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Appendix S2. Protocol for DNA extraction, PCR amplification, and gel electrophoresis

Developed by A. Avanesyan

1. Extract DNA from plants and grasshopper guts following the guidelines in QIAGEN DNeasy Plant Mini Kit (QIAGEN, Culver City, California, USA). Use the components of this kit for all extraction steps.

2. Prepare a primer mix (2 μM working solution) for each primer pair. For the total volume of 50 μL the components are as follows:

Component	Quantity
Primer 1 forward (stock solution, 200μM)	0.5 μL
Primer 2 reverse (stock solution, 200μM)	0.5 μL
Sterile water	49 μL
Total volume	50 μL

3. Prepare the number of PCR reactions which are needed. Each reaction (of 10 μL volume) consists of the following:

Component	Quantity
QIAGEN Master Mix (QIAGEN)	5 μL
Primer mix (working solution, see above)	1 μL
Sterile water	3.8 μL
DNA template	0.2 μL

		Total volume	10 μL

4. Run PCR in a thermocycler under the following conditions:

Step		Temperature	Time
Initial denaturation		95°C	15 min
35 cycles	Denaturation	95°C	15s
	Annealing	57 ° C	90 s
	Extension	72 ° C	60 s
Final extension		60 ° C	30 min

5. Prepare 1% agarose gel with ethidium bromide:

Component	Quantity
Agarose	1.5 g
1× TAE buffer (make it from 50× TAE stock)	150 ml
Ethidium bromide	3.6 μL

- Mix agarose and TAE buffer in a flask.
- Heat the mixture in microwave for 2 min until agarose is completely dissolved. Mix well every 15-20 s.
- Cool down the mixture until you can hold the flask with your hands.
- Add ethidium bromide. Mix well. Pour the solution in the gel tray.
- Insert combs to make wells. Let the gel cool down (approximately 20 min).

6. Load the gel and run an electrophoresis:

- Place the gel tray in an electrophoresis chamber.

- Add a TAE buffer to the gel chamber. Make sure the buffer completely covers the gel.
- Mix 1 μL of a loading dye and 3 μL of a PCR product on a parafilm, mix it well by pipetting, and load it in the gel.
- Load 6 μL of a DNA ladder.
- Run gel 45 min at 100A.
- Visualize gel *under* a UV *transilluminator*.

Chemicals check list:

QIAGEN DNeasy Plant Mini Kit (Cat. No. 69104, QIAGEN)

QIAGEN Multiplex PCR Master Mix, 2× (Mat. No. 1022829, Lot No. 133211862, QIAGEN)

Agarose (Cat. No. V3125, PROMEGA)

1 kb DNA ladder (Cat. No. G5711, PROMEGA)

Blue/Orange Loading Dye, 6× (Cat. No. G190A, PROMEGA)

Ethidium bromide

Primers (Integrated DNA Technology)

TAE buffer (ThermoScientific, 50X TAE Buffer [Tris-acetate-EDTA])