Supplementary Information (SI) for Lab on a Chip.
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Supplementary Information

An automated modular microfluidic platform for end-to-end mRNA synthesis and purification

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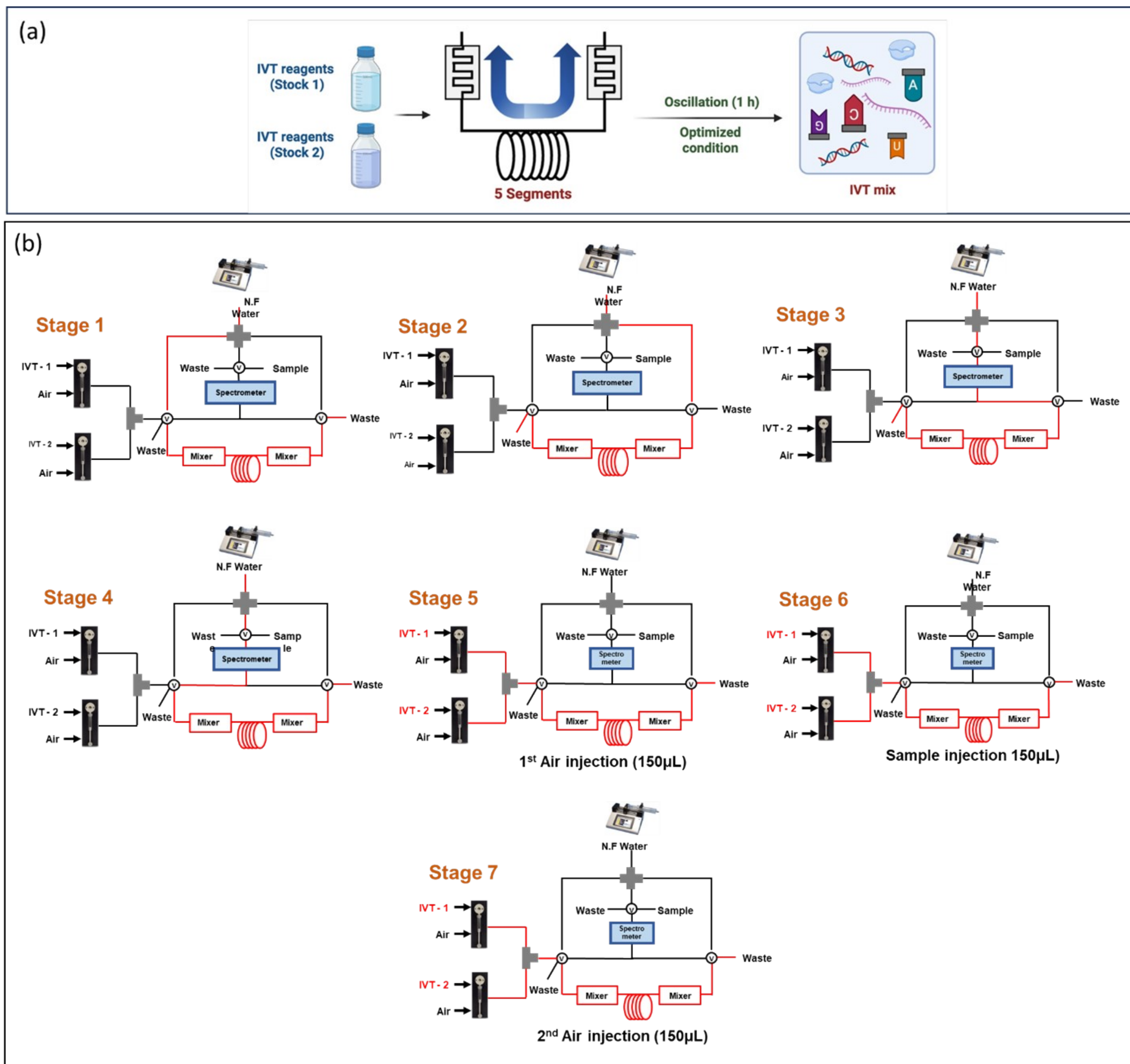
List of abbreviations

Supplementary Table S1. Composition of two independent feed stock solutions for IVT reaction.

Composition	**Solution 1**	**Solution 2**
10X transcription buffer	-	1 X
rNTPs (100 mM)	5 mM	-
Cap analog (100 mM)	4 mM	-
Template DNA	50 ng/μL	-
T7 RNA polymerase	-	60 U/μL
DTT (100 mM)	-	1 mM
RNase inhibitor (40 units/μL)	0.8 U/μL	-
Nuclease Free water	25 μL	40 μL

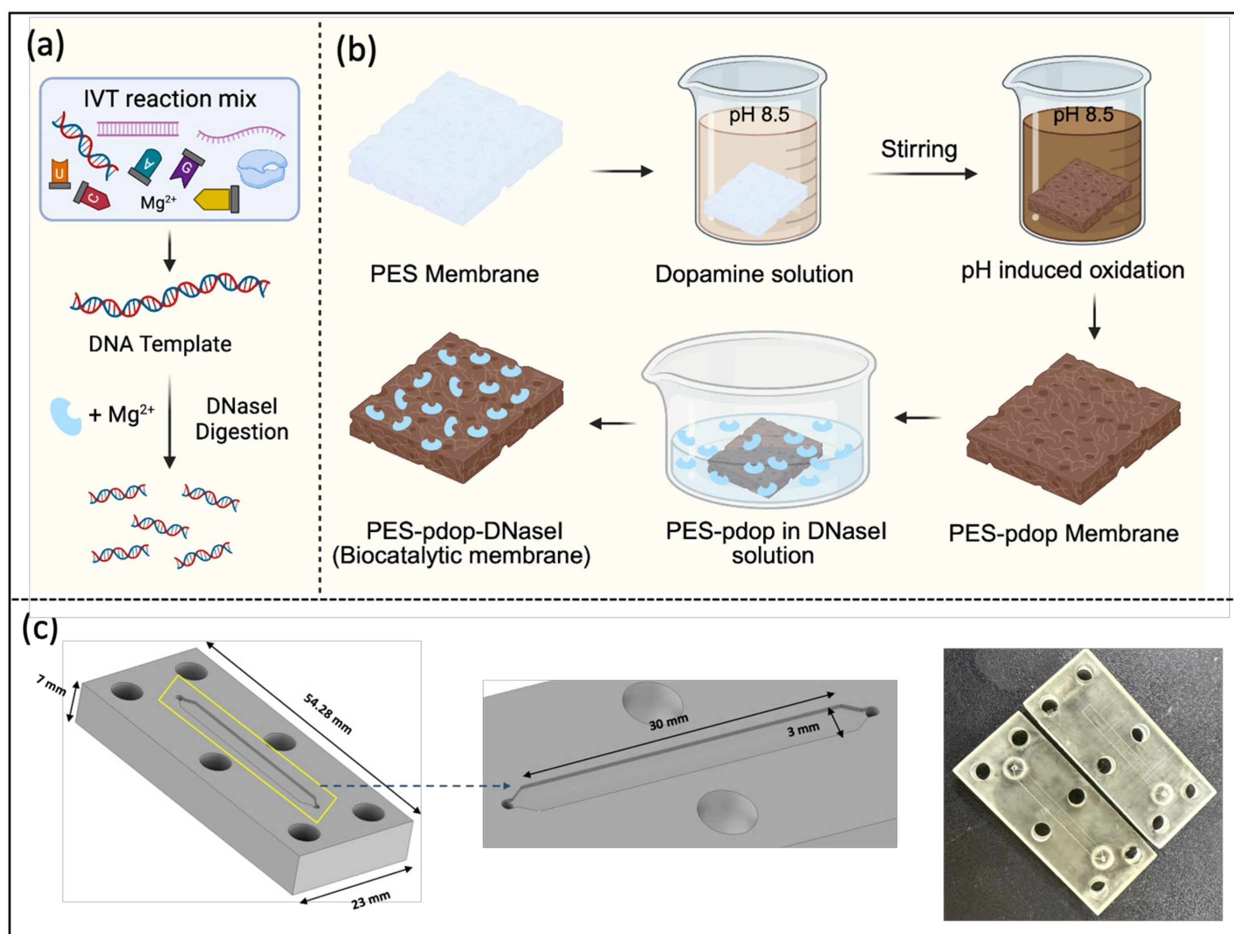


Supplementary Figure S1. (a) Oscillatory IVT module for mRNA synthesis; (b) Computer-controlled stepwise valve/pump sequence used to assemble and operate the (Os(IVT)) module (Unit Operation A). Stages 1-4 illustrate automated priming and formation of a five-segment plug comprising a leading nuclease-free water (NFW) segment, an air spacer, the IVT reaction segment

formed by simultaneous 1:1 co-injection of the two IVT feed solutions (total reaction volume 150 μL), a second air spacer, and a trailing NFW segment. The air spacers isolate the reaction segment from the carrier liquid and minimize dispersion. Stages 5-7 show the oscillatory reaction mode in which the plug is held within the capillary reaction zone and repeatedly shuttled back-and-forth through PTFE serpentine micromixers located at both ends of the reactor. Each flow reversal forces the reaction segment to traverse the serpentine channels, generating strong transverse advection for rapid homogenization. An in-line spectrometer monitors air/liquid interfaces and provides feedback to trigger valve switching and flow reversal, ensuring the plug remains in the intended mixing/reaction region for the programmed residence time before routing the crude IVT mixture to collection.

Supplementary Method S1. PTFE serpentine micromixer fabrication and assembly

The PTFE-serpentine micromixer consists of four layers: two aluminum plates (thickness: 1 cm) and two PTFE plates (thickness: 1 cm) with same width and height (5 cm x 3.5 cm). The upper aluminum plate includes threaded holes for connecting PTFE capillary tubing (outer diameter: 1.58 mm and inner diameter: 1 mm) for sample injection and ejection. The upper PTFE layer features a 2D-serpentine microchannel structure (width and height: 500 μm, length: 5 cm), precisely fabricated using computer numerical control (CNC) milling. The PTFE-serpentine micromixer was assembled by laminating four layers using a clamping method with six screws.

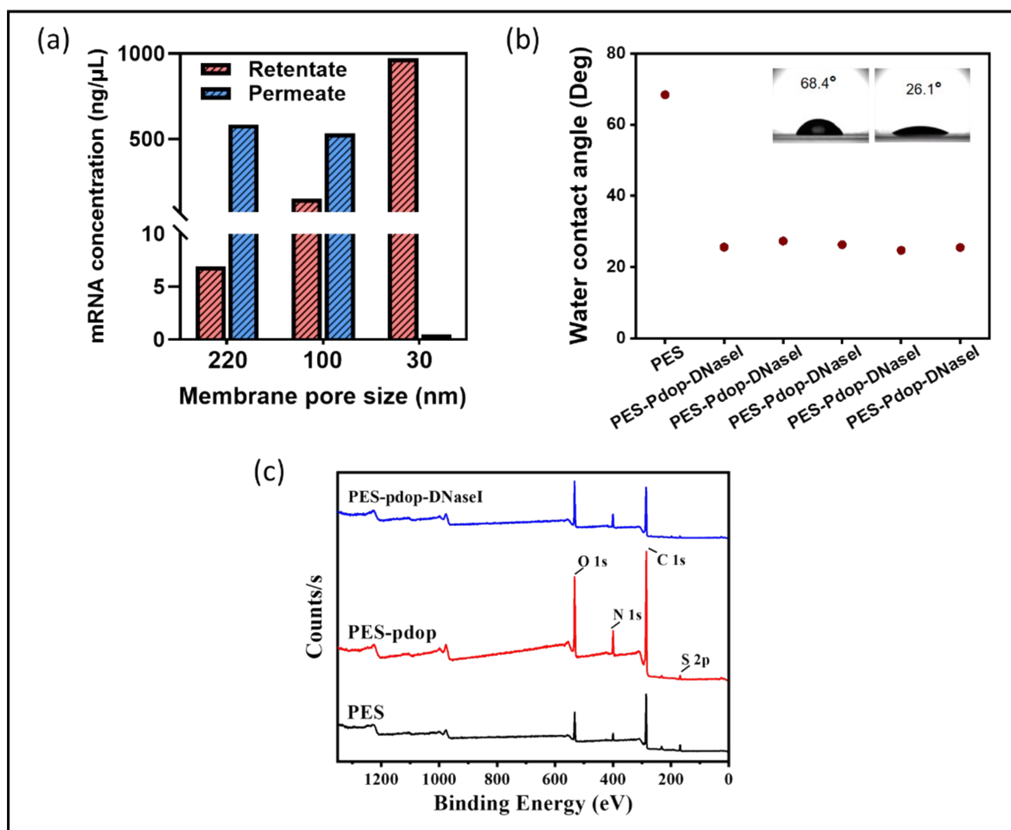


Supplementary Figure S2. Preparation of PES-Pdop-DNase I biocatalytic membrane and fabrication of μ-TFF device for μFD module, (a) mechanism of DNase I catalyzed digestion of DNA template; (b) step by step preparation of biocatalytic membrane (PES-Pdop-DNase I); (c) μ-TFF device design and dimensions.

Supplementary Method S2. PES-Pdop functionalization, DNase immobilization, μTFF device fabrication, and activity validation

A hydrophilic polyethersulfone (PES) membrane with 0.03 μm pore size (GVS S.p.A., Cat. No. 1235748) was used in the microfluidic biocatalytic membrane (μFD) module. The PES membrane was further functionalized with polydopamine (Pdop) via dopamine self-polymerization in Tris-HCl buffer (pH 8.5) to increase surface hydrophilicity and introduce catechol/amine functionalities for enzyme immobilization. Membranes were immersed in a 1 mg/mL dopamine solution and incubated at 37 °C for 24 h to form a Pdop coating that introduces amine and catechol groups for

enzyme binding. After coating, membranes were rinsed with diethyl pyrocarbonate (DEPC)-treated water and further incubated at 50 °C for 4 h to stabilize the functional layer. For enzyme immobilization, the Pdop-coated PES membranes were submerged in a DNase I solution and incubated for 8 h at 4 °C, using a dip-coating approach, followed by bovine serum albumin (BSA) treatment. BSA treatment served as a blocking/passivation step to reduce non-specific adsorption of biomolecules and to improve operational stability during digestion and filtration. The catalytic activity of the resulting PES-Pdop-DNase I membranes was validated using a fluorescence-based DNase activity assay (Abcam, UK). Briefly, membrane slices were incubated with a DNase I-specific fluorescent substrate in reaction buffer at 37 °C for 30 min. Fluorescence intensity was measured at excitation/emission wavelengths of 535/565 nm to confirm enzymatic activity. In addition, the 3D-printed μ-TFF unit was designed with AutoDesk Inventor software to create a virtual object and was fabricated using UV-curable acrylate resin (Polymethyl methacrylate, PMMA) through Digital Light Processing (DLP) desktop printer (Asiga Pico 2). The fabricated device was post-treated with UV radiation lamp with 2mW cm-2 intensity for 30 min to consolidate the structure. Potential adsorption to polymeric components (PMMA housing and PTFE tubing) was mitigated by Pdop-based membrane hydrophilization, BSA passivation, and short residence-time continuous-flow operation.



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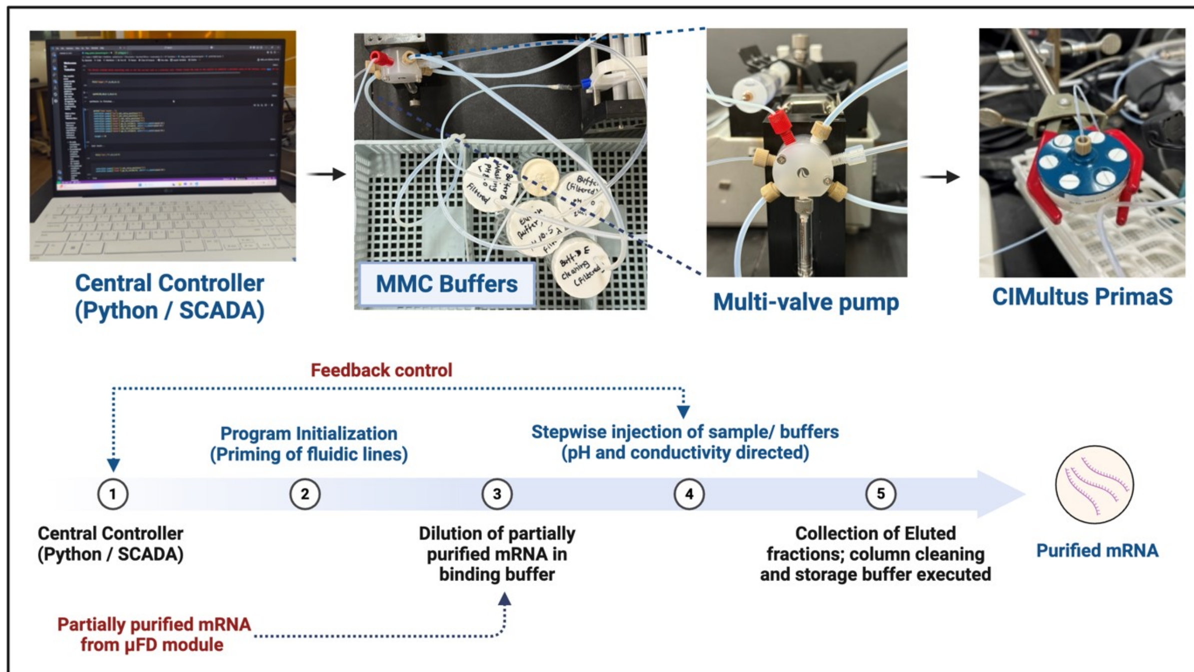
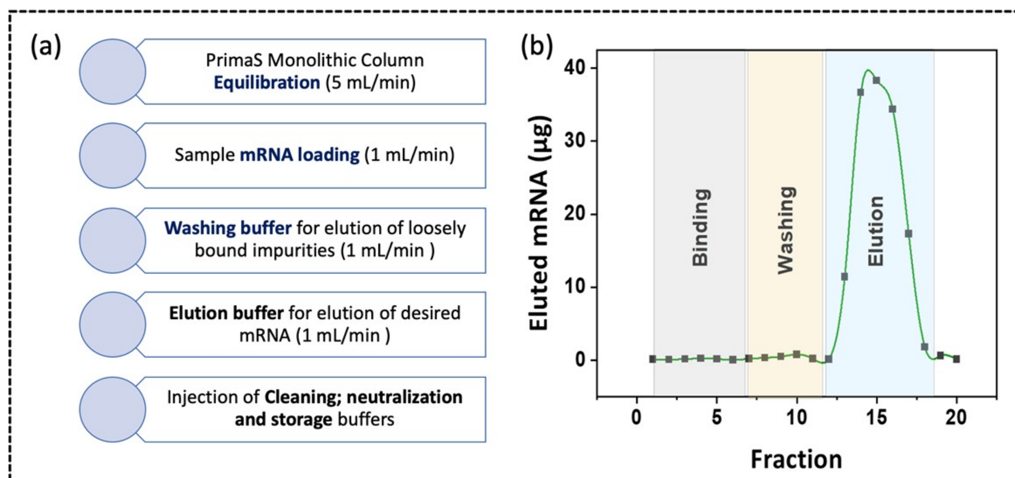


Supplementary Figure S4. Execution steps of automated multimodal chromatography (MMC) module.

Cleaning-in-Place (CIP) and Sterilization-in-Place (SIP) in the Integrated Platform

Cleaning-in-Place (CIP) and Sterilization-in-Place (SIP) are essential for closed-path bioprocesses to minimize cross-run contamination and RNase exposure while enabling rapid, repeatable turnarounds. In our integrated workflow, the chromatography step is programmed to execute a NaOH/NaCl CIP cycle followed by re-equilibration to the operating buffer, restoring column performance and reproducibility without disassembly. This sequence is triggered automatically as part of the stepwise run logic and immediately precedes the next cycle.

Beyond the column, a centralized Python SCADA/MES layer coordinates run-end procedures across valves, pumps, and sensors to perform automated CIP and SIP between cycles, enforcing valve states and residence times for each cleaning/sterilization reagent before re-equilibration. This closed, cleanable fluidic path reduces manual handling, helps control bioburden, and shortens changeover time—features that are directly aligned with GMP-style operation of disposable fluid paths and single-use components in microfluidic biomanufacturing.



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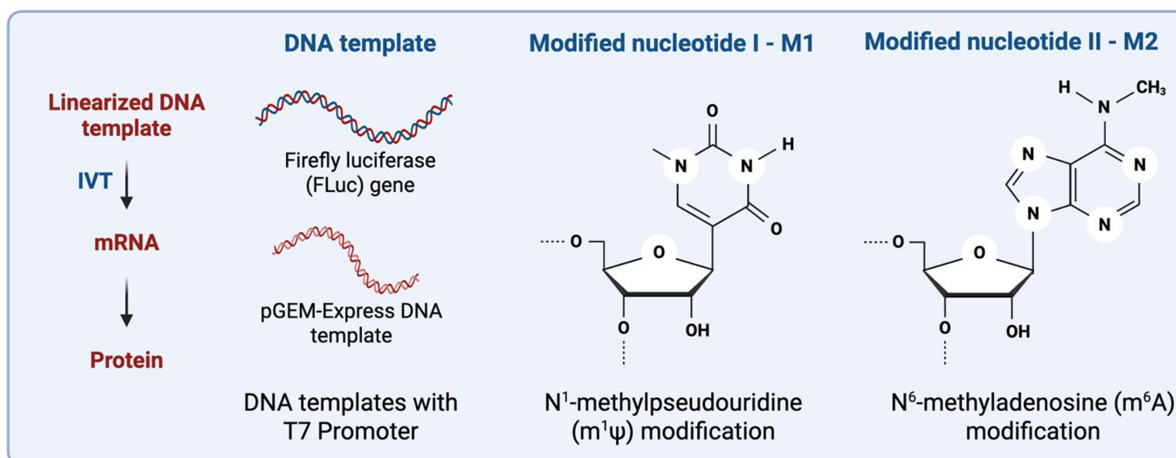

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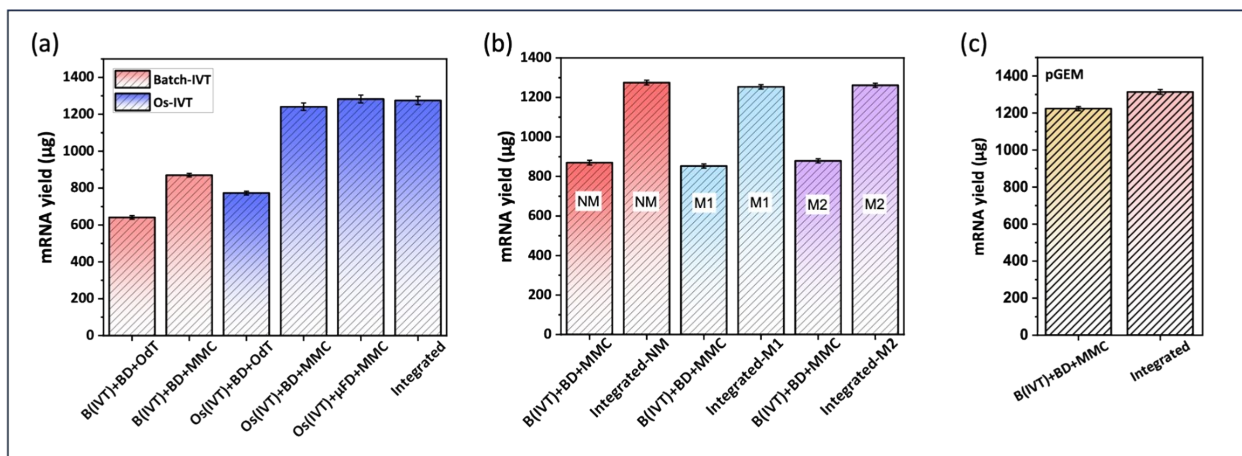

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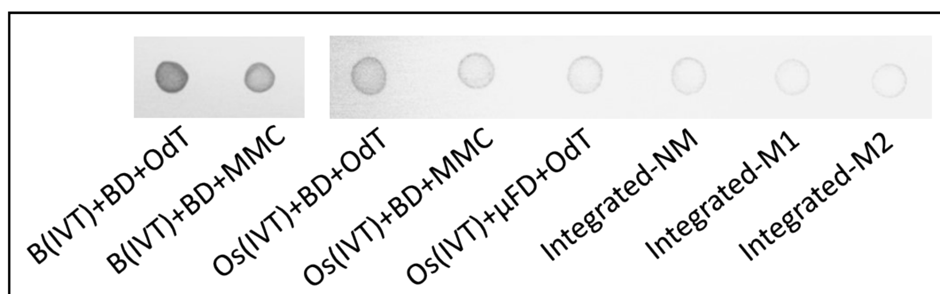

Supplementary Figure S8. Dot blot assay for presence of dsRNA impurities in the mRNA obtained from various discrete and integrated workflows by combining batch-mode and microfluidic-mode.

Supplementary Method S3. Oligo-dT bead purification (benchmark workflow)

The mRNA synthesized by Os(IVT) process was purified using Dynabeads™ oligo-dT. Briefly, mRNA samples were heated at 65°C for 2 min to disrupt secondary structures and then transferred to a microcentrifuge tube containing 200 µL of resuspended Dynabeads™ magnetic beads and binding buffer (20 mM Tris-HCl, pH 7.5, 1.0 M LiCl, 2 mM EDTA) suspension. The mixture was thoroughly mixed for 5 min, and the tube was placed on a magnet until all beads adhered to the tube wall. The supernatant was discarded, and the tube was removed from the magnet for two washing steps. The magnet was reapplied, the supernatant was removed again, and nuclease free water was added to elute the mRNA for subsequent analysis.

List of abbreviations:

Abbreviation	Description
AGE	Agarose gel electrophoresis
B(IVT)	Batch in vitro transcription
BD	Batch-mode DNase I digestion
CAPS	3-(Cyclohexylamino)-1-propanesulfonic acid
CE	Capillary electrophoresis
CIP	Cleaning-in-place
DLP	Digital light processing
DNase I	Deoxyribonuclease I
dsRNA	Double-stranded RNA
DTT	Dithiothreitol
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FDA	Food and Drug Administration
FLuc	Firefly luciferase
GMP	Good manufacturing practice
GUI	Graphical user interface
HF-TFF	Hollow-fiber tangential flow filtration
HRP	Horseradish peroxidase
IVT	In vitro transcription
LiCl	Lithium chloride
M1	N^1-methyl-pseudouridine-5'-triphosphate
M2	N^6-methyladenosine-5'-triphosphate
MES	Manufacturing execution system
MMC	Multimodal chromatography
MOPS	3-(N-morpholino) propanesulfonic acid
mPES	Modified polyethersulfone
MWCO	Molecular weight cut-off
NFW	Nuclease-free water
NM	Non-modified nucleotides
NTPs	Nucleotide triphosphates
OdT	Oligo-dT affinity purification
Os(IVT)	Oscillatory in vitro transcription
PDA/Pdop	Polydopamine
PES	Polyethersulfone
pGEM	pGEM DNA template / plasmid construct
PTFE	Polytetrafluoroethylene
RLU	Relative luminescence units
RRID	Research Resource Identifier
saRNA	Self-amplifying messenger RNA
SCADA	Supervisory control and data acquisition
SIP	Sterilization-in-place

TFF	Tangential flow filtration
XPS	X-ray photoelectron spectroscopy
μ-TFF	Micro-tangential flow filtration
μFD	Microfluidic digestion/clarification