

DISSECTION PROTOCOL

Conlon, R. A. Whole mount in situ hybridization to mouse embryos. In *A Laboratory Guide to RNA: Isolation, Analysis, and Synthesis* (P. Krieg, ed.) John Wiley and Sons, in press.

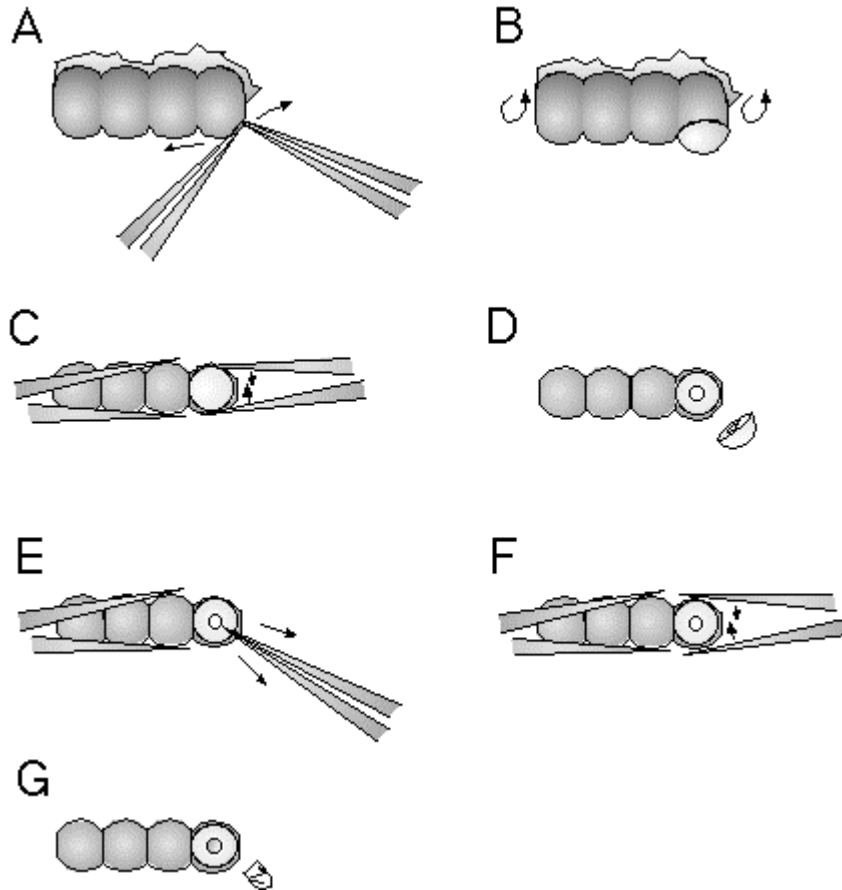


Figure 1. An alternative procedure for dissecting embryonic day 7 embryos. In contrast to previously published protocols (Hogan et al., 1994), the decidua are not removed from the uterus, allowing for more rapid dissection. Two pair of fine forceps (Dumont No. 5 or No. 5 Biologie forceps, Fine Science Tools, Foster City, CA), disposable petri dishes, a dissecting microscope and cold PBS are required. Place the dissected uterus in small petri dish and cover with cold PBS.. Arrange the uterus such that the membrane that attached the uterus to the body wall (the mesometrium) is facing away from you (A). Under a dissecting microscope grasp the uterus immediately next to the deciduum with both pairs of forceps and tear the uterus off the top of the deciduum--the uterus is tough and muscular, whereas the decidual tissue is soft and spongy (B). With one pair of forceps, clip off a portion of the exposed deciduum, amounting to about one quarter to one

fifth of the entire deciduum (C and D). The tip of the embryo (midventral or distal tip) should now be exposed to view (D). Reichert's membrane is usually still attached to the embryo at this point. To make a hole in the nearly invisible Reichert's membrane that covers the embryo, insert a pair of forceps immediately adjacent to the embryo, close and remove (E). Squeeze the embryo out of the deciduum and Reichert's membrane by placing the forceps on the overlying uterine wall and applying gentle pressure (E). The embryo will usually pop out intact, without Reichert's membrane and the ectoplacental cone. If they remain attached, they should be removed by careful dissection.

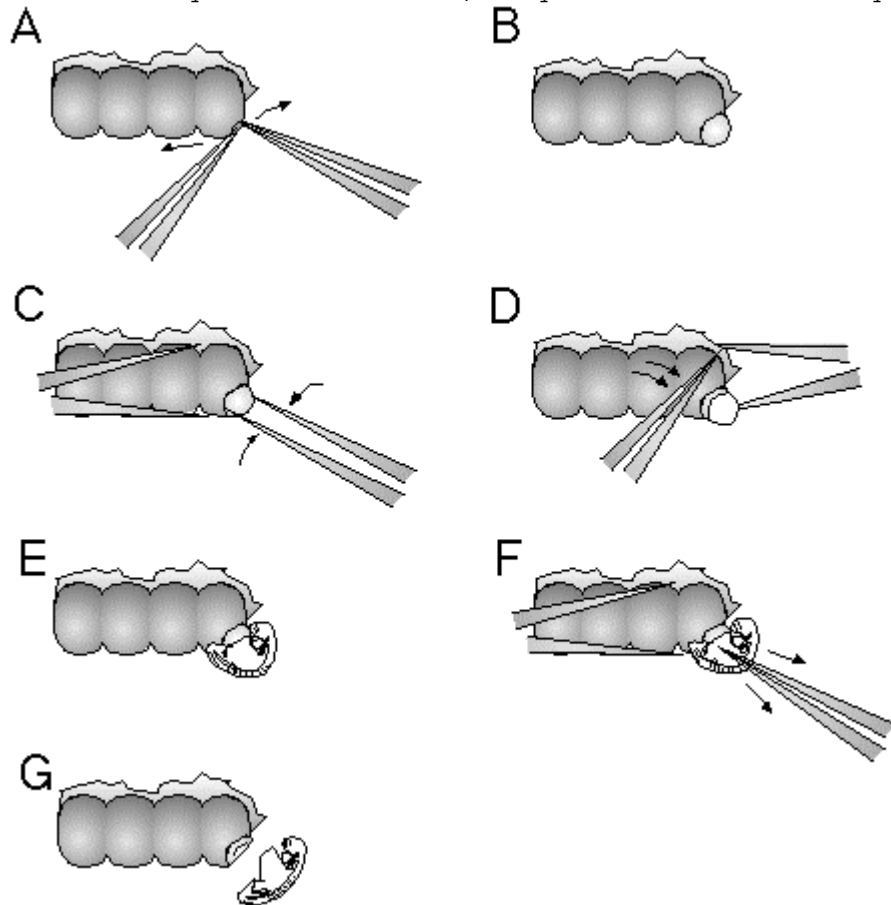


Figure 2. Rapid dissection of embryonic day 8 embryos. Arrange the uterus as described for Figure 1. Instead of exposing the entire antimesometrial pole of the deciduum, rip a hole in the uterus to expose a small portion of the side of the deciduum (A and B). With a pair of forceps make a shallow gash in the deciduum large enough for the embryo to pass through (C). Press gently on the deciduum through the covering wall of the uterus (D). A gentle push will cause the embryo to slide out (E). (If the embryo does not slide out, the cut in the decidual wall was not deep enough. However, if the cut is too deep, the embryo will be damaged. It will take some practice to determine the correct depth of the cut.) If the embryo does not come completely free (E), grasp the yolk sac of the embryo (F) to pull it free (F and G). Embryos that have finished turning (as shown in this figure) are ready to be fixed. Embryos that have not completed the inversion need to be dissected further (see Figure 3.).

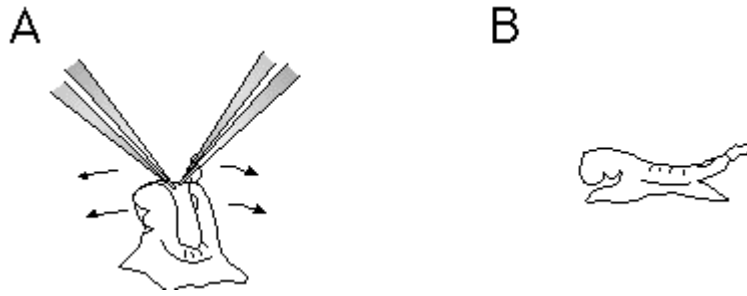


Figure 3. Further dissection of early embryonic day 8 embryos. Flatten the embryo by grasping the amnion between head and tail with both pairs of forceps and pulling towards the head and tail (A). Dorso-ventrally flattened embryos are easier to photograph (B).

Ronald A. Conlon, Department of Genetics, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-4955 TEL 216-368-1826; FAX 216-368-3432; EMAIL rac14@po.cwru.edu