Generation of riboprobes

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Digest plasmids O/N with a restriction enzyme downstream of the insert to be transcribed.
N.B. Do not use restriction enzymes leaving 3' overhang ends (e.g. KpnI)

Buffer	5 μl
BSA (10X), if need be	5 μl
Plasmid	10 μg
Enzyme	3 μl
Water	up to 50 μl

Check the linearization on an agarose gel.

Purify plasmids using a PCR purification kit and measure DNA concentration.

Set up the transcription reaction in the following order at **RT**:

Linearized DNA	1 μg
Nuclease-free water	up to 20 μl
10x NTP labeling mixture (Vial #7)	2 μl
10x Transcription buffer (Vial #8)	2 μl
Protector RNase inhibitor (Vial #10)	1 μl
RNA Polymerase SP6 / T7 (Vial #11 / 12)	2 μl

Flick the tube and spin briefly.

Incubate for 2 hr at 37 °C.

Add 1 μl of TURBO DNase and mix.

Incubate for 15 min at 37 °C.

Stop the reaction by adding 2 μl of 0.2M EDTA (pH 8.0).

Recover RNA using a Microspin G-25 Column or lithium chloride (LiCl) precipitation.

Microspin G-25 Column

Adjust the reaction volume to 50 μl with H_2O.

Resuspend the resin in the column by vortexing.

Loosen the cap one-quarter turn and snap off the bottom closure.
Centrifuge at 735 x g for 1 min, RT; discard tubes.
Pipet sample onto center of column gel, and place the column in a microfuge tube.
With column oriented with gel surface vertical, centrifuge at 735 x g for 2 min.

LiCl precipitation

Precipitate the RNA by adding the following:

Nuclease-free Water	30 µl
LiCl Precipitation Solution	30 µl

Mix thoroughly. Chill for ≥30 min at –20°C.
Centrifuge at 4°C for 15 min at maximum speed to pellet the RNA.
Carefully remove the supernatant. Wash the pellet once with 1 ml of 70% ethanol, and re-centrifuge to maximize removal of unincorporated nucleotides. Carefully remove the 70% ethanol, and resuspend the RNA in nuclease-free water.

Check the integrity of synthesized RNA by running on agarose gel.
N.B. Before running, wash electrophoresis chamber thoroughly and use fresh buffer and gel. Use the loading buffer provided in the kit.

Take 1 µl of RNA, add to 99 µl of H_2O, and measure the concentration.

Adjust the RNA concentration with Hybridization Buffer to 0.5-5.0 ng/µl depending on the concentration of mRNA you want to visualize.