Germline transformation of Tribolium

By Andreas Berghammer & Martin Klingler, 11/2001
with input for embryo handling from Sue Brown & Dick Beeman

Please cite Berghammer et al. 1999 for this protocol

Prepare:

let agar plates warm up to room temperature
Agar plates: mix 30 g agar (Roth 5210.2) and 1 liter tap water in a 2 liter beaker, autoclave; dissolve 1 g Nipagin in 4 ml ETOH, add to Agar shortly before pouring the plates

pull capillaries: for example World Precision Instruments "Pul-1",
settings: Delay=1, Heat=7

prepare DNA:
concentration should be 500 ng/ul vector plasmid, 350 ng/ul helper plasmid, 25% phenole red stock solution (Sigma, 0.5% in D-PBS)
To remove dirt that could clog the injection capillary tip: centrifuge injection mix through a filter (i.e. amicon Ultrafree-MC 0.45 micrometer filter unit)

store DNA at -20C

Tools:

simple microscope with 2.5x and 10x objectives
vibration free table
simple 3D micromanipulator
capillary holder attached to 20 ml syringe, helt in place by micromanipulator
2x1cm piece of 150 um polyamid gauze ("Monodur")
fine brush for lining-up embryos
bleech ("Klorox" diluted 1:10)
humid chambers (with wet paper towels; allow for some air exchange)
egg basket, petridish
large apple-juice agar plate (warmed to RT; 30g agar/litre)

Filling the capillary

break capillary tip (capillary with or without filament) by squeezing under a binocular with razor blade (as fine as you can, make the tip look like medical syringes)

Fill 3-4 µl of DNA mix into capillary: from behind with a capillary-filling filament-tip, or from front (place 3 ul DNA into yellow tip, align it with paraplast, slide on microscope)

put capillary into the injection apparatus and keep tip immersed in voltalef oil to prevent drying / clogging of DNA while you line up the embryos

Lining up the embryos

All manipulations of living embryos should be at temperatures above 23°C!

Collect Tribolium eggs for 3 hour at 24°C (or 1,5h at 32°C) from about 10 g of white-eyed beetles (p=pearl mutant), in fine white flour. Dechorionate eggs in 5% bleach for 2min. Swirl embryos to get them dechorionized/cleaned homogenously. Do not over-dechorionate;

wash well but not too rigidly with deion. water (avoid mechanical stress or strong temperature shifts for the eggs)

transfer devitellinized eggs with brush to a nylon mesh mounted on a slide and kept humid throughout

Use fine brush to line-up embryos directly on another microscope slide (residual chorion/flour will affix the eggs onto the slide when dry)

Injection

Place slide with embryos on a stage made of 3 slides stacked on top of each other, and place whole stack onto the microscope stage (this allows room for the injection capillary). Bring embryos and capillary tip into focus: first bring embryos into focus; move them out field of vision; move tip of capillary in the center and use micromanipulator to bring it to the same focal level; now move back in the embryos

Set constant air pressure in injection system such that DNA is leaking slightly (if you don´t have constant pressure, yolk will find its way into the capillary and clog it)

Inject by driving embryos into capillary using the microscope stage; you should see a red drop appearing in the egg, the egg may appear "growing" by turgor — but beware, if too much yolk leaks, the embryo is likely to die...

Rearing the embryos -

When done with injections for the day, place slides with embryos on agar plates (for humidity), put these plates into a closed plastic box with a small opening (box 15x15x12 cm, opening 2x3 cm)

Let develop at 32°C.

Check humidity daily: there should be no drops of condensation water around the embryos, nor should the air be so dry that the eggs shrink.

One day before embryos begin to hatch (i.e. after ~2 days at 32°C), remove lids of agar plates (but close again the large box) to let agar and remaining water on coverslip dry out completely. On the third day, also remove lid from box (embryos do not hatch well if they are too humid).

Collect larvae twice daily to prevent them from getting stuck on the agar; transfer with a needle onto whatman paper and place that into flour where larvae can grow.

Setting up transgenic lines

When injected eggs/larvae have grown to the pupal stage, separate sexes. Once the adults have emerged, set up crosses: 1 injected male x 1 injected virgin in small vials with about 8 gram of flour+yeast (small blocks). Transfer these single pair matings 3 times, after 1 week each (at 32°C).

Once the offspring of these crosses are adults, screen for green eyes. If dosage of your experimental construct does not matter, you can set up lines by combining all green-eyed offspring of one couple.

If dosage matters, i.e. if you want only one insertion per line, proceed differently: use only 1 male offspring and cross it with non-transformed pearl virgins. In our experience, most germ line precursers of the injected animals only carry one insertion. However, with small

constructs, many offspring can carry the transposon, i.e. several germ line precursors independently have received foreign DNA. If all these transformed offspring are combined into one stock, it can become difficult later to establish single-insertion lines.

Time table for germline transformation

day 1: Injection (room temp), then transfer to 33C

day 3/4: transfer larvae to flour (33C)

ca. day 27: sex pupae and set up single matings; after 2 more weeks, transfer to fresh flour, repeat this after 1 more week and once again after another week

ca. day 62: screen first egglay for green-eyed beetles, keep screening every week until done