition, suitable for the analysis of DNA transposition reporters and genomes engineered through the use of DNA transposition.

Reagents

1) Oligonucleotides generated by solid-phase synthesis and purified by HPLC: Biotinylated primer: 5'-[BioTEG]CATTTTGACTCACGCGGTCGT-3' Anchor: 5'-GTGGCACGGACTGATCNNNNNN-3', where NNNNNN is a random hexamer sequence Exponential: 5'-GTGGCACGGACTGCA-3' Transposon1: 5'-ATTGACAAGCACGCCTCACG-3' Transposon2: 5'-ATGCACAGCGACGGATTCTG-3' 2) PureLink Genomic DNA Mini Kit (#K1820-00, Invitrogen, Waltham, MA, USA) 3) Platinum PCR SuperMix High Fidelity (#12532-016, Invitrogen, Waltham, MA, USA) 4) Water, DNase and RNase free (#BP2484-100, Fisher, Waltham, MA, USA) 5) Dynabeads kilobaseBINDER Kit (#60101, Invitrogen, Waltham, MA, USA) 6) T7 DNA Polymerase (#M0274S, New England BioLabs, Beverly, MA, USA) 7) Deoxyribose nucleotide triphosphates dNTP (#18427-088, Invitrogen, Waltham, MA, USA) 8) PureLink PCR Purification Kit (#K3100-01, Invitrogen Corp., Carlsbad, CA, USA)

Equipment

1) DynaMag- 96 Well slide magnet (#12331D, Life technologies, Carlsbad, CA, USA) 2) GeneMate UltraFlux I, 8-Strip Standard PCR Tubes (#T-3135-1, Bioexpress, Kaysville, UT, USA) 3) Eppendorf Master cycler ProS (Eppendorf, Hamburg, Germany) 4) Eppendorf Thermomixer R (Eppendorf, Hamburg, Germany) 5) Amicon Ultra 0.5mL 100K (#UFC510096, EMD Millipore, Billerica, MA, USA)

Procedure

****Linear Amplification**** This step anneals biotinylated primer specific to the mobilized transposon and generates a linearly-extended single strand that spans the genomic junction of the transposon. Sequence of the biotinylated primer is specifically designed to hybridize with a unique sequence of the transposon reporter or transposition-mediated transgene, with the sufficiently long extension time to span the transposition breakpoint. In the case of this protocol designed to identify synthetic piggyBac transposon insertions, the extension time was determined by the 100 nucleotides separating the primer from the DNA transposon ITR (Fig. 1A). 1. Prepare the PCR mixture in GeneMate UltraFlux tubes by mixing: 1 µL of 5 µM Biotinylated primer (100 nM) 4 µL genomic DNA (2 µg) 45 µL Invitrogen Platinum high fidelity SuperMix 2. Carry out annealing and linear extension using the following PCR conditions: 95°C for 5 min 30 cycles of : 95 °C (45 seconds) 62 °C (45 seconds) 72 °C (3 min) 1 x 72 °C for 10 min 4 °C until next

step 3. Transfer PCR reaction products into Amicon Ultra 0.5 100K filter tubes, add 200 μ l of nuclease-free water 4. Centrifuge at 12,000 g for 10 minutes at room temperature, 42 μ l concentrated purified sample should remain 5. Invert the column in clean tubes and centrifuge at 1,000 g for 2 min ****Isolation of Biotinylated Amplicon**** 1. Transfer 3 μ L of Dynal magnetic streptavidin beads into GeneMate UltraFlux tubes 2. Place the tubes onto DynaMag 96 magnetic holder 3. Remove supernatant 4. Add 20 μ L kilobaseBINDER kit Binding solution 5. Place tube onto DynaMag 96 6. Remove solution 7. Resuspend magnetic beads in 10 μ L Binding solution 8. Add 5 μ L of isolated PCR product from the previous step 9. Add 5 μ L of nuclease-free water 10. Shake tube at room temperature for 3 hours ****Purification of Biotinylated Amplicon**** 1. Place tube onto DynaMag 96 2. Remove supernatant 3. Add 40 μ L of kilobaseBINDER kit Washing solution 4. Place tube onto DynaMag 96 5. Remove 40 μ L Washing solution 6. Add 40 μ L of nuclease-free water 7. Remove 40 μ L water 8. Add 20 μ L of 0.1M NaOH 9. Shake for 30 min at 37 °C 10. Place tube onto DynaMag 96 11. Remove NaOH 12. Add 40 μ L nuclease-free water 13. Place tube on DynaMag 96 14. Remove water ****Synthesis of Complementary Strand**** This step uses a degenerate primer to anneal and synthesize the complementary strand of the biotinylated amplicons. The sequence of the anchor primer was adapted from Pule et al. (1). The primer has a 3' degenerate sequence and a 5' adapter sequence that enables the anchor primer to prime to the 3' end of the linear product of the previous step, containing the unknown genomic sequence flanking the inserted transposon (Fig. 1B). Prepare the PCR buffer mixture by mixing: 15 μ L nuclease-free water 2 μ L 10x T7 DNA Polymerase Buffer 2 μ L of 100 μ M Anchor primer 1 μ L of 10 mM dNTP (0.5 mM) 1. Add 19 μ L of this mixture to tube with streptavidin beads and mix by pipetting 2. Incubate at 95°C for 1 minute and decrease temperature to 37 °C over 10 minutes 3. Centrifuge to collect any condensate and add 1 μ L of T7 DNA polymerase 4. Incubate at 37 °C for 1 hour At this point a linear double stranded DNA product has been synthesized, which spans the transposon terminal repeat as well as the flanking genomic sequence. ****Product Amplification**** This step uses exponential PCR to amplify the isolated double-stranded DNA sequences using adapter sequences included in the primers used for linear extension and complementary strand synthesis. As a result of this PCR, an amplicon is produced that includes sequences of the transposon terminal repeats and the flanking genomic sequences (Fig. 1C). Wash the streptavidin beads to remove remaining anchor primers and carry out PCR: 1. Place tube on DynaMag 96 and remove supernatant 2. Add 40 μ L of water 3. Place tube on DynaMag 96 and remove supernatant 4. Repeat 4 times 5. Prepare the PCR mixture: 1 μ L of 25 μ M Transposon1 primer 1 μ L of 25 μ M Exponential primer 45 μ L of Invitrogen Platinum High Fidelity SuperMix 6. Add 48 μ L of PCR mixture to the beads and mix by pipetting 7. Carry out PCR using the following conditions: 95 °C for 5 minutes 35 cycles of: 95 °C for 45seconds 62 °C for 45seconds 72°C for 3 minutes 1x 72 °C for 10 minutes 4 °C until next step ****Nested Product Amplification**** In order to increase the yield of specific amplicons spanning the breakpoint between transposon and flanking human genome, a nested PCR can be used (Fig. 1D). 1. Use PureLink PCR Purification Kit to purify PCR product amplicons. Elute in 50 μ L nuclease-free water 2. Prepare PCR reaction mixture: 1 μ L of 25 μ M Transposon 2 primer 1 μ L of 25 μ M Exponential primer 45 μ L Invitrogen Platinum High Fidelity SuperMix 3. Add 3 μ L of purified PCR product from previous PCR amplification to 47 μ L of PCR mixture in GeneMate UltraFlux tubes 4. Carry out PCR using the following conditions: 95 °C for 5 minutes 35 cycles of: 95 °C for 45seconds 62 °C for 45seconds 72°C for 3 minutes

1x 72 °C for 10 minutes 4 °C until next step 5. Purify PCR product and proceed with cloning for capillary Sanger DNA sequencing or library preparation for massively parallel single molecule DNA sequencing.

Timing

The entire procedure can be completed in two days.

References

1. Pule MA, et al. (2008) Flanking-sequence exponential anchored-polymerase chain reaction amplification: a sensitive and highly specific method for detecting retroviral integrant-host-junction sequences. *Cytotherapy* 10(5):526-539. 2. Fraser MJ, Cary L, Boonvisudhi K, & Wang HG (1995) Assay for movement of Lepidopteran transposon IFP2 in insect cells using a baculovirus genome as a target DNA. *Virology* 211(2):397-407. 3. Cary LC, et al. (1989) Transposon mutagenesis of baculoviruses: analysis of *Trichoplusia ni* transposon IFP2 insertions within the FP-locus of nuclear polyhedrosis viruses. *Virology* 172(1):156-169.

Acknowledgements

We thank Martin Pule for details regarding the original FLEA-PCR method.

Figures

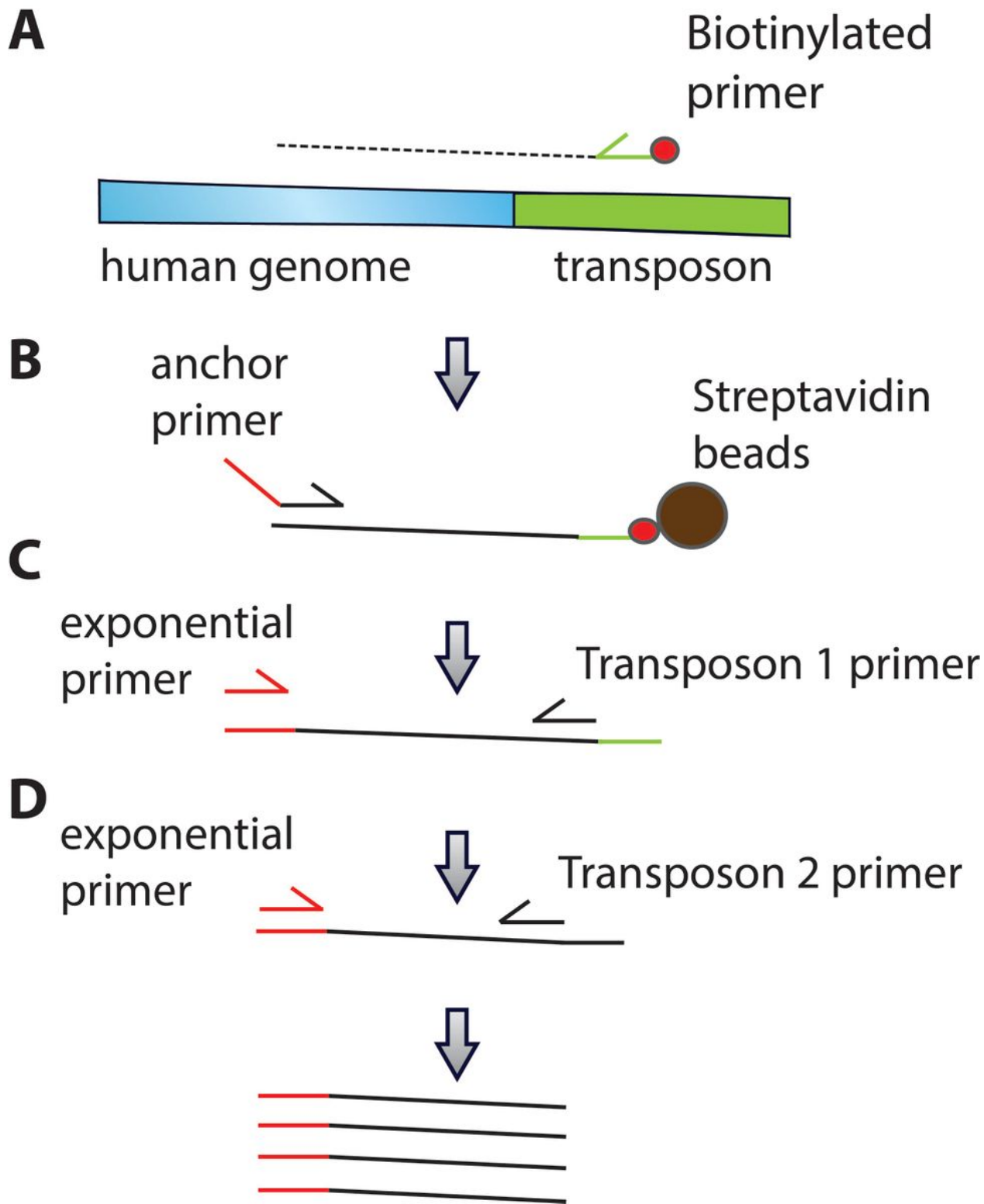


Figure 1

Schematic of transposon specific flanking-sequence exponential anchored–polymerase chain reaction amplification (FLEA-PCR) assay for amplifying genomic DNA transposon insertions.