10x Genomics - DNA Extraction from Single Insects (gDNA)

Summarized from 10x Genomics' protocol for retrieving high molecular weight DNA from whole insects, Rev A.

Materials

Supplier	Description	Amt./Sample	Part #
Teknova	1M Tris-HCl, pH 8.0	Buffers	T5088
Qiagen	Proteinase K, 20mg/ml	Buffers	19133
Sigma-Aldrich	Sodium Chloride Solution, 5M	250 µl	S6546
	Ethanol, Pure	1.2 ml	459836
	Pellet Paint Co-Precipitant	1 µl	69049
ThermoFisher	0.5M EDTA pH 8.0	Buffers	AM9260G
	10% SDS	Buffers + 40 µl	15553-027
	TE Buffer	35 µl	12090-015
	Fisherbrand RNase-Free Disposable Pellet Pestles	1	12-141-364
Eppendorf	DNA LoBind Tubes, 2.0ml (**note** - rxn will not fit in 1.5ml tube and cannot be substituted)	2	022431048
Misc.	Wide-bore pipette tips - 1000, 100 µl	1 each size	

Buffers

- Lysis Buffer - 600 µl per sample
  - 10 mM Tris-HCl
  - 400 mM NaCl
  - 100 mM EDTA, ph 8.0
- Proteinase K Solution - 100 µl per sample
  - 1 mg/ml Proteinase K
  - 1% SDS
  - 4 mM EDTA, pH 8.0

1) Homogenization & Overnight Lysis

1. Mix **600 µl Lysis Buffer**, **40 µl 10% SDS**, and 100 µl Protienase K Solution in a **2 ml Eppendorf tube**.
2. Fully **immerse sample** in solution.
3. **Grind the sample** with tube pestle using rolling motion against walls of tube.
4. **Vortex** for **5 seconds**, **centrifuge briefly**.
5. **Digest** the homogenized sample **overnight (12 - 18 h)** at **37°C** in **water bath** or ThermoMixer.

Do not store sample, proceed immediately with next step.

2) DNA Purification

~30-60min before starting: Cool temperature controlled centrifuge to 4°C

1. Add **250 µl 5M NaCl** to the tube containing the homogenized sample. Invert **5** times.
2. Centrifuge at **4°C** at **1100 x g** for **15 min**.
3. Using **wide-bore** pipette tip, transfer supernatant containing the DNA to a new **2 ml LoBind tube** and add **1.2 ml 100% ethanol**.
4. Add **1 µl Pellet Paint Co-Precipitant**.
5. Gently rock the tube and look for DNA strands.
6. Centrifuge at **4°C** at **6250 x g** for **5 min**.
7. Carefully **remove the supernatant**, retaining the DNA pellet in the tube.
8. Allow the DNA pellet to **air dry** for **5 min**
9. Using a **wide-bore** pipette tip, add **35 µl TE Buffer** and **resuspend** the DNA pellet with **gentle** pipette mixing.
10. Allow the solution to **homogenize** at **room temperature** for **1 h**.
11. Store the extracted gDNA at **4°C** for up to **2 weeks** or at **-20°C** for up to **6 months**.

Summarized by ZJ Nolen - 2020-09-10