



Jan 23, 2021

Karyotype on fixed mealybug tissue

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Works for me

dx.doi.org/10.17504/protocols.io.mnec5be

Mealybug Team



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DOI

dx.doi.org/10.17504/protocols.io.mnec5be

PROTOCOL CITATION

Isabelle Vea, Stevie Bain 2021. Karyotype on fixed mealybug tissue. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.mnec5be>

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CREATED

Jan 16, 2018

LAST MODIFIED

Jan 23, 2021

PROTOCOL INTEGER ID

9638

- 1 **Males testes:** Fix material in Bradley Carnoy (4:3:1 chloroform: ethanol: acetic acid) and keep at 4 degree celcius overnight (can keep up to 7 days for male testes)

Embryos: no need to fix

- 2 **Males testes:** Dissect the tissue in 45% acetic acid (add 20ul of 45% acetic acid on a slide coverslip, pipet out the males and place them on a filter paper, transfer the male in the acetic acid with a needle, dissect)

Embryos: use freshly laid embryos and transfer directly in 45% acetic acid on coverslip

- 3 Use a slide to catch the coverslip by surface tension

Embryos: reverse the slide and tap gently on the coverslip a few times so that the fixative goes in the embryos cells

- 4 Place a filter paper on the bench, then place the slide on top so you can fold the filter paper back to the coverslip, press firmly with thumbs (ideally, the fixative excess should squeeze out). AVOID SLIDING COVERSLIP ON THE SLIDE

- 5 Dip the slide with forceps in liquid nitrogen until the nitrogen doesn't bubble anymore
- 6 Snap the coverslip with a razorblade
- 7 Add 40 uL of Ethanol:Acid Acetic 3:1 on the slide where the sample is
- 8 Let the slide air dry **completely**
- 9 Add vectashield DAPI
- 10 add a coverslip and seal with nail polish and let it incubate in a drawer for 30 min before looking at the slide