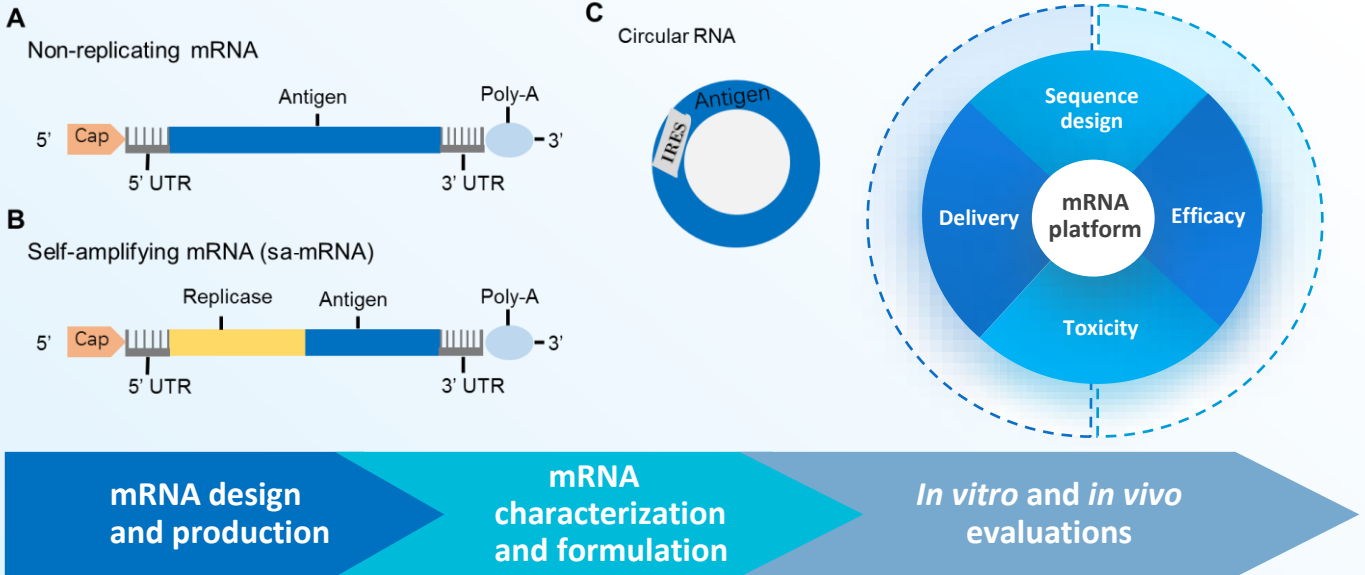


# Supporting Discovery of mRNA Drugs



## Design and Production of mRNA

### Design and optimization of mRNA sequences

- Utilizing the design and optimization techniques for mRNA sequences, UTR screening, and codon optimization

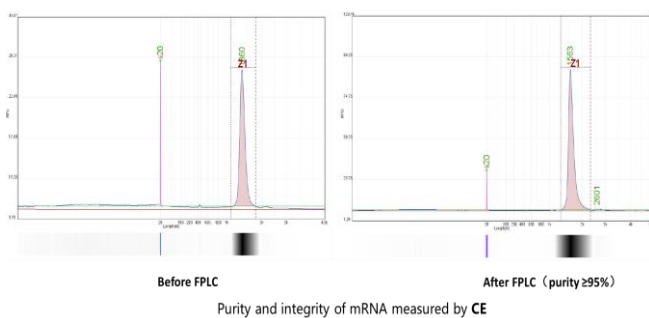
### Plasmid and DNA template productions for *in vitro* transcription

- Employment of a classical three-step purification method
- Implementation of mono-enzyme digestion and purification through chromatography

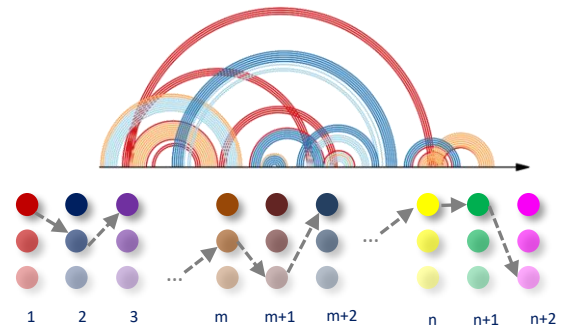
### Research scale mRNA production and characterization

- Generation of conventional 5' capped mRNA with chemically modified nucleosides (optional: a two-step method)
- Analysis of purity, integrity, capping efficiency, poly A length and dsRNA residue, and endotoxins residue

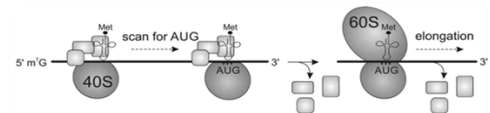
### Analysis of mRNA purity and integrity



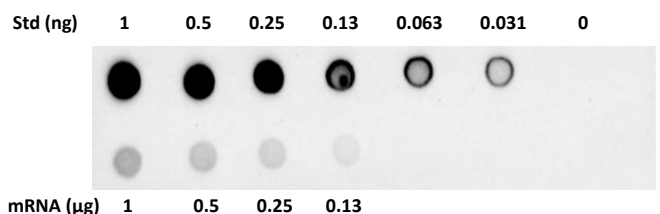
### Optimization of mRNA secondary structure



### Optimization of mRNA sequences at the translation level



### dsRNA content residue by dot-blot method



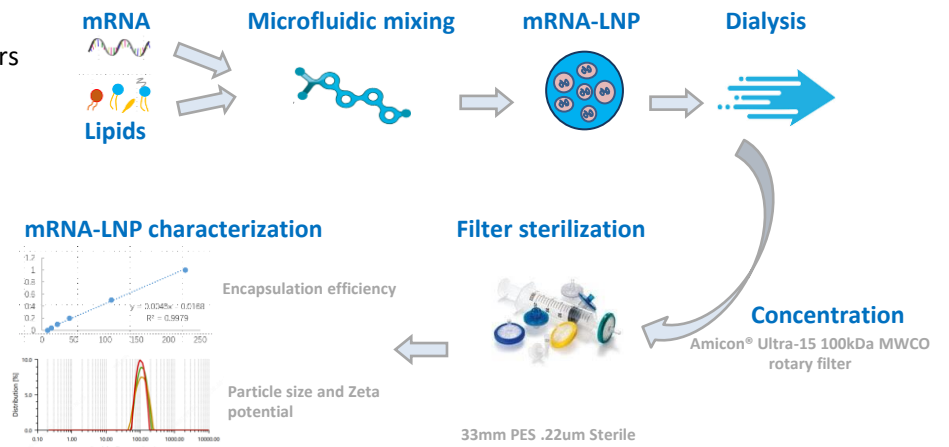
## mRNA Delivery System

### mRNA delivery

- LNP (microfluidic system) and others
  - ✓ Tissue targeted LNP
  - ✓ SORT strategy
- Lipoplexes (LPX)

### Characterizations

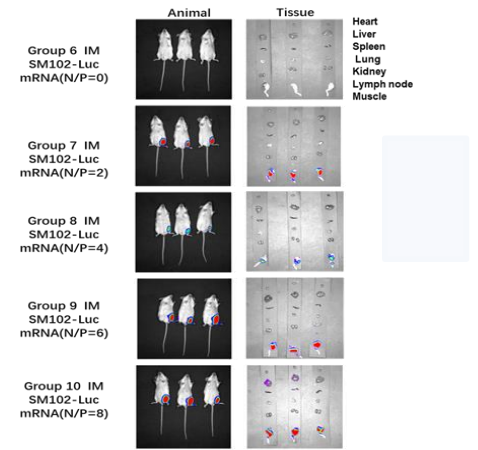
- Particle size distribution
- Polydispersity index (PDI)
- Zeta potential
- PKa
- Lipid concentration
- Encapsulation efficiency
- Particle morphology
- Endotoxin residue



## In Vitro and In Vivo Evaluations

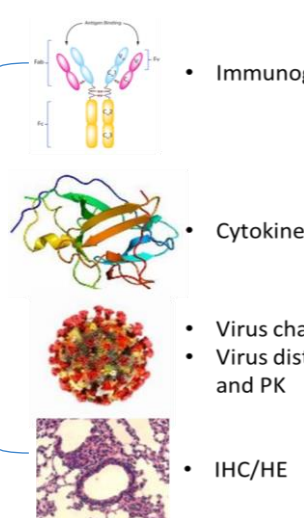
### In vitro and in vivo delivery and biodistribution

- Cell lines:** over 1500 cell lines and patient-derived fibroblasts, establishment of customized stable cell lines
- Primary cells:** primary hepatocytes of human, mouse, rat, dog and monkey; availability of other customized primary cells
- Animal models:** various species of rodents and NHP
- Methods for evaluations of mRNA uptake and protein expression:** bdNA, LC/MS, HCS, ELISA, western blot, flow cytometry, IF and more
- In vivo delivery and biodistribution:** live image system



### Evaluation of in vivo immunogenicity and efficacy of mRNA vaccines

- Mouse: IFV, RSV, HFMD, HSV-1, Zika, Dengue, etc.
- Rat: RSV
- Ferret (Mustela putorius furo): IFV
- Rabbit: EBV
- Rat: EBV
- Non-human primates: EBV



- Immunogenicity
- Cytokines
- Virus challenge
- Virus distribution and PK
- IHC/HE

### Humoral immune responses

IgG and subtypes, NAb, HAI, ADCC, ADCP, ADE, and other tests

### Cellular immune responses

Th1 and Th2 responses, T cell proliferation and cytokine profiling, CTL and cell activation by ELISpot and ICS

### Efficacy

clinical symptoms, survival rate, viral load, histopathology and more

### Early tox

