Materials

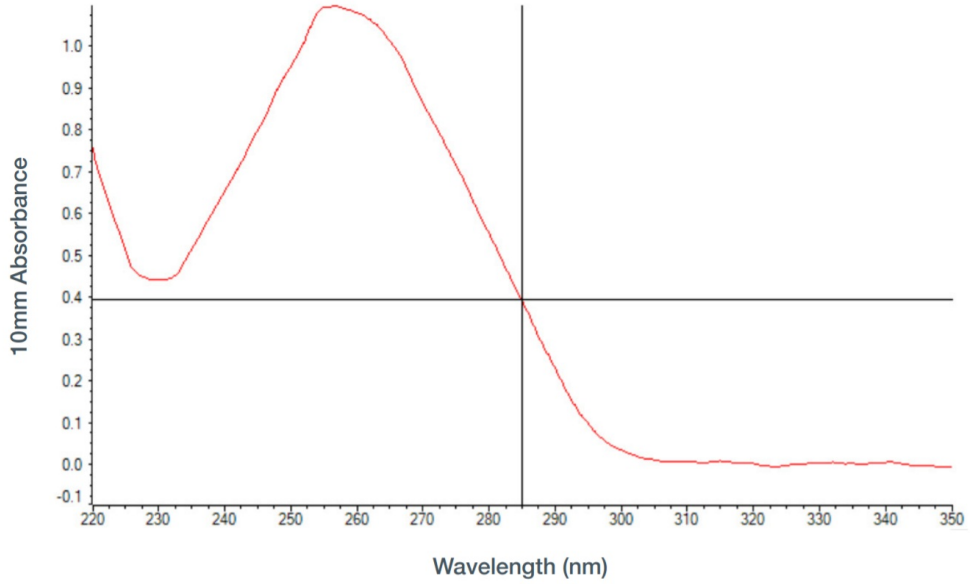
- 200 µl of chicken blood
- QIAamp DNA Blood Maxi Kit
- 1x phosphate buffered saline (PBS)
- QIAGEN Proteinase K
- TE buffer (1mM EDTA, pH 8.0)
- 96-100% ethanol
- SPRI beads (e.g. Agencourt AMPure XP beads)
- 1 M Tris-HCl
- 0.5 M EDTA pH 8
- 5 M NaCl
- 50% w/v PEG 8000
- Nuclease-free water
- 1.5 ml Eppendorf DNA LoBind tubes
- 50 ml Falcon tubes
- Centrifuge capable of taking 50 ml Falcon tubes
- Vortex mixer
- Incubator
- Magnetic rack
- Hula mixer (gentle rotator mixer)

Method

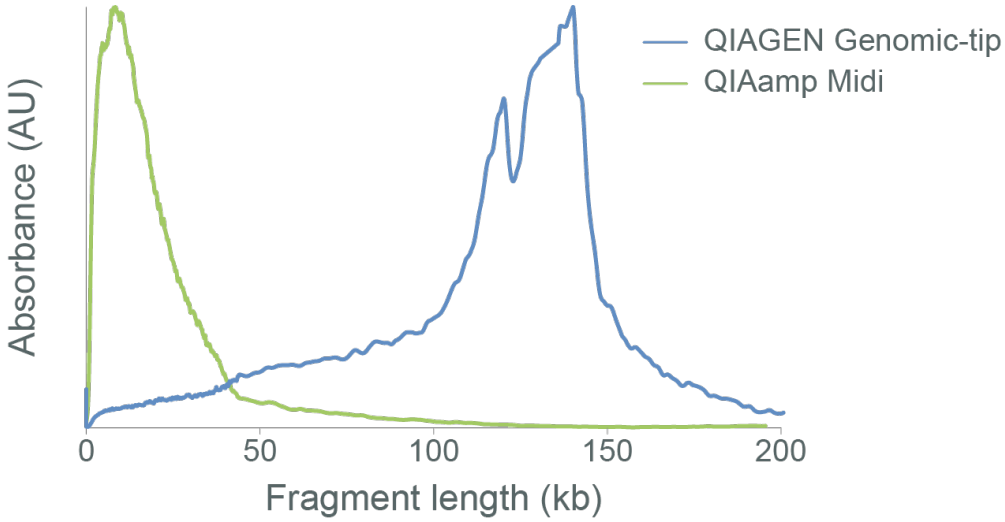
1. Transfer the chicken blood to a 50 ml Falcon tube containing 4.8 ml of 1x PBS, for a final volume of 5 ml. Pulse vortex the mixture to homogenise.
2. Add 500 µl of Proteinase K to the tube, and pulse vortex.
3. Add 12 ml of buffer AL and invert the tube multiple times, followed by a pulse vortex. The solution may not be totally homogenous at this point. If this happens, pulse vortex the sample every 15 minutes during the incubation time (next step).
4. Incubate the sample at 56° C for 2 hours, with agitation.
5. Purify the lysate according to the QIAamp handbook for volumes of blood of 5–10 ml (pages 24–25, steps 5–11).
6. **Critical Step** To maximize the DNA yield, we recommend adding 500 µl of TE buffer to the membrane followed by incubation at 37° C for 10 minutes. Centrifuge the column at 4500 rpm for 2 minutes and collect the eluted DNA. Repeat the above step once more, for a total elution volume of 1000 µl.
7. Take ~25 µl of eluate (corresponding to 3 µg of DNA) and perform a SPRI size selection.

Results

- **Yield:** 100–140 µg (before SPRI size selection)
- **OD 260/280:** 1.95 (after SPRI size selection)
- **OD 260/230:** 2.46 (after SPRI size selection)



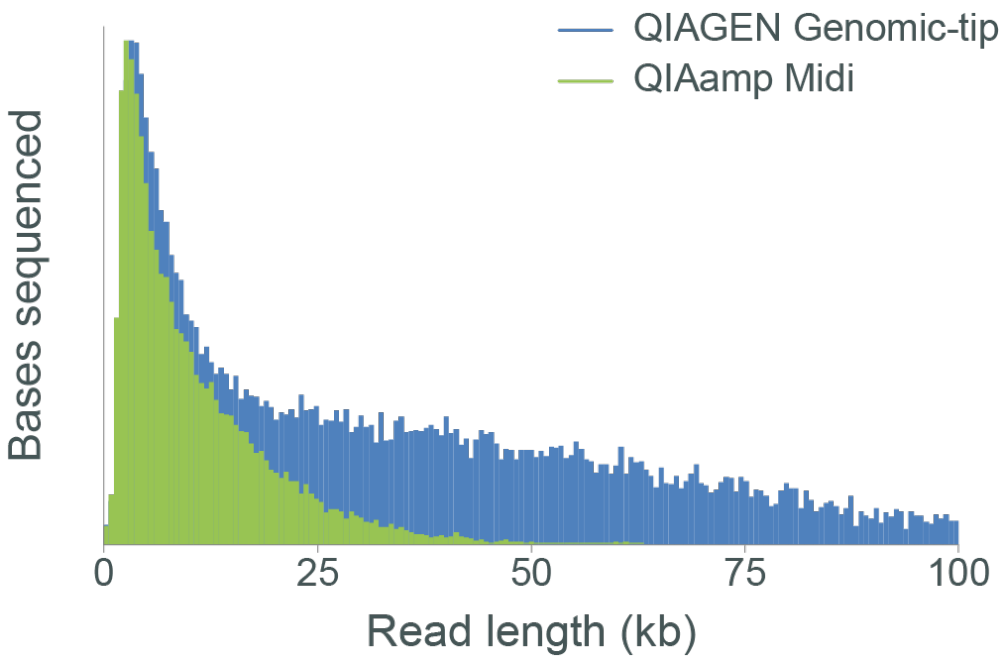
- **Fragment size** (FEMTO pulse):



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Output from the flow cell may be increased by performing a flow cell wash step (at the point where the rate of data acquisition begins to deteriorate due to the accumulation of pores in the “unavailable” or “recovering” state) and then adding a new library.
- Read length profile:



Change log

Version	Change
v1, 11th February 2019	Initial protocol publication