Qiagen Genomic Tip 20/G - Insect Pupae gDNA Extraction

Last revised 30 October 2020

Summarized from QIAGEN Genomic DNA Handbook (June 2015) to match methods used in our lab. According to manufacturer allows for the extraction of up to 20 µg gDNA between 20 to 150 kb in length from no more than 20 mg of tissue. This protocol version maintained by Zachary Nolen.

Sample preparation and lysis of tissue

Samples should be fresh or stored at -80°C.

1. Add **4 µl** of **RNase A** to **2 ml** of **Buffer G2** in a **>2.0 ml tube** for each sample to be prepared.
2. **Homogenize up to 20 mg pupal tissue** in the **Buffer G2 + RNase A** solution using a pestle.
3. Add **0.1 ml QIAGEN Proteinase K** solution. **Vortex**.
4. **Incubate** at **50°C** for **2 h** (or until lysate is clear, only pupal casing should remain).
5. After incubation, **remove pupal casing** with a **clean pipette tip** and discard. Solid material will clog the genomic tip.
6. Begin pre-cooling chilled centrifuge to 4°C for next section.

Genomic-tip protocol

1. Equilibrate **Genomic-tip 20/G** with **1ml** of **Buffer QBT**, allowing the tip to empty by gravity flow.
2. **Vortex** sample for at least **10 s** at **maximum speed** and **apply** to the equilibrated **Genomic-tip**. Allow it to enter the resin by gravity flow.
3. **Wash** the Genomic-tip with **1 ml Buffer QC**. **Repeat** wash **twice** more.
4. **Elute** the DNA with **1 ml** of **Buffer QF** that has been **prewarmed to 50°C** over a **clean 2.0 ml LoBind Tube**. Label as first elution.
5. **Elute** once more with **1 ml** of **prewarmed (50°C) Buffer QF** over a **clean 2.0 ml LoBind Tube**. Label as second elution.
6. **Precipitate** the DNA by adding **0.7 ml** (0.7 volumes) room-temperature **isopropanol** to each elution.
7. **Mix** and **centrifuge** at **20,000 x g** for at least **15 min** at **4°C** (>5000 x g called for in handbook, with higher speeds increasing pelleting efficiency). Carefully **remove the supernatant**.
8. **Wash** the centrifuged DNA pellet with **1 ml** of **cold 70% ethanol**.
9. **Vortex** briefly and **centrifuge** at **20,000 x g** for **10 min** at **4°C** (again, handbook calls for >5000 x g). Carefully **remove the supernatant**.
10. **Air-dry** for **5-10 min**, and **resuspend** the DNA in **0.2 ml** of **TE buffer, pH 8.0** (anywhere from 0.1 - 2 ml recommended by handbook depending on concentration and volume needs).
11. **Dissolve** the DNA overnight on a shaker or at **55°C** for **1-2 h**.

Required Materials and Reagents

QIAGEN Genomic-tip 20/G Kit (10223):

- Genomic tip 20/G

QIAGEN Genomic DNA Buffer Set (19060):

- Buffer QBT
- Buffer QC
- Buffer QF

User Supplied:

- Eppendorf LoBind Tubes (2.0 ml)
- RNase A (100 mg/ml)
- Proteinase K solution (20 mg/ml)
- Cold, 70% Ethanol
- Isopropanol
- TE Buffer