Staining Intact Embryos for HRP

(Rivera Lab)

Based on the protocol by: Beddington, R. S. P. and Lawson, K. A. (1990). Clonal analysis of cell lineages. In Postimplantation mammalian embryos. pp. 282. Eds. A. J. Copp and D. L. Cockroft. Oxford University Press.

1. Rinse the living embryo in rinse solution (0.1 M phosphate buffer, pH 5.5 containing 5% (w/v) sucrose).

2. Transfer the embryo to HRP staining solution (0.5 mg/ml solution of *p*-phenylenediamine-pyrocatechol (Hanker-Yates reagent, Polysciences) in rinse solution containing 0.02% H_2O_2) for up to 2 h. Protect from light. Use 0.5 ml reaction mixture/embryo. Although embryonic ectoderm cells that have gone through several generations require the full 2 h exposure, labeled endoderm will be visible within 15 min.

	1 ml	5 ml	10 ml	20 ml
Rinse solution	0.98 ml	4.9 ml	9.8 ml	19.6 ml
1% H_2O_2	0.02 ml	0.1 ml	0.2 ml	0.4 ml
Hanker-Yates reagent	0.0005 g	0.0025 g	0.005 g	0.01 g

3. Fix the embryo in fresh 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h. Rinse in buffer and examine in the dissection microscope.