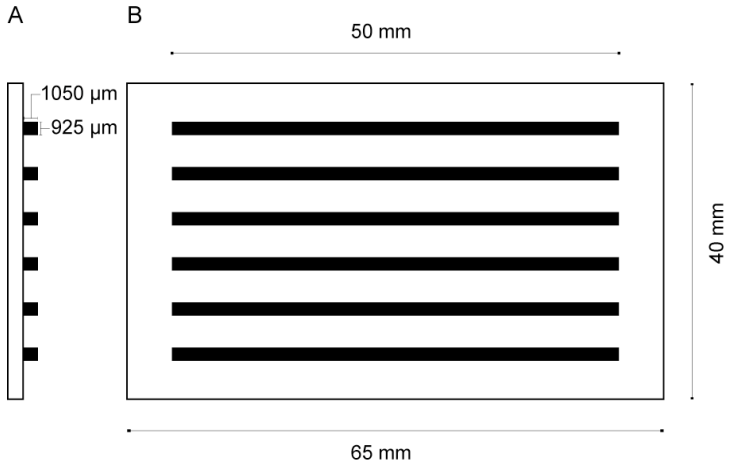
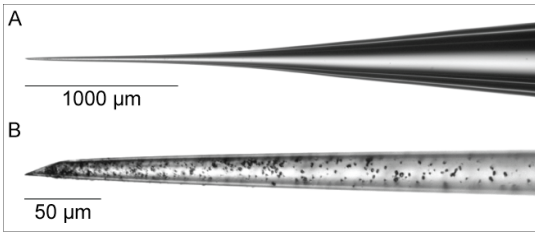
Protocol for injection of *Gryllus bimaculatus* eggs for zygotic RNAi

- Remove cotton wool from cricket cages >12h prior egg collection, in order to increase egg numbers, feed crickets with dried cat food (optionally: canned cat food)
- Collect eggs for 1h in wet cotton wool
- Incubate cotton wool in petri-dish for 30min @28°C
- Wash out eggs from cotton wool and place eggs on moist filter paper
- Air dry eggs for 10 min in order to desiccate eggs (in microscope room, without lid)
    - no longer though, because egg shells become too hard otherwise and survival rate is poor
- add new PBS (+100 I.U./ml Penicillin and 100 µg/ml Streptomycin (VWR 45000-650)) to agarose wells
- Transfer eggs in 2% agarose wells containing 1 mg/l Methyleneblue
- cover remaining eggs on filter paper with petri-dish lid
- Inject eggs within 1-4h after egg collection (age of eggs at the time of injection 2-5h)



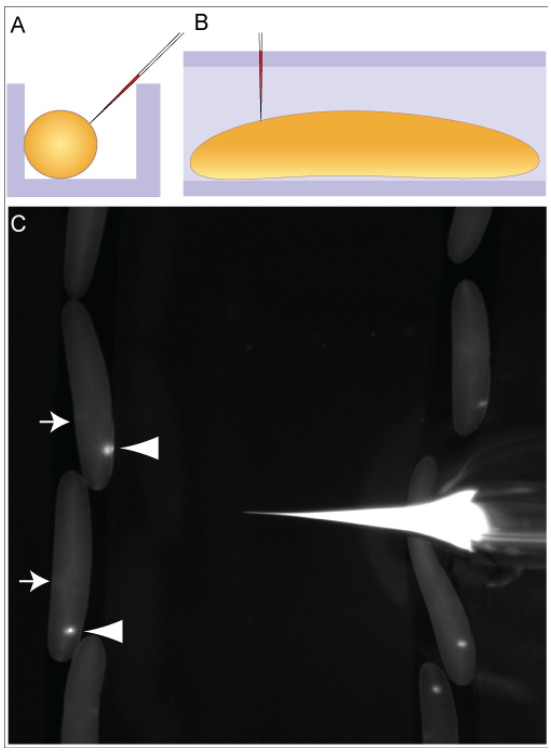
Schematic of lateral (A), and top view (B) of custom-made mould for preparing agarose wells.



Microinjection needles for *Gryllus bimaculatus* eggs. The short taper length guarantees a sufficient stability during injection (A). Close-up of the needle shows the tapered tip after bevelling with a micro grinder (B).

	heat	pull	velocity	time
1.	566	100	20	250
2.	566	100	20	250
3.	566	100	100	250

Settings for the Sutter instrument horizontal needle puller model P-97. Ramp temperature of heating filament used: 566.



Schematic (A, B) and photograph (C) of microinjection of *G. bimaculatus* eggs. The nucleus serves as a marker for the posterior pole, visible as a bright spot under fluorescence illumination (marked with arrows). Eggs are injected at the posterior pole from the lateral side. (A) Anterior/posterior view. (B) Lateral view. Eggs in (C) were injected with A488-coupled dextrans (marked with arrowheads).