# Preparation for Vibratome Sectioning

Embedding medium: Gelatin/Albumin

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|---|---|
| PBS (10x) | 22.5 ml |
| Gelatin | 1.1 g |
| $dH_20$ | 225 ml |

Stir with heating at 60°, approx. 1h, then cool down to room temp.
Add Albumin 67.5g
Stir for a long time at RT till it is dissolved.
Cover the beaker and store at 4°C until the next day.
Then, heat up to 37°C in water bath.
Make 40 ml aliquots and store at -20°C.

Gelatin: Sigma Type A, item number = G1890
Albumin, Bovine: Sigma, 50g, item number = A3912
Glutaraldehyde: Sigma, Grade I, 25% aqueous, C5H8O2, FW 100.1

## Vibratome sectioning

1. Take 1.5 ml of thawed gelatin/albumin solution and put it on ice
2. Add 105 μl of glutaraldehyde
3. Stir quickly and thoroughly with a plastic pipette and let it solidify inside a plastic mold.
4. Put some embryos (in a minimum of PBS) for 10-15 min into albumin/gelatin mixture (without glutaraldehyde) until they sink to the bottom.
5. Take them out and put them onto solidified block. Don't put too many! Orient them the way you want them. Leave them oriented, in the medium they came with, for about 5-10 minutes: their bottoms will stick, and they'll stay oriented when you remove their medium and add medium+glutaraldehyde.
6. Once they're stuck, remove the medium.
7. Apply steps 1, 2 and 3 for another 1.5 ml aliquot of gelatin/albumin solution and pour it onto the embryos, so that they are embedded completely. You have to do this very quickly because the glutaraldehyde will cause the thing to solidify within 20 seconds or so. Add the medium gently so as not to disturb the orientation of your

embryos. If you wait too long before adding the top layer, the embryos may dry out and lift off when you try to drown them.

8. Alternative: if you're going to use a fluorescent antibody, don't use glutaraldehyde for the part 6. Instead, mix 220 μl of formaldehyde (Sigma stock 37.5%) with the gelatin albumin.
9. Let it solidify for 10-15 min at room temp (unless you used formaldehyde instead of glutaraldehyde, in which case you have to leave it for some hours, or even overnight).
10. Cut the embryo from the gelatin block out so that the distance between the edge of the new block and embryo is about 7 mm. Leave a decent distance on the bottom (the part which will attach to the block), but trim the top so that you don't waste too much time sectioning empty block.
11. They can be stored for a few days at 4 °C if need be. If you do this, put them into an airtight Ziploc bag with a wet paper towel in it, to prevent dehydration.
12. When you're ready to section, attach the block with embryo to the metal block with Crazy Glue.
13. Let glue harden at room temperature 10-15 min.
14. Put the block with embryo into vibratome chamber.
15. Fill the vibratome chamber with 1X PBS.
16. Cut 30-50 μm sections, speed 2, frequency 3 (this is for Leica VT1000S).
17. You can pick up the sections (by the corner) using forceps.
18. If using the sections for immunohistochemistry, put them into a vial with PBST + 0.1% sodium azide. If using the sections for *in situs*, put them into vials of 4% Paraformaldehyde for 30 minutes, and then transfer them through a methanol series into 100% methanol like you would for wholemount *in situs*. Note: to do *in situs*, don't store the blocks over 1 day – do embedding, cutting, and methanol series on the same day. If mounting, put the sections on slides (in which case, let sections dry at room temperature – but don't overdry!) and coverslip them with some sort of mounting medium.