mRNA hub meeting
Cape Town, April-2023

medicines patent pool

World Health Organization

Pan American Health Organization

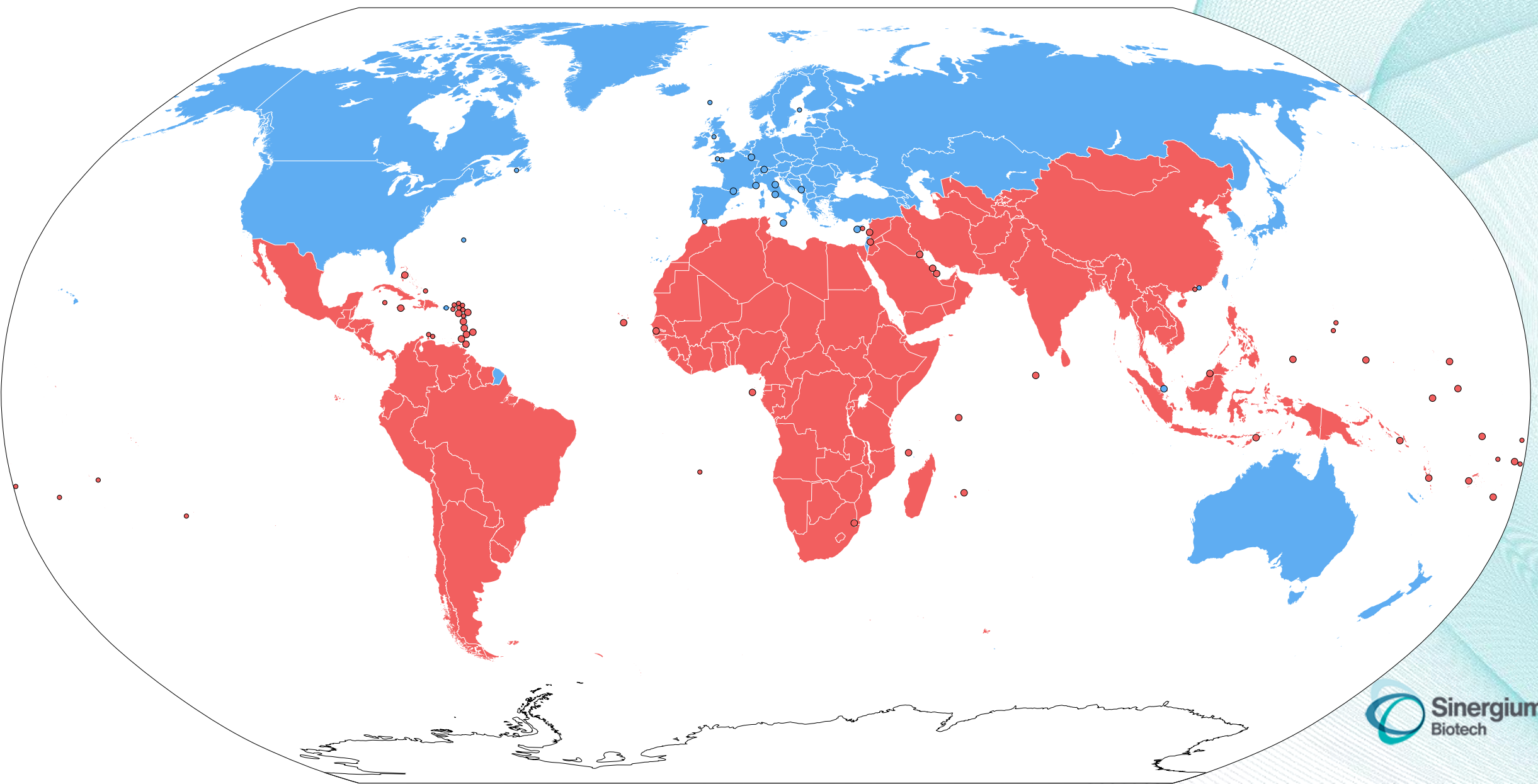
Afrigen

Sinergium Biotech

Cuidamos la salud, preservamos la vida.

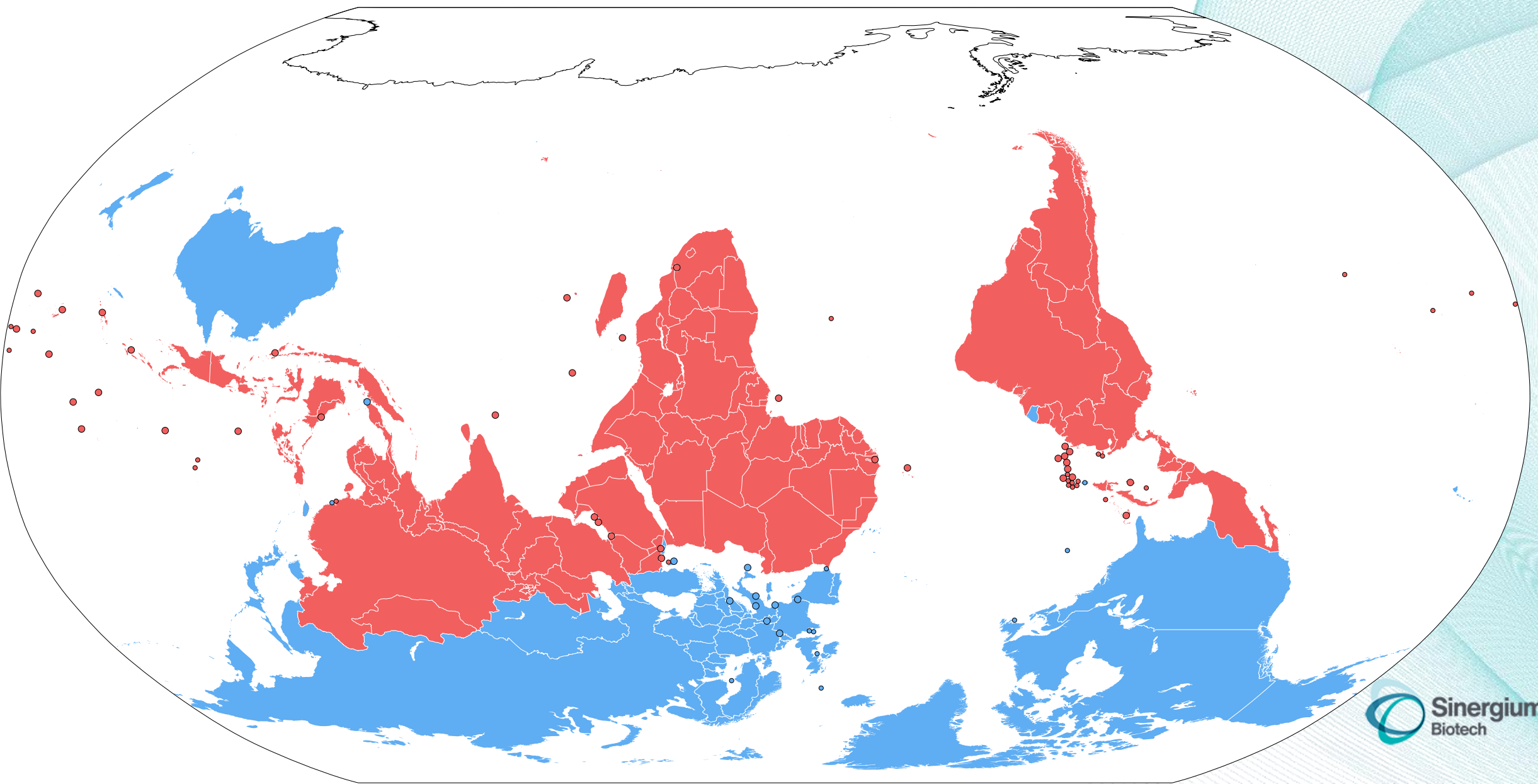
Argentina in the world

Global South



Sinergium Biotech

Global South



Sinergium Biotech

Our History: Sinergium Biotech S.A.

INSUD PHARMA

Bagó

ELEA

Biggest pharma lab in Argentina

Biogénesis Bagó

Larger FMD veterinarian vaccine in the World

Sinergium Biotech

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Biotech Hub: 20.000 sqm, 1.500 specialized employees, three companies.

Sinergium Biotech in numbers

200
Million doses produced

>700
Batches manufactured

0
Rejected batches

350
Direct employees

150
Indirect employees

45 %
Women in our staff

7
Agreements with multinational pharma companies

8
Successful tech- transfers

6
Public-private R&D projects

Cuidamos la salud,
preservamos la vida

Vaccines Portfolio

Sinergium Biotech

CSL Seqirus

VIRAFLU – 4FLU

Inactivated Sesonal Trivalent and Quadrivalent influenza

Pan American Health Organization

CSL Seqirus

FLUXVIR

Inactivated Seasonal Trivalent Influenza with adjuvant MF59C.1

Pfizer

PREVENAR 13

13-Valent Pneumococcal Vaccine

Vaccines Portfolio

Sinergium Biotech

MSD

SILGARD VPH

Human Papillomavirus (HPV) Vaccine
Type 6, 11, 16, 18

SERUM INSTITUTE OF INDIA PVT. LTD.
Cyrus Poonawalla Group

CARCIVAC

Onco BCG Vaccine Bacillus Calmette Guerin

SINOVAC

VAXIPAT

Inactivated Hepatitis A Vaccine

mAbs Portfolio

mAbxience
From lab to life

NOVEX (Rituximab)

100 mg
500 mg

LUMIERE (Bevocizumab)

0.2 ml with
5 mg Bevacizumab

BEBAX (Bevocizumab)

100 mg
400 mg

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Manufacturing site

Sinergium Biotech

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The Production Site

Strong know-how in formulation and filling

Pre-filled syringes line	• Capacity for **50 million doses** • 60% available capacity (30 million doses)
Vials line	• Capacity for **400 million doses** (estimated for 10-dose vials) • Project under execution – 100% available for future projects from 2024
Site and industrial area	• Biotech Hub (Sinergium, Biogenesis Bago, Mabxience) • 1.500 employees in the area • 20.000 sq meters (only Sinergium site)
Antigen production	• Mammalian/insect cells capacity • Adenovirus production • **24.000 L** biorreactor capacity

The Production Site

Formulation areas - Grade C

- Fixed stainless Steel 316 AISI tank - Manufacturer: GEA Diessel GmbH (Germany)
  - 400 Liter capacity
- Mobile stainless steel 316 AISI tank - Manufacturer: GEA Diessel GmbH (Germany)
  - 200 Liter capacity
- Expertise in single use technology for vaccines & mABS
- Double jacket / Rupture disk included / Vesta Sterile Valve GEA / Temperature sensor / Preassure sensor
- Filter Integrity test equipment: Millipore IT5 / Biowelder and Biosealers: Sartorius
- Automatic CIP/SIP and formulation récipe : Simatic Batch (SIEMENS)

Quality Control

- HPLC/UPLC
- Capillary Electrophoresis
- UV
- QPCR
- Osmometer
- TOC
- Cell Bioassay Lab. (BSC, Incubators, Plate readers)
- Full Microbiology Lab. (BSC, Strain identification ViTek, particle counter)
- Sterility Test Area
- Hygienic control Area
- Physicochemical / Raw Material Lab. (pH meter, Conductivity meter, IR)
- Stability Areas (5±3ºC / 25±5ºC / -15±5ºC)

Process development team

- 2 R&D centers, 1 dedicated tech-transfer team for new products
- 27 dedicated people
- AMBR High Throughput Microbioreactors Technology.
- 1 to 10L Bench Scale reactors.
- ATF Systems
- TFF, Chromatography and Filtration Scale Down Technology.
- Independent Analytical capacity

Sinergium Strategic Vision

Our objectives

- Foster strong long-term partnerships.
- Expand our reach globally.
- Expand our portfolio.
  New partnerships.
  New products.
- Develop new products under different platforms
  - Projects ongoing:
    - Baculovirus recombinant platform
    - mRNA platform

Advances in mRNA platform Development.

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Sinergium Biotech in mRNA Program: Key events

Jul-2021

mRNA hub lunch (Afrigen-Biovac-WHO-MPP)

Abr-2021

WHO-EOI for mRNA techology

Sept-2021

Sinergium Selected as a “Partner”

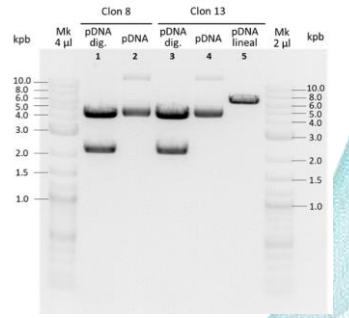
Dec-2021

Kick off Tech transfer Meeting-WHO-PAHO

Training in RNA tech at UBA

Mar-2022

Training on Afrigen



Apr-2022

Starts uL tests. mRNA for research grade

Apr-2023

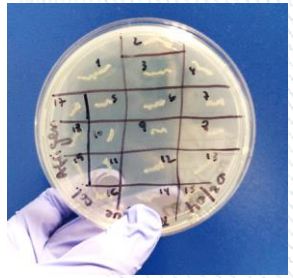
Package 1a

Feb-2023

WHO-PAHO-MPP visit to Argentina.

PAHO selects centers in Argentina, Brazil to develop COVID-19 mRNA vaccines

21 Sep 2021



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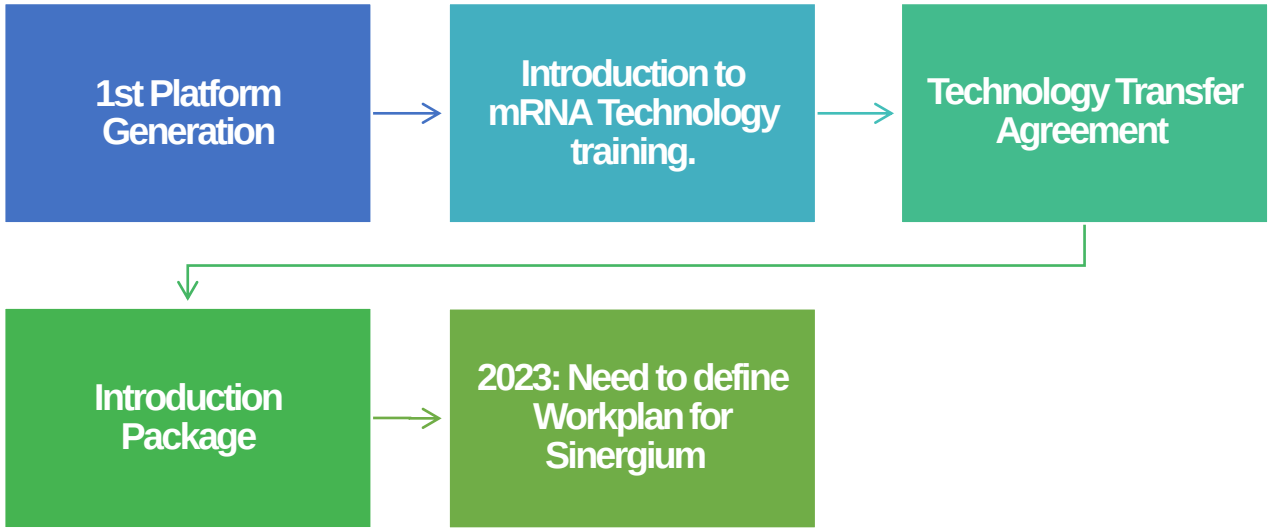
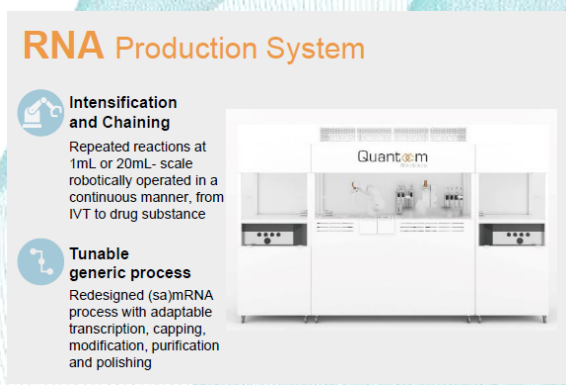
Sinergium Biotech in mRNA Program: Platform technologies

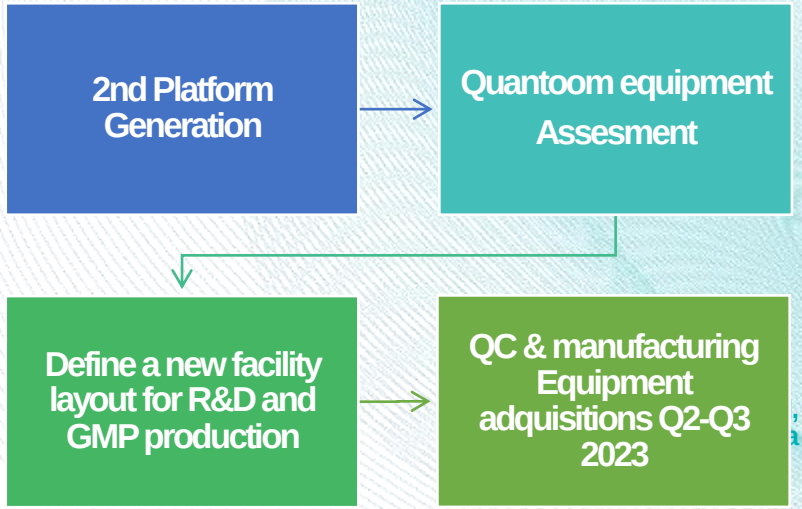
1st generation mRNA technology – focusing on being able to demonstrate region capabilities to developed mRNA vaccines – base on mRNA Technology Transfer HUB Program (WHO/MPP)

- Traditional Approach

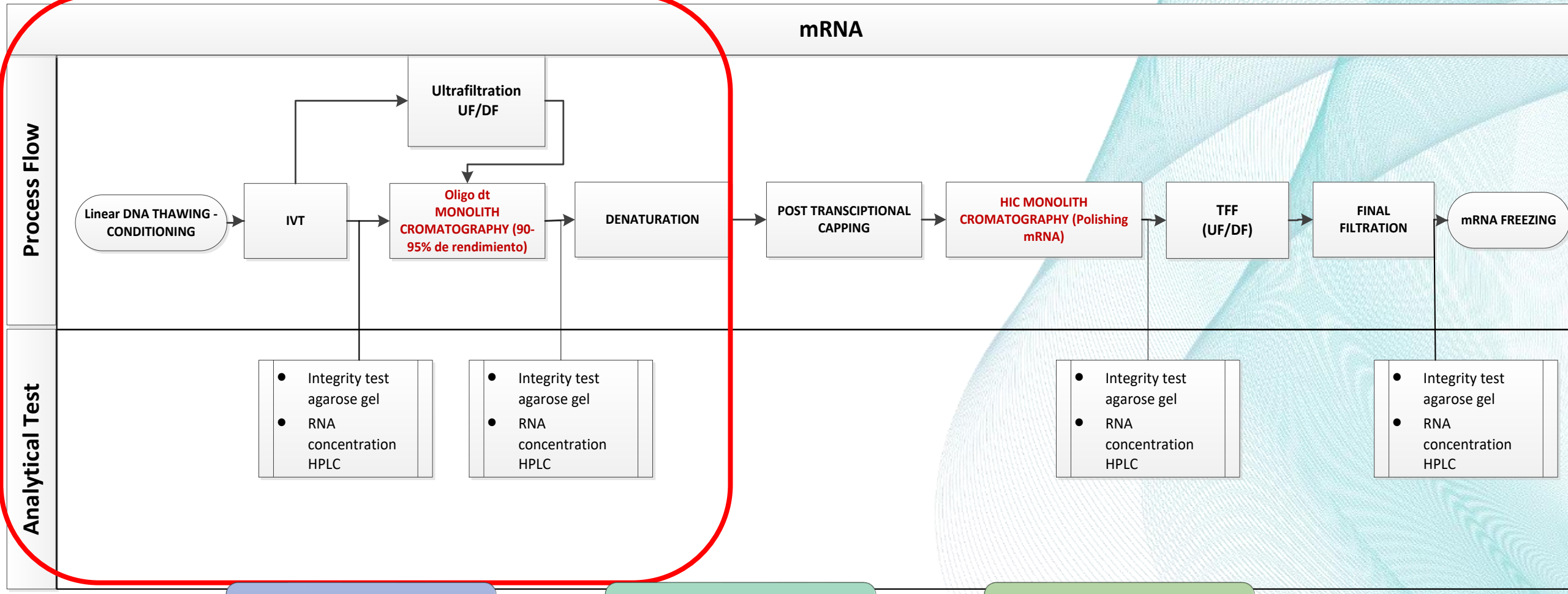


2nd generation mRNA technology – Intensified Continuous manufacturing.





mRNA Platform development: Process flow (mL scale)



Lab Scale (20 & 100µL)
*pDNA
*mRNA
*LNP

Lab Scale (0,5-1 ml)
*pDNA
*mRNA
*LNP

Lab Scale (TBD ml)
*pDNA
*mRNA
*LNP

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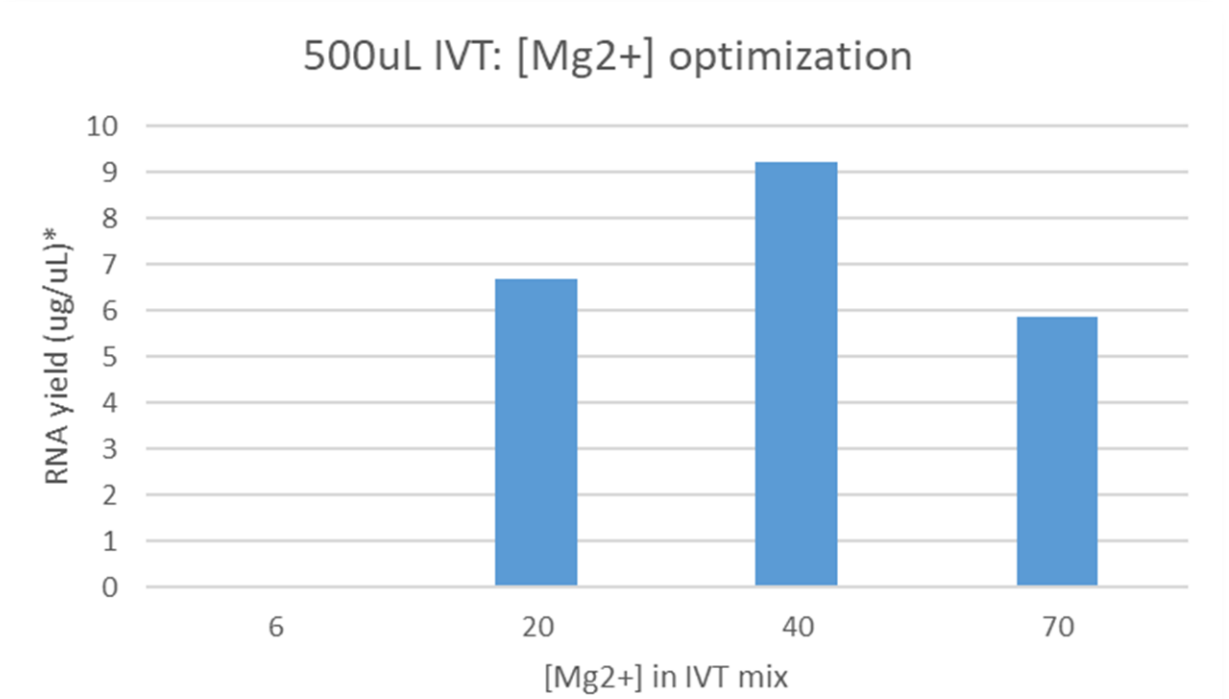
mRNA Platform development: IVT optimization

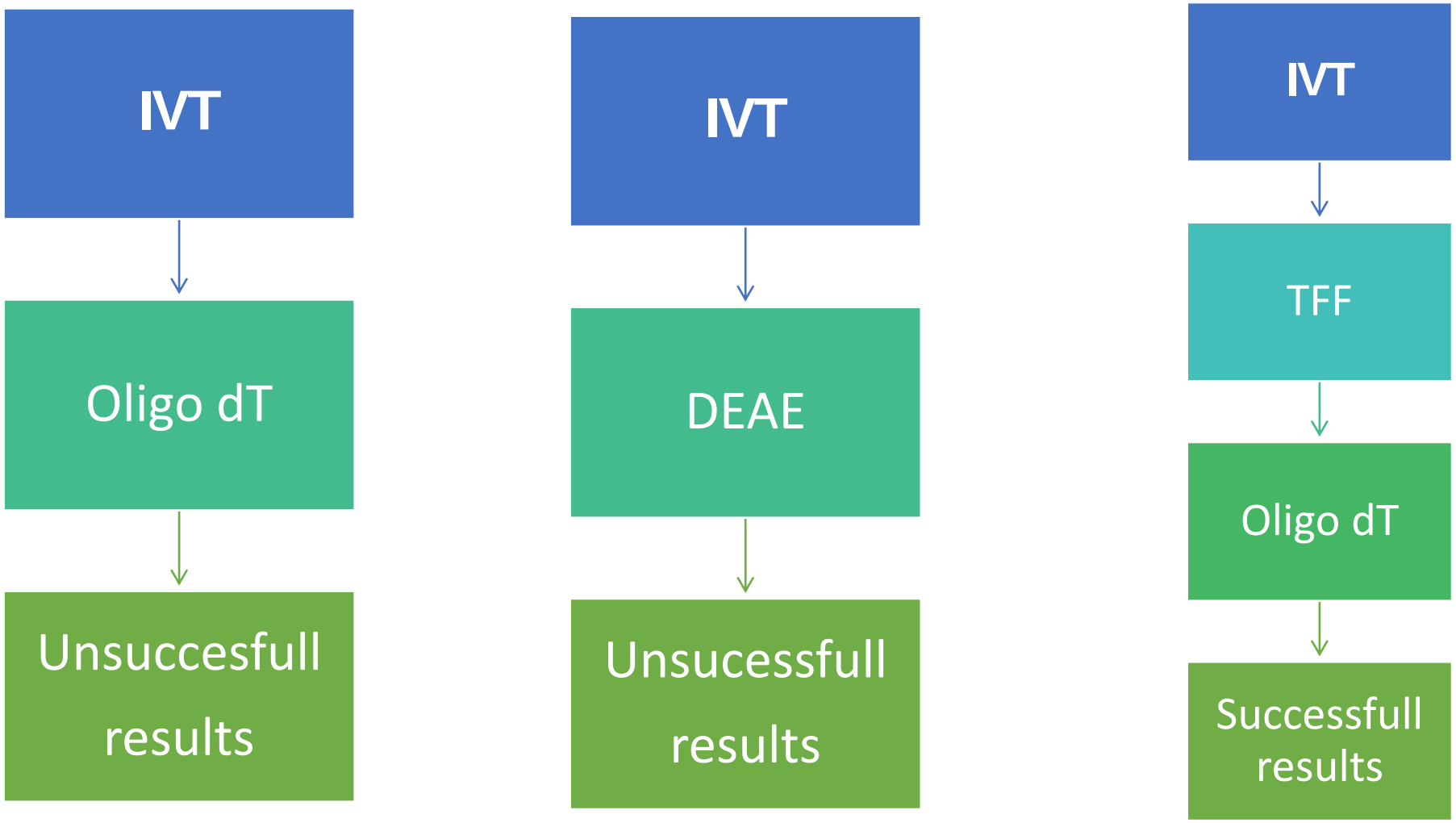
- **From kit to bulk reagents: need to optimise cc of reagents in IVT mix (500 µl) to get high yields.**

[Mg2+]: 6mM to 70mM



→ [Mg2+]= 40mM final (ratio Mg2+:NTPs = 1) shows the highest yield (~9,22 ug/uL)

mRNA Platform development: Downstream process



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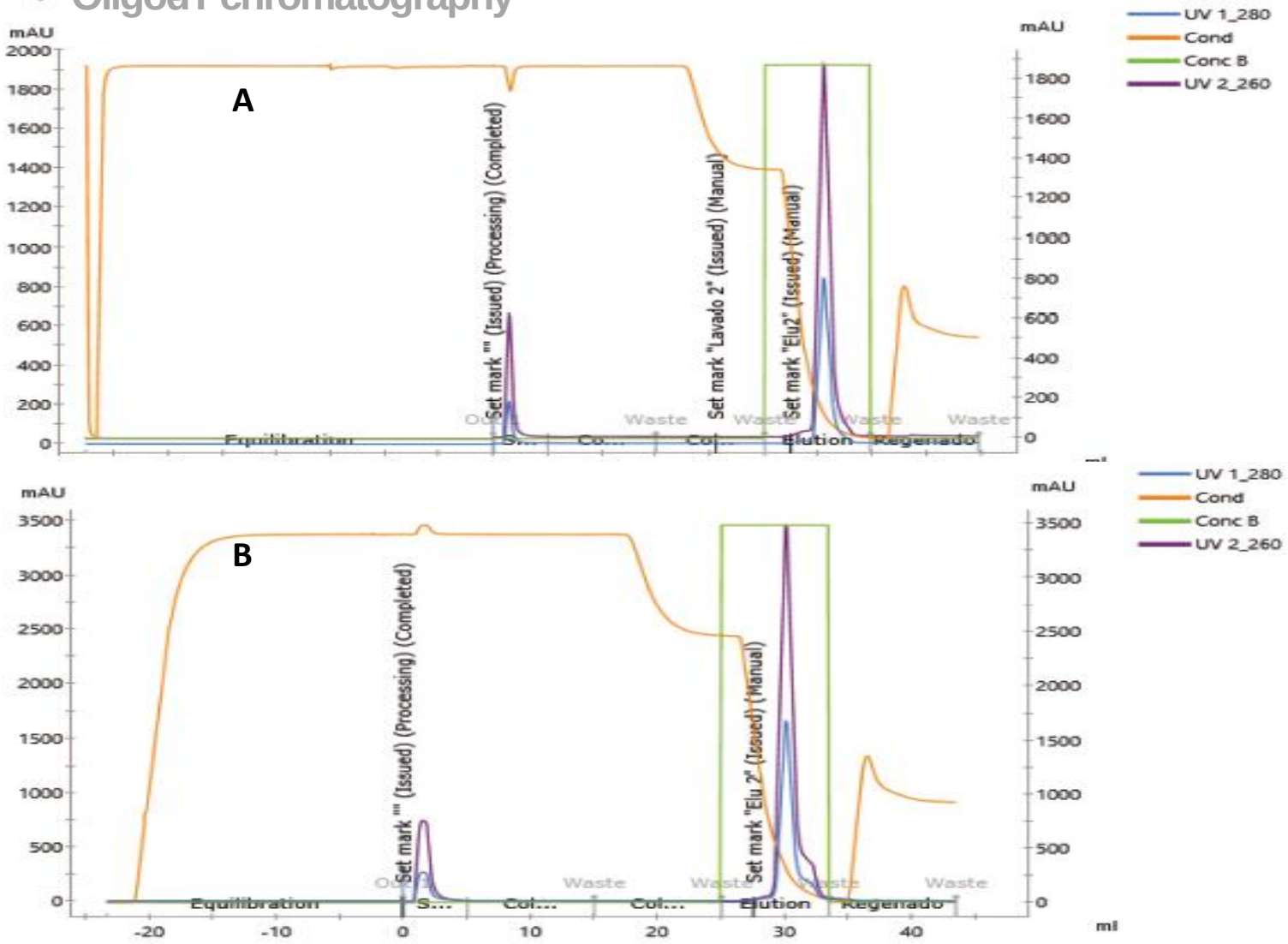
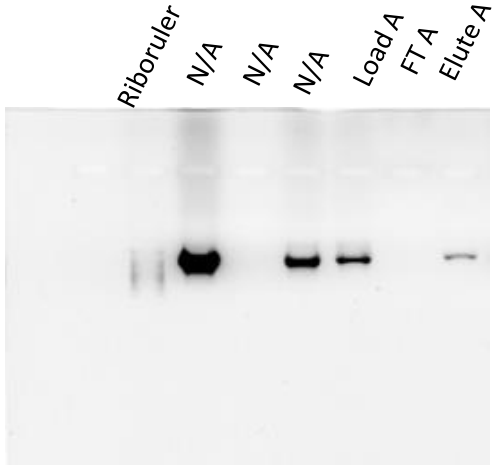
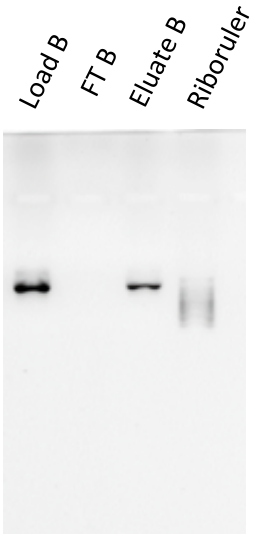
mRNA Platform development: Downstream process

OligodT chromatography



CIMmac Oligo dT (18) monolyth of 1 mL -BIA Separations





	% Recovery	% Recovery Eluate + FT
Chromatography A (load of 500 µL)	70	85
Chromatography B (load of 1000 µL)	61	72

➢ Better % Recovery & mRNA shows the predicted size.

mRNA Platform development: LNP manual formulation

- Pipetting method
- Formulation Volume= **0,25mL**
- Lipids molar ratio= **38.5: 10: 50: 1.5** for **cholesterol: DSPC: SM-102: DMG-PEG2000**
- N:P= **6**
- Aquose phase: Organic phase= **3**
- Lipids mix concetration= **12,5mM** vs **25mM**

Usando master mix 25 mM			Usando master mix de 12,5 mM	
Vol. Total (ml)	0,25 ml	mRNA-LNP	Vol. Total (ml)	0,25 ml
mRNA:Ethanol volum ratio v/v	3	(si es 1 aumeta el tamaño molecular)	mRNA:Ethanol volum ratio v/v	3
vol total partes	4		vol total partes	4
mRNA:total ratio v/v	0,75		mRNA:total ratio v/v	0,75
Ethanol:total ratio v/v	0,25		Ethanol:total ratio v/v	0,25
Cc Mix lipidos (mM)	25		Cc Mix lipidos (mM)	12,5
Volumen de mRNA (ml)	0,188 ml		Volumen de mRNA (ml)	0,188 ml
Volumen de Etanol o Mix lipidos (ml)	0,063 ml		Volumen de Etanol/ Mix lipidos (ml)	0,063 ml
Cantidad de lipidos totales en mix (µmol)	1,5625		Cantidad de de lipidos totales en mix (µmol)	0,7813
N:P ratio (mol a mol)	**6**		**N:P ratio (mol a mol)**	**6**
mol P necesario	0,13		mol P necesario	0,07
MW RNMP promedio (g/mol)	321,48		MW RNMP promedio (g/mol)	321,48
MASA de mRNA para formular (mg)	0,04		MASA de RNA para formular (mg)	0,02
MASA de lipidos para formular (mg) usando Master Mix 25 mM	0,97		MASA de lipidos para formular (mg) usando Master Mix 12,5 mM	0,48

Lipids mix concentration	12,5mM		25mM	
DLS Parameter	Z (nm)	IP	Z (nm)	IP
mRNA-LNP	165.3	0.084	136.8	0.099
mRNA-LNP diluted	159.3	0.095	132.7	0.120
mRNA-LNP concentrated	165.7	0.094	133.6	0.099
mRNA-LNP concentrated + Sucrose	194.6	0.142	181.1	0.134

PDI ≤0.1 highly monodisperse

PDI de 0.1-0.4 slightly polydisperse

PSD > 0.4 highly polydisperse

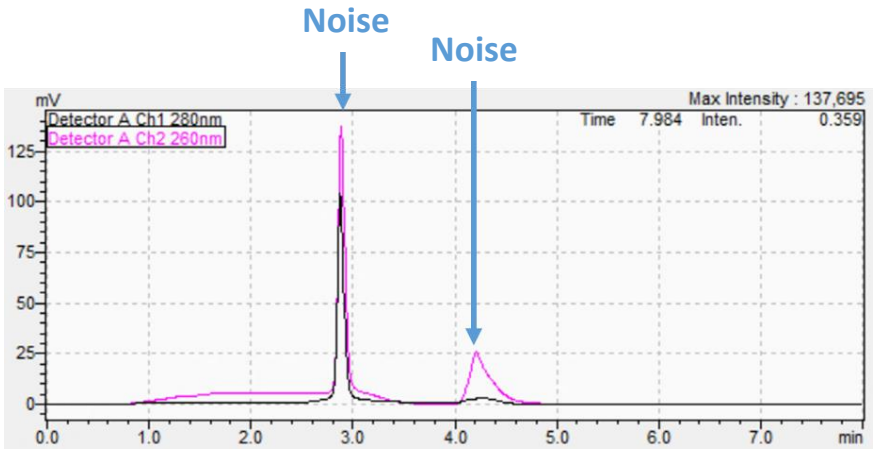
mRNA analytical tools development: RNA quantification using HPLC

- Testing made with **PrimaS analytic monolith (Sartorius)**
- **Optimization of initial washing steps** to reduce “noise”
- High quality salts and buffers needed

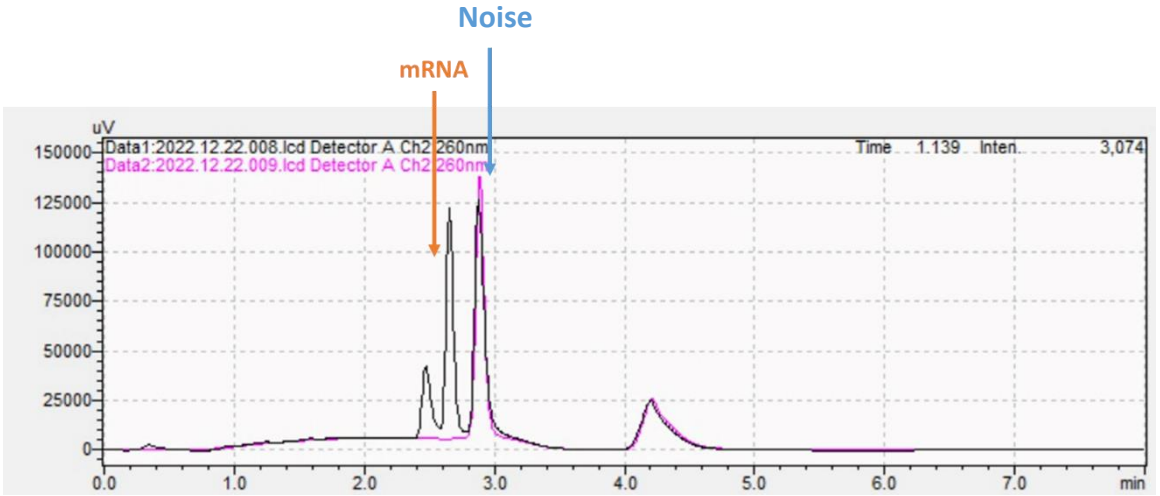
Table 1: Method details and gradient.

Mobile phase A	50 mM HEPES, pH 7.0
Mobile phase B	50 mM HEPES, 100 mM $Na_4P_2O_7$, pH 8.3
Mobile phase C	100 mM NaOH, 1 M NaCl
Mobile phase D	0.5 M HEPES, pH 7.0
Detection	UV 260 nm & 280 nm, conductivity, pH
Sample amount	1 µg
System	PATfix™ mRNA analytical HPLC system

Time [min]	MPA [%]	MPB [%]	MPC [%]	MPD [%]	Flow [mL/min]
0.00	100	0	0	0	2
0.10	100	0	0	0	2
1.10	55	45	0	0	2
1.80	55	45	0	0	2
1.82	0	40	60	0	2
2.50	0	40	60	0	2
2.52	0	0	100	0	2
3.40	0	0	100	0	2
3.42	0	0	0	100	2
3.52	0	0	0	100	2
3.54	100	0	0	0	2
8.00	100	0	0	0	2



Mobile phase A (baseline)



mRNA sample purified by LiCl precipitation

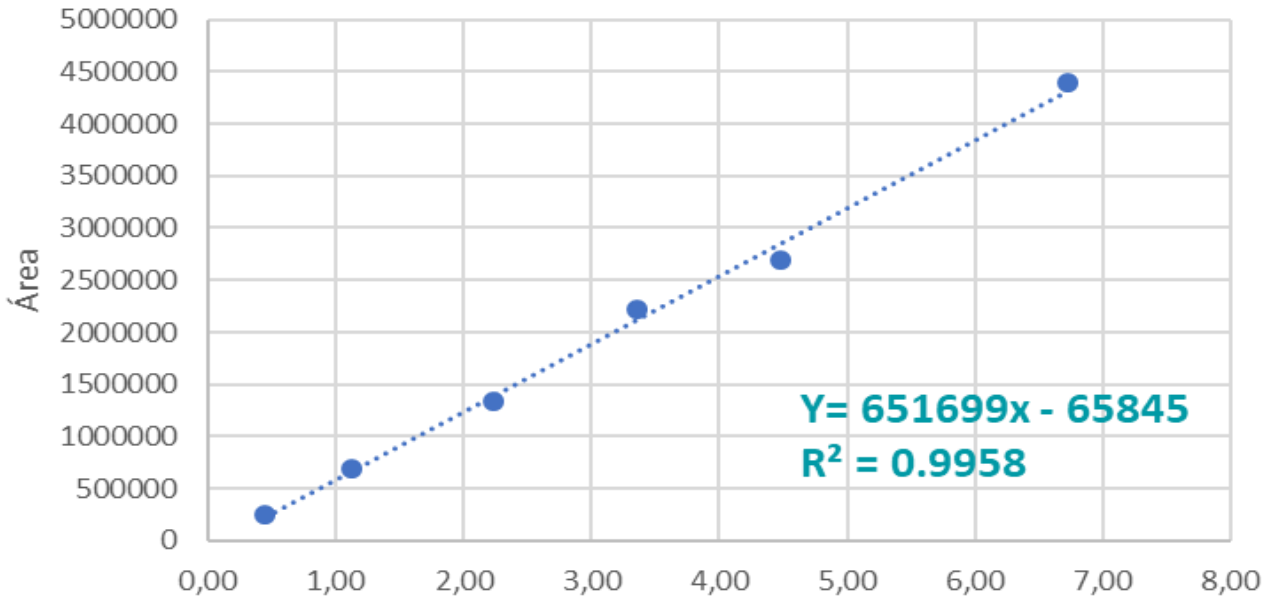
mRNA analytical tools development: RNA quantification using HPLC

Calibration curve using RNA sample purified by LiCl and quantified by spectrophotometry:

Curva de calibración 2

(µL)	(µg)	Area
4	0.45	250803
10	1.12	691430
20	2.24	1334608
30	3.36	2222670
40	4.48	2686709
60	6.72	4389118

ARN011 M2 CC = 0.112 ug/uL

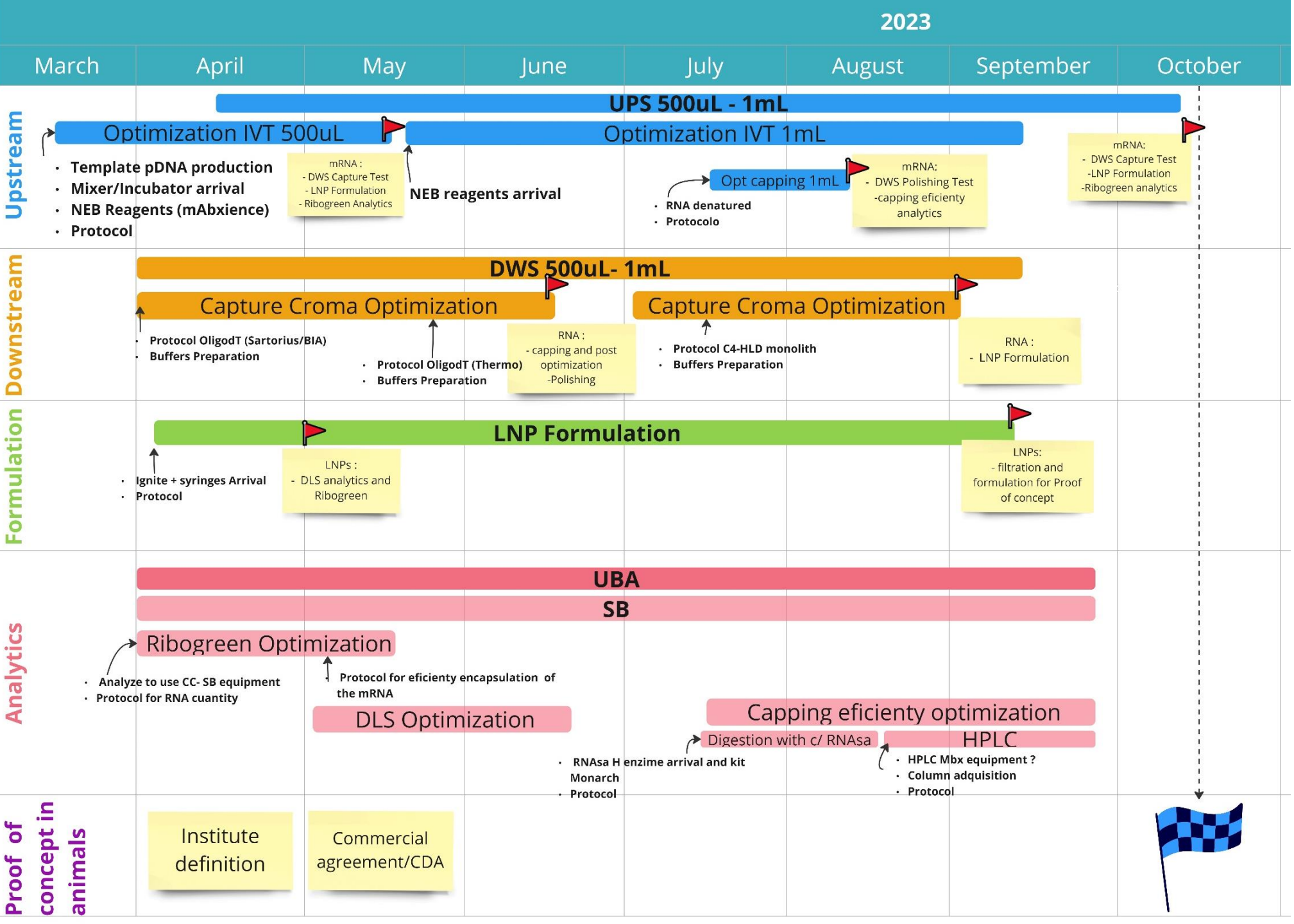


RNA mass determination using calibration curve above : 10µL injection of 1/20 sample dilution

RNA samples purified by LiCl & quantified by spectrophotometry

RNA samples post IVT- non purified

Sample	Area	RNA mass PrimaS (ug)	RNA mass spectro (ug)
ARN012 ARN M3	2906471	4.56	4.61
ARN012 ARN M2	2210289	3.49	3.34
ARN012 ARN M1	0	0.10	0
ARN012 IVT M2	2583047	4.06	-



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New mRNA capabilities

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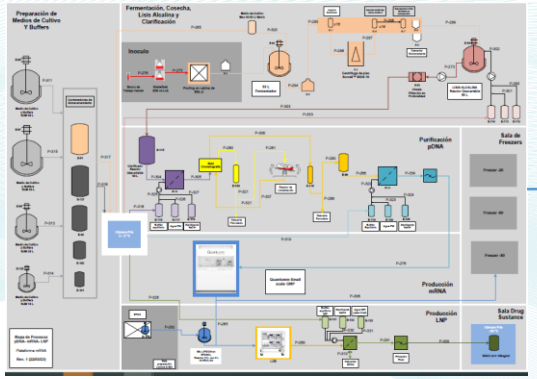
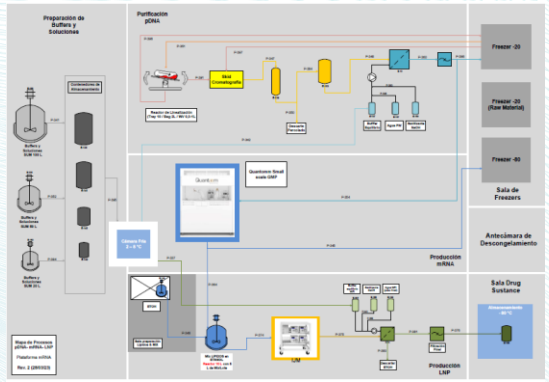
New R&D / GMP RNA facility

Option	Surface	Estimated cost of construction (USD)
R&D	350 m2	Aprox. 2 M
R&D + pDNA manufacturing + mRNA manufaturing + LNP manufacturing (**Process diagram 1)**	900 m2 manufacturing, 200 m2 Warehouse y 100 m2 annex areas.	Out of Budget
R&D + mRNA manufaturing + LNP manufacturing (**Process diagram 2)**	~500 m2	TBD



Process diagram 1



Process diagram 2

Process Flow Diagram & mass balance

Equipment dimensions

Conceptual layout

pDNA linealization

Quantoom equipment Midi

Knauer system

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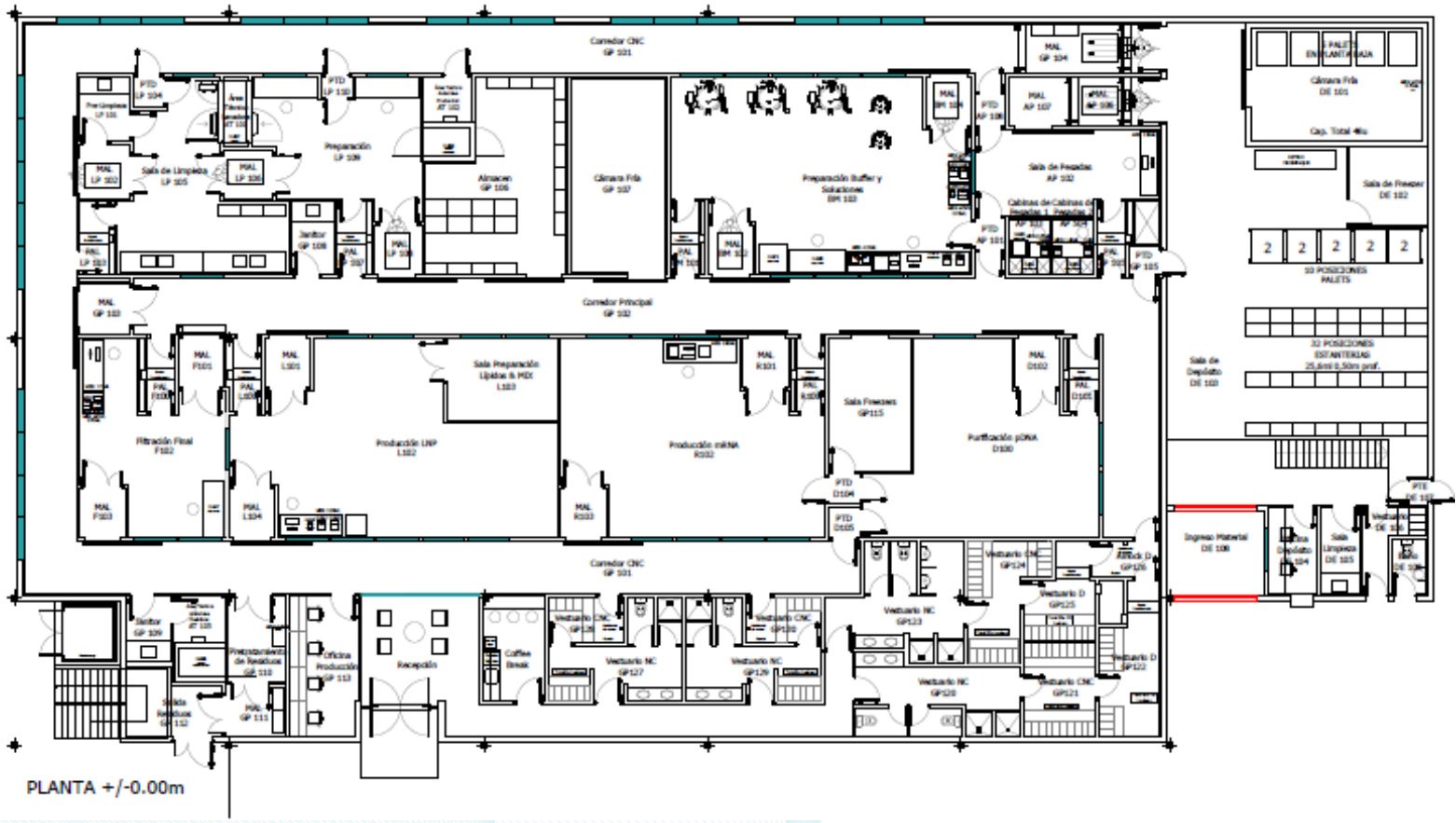
New R&D and GMP RNA facility

- Start with a R&D layout **(Q1 2023)**
- Finish with a GMP mRNA layout **(Q1 2023)**
- Started construction: Ground preparations **(March 2023)**
- Detail engenieering **on going**.
- Equipment quotations & adquisitions **on going**
- Estimated end construction: **(Q3 2024).**



Thank you!

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medicines patent pool

Pan American Health Organization

World Health Organization

Afrigen

Sinergium Biotech

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