

5 Fly *Drosophila* Genomic Prep for iPCR in 96-well Format

This protocol is an adaptation of

"Single-Fly DNA Preps for PCR" by Greg Gloor and William Engels

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I. Reagents

This protocol optimized with the following reagents:

<u>Reagent</u>	<u>Manufacturer</u>	<u>Catalog</u>
96-well flat bottom plate (chimney style)	Greiner	655080
96-deep well (2.4 ml) plate	E&K Scientific	EK-22270
Aluminum Sealing Film	Axygen	47734-816
Tape Pad	Qiagen	19570
Multiple Homogenizer (96 Pin)	Burkard Scientific	BA/MH/96
96-well (0.3 ml) U-bottom plate	Nalge Nunc International	268152

multichannel pipet, vortex, 65°C incubator, 37°C incubator, centrifuge, speed-vac

3 M Potassium Acetate, isopropanol, 70% ethanol, TE Buffer

Buffer A

100 mM TrisHCL pH 7.6

100 mM EDTA

100 mM NaCl

0.5% SDS

II. Notes

Use of chimney style flat bottom 96-well plates allows the spaces between the wells to be filled with water and frozen. This is not essential, however it helps to keep the wells cold while pootling flies, and thus the flies stay put.

Wash Multiple Homogenizer after use to remove any fly tissue. Soak Homogenizer in 1:10 Clorox Bleach:H₂O for 10 minutes. Rinse with water. Rinse with 70% ethanol. Dry on paper toweling with pins down.

III. 5 Fly Genomic Prep

- 1) Seal the top of a Greiner 96-well flat bottom plate with aluminum film. Fill the underside of the plate with water and freeze at -80°C for 20 min (or 1 hour at -20°C)
- 2) Place frozen plate on ice (a pipet box lid works well) for duration of pootling flies.
- 3) Knockout flies with CO₂ and place 5 flies per well.
- 4) Add 100 μl Buffer A to each well. Smash flies with Burkard Scientific Multiple Homogenizer (do not splash and cross-contaminate wells).
- 5) Cover with Tape Pad and incubate at 65°C for 15 min.
- 6) Quick spin to pull down any condensation.
- 7) Add 100 μl 3M Potassium Acetate. Cover with Tape Pad. Gently mix on a vortex and place on ice for 10 min.
- 8) Spin covered plate 20 min @ 2,470 RCF
- 9) Transfer 150 μl supernatant with a multichannel pipet and aerosol tips to a 96-deep well plate
- 10) Add 90 μl isopropanol. Cover with Tape Pad. Gently mix on vortex and incubate at room temp for 5 min.
- 11) Spin covered plate 30 min @ 4,200 RCF.
- 12) Pour out supernatant by quickly inverting the plate over a sink.
- 13) Add 200 μl cold 70% ethanol and cover with a Tape Pad
- 14) Spin covered plate 10 min @ 4,200 RCF.

- 15) Pour out supernatant and invert briefly on a paper towel.
- 16) Speed vac to dry plates on medium heat about 10-15 min. Confirm by eye that all ethanol is evaporated.
- 17) Add 100 μ l TE buffer. Gently mix on vortex. Place at 37°C for 10 min. to help resuspend pellet. Vortex again and quick spin to pull down liquid.
- 18) Transfer 100 μ l with aerosol barrier tips to a 96-well Nunc plate. Seal with aluminum film for storage at -80°C.