DIG-labeling of RNA Probes for In Situ Hybridization

by Tatsuya Okafuji, Kevin Mitchell Lab, Smurfit Institute of Genetics, Trinity College Dublin

Updated on 20/10/2009

1. Add the following reagents in an RNase-free tube. Mix gently by pipetting.

10x transcription buffer	2 μl
Template DNA	1 μg (plasmid) or 0.25 μg (PCR product)
10x DIG labeling mix (Roche)	2 μl
RNase inhibitor (40 U/μl)	0.5 μl
T7, T3 or SP6 RNA polymerase (*1)	20U
Nuclease-free H_2O (Sigma)	to 20 μl

2. Incubate at 37°C for 2-4 hr. Prepare an agarose gel for electrophoresis.
3. After 1.5 or 3.5 h*, take a 1 μl aliquot to run on an agarose gel for quality check of RNA.
4. Add 1 μl of RNase-free DNase I and incubate for 15 min at 37°C.
5. Stop reaction by adding 2 μl of 0.2 M EDTA pH 8.0.
6. Column-purify RNA [We use NucleoSpin RNA Clean-up XS (Macherey-Nagel) and elute RNA in 30 μl of RNase-free H_2O.
7. Quantify RNA (We usually do that with Nanodrop using 1.5 μl of eluted RNA).
8. Adjust the concentration of RNA to 0.1 μg/μl with DEPC-treated H_2O.
9. Add 1/100 volume of 100 mM sodium citrate pH 6.35.
10. Store at -20°C until use.

* RNA probes can be checked after finishing all the procedures.