

EMBRYO STAINING (FINNEY)

For arrays only:

1. Enrich population for array-bearing animals.
2. Heat shock gravid hermaphrodites at 30 degrees for 30 minutes.
3. Let worms recover for 1-2 hours on the benchtop

MODIFIED FINNEY PROTOCOL

1. Wash worms off of plates with M9, wash in M9 1-2 times.
2. Lyse worms in 20% bleach, 5% 10N NaOH. Do not overbleach.
3. Wash in M9 2x.
4. Add Finney fix:
 - ♦ 125 μ L paraformaldehyde 16%
 - ♦ 112 μ L Witches' Brew
 - ♦ 180 μ L Methanol
 - ♦ 10 μ L 100 mM EGTA
 - ♦ 573 μ L ddH₂O
5. Freeze at -80°C after adding fixative. Can store at -80 forever.
6. Thaw under tap water. Rock at RT for 20 min.
7. Incubate for 15 min in PBST.
8. Incubate in primary antibody 4 hrs to O/N, wash in PBST 3X for at least 15 min.
9. Incubate in secondary antibody 4 hrs to O/N, wash. Add 0.1 μ L DAPI (1 mg/mL) to last wash.
10. Place 10 μ L stained embryos on slide. Mount with Vectashield.
11. Store remainder at 4 degrees in PBST

1 L PBST

100 mL 10x PBS
 2 mL 0.5 EDTA
 5 mL Triton-X 100 or (25 mL 20%)
 H₂O to 1L

Witches' Brew: (store at 4°C)

For 7 mL stock:

- 4 mL 1 M KCl
- 100 μ L 5 M NaCl
- 2 mL 50 mM EGTA
- 25 μ L 1 M spermidine
- 10 μ L 1 M spermine
- 250 μ L B-ME
- 750 μ L 1 M PIPES pH 7.5