Producing low-immunogenicity, high-stability mRNA with dsRNA reduction to <0.005% (LLOQ)

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Optimizing mRNA design for therapeutic applications is critical to ensure maximum protein expression, low impurities and a reduced immune response. This poster describes how to efficiently generate lower immunogenicity mRNA for downstream applications while also reducing dsRNA content using the INCOGNITO™ mScript™ Complete mRNA Production Systems.

CONSIDERATIONS FOR OPTIMAL mRNA DESIGN

Optimizing mRNA design enables robust protein expression, minimizes immunogenicity, and provides mRNA stability and longevity in cells.

The most important design feature for therapeutic applications is nucleoside selection—particularly the incorporation of modified nucleosides, which can increase mRNA stability and reduce innate immunogenicity.

5′ capping provides stability and recognition by cellular translational machinery. Generally, either a Cap 0 or Cap 1 cap is used. The addition of Cap 1 marks the mRNA as 'self', thus reducing innate immune responses. Caps can be added either post-transcriptionally or co-transcriptionally. Post-transcriptional capping uses enzymes to cap *in vitro* transcribed (IVT) RNA by first adding Cap 0, which is then methylated to Cap 1. This process has a high capping efficiency of almost 100%. In contrast, co-transcriptional capping incorporates a cap analog during transcription, which results in a lower capping efficiency.

At the 3′ end, longer poly(A) tails protect from enzymatic degradation, thus improving mRNA stability. Exonuclease digestion in cells shortens the tail eventually, leading to instability of the 5′ cap and de-capping. Poly(A) tails can be added either post-transcriptionally, which can generate tails of over 300 bases, or co-transcriptionally, which limits tail lengths to approximately 60–120 bases.

INCOGNITO mScript Complete Kits provide all reagents to generate a functional post-transcriptionally capped and tailed mRNA with reduced immunogenicity due to incorporation of modified nucleosides and dsRNA removal.

dsRNA REMOVAL IS CRUCIAL FOR REDUCING IMMUNOGENICITY

dsRNA is an unwanted byproduct of IVT, and all IVT RNA and mRNA preps will contain some degree of dsRNA contamination. As cells have evolved pattern recognition sensors to detect dsRNA from viral infections, the presence of dsRNA will trigger an immune response. Removal of dsRNA is essential for reducing immunogenicity and has traditionally been performed using HPLC or chromatography columns.

A new highly efficient enzymatic and scalable method for dsRNA removal is provided by the Min-Immune™ Gold dsRNA Removal Kit, which reduces dsRNA to <0.005% LLOQ per sample (**Figure 1**). This is achieved without any reduction in the ssRNA sample, unlike chromatographic methods. The INCOGNITO mScript Complete Kits includes a Min-Immune Gold module for dsRNA removal.

mRNA QUALITY ANALYSIS

To ensure a high quality of mRNA, 5′-capping efficiency and 3′-poly(A) tail lengths should be analyzed. The EZ-QC™ mRNA Assay Kits can be used to perform these analyses using polyacrylamide gel electrophoresis at a standard laboratory bench. The assays are cost effective and only require low input mRNA amounts at picomolar levels. **Figure 2** shows an example of the results from the EZ-QC mRNA Capping Efficiency Assay Kits.

SUMMARY

INCOGNITO mScript Complete Kits simplify the synthesis of ultra-low immunogenicity, high-stability mRNA. They provide high yield transcription as well as approximately 100% capping efficiency via post-transcriptional capping. Post-transcriptional tailing offers an expanded poly(A) tail-length range with the standard protocol generating poly(A) tails of approximately 150–200 bases, and the modified protocol enables tails of over 300 bases. The Min-Immune Gold dsRNA Removal Module can remove dsRNA to less than 0.005% LLOQ per sample.

Figure 1. Comparison of Min-Immune Gold-treated 1.4-kb pseudouridine (Ψ)-containing RNA sample to an untreated sample (right) and dsRNA standards (left panel). Min-Immune Gold-treated dsRNA is reduced below the limit of quantitation (less than 0.005% LLOQ) after treatment.

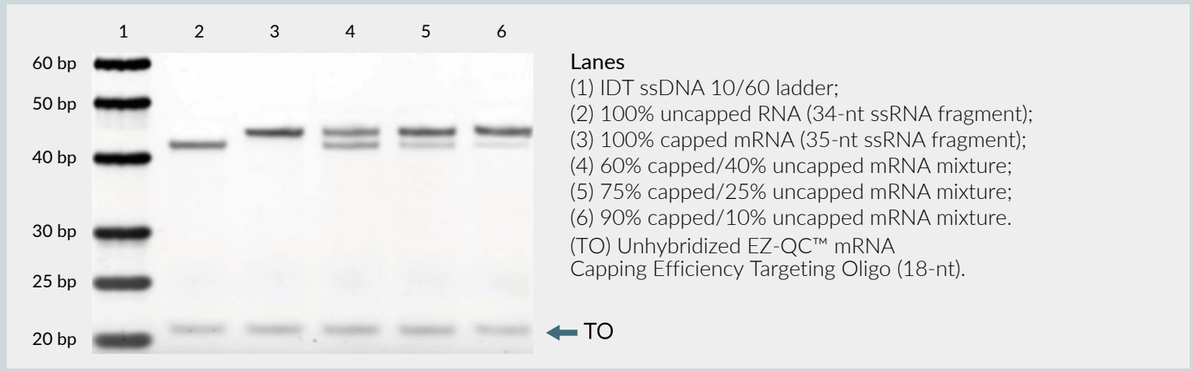

Figure 2. Mixtures of capped and uncapped mRNA were assayed using the EZ-QC mRNA Capping Efficiency Assay Kit and resolved on a polyacrylamide gel. The targeting oligonucleotide facilitates cutting the mRNA at a single, repeatable location allowing for greater confidence in determining the percentage of capped and uncapped content of the assayed samples.

Jen Dennin is a Product Manager at CELLSCRIPT™. Prior to joining CELLSCRIPT she spent over 9 years in R&D, including positions at Exact Sciences and Illumina. She earned her MSc in Biotechnology from the University of Wisconsin–Madison, WI, USA and CAPM certification through the Project Management Institute, PA, USA.