

Emerging trends for mRNA / mRNA-LNP manufacturing

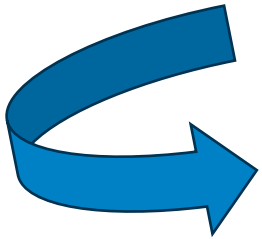
1st November 2023

Disclosure

- This review was conducted based on publicly available information.
- This review includes some technologies as examples and is not comprehensive – mRNA manufacturing is a field in great expansion.
- WHO and MPP do not have any financial interest in any of the technologies included in this review and cannot endorse any specific system.
- It is essential that partners acquire and understand the manual process for mRNA /mRNA-LNP manufacturing (Technology Transfer from mRNA hub in South Africa) prior to selecting and acquiring automated systems.
- Partners are encouraged to consider the automated systems as an addition to the “conventional multi-steps” manufacturing process, rather than as a replacement.
- Partners interested in acquiring automated systems for mRNA manufacturing are advised to conduct a detailed analysis of such systems including long-term supply of proprietary reagents, maintenance, etc. and how this would be affected in the event of a pandemic.

WHY: optimizing the manufacturing process for mRNA-products

- **Increased flexibility** → modular technologies rapidly scaled up/scaled out
- **Increased efficiency** → closed continuous flow process with reduced operator action
- **Smaller facilities** → disposable technologies in fully closed systems
- **Simplified supply chain** → consumable kits, enzymes and reagents provided by equipment supplier



- **Reduced cost and time** of manufacturing
- **Improved** process **reproducibility**
- **Optimized** qualified workforce **occupation**

1. Systems for mRNA manufacturing (in vitro transcription)

Example 1: Nature's Toolbox, Inc.

<https://ntxbio.com/technologies/#ntxscribesection>



- **Proprietary continuous-flow, fully recombinant, in vitro, cell-free, transcription system of RNA** utilizing **hollow fiber bioreactors**.
 - 5'-capping and 3'-poly(A) extension of the mRNA are accomplished in continuous flow (directly coupled mRNA synthesis)
 - Minimized product handling
- **Integrated analytics** for real time in-process monitoring
- It allows for manufacturing scaling **from R&D quantities to commercial needs** in a small, economical footprint.
- Vertical integration of supply chain
 - **Proprietary enzymes/buffers**, supplied by NTx.

Example 2: Quantoom Bioscience

<https://quantoom.com/nfinity/>



- **Automated production** technology able to **synthesize and purify mRNA**, ready to be formulated into a drug product
 - Optimized process for **high performance** (yield and quality)
 - **Quantoom's proprietary reagent** pre-mixes
 - Specific **pDNA** design for **co-capping**
 - **Quantoom's proprietary reactors** using **single-use** consumables (ease of use)
- Available as **R&D-grade** system and **GMP-grade** system (modular for clinical / commercial scale)
 - **No scale-up** needed : (pre-)clinical development at the final scale. Process validation can occur at 200 ml-scale and production of batches can range from 200 ml to x L.

Note: Ntensify™ for RNA production is part of the **Nfinity™ Production Platform**, which includes also: **Nplify™ for DNA production**, and **Ncapsulate™ for LNP formulation** (under development)

Example 3: King's College - “factory-in-a-box”



https://media.kcl.ac.uk/media/COVID+Heroes+-+Mass+Manufacturing+a+VaccineA+Factory-in-a-box/1_rcu7toub

- **End to end GMP process** (from DNA to bulk drug product, without fill and finish) in <35m²
- **Same process (no adaptation)** for **vaccines** and **therapeutic products**
- **Same process (no scale-up / tech transfer)** from 1mg to 50g / day
- **Multiple products in the same day**, without re-validation
- **Strong dose reduction (Vx)** and **cost reduction (Tx)**
- **universal product design rules to**
 - increase biological activity
 - predict and improve product manufacturability

Where it is today ...

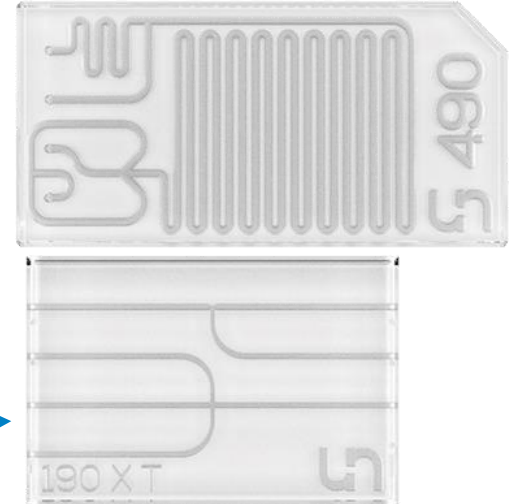
Duccio Medini's dedicated presentation following shortly

2. Systems for LNPs manufacturing

Example 1: Sunscreen and Sunshine



- **Sunscreen**: Automated LNP formulation **screener**
 - 96 formulations in <6 hours
 - 200 μ L – 2 mL per experiment
 - 0.1 – 30 mL/min flow rates
 - Reusable microfluidics
 - Scalable method
- **Sunshine**: Automated LNP process **developer and preclinical scale**
 - 10 experiments in 15 minutes
 - Fractional & total flow rates
 - 1 mL to continuous flow
 - 0.1 – 30 mL/min flow rates
 - Reusable microfluidics
 - Protocol transfer from Sunscreen
- **Key features**
 - No consumable neither proprietary reagents
 - Flexibility in the mixing methods (T-mixer or microfluidics)
 - Scalable from 96 well plates to preclinical scale (same mixing cell)
 - No GMP equipment available yet,



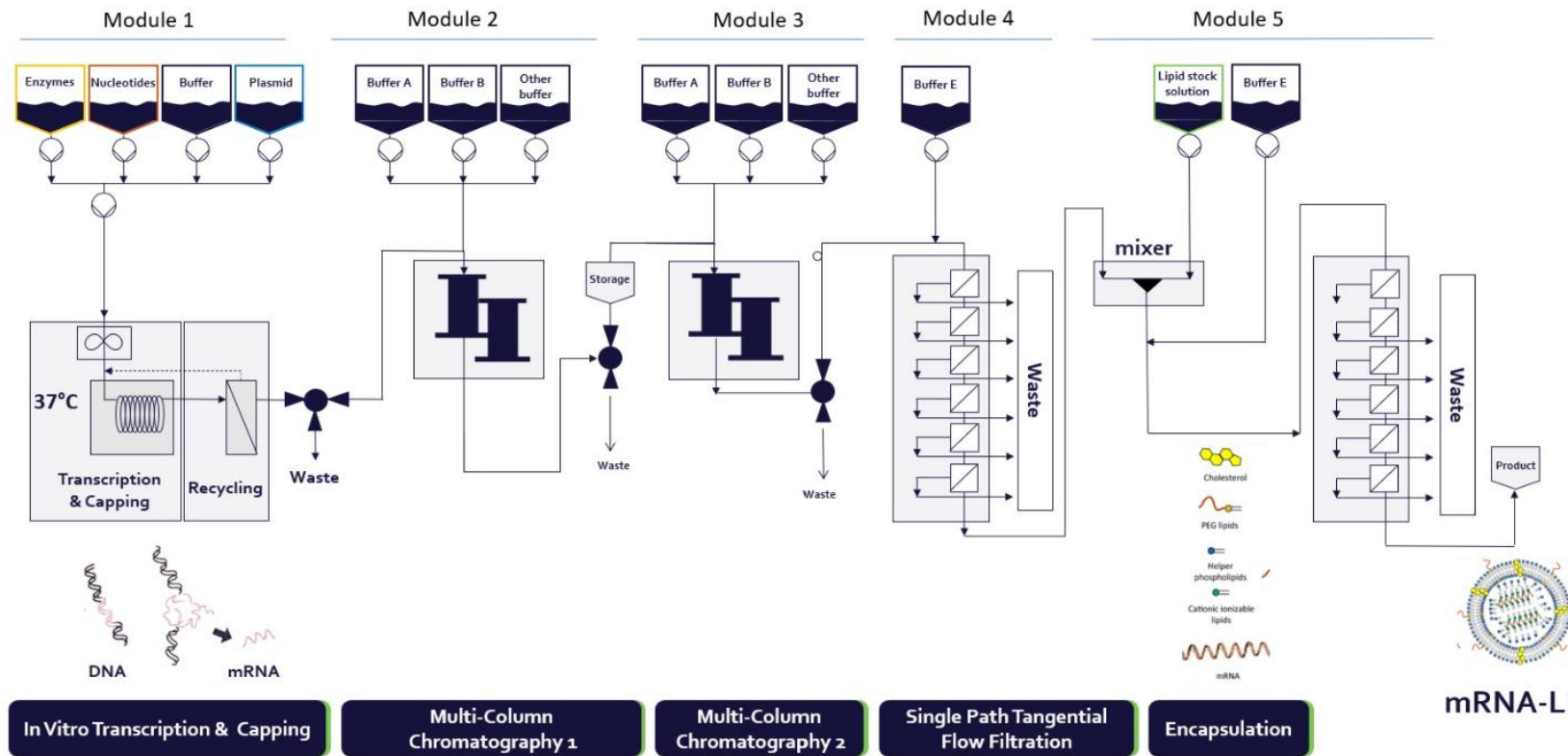
3. Systems for mRNA/LNP manufacturing (in vitro transcription & encapsulation)

Example 1: Dillico

<https://dillico.com/technology/>



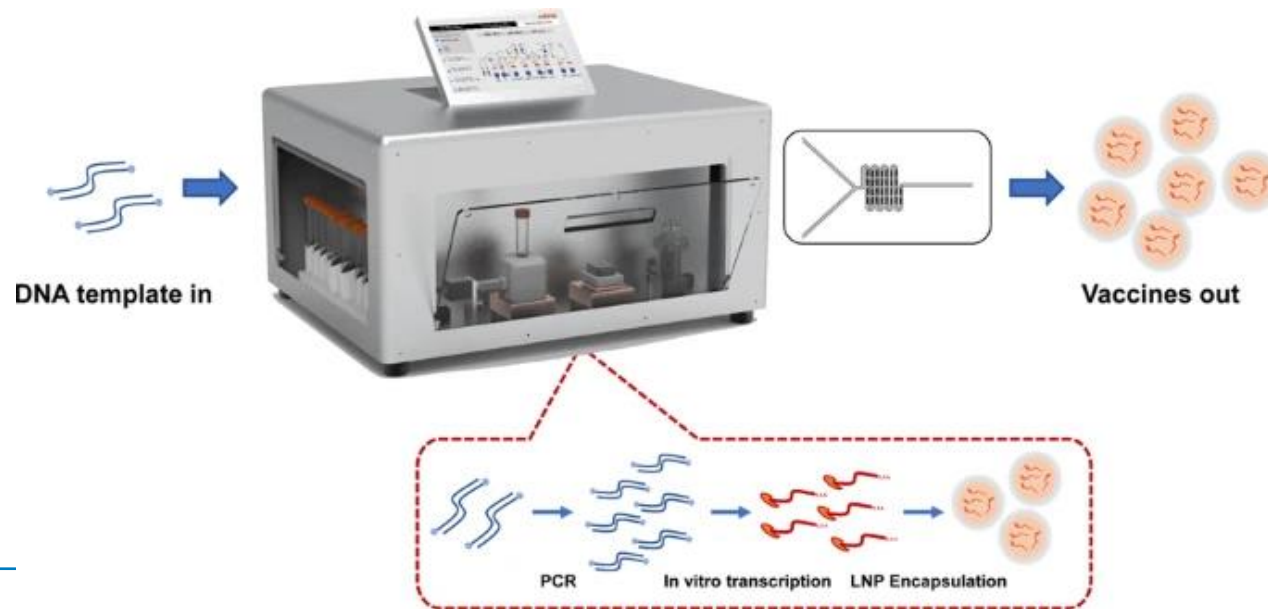
- The **continuous All-ScaleFlow™ technology** enables end-to-end production of **GMP-grade mRNA-LNP**
 - volumes per batch: **1,000 doses for pre-clinical / phase 1 to 10 million doses** for commercial production
 - real-time and remote monitoring** capabilities, facilitating operations and technology transfers



Example 2: Bioinformatics Center of AMMS, Beijing, China

<https://www.nature.com/articles/s41378-023-00538-8>

- **Universal integrated platform** with a corresponding **control system** for the streamlined and on-demand preparation of mRNA products
- **Three main components:** **(1) a PCR module** to amplify the target DNA templates; **(2)** a heating–magnet separating–mixing (HMM) module to provide a mixing platform for thermostable, magnetically separable reaction components for **IVT**; and **(3) an LNP module** to directly encapsulate mRNA into LNPs by using staggered herringbone micromixer (SHM) microfluidic chips.



Research prototype –
lab scale

4. Technological investments at RNA therapeutics companies

Example 1: CureVac - The RNA Printer®



<https://www.curevac.com/en/curevac-establishes-fully-owned-company-dedicated-to-advancing-the-rna-printer/>

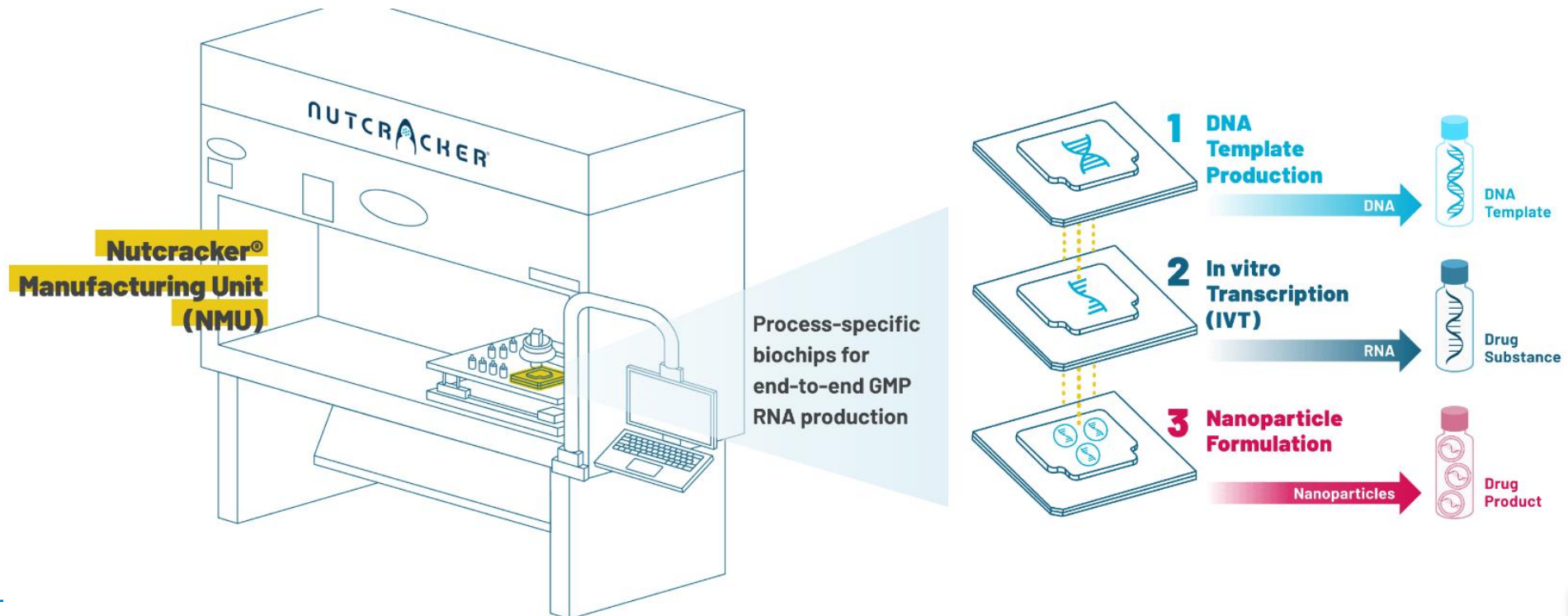
- **Integrated and automated** manufacturing of **GMP-grade** RNA vaccines and therapeutics.
- Proprietary and advanced manufacturing technology designed to cover all steps for **rapid and standardized manufacturing** of mRNA medicines.
- **Facilitate broad access to mRNA technology** and to **accelerate the transition** of innovative products concepts from science **to the clinic**.

CEPI awards US \$34million contract to CureVac to advance The RNA Printer™ —a mRNA vaccine platform that can rapidly combat multiple diseases

Example 2: Nutcracker Therapeutics - Nutcracker[®] Manufacturing Unit (NMU)

<https://www.nutcrackerx.com/platform/>

- **Single hardware system** powered by **process-specific biochips**.
- RNA therapeutic manufacturing **from DNA sequence to fully formulated nanoparticles** on a versatile, **software-controlled platform**.



Conclusion

Points for consideration

- Need for unique/tailored pDNA
 - Objective of hub/partners partnership is to minimize the effort of each partner to undergo end-to-end development. If the automated system is not compatible with the pDNA design /pDNA manufacturing SOPs provided by the hub in SA, the procurement of tailored pDNA and respective manufacturing process development will lie with the partner
 - Ideally hub and partners should ensure compatibility of the pDNA across systems
- Access to proprietary reagents and service
 - What are the guarantees to reagent supply if sole manufacturer is purchased/ceases to exist?
 - What is the maintenance and repair service? Will this be available locally and in the event of a pandemic? How much time will be lost if a machine breaks down?
- Infrastructure requirements (Class A/B/C); electricity; water; multi-purpose facilities; ...
- Installation/qualification; GMP-compatibility; in-process controls; cleaning validation; ...
- Regulatory acceptance



THANK YOU!