

FISH Probe Preparation

1. Mix 30 λ resuspended labeled probe (biotin or DIG)
15 λ Cot1 DNA
1 λ sonicated salmon sperm DNA
2. Add 454 λ H₂O
50 λ 3M sodium acetate
550 λ cold isopropanol
3. Microcentrifuge at maximum velocity for 30 min. at room temp.
4. Carefully remove liquid, leaving pellet undisturbed. No need to ethanol wash, just make sure pellet is completely dry before proceeding.
5. Air dry pellet
6. Resuspend dried pellet in 25 λ hybridization buffer. Allow to resuspend for at least 2 hrs at 56°C
7. Use for FISH hybridization or store at -20°C.

Hybridization Buffer

50% deionized formamide (DI-FA)
10% dextran sulphate
2X SSC
0.15% SDS