

## User-Developed Protocol:

### Isolation of genomic DNA from mosquitoes or other insects using the QIAGEN® Genomic-tip

This procedure has been adapted by customers from the *QIAGEN® Genomic DNA Handbook*, and is for use with QIAGEN Blood and Cell Culture DNA Kits. **It has not been thoroughly tested and optimized by QIAGEN.**

The lysis time will vary depending on the size and density of the source material.

Please be sure to read the *QIAGEN Genomic DNA Handbook* and the detailed Genomic-tip Protocol carefully before beginning the procedure.

## Procedure

1. **Estimate the approximate DNA content per unit weight (e.g., mg or g) of tissue. Which particular QIAGEN-tip is used will depend on the amount of DNA required (Genomic-tip 20/G: up to 20 µg; Genomic-tip 100/G: up to 100 µg; Genomic-tip 500/G: up to 500 µg). Freeze the appropriate amount of tissue in liquid nitrogen and pulverize.**
2. **Suspend powder in lysis buffer (Genomic-tip 20/G: 2 ml; Genomic-tip 100/G: 15 ml; Genomic-tip 500/G: 40 ml) containing:**
  - 20 mM EDTA
  - 100 mM NaCl
  - 1 % Triton® X-100
  - 500 mM Guanidine-HCl
  - 10 mM Tris, pH 7.9.
3. **Supplement with DNase-free RNase A (20 µg/ml) and incubate for 30 min at 37°C.**
4. **Add Proteinase K to 0.8 mg/ml and incubate for 2 h at 50°C with gentle agitation.**
5. **Centrifuge for 20 min at 12–15,000 x g to pellet insoluble debris.**
6. **Transfer clarified lysate to the appropriate Buffer QBT-equilibrated QIAGEN Genomic-tip (Genomic-tip 20/G: 1 ml; Genomic-tip 100/G: 3 ml; Genomic-tip 500/G: 10 ml).**
7. **Wash with Buffer QC (Genomic-tip 20/G: 4 x 1 ml; Genomic-tip 100/G: 2 x 10 ml; Genomic-tip 500/G: 2 x 30 ml).**
8. **Elute with Buffer QF (Genomic-tip 20/G: 0.8 ml; Genomic-tip 100/G: 5 ml; Genomic-tip 500/G: 15 ml).**
9. **Add 0.7 volumes of room temperature isopropanol to precipitate the DNA.**
10. **Centrifuge for 20 min at 15,000 x g to pellet DNA, or spool out DNA on a glass rod.**
11. **Wash with 70% ethanol.**
12. **Air-dry and resuspend in TE.**

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.

Selected handbooks can be downloaded from [www.qiagen.com/literature/handbooks/default.asp](http://www.qiagen.com/literature/handbooks/default.asp).

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from [www.qiagen.com/ts/msds.asp](http://www.qiagen.com/ts/msds.asp).

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