Product Development and Manufacturing of oRNA™ Therapies

Ben Maynor

CASSS CELL & GENE THERAPY PRODUCTS (CGTP): MANUFACTURING, QUALITY AND REGULATORY CONSIDERATIONS

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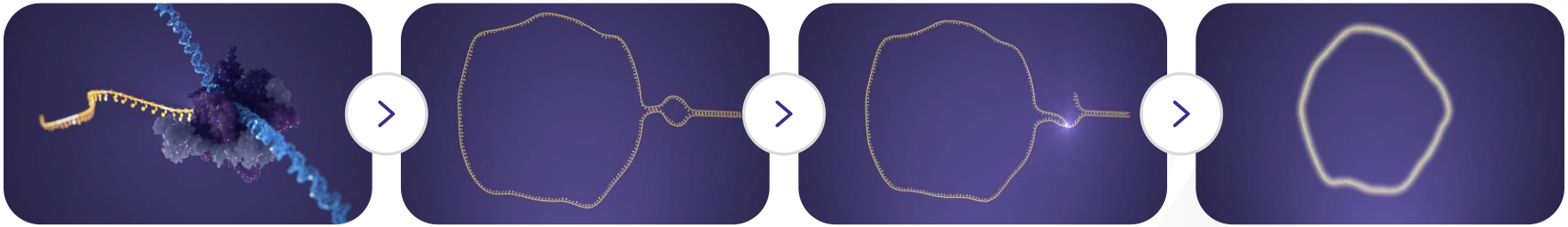
What is oRNA™ technology?

Synthetic circular RNAs combining nature-driven insights with rational design to **deliver superior therapeutic potential**

Self-circularization affords **key functional advantages** over mRNA and other circularization methods

Readily scalable to address manufacturing needs



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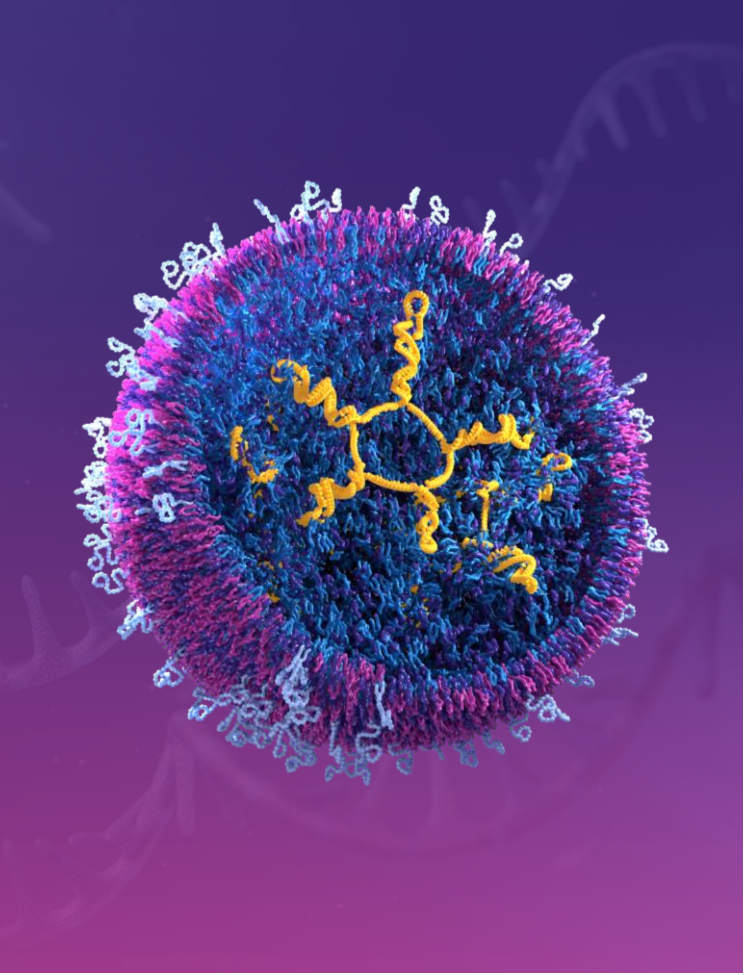

Lipid Nanoparticle Technology

- LNPs are clinically and commercially validated delivery vehicles for long (coding) and short RNAs
- Classic LNPs have 4 lipid components and a payload
- The most important lipid is the ***ionizable lipid***, which determines cell uptake and payload escape from the endosome

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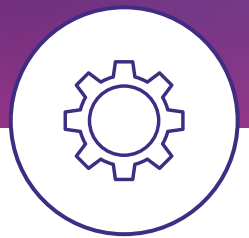
Platform Overview

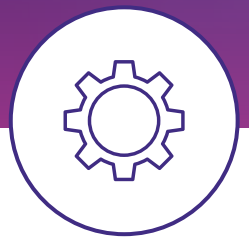


Production

Expression

Delivery

Platform Overview



Production

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Delivery

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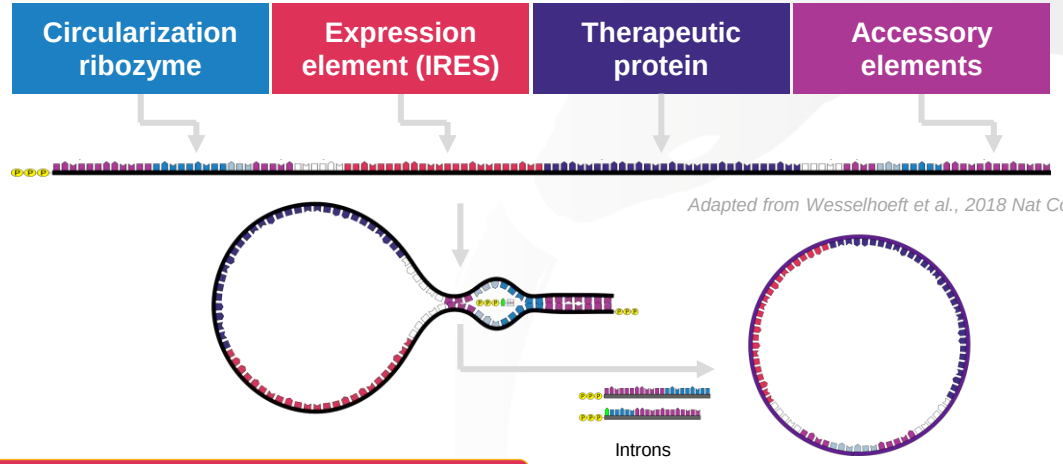
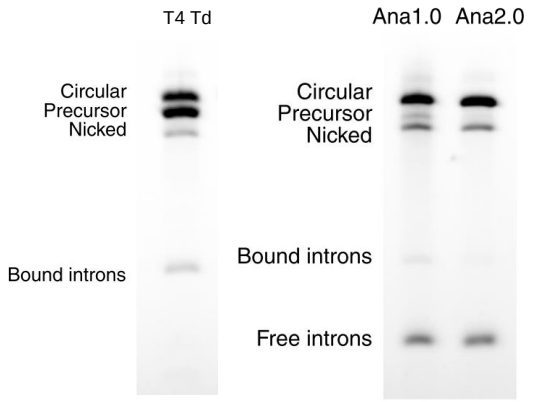
Production: oRNA Self-circularizes

- Production is by *in vitro* transcription of a linearized plasmid
- We use **no** modified nucleotides
- Co-transcriptional circularization *via* a proprietary, autocatalytic split ribozyme
- All circles are full-length
  - Only full-length transcripts can reconstitute the ribozyme
  - No *N-1* molecules
- High payload capacity with robust circularization efficiency beyond 10 kb
- Circular topology allows for robust purification

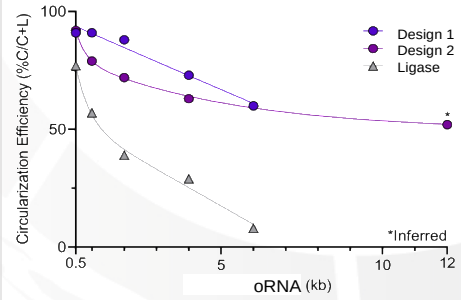


Adapted from Wesselhoeft et al., 2018 Nat Comm.

Wesselhoeft *et al*. 2018



Orna's enhanced circularization



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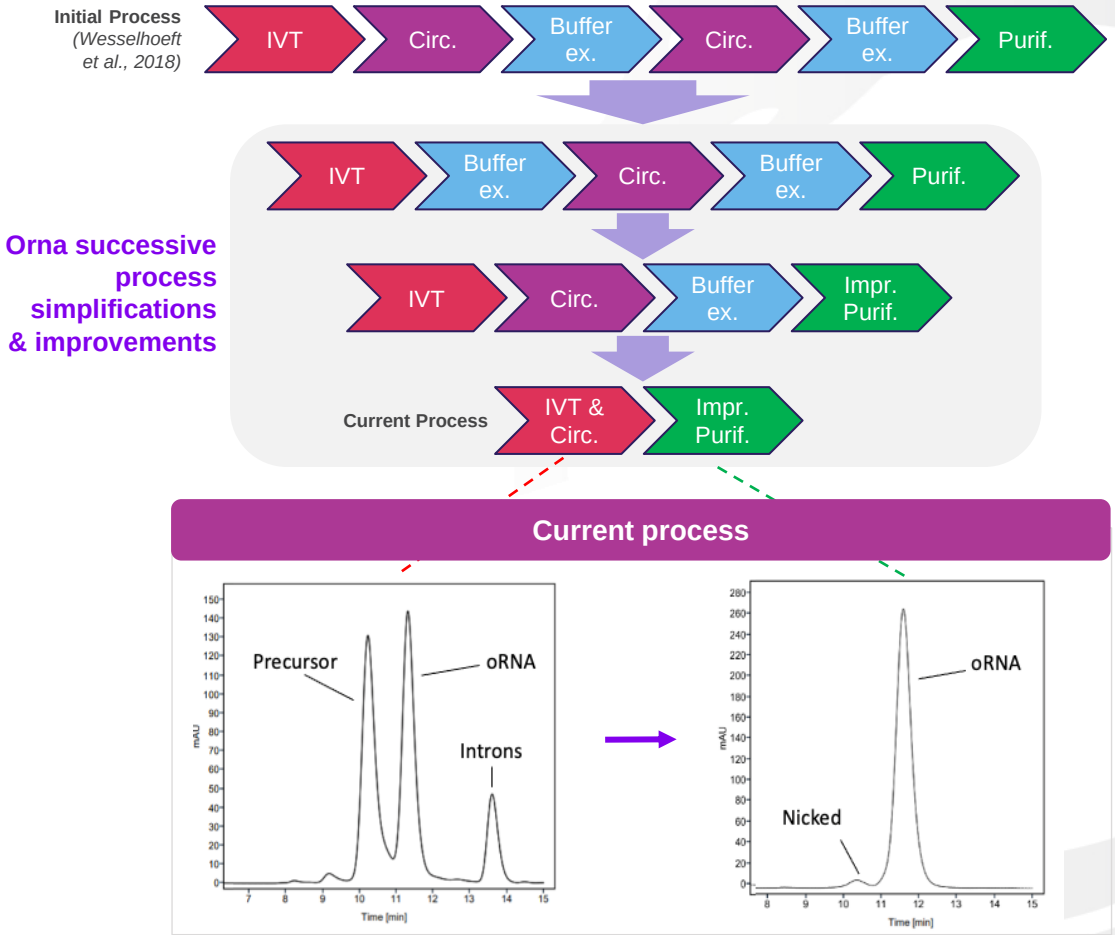


oRNA is simpler & cheaper to make

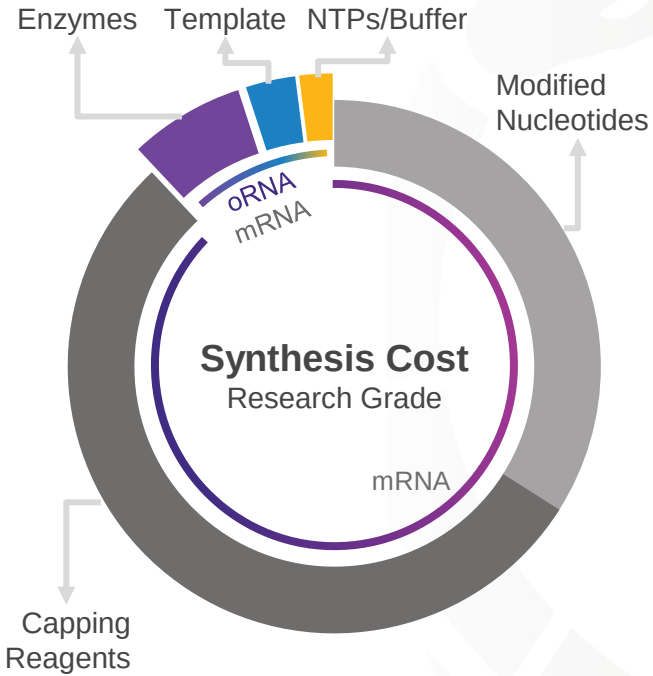
oRNA synthesis requires only **standard IVT reagents**

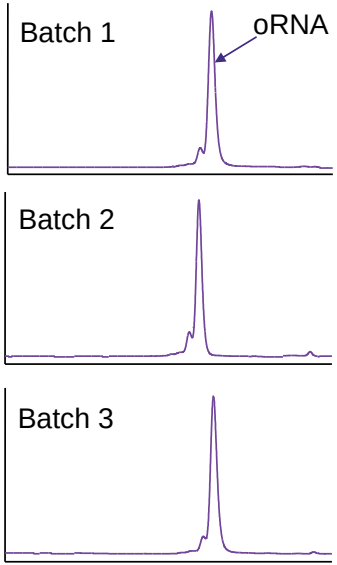
- No additional proteins, splints, or crowding agents needed

Absence of cap, tail, & modified nucleotides dramatically **reduces overall complexity & synthesis costs by ~10x**

Mobility shift upon circularization allows isolation of product at high purity and reproducibility

Process scalability augurs well for clinical- and commercial-scale production





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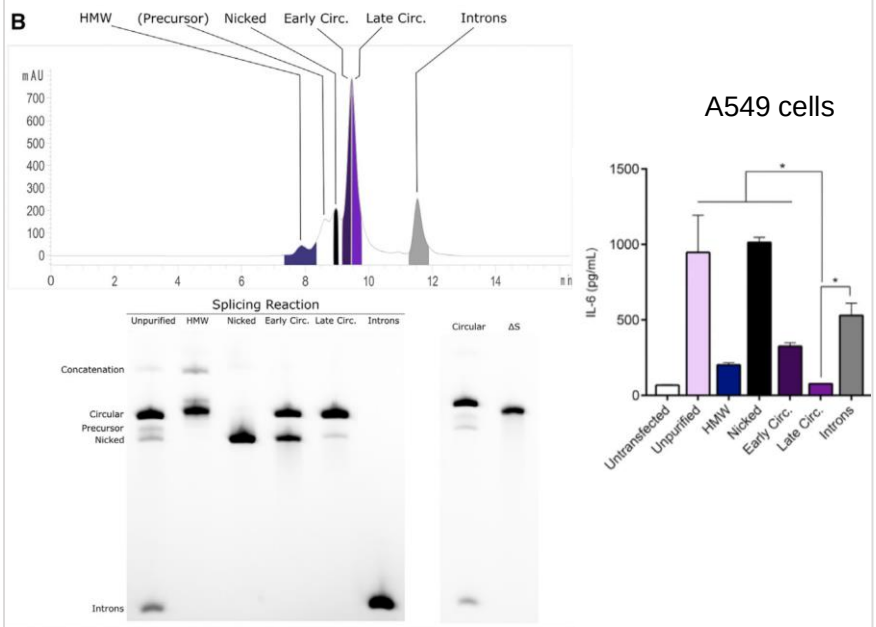
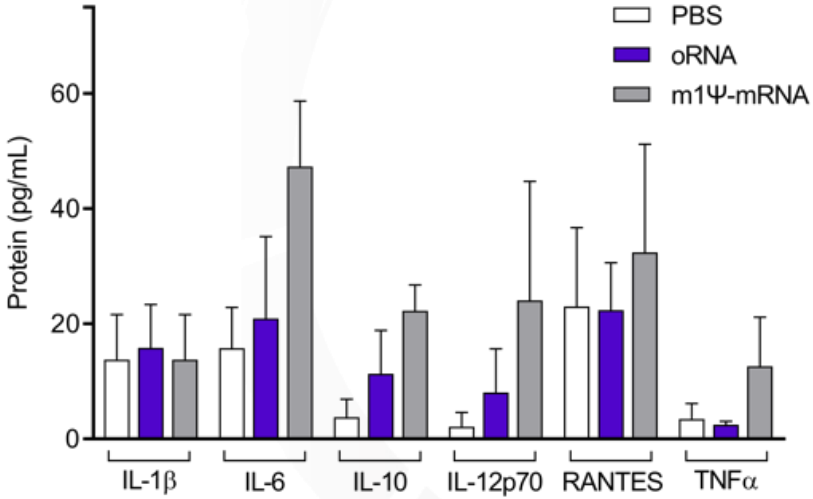
Pure unmodified oRNA is immunoquiescent

Cytokine response to IVT fractions *in vitro*



Cytokine response to LNP (MC3) / oRNA *in vivo*



Wesselhoeft et al., 2019 Mol. Cell

Platform Overview

Production

Expression

Delivery

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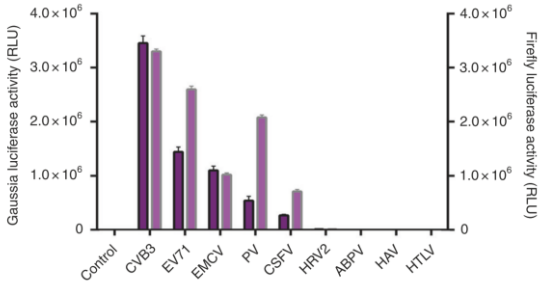
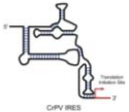
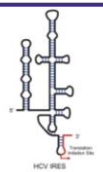
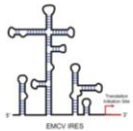
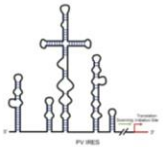
Expression: Cap-independent translation – the IRES

- Wesselhoeft *et al*. 2018 began to explore IRES functional diversity
  - Variable activity in human cell lines; many inactive

- IRES diversity is large and only loosely classified
  - Naturally occurring protein-binding aptamers
  - Adapted to host, cell & natural history of source



Wesselhoeft et al., 2018 Nat. Comm.

	Group I	Group II	Group III	Group IV
IFs	None	eIF2 eIF3	eIF2 eIF3 eIF4A eIF4B eIF4G	eIF2 eIF3 eIF4A eIF4G
ITAFs	None	None	PTB $ITAF_{45}$	PTB PCBP1 PCBP2
40S recruitment	Direct binding to40S ribosomal subunit, no IFS requirement	Direct binding to40S ribosomal subunit, 40S does not scan	Binding to 40S ribosomal subunit through ITAFs, 40S does not scan	Binding to 40S ribosomal subunit through ITAFs, 40S scanning to the downstream AUG
Examples	CrPV, PSIV, TSV	HCV, CSFV, PTV-1	EMCV, FMDV, TMEV	PV, rhinovirus
Secondary Structure				

Compact Structure → **Factor requirement**

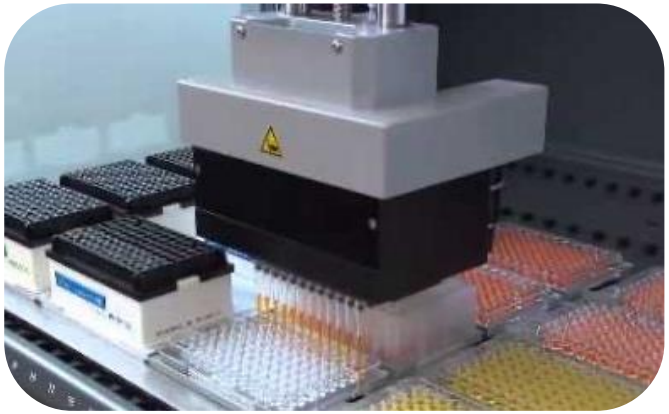
Yang & Wang 2019 J. Mol. Cell. Biol

The IRES as a “promoter” for translation

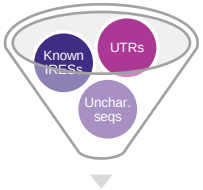
We have computationally identified and compiled the universe of candidate IRESs

Activity screening in cell-type specific assays

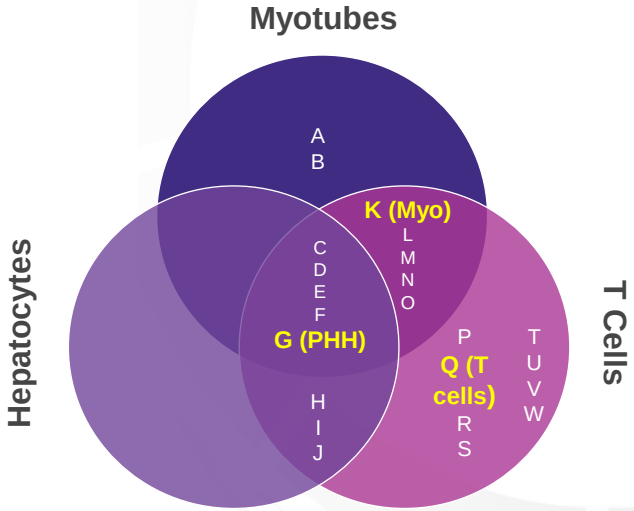
IRESs can be universal or cell- or species-specific



FoRCE™ Process



Cell Type-specific IRESs?



**Yellow: #1 hit for the indicated cell type*

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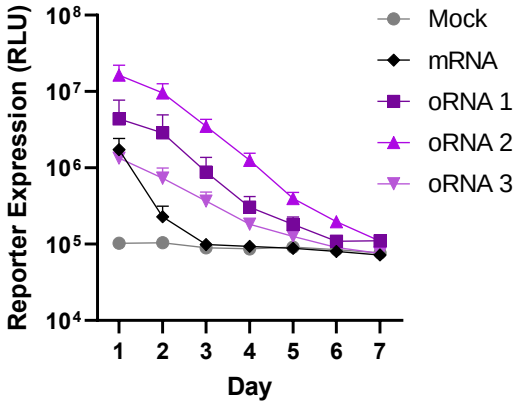
oRNA expression in human T cells

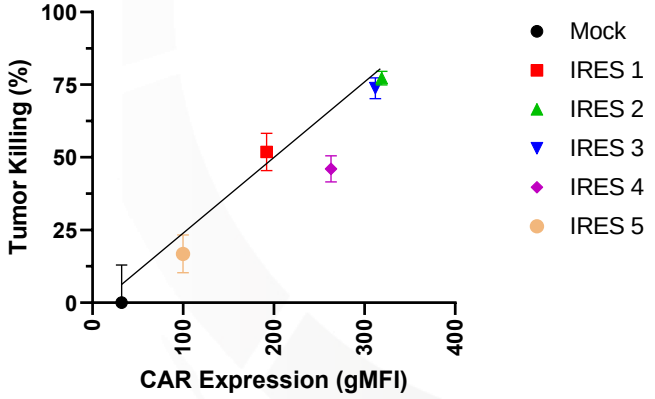
IRES-driven oRNA expression in proliferating human T cells outperforms mRNA in C_{max}, AUC, and $t_{½}$

Orna's proprietary immune IRESs provide a new level of functional tuning

Improved peak and duration of expression



IRES choice improves CAR killing



Platform Overview

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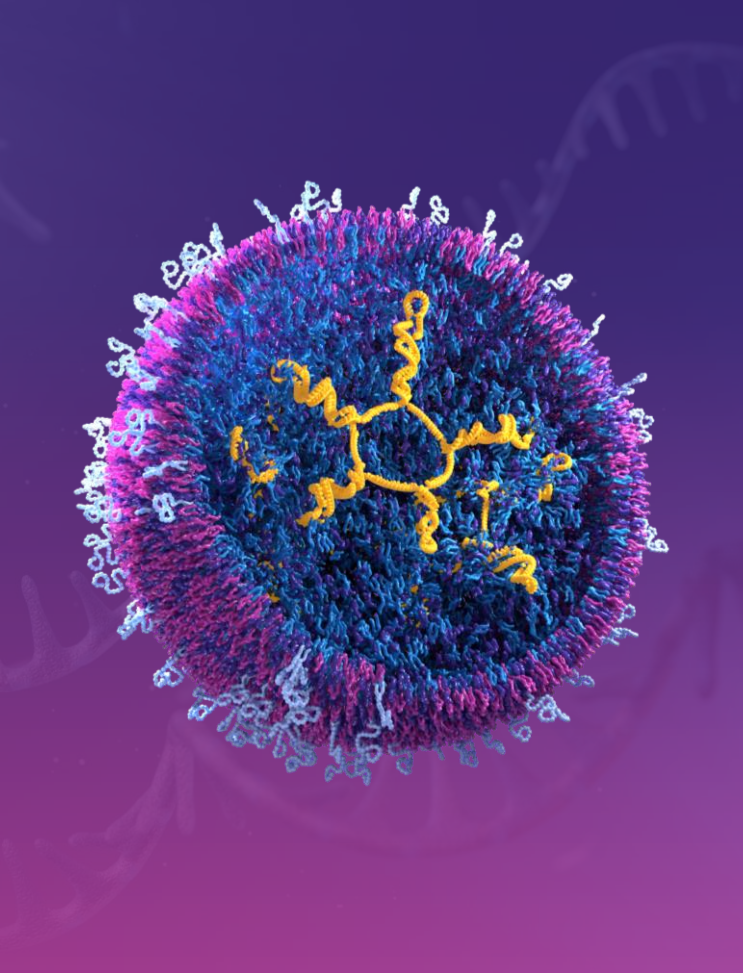

Lipid Nanoparticle Technology

- LNPs are clinically and commercially validated delivery vehicles for long (coding) and short RNAs
- Traditional LNPs are “**4** Component” nanoparticles (+ Payload)
  - **Ionizable lipid (IL)**
    - Neutralize the negative charges of phosphate backbone
    - Drive tropism and endosomal escape
    - Major determinant of toxicity
  - Helper Lipid
    - Structure and stability
  - Cholesterol
    - Structure and stability
  - PEG Lipid
    - Enhance circulation time, storage stability

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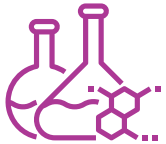
Delivery Strategy: Building on success of established lipids

1 | Lipid access

- Access chemical matter (validated & novel) by license or public domain
- Tap internal and external experts
- Seek diverse tissue tropisms



2 | Industrial formulation & validation

- Validate formulatability to an industry standard
- Generate novel IP and know-how
- Establish tropism in rodents, NHPs and human tissue models

3 | Chemical optimization

- Improve ionizable lipid *via* chemistry
- Generate novel IP and know-how
- Avoid risky and time-consuming *de novo* lipid discovery

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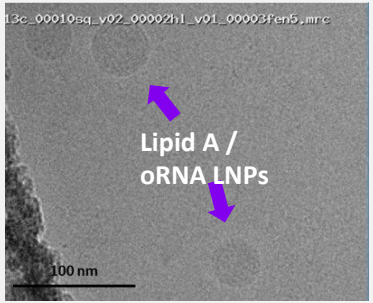
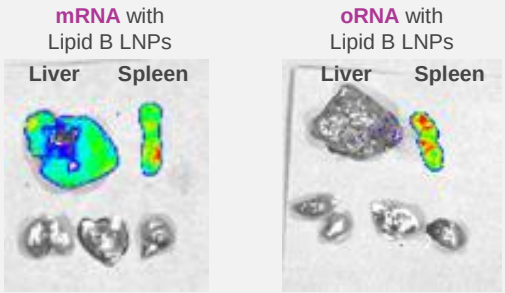
oRNA exhibits superior formulation properties

Lipid A / oRNA NPs are well-formed



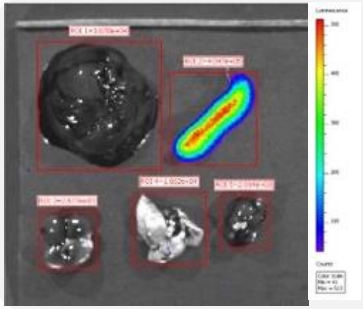
- Lipid A is a validated hepatotropic lipid
- Liver tropism is preserved with oRNA

Lipid B NPs change tropism with oRNA



- oRNA shifts Lipid B LNPs to the spleen
- Identical particle sizes

Lipid C NPs formulate only with oRNA, not mRNA



- Lipid C formulates with siRNA but not mRNA
- oRNA successfully formulated; preserves spleen tropism

Better formulatability leads to a greater, differentiated set of lipid options

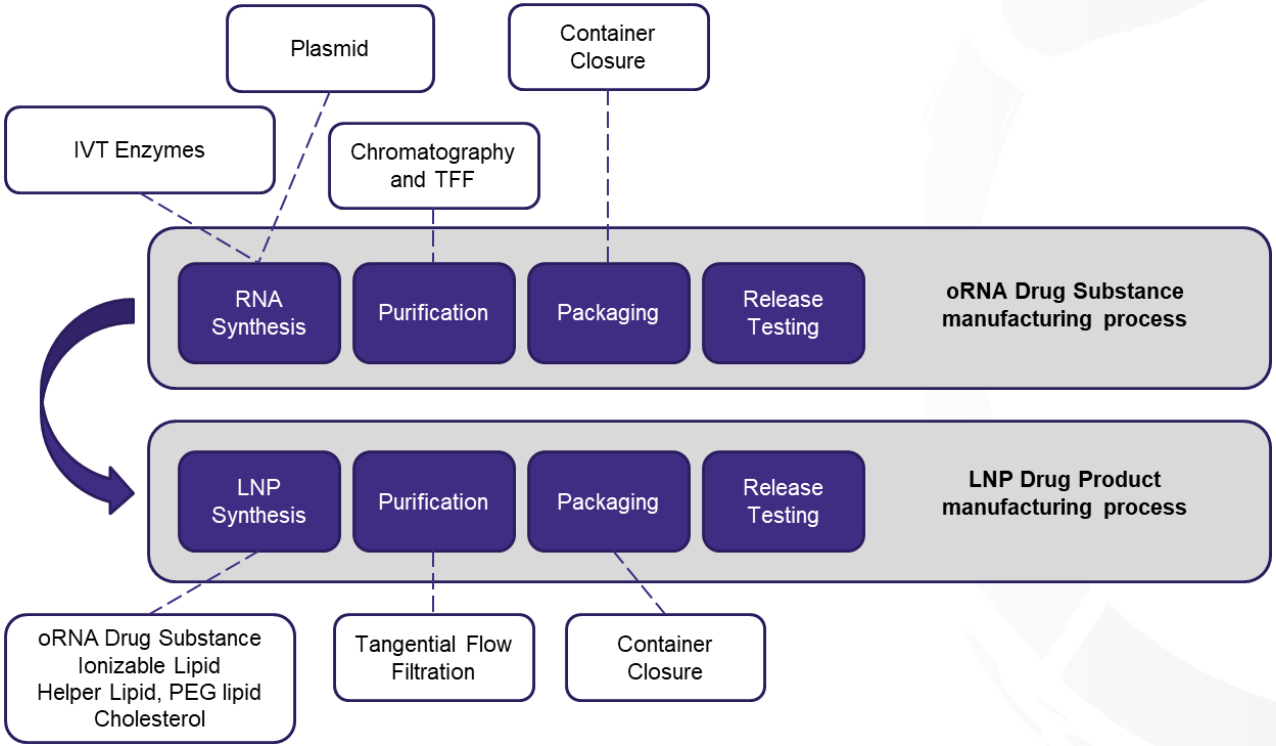
CMC considerations for production of oRNA therapies

Drug Substance

Drug Product

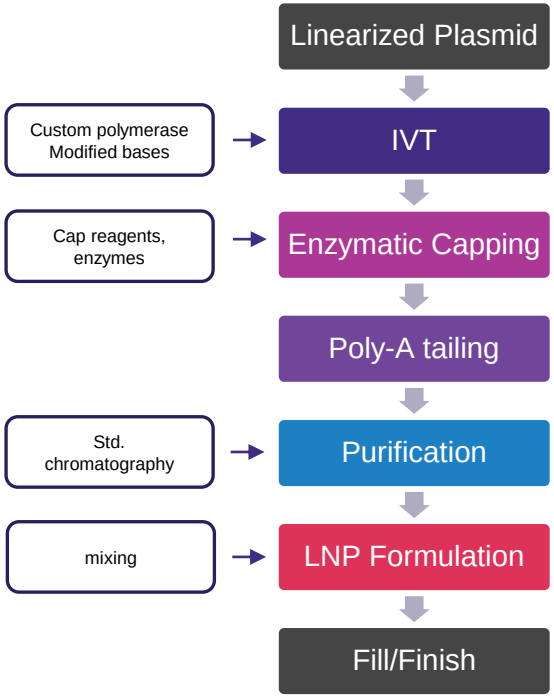
Quality

Orna's technology brings together multiple manufacturing platforms

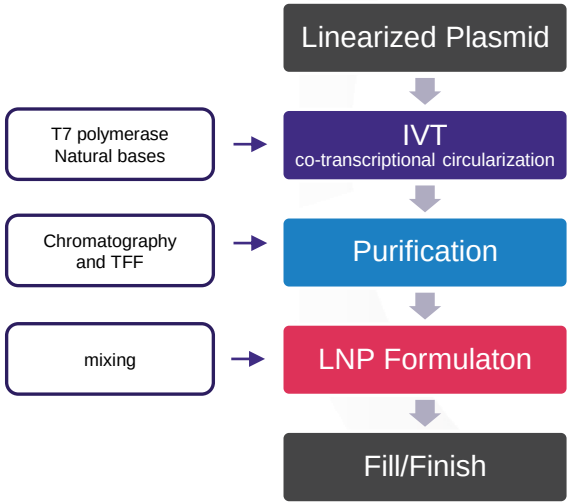


oRNA manufacturing is compatible with current mRNA mfg. infrastructure

mRNA production



oRNA production



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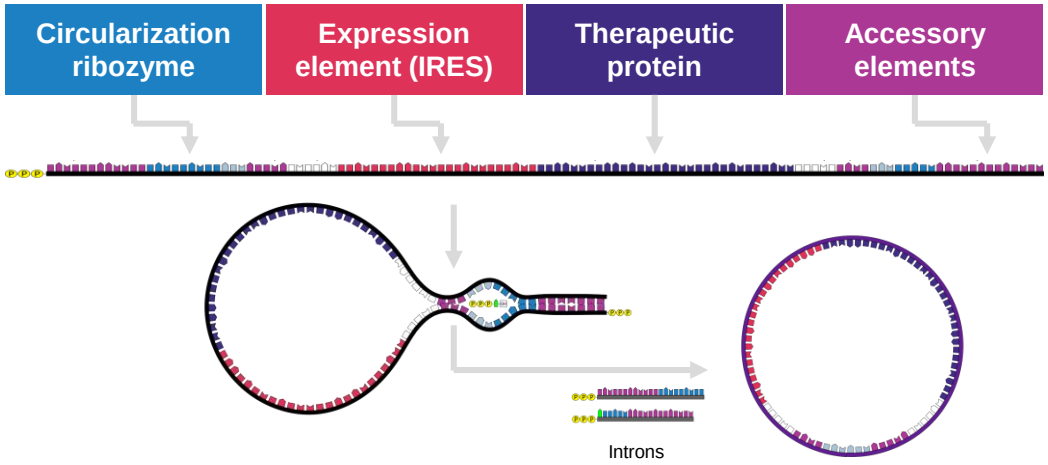
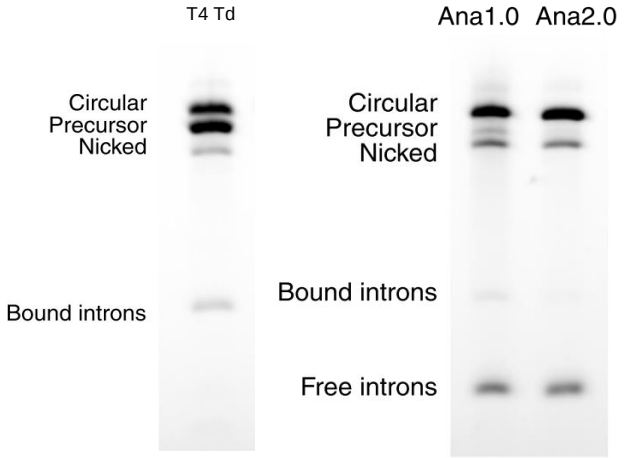
Circularization happens with >90% efficiency



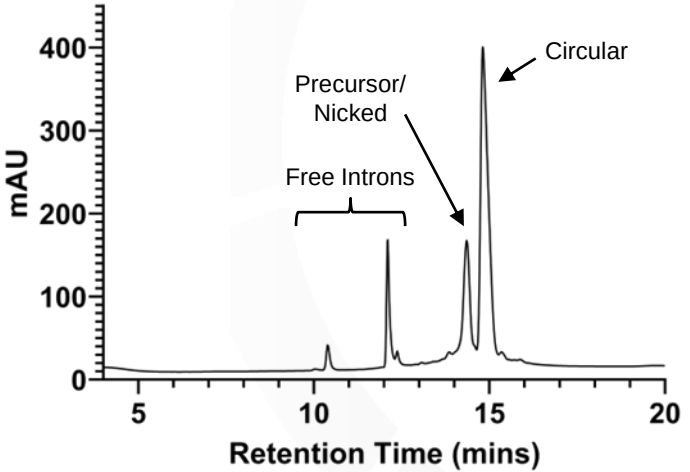
	% of circular oRNA DS
Batch 1	90.9
Batch 2	94.7
Batch 3	92.9
Batch 4	90.5
Batch 5	90.3

Topological differences in circles and lines allow efficient separation and chromatographic purification

- Agarose Gel



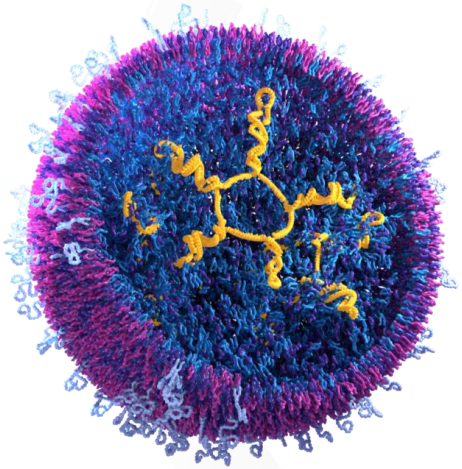
- HPLC Chromatogram



Microfluidic mixing offers an attractive option for rapid manufacturing of complex LNP formulations

Aqueous components:
oRNA DS
Buffer

Non-aqueous components:
Ionizable lipid
Helper lipid
Cholesterol
PEG Lipid
ethanol



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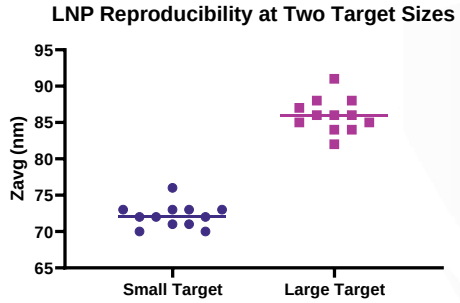
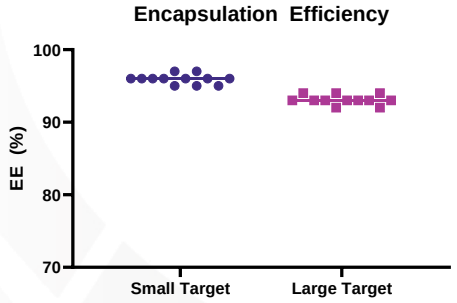
oRNA LNPs are compact and reproducible

- oRNA LNPs can be >2x more compact per nucleotide
- oRNA LNP characteristics are consistent across multiple batches
  - Particle size, encapsulation efficiency, and polydispersity are all highly reproducible
  - Particle characteristics maintained using clinical scale manufacturing equipment
- The oRNA/LNP process can be controlled to dial in desired LNP characteristics

LNP	RNA	Z-Avg (nm)	Vol. Ratio	PDI	EE (%)
B	mRNA	73	1	0.04	94
	oRNA	69	0.84	0.03	94
C	mRNA	98	1	0.18	99
	oRNA	83	0.61	0.13	96
D	mRNA	99	1	0.22	99
	oRNA	62	0.25	0.07	99





Potential oRNA Drug Substance CQAs

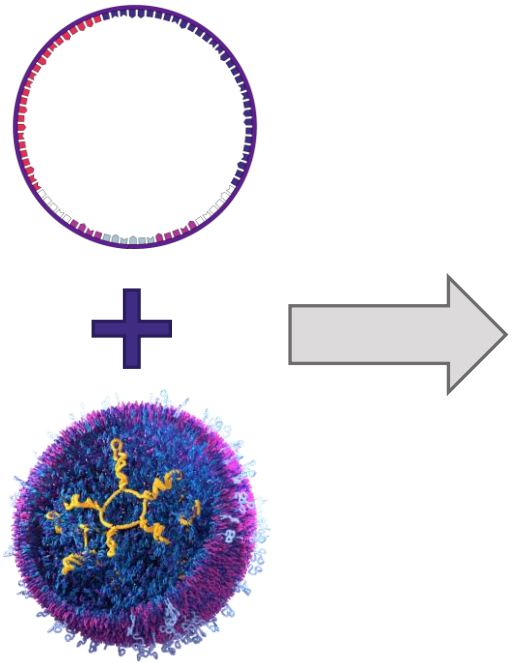
Assay	Method
Appearance	USP<1>
pH	USP<791>
Length	Chromatography
Identity (sequence confirmation)	RT-Sanger
Total RNA concentration	UV
Purity	RP-HPLC/SEC-HPLC
Product related impurities	RP-HPLC/SEC-HPLC
Total residual protein/enzymes	multiple
Residual DNA template	qPCR
Osmolality	USP<785>
Translational fidelity (Western)	Western Blot
Potency	ELISA
Bacterial endotoxin	USP<85>
Bioburden	USP<61>
Elemental Impurities	USP<232/233>
Residual dsRNA	ELISA

Potential oRNA-LNP Drug Product CQAs

Test	Method
Appearance	USP<1>
pH	USP<791>
Osmolality	USP<785>
Vesicle Size	DLS
Polydispersity Index	DLS
Zeta Potential	DLS
Lipid X, Y, Z, Identity	Chromatography
Lipid X, Y, Z, Content	Chromatography
Total Lipids Content	Chromatography
Buffer Identity	Chromatography
Buffer Content	Chromatography

Test	Method
RNA Content	RiboGreen
RNA Encapsulation	RiboGreen
Residual RNase	Fluoresence Assay
Length	Fragment Analyzer
Sequence Confirmation	RT-Sanger
Total RNA Content	UV
Purity	RP-HPLC/SEC-HPLC
Translational fidelity	Western Blot
Potency	various
Volume of Injection	Gravimetry
Sterility	USP<71>

Summary: Two technologies combine to make a broad Platform



Immunotropic delivery	**Oncology** *(in situ CAR)*	**Autoimmune diseases**	**Cancer vaccines**
Solid organ delivery	**Genetic diseases** *(protein replacement)*	**Gene editing**	**Regenerative Medicine**
Other classes of delivery	**Vaccines**	**Antibodies** *(in situ mAbs)*	

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Acknowledgements


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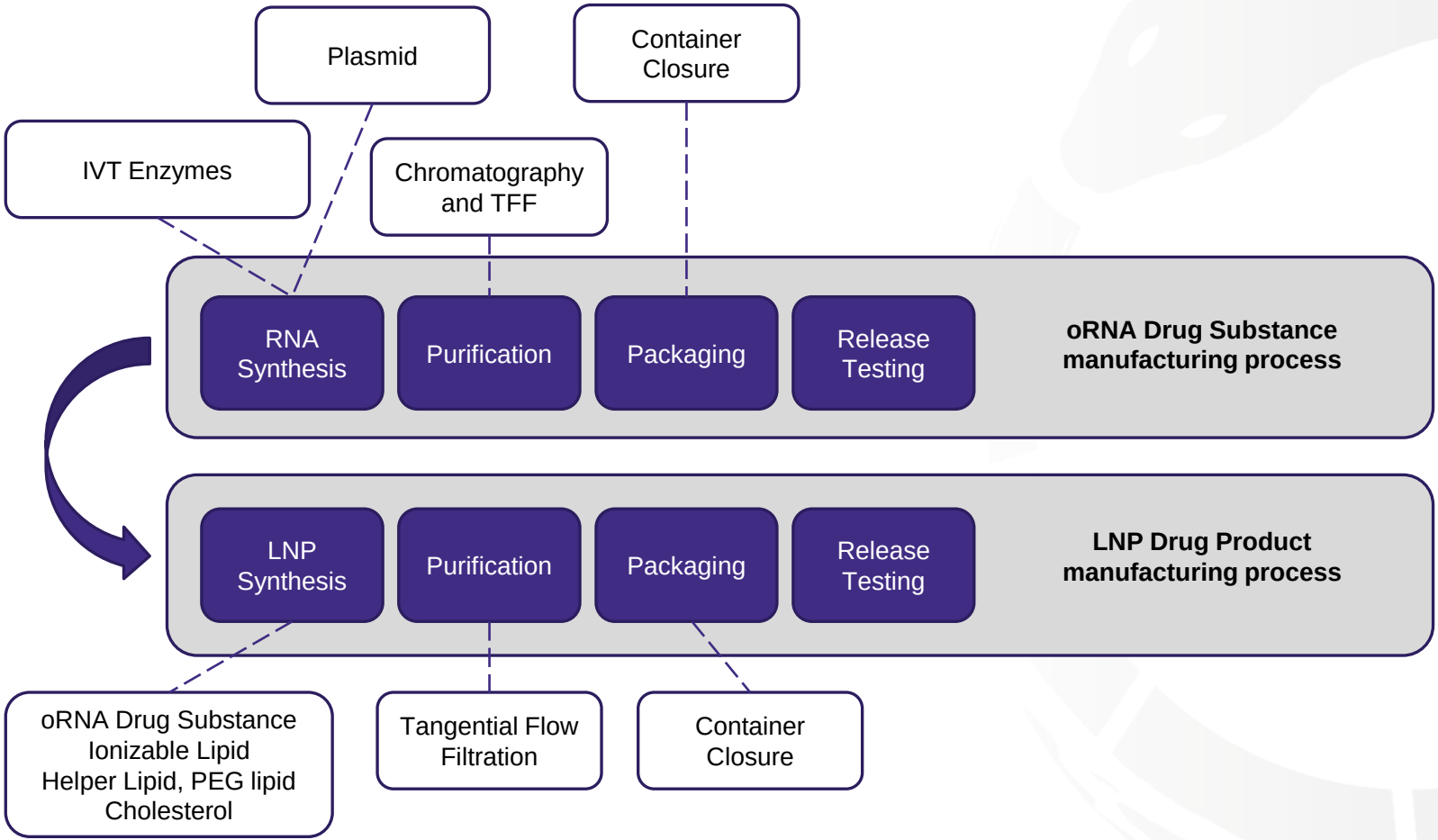
Plasmid
Container Closure
IVT Enzymes
Chromatography and TFF
RNA Synthesis
Purification
Packaging
Release Testing
oRNA Drug Substance manufacturing process
LNP Synthesis
Purification
Packaging
Release Testing
LNP Drug Product manufacturing process
oRNA Drug Substance
Ionizable Lipid
Helper Lipid, PEG lipid
Cholesterol
Tangential Flow Filtration
Container Closure