Collecting and staining embryos

Gongping Sun

Materials

Reagents

1) Paraformaldehyde 16% solution, EM grade (VWR, Cat: 15710)
2) DPBS modified (1X), without calcium, without magnesium (VWR, Cat: 82020-066)
3) NaCl (Fisher Scientific, Cat: BP358-1)
4) Triton X-100 (Sigma, Cat: T8787)
5) Hoechst 33342, trihydrochloride trihydrate (Molecular Probes, Cat: H-1399)
6) VECTASHIELD mounting medium (Vector Laboratories, Cat: H-1000)
7) Clear nail polish (Electron Microscopy Sciences, Cat: 72180)
8) N-heptane (Fisher Scientific, Cat: H350SK-1)
9) FlyStuff grape agar premix (Genesee Scientific, Cat: 47-102)
10) Bleach
11) Bovine serum albumin, fraction V, heat shock treated (Fisher Scientific, Cat: BP1600)

Equipments

1) Nutator mixer
2) Camel's hair brush #4 (Ted Polla, Cat: 11862)
3) Fisherbrand superfrost disposable microscope slides (Fisher Scientific, Cat: 12550143)
4) Fisherbrand cover glasses 22X22 (Fisher Scientific, Cat: 12-542-B)
5) Embryo collection cage-small (Genesee Scientific, Cat: 59-100)
6) Thermo Scientific Samco fine tip transfer pipets (Fisher Scientific, Cat: 1371125)
7) Falcon cell strainer (Fisher Scientific, Cat: 08-771-1)

Reagents setup

1) Fixative
   Dilute 1ml 16% paraformaldehyde (PFA) solution with 3ml 1X PBS to a working concentration of 4%. Store @4C. The 16% PFA stock solution should be aliquoted and stored @-20C.
2) 0.2% PBT
   Add 0.2ml Triton X-100 to 100ml PBS. Stir until fully dissolved. Store @ room temperature.
3) Blocking solution
   Dissolve 1g BSA in 50ml 0.2% PBT. Store @4C.
4) Hoechst solution
   Dissolve 2mg Hoechst 33342 in 1ml ddH_2O to make 2mg/ml stock solution. Protect from light. Store @4C. Before use, add 1ul stock solution to 400ul 0.2% PBT to make 5ug/ml working solution.
5) Grape juice agar plate
   See instruction of the FlyStuff grape agar premix (http://flystuff.com/wp-content/uploads/2014/02/Grape-Agar-Instruction.pdf)

Procedures

Collecting embryos

1. Set up an embryo collection cage. Put flies inside, and cover it with a grape juice plate with yeast on it. Secure the plate with a rubber band. Give the flies overnight to acclimate in the cage before beginning your collection with a new grape juice plate.
2. Determine the laying time you need to get embryos at the stage(s) you desire. The developmental timing of fly embryos at 25C is shown in Table 1.
   Note: At 18C embryos develop slower, roughly following the formula:
   (time @18C) = 1.82*(time @25C)
   At 29C embryos develop faster, roughly following the formula:
   (time @29C) = 0.86*(time @25C).
3. Put the cage plate side down in the fly incubator for the desired laying time.
4. When the laying time is complete, remove the old plate and put a new plate on the cage if more embryos are needed.
5. To remove yeast and dechorionate embryos, cover the old plate with 50% bleach for 2-3min.
   Note: Do not keep the embryos in bleach longer than the recommended time.
6. Filter the bleach solution with embryos through a cell strainer to collect embryos from bleach solution. Use a brush to transfer the embryos.
7. Rinse the embryos on the cell strainer thoroughly with ddH_2O until you cannot smell any bleach.

Staining embryos

8. Mix fixative and N-heptane at 1:1 in an eppendorf tube. Then use a brush to transfer the embryos from the cell strainer to the tube.
9. Shake vigorously embryos for 30s in order to allow the fixative to penetrate. Embryos will be between the two phase layers.
10. Place embryos on a nutator mixer for 25min @RT to fix.
11. Remove the lower phase (fixative) and add equal amount of methanol. Immediately shake for 15s. This step removes the viteline membrane. Embryos should sink to the bottom after shaking.
12. Discard all the liquid and embryos that did not sink. Rinse the embryos at the bottom with methanol twice.
    Stop point: At this point, embryos can be stored in methanol @-20C until needed.
13. Rehydrate the embryos by washing with 0.2% PBT for 3X5min.
14. Incubate embryos with blocking buffer for 1h @RT.
15. Incubate embryos with primary antibody diluted in blocking buffer @4C overnight.
16. Wash embryos with 0.2% PBT for 4X15min.
17. Incubate embryos with blocking buffer for 30min @RT.

18. Incubate embryos with secondary antibody diluted in blocking buffer for 2h @RT.
19. Wash embryos with 0.2% PBT for 15min.
20. Incubate embryos with 5ug/ml Hoechst solution for 15min.
21. Wash embryos with 0.2% PBT for 3X15min.

Mounting embryos

22. Take a slide, wipe and label it.
23. Use a P20 pipet or a transfer pipet to transfer the dissected tissues from the dissecting well onto the slide. Carefully remove the extra liquid using the pipet or a small piece of Kimwipe.
24. Immediately add 20ul or 30ul mounting medium onto the tissues on slide. Use the forceps to gently position the tissues on the slide.
25. Put the coverslip on. Seal with nail polish.

Table 1. Embryonic stages of Drosophila

Stage	t_1	t_2	**Stage**	t_1	t_2	**Stage**	t_1	t_2
S_1	t_0=0	0:25	**S_7**	3:00	3:10	**S_{13}**	9:20	10:20
S_2	0:25	1:05	**S_8**	3:10	3:40	**S_{14}**	10:20	11:20
S_3	1:05	1:20	**S_9**	3:40	4:20	**S_{15}**	11:20	13:00
S_4	1:20	2:10	**S_{10}**	4:20	5:20	**S_{16}**	13:00	16:00
S_5	2:10	2:50	**S_{11}**	5:20	7:20	**S_{17}**	16:00	Hatch
S_6	2:50	3:00	**S_{12}**	7:20	9:20			

Reference

Modified from embryos collection protocol from Stephen Crews Lab (available online) and an online embryos staining protocol (http://www3.mpibpc.mpg.de/groups/shcherbata/protocols/Embryo%20staining.pdf)