January 2025

Edita Botonjic-Sehic PhD

Faster, smaller, smarter:

Empowering 5.0 BioSolutions Through Realtime PAT and Computational Controls

ReciBio Pharm

AGENDA

01 WHO WE ARE
A Leading CDMO | Capabilities

02 WHERE WE'RE GOING
Integrated & Intelligent Manufacturing | Partnership with MIT

03 WHAT WE ARE UP TO
Leading PAT in xRNA manufacturing | Real time monitoring

A GLOBAL CDMO

5,200+
Employees worldwide at 3/09/24

100+
Supplying over one hundred markets around the world

18
Development and manufacturing facilities in Europe, Israel, USA and India

1,000+
Every minute over one thousand people use one of our products

€ 1.3 bn
Net sales (FY23)

400+
Customers

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FOCUS AND HARMONIZATION ACROSS SITES ENABLES CROSS-CONTINENT MANUFACTURING AND CENTERS OF EXCELLENCE

			USA	Portugal	Germany
MODALITY	pDNA, xRNA, LNP	End-to-end, dual continent GMP manufacturing	●	●	
	Virus and Viral Vectors (AAV, LV, OV)	Virus experts, with a companion delivery option for DNA/RNA	●	●	●
	Microbiome and Live Biotherapeutics	Commercial FMT product & deep LBP development & GMP experience	●	●	
	Recombinant Proteins	Traditional protein scale-up & clinical scale manufacturing		●	
	ATMP Fill-Finish	Both automated & manual filling with best-in-class controls	●	●	●
SERVICES	PROCESS DEVELOPMENT	Rapidly scale for tox and clinical manufacturing + characterization	●	●	●
	CGMP MANUFACTURING	Cross-modality technical expertise and quality systems in execution	●	●	●
	REGULATORY SUPPORT	Clinical phase appropriate approaches to CMC	●	●	●
	COMMERCIAL READINESS	Commercial systems with clinical toggles, fit for purpose	●	●	●
			RUO, Clinical, Commercial Ready	RUO, Clinical, Commercial	RUO, Clinical, Commercial Ready

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ADVANCING INNOVATIONS ACROSS NEW AND EMERGING MODALITIES

EXPERIENCE

Therapeutic Areas

Gene Therapy & Editing, Rare & Infectious Disease, Oncology

Nucleic Acids

pDNA & xRNA in LNP, PNP, or AAV

Viruses

Oncolytic Virus, Adenovirus, AAV, LV

Microbiome

Aerobic, Anaerobic, Single-strain, Consortia

CUSTOMERS

55

Active Customers

50

Programs in Clinic

3

Commercial Programs*

MANUFACTURING

400+

Batches Manufactured**

200+

CGMP Batches

90+

Sterile/Aseptic Batches

*1 active, 2 in PPQs. **From 2019-2024.

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RBP IS DRIVING NEXT-GENERATION RNA BIOMANUFACTURING WITH MIT THROUGH FDA CBER'S LARGEST GRANT

CORE PROJECT AMBITIONS

Increase **Speed** to the Patient

Continuous & Integrated production

Compatibility with Multiple xRNA Modalities and LNP Formulation

Scalability from bench top to pandemic scale

Flexible for capability swapping to next-gen technology

CORE PROJECT AMBITIONS

- Grant spearheaded by Peter Marks (CBER)
- $82M over three years awarded to MIT
- $62M sub-awarded to ReciBioPharm (2023)
- RBP deliverables focused on industry use

OUTPUT

- cGMP manufacturing platform capable of 40g/day
- Digital PD simulator
- Process Analytics with predictive modeling, machine-to-machine communication, Real-Time Release
- Alignment with ICH Q13 guidance

A NEW APPROACH TO RNA MANUFACTURING IS CRITICAL TO ENHANCE PATIENT'S LIVES AND EXPAND THE REACH OF RNA THERAPIES

Costs and development times are hindering access to advanced therapies

- Biologics account for 46% of pharmaceutical spending but only 2% of all prescriptions
- Costs are too high to enable development of many orphan indications
- Even at Covid-scale vaccines cost $2.70/100ug dose

ReciBioPharm's Approach to industry's needs

Digital PD	Development times drop from 6-9 months to <1 month
Integrated Manufacturing	Manufacturing times drop from 3 months weeks to 7-14 days
Process Analytical Technologies	Real time QC removes CQA-based gating
Machine Learning Knowledge Hub	Predictive control

Partners

MIT

PennState

Rensselaer

The speed and accuracy of this platform will enable access to advanced therapeutics and bring speed to vaccines in infectious disease outbreaks

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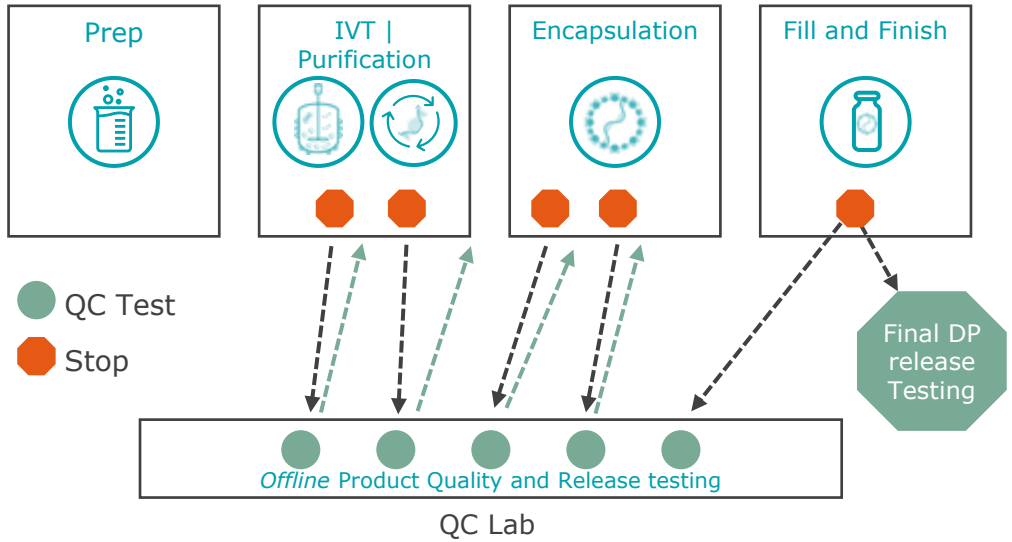
CONTINUOUS PRODUCT FLOW | GREATER EFFICIENCY IN A SINGLE ROOM THROUGH PAT AND DIGITAL ARCHITECTURE

Traditional Batch Process

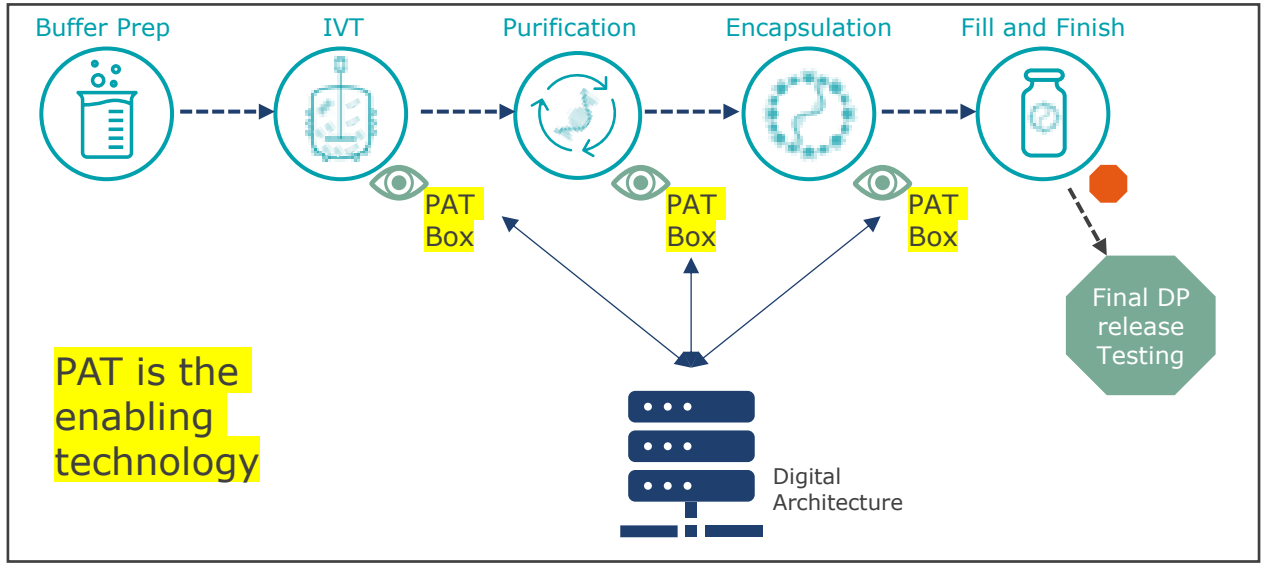
Five Manufacturing Suites



- Process steps are gated by QC release
- Limited in-process knowledge → rework and manufacturing risk
- QC lab can be on a different floor, building or a 3rd party

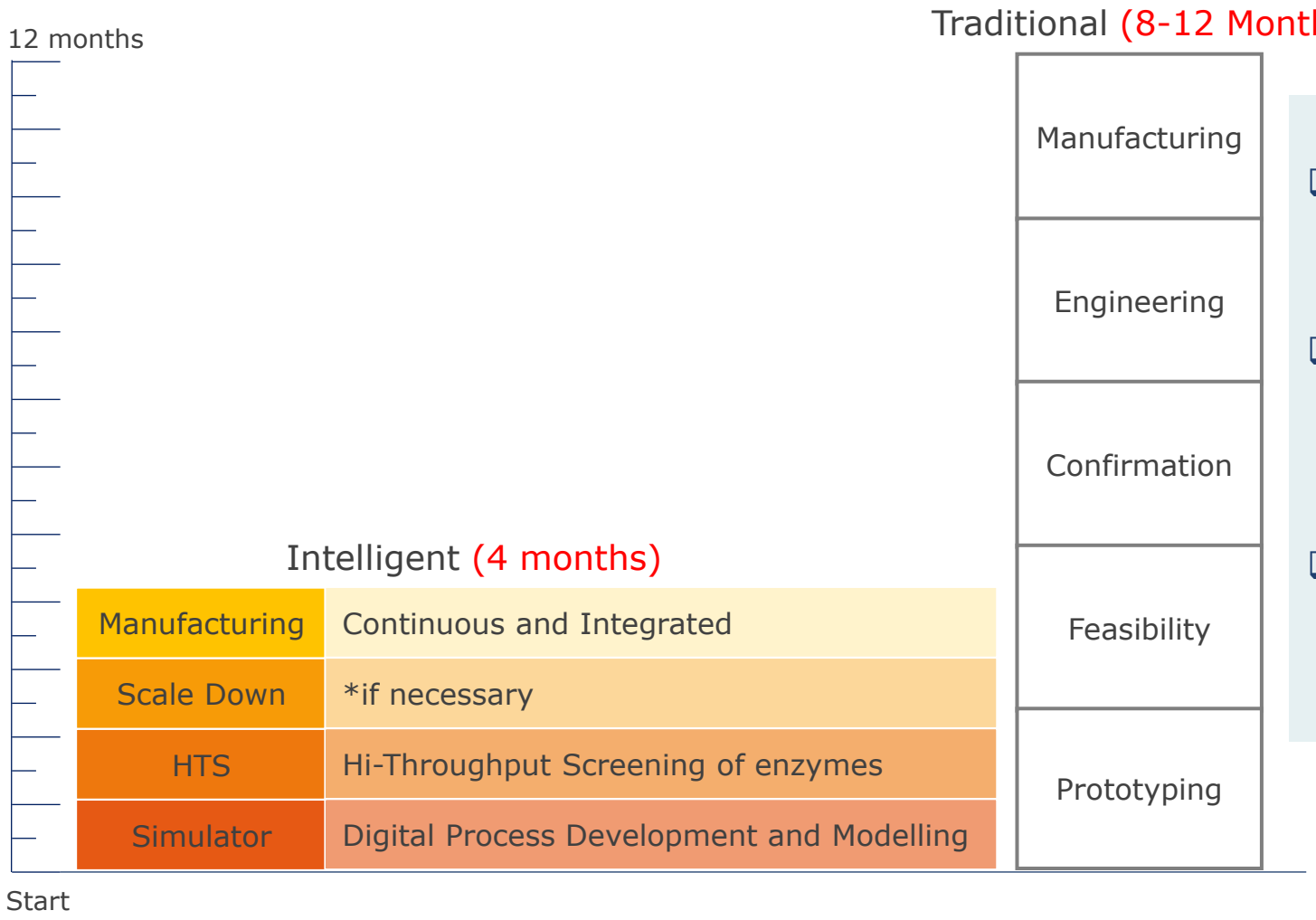
Continuous and Integrated Process

One Manufacturing Suite



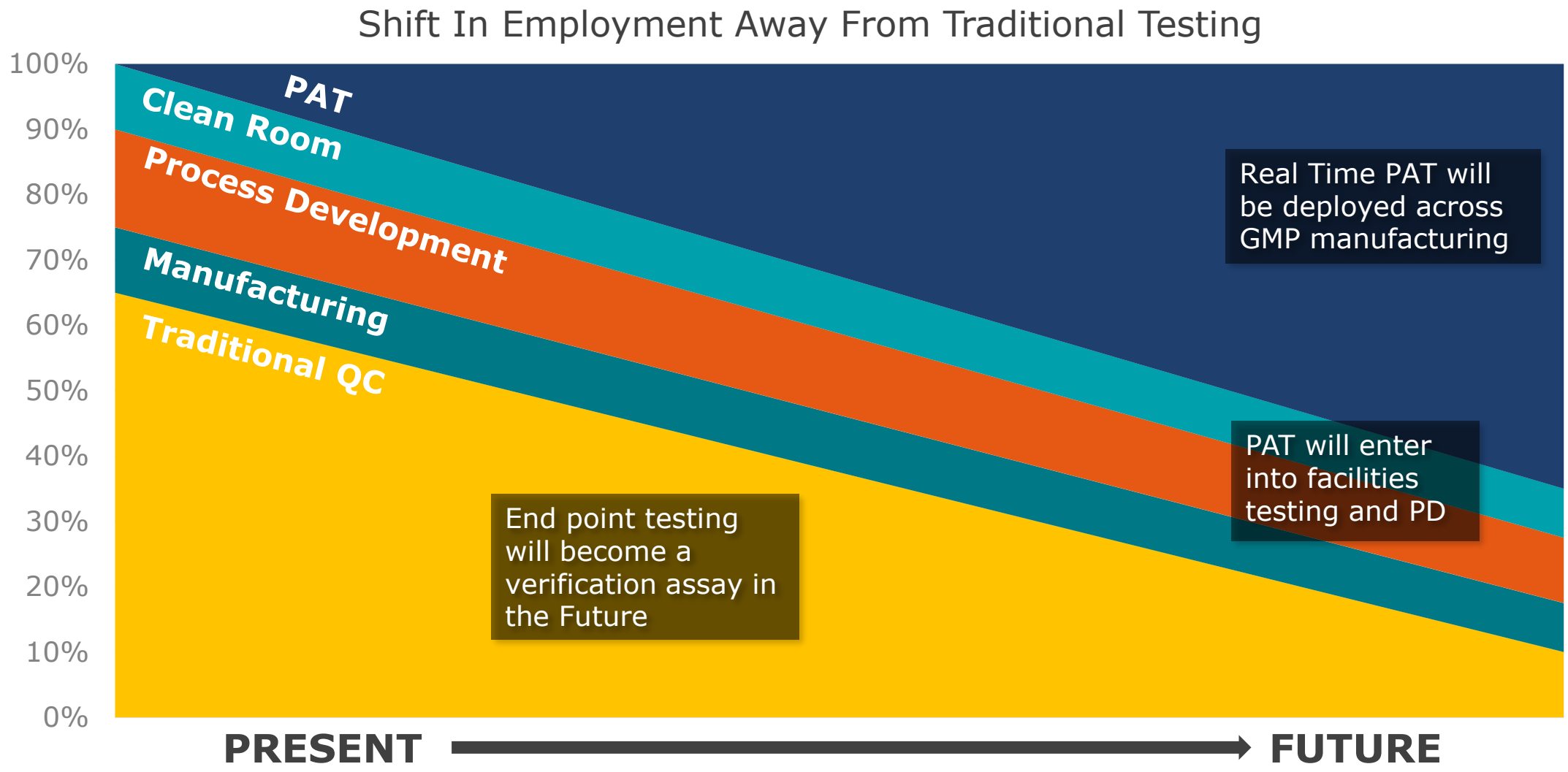
- Processes and QC testing occur in a single suite
- QC results are fed to automation control software
- Constant monitoring and real time product characterization

PAT OFFERS A MORE INTELLIGENT APPROACH TO PD | A HOLISTIC APPROACH TO TECHNOLOGY AND PROCESS DEVELOPMENT



- **A PARADIGM SHIFT IN HOW PROGRAMS WILL DEVELOP**
- **PAT RELIEVES PAIN POINTS FROM TRADITIONAL PD AND TECH TRANSER**
- **FASTER WORKFLOW, LOWER COGS AND PEACE OF MIND THROUGH REAL-TIME MONITORING**

IN THE FUTURE, THE USE OF PAT WILL OVERTAKE TRADITIONAL END-POINT QC TESTING



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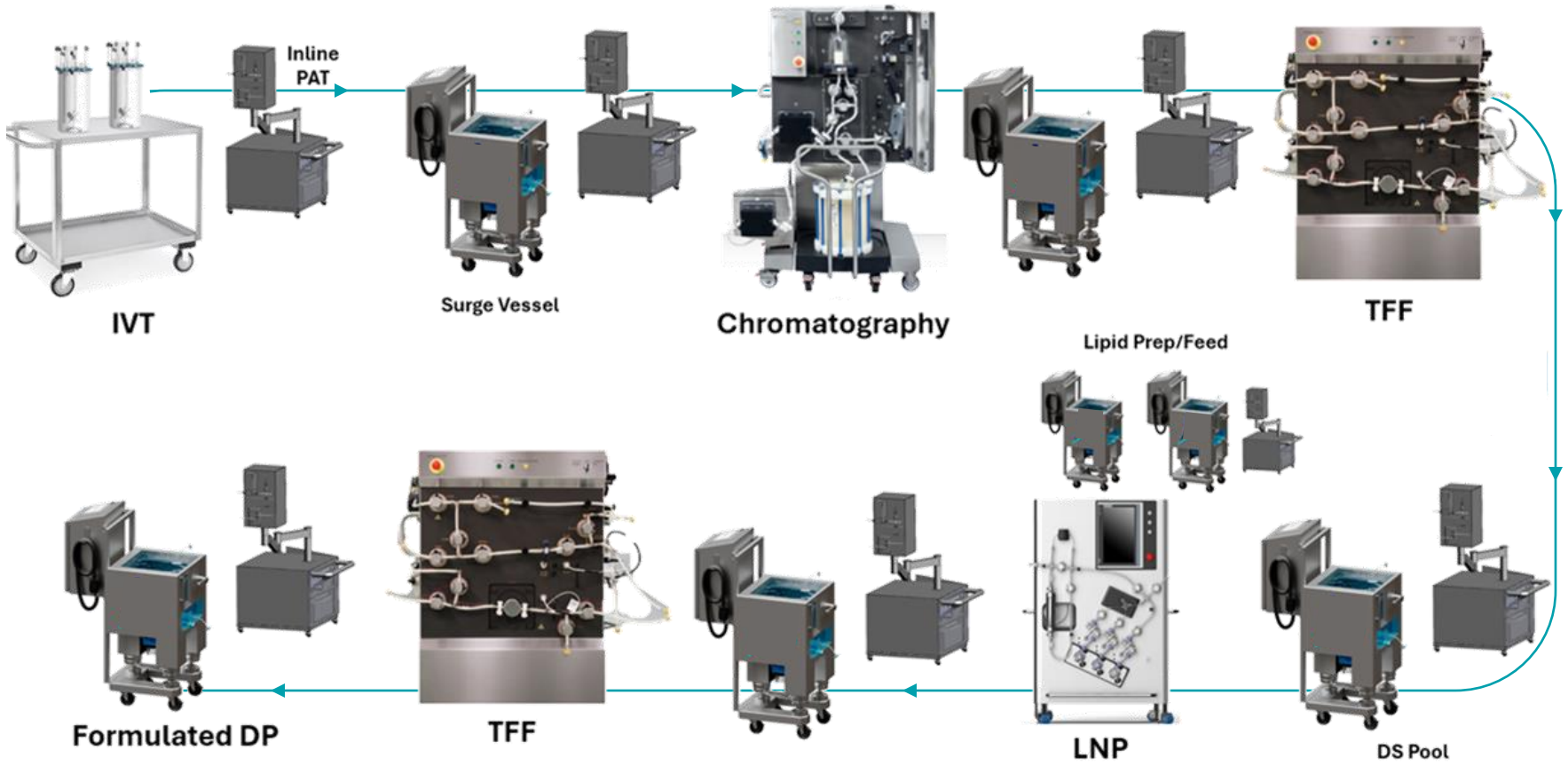
SMART MANUFACTURING OF xRNA THROUGH ADVANCED HARDWARE, SOFTWARE, AND PATENTED PROCESS ANALYTICAL TECHNOLOGIES



Integrated IVT

Formulation with ***inline PAT***

Images are for illustrative use only

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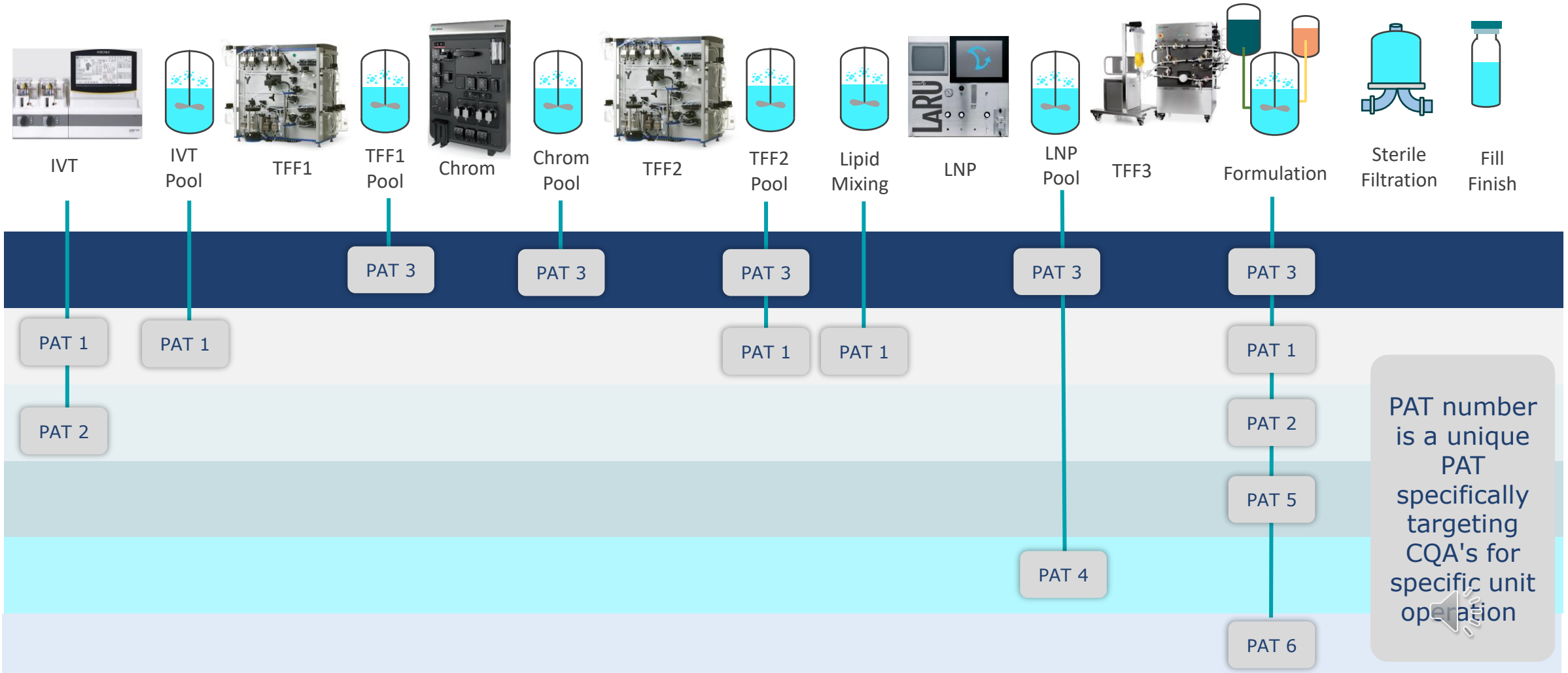
PAT FOR CONTINUOUS TO SUPPORT DIGITAL TWIN DEVELOPMENT AND REAL TIME ANALYTICS



IN-PROCESS PAT (ASSAY COVERAGE) / MOST CQA'S HAVE A PAT SOLUTION WITH THE OTHERS STILL BEING EXPLORED

In-process PAT assay coverage

Step	mRNA concentration - A260	mRNA concentration (Nanodrop)	Encapsulation - Florescence (RiboGreen)	mRNA purity - CE (Frag A)	mRNA integrity - CE (Frag A)	mRNA intactness	percentage of fragment mRNA	aggregate quantitation	dsRNA content - Dot blot	LNP size, polydispersity - DLS	LNP surface charge, morphology, ζ-potential	Residual Total Protein - Florescence (NanoOrange)	Residual plasmid DNA - qPCR	Visual Appearance - USP⟨790⟩	Subvisible particles	Particulate matter	Sucrose concentration - LC/CAD	Total lipid (Ionizable, PEG, DSPC, cholesterol)	Osmolality - mOsm/kg H_2O	Viscosity - USP⟨911⟩	Residual ethanol - GC	Residual solvents	Residual *E.coli* HCP - ELISA	Residual nucleotides, NTP - LC/MS	pH	Bioburden, Sterility - USP⟨1⟩,⟨61⟩,⟨71⟩	Endotoxin - USP⟨85⟩	5' Capping Efficiency - LC/MS	3' Poly(A) tail length, variant distribution - LC/MS	Sequence identification - Illumina, Sanger	IVT potency - cell-based assay	mRNA purity - LC, CE, Bioanalyzer?	mRNA content - UPLC and Rib green	mRNA/Lipid Mass (N:P ratio)	Extractable Volume	Container Closure integrity (CCIT)	Immunogenicity
In-line PAT (2025/2026)	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X			X	X		X	X	X		X	X	X		X		X		
IVT Reaction	X																																				
IVT Pool	X				X				X			X	X										X	X		0	X	X	X	X						
IVT Pool Dilution	X																																			
TFF1 Pool	X				X				X			X	X										X	X		0	X									
TFF1 Dilution	X																																			
Oligo dT Pool	X			X	X				X			X	0										X	X		0	0									
TFF2 Pool/Release DS	X			X	X	X		X	X			X	X	X								X	X	X	X	X	X	X	X	X	0	X				
Lipid Solution																		X																		
DS Dilution	X			X																					X											
LNP Pool		X	X							X																	0						X			
TFF3 Pool		X	X							X																X	0									
Post-Sucrose		X	X							X							X		X							X	X									
Bulk DP		X	X		X					X				X			X								X	X	X									
Release DP		X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X

Assay coverage for mRNA process

Steps of the mRNA process aligned to assays per unit operation

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KNOWLEDGE BIOHUB

(Intelligence Hub)

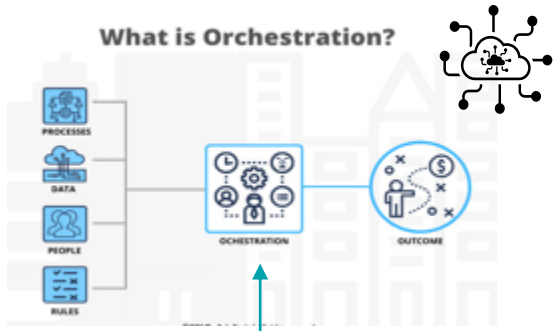
Structured, semi-structured, unstructured data

Data Lake

Knowledge Hub

PD Simulator (Digital DOE calculator)

Streaming analytics/PAT Management Software and data orchestration & diagnostics for preventive maintenance



Digital Twin/Machine Learning

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PAT SOFTWARE MANAGES INSTRUMENTS, DATA, AND MODELS IN REAL-TIME

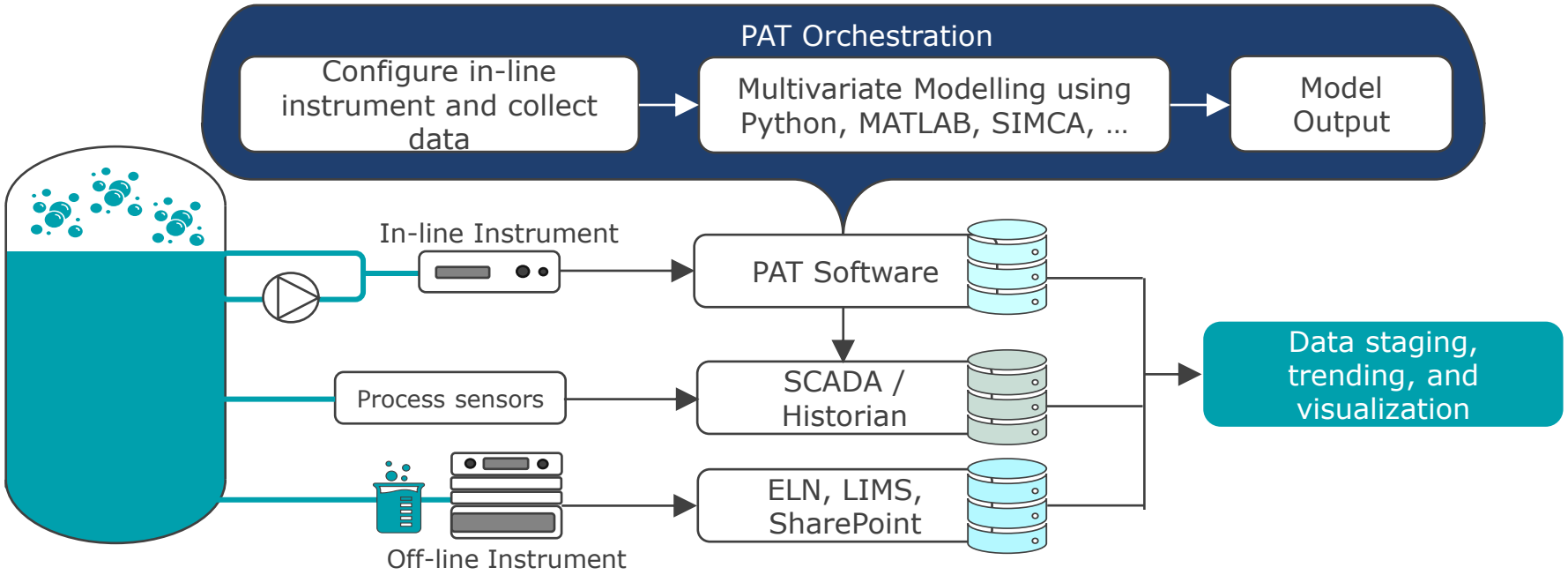
A bespoke PAT software solution reducing cost, allowing for modularity, and reducing time for Data Scientists.

PAT software roles:

1. Control inline equipment
2. Data collection and management
3. Execute CQA models
4. Process Monitoring and Control

Benefits:

1. Long term Cost
2. Maximum Flexibility
3. Reduction of time for integration of new instruments



Knowledge Hub/Simulator

IVT Mechanistic Model and Simulator GUI (Advanced Window)

Simple Simulation | Advanced Simulation

IVT Model Advanced Simulator!

Process, Product and Technology Selection

Reactor Type	Batch
Product Type	mRNA
Capping Technology	Co-transcriptional
Tailing Technology	Co-transcriptional

RNA Sequence Properties or Sequence

RNA Sequence Properties:

RNA Sequence Length	9552
Adenine proportion	0.26
Cytosine proportion	0.27
Guanine proportion	0.28
Uracil proportion	0.18

RNA Sequence:

Import Sequence

Paste Sequence

Ouputs (KPIs, e.g., CQAs)

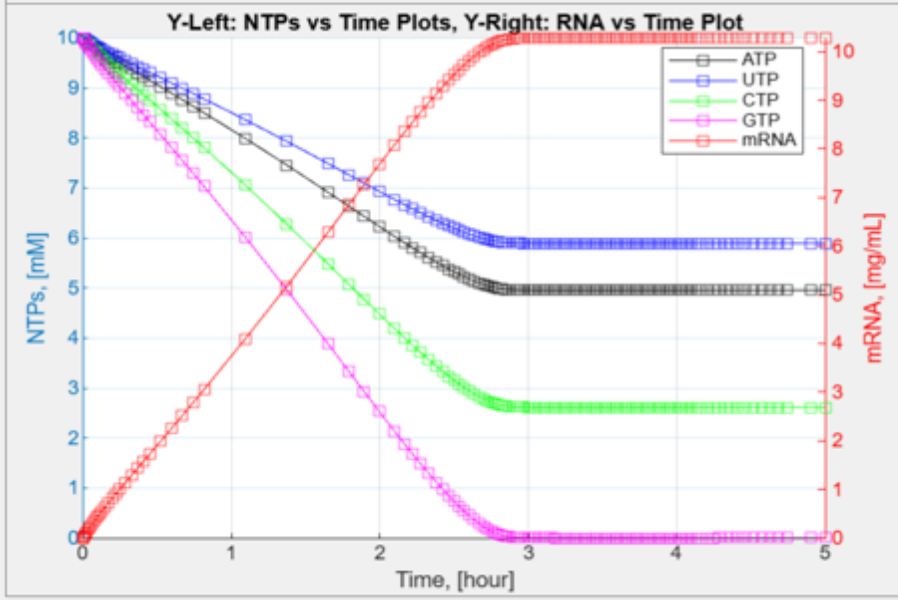
Yield	90
Integrity	10

Reaction Duration

Reaction Duration: 5 Unit: hr

Inputs

ATP	10	mM	RNAP	25	kU/mL
CTP	10	mM	PPiase	0.001	kU/mL
GTP	10	mM	RNase Inhibitor	0.25	kU/mL
ΨTP	10	mM	Spermidine	2	mM
DNA	0.025	mg/mL	Dithiothreitol	10	mM
Mg	25	mM	Temperature	37	degC
Cap	4	mM	Stirring Rate	100	RPM
			pH	7.5	



mRNA Yield [mg/mL]	10.28
ATP Conversion [%]	50.45
CTP Conversion [%]	73.86
GTP Conversion [%]	100.00
ΨTP Conversion [%]	41.06

RESET

HELP

A chatbot interface for users to interact with the results of the simulation, and historical data!

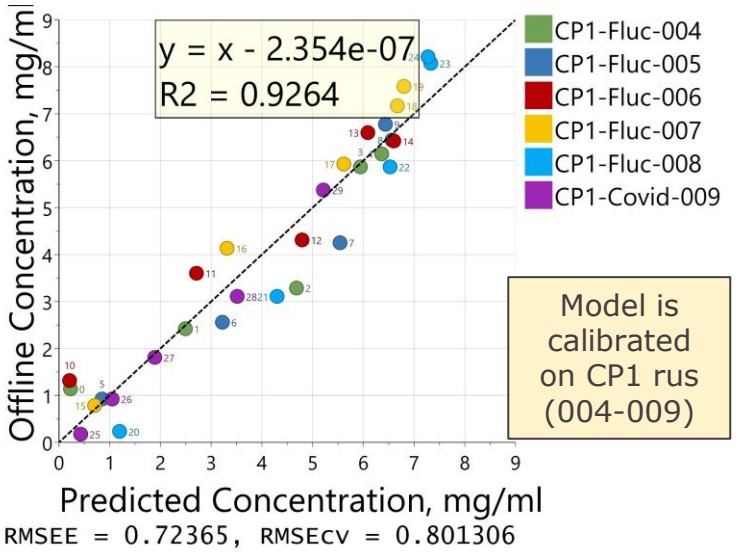
Send/Enter

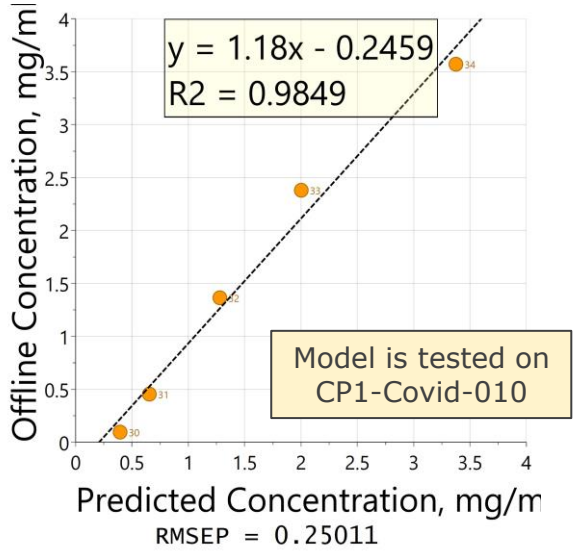
IVT RAMAN + PLS MODEL TO MONITOR MRNA CONCENTRATION

Real-time model performance

Raman model for mRNA prediction in IVT

- Trained on five Fluc and one Covid runs.
- RMSECV = 0.8 mg/mL
- R2 = 0.926
- Used the newest Covid run as an independent test dataset with RMSEP = 0.250
- The generic Raman model adequately predicts the latest run CP1-Covid-010.

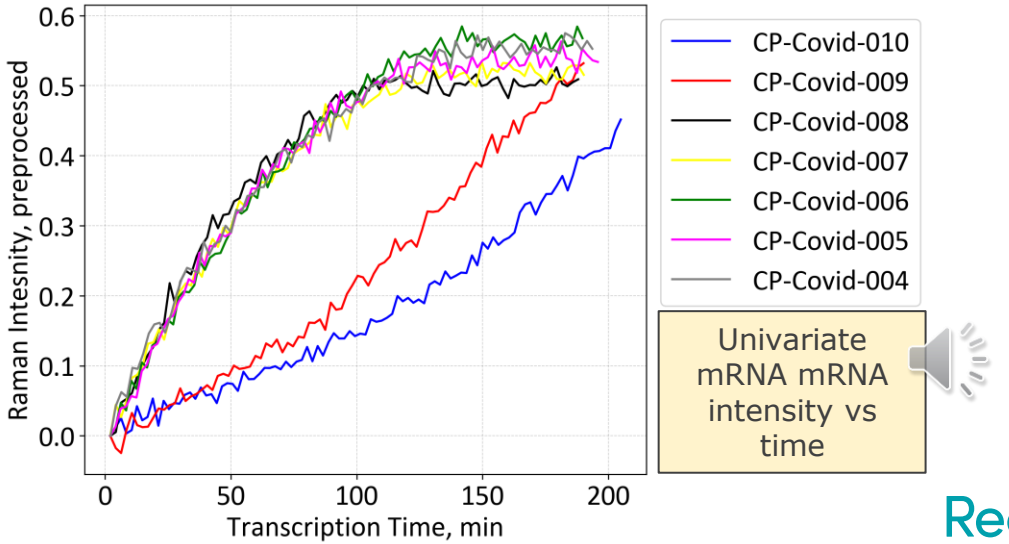




Univariate Analysis

Univariate trending:

- Is used to determine mRNA growth in the current run vs previous runs (batch comparison).
- Determines transcription performance: pace and endpoint.
- Provides insight on if the reaction needs to continue or plateaued.
- Could be used to determine the cause of plateau if related to NTP concentration and shortage.



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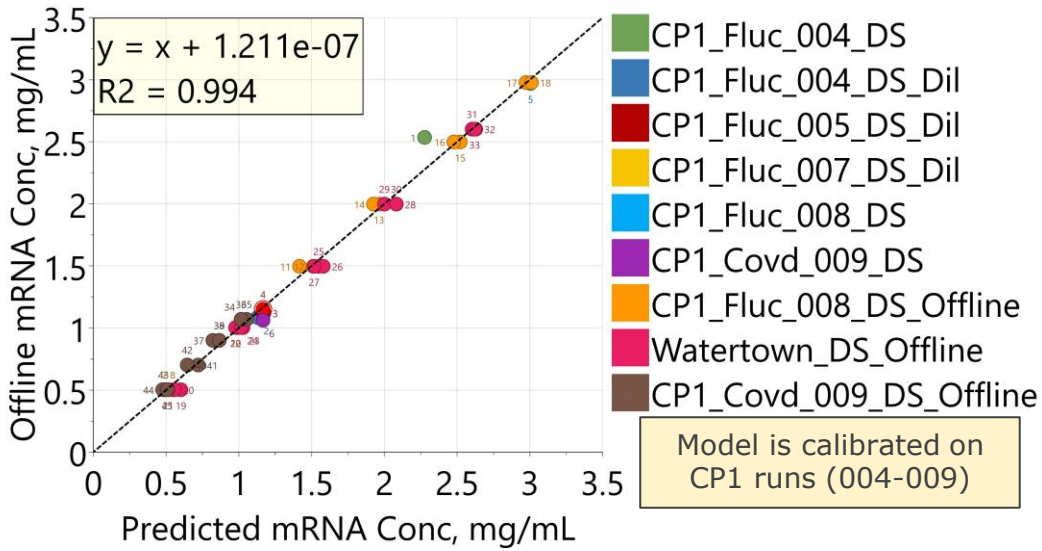
DS POOL + PLS MODEL TO MONITOR MRNA CONCENTRATION

Real-time model performance

Raman model for mRNA prediction in DS Pool:

- Calibrated on four Fluc, one Covid run, and three DOE studies.
- RMSECV = 0.089 mg/mL
- R2 = 0.99



Model is calibrated on CP1 runs (004-009)

Model is tested on CP1-Covid-010:

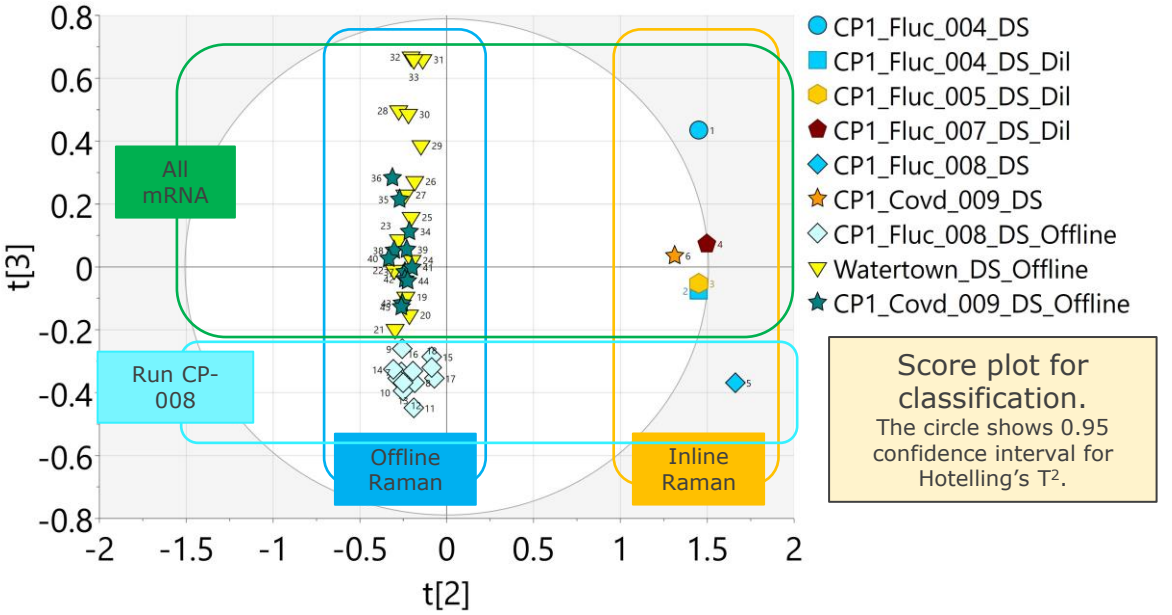
- RMSEP = 0.078 mg/mL

Measurement	Sample 1	Sample 2
Predicted Conc, mg/mL	1.26	1.04
Offline Conc, mg/mL	1.215	0.939

Real-time batch classification of runs

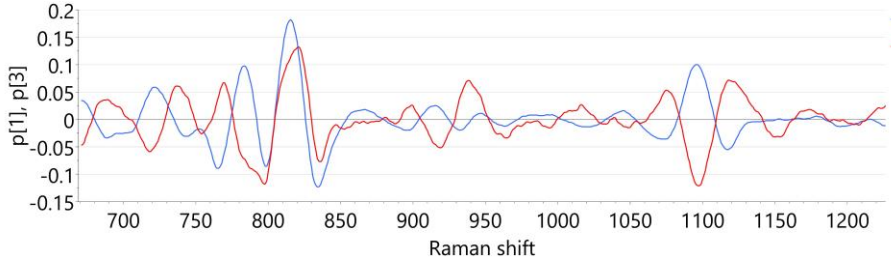
Classification of samples:

- PCA analysis was performed with 4 components.
- Raman classified runs and measurements based on variations such as concentration, instrument, and mRNA Raman signal (possibly secondary structure).



Score plot for classification.
The circle shows 0.95 confidence interval for Hotelling's T^2.

- Loading plot (relationship b/w variables) points out variations on a mRNA Raman signal.

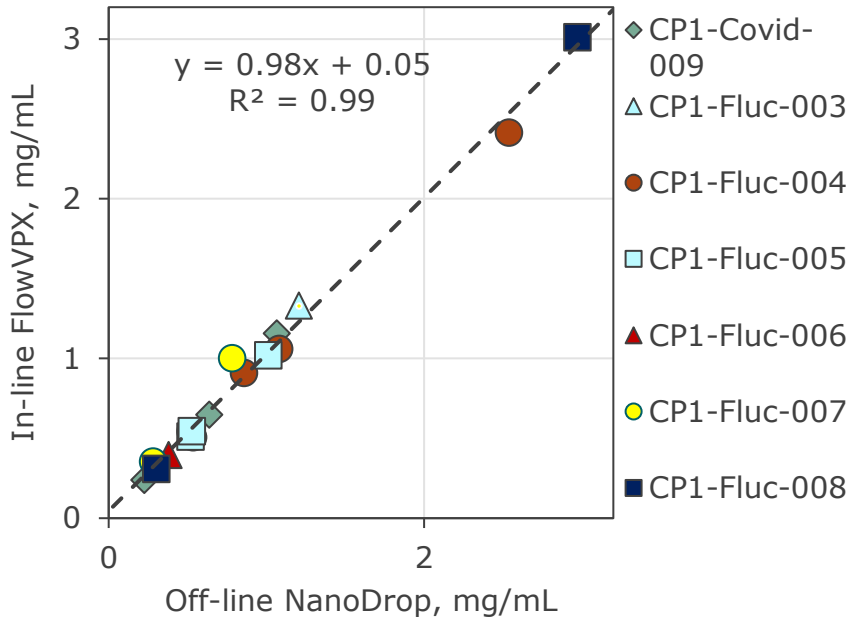


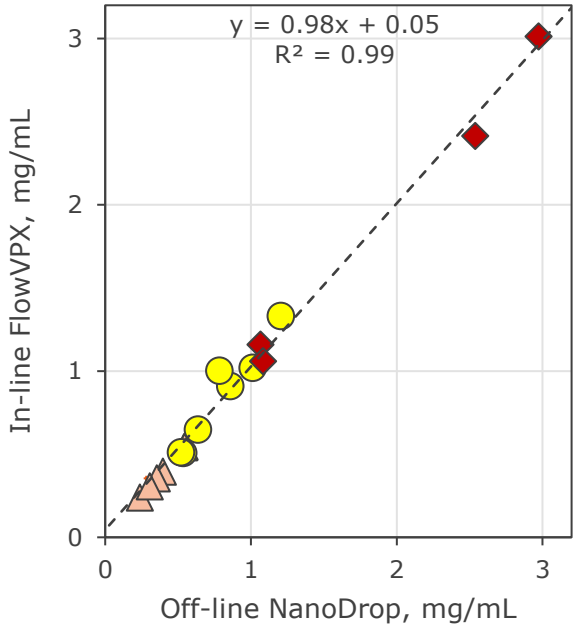
Loading plot for comp #1 and #3.

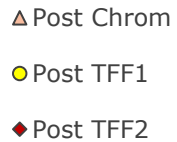
IN-LINE UV-VIS TO MONITOR MRNA CONCENTRATION IN DOWNSTREAM

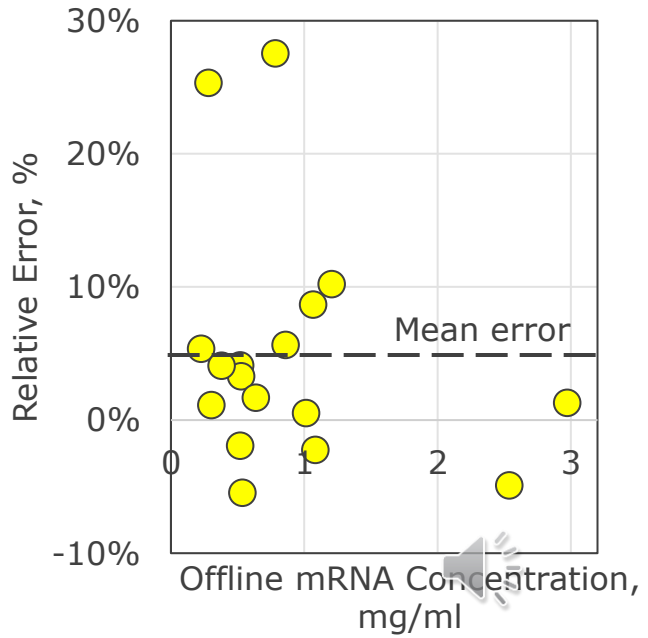
In-line PAT is monitoring mRNA concentration Downstream with less than 5% relative error.

1. FlowVPX prediction showed similar accuracy for both Covid and Fluc without a change in the calibration.
2. FlowVPX successfully predicted mRNA concentration in all downstream samples.
3. Regression between the in-line and off-line measurement showed R2 =0.99 and relative error of 5%. By removing two samples that seems to be outlier, the relative error decreases to 2%.





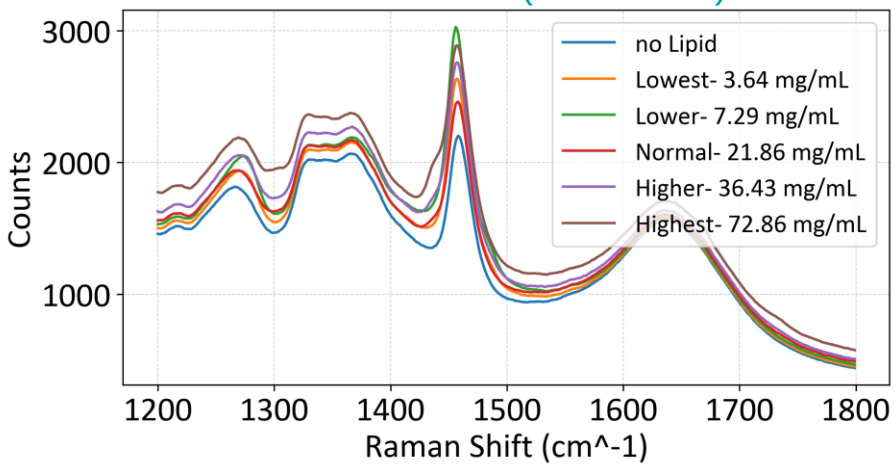




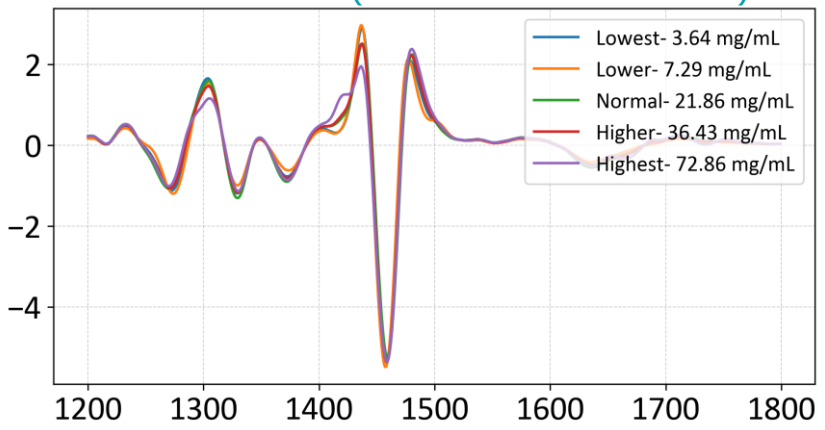
WELL CHARACTERIZED MODELS ENSURE LNP FORMATION REMAINS CONSISTENT DURING PRODUCTION

- Monitor lipid component concentration during LNP production(mixing, TFF3, Drug Product formulation) via Raman

Raman shifts (unmodeled)

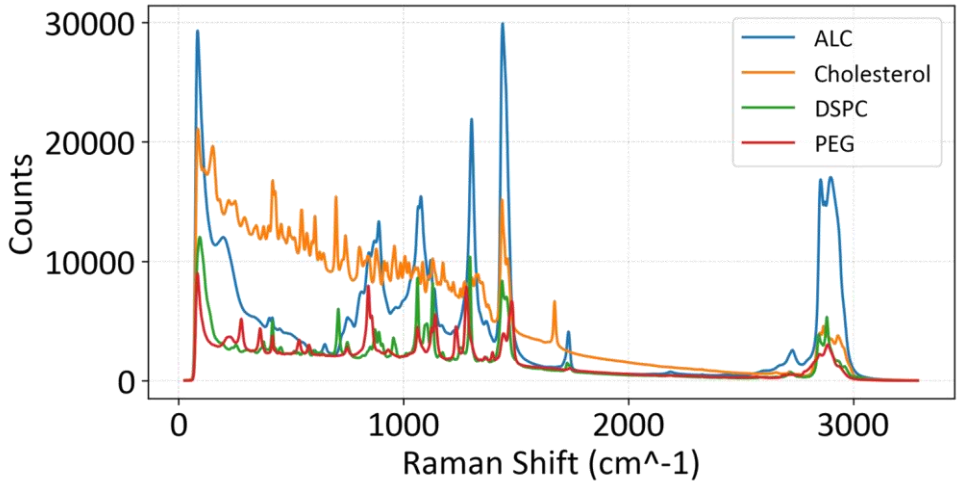


Raman shifts (filtered and normalized)



Optimal LNP ratio:
- 50% ALC
- 10% DSPC
- 35% Cholesterol
- 1.5% PEG
- 3.5% ETOH (by weight)

Isolated Lipid Component Spectra



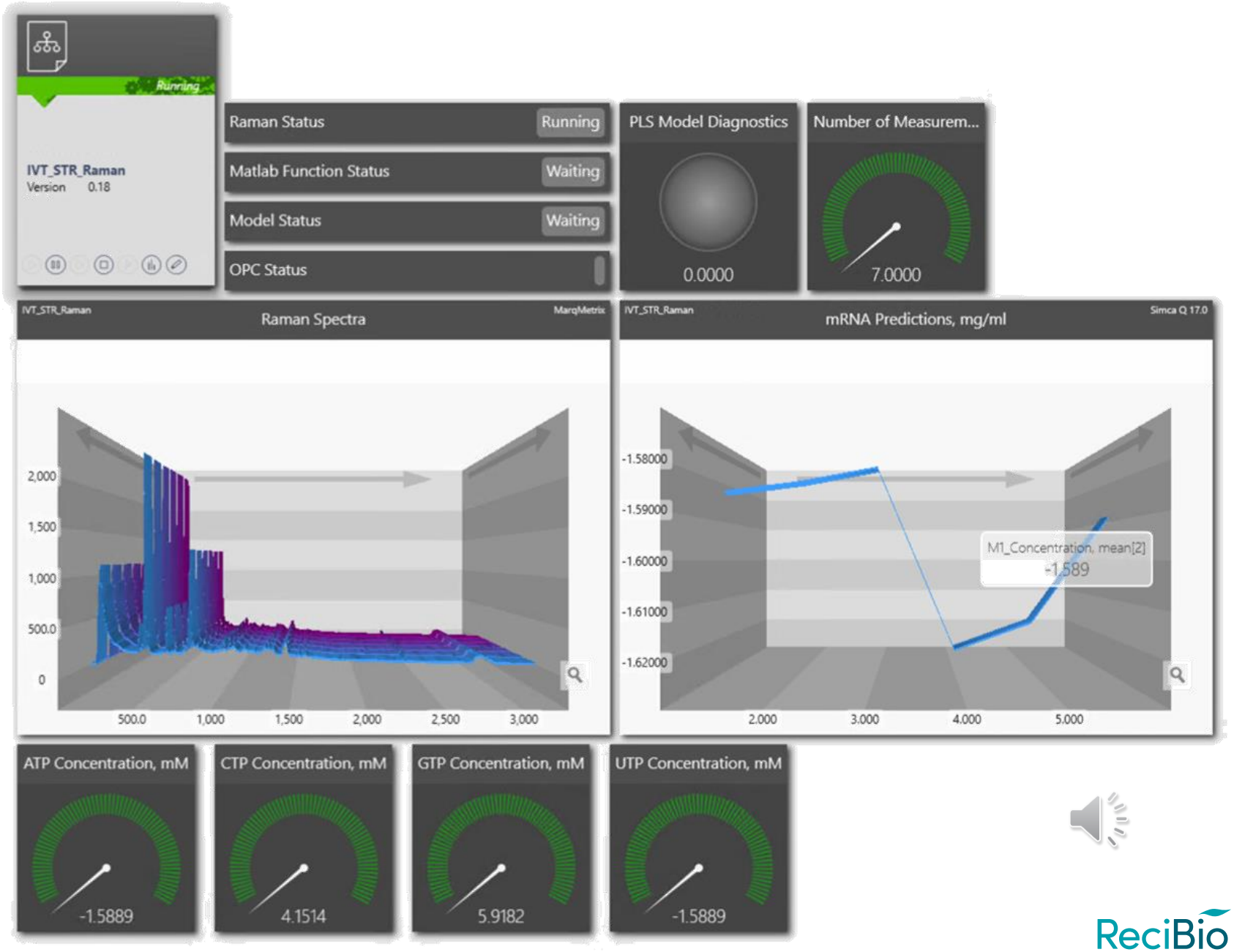
Area of focus: 1200-1800 cm-1 region
- Major mRNA signals occur at 700-1200 (812, 1097)
- DSPC: 949, 1700
- Cholesterol: 1440, 1673
- PEG: 1700

PREDICTIVE MODELING AND REAL TIME MONITORING—IVT

Orchestration status

Raman spectra and mRNA conc. mg/mL

NTP Conc. mM



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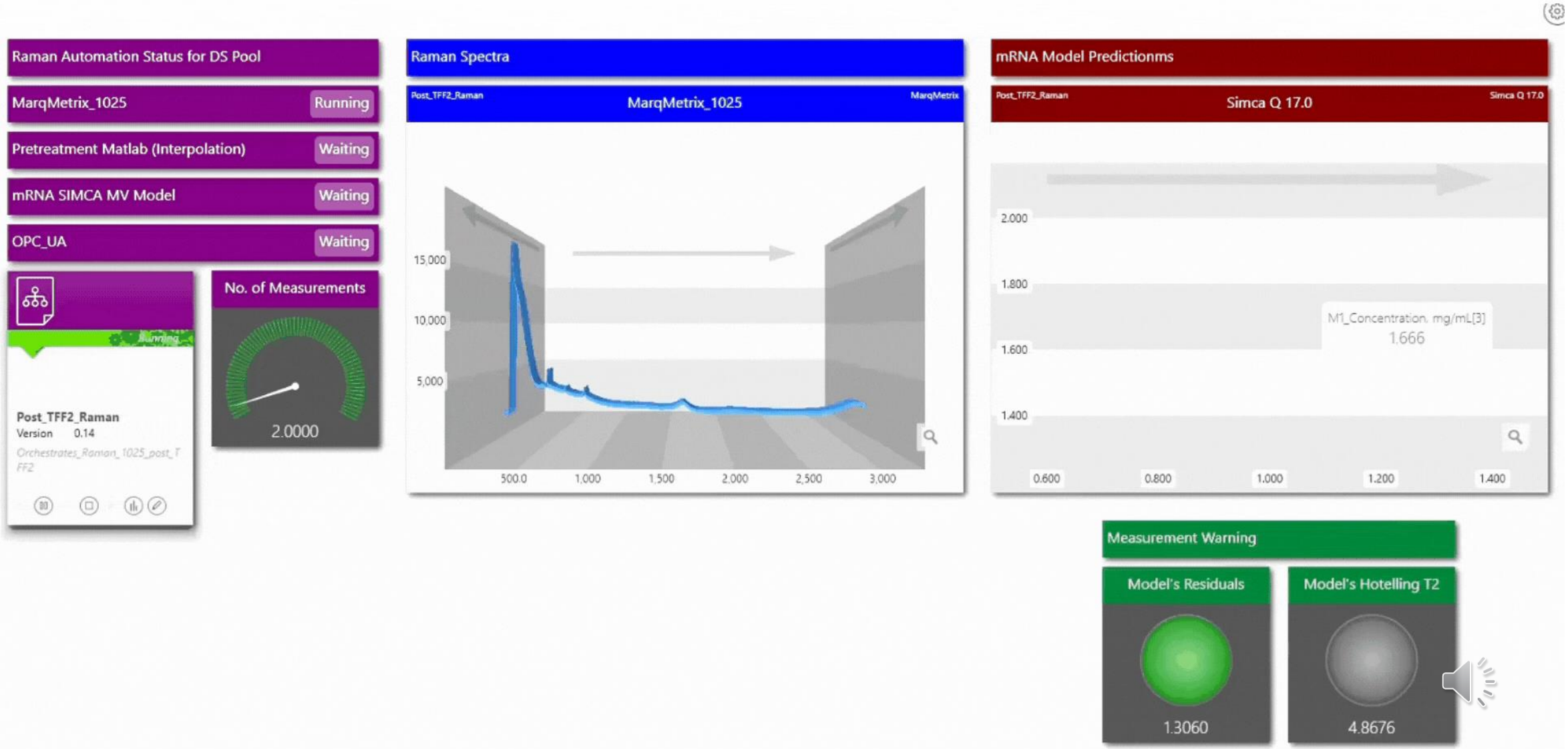
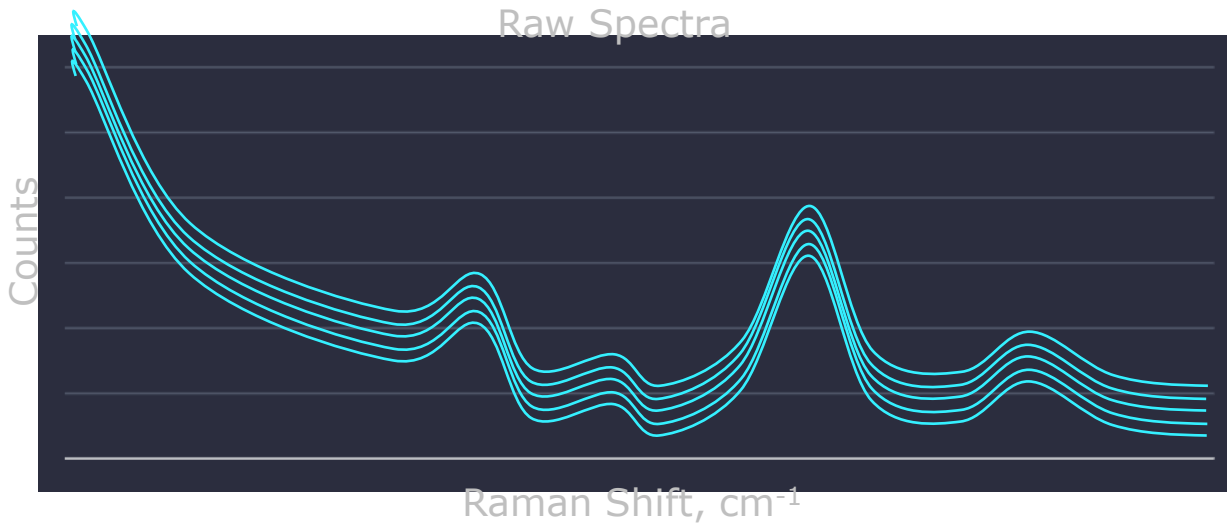
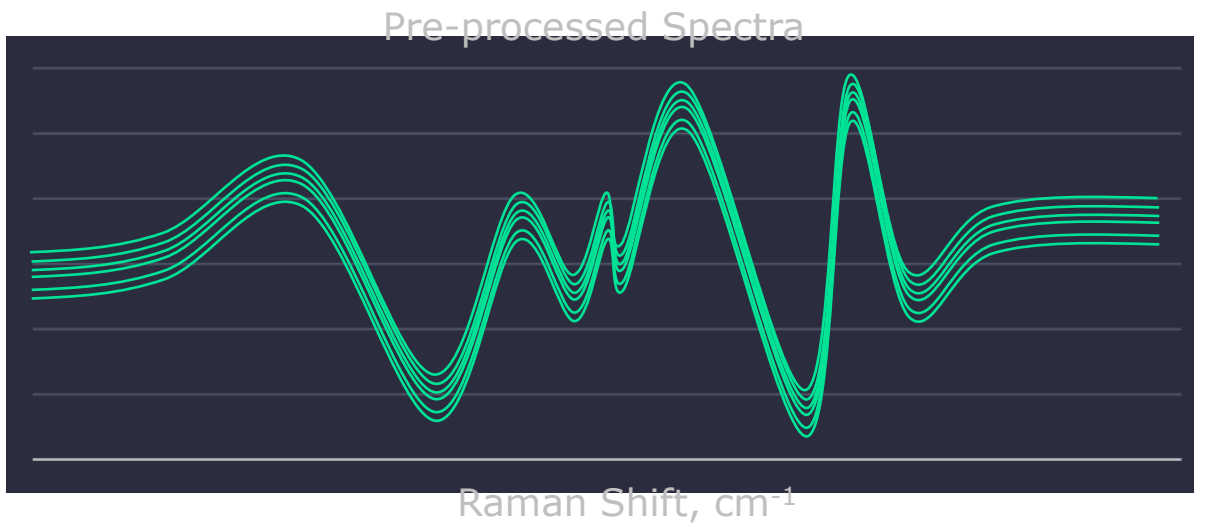
PREDICTIVE MODELING AND REAL TIME MONITORING—DS POOL

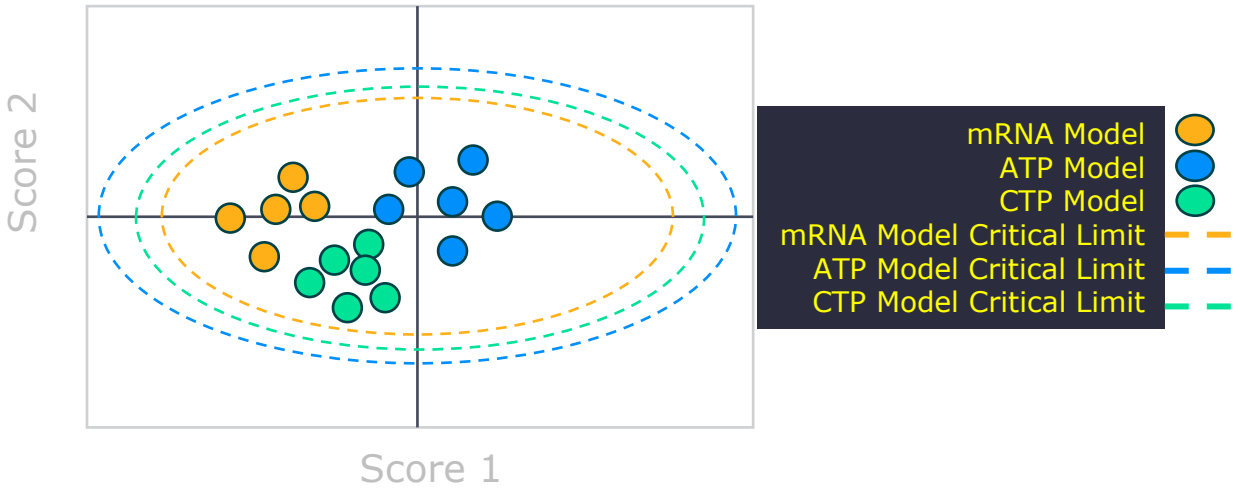


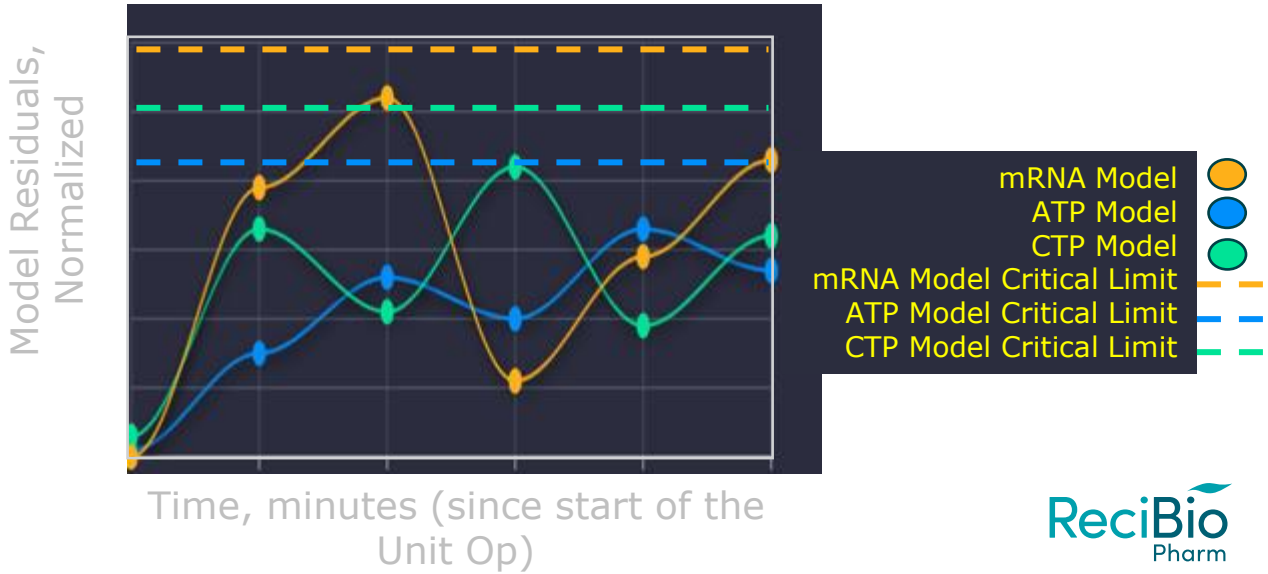
Advanced Dashboard

CP1-FLuc-008 | IVT | Raman | Multivariate PLS Model









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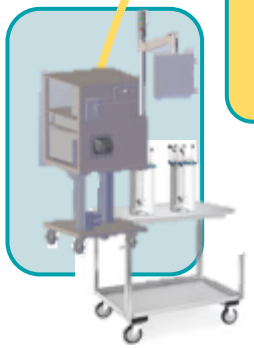
CP2/PAT SKID DATA FLOW: REAL TIME MONITORING AND RELEASE

PAT SKID: (THE "MICRO-CHIP")

ReciBioPharm Command Center

IVT

☑ CQA
☑ CQA
☑ CQA

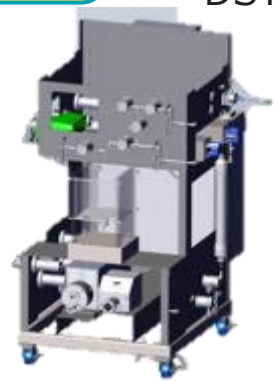


Chrom

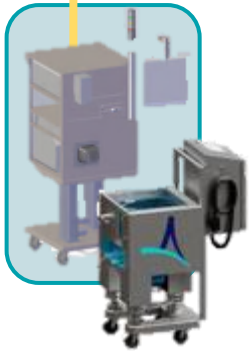
☑ CQA
☑ CQA
☑ CQA

DSTFF

☑ CQA
☑ CQA
☑ CQA

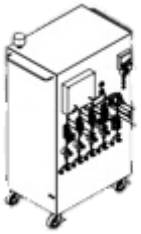


☑ CQA
☑ CQA
☑ CQA



☑ CQA
☑ CQA
☑ CQA

LNP



☑ CQA
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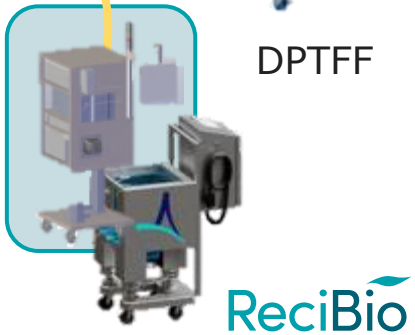

DPTFF

☑ CQA
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QUALITY | INTEGRATED CP PROCESS DEMONSTRATES EQUIVALENT PERFORMANCE FOR DS AND DP MANUFACTURING COMPARING TO BATCH MODEL

mRNA construct	Process model	mRNA					mRNA/LNP			
		Purity, A260/A280	mRNA integrity	dsRNA residue	Enzyme residue	DNA residue	mRNA integrity	EE%	Particle size	PDI
Construct #1 (2400nt)	Batch model	1.82	91%	<1%	<1 ug/mL	0.17 ng/mg	91%	93%	64 nm	0.02
	Continuous model	1.91	93%	<1%	1.96 ug/mL	0.37 ng/mg	91%	94%	60 nm	0.05
Construct #2 (4500nt)	Batch model	1.74	89%	<1%	1.8 ug/mL	0.05 ng/mg	87%	90%	62 nm	0.03
	Continuous model	1.77	88%	<1%	1.2 ug/mL	0.22 ng/mg	n/a	88%	60 nm	0.04

ACKNOWLEDGEMENT

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Thank you for your attention!

ADVANCING TOGETHER

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