Protocol P *(PRINS Probe)*
Fluorescence *IN SITU* Hybridisation Protocol

(1490-T)

Approx time:

Probe Preparation: 1 hr

Solutions to be prepared:	Stop Buffer

Stop Buffer: 50mM NaCl
50mM EDTA, pH8.0

Reaction Mix contains dNTPs, primer, glycerol and reaction buffer.

Procedure:

1. Slides can be made up to 24 hours in advance dehydrated through an ethanol series air dried and stored at 4°C. No pretreatment is necessary.
2. Warm Reaction mix to 37°C for 5 min and mix well.
3. Combine 25µl of reaction mix, 1nmole of Cy3 or FITC-dUTP, or 0.04mmole biotin-dUTP and 2 units of Taq polymerase per slide.

Note: L*abels and Taq polymerase are not supplied with this product.*

4. Add to slide. Seal with Fixogum and dry for 5 min in an oven at 37°C.
5. Place slide on a preheated block at 94˚C for 5 min.

Note: *Slides have to be in direct contact with the hot surface.*

6. Transfer to an oven at 58°C for 30 min.
7. Wash in stop buffer for 5 min at 58°C.
8. Wash in stop buffer for 5 min at room temperature.
9. Dehydrate through an ethanol series and air dry.
10. Dilute 1µl Counterstain 1 (DAPI) with 9µl distilled water. Add 5µl diluted counterstain to 200µl Mountant. Mix well.
11. Mount slides with 20µl Mountant/Counterstain. Overlay with a coverslip and seal with nail varnish.
12. Slides should be stored in the dark at 4°C.
13. View slides using epifluorescence filters as appropriate.