General plasmid design guidelines

for **saRNA** Ntensify® process

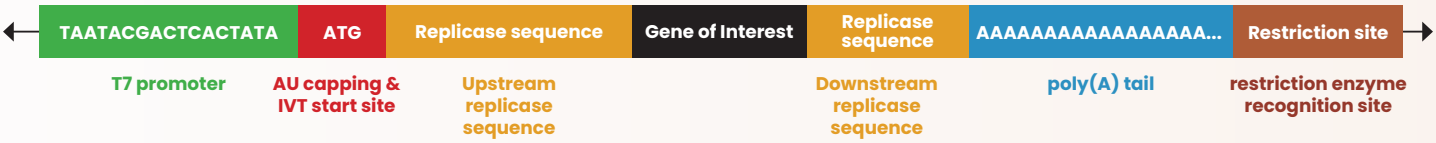
Quantoom Biosciences

The following document provides general guidelines for DNA design for saRNA 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 saRNA production

- **T7 RNA Polymerase promoter:** TAATACGACTCACTATA
- **Co-transcriptional capping (AU cap) & IVT start site** after T7 promoter: ATG
- **Replicase sequences** flanking the gene of interest (for self-amplification)
- **Poly(A) tail** after the downstream replicase sequence (for protein expression)
- A **restriction enzyme recognition site** after the poly(A) tail (when using a plasmid)
- The complete sequence should be <12000nt in length



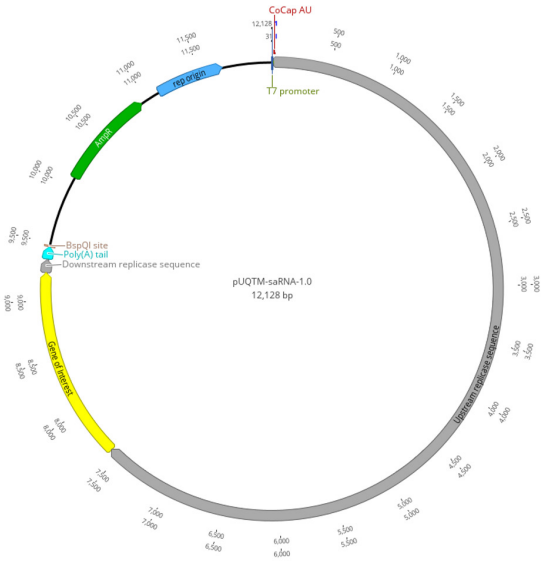
T7 promoter | AU capping & IVT start site | Upstream replicase sequence | Downstream replicase sequence | poly(A) tail | restriction enzyme recognition site

DNA sequence recommendations for saRNA production

- Use a Type IIS enzyme for plasmid linearization as they cleave outside of their recognition site
- Do not replace the natural replicase untranslated regions (also called conserved sequence elements) with other UTR sequences
- 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 saRNA production

View the full sequence



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

About Quantoom Biosciences

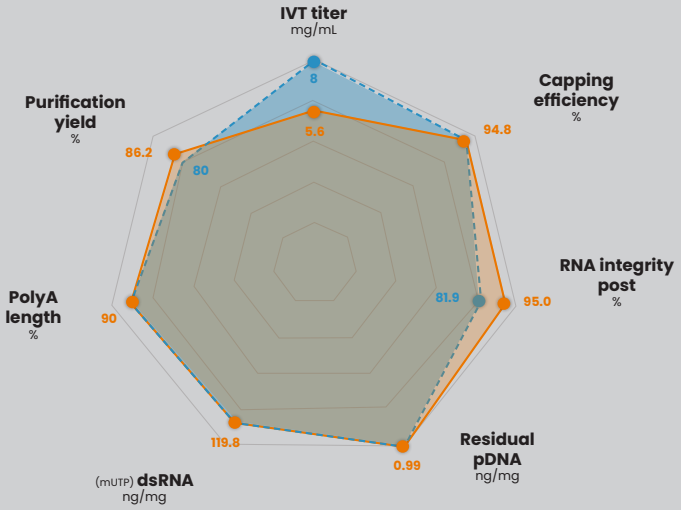
Quantoom Biosciences is a full-stack RNA partner for mRNA- and saRNA-based vaccines and therapeutics. Its N-Force toolbox relies on 3 core elements to turn any antigen into a (sa)mRNA-LLP drug product: Ncode for sequence design and optimization, Ntensify® for RNA production and Ncapsulate® for RNA-LLP 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 (sa)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)

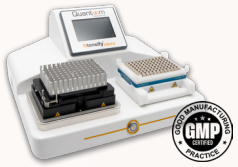


Average performances of the Ntensify process related to process parameters and mRNA quality attributes.

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.

Ntensify product line

mano	micro	mini	midiflex
			
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
mRNA capacity per batch (purified naked mRNA)			
125 µg – 3 mg	400 µg – 100 mg	8 mg – 92 mg	0.1 g – 4 g
saRNA capacity per batch (purified naked saRNA)			
320 µg – 2.5 mg	640 µg – 128 mg	10 mg – 123 mg	0.128 g – 5 g
Level of automation & run duration			
Manual in 4 hours	From 4 to 6 hours	Automated in under 1 day	