RNA Synthesis of Cloned Insert Transcripts

Overview

The NEB PCR Cloning Kit vector, pMiniT 2.0, has *in vitro* transcription capabilities. NEB has a variety of T7 RNA polymerase-based high yield transcription kits (NEB #E2040, #E2050) and mRNA synthesis kits (NEB #E2065, #E2060). We suggest using the "Recommended HiScribe®; RNA Synthesis Kits by Application" selection chart in our Tools & Resources section.* These kits include optimized protocols to synthesize high yield RNA for a variety of applications. For RNA synthesis using the stand-alone enzymes T7 (NEB #M0251) or SP6 (NEB #M0207) RNA Polymerases, the protocol below can be used; companion products are listed with product.

**Chart can also be located by use of the search window on the main landing page using this title.*

RNA Synthesis Protocol

1. Linearize the purified plasmid containing your cloned insert downstream of the insertion site utilizing any of the restriction enzyme sites flanking the insertion site after confirming your insert does not contain any internal sites for your chosen restriction enzyme. We recommend restriction digests leaving blunt or 5´ overhang ends.
2. Purify and quantitate your DNA by use of Nanodrop, Qubit or UV absorption approaches.
3. Assemble the RNA synthesis reaction at room temperature in the following order:

COMPONENTS	AMOUNT	FINAL CONC.
Nuclease-free Water	X µl	
RNAPol Reaction Buffer (10X)	2 µl	1X
Ribonucleotide Solution Mix (25 mM each)	3.2 µl	4 mM each
Template DNA	X µl	0.2-1 µg
MgCl2 (100 mM)	2.8 µl	additional 14 mM*
RNase Inhibitor, Murine or Human Placenta (40 units/µl)	0.5 µl	1 unit/µl final
Fresh DTT (100 mM) (optional)	1 µl	5 mM final
T7 RNA Polymerase (50 units/µl) or SP6 RNA Polymerase (20 units/µl)	2 µl	100 units (T7) or 40 units (SP6)
Total Volume	20 µl	

*1X reaction buffer contributes 6 mM for a final concentration of 20 mM.

4. Incubate at 37°C for 1 hour. For shorter (< 300 nt) transcripts incubate at 37°C for 2-4 hours.

Note: Higher yields of RNA using this standard protocol may be obtained by adding higher levels of RNA polymerase and increasing the amount of template in the reaction.