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Protocol: Routine Authentication of Drosophila using RFLP Markers

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Lauren McCann[1], Natalia Rivera Rincón[1], Emma Saurette[1], Melika Ghasemi Shiran[1], Laurie Stevison[1]

[1]Auburn University

Drosophila Stock Validat...

Melika Ghasemi Shiran

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We use this protocol and it's working

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Abstract

Stock validation is an important factor in scientific research; by authenticating the resources used there is an increase of the quality of research to be performed. This protocol was designed to validate ten stocks of *D. melanogaster* and four stocks of *D. pseudoobscura* in the DGRP. Designed for *DMEL_42, DMEL_57, DMEL_217, DMEL_357, DMEL_391, DMEL_399, DMEL_437, DMEL_491, DMEL_508,* and *DMEL_810_* of *D. melanogaster; in addition to MV2-25, TreeLine, Flagstaff14,* and *Flagstaff16* of *D. pseudoobscura.* Following sample collection, DNA Extraction, PCR Amplification, EcoR1 Enzyme, and Gel Electrophoresis you will be able to confirm the identities of your samples in comparison to the expected results.

Materials

Step 1: Sample Collection

- Fly stocks of interest
- CO_2 to anesthetize flies
- Standard Dissecting Microscope
- -20°C freezer
- 96-well plate with sealer or PCR strip tubes with caps
- Labels

Step 2: DNA Extraction (Gloor and Engels 1992)

- Squish Buffer (SB)
  - 100 µL 1M Tris-HCl pH 8.2
  - 20 µL 0.5M EDTA
  - 50 µL 5M NaCl
  - 9.9 mL dH_2O
  - Stored at -20°C
- 20 mg/mL proteinase-K solution
- Thermocycler

Step 3: PCR Amplification

- DNA template to be added to master mix
- Master mix
  - Promega GoTaq™ G2 Green Master Mix (Promega M7822)
  - Upstream primer (10µM)
  - Downstream primer (10µM)
  - Nuclease-free water
- New 96-well plate with new sealer and labels
- Thermocycler

Step 4: ECOR1 Enzyme

- Nuclease-free water
- 10X NEBuffer EcoR1/SspI
- DNA sample from the previous step
- EcoR1 enzyme
- Purple loading dye
- New 96-well plate with new sealer and labels
- Thermocycler

Step 5: Gel Electrophoresis

- Agarose gel
- 1x TAE Buffer
- EtBr (Ethidium Bromide)

- Microwave
- Erlenmeyer flask
- Gel Electrophoresis system
- Well combs
- DNA Ladder

Step 6: Azure Pictures

- Azure Imaging Systems (AZI200-01 - AZI600-01)

SAMPLE COLLECTION

1 Prepare and label your tubes for stocks of interest, collecting 2 females of each stock.

42	57	217	357	391	399	437	491	508	810
42	57	217	357	391	399	437	491	508	810

MV	MV	TL	TL	FS14	FS14	FS16	FS16

KEY
DMEL_42 (42), *DMEL*_57 (57), *DMEL_217* (217), *DMEL*_357 (357), *DMEL_391* (391), *DMEL*_399 (399), *DMEL_437* (437), *DMEL*_491 (491), *DMEL_508* (508), *DMEL*_810 (810), *MV2-25* (MV), *TreeLine* (TL), *Flagstaff14* (FS14), *Flagstaff16* (FS16)

2 Anesthetise flies with CO_2, once all flies are anesthetised, continue to Step 3.

3 Pour flies onto CO_2 table of your microscope, then sort out 2 females and place into pre-labeled tubes (see step 1 chart). Then move onto next variety, making sure to not mix up any stocks.
- 2 female samples of each stock variant
- 1 fly per tube

4 Place flies in -20°C freezer one day prior to following DNA extraction step

DNA EXTRACTION

5 Create a master mix by combining Squishing Buffer with Proteinase-K Solution for the following **50µL** reaction, amplifying by 1 plus the number of samples
- **49.5 µL Squishing Buffer**
- **0.5 µL Proteinase-K Solution**

- This solution must be mixed fresh

6 Briefly vortex your master mix until homogeneous

7 Draw up 50 µL master mix solution and begin to stab one fly repeatedly with the tip of the pipette, slowly release the master mix solution to moisten the fly so it will stick to the tube.

8 After the fly is mashed (~20 seconds and the liquid has turned red) eject any remaining master mix liquid into the tube over the carcass. Replace pipette tip and repeat for the rest of the samples.
*Keep on ice until all preps are done

9 When finished with all samples, run the following thermocycler protocol as follows:
- 37°C for 30 minutes
- 95°C for 2 minutes
- Volume 50 µL

10 When thermocycler is finished, samples can be placed in -20°C freezer or continue to next stage.
- Preps are often good for a year (or longer) if amplifying small DNA fragments, though repeated freeze/ thaw will shorten its lifespan.

PCR AMPLIFICATION

11 Select Primers
DPSE → 2 markers
DMEL → 4 markers

12 2 Samples Per Stock
1 DPSE sample → will be allocated into 2 seperate wells with appropriate primers
1 DMEL sample → will be allocated into 4 seperate wells with appropriate primers

1-42	1-57	1-217	1-357	1-391	1-399	1-437	1-491	1-508	1-810	1MV	2MV
1-42	1-57	1-217	1-357	1-391	1-399	1-437	1-491	1-508	1-810	1MV	2MV
2-42	2-57	2-217	2-357	2-391	2-399	2-437	2-491	2-508	2-810	1TL	2TL
2-42	2-57	2-217	2-357	2-391	2-399	2-437	2-491	2-508	2-810	1TL	2TL
3-42	3-57	3-217	3-357	3-391	3-399	3-437	3-491	3-508	3-810	1FS14	2FS14
3-42	3-57	3-217	3-357	3-391	3-399	3-437	3-491	3-508	3-810	1FS14	2FS14
4-42	4-57	4-217	4-357	4-391	4-399	4-437	4-491	4-508	4-810	1FS16	2FS16
4-42	4-57	4-217	4-357	4-391	4-399	4-437	4-491	4-508	4-810	1FS16	2FS16

PSE-1 NC	PSE-2 NC	MEL-1 NC	MEL-2 NC	MEL-3 NC	MEL-4 NC

1- 96 well plate for samples. Colors represent the different primers assigned to each sample

13 Prepare master mixes for each marker according to the following quantities for a single **12.75 µL** reaction based on 1 plus the number of samples to provide a negative control.

6.5 µL Promega GoTaqGreen
0.5 µL Upstream primer (10µM)
0.5 µL Downstream primer (10µM)
5.25 µL Nuclease-free water

14 Pipette **11.75 µL** of the master mix into wells as assigned (see Diagram in Step 12)

15 Add **1 µL** of DNA sample into appropriate wells, again following the diagram in Step 12.
- A multi-pipette can be used to transfer DNA samples

16 Seal well plate and run the thermocycler protocol for touchdown PCR as follows:
- 2 min. 95 °C
- 10 Cycles of:
  - 30 sec. 95 °C
  - 30 sec. 65 °C (decreasing by 1°C each cycle)
  - 30 sec. 72 °C
- 20 Cycles of:
  - 30 sec 95°C
  - 30 sec 55°C
  - 30 sec 72°C
- 2 min. 72°C
- Hold 4 °C

17 After the program is done, the samples can be stored in -20°C freezer, or continue to next stage.

ECOR1 ENZYME PROCEDURE

18 In a new 96 well plate, add the following into each well **IN ORDER AS LISTED ENSURING TO ADD THE ENZYME LAST**
- **3.4µL** Nuclease-free water
- **1µL** 10X NEBuffer EcoR1/SspI
- **5µL** DNA sample from the previous step
- **0.5µL** EcoR1 enzyme

19 Seal well plate and run thermocycler program for ECOR1 as follows:
- 60 min 37°C
- Volume 10 µL

20 After the program is done, add **2µL** of purple loading dye into each well to stop the reaction.

21 Once dye is added, the samples can be stored in the -20°C freezer, or continue to next stage.

GEL ELECTROPHORESIS

22 Making the gel
- Measure **2g** of agarose and add to an Erlenmeyer flask
- Add **100mL** of 1x TAE buffer into the flask and swirl
- Microwave this solution in 30 second increments and swirl until color is clear and all powder is dissolved
- Let your gel mixture cool before adding Etidium Bromide, you should be able to touch the flask and feel warmth without burning
- Add **5µL** of EtBr to flask and swirl
- Ready to pour into gel tray

23 Pour prepared gel into the gel tray, with the well comb in place, and let gel solidify

24 Once gel is solidified: Remove the tray and leave the gel in the gel box (electrophoresis unit), with the wells on the black side
- DNA will run to the red side

25 Add 1x TAE buffer to cover the gel in the gel box
- Buffer must correspond to the buffer in the gel or it will not work

26 Add **5µL** of the ladder into the first well and Pipette **5µL** of each DNA sample into corresponding wells
- When dispensing, make sure the pipette tip does not stab the gel and that all the sample goes into the well. Keep track of the order in which the samples are placed so they can be identified once the gel has run.

27 Place cover on and attach power source
- DNA sample will run toward the positively charged elecctrode (red) since DNA is negatively charged
Set voltage and time accordingly (100v for 30 min)

28 Ensure everything is running smoothly, small bubbles will appear
Once gel is run, take photos as needed and analyze results

AZURE PICTURES

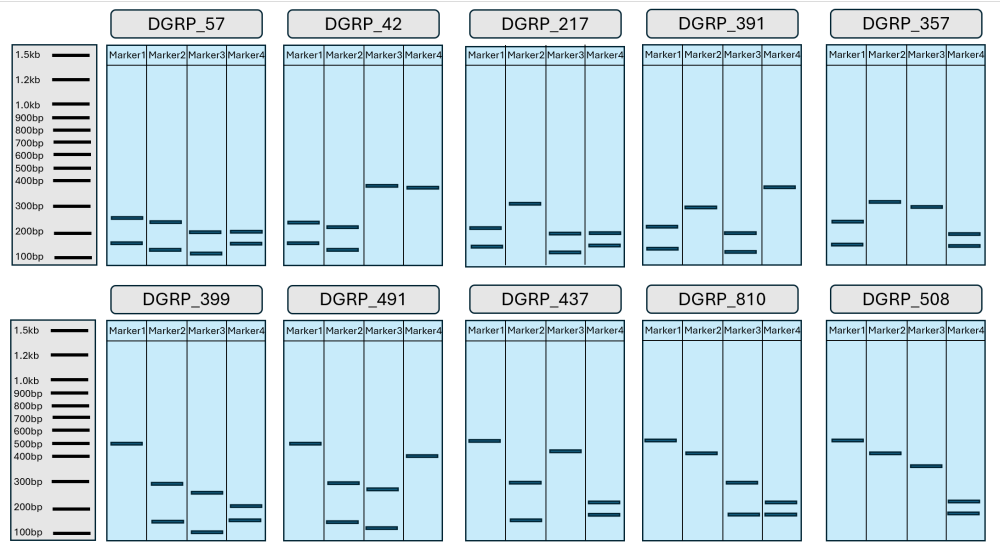
29 Turn on the machine, place the gel into the tray and close the door.
Select Nucleic Acid dye: Ethidium bromide

30 Take the picture, edit as needed to view the bands

31 Discard gel and clean tray

ANALYSIS

32 Compare your gel results to the guide below, ensuring your stamples performed as anticipated.



Protocol references

Gloor GB, Engels WR (1992) Single-fly DNA preps for PCR. Drosoph. Inf. Serv. 71:148-149

GoTaq® Green Master Mix (M712) Protocol

R.H. Don, P. T. Cox, B.J. Wainwright, K. Baker, J. S. Mattick, 'Touchdown' PCR to circumvent spurious priming during gene amplification, *Nucleic Acids Research*, Volume 19, Issue 14, 25 July 1991, Page 4008, https://doi.org/10.1093/nar/19.14.4008