Materials

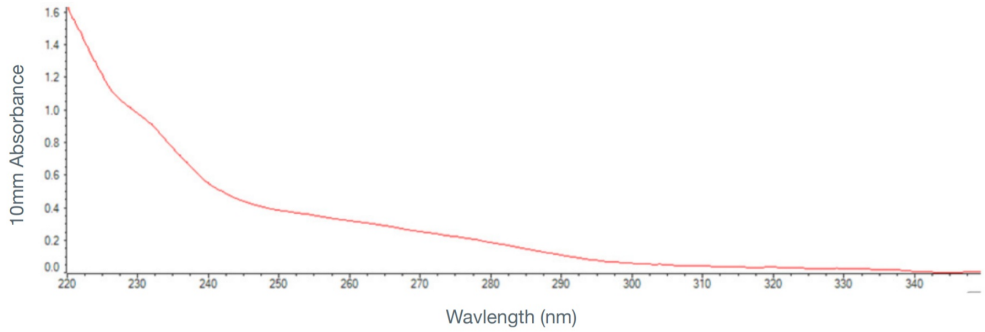
- *Oryzias sarasinorum* brain
- QIAGEN Blood and Cell Culture DNA Midi Kit
- 50 ml Falcon tubes
- Liquid nitrogen
- RNase A
- 70% ethanol
- Isopropanol
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Agencourt AMPure XP Beads (optional)
- Mortar and pestle
- Incubator or water bath at 50°C
- Centrifuge with capacity for 50 ml Falcon tubes

Method

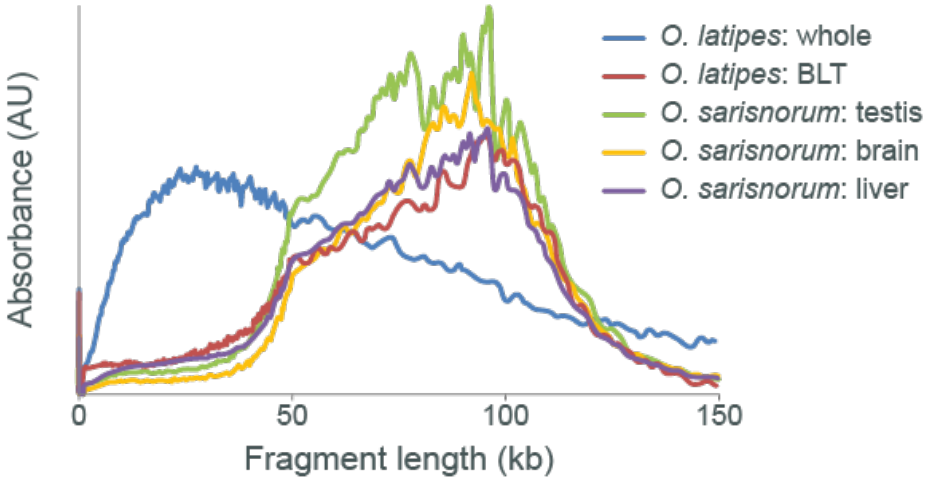
1. Snap freeze the sample in liquid nitrogen.
2. Grind the frozen sample using a pre-cooled (e.g. stored in the freezer for 30 min) mortar and pestle.
3. Lyse up to 100 mg of the ground, frozen sample according to thestandard protocol (steps 3B, 4B, and 5, pages 35-36).
4. Purify the lysate (steps 1 to 6, pages 49-52).
5. **Critical Step:** To maximize the DNA yield, we recommend eluting the DNA overnight in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Results

- **Yield:** 13.7 µg
- **OD 260/280:** 1.76
- **OD 260/230:** 0.30



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



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile: