



Unlocking iPSC Innovations: Next Generation Reprogramming, Engineering, & Manufacturing Solutions



Presenter:

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Logistics:

- You will be put on mute during the webinar
- We welcome you to ask questions using the “Q&A” on the top right corner of the platform
- Your questions will be addressed after the presentation

Agenda

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Introductions

02

uBriGene Overview: Global & N America

03

uBriGene's iPSC Solutions Portfolio

04

Q & A

Established in 2015, uBriGene is committed to advancing and implementing cutting-edge CGT technologies and offering comprehensive solutions for the CGT industry.



CRO Services

- Plasmid
- Lentivirus
- AAV
- Autologous Cell Therapies
- Allogeneic Cell Therapies
- Gene Editing
- mRNA/circRNA

CDMO Services

- Plasmid
- Lentivirus
- AAV
- Autologous Cell Therapies
- Allogeneic Cell Therapies
- Gene Editing
- mRNA/circRNA
- QC/Release Testing
- Stability Testing

Chinese IIT Coordination

- Preliminary Communication
- Protocol Preparation
- Process Initiation
- Patient Management
- Clinical Process Management
- Data Management
- Work Completion & Reporting

uBriGene is your one-stop-shop from concept to clinical proof-of-concept

QUALITY

Customer Support
Clear, timely, accurate
technical support & project
management updates

GMP excellence
Team with a track record
of GMP success across
multiple modalities

QMS rigor
Ensures our personnel,
processes, and products
exceed client & regulatory
expectations

COLLABORATION

CRO & CDMO Services
One-stop shop of PD, AD &
GMP platform solutions for
ATMP developers

Regulatory Support
Experienced Reg Affairs
team & 10 FDA DMFs in-
place to help clients
navigate regulatory body
engagements

Chinese IIT Coordination
Rapid and cost-effective
path to first-in-human
clinical data

INNOVATION

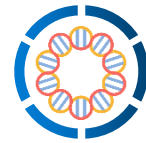
Upstream Processes
Proprietary vectors, RNA
methodologies, cell lines, &
cell culture processes

Downstream Processes
Cost & time saving
purification processes for
cells, viruses, and RNA
assets

Novel Technologies
Constant commitment to
optimizing every unit
operation of our GMP
manufacturing platforms

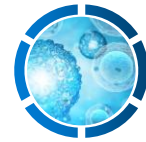


Platforms



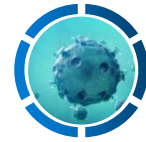
Plasmids

- ◆ Plasmid construction
- ◆ Strain banking
- ◆ Plasmid PD & manufacturing



Cell therapy

- ◆ CAR-T, TCR-T
- ◆ CAR-NK
- ◆ MSC, iPSC, cell banking



Viral Vectors

- ◆ LVV, RVV
- ◆ AAV
- ◆ Adenovirus
- ◆ OV



Gene Editing

- ◆ IVT-sgRNA
- ◆ Nuclease
- ◆ RNP PD



RNA-LNP

- ◆ mRNA
- ◆ circRNA, saRNA
- ◆ IVT-sgRNA



QC release testing

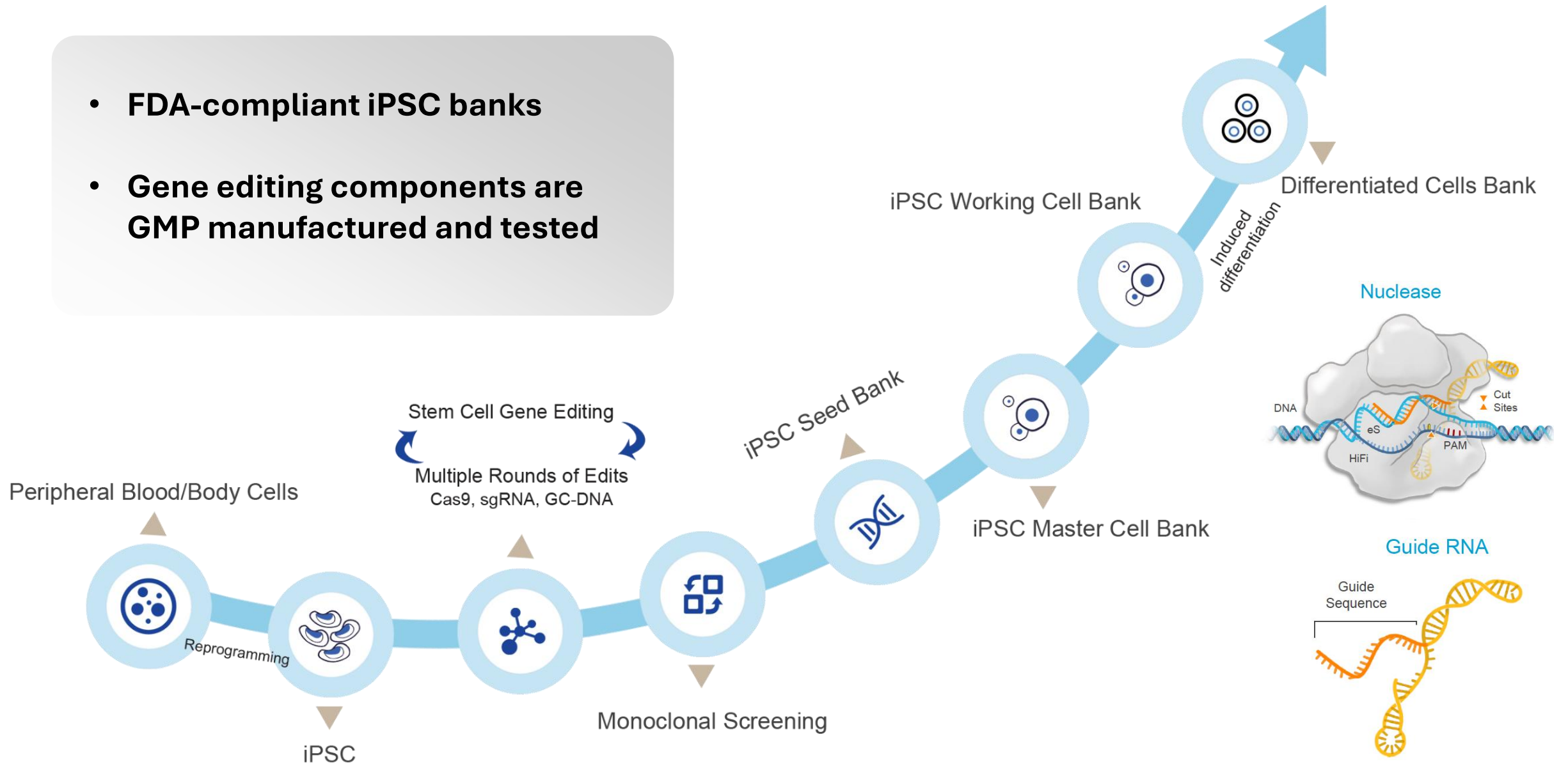
- ◆ Safety
- ◆ Identity
- ◆ Potency



uBriGene iPSC
Solutions

iPSC-Derived Cell Therapy Workflows

- **FDA-compliant iPSC banks**
- **Gene editing components are GMP manufactured and tested**



Bottleneck	Common, but Inadequate Approaches	uBriGene Biosciences' Solution
Donor cell access hurdles	Complex donor cell access & screening logistics	FDA-compliant sourcing & screening infrastructure
Safety – Genomic Instability	Viral & transfection approaches	mRNA-LNP reprogramming cocktail
Reprogramming inefficiencies	Methods requiring extensive downstream clone screening & characterization	Optimized high efficiency products & protocols
Cell engineering challenges	Ala carte methodologies, requiring a breadth of suppliers & technical expertise	Multiple GMP-ready time- and cost-effective cell engineering platform offerings

Bottleneck	uBriGene End-to-End Solution
CMC dependencies on multiple products & services partners	Reliable One-Stop Shop for scalable RUO & GMP iPSC workflows

FDA-Compliant Donor Sourcing

- Medical Centers
- CMS-certified CLIA testing labs
- Courier logistics

SpCas9-mRNA/SpCas9 protein

- GMP mRNA from in vitro transcription
- GMP SpCas9 protein from prokaryotic expression system

iPSC mRNA-LNP Reprogramming Cocktail

- Ready-to-use mRNA-LNP product
- No need for viral transduction or transfection
- Non-integrating/footprint-free

iPSC Banking

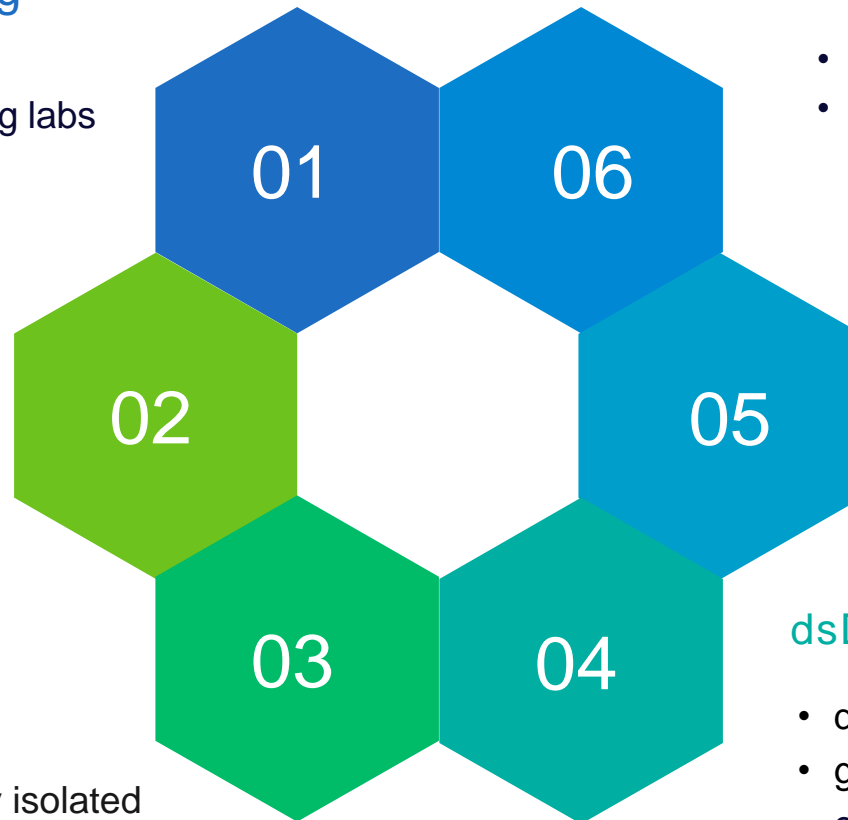
- Class A production environment - fully isolated production process with zero cross contamination.
- Extensive experience in cell therapy production.

IVT-gRNA Process

- Simple procedure, high throughput, inexpensive equipment
- No residual organic solvent (compared to the chemical synthesis process)

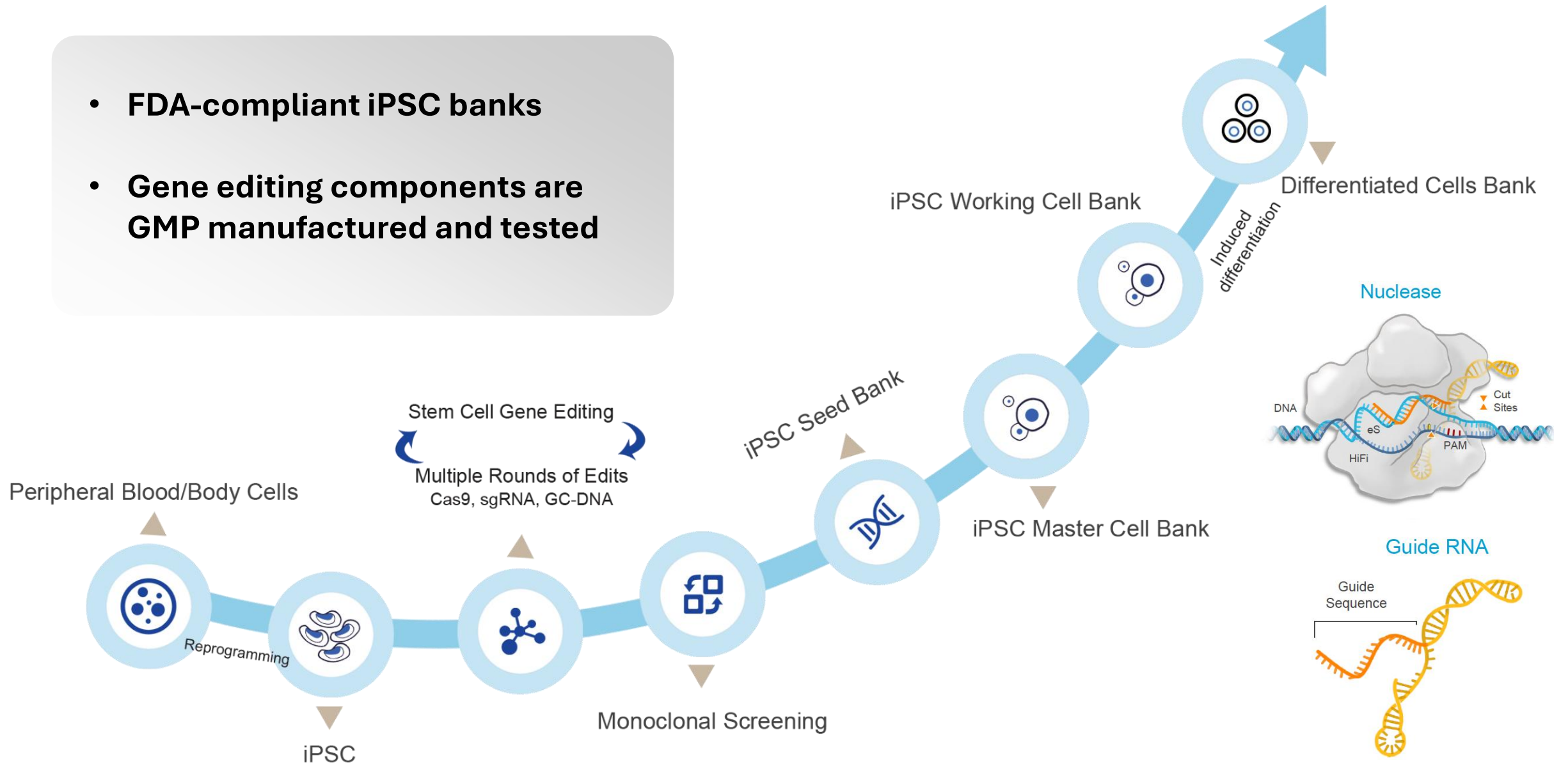
dsDNA/gcDNA/AAV

- dsDNA: Linearized plasmid
- gcDNA: Internally-developed patented method of closed-ended dsDNA reduces non-closed dsDNA fragments and thereby increasing yield.
- AAV: GMP grade AAV packaging, high nucleus entry efficiency and editing efficiency.



iPSC-Derived Cell Therapy Workflows

- **FDA-compliant iPSC banks**
- **Gene editing components are GMP manufactured and tested**



Fully FDA-compliant

Governing Documentation
Trial Master Files



Qualified Service Partners



Medical Clinics

- PI & Staff: Adequate experience and training
- Facility: Certification/Accreditation
- Patient recruitment access
- Investigational Review Board

CMS Certified Testing Lab with CLIA license

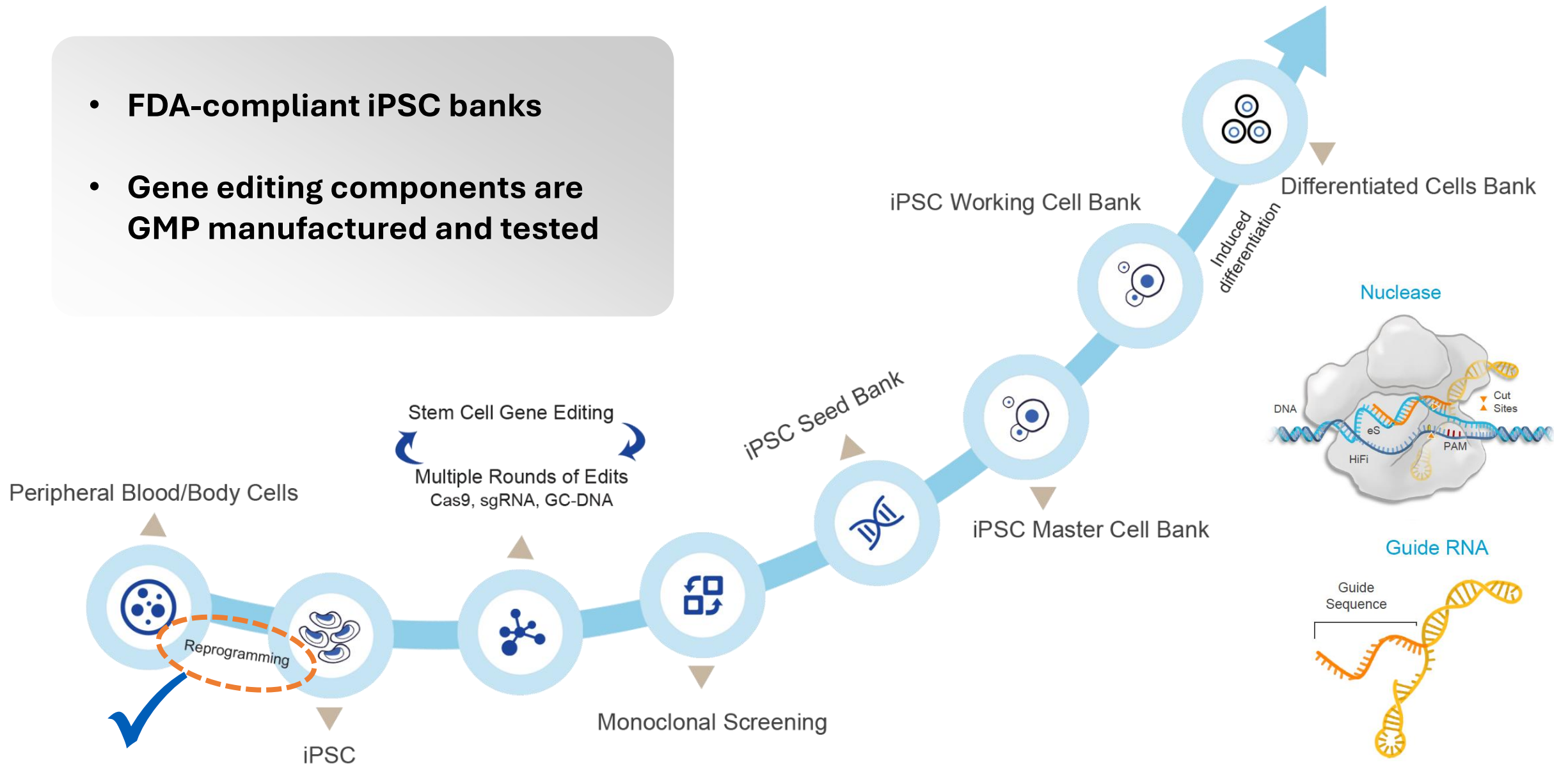
- Accuracy
- Precision
- Reportable range
- Reference intervals/range (normal values) for the laboratory's patient population

Courier

- Time-critical nature of customized requirement for clinical specimens
- Expert coordination between patients, clinics, and laboratories
- Door-to-door supply chain meets customer demand
- Provide on-demand packaging solutions from small parcels to multi-pallets across all temperature ranges

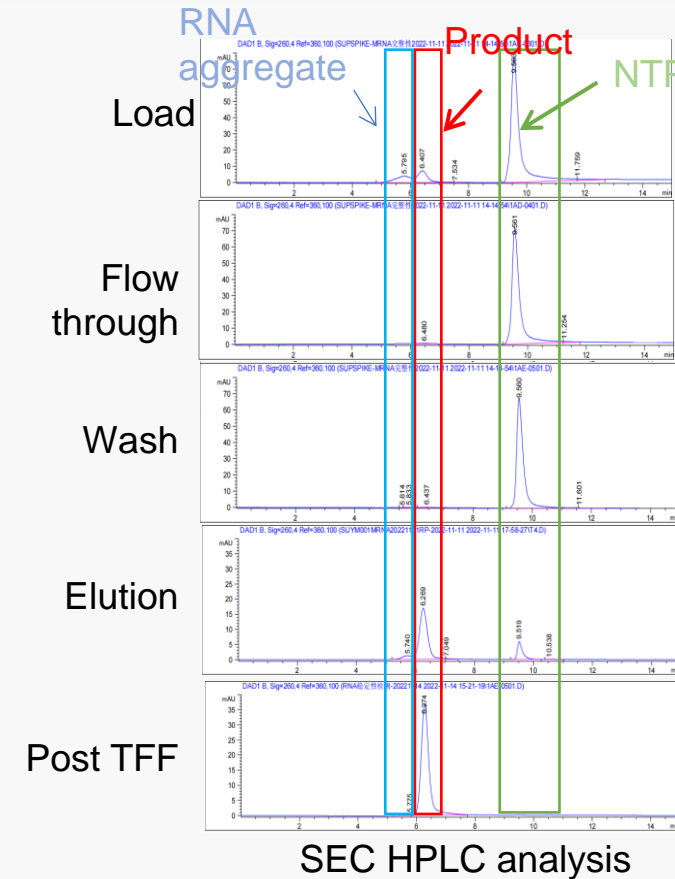
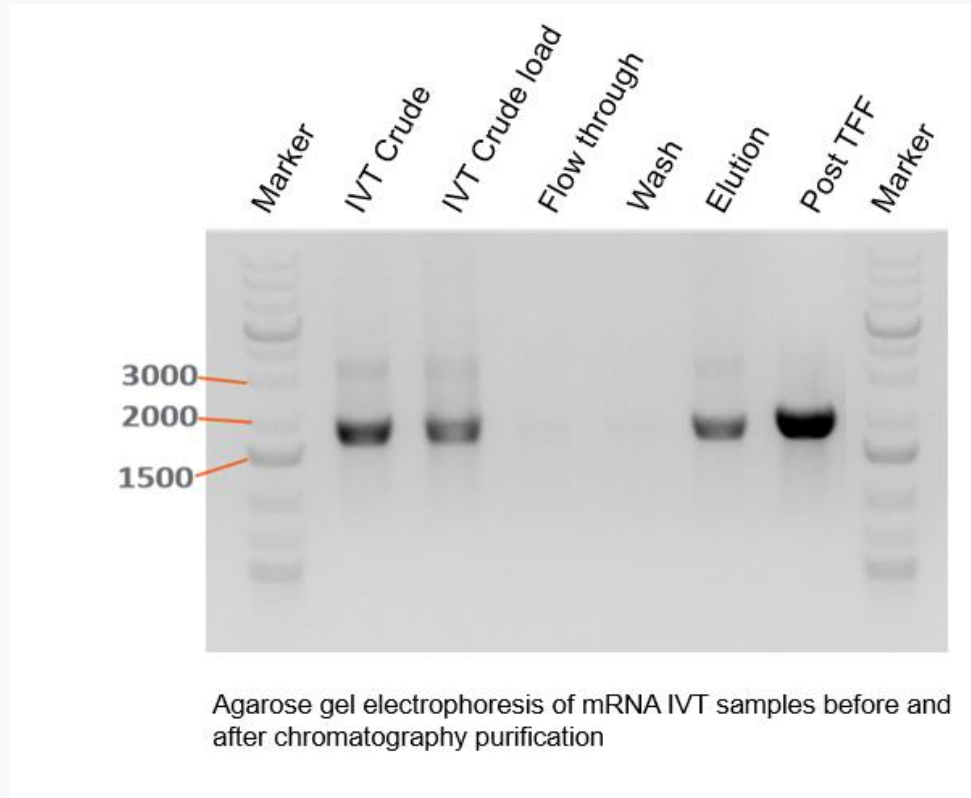
iPSC-Derived Cell Therapy Workflows

- **FDA-compliant iPSC banks**
- **Gene editing components are GMP manufactured and tested**



Highly Efficient iPSC Reprogramming mRNA-LNP Cocktail

- Reprogramming with mRNA, no risk of gene integration
- Convenient, Ready-to-use LNP mix, can be directly added to cells, eliminating the need for transfection reagents or viral manipulation
- Low cytotoxicity, high iPSC reprogramming efficiency, 4-10% (fibroblasts)
- System flexibility: suitable for feeder or feeder-free conditions



mRNA Production Summary

- High conversion rate: mRNA:DNA mass ratio in IVT reaction bulk reaches 200:1, mRNA reaches **10 mg/mL**
- High purity: after rough and fine purification, the final product's main peak accounts for **>95%**
- Low residual amount: T7 RNA Polymerase, dsRNA, and dsDNA residuals are well under industry standards.

- High yield: total yield is 76.5%

Efficient Production of mRNA-LNP Using Microfluidics

- Innovative microfluidics technology to encapsulate mRNA
- $\geq 90\%$ encapsulation efficiency
- Scalable mRNA-LNP process
- Y-mixer to 20L scale

Features of mRNA-LNP process:



Good batch consistency



Controllable particle size (within 50-200nm)



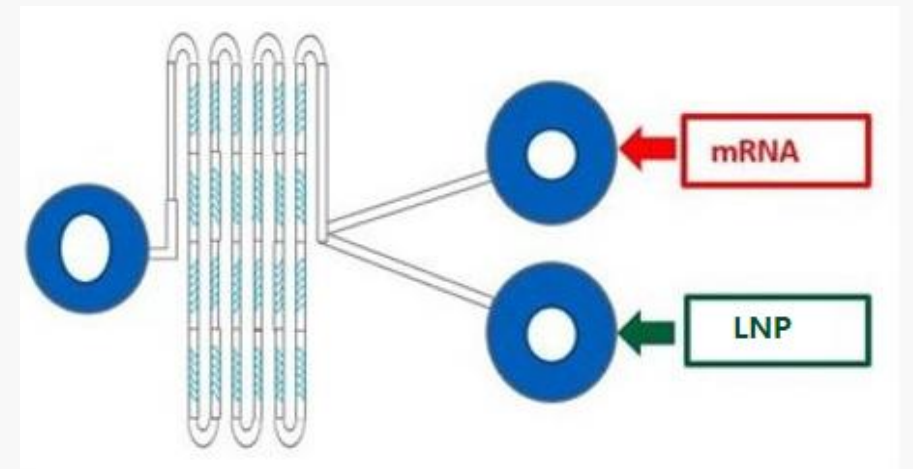
Packaging efficiency above 90%



Can provide mg-g level mRNA products



High efficiency payload delivery into primary T-cells & HSCs



Microfluidics mRNA-LNP packaging

COA for GMP manufactured mRNAs



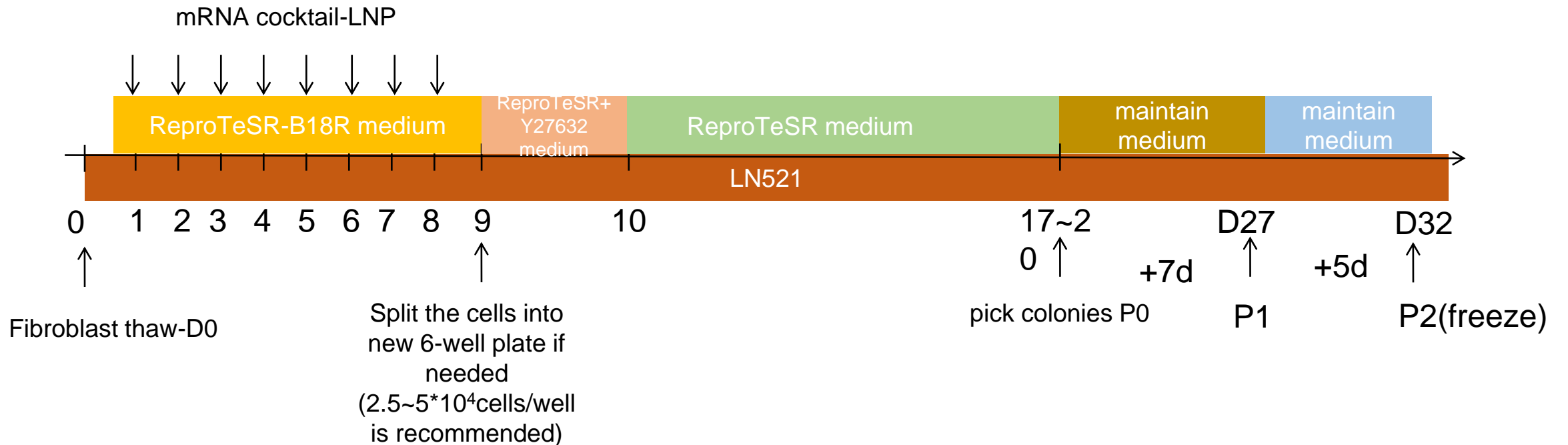
Testing items	Test Methods	mRNAs for reprogramming cocktail					Release Standard
		iPSCO0001m RNA	iPSCS0002 mRNA	iPSCK0003 mRNA	iPSCM0004 mRNA	iPSCS0005 mRNA	
Appearance	Visual method	Clear colorless	Clear colorless	Clear colorless	Clear colorless	Clear colorless	Clear colorless
RNA Concentration	UV absorbance	1.16 mg/mL	1.14 mg/mL	1.07 mg/mL	1.00mg/mL	1.22 mg/mL	≥1mg/ml
Purity	HPLC	94.6%	92.2%	94.9%	94.1%	95.4%	≥90.0%
Purity	A260/A280	2.1	2.1	2.0	2.1	2.1	A260/A280: 1.9~2.2
Endotoxin Detection	BDBU GEL-CLOT Method	< 10EU/mg	< 10EU/mg	< 10EU/mg	< 10EU/mg	< 10EU/mg	< 10EU/mg
Sterility	Culture method	Not detectable	Not detectable	Not detectable	Not detectable	Not detectable	Not detectable
pH	Multi-Parameter Tester	5.8	6.0	5.8	5.9	6.1	5.0-7.0

COA for Reprogramming mRNA-LNP



Testing items	Test Methods	mRNA-LNP	Release Standard
Appearance	Visual method	translucent, light milky white solution	translucent, light milky white solution
mRNA Concentration	Fluorescence-based assay	0.1 mg/mL	≥0.1mg/ml
Encapsulation efficiency	Fluorescence-based assay	95.6%	≥90.0%
Endotoxin Detection	GEL-CLOT Method	< 10EU/ml	< 10EU/ml
Sterility	Culture method	Not detectable	Not detectable
LNP size and polydispersity	Dynamic light scattering (DLS)	Z-Average: 99nm, PDI: 0.1	70nm-160nm, PDI < 0.3
Reprogramming potency	Reprogramming test	Pass	iPSC reprogramming ability from fibroblast

Patented mRNA cocktail for easy and highly efficiency iPSC reprogramming



4-10% reprogramming efficiency from fibroblasts

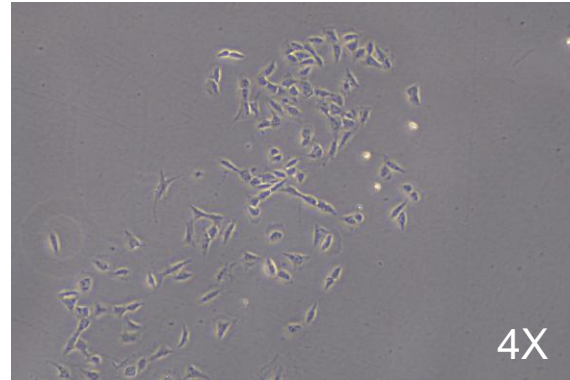
Patent pending

iPSC Clones Using Reprogramming mRNA-LNP

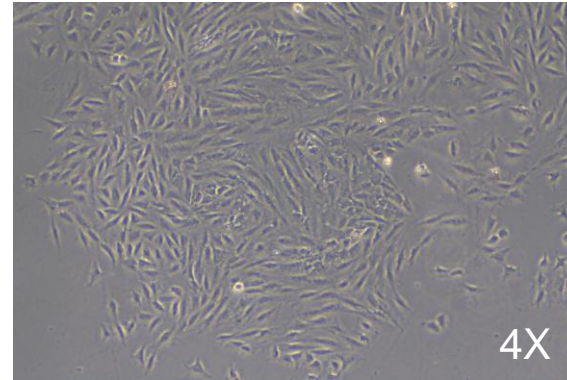
Day3



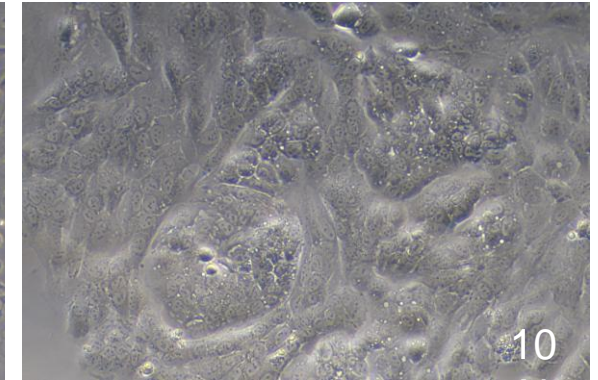
Day5



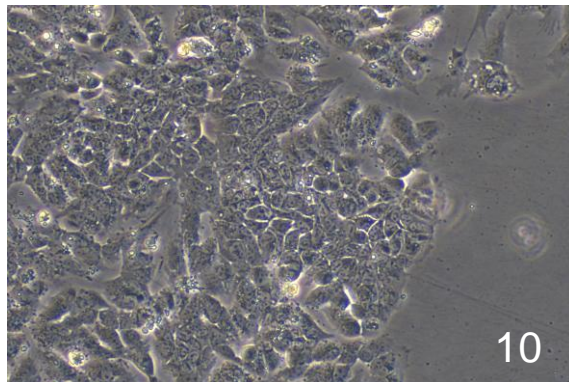
Day7



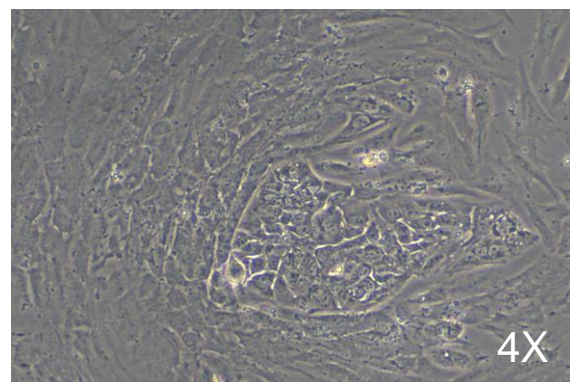
Day9



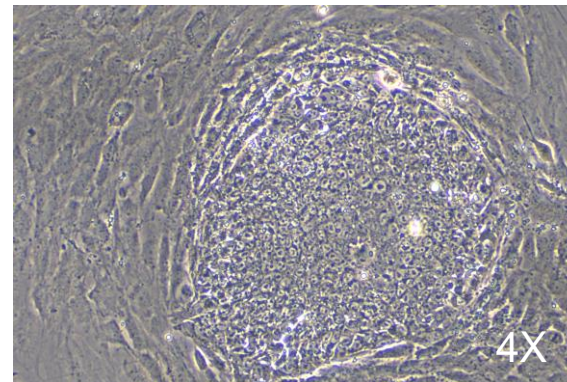
Day11



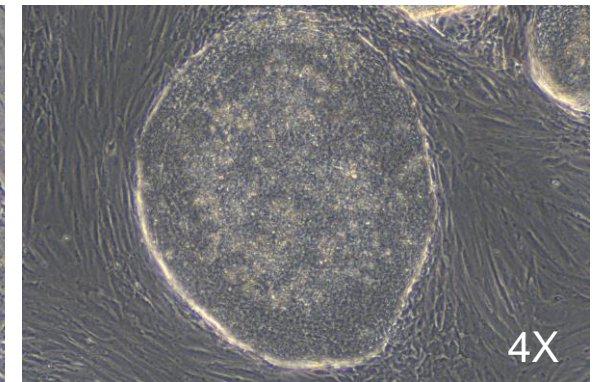
Day13



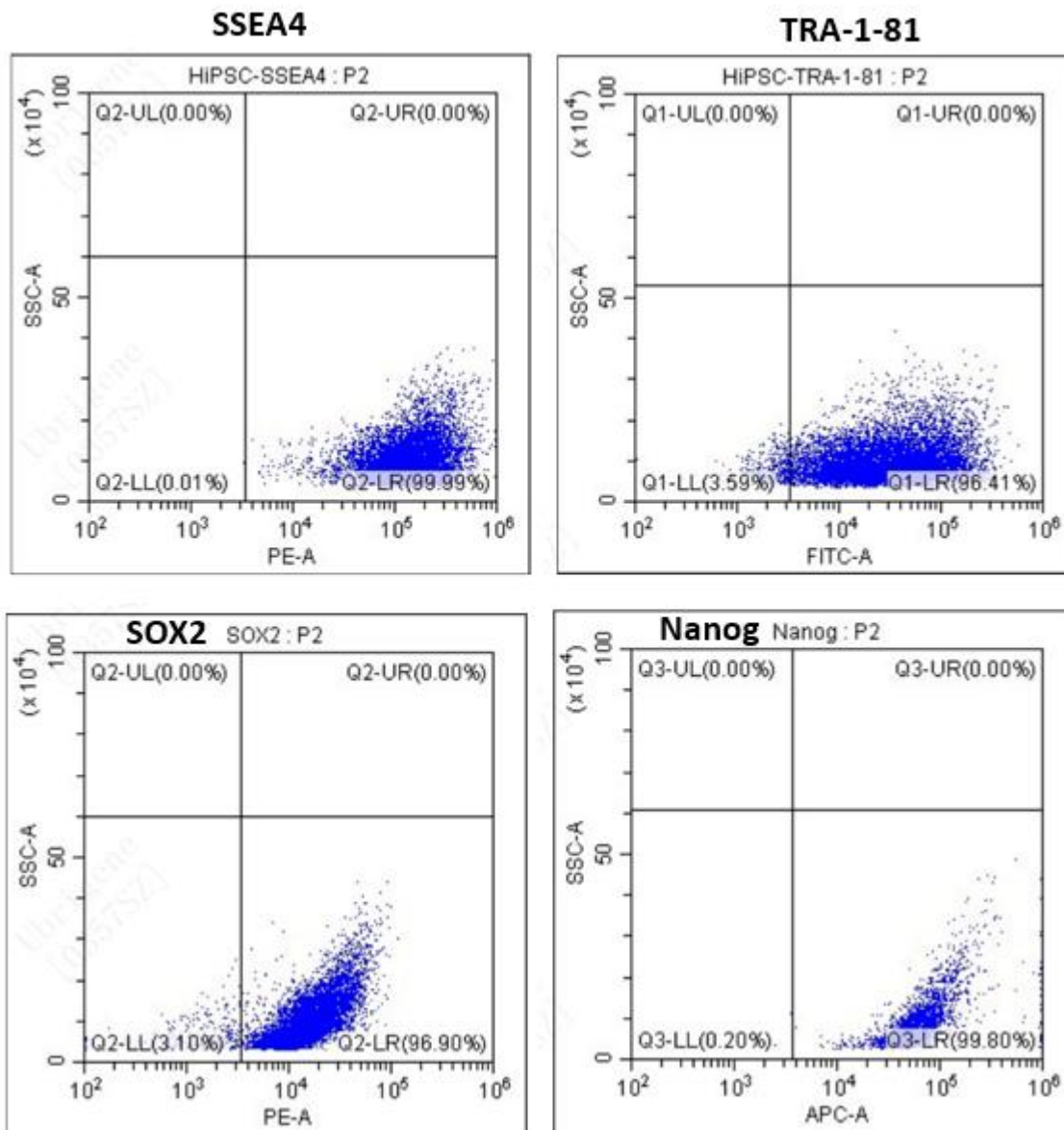
Day15



Day18

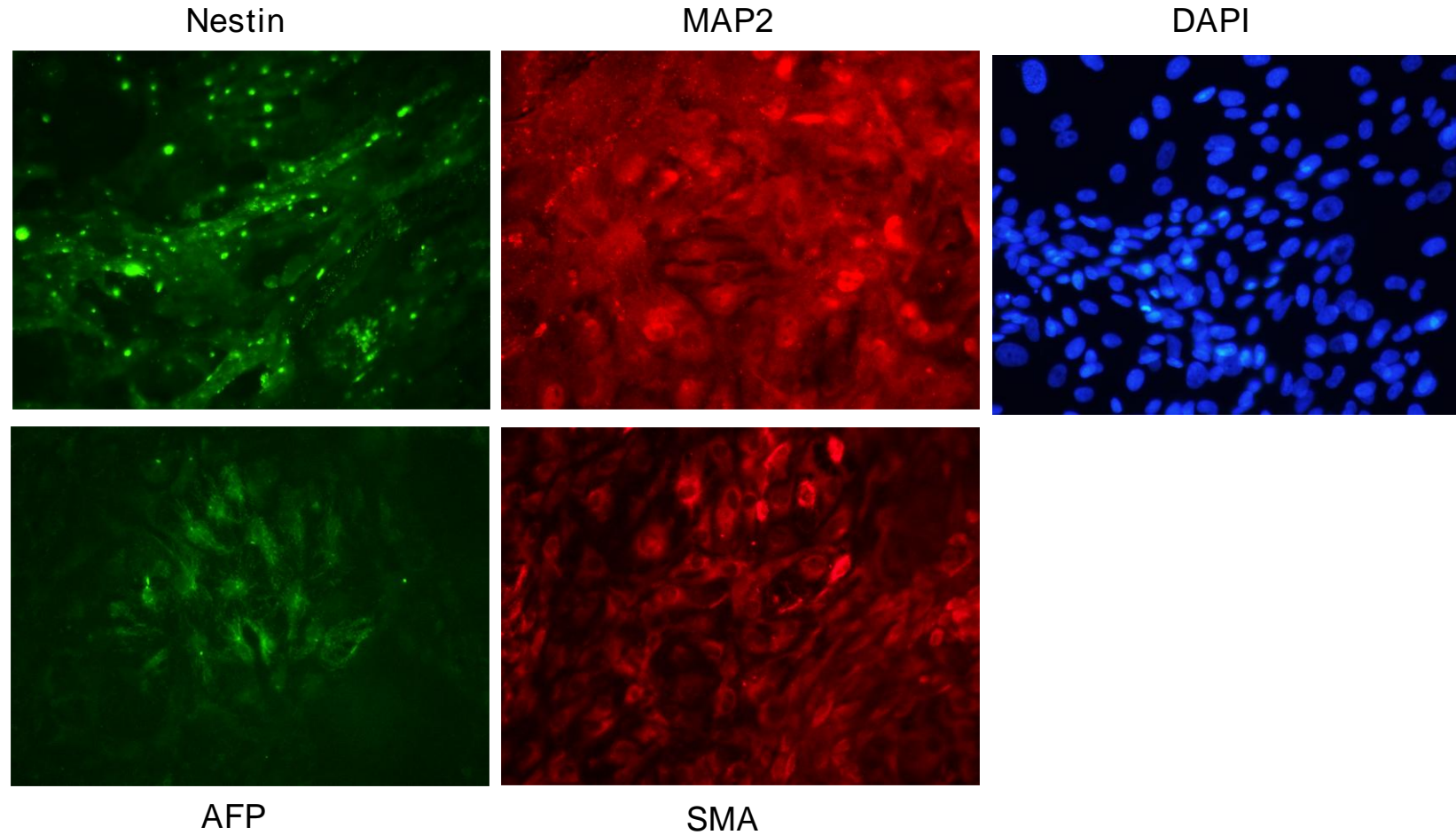


iPSC Identity by Flow Cytometry



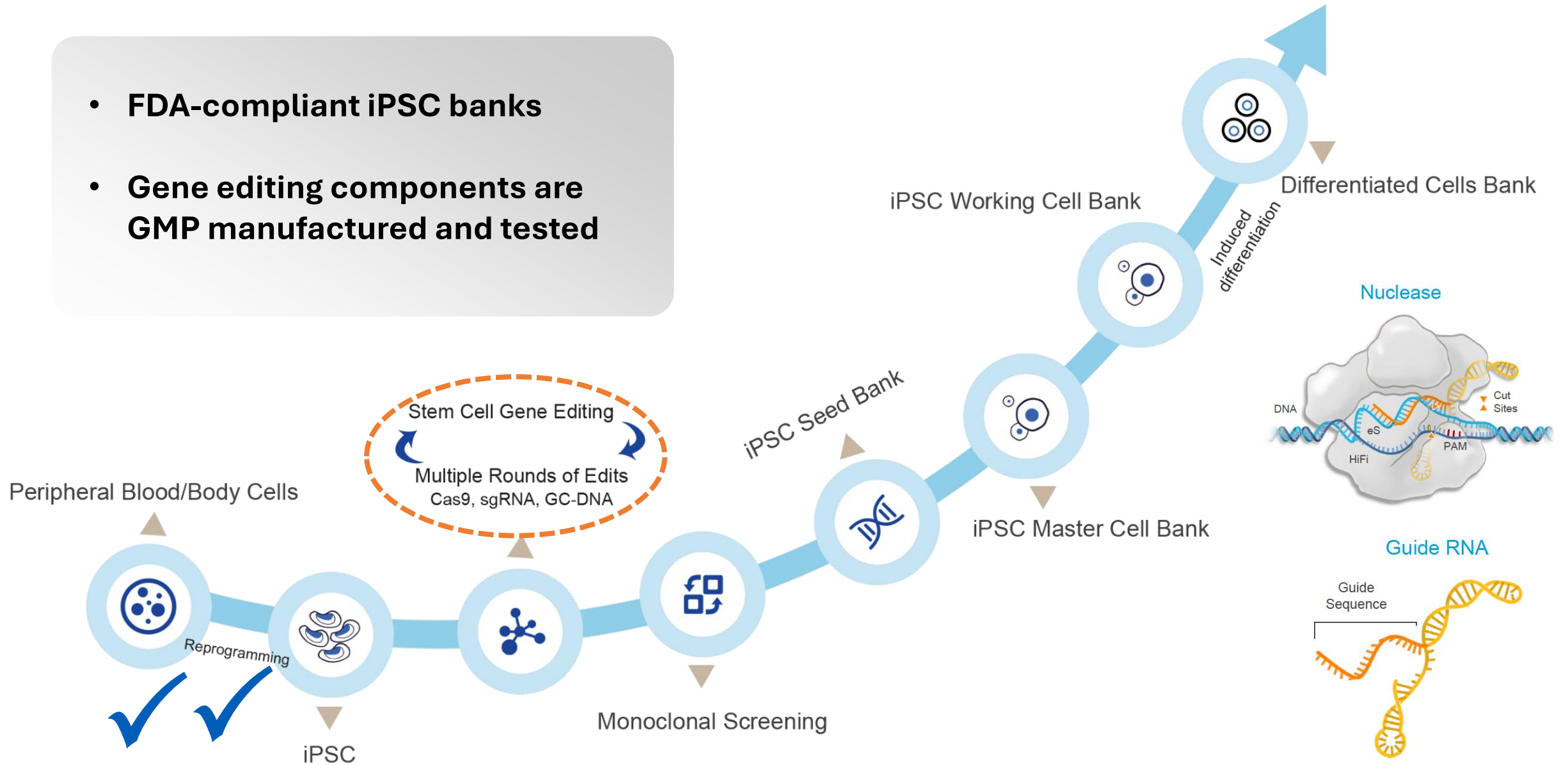
Confirmation of Stem Cell Markers by Flow Cytometry

Embryoid Body Formation Assay-iPSC potency



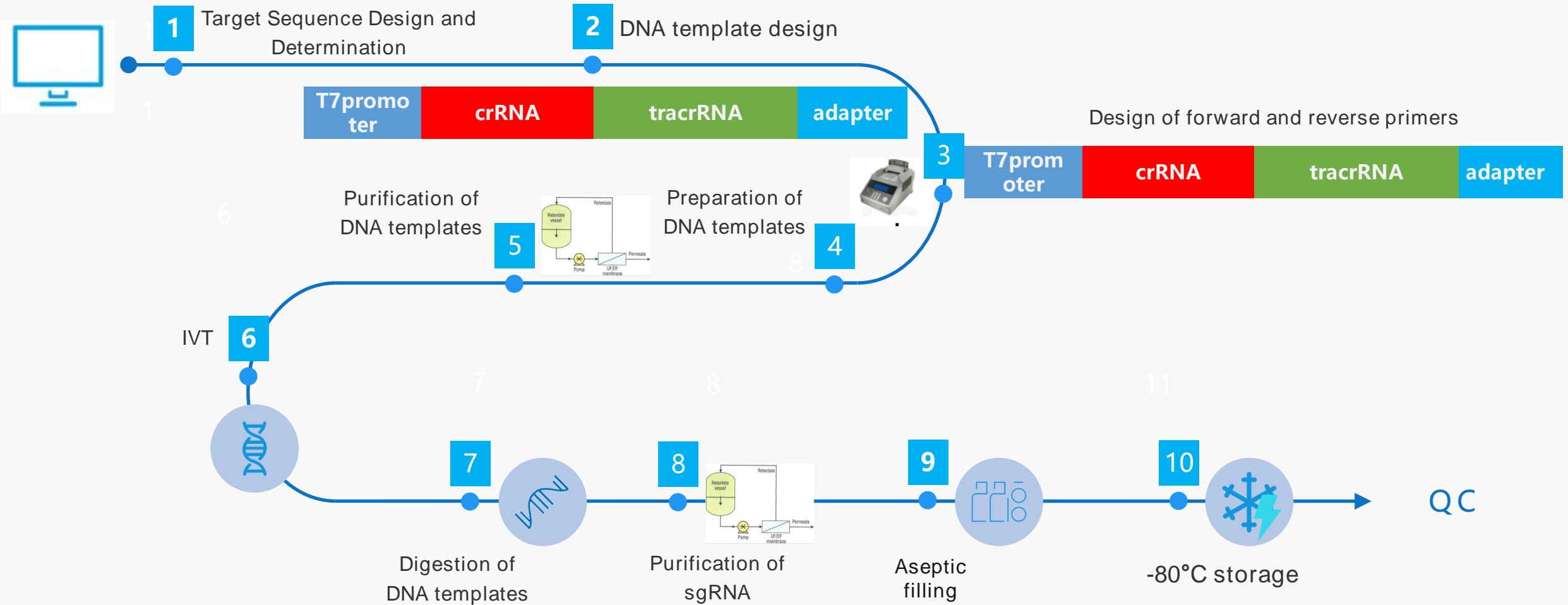
iPSC cells were differentiated into embryoid bodies to demonstrate the pluripotency of the iPSCs generated using our iPSC reprogramming mRNA-LNP cocktail. Immunostaining was performed for Nestin, MAP2, AFP, and SMA,

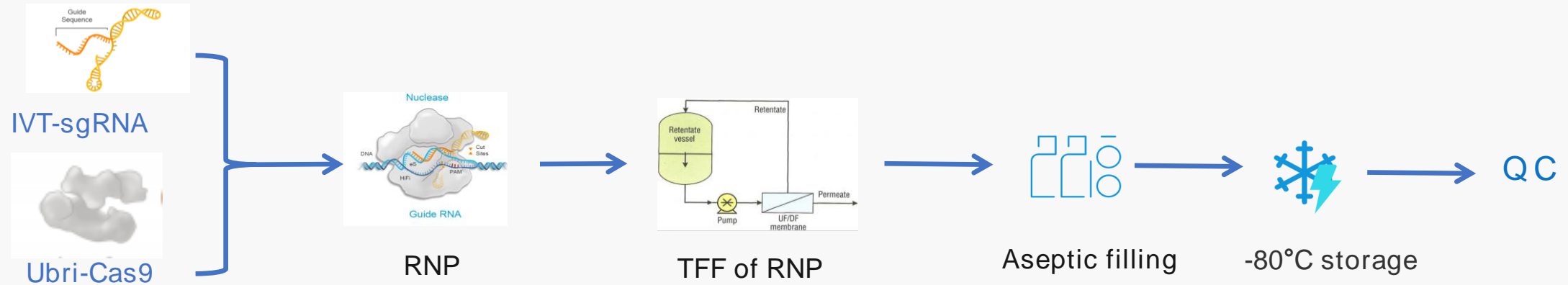
- **FDA-compliant iPSC banks**
- **Gene editing components are GMP manufactured and tested**



- Viral-based delivery
 - Lentiviral delivery of Cas cDNA (Cas9, Cas12a, Cas13a, Cas14a,...)
 - AAV delivery of donor KI template and/or compact Cas nucleases
 - sgRNA transfection
- mRNA-LNP
 - Cas mRNA-LNP
 - sgRNA-LNP
 - Donor DNA template-LNP (?)
- RNP (precomplexed Cas-sgRNA)
 - Transfection/Electroporation
 - RNP-LNP (PD stage)

IVT-sgRNA Platform





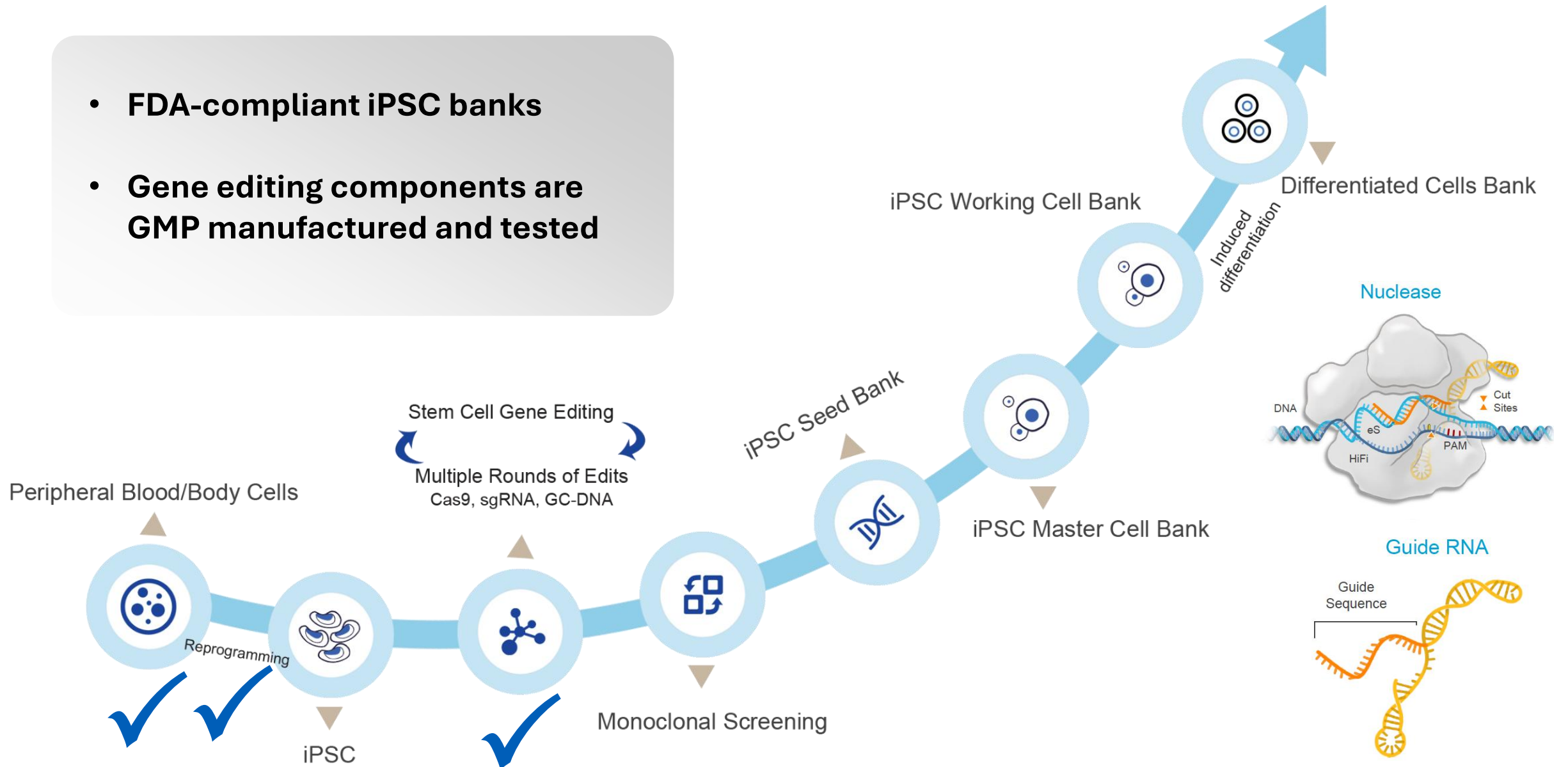
Process optimization

- Optimize the concentration and ratio of gRNA and cas9 protein to enhance RNP formation
- Optimize different buffer systems to enhance RNP formation and maintain RNP stability
- Optimization of the TFF process to enhance the removal of free sgRNA

IVT-sgRNA vs. Chemically Synthesized sgRNA

sgRNA name	Characteristic of Gene editor	Cell type	Transfection method	Method for detecting gene editing efficiency	IVT-sgRNA Gene editing efficiency(%)	Chemically Synthesized sgRNA Gene editing efficiency(%)
B2M-sgRNA	RNP	iPSC	Cell electroporation	Sequencing and TIDE analysis	72.5	57.45
XXXX-sgRNA1	RNP	iPSC	Cell electroporation	Sequencing and TIDE analysis	43	45.4
AAVS1-sgRNA	RNP	iPSC	Cell electroporation	Sequencing and TIDE analysis	62.5	41.45
TRAC-1-sgRNA	RNP	T cell	Cell electroporation	Flow cytometer	89.6	95.39
B2M-1-sgRNA	RNP	T cell	Cell electroporation	Flow cytometer	89.58	88.8

- **FDA-compliant iPSC banks**
- **Gene editing components are GMP manufactured and tested**

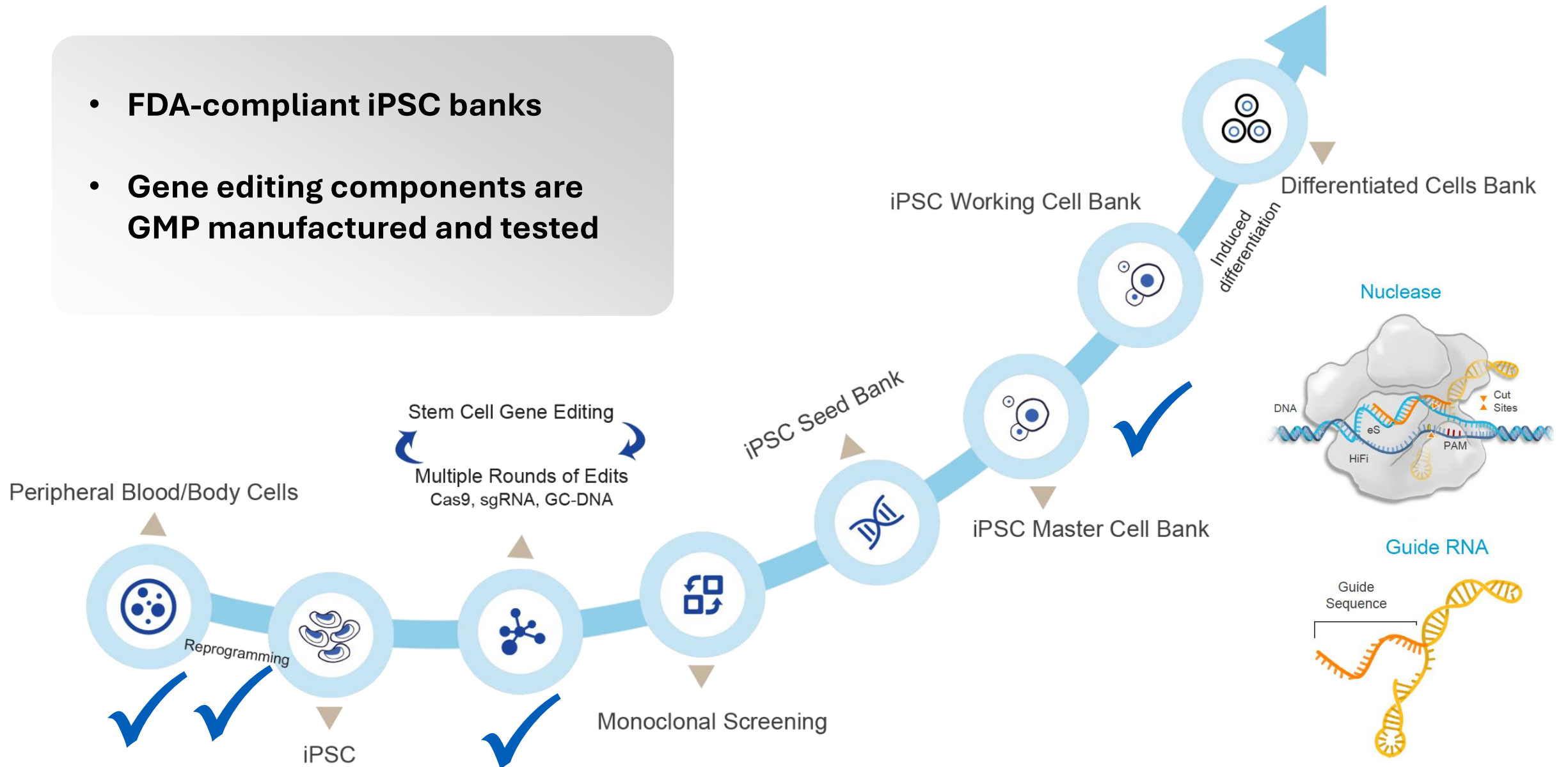


iPSC Cell Bank QC Panel



Objective	Assay	Acceptance Results	Parent cell	iPSC seed	iPSC MCB
Sterility	Immersion Sterility tests(B/F)	No organisms	✓	✓	✓
Mycoplasma Testing	Culture and cell indication assay	Negative	✓	✓	✓
Endotoxin	Kinetic chromogenic LAL	<2EU/ml	✓	✓	✓
Cell Viability	Trypan Blue	>70%	✓	✓	✓
Genotyping	STR Genotyping		✓	✓	✓
	Karyotype Analysis		✓	✓	✓
Adventitious virus	In vitro Assay for Adventitious Virus Contaminant	Negative			✓
	Fluorescent Product Enhanced reverse Transcriptase (FPERT) method	Negative			✓
	Transmission Electron Microcopy (TEM)	No viral particles			✓
Multipotency assay	Flow cytometry/ Immunostaining	SEA4 > 70% Tra-1-60 > 70% Tra-1-81 > 70%		✓	✓
		NANOG > 70%; OCT3/4 > 70% SSEA4 > 70%		✓	✓
		Tra-1-60 > 70%; Tra-1-81 > 70%		✓	✓
	Embryoid body formation assay	Detect of at least one marker per germ layer		✓	✓

- **FDA-compliant iPSC banks**
- **Gene editing components are GMP manufactured and tested**



- FDA-compliant donor cell sourcing & screening processes established
- Generated using mRNA-LNP reprogramming - no genomic footprint
- Access to multiple uBriGene gene editing platforms
- iPSC banks are subject to rigorous QC metrics
- Multiple iPSC banks available for RUO and/or GMP applications

A large white circle with a soft drop shadow, containing the text 'Thank You' in a blue sans-serif font. The background of the slide is a blue gradient with a glowing molecular structure pattern.

Thank You

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