IN SITU HYBRIDIZATION PROTOCOL
IN VITRO TRANSCRIPTION USING DIGOXIGENIN-UTP

DAY 1 OF TRANSCRIPTION:

1. Add the following to a microtube:

   A) 5X TRANSCRIPTION buffer: **4 μl**

   B) RNasin inhibitor : **0.5 μl**

   C) NTP mix: **8 μl**
   Preparation of NTP mix: **3.5 μl** DIG-UTP
   6.5 μl UTP (normal)
   10.0 μl ATP +GTP + CTP each
   TOTAL: **40.0** μl - vortex and spin.

   D)Human Plasmid DNA (1μg/μl): **1 μl**

   E) DEPC H_2O: **4.5 μl**

   F) RNA polymerase: **2 μl**

   TOTAL VOLUME: **20 μl**

2) Incubate for **2 hours** at 37°C in waterbath.
3) Add 1 μl Rnase-free Dnase to mixture.
4) Incubate in waterbath for **15 minutes** at 37°C.
5)Add 2.5 μl 4 M NACL to mixture.
6) Add 70 ml 100% Ethanol.(**Precool the alcohol to -20°C**)
7) Precipitate overnight at -20°C.

Next day:

1.Spin down at 12000 RPM at 4oC for **10 minutes**;discard supernatant.

2.Wash with 70% icecold ethanol 100μl.

3.Spin down 12000 RPM at 4oC for **5-10 minutes**; discard supernatant.

4. Dry RNA pellet in speed vacuum for **5 minutes** maximum.

5) Resuspend in DEPC H_2O to 100 ng/μl . Final volume is 20 μl.

6) Put for 5 min. at 65 C (to dissolve better) **Store at - 20 C**

7)To check the concentration : Run a 2 % gel in 0.5 x TBS .

a) HAE marker o.5 μg/μl : use 1 μl.
DEPC 0.1 % 3 μl
6x Dye 2 μl

b) Unlabelled linearized Plasmid with knownconc. 7 μl (use several dilutions)
DEPC o.1 % 3 μl
5 x Dye 2 μl

c) Labelled (linearized) sample : same volumes as b).

This will give you the estimated concentration of your sample.
You will need 3 ng/section.