

EMBRYO EXPLANT CULTURE (Black Lab, adapted from Rojas et al., Development 2005)  
(everything should be sterile or sanitized)

- 1) set up a 37°C warm plate by the dissection scope
- 2) prepare fresh DMEM+1%FBS  
    aliquot 400ul DMEM+1%FBS to each well in a 24-well-plate  
    (optional: add 1%antibiotics in DMEM)
- 3) prewarm the 24-well-plate with DMEM on the warm plate
- 4) dissect E9.5 embryos in room temperature PBS  
    (optional, save yolk sac for genotyping)
- 5) pinch off head (cut just above first branchial-arch) and tail (anywhere below hindlimb level is fine) with forceps
- 6) transfer the embryo explant to the pre-warmed DMEM on a warm plate
- 7) proceed to the next embryo until dissection is complete
- 8) transfer the 24-well-plate to a cell culture incubator (37°C, 5% CO<sub>2</sub>) to recover for 1-hour
- 9) check heartbeat under a dissection scope, make sure embryos are alive. Add drug(s) of interest and vehicle controls and return plate back to cell culture incubator
- 10) culture 12-15 hours
- 11) check heart-beating again. Harvest "healthy" (-in terms of heart beating) embryo explants for further analysis

Cultures can be maintained with contractile hearts for up to 72 h but 24-48 h is more typical.