RNA-probe synthesis (In-vitro transcription)

Materials:

1. RNase-free tubes and filter tips.
2. 10x Transcriptional buffer (BioLabs M0460)
3. SP6 RNA polymerase (antisense probe) and T7 RNA polymerases (sense probe) (BioLabs M0460)
4. 10X DIG RNA Labeling MIX (or Fluorescein RNA Labeling Mix)[1]
5. RNAsin (Promega N2615)
6. DNA Template from the 2nd PCR
7. Sterile, RNase-free water.
8. DNase I, RNase-free 1U/µL (ThermoFisher EN0521)
9. $MgCl_2$ (BioLabs M0460)

Method:

This procedure should be performed at RT

2.1 Maintain an RNase-free environment (gloves, RNA free labware, and nuclease-free water.

2.2 Set up a 30 µL reaction for the in vitro transcription of each probe. To calculate the amount of DNA and water to the mix, divide the amount of DNA that you have measured with Nanodrop to 1000. For example, if your DNA is with concentration of 76.4ng/µL, you must divide 1000/76.4 and the result is 13.08 µL. So, this is the amount of template DNA that you are going to use.

The final concentration of the DNA in the reaction should be 1000 ng

The 30 µLreaction must include the following reagents

1. Template DNA (from 2nd PCR)[2] 13.08 µL
2. 10x Buffer 2.5 µL
3. 10x DIG MIX 2.5 µL
4. RNAsin 1 µL
5. Sp6 Polymerase 1.5 µL
6. H_2O to 30 µL

2.3 Incubate at 38^0 C for 2h.

2.4 Degradation of DNA templates in transcription reaction

For the degradation of the leftover DNA, combine 1 µL of DNAse1 with 11 µL of H_2O and 8 µL of $MgCl_2$ (300mM).

1. H_2O 11 µL
2. DNAse 1 1 µL
3. $MgCl_2$ 8 µL

Combine them and use 2 µL of the mixture per tube of RNA probe.

2.5 Incubate for 30min at 37^0 C.
2.6 Proceed to RNA precipitation step

NOTES

1. If you want to perform a double labeling, FISH for example, both digoxigenin and fluorescein probes should be prepared. Note that for gene with high expression the preferred method for labeling is fluorescein and the weaker gene should be labeled with digoxigenin.
2. Before using in this reaction the template DNA should be purified with a column-based method PCR purification kit, Qiagen) and eluted in nuclease-free water