USER MANUAL PIGGYBAC®

Hera BioLabs

PiggyBac® Splinkerette Kit

2022 User Manual
Version 6.0

Hera BioLabs, Inc.
services@herabiolabs.com
1-859-414-0468

2277 Thunderstick Drive, Suite 500
Lexington, KY 40505

Splinkerette PCR (spPCR) can be used to map the insertion sites of transposable elements. It is especially useful when inverse PCR (iPCR) proves ineffective. Splinkerette was developed in response to iPCR limitations such as generation of short sequence reads that can be difficult to unambiguously map.

This kit includes an optimized protocol and verified primers required for identification of piggyBac™ transposon integration sites.

Splinkerette Protocol:

A) Prepare the Splinkerette Adaptor

50 µM Adapter Primer Mixture	25 µl
10x Adapter Buffer	25 µl
Total 25µM Adaptor Mix	**50 µl**

Using a thermal cycler, heat the splinkerette adaptor to 95°C for 5 minutes, then cool by 1°C every 15 seconds until it reaches 24°C. Afterwards, keep at 4°C.

Day One

1.Collect cell or tissue samples; 1 to 5X10^6 cells or <25 mg tissue. For rodent tail, cut tail to 0.4 to 0.6 cm length, use one piece for rat and two pieces for mouse. Sample can be stored at -80°C after collected.

2. Extract tissue or cell gDNA by Qigen DNeasy Blood &Tissue Kit (cat: 69504) or other suitable kits according to manufacture protocol. Prepare gDNA in 50 µl volume (4 hours).

3. Quantify gDNA concentration by UV spectrophotometer or nanodrop.

4. Sau3AI restriction digestion: Set digestion reaction as listed in a 1.5 ml tube.

Component	Amount
Genomic DNA	**2 µg**
Sau3AI enzyme	**1 µl [20U/µl]**
EcoRV a Sau3AI Buffer	**3 µl**
ddH20 to Final Volume	**30 µl**

Digest at 37°C for at least 2 hours. Digestion can be carried out overnight. Heat inactivate at 65°C for 20 minutes and store at -20°C indefinitely.

5. Optional: Purify Sau3AI digested gDNA with column (QIAquick PCR purification kit or QIAquick gel purification kit) following manufacturer protocols. Elute DNA in 30 µl ddH20.

6. Set ligation reaction as follows:

Component	Amount
Sau3AI Digested DNA (from Step 4)	**300 ng**
Adaptor mix (25µM)	**1 µl**
10XT4 DNA-ligase buffer	**4 µl**
T4 ligase [20u/µl]	**1 µl**
ddH2O to Final Volume	**40 µl**

Incubate the ligation reaction at 4°C overnight (12 to 16 hours).

Day Two

7. Heat inactive the Step 6 Reaction at 65°C for 20 minutes. Cool down sample to room temperature.

8. EcoRV digestion as follows in a 1.5 ml tube:

Component	Amount
Step 6 Reaction Mixture	**40 µl**
EcoRV enzyme [20U/ µl]	**1 µl**
EcoRV & Sau3AI Buffer	**10 µl**
ddH2O	**49 µl**
Final Volume	**100 µl**

Incubate at 37°C for at least 4 hours. Overnight incubation is fine.

9. Column purify the digestion reaction (as in Step 6). Elute DNA in 32 µl TE buffer.

10. Primary PCR amplification with PCR Master MIX (We suggest using 2X Green GoTaq from Promega, M712) or any other DNA polymerase. For 2XGoTaq, set PCR reaction mixture and reaction as follows:

Component	Amount
2X Go Taq	**10 µl**
Spkt-Primer Mix-1	**2 µl**
Template from Step 10	**5 µl**
ddH2O	**3 µl**
Final Volume	**20 µl**

PCR reaction
95°C 3 min. 25 cycles of 94°C 30 sec. 58°C 30 sec.
72°C 2 min. Final extension of 72°C 5 min. to pause at 4°C

PCR products can be stored at -20°C.

11. Secondary nested PCR:

Component	Amount
2X Go Taq	**10 µl**
Spkt-Primer Mix-2	**2 µl**
Template from Step 10	**1 µl**
ddH2O	**7 µl**
Final Volume	**20 µl**

95°C 3 min. > 20 cycles of 94°C 30 sec. > 60°C 30 sec.
> 72°C 2 min. > Final extension of 72°C 5 min. to pause at 4°C

PCR products can be stored at -20°C.

Day Three

Analysis of Splinkerette-PCR products:

a. Prepare a 3% agarose gel in 1XTAE buffer with 0.5 µg/ml Ethidium Bromide.

b. Loading 10 µl secondary PCR products. Run gel in 1XTAE buffer. There should be multiple bands (like a DNA ladder) generated from your sample.
Positive Control Note: Should get a single band ~200bp.

c. Optional: Column purify the remaining PCR products and store at -20°C.

Shotgun Topo cloning for sequencing:

a. Using Invitrogen Topo TA cloning Kit (K4530-20) for cloning PCR products. Set up ligation reaction in PCR tube as following:

Component	Amount
Go Taq PCR products	**0.4 µl (0.4 to 1.6µl)**
Salt	**0.4 µl**
Topo Vector	**0.4 µl**
Double-distilled water	**1.2 µl (1.2 µl to 0 µl)**
Final Volume	**2.4 µl**

Incubate at room temperature for 30 minutes. Move samples onto ice after incubation or store at -200C for later use.

b. Heat shock transformation: Pre-chill a new PCR tube on ice. Mark sample properly. Thaw chemical competent cells (NEB 5-a C2987H) from -80°C on ice. Mix 1 µl ligation products with 20µl competent cells on ice gently. Incubate on ice for 30 min. Heat shock at 42°C for 45 seconds then move tube back on ice for another 2-5 minutes.

c. In a 1.5 ml tube, mix 200 µl room temperature SOC medium and transformed competent cells. Incubate at 37°C in an orbit shaker at speed of 200-250rpm/min for 1 hour. At the same time, warm up LB-amp plate in a 37°C incubator by setting plate bottom up.

d. Spread two plates for each transformation, one 20 µl another 100 µl.

e. Culture LB-amp plate overnight.

f. Randomly pick a suitable number of single clones for sequences the next day.

Sequence Analysis:

Scan TOPO clones for presence of piggyBac ITRs preceded by TTAA. The sequence immediately preceding the TTAA should be the genomic integration site of the transposon. Copy this sequence and perform a BLAST search to determine specific genetic location of integration.