Production of mRNA by *in vitro* transcription using a single-use system and ReadyToProcess™ WAVE™ 25 rocker

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Summary

Single-use (SU) components were evaluated for production of messenger RNA (mRNA) by *in vitro* transcription (IVT). An initial test was performed using a Cellbag™ bioreactor container and a ReadyCircuit™ jumper Y manifold to investigate the presence of RNase activity and the suitability of the SU format for IVT reactions. This test was followed by three separate mRNA transcriptions in Cellbag bioreactor containers at 150-mL scale using a linearized 7 kb DNA template that provides the sequence to be transcribed into 2000 nt mRNA. No difference in the assessed quality attributes was observed between IVT reactions performed in Cellbag bioreactor containers and control tubes. Yields were comparable and integrity analysis by capillary gel electrophoresis (CGE) showed no apparent sign of degradation. These results indicate Cellbag bioreactor containers can be used for IVT reactions, at 150 mL scale but most likely also smaller and larger volumes. A single test of tangential flow filtration (TFF) was also performed using a Centramate™ omega cassette (100 kDa NMWCO). The recovery was > 90%. No sign of degradation was detected by CGE analysis and no loss of material in permeate was observed. These results indicate that the chosen nominal molecular weight cutoff (NMWCO) is appropriate for this size of mRNA and larger constructs.

Pretest of the SU format for IVT

A single-point mRNA stability test was performed using a ReadyCircuit jumper Y manifold and a 2 L Cellbag bioreactor container with material obtained from a previous IVT reaction. In addition, a 1-mL scale IVT reaction was performed without presence of RNase inhibitor in the Cellbag bioreactor container. Each sample was analyzed using the RNaseAlert RNase detection kit. The positive control (RNase + water) displayed significant RNase activity while no RNase activity was observed in any of the tested SU components or the controls (Fig 1). RiboGreen analysis of the stability of mRNA in the tested tubing showed no difference compared to the controls (Fig 2), and the single-point experiment comparing IVT in a Cellbag bioreactor container and the control indicated no difference in concentration (Fig 3).

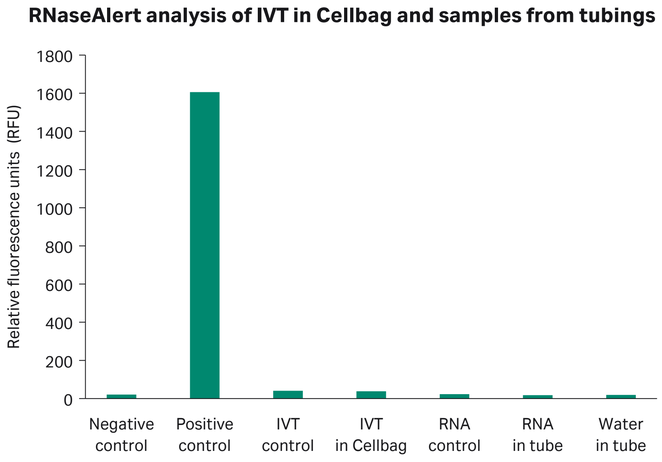

Fig 1. Results from RNase activity analysis.

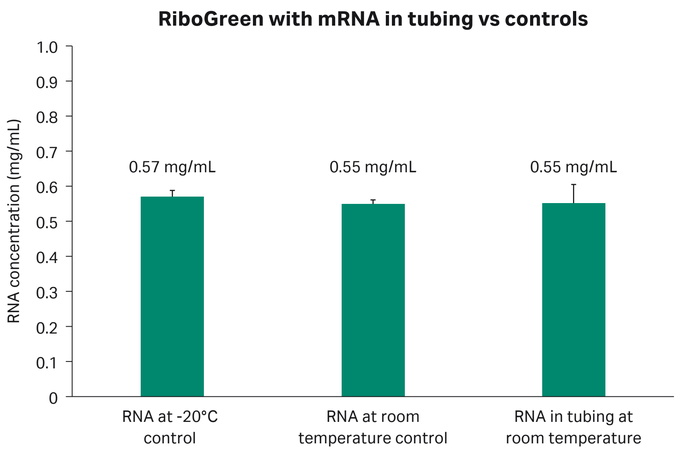

Fig 2. Controls incubated in Eppendorf® tubes at -20°C and room temperature, respectively, compared to incubation in the tubing of a tubing kit at room temperature.

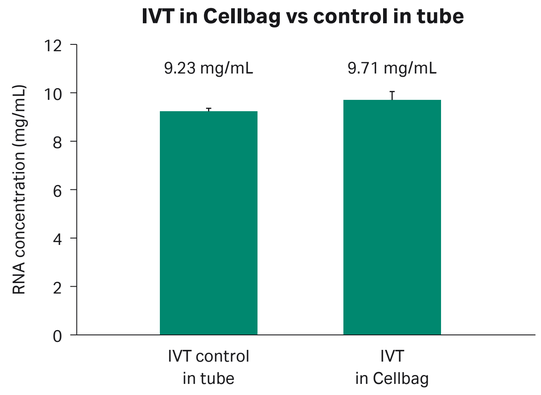

Fig 3. RiboGreen analysis of crude IVT mRNA from control and Cellbag bioreactor container.

IVT reaction

Three 150-mL IVT reactions were performed using the ReadyToProcess WAVE 25 rocker using a target temperature of 37°C, a tilt angle of 6° to 7°, and a rocking speed of 40 rpm. A master mix of all IVT components, except T7 polymerase, was preheated for approximately 30 min to reach 37°C before T7 polymerase was added. The reaction was performed in the presence of RNase inhibitor to reduce the risk of failure. All liquids were transferred to the Cellbag bioreactor container with a ReadyToProcess™ Pump 25. Control IVT reactions were performed at 1-mL scale at 37°C. The reaction time was approximately 2.5 h. No 5'-capping was performed. DNase template digestion was performed for 60 min at 37°C before EDTA was added to quench the enzymatic reaction. Buffer was added to the bag to minimize losses before the solution was pumped out. Additional buffer was added to the IVT to a final five-fold dilution of the crude reaction. Total duration of all steps was 5–6 h, including setup, reaction, and digestion of DNA. Analysis of the final concentration of mRNA was performed by RiboGreen assay and LiCl precipitation (Table 1). No apparent difference was observed between IVT performed in Cellbag bioreactor containers and the IVT control tubes.

Table 1. Concentration analysis of IVT reaction product following 5× dilution

Sample	RiboGreen (mg/mL)	LiCl (mg/mL)
Cellbag run 1	3.79	2.82
Cellbag run 2	1.79	2.70
Cellbag run 3	2.33	2.33
Cellbag average	2.64	2.62
%CV Cellbag	32%	8%
Control run 1	0.71	2.59
Control run 2	2.50	2.74
Control run 3	2.61	2.55
Control average	1.94	2.62
%CV Control	42%	3%

Tangential flow filtration (TFF)

A single TFF evaluation experiment was performed using a Centramate omega cassette (100 kDa NMWCO, 93 cm^2 surface area) mounted to a Centramate LV holder using a PSG Biotech Quattroflow® 150S pump. The material used was a diluted sample (0.20 mg/mL) from Cellbag bioreactor container IVT1 with a volume of 70.7 mL. The final diafiltration (DF) factor was 7.0. Prior to product recovery, the retentate was concentrated 1.5× to 46.4 mL to accommodate wash out with 25 mL of buffer. The final volume was 73.1 mL. Average permeate flux was 45.1 LMH ($L/m^2/h$) and the average transmembrane pressure (TMP) was 0.15 bar (Fig 4).

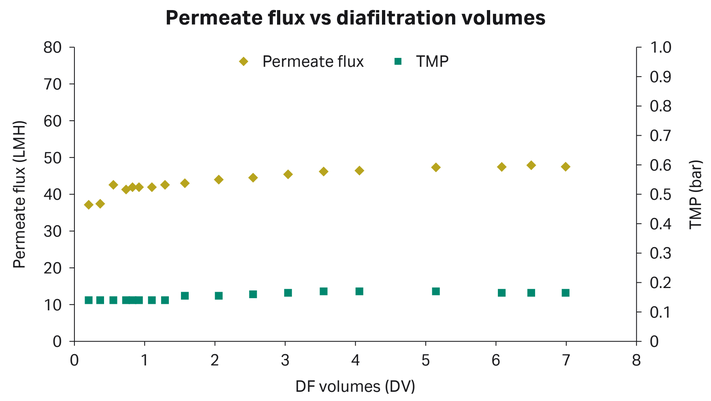

Fig 4. Permeate flux and TMP curves from DF using a 100 kDa NMWCO cassette.

Integrity analysis

Analysis of mRNA integrity was performed using CGE on the three 150-mL IVT Cellbag bioreactor container reactions (Fig 5) and the samples from TFF evaluation (Fig 6). No visible degradation of mRNA was detected. Differences in band intensity in Figure 6 are due to the difficulty in preparing the required dilutions for samples with unknown concentrations. The retentate recovery was > 90% with no detectable loss of material in the permeate.

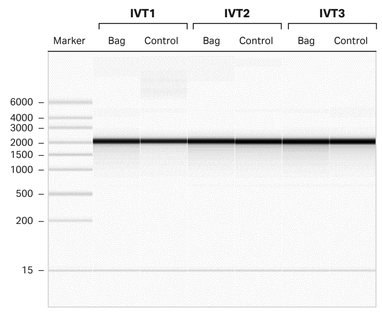

Fig 5. Integrity analysis of mRNA from the three Cellbag IVT reactions by CGE.

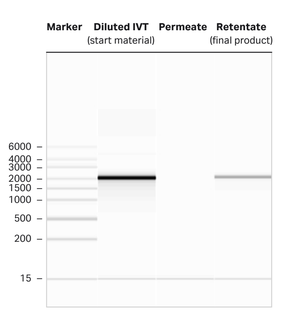

Fig 6. Integrity analysis of mRNA from TFF by CGE.

Conclusions

- IVT reactions performed in Cellbag bioreactor containers demonstrate comparable performance to small-scale tube reactions.
- Single-use components demonstrate low background RNase activity.
- TFF with 100 kDa cut-off cassettes indicates purification potential with maintained integrity and good recovery.

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