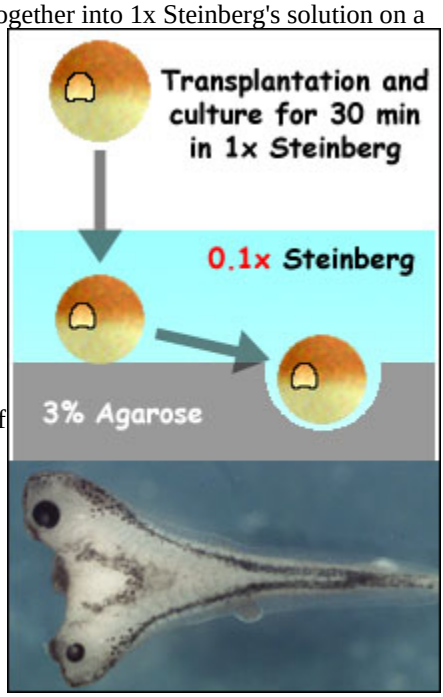
Spemann's organizer transplantation - Hiroki Kuroda

Note: All surgery steps are done with forceps.
Note: Use 50 mm sized plastic plate.
Note: Use an embryo between stage 10-10.5 if you really want to have a complete secondary axis.

- Put donor and host embryos together into 1x Steinberg's solution on a plate.
- Remove the chorion membrane from the dorsal animal side for host and ventral animal side for donor embryos.
  Note: If the embryos are significantly damaged, discard.
- Cut dorsal cap and remove yolk rich cells and the ectodermal region.
- Make slit at the ventral side of the host embryo. Note. The slit should reach to the blastcoel (this greatly helps healing).
- Transplant donor explant into ventral region in the host embryo.
- Leave transplanted embryos for 30 min (or until healed).



- Move transplanted embryo into a hole in 3% agarose plate in 0.1 x Steinberg's solution. Note: Do not use a high salt buffer, or you can get exogastrulation. Note: The hole in the agarose can be made with a heated glass ball at the tip of a glass pipet.
- Culture the embryo.

10 x Steinberg's solution
NaCl 34g (580 mM), KCl 0.5g (6.7 mM), $CaNO_3$-$4H_2O$ 0.8g (3.4 mM), $MgSO_4$-$7H_2O$ 2g (8.3 mM), Kanamycin 0.1 g, Tris (MW=121) 6.0g (50 mM), adjust pH between 7.35-7.45 by HCl, adjust volume to 1L with H_2O and autoclave.

1 x Steinberg's solution
Kanamycin 0.1g, 10x Steinberg's solution 100 ml, adjust volume to 1L H_2O and autoclave.