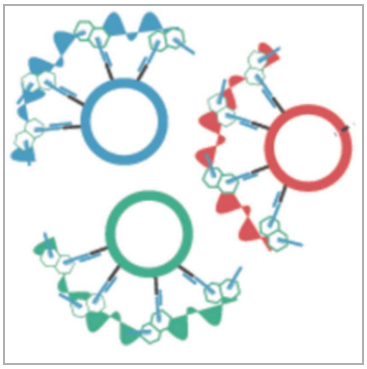

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ABSTRACT

This protocol is used to clarify the process of stLFR library preparation for the sequencing of high-quality snake genomes.

1 Sample preparation

Remove the long-fragment gDNA from 4 °C and store on ice.

2 Transposon Insertion

2.1 Transfer 10 ng long-fragment gDNA gently into a 0.2ml PCR tube. Without mixing, add Nuclease Free Water to a total volume of 36.8 µL. Collect and dispense long fragment DNA slowly (the process should take > 00:00:10) each time when pipetting. 10s

2.2 Prepare the stLFR_SamBarTIE and TE Buffer. Dilute the stLFR_SamBarTIE 16-fold with TE Buffer. Add 6 µL TE Buffer into a new 0.2 mL PCR tube and then transfer 2 µL stLFR_SamBarTIE to the tube. Vortex intermittently for 4 times (2s each) to mix. Label it as “4× dilution stLFR_SamBarTIE”.

2.3 Add 18 µL TE Buffer into a new 0.2 mL PCR tube and transfer 6 µL of the 4× dilution stLFR_SamBarTIE into the tube. Vortex intermittently for 4 times (2s each) to mix the tube. Label it as “stLFR_SamBarTIE (Working Mix).” stLFR_SamBarTIE (Working Mix) can be used for 12 reactions.

2.4 According to the requirements of the sample sequencing pooling strategy, mix the stLFR_SamBarTIE Working Mix.

2.5 Prepare the Transposon Insertion Reaction Mix on ice as shown. Vortex intermittently for 4 times (2s each) to mix.

2.6 Transfer 13.2 µL Transposon Insertion Reaction Mix to the DNA sample from step 2.1(total volume is 50 µL). Mix by very gently pipetting 10 times using wide-bore tip. Briefly centrifuge the tube. Transfer the tube to the thermocycler and start the Transposon Insertion Reaction Program.

2.7 Store the tubes on ice after the transposon insertion step has finished.

2.8 Pipette 42.5 µL TE buffer to a new 0.2 mL tube then transfer 7.5 µL transposon-inserted product (from step 2.6) to this new 0.2 mL tube. Mix by inverting the tube very gently and collect liquid to the bottom of the tube by briefly centrifuging (1 second) on a microcentrifuge. Label this new tube as the sample tube.

3 Capture

3.1 Vortex Capture Beads to mix thoroughly before use. Pipette 30 µL Capture Beads per sample to the tube.

3.2 Place the tube on a magnetic separation rack. Once the liquid is clear, carefully collect and discard the supernatant.

3.3 Pipette 50 µL Wash Buffer I per sample into the 0.2 mL PCR tube or 1.5 mL EP tube. Ensure that Wash Buffer I can cover all of the Capture Beads.

3.4 Rotate the tube 180 degrees within the rack such that the beads are forced to pass through the Wash Buffer I. Repeat the tube rotation once. Carefully remove and discard the supernatant once the liquid in the tube is clear. Pipette 50 µL Capture Buffer per sample to resuspend the Capture Beads.

3.5 Transfer 50 µL Capture Beads from step 3.4 to the sample tube from step 2.7 and mix thoroughly by inverting gently at least 10 times.

3.6 Centrifuge the product from step 3.5 for 1 second and place on the rotator in the incubator. 1h

Immediately start rotating the sample. In this experiment, After the first 00:10:00 of incubation at 60 °C, switch the temperature of the incubator to 45 °C. Open the door of the incubator to accelerate cooling, then close the door of the incubator and start the 00:50:00 countdown once the temperature drops to 48 °C.

4 Ligation Reaction 1

4.1 Centrifuge the product from step 3.6 and allow the product to cool to room temperature.

4.2 Ensure the product has cooled to room temperature, then transfer 30 µL Ligation Reaction 1 Mix to the 100 µL sample. Mix by inverting the tubes gently at least 10 times then briefly centrifuge (1s). Place the sample tube on the rotator and turn it on.

4.3 Perform Ligation Reaction 1 with the incubation in Room temperature (20°C to 25°C) 00:10:00. After incubation, centrifuge the sample and place it on the magnetic separation rack. Carefully remove and discard the supernatant once the liquid is clear. 10m

4.4 Pipette 180 µL of Wash Buffer II into the sample tube. Rotate the tube 180 degrees while on the magnetic separation rack to let the beads move through the Wash Buffer II. Repeat the tube rotation once. Carefully remove and discard the supernatant once the liquid is clear. Keep the Capture Beads in the Wash Buffer II until the Digestion Reaction Mix 1 is ready.

5 Digestion Reaction 1

5.1 Transfer 100 µL Digestion Reaction 1 Mix to the sample tube from step 4.4. Mix by inverting the tube gently at least 10 times followed by an instantaneous centrifugation (1s).

5.2 Place the sample tube on the rotator and turn it on. Perform the Digestion Reaction 1 incubation in 37 °C 00:10:00. Remove the sample tube from the incubator at the end of the reaction. Immediately add TIS Buffer. 10m

6 Termination Reaction

6.1 Remove the sample from 37 °C, centrifuge briefly, and store at room temperature. Immediately add 11 µL TIS Buffer to each sample from step 5.2.

6.2 Ensure the sample tube is sealed tightly. Mix the sample tube by vortexing at medium speed for 3 to 5 seconds to make sure the beads are fully resuspended. Centrifuge the tube for 00:00:01 and place on the rotator. Start the rotator and perform the incubation in Room temperature (20°C to 25°C) 10 minutes. 1s

6.3 After incubation, centrifuge the sample and place on the magnetic separation rack. Carefully remove and discard the supernatant once the liquid is clear. Keep tube on the magnetic separation rack and pipette 150 µL Wash Buffer II into the sample tube. Mix the beads by vortexing for 00:00:05. Centrifuge briefly and place the tube back onto the magnetic rack for 00:02:00. Carefully remove and discard the supernatant once the liquid becomes clear. Repeat this step twice. 2m 5s

7 Pre-Ligation Reaction 2

7.1 Pipette 24 µL Pre-Ligation 2 Reaction mix into the sample tube from step 6.3. Mix the sample tube by vortexing for 3-5 seconds to ensure the beads are fully resuspended. Centrifuge the tube for 00:00:01 and place on the rotator stored in the 37 °C incubator. Perform the incubation in 37 °C 00:10:00. 10m 1s

7.2 When the Pre-Ligation 2 Reaction is complete, remove the product from incubator immediately

and centrifuge briefly. Keep it at Room temperature and proceed to next step.

8 Ligation Reaction 2

8.1 Pipette all 76 µL Ligation Reaction 2 mix into the sample tube for a total volume of 100 µL. Mix the sample tube by vortexing for 00:00:10. Centrifuge the tube for 00:00:01 to make sure beads are fully resuspended. 11s

8.2 Place the tube on rotator and perform the incubation at Room temperature (20°C to 25°C) 02:00:00. 2h

8.3 After incubation, centrifuge the sample and pipette 80 µL Wash Buffer II into the sample tube. Place the sample tube on the magnetic separation rack for 00:02:00. Carefully remove and discard the supernatant when the liquid becomes clear. 2m

8.4 Keep the sample tube on the magnetic separation rack and pipette 180 µL Wash Buffer II into the sample tube. Rotate the tube 180 degrees within the magnetic separation rack to allow the beads to move through Wash Buffer II. Repeat once. Carefully remove and discard the supernatant when the solution becomes clear. Make sure Wash Buffer II is completely removed.

9 PCR

9.1 Pipette 150 µL of the PCR mix into sample tube. Use the pipet to mix the beads until fully resuspend. Transfer 75 µL of the sample to a different 0.2 mL tube.

9.2 Place all samples on the thermocycler. Make sure the beads are fully resuspended before starting.

9.3 Centrifuge the sample and place on the magnetic separation rack. Transfer all the supernatant of the two PCR tube from the same sample into a new 1.5 mL EP tube and mix together.

9.4 After confirming complete recovery of the supernatant, discard the original PCR tube.

10 PCR Product Purification

10.1 Remove the DNA Clean beads from 4 °C and equilibrate to Room temperature for 30m 10s least 00:30:00 before use. Vortex at full speed for 00:00:10 to ensure the beads are completely resuspended.

10.2 Measure the volume of the PCR product from step 9.3. Add 0.7-fold DNA Clean beads to the PCR product. Vortex the tube to mix the beads with the sample.

10.3 Incubate the sample at Room temperature for 00:10:00 . 10m

10.4 75 µL Centrifuge the tube and place on the magnetic separation rack. Wait for 2m 00:02:00 or until the solution is clear. Discard the supernatant.

10.5 Keep the sample tube on the magnetic separation rack and transfer 500 µL 80% (v/v) ethanol into the tube. Let stand for 00:00:30 . Carefully remove and discard the supernatant. Repeat this step once more. 30s

10.6 Keep the sample tube on the magnetic separation rack, open the cap of tube and air-dry the beads for 3 to 5 minutes until no wetness is observed (the surface of the beads will dim). Do not over dry the beads as this will significantly decrease the elution efficiency (cracks can be observed on pellet).

10.7 Remove the sample tube from the magnet and add 33 µL TE Buffer for elution. Vortex for 00:00:03 to resuspend the beads, then briefly centrifuge. 3s

10.8 Incubate the sample at Room temperature for 00:05:00. 5m

10.9 Place the sample tube on the magnetic separation rack and wait for 00:02:00 or until the supernatant is clear. Transfer 31 µL of supernatant from the sample tube to a new 1.5 mL EP tube. Do not disturb or pipette the beads. 2m