Introduction

This protocol describes a method to extract high molecular weight genomic DNA from the green anole lizard (*Anolis carolinensis*), as an example of reptile tissue. A lizard's tail was collected (the anole lizard drops its tail when manipulated) and stored in ethanol 96-100% for one week at room temperature until extraction. The attached protocol will also work with fresh tissue. Sequencing performance was determined by MinION™. Prior to library preparation, 3 µg of extracted DNA was size selected using the size selection of HMW DNA by semi-selective DNA precipitation protocol. The Ligation Sequencing Kit was used to generate sequencing libraries from both 1 µg of the extracted DNA and 1 µg of size selected DNA.

Materials

- 100 mg lizard tail tissue
- QIAGEN Blood and Cell Culture DNA Midi kit
- RNase A
- QIAGEN Proteinase K
- 2X "size selection buffer" (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- 15 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- 100% Isopropanol
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Centrifuge and rotor with capacity for 15 ml Falcon tubes
- Incubator or water bath set to 56°C
- Tweezers and scalpel
- Vortex mixer

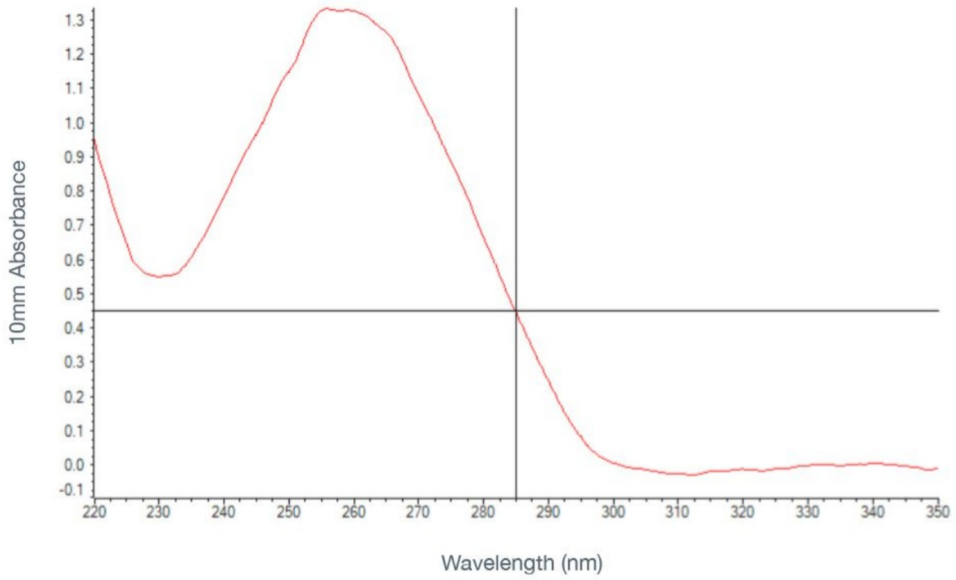
Method

1. To a 15 ml Falcon tube, add 19 µl of RNase A and 9.5 ml of G2 buffer.
2. Grind up to 100 mg lizard tail tissue using tweezers and a scalpel, and transfer the resulting pulp to the Falcon tube containing the G2 buffer. Alternatively, users may grind the tissue with liquid nitrogen, mortar and pestle, or using a sample disruption device (such as the TissueRuptor), however we have not validated these methods for this tissue.
3. Add 250 µl of Proteinase K, and vortex the 15 ml Falcon tube thoroughly. It is important that the suspension is well mixed for the lysis to be efficient.
4. Incubate the tissue suspension overnight at 56°C.
5. If after the incubation the lysate is not homogeneous, or if pieces of tissue are still visible (if the lysate is not homogeneous, it is likely that it will block the genomic-tip column), centrifuge the tube at 2000 x g for 10 minutes at 4°C. Discard the pellet and retain the homogeneous supernatant.
   Note: If after the spin step the lysate is still not homogeneous, add the same volume of QBT buffer and invert 10 times before pouring it into the column.
6. Purify the lysate according to the QIAGEN Genomic-tip protocol (steps 1–6b, pages 49–52).
7. To maximize the DNA yield we recommend that the elution is performed at 50°C for 2 hours, using 150 µl TE (1 mM EDTA, pH 8.0), occasionally mixing the tube's contents by gentle inversion.
8. **Optional Step** 3 µg of extracted DNA was size selected using the size selection using the size selection of HMW DNA by semi-selective DNA precipitation protocol.

Note:~40% of input DNA was recovered after size selection.

Results

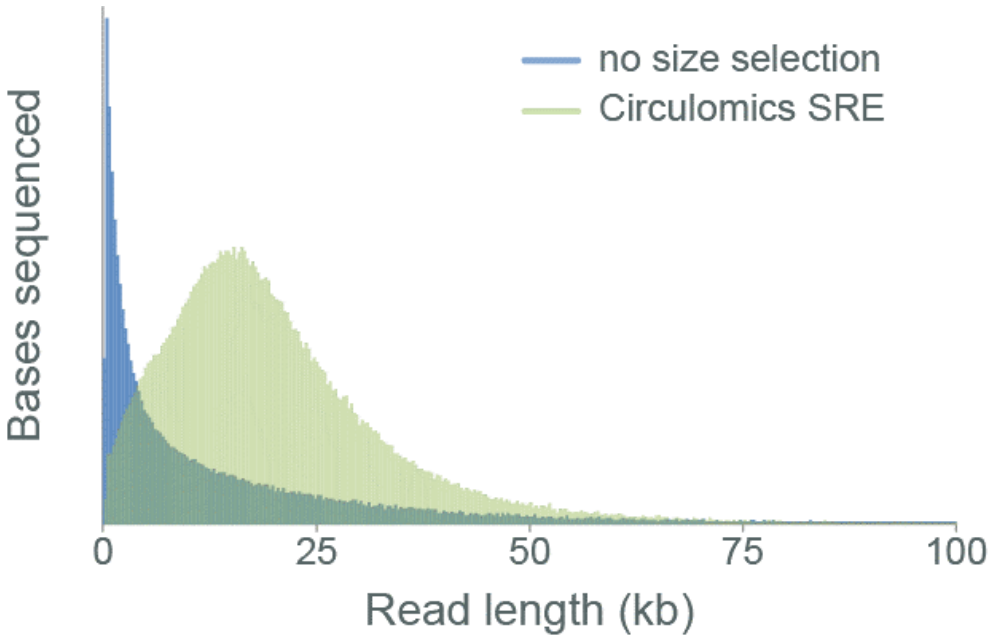
- **Yield:** 15-20 µg
- **OD 260/280:** 1.85
- **OD 260/230:** 2.18



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



Change log

Version	Change
v2, September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol.
v1, June 2019	Initial protocol publication