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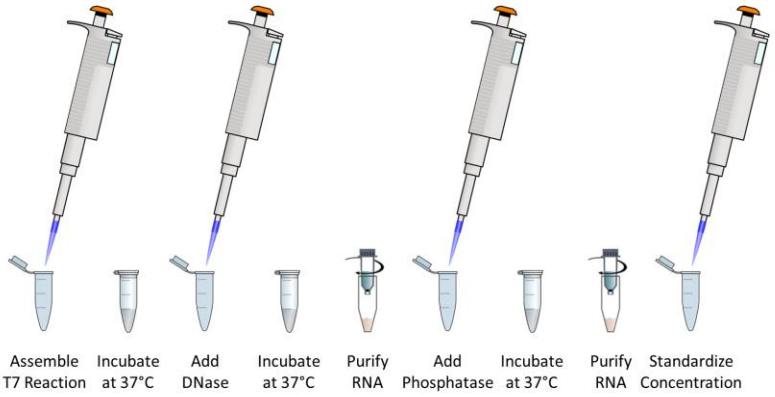
Production of Synthetic Messenger RNA

General Points

1. Thaw T7 reaction buffer at room temperature. Vortex and/or warm the buffer to ensure that any precipitate is dissolved. Thaw nucleotide mix and PCR product template on ice.
2. Assemble reaction components in the order listed to prevent precipitation of the template DNA.

Protocol (40 uL Reactions)

1. Assemble *in vitro* transcription (IVT) reactions using 16 uL nucleotide mix with cap analog, 4 uL 10X T7 buffer, 16 uL PCR product template and 4 uL T7 enzyme.
2. Incubate the reactions for 4–6 hours at 37°C.
3. Add 2 uL TURBO DNase to each reaction and incubate a further 15 minutes at 37°C.
4. Purify RNA on MEGAclear columns per the manufacturer's protocol, doing two 50 uL elution steps.
5. Add 10 uL Antarctic Phosphatase buffer and 3 uL enzyme to eluent and incubate 30 minutes at 37°C.
6. Re-purify the RNA from the phosphatase reaction using MEGAclear columns.
7. Quantitate RNA by A_{260} (e.g., Nanodrop) and adjust to 100 ng/uL concentration by adding TE pH 7.0.



Materials

2.5X Nucleotide Mix with Cap Analog (Allele Biotechnology ABP-PP-NTPMIX)
PCR product templates (Allele Biotechnology catalog or custom order)
MEGAscript T7 IVT Kit (Ambion AM1333)
MEGAclear RNA Purification Kit (Ambion AM1908)
Antarctic Phosphatase (NEB M0289S)
Pre-aliquoted TE pH 7.0 (Ambion AM9860)
2% SYBR Safe E-gels (Life Technologies G521802)

Notes

1. Extended or low-temperature incubation of *in vitro* transcription reactions can lead to excessive side-product formation, folding and aggregation, increasing the immunogenicity of the RNA.

2. When using UV absorbance assays such as Nanodrop, RNA can only be quantitated once residual nucleotides are removed by purification.

3. The expected RNA product yield when using this protocol is ~1 ug per ul of reaction volume (30-50 ug per 40 uL reaction).

4. Non-denaturing E-gels can be used to check for the consistency of primary product yield and the absence of any visible secondary bands. Run 5 uL of concentration-adjusted RNA (500 ng) per lane to get a good band intensity. Dilute the RNA 4X in TE 7.0 before loading it on the gel, and run the gel for 10-15 minutes. An example E-gel showing several different RNA products is shown below.