Considerations for the Development, Scale-up and Manufacturing of mRNA Therapeutics

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Abstract

Recently, there has been significant interest in the use of messenger RNA (mRNA) as an *ex vivo* and *in vivo* therapeutic. Since mRNA is expressed in the cytoplasm it may be particularly useful for improving gene expression in difficult-to-transfect non-dividing cells. In contrast to plasmid or viral vectors, there is no risk of insertional mutagenesis or subsequent oncogenesis upon mRNA transfection and the transient nature of mRNA expression is desirable for genome editing (CRISPR/Cas Systems, ZFNs and TALENs) and vaccines. In each case, the goal is to produce a synthetic RNA that mimics a natural mRNA.

Many Biotech, Biopharmaceutical, and Pharmaceutical companies have initiated programs to investigate mRNA therapeutic applications. Their target centric research has identified thousands of potential mRNA candidates, but many companies struggle with determining the optimum path forward to progress identified candidates through the drug development process. Contract Development and Manufacturing Organizations (CDMOs) like TriLink BioTechnologies with focused expertise in mRNA and nucleic acid manufacturing optimization can greatly assist both virtual and established companies achieve their goals.

Multiple compound attributes and manufacturing parameters must be determined, optimized, and rigorously tested. It is essential to consider sequence design, raw material identification and sourcing, and manufacturing processes that are inherently scalable. Critical decisions must be made about:

» Project management
» Technology transfer process
» Transcription optimization
» Purification optimization
» Process scale-up
» Analytical development

We will provide a broad roadmap for the application of these principles to the design and manufacturing of novel mRNA therapeutics. Data from the development, optimization, manufacturing, and scale-up of mRNA will be presented.

Project Management Support

It is important to know that TriLink has a proven history, a robust project management team and technical platform in place. This helps ensure the ability to understand and execute against the project requirements, mitigate risk, deliver the project on time, and right the first time.

To ensure project success, the project lead will establish a cross-functional team comprised of Subject Matter Experts (SME) for each function.
It is important the team establishes a strong partnership and lines of communication such that key roles can work closely with their counterparts throughout the project.

Technology Transfer Process

TriLink has a proven track record of successful technology transfers

The technology transfer process ideally starts early in the process development life cycle. Best practices can be incorporated into early stage development. In general, the development stage identifies the manufacturing process and can be made more efficient if "platform" processes are defined for selection and leveraged with historical knowledge.

The second step is the process and facility fit assessment. Effective teamwork and knowledge sharing between the client and TriLink considerably streamlines the transfer.

When selecting a team to deliver a process transfer, members are selected from both the originating and implementation sites with aligned and overlapping cross functional technical and operational expertise. All team members have responsibility to the overall mission, including individual ownership for key deliverables within the process transfer.

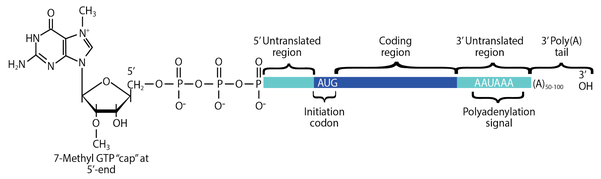
Processes are transferred to the implementation site through the integration of process requirements into local business processes, and associated process and operational documentation. Early development of local process deliverables during the technology transfer process enable complete risk identification with agreed resolution by the process transfer team including:

» Process descriptions for each unit operation
» Process and operating control strategies
» Process and equipment risk assessments
» Subsequent product/process requirements

Research and GMP Grade Manufacturing

TriLink offers high quality custom and standard long RNA and mRNA

» Lengths from 100 bases to >12 Kb
» Microliter to liter production scales
» Variety of post *in vitro* transcription modifications
  » Enzymatic tailing
  » Enzymatic capping
  » Phosphatase treatment



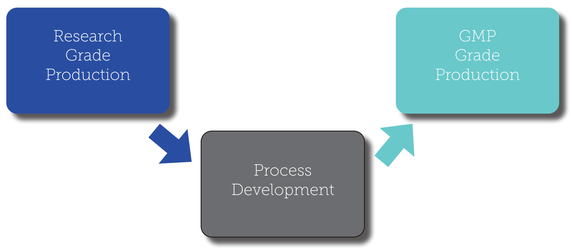
Traditional Interdepartmental Workflow

TriLink has developed a robust platform process for mRNA synthesis

Process optimization is critical, every construct has unique requirements

Differences result from the primary sequence
» Lengths from 100 bases to >12 Kb
» Microliter to liter production scales
» Variety of post *in vitro* transcription modifications
  » Transcriptional start
  » Codon optimization
  » Length of mRNA

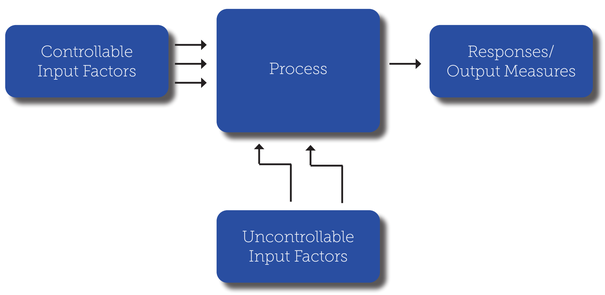
CDMO at TriLink



Preparation for pharmaceutical grade mRNA production

» Focus on developing an efficient and scalable process to ensure consistent quality
  » Increase inprocess material (IPM) yield
  » Increase IPM purity
  » Reduce process related contaminants
  » Upstream and downstream throughput optimized cycle times

Design of Experiment (DoE)



In order to quickly identify optimal transcription conditions we use DoE software to comprehensively screen multiple reaction parameters (Input Factors) resulting in optimal "Response" or "Output" conditions to ensure all identified critical quality attributes are addressed.

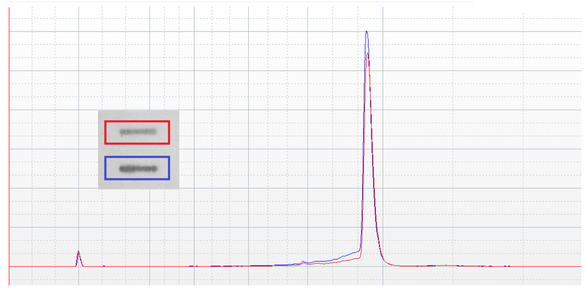
Transcription Optimization

Test a matrix of conditions to establish optimal conditions for cost, yield and product quality

» Establish magnesium donor
» Establish transcription buffer
» Establish transcription duration
» Establish optimum enzyme, nucleotide and DNA template concentrations

Monitor in process quality by UV Spec, Fragment Analyzer and Slot Blot

Bioanalyzer and Slot Blot Pre and Post Transcription Optimization



Red = Post Transcription Optimization
Blue = Pre Transcription Optimization

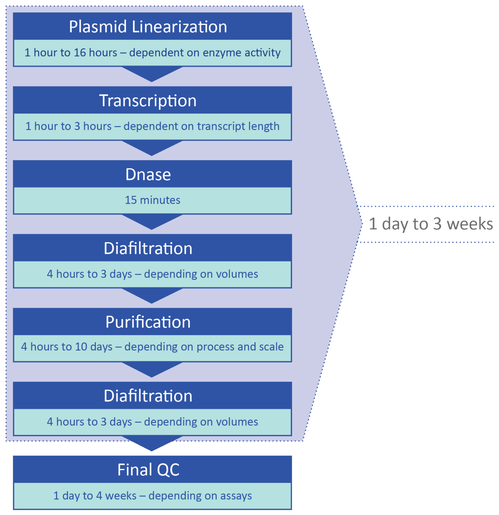
HPLC Development

Test a matrix of conditions to establish optimal conditions for cost, yield and product quality

» Establish column chemistry and dimensions from prescreened options that have proven successful for mRNA purification for various constructs
» Establish temperature
» Establish concentration of buffer and gradient profile
» Establish column capacity

Report results of each reaction including yield, Fragment Analyzer and Slot Blot

Streamlined mRNA Manufacturing



Enzymatic Reaction Scale up

Bioreactor

» Single use
» Monitor temperature/pH
» Fit into current and future processes
  » Close system transcription and purification
» Direct transition into cGMP production

Vessels from 100 mL – 3.75 L
(theoretical crude transcription yield of 500 mg – 18 grams)

Analytical Development and Method Transfer

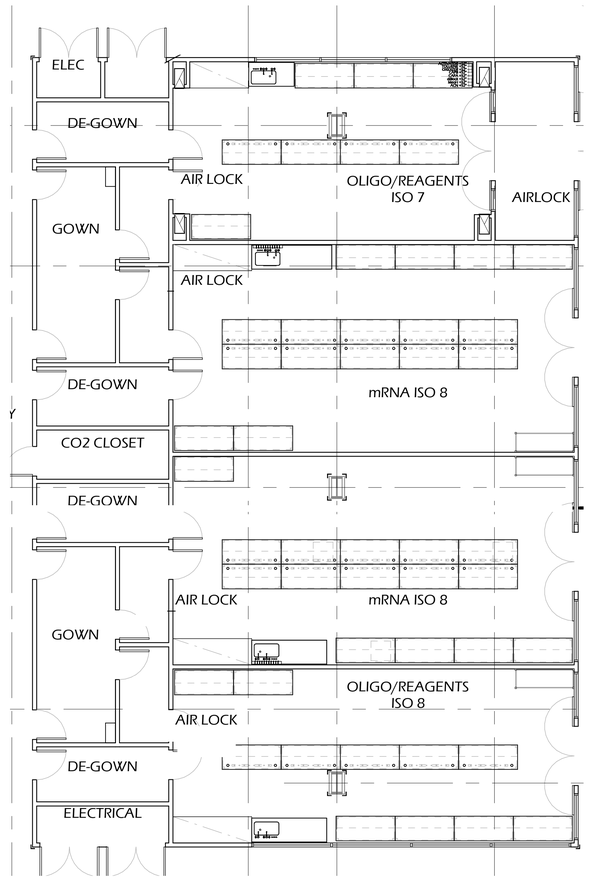
Characterization of mRNA by mass spectrometry, HPLC, qPCR, Spectrophotometric, Fragment Analyzer and UPLC techniques
» mRNA specific methods including:
  » Capping efficiency/methylation
  » Double stranded RNA
  » Poly A tail length
  » Residual protein
  » Residual plasmid
  » Concentration
  » Identity
  » Purity

The New TriLink Facility to Accommodate Scale Up

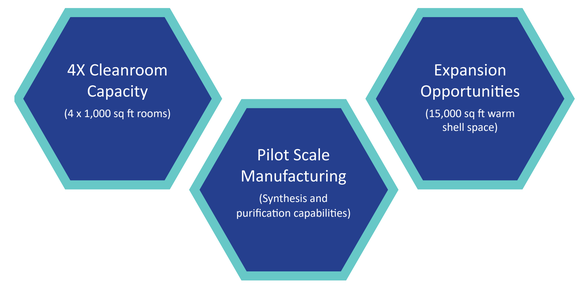
Located at 10770 Wateridge Circle, San Diego, 92121 (distance: 3 miles)

95,000 sq ft Facility

50,000 sq ft Manufacturing/Lab Space



Highlights and Opportunities:



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