

New!

Transcribe ssDNA Templates with Single-Stranded Promoters Using the MiniV™ *In Vitro* Transcription Kit

EPICENTRE, the leader in *in vitro* transcription technology, is pleased to introduce the new MiniV™ *In Vitro* Transcription Kit. The MiniV *In Vitro* Transcription Kit enables users to produce RNA transcripts *in vitro* from single-stranded DNA (ssDNA) templates that are joined to a single-stranded N4 bacteriophage promoter.

MiniV™ RNA Polymerase* is the transcriptionally-active 1,106-amino acid domain of the N4 virion RNA polymerase. The enzyme lacks RNA strand displacement and unwinding activity on RNA:DNA hybrids and requires *E. coli* Single-Stranded DNA Binding Protein (EcoSSB) to displace the RNA strand from the DNA template for efficient *in vitro* transcription.

Linear templates of any length, including oligodeoxynucleotides and single-stranded cDNAs, that contain an N4 promoter can be transcribed. Functional N4 transcription promoter sequences (19 to 21 bases in length) are provided in the product

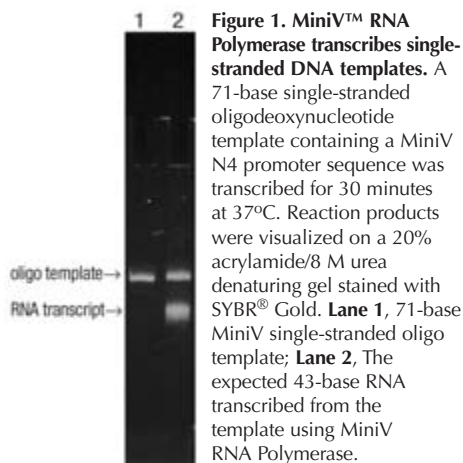


Figure 1. MiniV™ RNA Polymerase transcribes single-stranded DNA templates. A 71-base single-stranded oligodeoxynucleotide template containing a MiniV N4 promoter sequence was transcribed for 30 minutes at 37°C. Reaction products were visualized on a 20% acrylamide/8 M urea denaturing gel stained with SYBR® Gold. **Lane 1**, 71-base MiniV single-stranded oligo template; **Lane 2**, The expected 43-base RNA transcribed from the template using MiniV RNA Polymerase.

literature and on EPICENTRE's website at www.epicentre.com. The yield of RNA from a MiniV transcription reaction varies depending on the sequence of the DNA template. Typically 1 to 5 moles of RNA are produced per mole of ssDNA template.

www.epicentre.com/miniv.asp

MiniV™ *In Vitro* Transcription Kit

MV41025 25 Reactions

Contents:

MiniV™ RNA Polymerase (with RNase Inhibitor)

MiniV™ 5X Transcription Buffer

ATP, CTP, GTP, UTP

DTT

E. coli SSB Protein

RNase-free DNase I

MiniV™ ssDNA Control Template

RNase-free Water

*Covered by U.S. patent Application No. 2003/0096349 and related patent applications in other countries.

SYBR® is a registered trademark of Molecular Probes Invitrogen Detection Technologies.

New!

Produce Templates for Rolling Circle Replication and Transcription in 1 Hour Using CirLigase™ ssDNA Ligase

CirLigase™ ssDNA Ligase is a thermo-stable, ATP-dependent ligase that catalyzes intramolecular ligation (circularization) of single-stranded DNA (ssDNA) with 5'-phosphate and 3'-hydroxy termini. Linear ssDNA >30 bases can be circularized by CirLigase ssDNA Ligase.

Traditional methods for preparing circular ssDNA involve the use of oligo "splints" spanning the 5'- and 3'- termini of the oligonucleotide to be circularized. Even under the best of conditions, both linear and circular concatamers are produced and extensive purification is necessary to remove them. In contrast, the standard 60-minute CirLigase reaction, produces virtually no linear or circular concatamers, making the enzyme ideal for easily and rapidly preparing closed-circular ssDNA templates for rolling circle replication or rolling circle transcription.

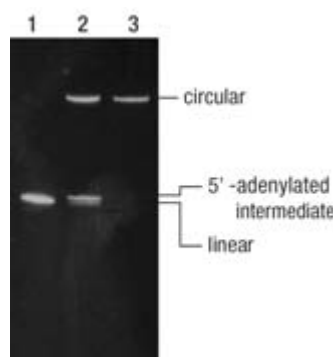


Figure 1. Under standard reaction conditions, CirLigase™ ssDNA Ligase converts linear single-stranded DNA (ssDNA) into circular ssDNA.

A 71-base ssDNA oligo was converted to circular DNA using CirLigase ssDNA Ligase. **Lane 1**, 71-base ssDNA oligo; **Lane 2**, Circularization proceeds through an adenylated intermediate; **Lane 3**, To confirm that the reaction product was a closed circle, the reaction was treated with exonuclease I, a DNase that specifically digests linear ssDNA.

Applications

Produce circular ssDNA templates for:

- Rolling circle replication experiments.
- Rolling circle transcription experiments.
- RNA Polymerase activity and inhibitor screening assays. (See Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit on p. 22)

www.epicentre.com/circligase.asp

CirLigase™ ssDNA Ligase

CL4111K 1000 Units

CL4115K 5000 Units

Contents:

CirLigase™ ssDNA Ligase

CirLigase™ 10X Reaction Buffer

ATP

50 mM MnCl₂

CirLigase™ Linear ssDNA Control Substrate Water