Supplementary Information (SI) for Lab on a Chip.

Supplementary Information

An Integrated Microfluidic System for Automated Extraction of *In-vitro* Transcribed mRNAs via Probe-Coated Magnetic Beads

*Swati T. Gurme[1], Yu-Ting Su[1], Bhushan Koparde[1], Lily Hui-Ching Wang[2**] and Gwo-Bin Lee[1,3*]*

[1]Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu, Taiwan

[2]Institute of Molecular and Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan

[3]Institute of NanoEngineering and Microsystems, National Tsing Hua University, Hsinchu, Taiwan

***Corresponding author details:**

Gwo-Bin Lee

Department of Power Mechanical Engineering,

National Tsing Hua University, Hsinchu, 30013, Taiwan.

E-mail addresses: gwobin@pme.nthu.edu.tw

****Co-corresponding author details:**

Lily Hui-Ching Wang

Institute of Molecular and Cellular Biology,

National Tsing Hua University, Hsinchu, 30013, Taiwan.

E-mail addresses: wang.huiching@gmail.com

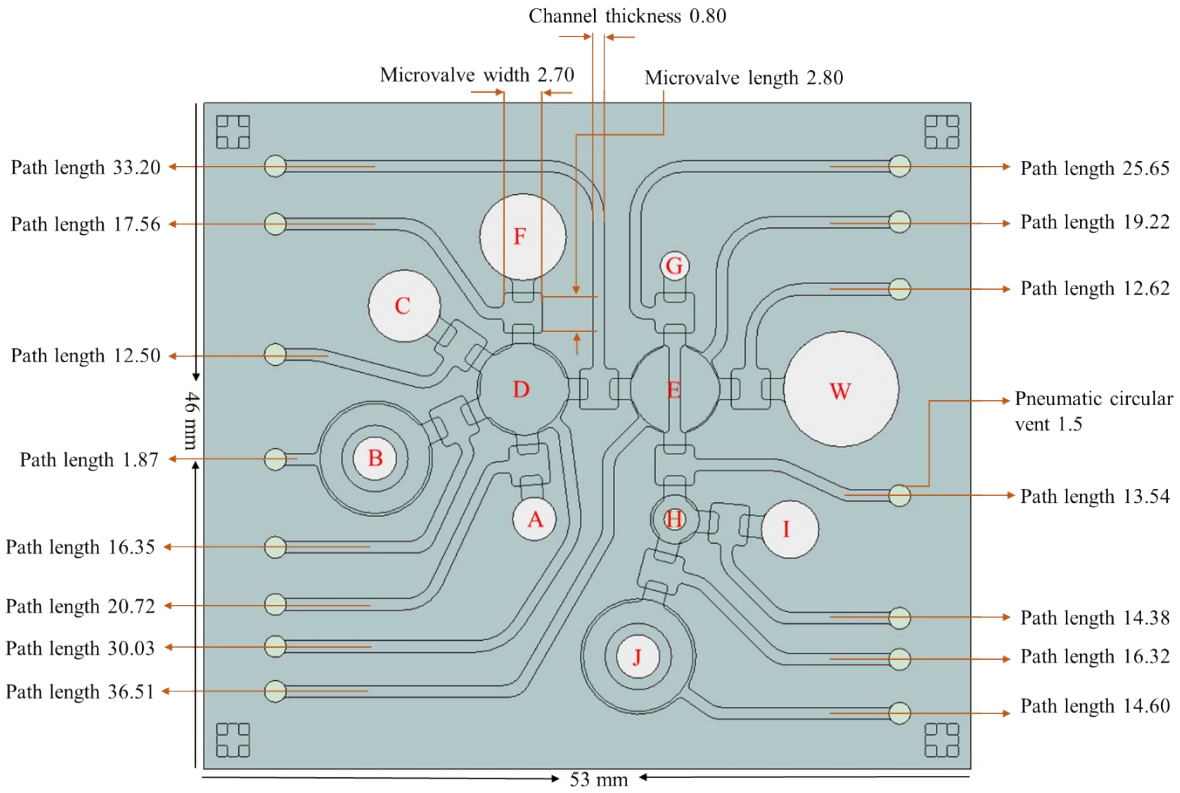

Figure S1: Two-dimensional schematic layout of the microfluidic chip (52 mm × 43 mm) designed for automated mRNA extraction, with all the dimensions (mm) of the microvalve, microchambers, and microchannel thickness. The device incorporated pneumatically-actuated microvalves and micropumps to control reagent flow across functional chambers: (A) in-vitro transcription inlet, (B) DNase treatment chamber/micromixer, (C) probe-coated MBs chamber, (D) micropump, (E) micropump/micromixer, (F) washing buffer chamber, (G) release buffer chamber, (H) micropump, (I) puromycin linker chamber, (J) reverse transcription chamber/micromixer, and (W) waste chamber. The orange-coloured arrows indicated the approximate path length of the respective channel. All the dimensions are in mm.

Table S1: *In vitro* transcription reaction details

Reaction components	Sample reaction volume (µL)	Control reaction volume (µL)
RiboMax Express T7 2x buffer	10	10
Linear DNA template	8	0
pGEM express positive control template (1 µg)	0	1
Nuclease-free water	0	7
T7 express enzyme mix	2	2
Final volume	20	20

Table S2: Reverse transcription (RT) and quantitative PCR (qPCR) reaction details

Reaction components	RT reagent volume (µL)	qPCR reagent volume (µL)
T7SD8M2.F44 primer (10 µM)	0	0.20
G5S-4.R20 primer (10 µM)	0.25	0.25
KAPA RT mix	0.40	0
KAPA SYBR® FAST qPCR master mic (2X)	0	10
ddH_2O	17.35	7.55
Test samples	2	2
Total volume	20	20

Table S3. The operating procedures of the microfluidic chip. RT=reverse transcription

Step	Volume (μL)	Chamber(s) used	Applied gauge pressure (kPa)
IVT	20	A, D	-35/20
DNase treatment	35	B	-35/20
Hybridization	35	C, D	-35/20
Washing	25	F→D→E→W	-20/20
Thermal release	10	E	-35/20
Puromycin linker annealing	10	I	-
RT	20	J	-35/20