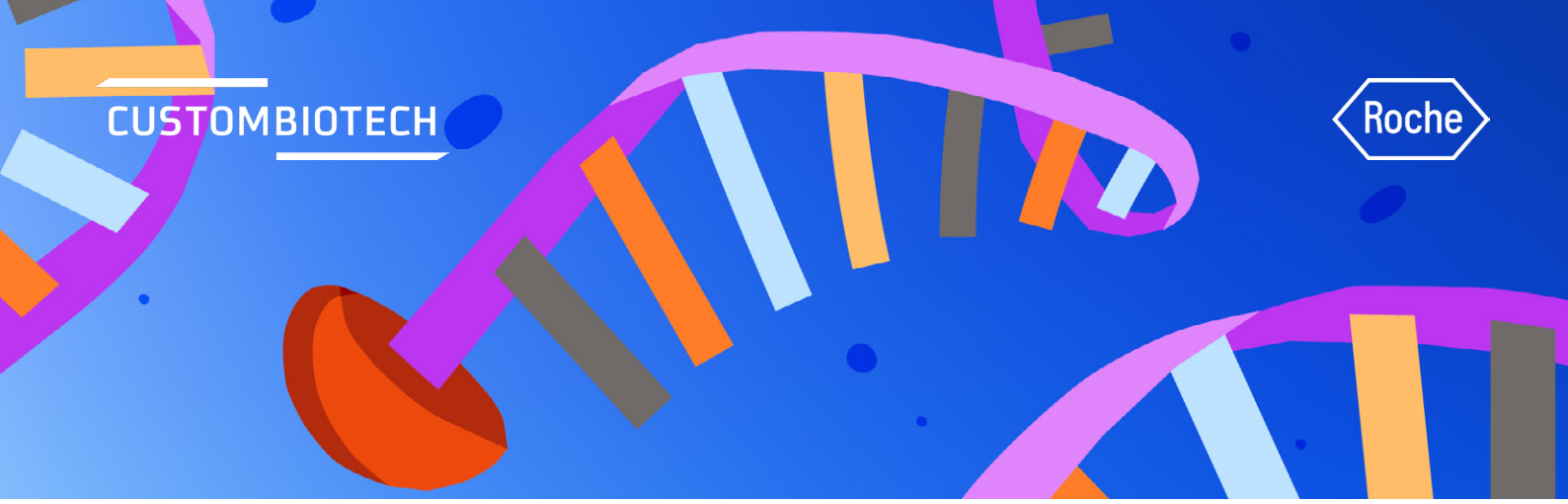


Expand your toolbox for quality mRNA manufacturing with SP6 RNA Polymerase

Starting guidelines for the use of SP6 RNA Polymerase for mRNA synthesis

In vitro synthesis of mRNA molecules is a widely used laboratory procedure that is central to RNA research and for the development of therapeutic applications. Although already decades in development, mRNA was first validated as new drug class with the approval of mRNA COVID-19 vaccines. mRNA is now being considered for diverse applications like protein replacement, cell reprogramming, and gene editing. Individual constructs and intended uses require a tailored manufacturing process. Moving forward, expanded tool-box to produce mRNA will be needed.

In response to customer requests, we now added SP6 RNA Polymerase in a GMP Grade and fit-for-purpose format to our portfolio. This expands options in mRNA manufacturing for our customers. Here we present some starting guidelines for the use of SP6 RNA Polymerase.

Overview

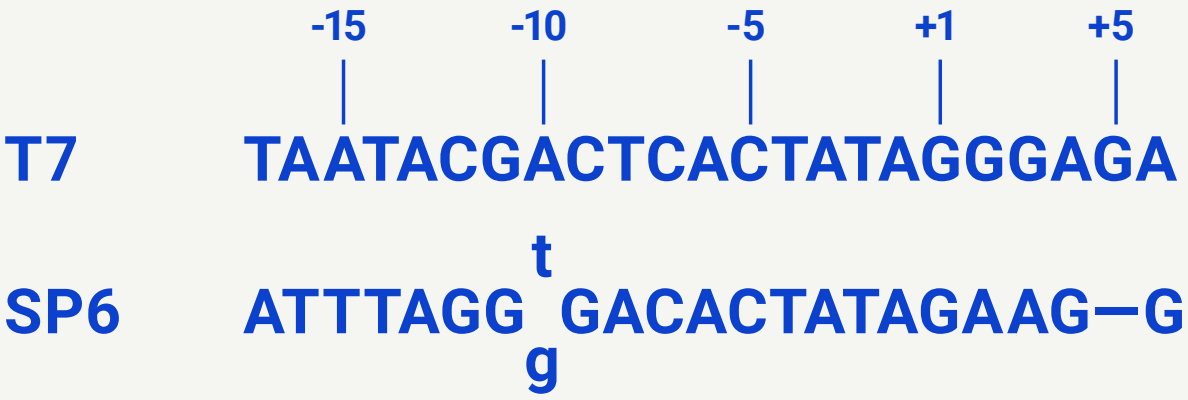
Synthesis of mRNA through in vitro transcription (IVT) utilizes a diverse mix of enzymes, proteins, and ribonucleotides. However, RNA polymerase is the main driver of the reaction to produce mRNA. In simple terms, mRNA synthesis starts when an RNA polymerase recognizes and binds to a highly specific promoter sequence encoded in the DNA template. This is followed by the elongation of the mRNA strand through the incorporation of ribonucleotides by the RNA polymerase.

While T7 RNA polymerase is a popular choice for mRNA production (Comirnaty by Pfizer/BioNTech and SpikeVax by Moderna[1], other polymerases also work well for mRNA synthesis. SP6 RNA Polymerase is commonly used in molecular biology research along with T7 and T3 RNA polymerases.

As a starting point, the following is a first technical guidance to test SP6 RNA Polymerase for your mRNA production process.

Introduction

SP6 RNA polymerase is similar to T7 RNA polymerase in that both are single subunit enzymes and do not require any other additional transcription factors to function. Both enzymes also provide stringent promoter specificity and very high efficiency.[2-5] Where they differ, however, is in their unique promoter sites. (Fig. 1) That difference expands options in mRNA synthesis. For example, SP 6 prefers the sequence 5'GAAGA at the start site while T7 RNA polymerase prefers 5'GGGAG.[7]



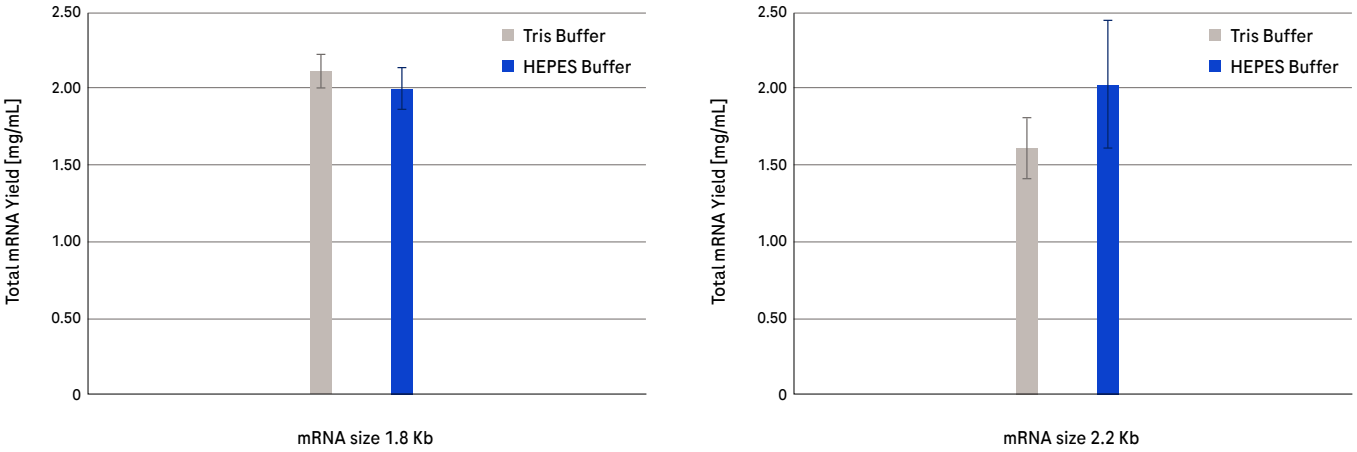
01
Comparison of the promoter sequences for T7 and SP6 RNA Polymerase.

Both promoters share a core sequence that extends from -7 to +1. This region may play a common function in promoter function. They diverge in the region from -8 to -12, suggesting that promoter-specific contacts are being made in this region. By convention, the nontemplate strand is presented.[6]

Follow these technical guidelines to test if SP6 RNA Polymerase is a good fit for your specific template and *in vitro* transcription process (technical guidance for T7 RNA Polymerase).[8]

General guidelines for reaction set up

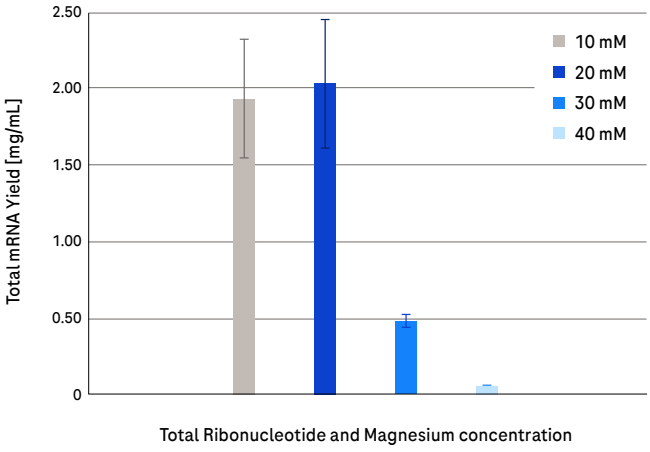
Multiple buffer options give good overall yield with different constructs
In our experiments, SP6 RNA Polymerase provided good yields using using either Tris or HEPES-based IVT reaction buffers (Fig 2). Similar observations are reported in the literature. Combined with high-capacity buffers that maintain the pH throughout the reaction, SP6 RNA Polymerase achieves maximum yield in the IVT reaction. The optimal reported pH range is between pH 7.0 and 8.5.2,9



02
Testing the IVT reaction in HEPES and Tris buffer systems using two different plasmid constructs. (Data on file at Roche: Latta, Application Lab, May 2023)

Optimization of ribonucleotide and Mg2+ concentration for yield

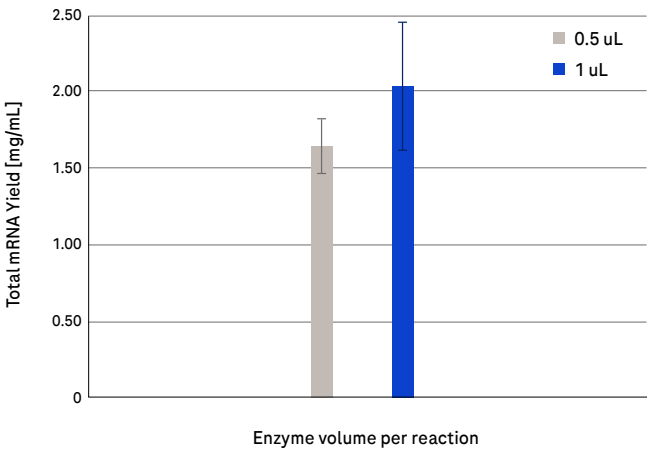
Total ribonucleotide and magnesium concentrations have the most significant impact on the RNA yield. In our experiments, a 1:1 ratio of magnesium to total ribonucleotide concentration showed the greatest yields, as long as the total ribonucleotide concentration did not exceed 20 mM (Fig. 3). These results differ from T7 RNA polymerase, which tolerates towards higher ribonucleotide and magnesium concentrations of up to 40 mM.[9]



03
Titration of the Mg^{2+}/ribonucleotide concentration in the IVT reaction. Mg^{2+} and total rNTP concentrations varied between experiments but were kept at a constant ratio of 1:1 for a given experiment. (Data on file at Roche: Latta, Application Lab, May 2023)

SP6 enzyme concentration has moderate influence on yield

SP6 RNA Polymerase showed robust performance even at low enzyme concentration, under optimized conditions for other factors like ribonucleotide: magnesium ratio and template concentration. Increasing the enzyme concentration improved performance slightly for the 2.2 Kb construct, but optimization with higher enzyme concentration might be crucial for longer mRNA transcripts.



04
Testing the IVT reaction with different amounts of enzymes. (Data on file at Roche: Latta, Application Lab, May 2023)

Experimental conditions

The reaction mix used in the described experiments consisted of 500 ng DNA template of a linearized plasmid, 1 µl SP6 RNA polymerase (CustomBiotech 09788131103), 1 µl pyrophosphatase (CustomBiotech 08140677103), 20 U RNase inhibitor (CustomBiotech 09537643103), 2 µl reaction buffer, and RNase-free water to a final volume of 20 µl. The reaction buffer was prepared from a 10X transcription buffer master mix.10X Tris reaction buffer: 400 mM Tris-HCl, 20 mM spermidine,100 mM DTT, pH 7.9 OR 10X HEPES reaction Buffer: 1 M HEPES-KOH, 20 mM Spermidine, 400 mM DTT, pH 7.5.

The reaction was incubated for 1 h at 37 °C. RNA was subsequently purified using the High Pure FFPET RNA Isolation Kit (Mat # 06650775001, sold by Roche Molecular Systems; www. lifescience.roche.com), including proteinase K digestion and DNase I treatment according to the manufacturer's instructions. Experiments were performed in triplicates. Subsequently, the resulting RNA was analyzed using the Agilent® Bioanalyzer and quantified using a UV/Vis spectrophotometer at 260 nm (NanoDrop™). Experiment-specific adaptations are mentioned in the respective figure descriptions. Experiments shown in figures 3 and 4 were performed in HEPES buffer with a 2.2 kb mRNA generating template construct.

Key takeaways

When working with SP6 RNA Polymerase, some components of the IVT reaction – buffers, RNAse inhibitor and pyrophosphatase concentrations – can be used in similar amounts as in T7 RNA polymerase reactions.

Optimization of the total ribonucleotide and magnesium concentrations seems to be a critical factor for SP6 RNA Polymerase. The enzyme is sensitive to higher concentrations of ribonucleotide: magnesium.

Use these starting guidelines to determine if SP6 RNA Polymerase is a good alternative for your specific template and optimize your process.

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