DNA extraction protocol for snake genomic sequencing by using AxyPrepTM Multisource Genomic DNA Miniprep Kit V.2

In 2 collections

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ABSTRACT

Genomic DNA was extracted from the muscle of a snake using the AxyPrep^{TM} Multisource Genomic DNA Miniprep Kit (Axygen, China), with some modifications from the kit instructions as outlined in this protocol. The extracted DNA was then suitable for WGS sequencing.

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Keywords: DNA extraction, AxyPrepTM Multisource Genomic DNA Miniprep Kit, nucleic acid isolation

1 The following protocol is a modified DNA extraction protocol using the AxyPrep^{TM} Multisource Genomic DNA Miniprep Kit.

Approximately 10mg of snake tissue was minced into small pieces using sterilized scissors on ice.

On ice

2 Add 350 µl Buffer PBS and 15 µl SDS Lysis Buffer and shake for 30s. 30s

00:00:30

3 Add 150 µl Buffer C-L and 20 µl Proteinase K. Immediately vortex for 1 min to mix well. After brief centrifugation, incubate at 56°C for 10 min. 11m

00:01:00

56 °C 00:10:00

4 Add 350 µl Buffer P-D, vortex for 30s to mix well, and centrifuge at 12,000×g for 10 min. 30s

00:00:30

12000 rpm, 00:10:00

5 Place the DNA preparation tube in a 2 ml centrifuge tube, transfer the mixture in step 4 to the preparation tube, and centrifuge at 12,000×g for 1 min.

12000 rpm, 00:01:00

6 Discard the filtrate, put the preparation tube back into the original 2 ml centrifuge tube, add 500µl Buffer W1, and centrifuge at 12,000×g for 1 min.

12000 rpm, 00:01:00

7 Discard the filtrate, put the preparation tube back into the original 2 ml centrifuge tube, add 700µl Buffer W2, centrifuge at 12,000×g for 1 min.

12000 rpm, 00:01:00

7.1 In the same way, cleanse again with 700µl Buffer W2.

> **Note**
>
> 1. Confirm that absolute ethanol has been added to Buffer W2 concentrate according to the volume specified on the reagent bottle.
> 2. Cleaning again with Buffer W2 can ensure that the salt is completely removed and eliminates the impact on the enzyme digestion reaction.

8 Discard the filtrate, put the preparation tube back into the original 2ml centrifuge tube, and centrifuge at 12,000×g for 1 min.

12000 rpm, 00:01:00

9 Place the DNA preparation tube in another clean 1.5 ml centrifuge tube, add 100-200µl Eluent or deionized water to the center of the preparation tube membrane, stand at room temperature for 1 min, and centrifuge at 12,000×g for 1 min to elute the DNA. 1m

00:01:00 Room temperature

12000 rpm, 00:01:00