General plasmid design guidelines

for **mRNA** Ntensify® process

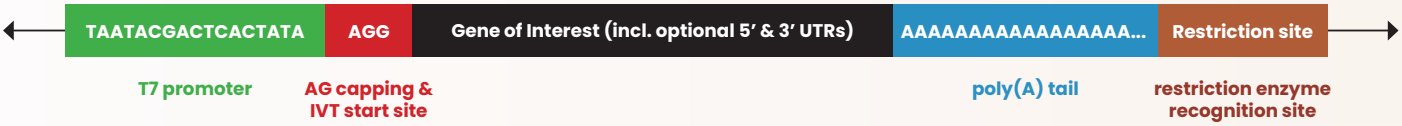
Quantoom Biosciences

The following document provides general guidelines for DNA design for mRNA production and protein expression. It also lists the plasmid specifications and minimum quality criteria to be met for use in Quantoom's Ntensify Process.

The DNA used as template in the IVT reaction must be **linear DNA** (linearized plasmid or synthetic linear DNA) at a concentration of **0.5mg/mL.**

DNA sequence requirements for mRNA production

- **T7 RNA Polymerase promoter:** TAATACGACTCACTATA
- **Co-transcriptional capping (AG cap) & IVT start site** after T7 promoter: AGG
- **Poly(A) tail** after the gene of interest and 3' UTR (for protein expression)
- A **restriction enzyme recognition site** after the poly(A) tail (when using a plasmid)
- Length:
  - The sequence transcribed into RNA (gene of interest, optional UTRs, and poly(A) tail) should be >900nt in length
  - The remaining non-transcribed sequence should be >1000nt in length

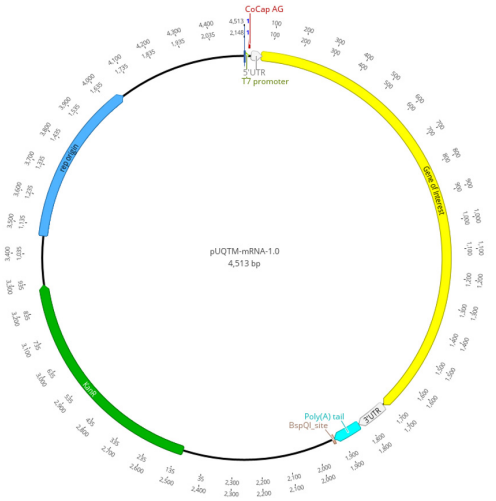


DNA sequence recommendations for mRNA production

- Use a Type IIS enzyme for plasmid linearization as they cleave outside of their recognition site
- Add untranslated regions (UTRs) to promote protein expression
  - 5' UTR immediately before the gene of interest
  - 3' UTR immediately after the gene of interest
- Use a poly(A) tail of 90-120A
- 40-60% GC content (global and in 100nt windows)
- Avoid short repeated sequences which can lead to complex structures

This plasmid map provides an example of a plasmid design for mRNA production

View the full sequence



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Quality attributes for linear plasmids (minimum R&D grade quality)

Attribute	Specification & testing method
Appearance	Clear, colorless, free of visible particles (Visual inspection)
Concentration	≥ 0.5 mg/mL (Spectrophotometry UV)
Restriction control (identity) - Any restriction enzyme	Expected digestion profile and narrow bands (Restriction digest + agarose gel electrophoresis)
Sequence identity	100% alignment with reference (Sequencing)
PolyA tail size	Narrow band at expected size after digestion around poly(A) tail (Restriction digest + polyacrylamide gel), **or** Expected length +/- 10% (Sequencing)
A260/280 ratio	1.80 - 2.0 (Spectrophotometry UV)
A260/230 ratio	≥2.0 (Spectrophotometry UV)
Residual bacteria gDNA	Not visible on gel (Agarose gel electrophoresis), **or** <5% (qPCR)
Residual RNA	Not visible on gel (Agarose gel electrophoresis)
Endotoxin	< 20 EU/mg of DNA (optional; depends on end purpose)
DNA Integrity	>80% (Capillary gel electrophoresis)
Linearization efficiency	Narrow band at expected size (Agarose gel electrophoresis)
RNAse	No RNAse
DNAse	No DNAse

Please note that the final RNA concentration is influenced by the size of the RNA being produced. A shorter RNA will lead to less mass production due to the shorter length of each molecule produced and increased abortive transcriptions.

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About Quantoom Biosciences

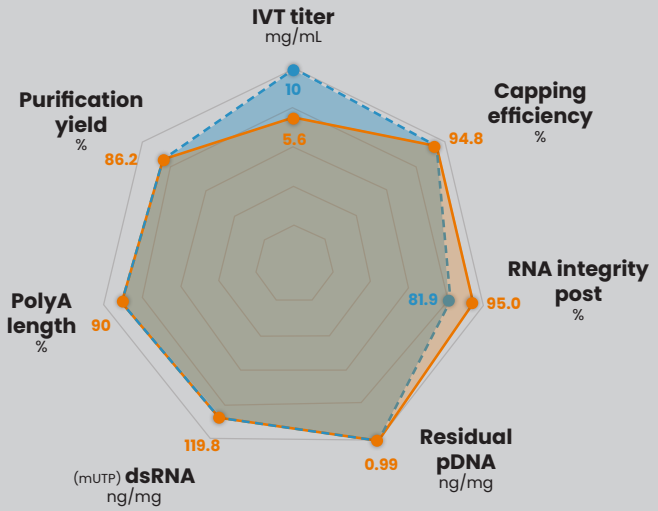
Quantoom Biosciences is a full-stack RNA partner for mRNA- and saRNA-based vaccines and therapeutics. Its N-Force toolbox relies on 3 core elements to turn any antigen into a (sa)mRNA-LNP drug product: Ncode for sequence design and optimization, Ntensify® for RNA production and Ncapsulate® for RNA-LNP formulation. Launched in 2023, the Ntensify solution enables fully integrated, scalable RNA production by combining processes, equipment, reagent mixes, and disposables and has gained global adoption, being recognized for performance and ease-of-use. Beyond technology, Quantoom Biosciences assists its partners by providing extensive enabling solutions, ranging from strategic R&D partnerships to sequence design & optimization.

Quantoom is committed to providing its customers with the best possible solution for mRNA production and purification. To this end, it has developed Ntensify process: a construct agnostic process with redesigned and optimized process.

Data obtained with Quantoom's Ntensify process* tested on numerous mRNA constructs

Demonstrated by robust DoE

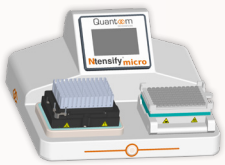
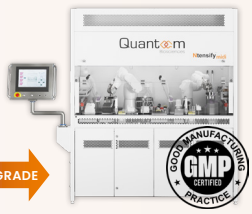
- > 2000 individual mRNAreactions
- > 500 individual saRNA reactions
- 100+ different constructs tested
- Different mRNA Sizes (1 to 4kb)
- Different saRNA sizes (8 to 12Kb)



Average performances of the Ntensify process related to process parameters and mRNA quality attributes.

mRNA (Version 4.3 v2) saRNA (Version 6.0 v1)

All the production technologies in our product line leverage the same Ntensify process, at any scale. This confers reproducibility to our product line and enables consistency of CQA's and CPP's at every scale, from R&D to commercial production in GMP facilities. Please find the **Ntensify product line** overview below.

mano	micro	mini	midi	midi flex
				SOON
				
Entry point for RNA construct assessment *In vitro & in vivo* studies	Drug discovery & pre-clinical phase Up to 96 constructs	Drug discovery & pre-clinical phase Up to 48 constructs	Clinical trials & commercial production	Pandemic readiness at maxi capacity
IVT volumes				
50 - 200 µL	100 µL – 20 mL	2 mL – 100 mL	200 mL - 800 mL	20 mL - 3.2 L
mRNA capacity per batch (purified naked mRNA - showcased in the process perfomance spider chart)				
250 µg - 1 mg	500 µg – 100 mg	4 mg – 100 mg	1 – 5 g	0.1 – 16 g
saRNA capacity per batch (purified naked saRNA)				
400 µg – 1.6 mg	800 µg – 160 mg	3.2 mg – 160 mg	NA	0.16 – 26 g
Level of automation & run duration				
Manual in 4 hours		Automated in under 1 day		

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