Optimized IVT Platforms: Advancing RNA Quality and Scalability for Animal Biologics Innovation

Hélène Boyer[1], Laurence Delaurière[1], Dana Brecklin-Benassi[1], Daniela Torres Campana[1], Laura Alexander[1], Maria Dashek[1]

[1]Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711

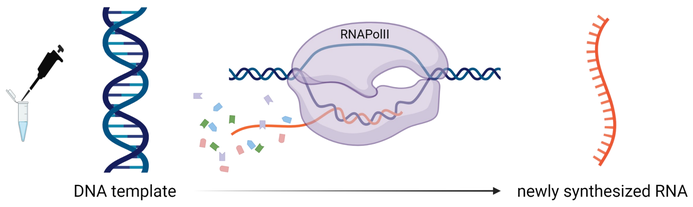
1. Introduction: Tackling the Challenges in Animal Biologics

In the rapidly evolving field of animal biologics development, producing high-quality RNA is essential for developing next-generation vaccines and therapeutic interventions. Advanced in vitro transcription (IVT) methodologies provide researchers and process engineers with scalable options to generate large quantities of high-integrity RNA. From efficient transcription systems to precise clean-up and automated workflows, these approaches address the rigorous demands of animal health applications by minimizing immunogenic risk and ensuring consistent results.

2. Advanced RNA Production Technologies

The RiboMAX™ Large Scale RNA Production Systems enable the production of **milligram amounts of RNA** from a linear DNA template. They are typically used to produce transcripts up to **5-6kb** in size, although much longer transcripts have been successfully generated.

Capped and **uncapped** RNA transcripts can be produced.



Main Applications:

- In vitro translation
- In vivo translation
- Synthesis of tRNA, rRNA, siRNA…
- Synthesis of RNA virus genomes
- Synthesis of ribozymes
- Synthesis of guide RNA for CRISPR gene editing[1]
- cDNA library preparation[2]
- Study of RNA:protein interaction[3,4]
- Study of RNA splicing
- Drug discovery
- mRNA therapeutics/vaccine
- …

3. Ensuring RNA Integrity and Purity: Comparative Clean-Up Solutions

ReliaPrep™ RNA Clean-Up and Concentration System

After in vitro transcription (20µl reaction volume), RNA was cleaned-up using either the standard protocol recommended in the technical manual (Phenol chloroform; N=3) or using the ReliaPrep™ RNA Clean-Up and Concentration System (N=4).

➔ *Yields were lower with the ReliaPrep™ system, but RNA integrity remained comparable across methods.*

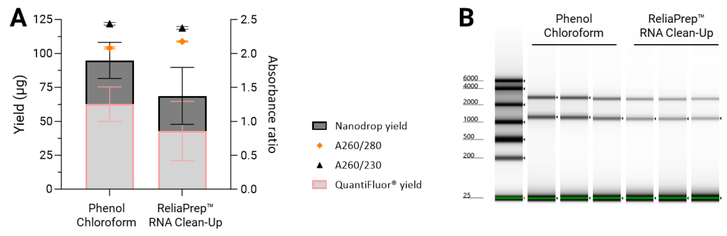


Figure 1. Comparison of phenol chloroform and ReliaPrep™ RNA Clean-Up methods. A. Yields (in µg) expressed as mean ± standard deviation, determined using Nanodrop™ or QuantiFluor® RNA System (left y axis). Absorbance ratios (mean ± standard deviation) are represented on the right y axis. **B.** RNA integrity was assessed using an RNA ScreenTape.

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Maxwell® RSC miRNA Tissue Kit

After in-vitro transcription (100µl reaction volume), RNA was cleaned-up using the Maxwell® RSC miRNA Tissue Kit, from an input volume of 20µl, 50µl or 100µl (N=3 for each).

➔ *Under comparable conditions, yields matched those from the phenol chloroform protocol, with linear scalability from 20 to 100µl.*

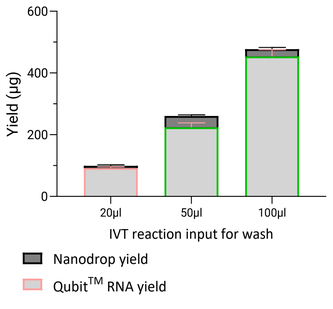

Figure 2. Clean-up of IVT reaction using the Maxwell® RSC miRNA Tissue Kit. Yields (in µg) expressed as mean ± standard deviation, determined using Nanodrop™ or Qubit™ RNA BR Assay Kit. Yields are linear between 20 and 100µl (R^2≥0.997 for both quantitation methods).

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ProNex® Size-Selective Purification System

After in vitro transcription (20µl reaction volume), RNA was cleaned-up using the ProNex® Size-Selective Purification System (N=2).

➔ *The ProNex® Size-Selective Purification System produced higher yields than phenol chloroform, with good RNA integrity.*

Sample #	Concentration (ng/µl)	A260/280	A260/230	Total yield (µg)
1	1418	2.16	2.44	141.8
2	1391.7	2.15	2.43	139.2

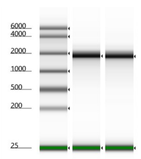

Figure 3. Clean-up of IVT reaction using the ProNex® Size-Selective Purification System. Concentrations and yields (in µg) were determined using Nanodrop™, and RNA integrity was assessed using an RNA ScreenTape.

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4. Innovative Workflows: From Circular RNA to Long ssDNA

In vitro transcription reaction volume scale-up

RNAs were generated by in vitro transcription in a 100µl, 1ml, 5ml or 10ml reaction volume (N=2 for each), and dye-based quantified.

➔ *In vitro transcription can be scaled to 10 ml while maintaining consistent RNA yields.*

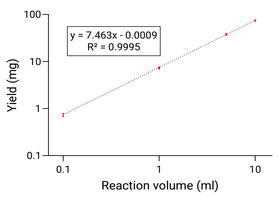

Figure 4. IVT reaction volume scale-up. Yields (in mg) determined using Qubit™ RNA BR Assay Kit. Yields are linear between 100µl and 10ml reaction volume.

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Synthesis of circular RNA using the RiboMAX™ System

Circular RNAs can be synthesized in vitro as shown by sequencing of the circRNA junction and ScreenTape analysis.

➔ *Reaction products are a mix of circRNA and linear precursors, which can be isolated by gel purification or RNase R digestion.*

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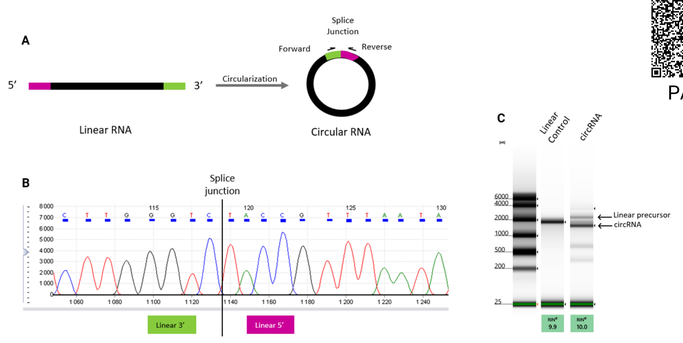
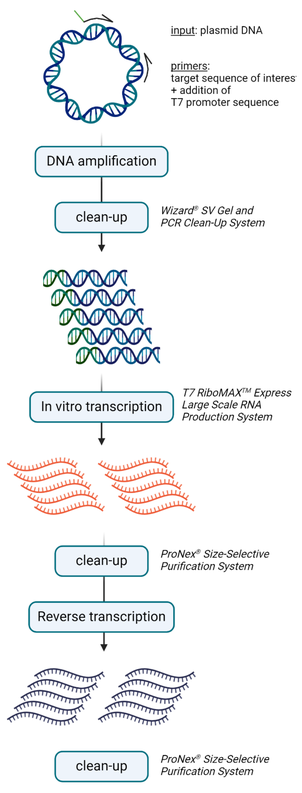


Figure 5. circRNA synthesis. A. Schematic Representation of the junction and primers used for sequencing. **B.** Electropherogram obtained by Sanger sequencing confirming the RNA circularization. **C.** RNA circularization was visualized using an RNA ScreenTape.

Workflow: long ssDNA template synthesis for CRISPR insertion

Long ssDNA can be synthesized using a multistep workflow that includes in vitro transcription, followed by appropriate purification and reverse transcription steps.



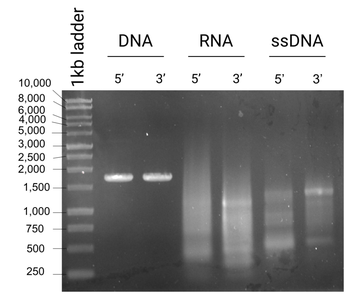


Figure 6. long ssDNA synthesis. Right. Schematic workflow representation. Plasmid DNA (input) is amplified by PCR with the addition of the T7 promoter sequence (in 5' or 3' position) and cleaned-up using the Wizard® SV Gel and PCR Clean-Up System. Then, in vitro transcription is performed using the T7 RiboMAX™ Express Large Scale RNA Production System. Synthesized RNAs are cleaned-up using the ProNex® Size-Selective Purification System and reverse transcribed. A final clean-up step using the ProNex® Size-Selective Purification System enables the obtention of clean ssDNA. **Left.** Example of intermediate and final products. 'DNA' was generated using the Q5® High-Fidelity 2X Master Mix, 'RNA' was generated by in vitro transcription, and 'ssDNA' corresponds to reverse transcription output.

5. Quality Control: Reducing Immunogenicity with dsRNA Detection

dsRNA is a common by-product of in vitro transcription responsible for immunogenicity and decreased protein translation efficiency[5]. The Lumit® dsRNA Detection Assay was developed to quantitate dsRNA in biological samples.

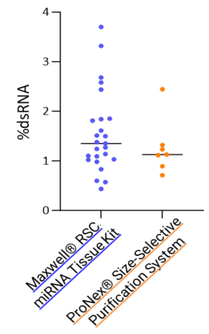


Figure 7. dsRNA detection using the Lumit® dsRNA Detection Assay, for in vitro transcribed RNAs cleaned-up using the Maxwell® RSC miRNA Tissue Kit or the ProNex® Size-Selective Purification System.

6. Automation for Consistency and Scale

Automation streamlines production, reduces hands-on time, and ensures reproducibility in process development. An automated method has been developed on the CyBio FeliX Liquid Handler from Analytik Jena, enabling the automation of the synthesis of RNA from a linear DNA input.

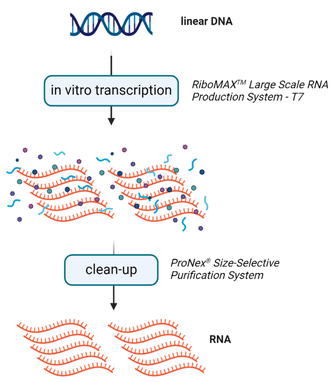


Figure 8. Automated synthesis of RNA from a linear DNA input. Schematic representation of the automated workflow. Linear DNA is used as input. In vitro transcription is carried out using the RiboMAX™ Large Scale RNA Production System T7 (automated dispensing of the reaction mix onto the linear input plate). The synthesized RNA is cleaned-up using the ProNex® Size-Selective Purification System.

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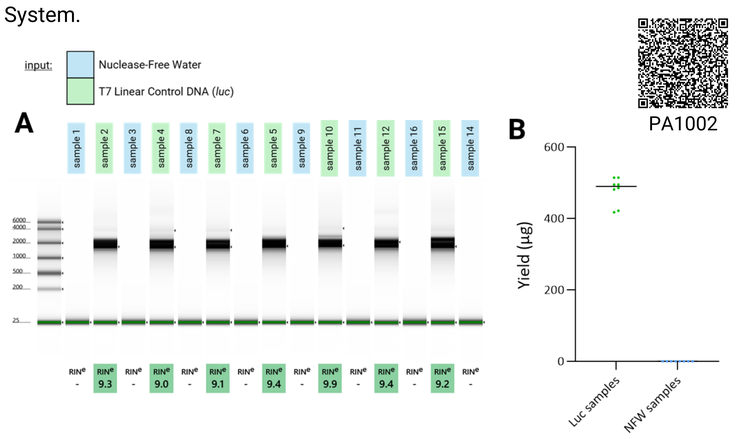

Figure 9. Automated transcription and clean-up on the CyBio FeliX. Control RNA was generated and cleaned-up according to the workflow represented above in an automated fashion on the CyBio FeliX, according to a checkerboard pattern (linear control RNA and Nuclease-Free Water were used as input). Eluates were analyzed using the TapeStation 4200 and quantified using the Qubit™ RNA HS Assay Kit on the Quantus™ Fluorometer.

➔ *RNA was successfully produced in an automated fashion on the CyBio FeliX, with no cross contamination detected.*

7. Conclusions

- Unlock scalable, high-quality RNA production tailored for animal biologics development.
- Leverage advanced clean-up methods, dsRNA detection, and automation to streamline workflows and boost reproducibility.
- Drive innovation in animal biologics development through these integrated technologies.

8. References

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2. Fujimori, S. *et al.* (2012) Next-generation sequencing coupled with a cell-free display technology for high-throughput production of reliable interactome data. *Sci Rep* **2**, 691.
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Corresponding author: helene.boyer@promega.com