

Rapid and Highly Sensitive Screening of Bacterial RNA Polymerase Activity and Inhibitors Using the Kool™ NC-45™ Universal RNA Polymerase Templates

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Kool™ Universal RNA Polymerase Templates are small (28 – 150 nucleotide) circular single-stranded DNA molecules (ssDNA nanocircles). As observed in the laboratory of Dr. Eric Kool, DNA nanocircles can be efficiently transcribed *in vitro* by DNA-dependent RNA polymerases (RNAP) by a rolling circle transcription (RCT) mechanism* without the requirement for canonical promoter sequences or a primer.^{1,2,3} The product of a Kool RCT reaction can be detected by a variety of methods, including radioactive or non-radioactive end-point detection, or real-time monitoring using fluorescent dyes or molecular beacons.³

The Kool™ NC-45™ Universal RNA Polymerase Template (included in the Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit and also sold separately) is a 45 base ssDNA nanocircle that efficiently functions as a template for *in vitro* transcription by bacterial and bacteriophage RNA polymerases, such as *E. coli* RNA polymerase (both core and holo-enzyme), and phages T7, T3, SP6 and N4. In addition, the fact that RCT from a Kool NC-45 Template does not require canonical promoter sequences makes it possible to utilize Kool NC-45 Templates for detecting RNA polymerases. The system is also suitable for the high-throughput screening of potential RNAP inhibitor compounds (see FIG 1).

Here we demonstrate the use of the Kool NC-45 Template as a tool for *E. coli* RNAP inhibition studies (FIG 2).

Method and Results

Real-Time detection of inhibitors of *E. coli* RNA polymerase (core enzyme)

To verify the suitability and specificity of the Kool NC-45 Template for screening RNAP inhibitors, the activity of *E. coli* RNAP (Core enzyme; EcRNAP) was tested in the presence and absence of RNAP inhibitors using the Kool RCT assay. The RNAP inhibitors chosen were: rifampicin (a strong inhibitor of EcRNAP), α -amanitin (an inhibitor of eukaryotic RNA polymerase II), and Tagetin™ RNA Polymerase Inhibitor (EPICENTRE

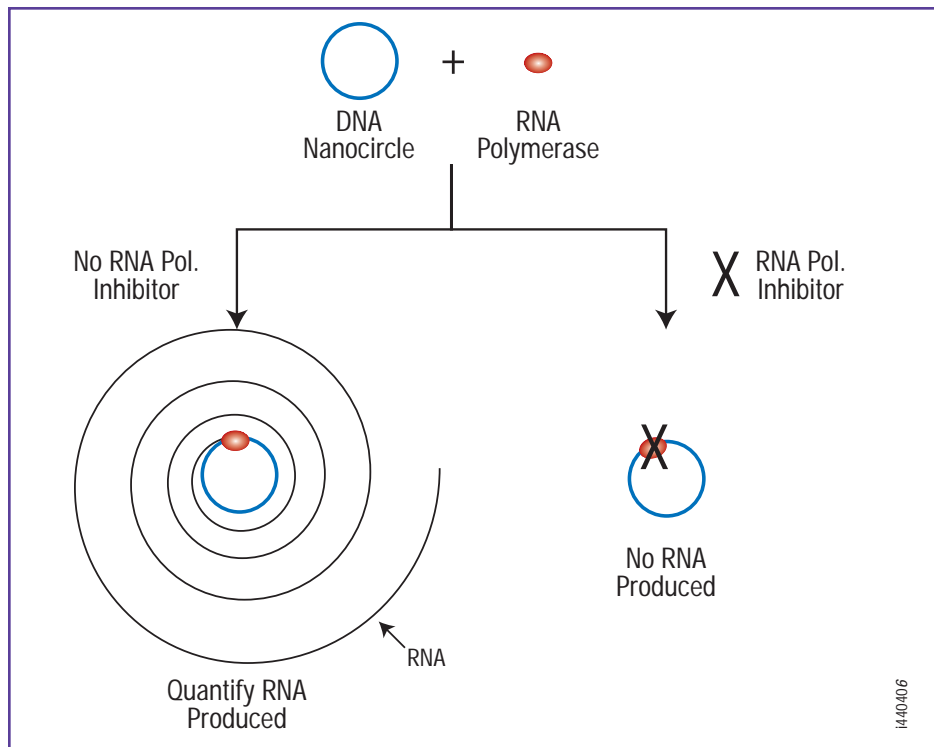


FIG 1. An outline of the Kool™ rolling circle transcription (RCT) assay for RNA polymerase inhibition.

Biotechnologies; the only compound known to potently and selectively inhibit RNA Polymerase III from a variety of eukaryotic organisms, including mammalian cells).

Half a unit of EcRNAP was pre-incubated at 37°C for 10 minutes with or without each inhibitor (25 picomoles of rifampicin, 20 U of Tagetin, or 0.5 ng α -amanitin) in the presence of *E. coli* RNA Polymerase Reaction Buffer, DTT, and 20 U of RNase Inhibitor. Following incubation, 2.5 μ l of SYBR® Green I dye

(1:150 dilution of the 10% stock solution provided in the Kit), and 2 picomoles of Kool NC-45 template were added to the reaction. The reaction was initiated by addition of a mixture of ATP, CTP, GTP, and UTP. The reaction contained 0.5mM NTPs, 8 mM DTT, 40 mM Tris-HCl pH 7.5, 50 mM KCl, 10 mM MgCl₂, and 0.01% Triton®-X 100 (final concentration), plus the proteins and inhibitors, in a 25 μ l volume. RNAP activity was monitored by following the increase of SYBR Green I dye fluorescence at 490

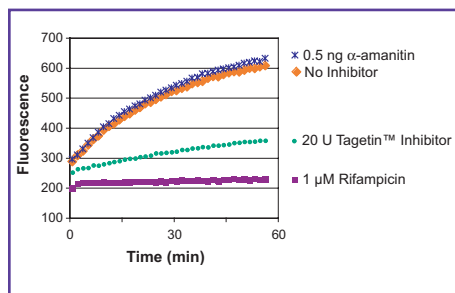


FIG 2. Inhibitors of bacterial RNA polymerases can be rapidly screened using the Kool™ NC-45™ RNA Polymerase Template and the Kool™ NC-45™ RNAP Activity & Screening Kit. The activity of *E. coli* RNA polymerase (Core Enzyme; EcRNAP) was assayed in the presence of the RNAP inhibitors rifampicin, α -amanitin, and Tagetin™ RNA Polymerase Inhibitor, using the Kool NC-45 Template in the Kool Rolling Circle Transcription assay with fluorescent SYBR Green I dye detection. See text for details.

and 530 nm excitation and emission wavelengths using a Bio-Rad iCycler iQ® Real-Time PCR Detection System. For high throughput screening, fluorescence can be measured using 96-well plates and a microplate fluorimeter. As shown in FIG 2, strong inhibition of EcRNAP activity by rifampicin, a known inhibitor of bacterial RNA polymerase,⁴ and partial inhibition by Tagetin⁵ can be detected, while α -amanitin, an inhibitor of eukaryotic RNA polymerase II, has no effect.

Conclusions

The Kool™ NC-45™ Universal RNA Polymerase Template and the Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit enable rapid and sensitive real-time screening of inhibitors of *E. coli* RNA polymerase.

References

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5. Mathews, D.E. and Durbin, R.D. (1990) *J. Biol. Chem.* **265**(1), 493.

* Use of Kool™ Templates in Rolling Circle Transcription reactions is covered by U.S. Patent Nos. 5,714,320; 6,077,668; 6,096,880; 6,368,802; and other patents pending in the U.S. and foreign countries, licensed or assigned to EPICENTRE Biotechnologies. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased product(s) solely for life science research. Contact EPICENTRE concerning licenses for other uses.

www.EpiBio.com/kool.asp

Kool™ NC-45™ RNA Polymerase Template	
KN411100	100 pmoles
Kool™ NC-45™ RNAP Activity & Inhibitor Screening Kit	
KNK49025	25 Reactions

www.EpiBio.com/holoenzyme.asp

<i>E. coli</i> RNA Polymerase Core Enzyme	
C90100	100 Units
C90250	250 Units
C90500	500 Units

<i>E. coli</i> RNA Polymerase Sigma-Saturated Holoenzyme	
S90050	50 Units
S90100	100 Units
S90250	250 Units

www.EpiBio.com/tagetin.asp

Tagetin™ RNA Polymerase Inhibitor		
T9705H	20 U/μl	500 Units
T9701K	20 U/μl	1,000 Units
T9702K	20 U/μl	2,500 Units

Obtain Higher Molecular Weight Soil DNA without Bead-Beating

Bruce W. Jarvis, EPICENTRE Biotechnologies

The SoilMaster™ Kit is ideal for extracting high quality PCR-ready soil DNA without bead-beating.

DNA fragment size, yield, and quality, are important considerations in research. Consequently, when working with soil DNA, it is essential to choose an extraction procedure that does not include bead-beating, which can shear DNA into smaller fragments. EPICENTRE Biotechnologies' SoilMaster™ DNA Extraction Kit is ideal for extracting high quality PCR-ready soil DNA without bead-beating.

FIG 1 shows the effect of bead-beating on soil DNA fragment length. Lanes 2 and 3 were loaded with DNA extracted from an equivalent amount of the same garden soil. In lane 3, the soil DNA was extracted using another vendor's kit (vendor M), which includes bead-

beating. As can be seen, the soil DNA fragment size in lane 3 has been reduced significantly. In contrast, the DNA extracted using the SoilMaster DNA

Extraction Kit in lane 2 is much larger and more typical of the size used to make fosmid libraries (additional field inversion gel electrophoresis data not shown). Fosmid libraries are used for soil metagenomics work—the study of genomes recovered from environmental samples as opposed to those from pure cultures. Furthermore, the results show that the SoilMaster DNA Extraction Kit provides a greater yield of DNA than the kit from vendor M. High yield is important for obtaining representative DNAs from a mixed soil microbial population.

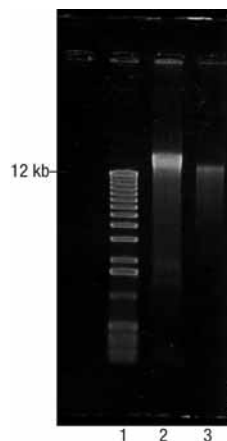


FIG 1. Agarose gel (1%) of DNA extracted from replicate 33 mg samples of garden soil. Lane 1, molecular weight markers; Lane 2, DNA extracted using EPICENTRE's SoilMaster™ DNA Extraction Kit; Lane 3, DNA extracted using vendor M's kit.

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SoilMaster™ DNA Extraction Kit	
SM02050	50 Reactions
Contents: Soil DNA Extraction Buffer, Proteinase K, Soil Lysis Buffer, Protein Precipitation Reagent, Inhibitor Removal Resin, Spin Columns, DNA Precipitation Solution, Pellet Wash Solution and TE Buffer.	