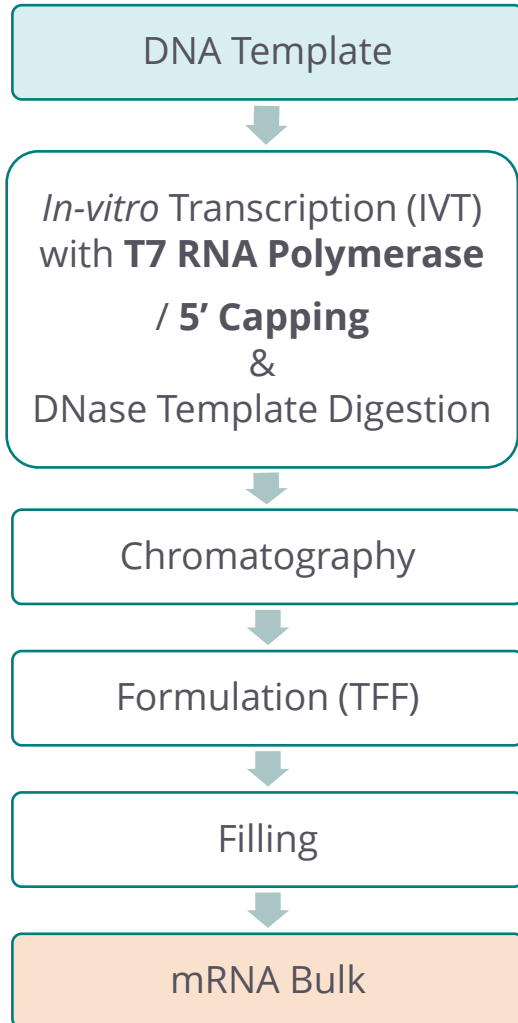




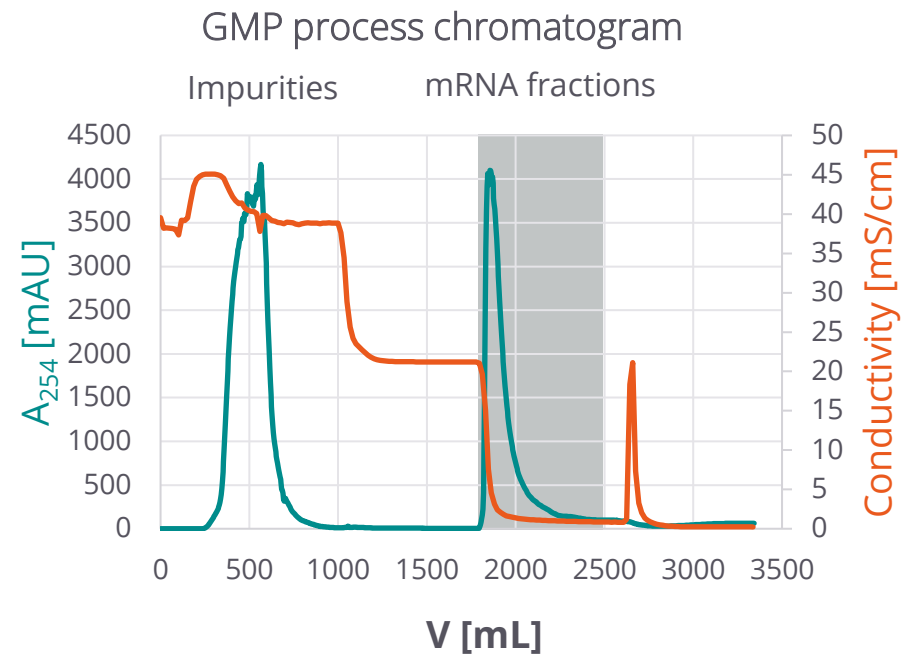
Manufacturing of mRNA by fed-batch *in-vitro* transcription:
optimization of production costs, space-time yield & product quality

mRNA cGMP Manufacturing



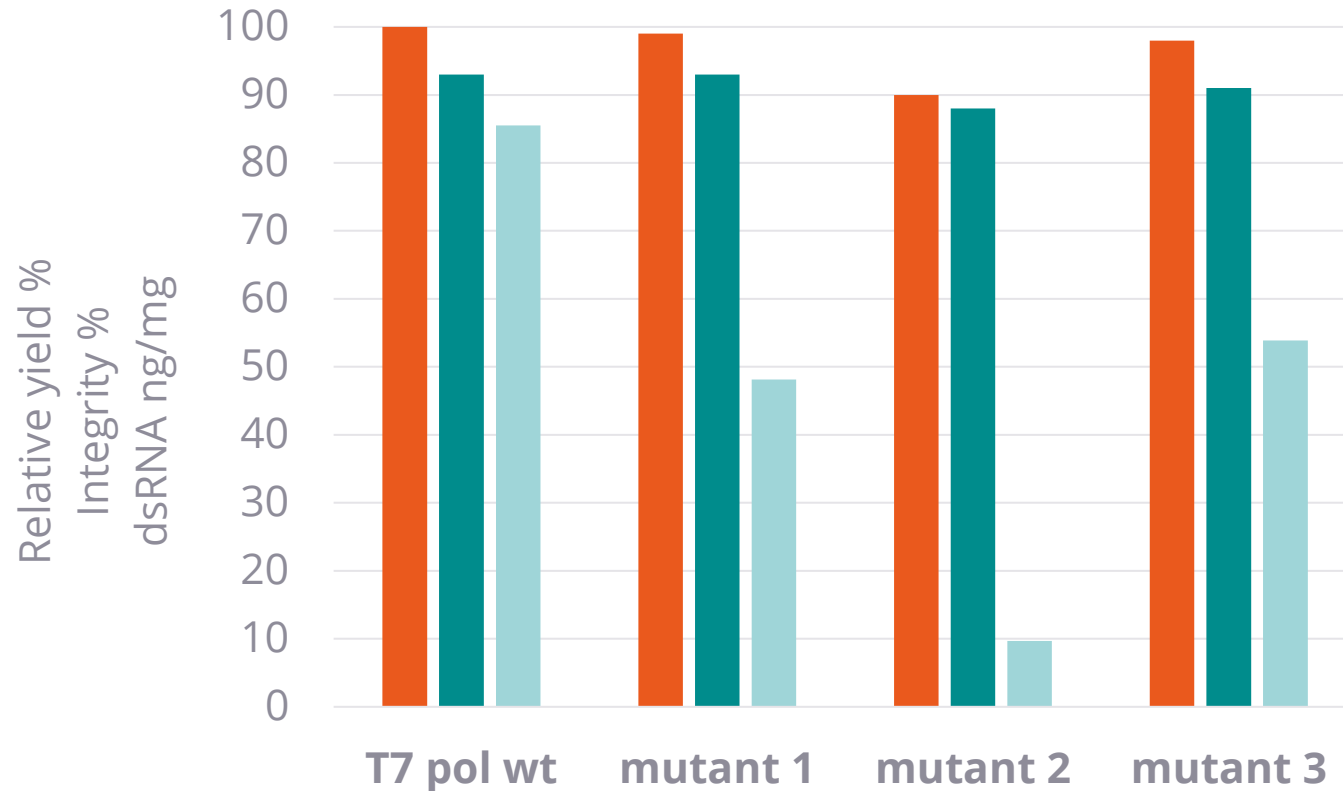
Goals for process intensification at GMP scale:

- (1) Increase IVT yield / reduce COGs
- (2) by maintaining mRNA quality / integrity
- (3) and limit double-stranded RNA



Impact of T7 Polymerase on mRNA Yield and Quality

Comparison different T7 RNA-polymerases (batch mode)

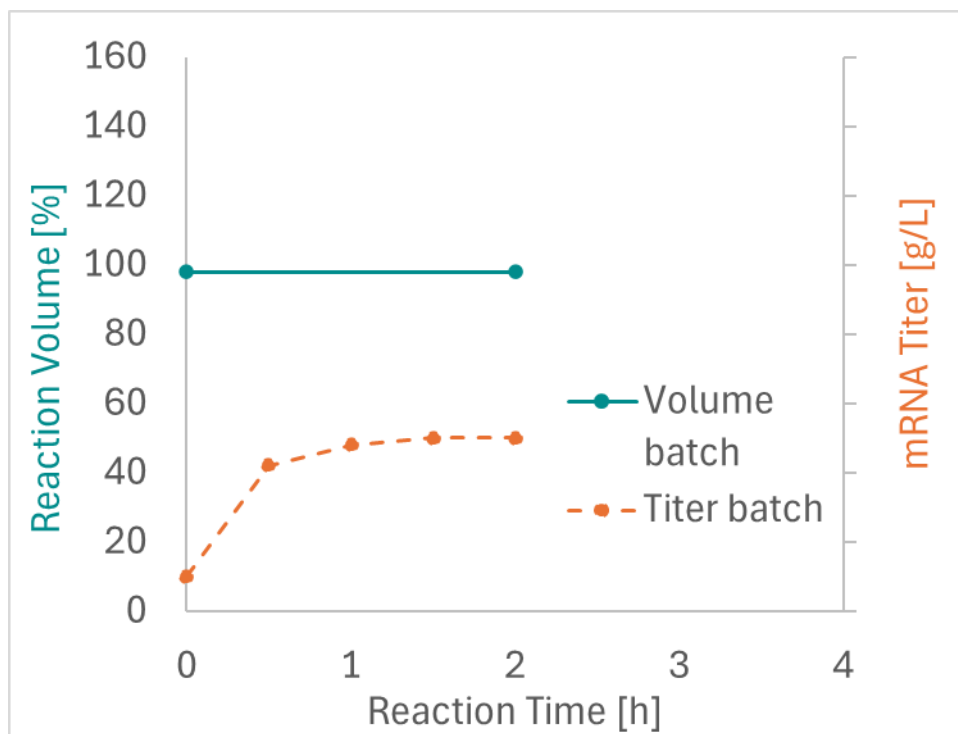


- Wild type T7 RNA polymerase
- vs. 3 different mutant polymerases
- Testing for:
 - mRNA IVT titer/yield (RP HPLC)
 - mRNA integrity (CGE)
 - dsRNA (ELISA)

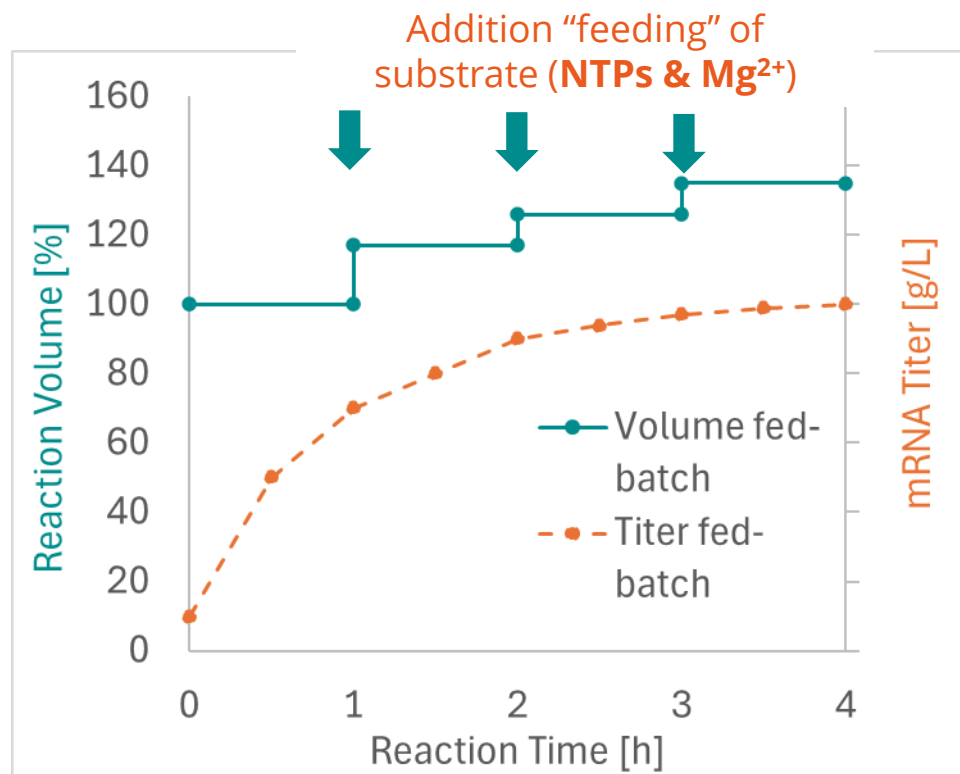
- Relative IVT yield [%]
- mRNA integrity [%]
- dsRNA content [ng/mg RNA]

Batch vs. Fed-batch In-vitro Transcription

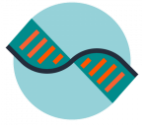
Batch mode IVT



Fed-batch mode IVT



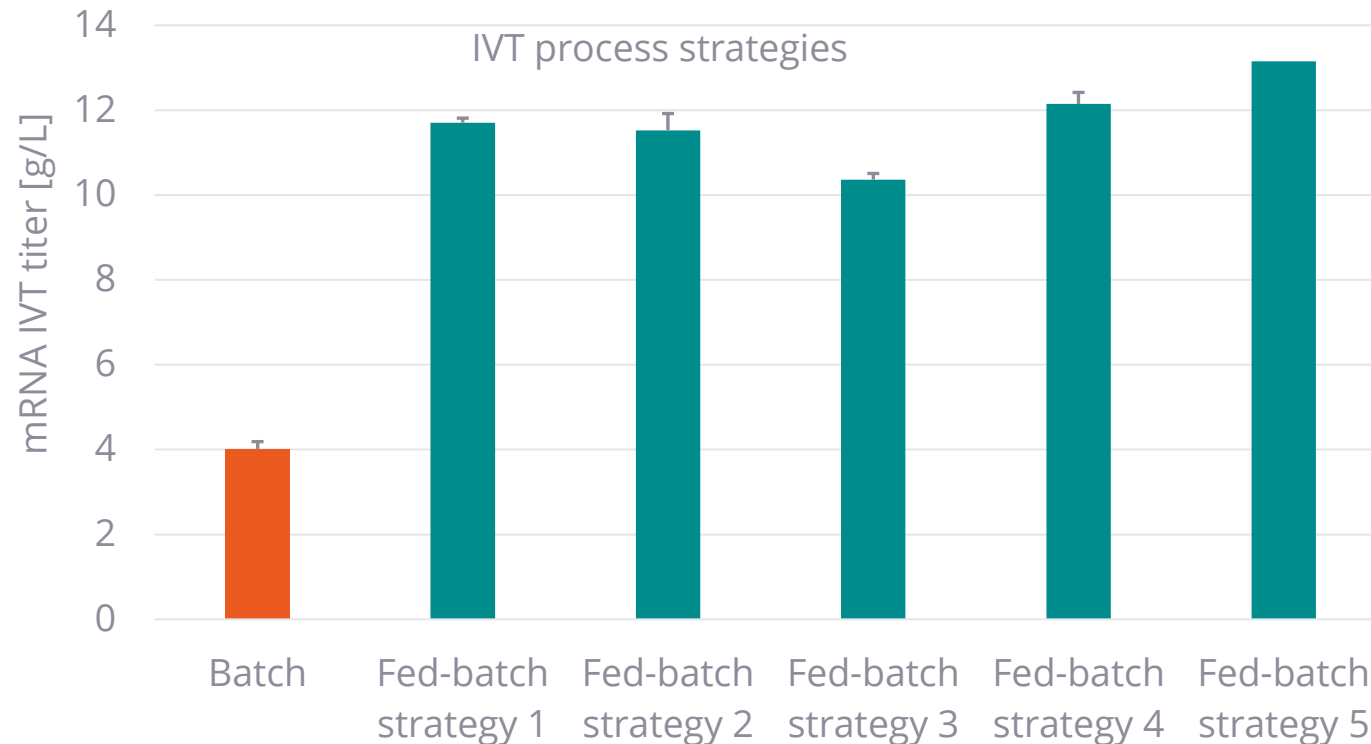
- Feeding of NTPs prevents overconcentration
- Prevents inhibition of T7 RNA-polymerase (PP_i)
- Increases mRNA titer
- Reduces cost of goods



IVT Optimization: Fed-batch Processing

1. Optimize fed-batch & feeding strategy with wild-type T7 polymerase

- NTP ratio & concentration,
- Mg^{2+} concentration
- Number & timing of feed-additions
- Reaction time

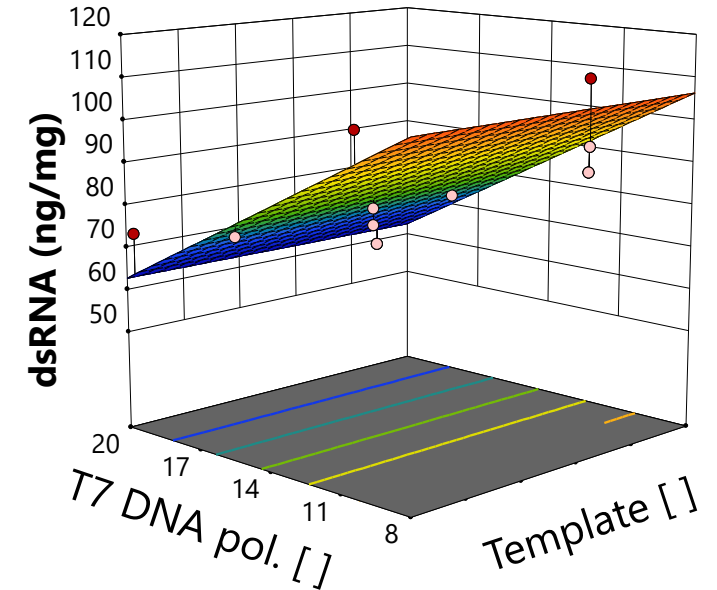
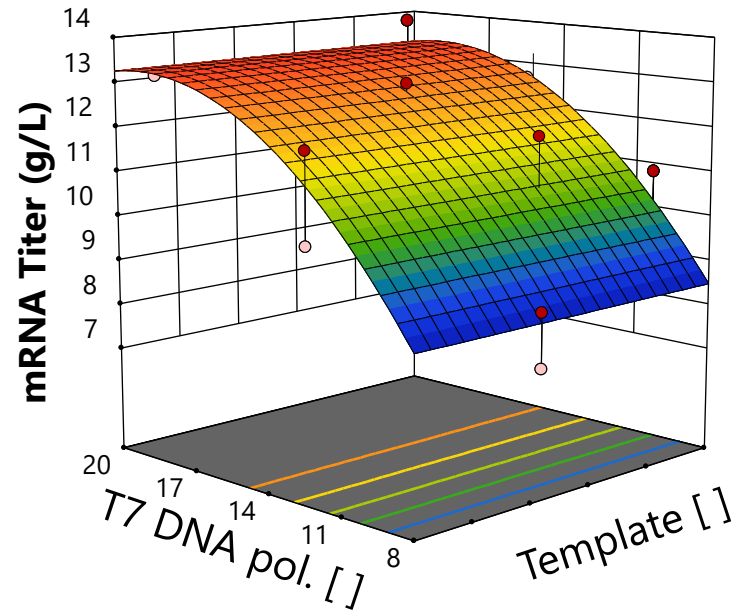


→ 3-fold increase of mRNA titer (12 g/L) as compared to batch mode

IVT Optimization: Design of Experiments

2. DoE / Response Surface Analysis of different fed-batch IVT conditions

- Template conc.
- T7 RNA polymerase conc
- Pyrophosphatase conc.
- Mg^{2+}
- Temperature

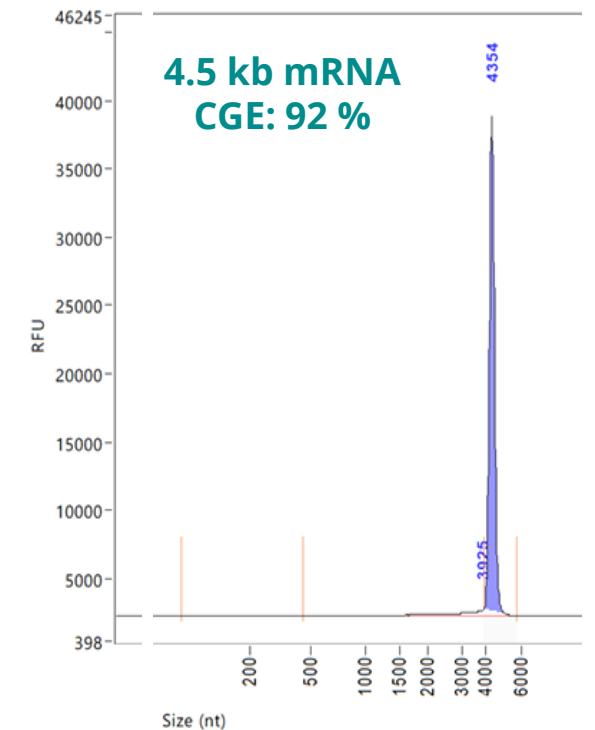


→ A higher T7 polymerase concentration supported a higher mRNA titer, and a lower dsRNA

Scale-up and GMP Manufacturing Cas9-mRNA

- Comparable results after ~1500-fold scale up
- Reduction of cost of goods by 65 %

mRNA Parameter	QC assay	Specification	R&D	cGMP batch
Volume [mL]		-	0.2	300
Titer IVT [g/L]	RP HPLC	-	12.6	11.6
Integrity [%]	CGE	≥ 85	93	92
Capping efficiency [%]	IP RP-HPLC / MS	≥ 90	96	95
dsRNA [%]	ELISA	< 0.005 (50 ng/mg)	0.0006 (6 ng/mg)	0.0006 (6 ng/mg)
Residual template DNA [ng/mg]	qPCR	< 0.1	0.08	0.02

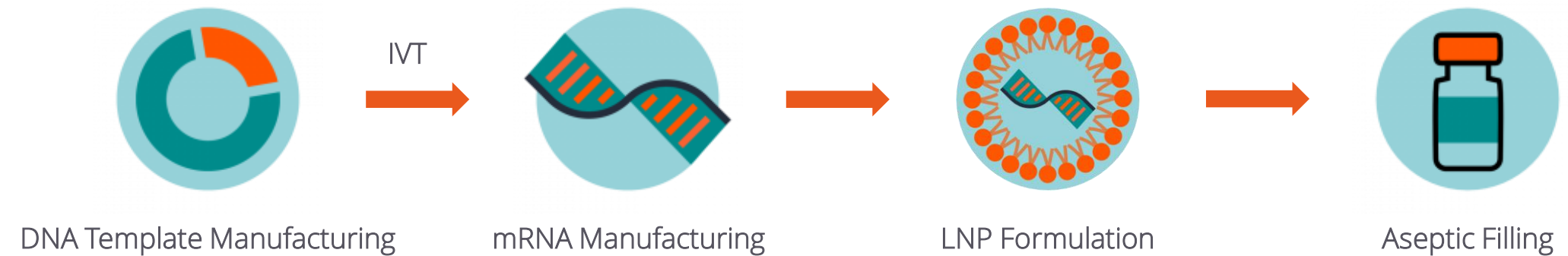


Summary

- Fed-batch processing resulted in **3-fold increase of mRNA titer (12 g/L)**
- Low dsRNA T7 polymerase mutants showed poorer mRNA quality as **wild-type T7 pol**
- Comprehensive set of **analytical assays** applied for **DOE** studies is key
- **Cost of goods** decreased by 65 %
- **Comparability and scalability** (1500x) proven at cGMP batch

End-to-End GMP Manufacturing of mRNA

- Integrated concept / mostly in-house
- Significant time / cost saving (no interfaces)
- Single point of contact



4 to 5 months from scratch

1 to 2 months mRNA resupply batches