Materials

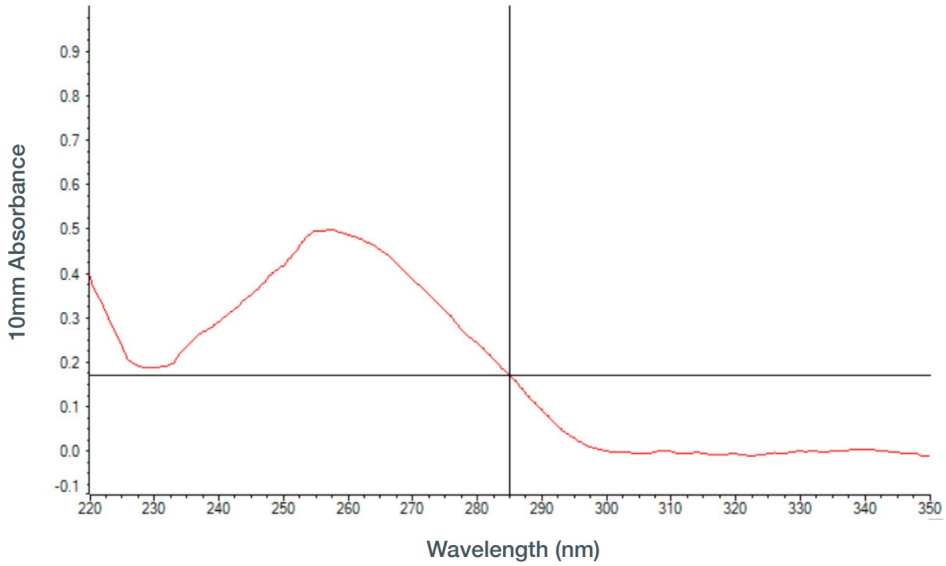
- 7.5 µl of chicken blood
- QIAGEN Genomic DNA Buffer Set
- QIAGEN Genomic-tip 100/G
- QIAGEN Proteinase K
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 70% ethanol in nuclease-free water
- Isopropanol
- SPRI beads (e.g. Agencourt AMPure XP beads)
- 1 M Tris-HCl
- 0.5 M EDTA pH 8.0
- 5 M NaCl
- 50% w/v PEG 8000
- Nuclease-free water
- 1.5 ml Eppendorf DNA LoBind tubes
- 15 ml Falcon tubes
- Centrifuge capable of taking 15 ml Falcon tubes
- Vortex mixer
- Incubator
- Magnetic rack
- Hula mixer (gentle rotator mixer)

Method

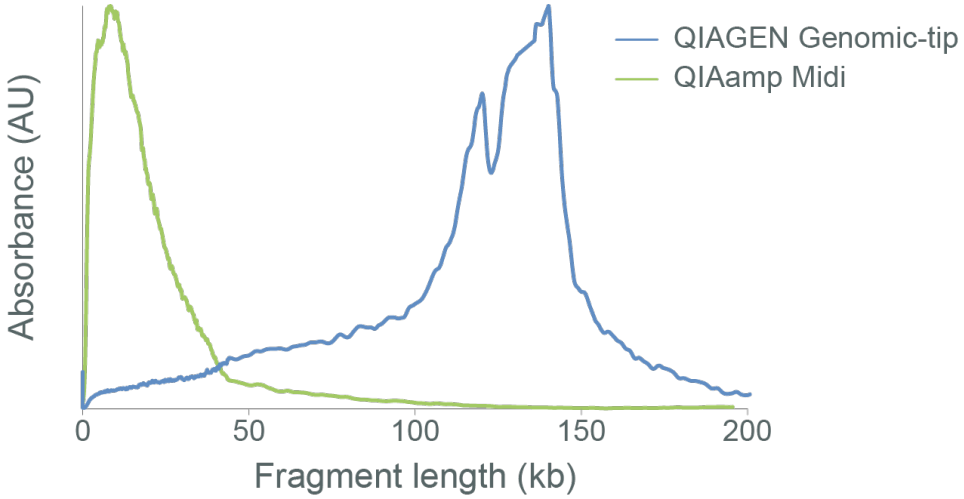
1. Transfer the chicken blood to a 15 ml Falcon tube containing 190 µl of 1x PBS. Pulse vortex the mixture to homogenise.
2. Add 5 ml of G2 buffer and 95 µl of Proteinase K to the tube, and pulse vortex. The solution may not be totally homogenous at this point. If this happens, pulse vortex the sample every 15 minutes during the incubation time (next step).
3. Incubate the sample at 56°C for 2 hours, with agitation.
4. **Critical Step** Flick the tube (instead of vortexing) before loading the sample into the Genomic-tip. If the lysate has a viscous consistency, add 5 ml of QBT to the lysate and mix by flicking the tube. Keep the lysate in the incubator until the Genomic-tip is equilibrated with QBT and ready to be loaded with
   the lysate.
5. Purify the lysate according to the standard protocol (pages 50–52, steps 3–6).
6. **Critical Step** To avoid DNA fragmentation, we recommend spooling the DNA (e.g. using one arm of disposable tweezers) instead of centrifugation.
7. To maximize the DNA yield, we recommend eluting the DNA overnight in 500 µl TE (10 mM Tris HCl, 1 mM EDTA, pH 8.0).
8. Take ~150 µl of eluate (corresponding to 3 µg of DNA) and perform a SPRI size selection.

Results

- **Yield:** 8-13 µg (before SPRI size selection)
- **OD 260/280:** 2.01 (after SPRI size selection)
- **OD 260/230:** 2.62 (after SPRI size selection)



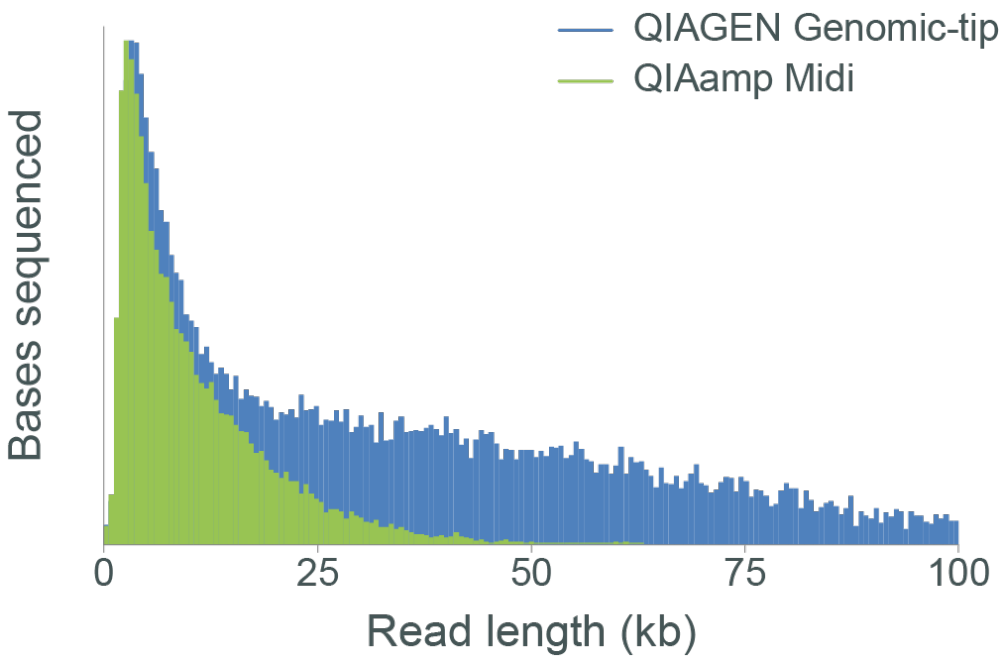
- **Fragment size** (FEMTO pulse):



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