In vitro transcription

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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**:

Nuclease-free water	up to 20 μl
2x NTP/CAP	10 μl
10x Reaction buffer*	2 μl
Linear template DNA	1 μg
RNase inhibitor	1 μl
Enzyme mix	2 μl

* should be kept at RT.

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.

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

Microspin G-25 Column

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.

N.B. If the RNA is to be injected into embryos, repeat this wash step twice, since LiCl stimulates Wnt signaling.

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.