Electronic supplementary information for

Automated centrifugal microfluidic system for the preparation of adaptor-ligated sequencing libraries

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Supplementary videos

Supplementary video 1: DNA cleanup by SPRI bead

File name: “Guo et al. - Supp. video 1 - DNA cleanup by SPRI bead.mp4”

Video presenting the various steps of the on-chip DNA clean-up protocol. Refer to table S1, step 4 for details.

Supplementary video 2: Repeatability of SPRI bead transfer

File name: “Guo et al. - Supp. video 2 - Repeatability of SPRI bead transfer.mp4”

Mosaic of five different videos showing repeatability of SPRI bead transfer process from one test to another. Refer to Figure S1a for details.

Supplementary video 3: Volume control of SPRI bead transfer

File name: “Guo et al. - Supp. video 3 - Volume control of SPRI bead transfer.mp4”

Mosaic of five different videos showing the capacity of the system to control the volume of SPRI bead transferred by adjusting the duration of the pressure pulse. Refer to Figure S1b for details.

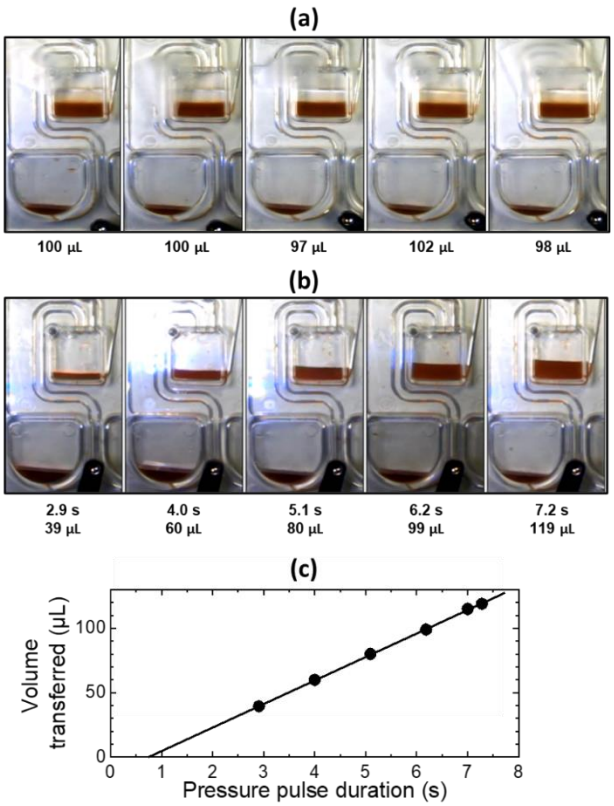

Figure S1. (a) Series of images showing the repeatability of the SPRI bead transfer step for five independent tests. Numbers indicate the measured volume, as determined by image analysis (target volume: 100 µL). See also supplementary video 2. **(b)** Series of images showing the capacity to control the volume of SPRI bead transferred by adjusting the duration of the pressure pulse. Numbers indicate the duration of the pressure pulse and measured volume, as determined by image analysis. See also supplementary video 3. **(c)** Effect of the pressure pulse duration on the volume of SPRI bead transferred to the main chamber. Rotation speed was set to 400 rpm and positive air pressure applied was 3.0 psig.

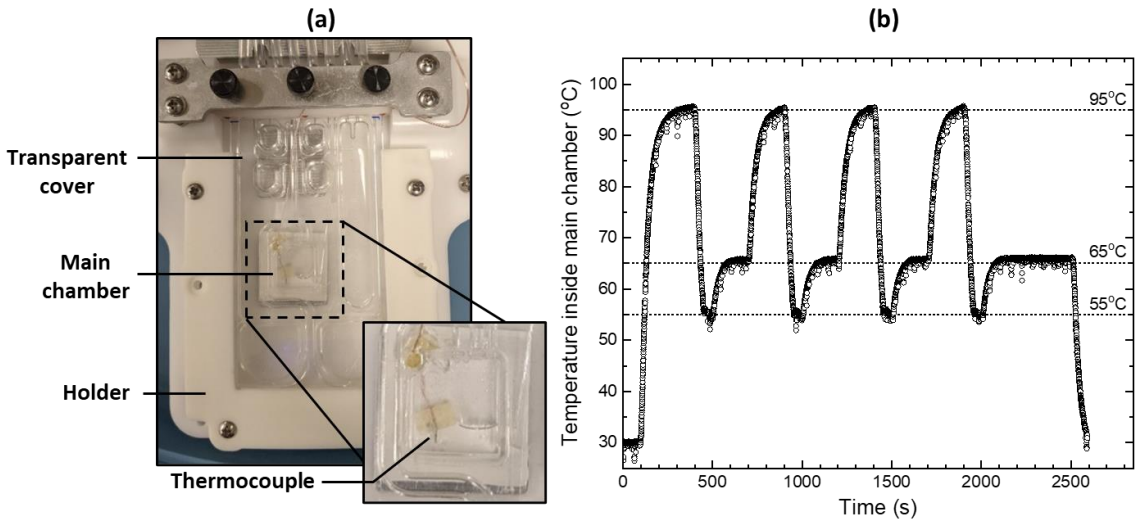

Figure S2. (a) Picture of the holder with a transparent cover used to isolate the microfluidic device from the cold air flow during centrifugation. Inset shows an enlarged view of the thermocouple inserted inside the main chamber of the microfluidic device during temperature calibration. **(b)** Example of the temperature profile measured inside the main chamber during the PCR amplification step.

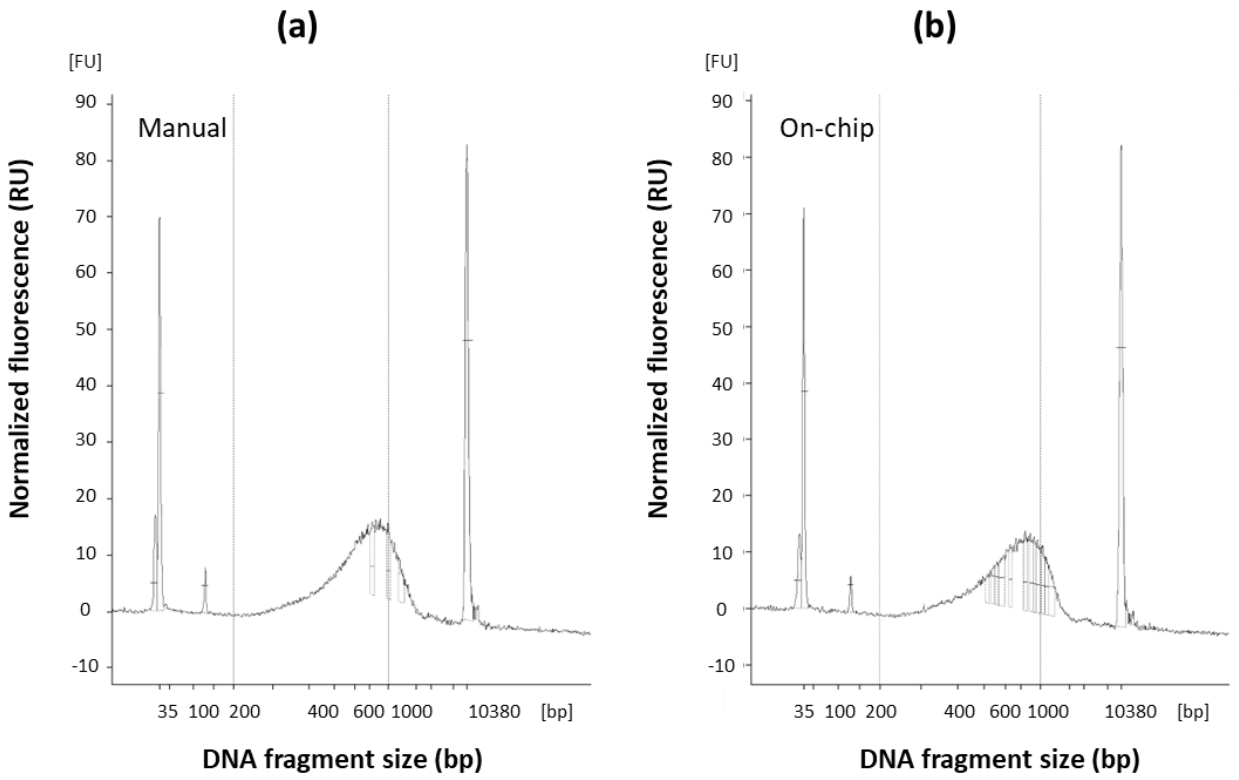

Figure S3. Bioanalyzer traces obtained after PCR amplification of a diluted Illumina-compatible library for 4 cycles performed using both **(a)** a conventional thermal cycler (manual) and **(b)** a microfluidic device heated by the centrifugal platform (on-chip).

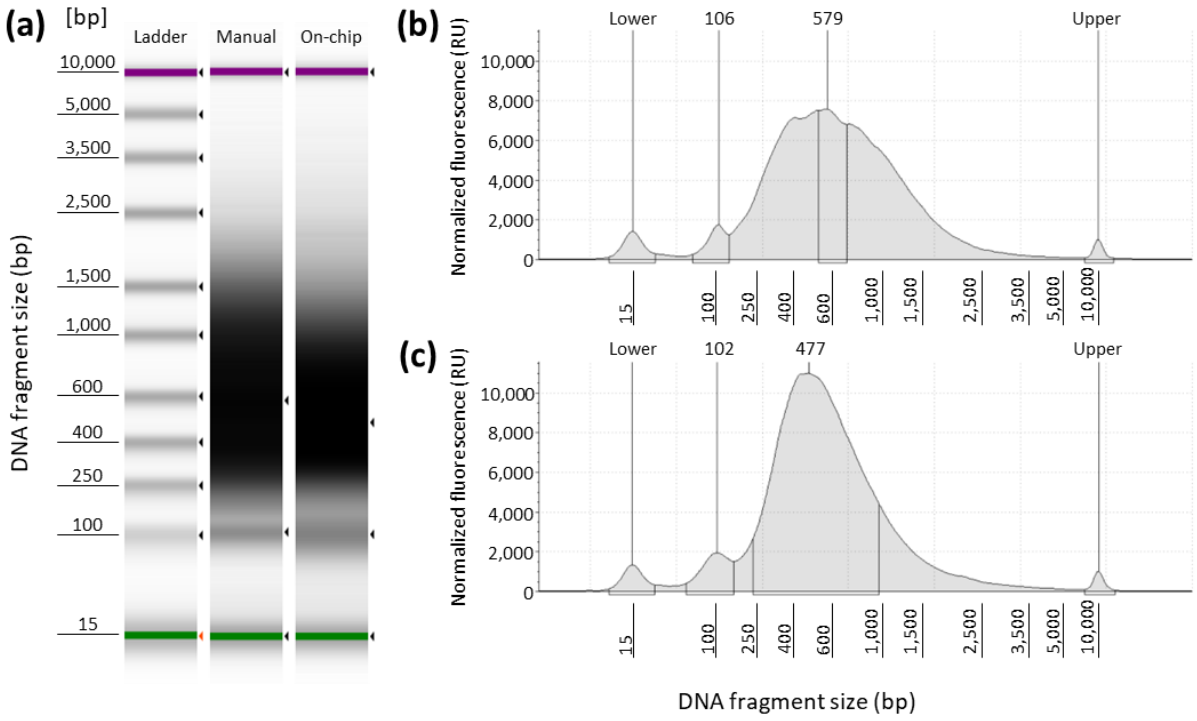

Figure S4. (a) Comparative size analysis of libraries created by the manual and on-chip methods using TapeStation HS D5000 assay. More detailed analysis of the manual **(b)** and on-chip **(c)** libraries show their respective fragment size distributions. Fragment size values from TapeStation results, along with Qubit DNA quantification values, were used to calculate library molarity for pooling. Representative data from replicate 2 is shown.

Table S1. Microfluidic implementation of the automated library preparation protocol

Step	Operation	Active ports (#)	Applied pressure (psig)	Temperature (°C)	Rotation speed (rpm)	Duration
1	**Fragmentation and end preparation**					
a	Transfer	3	2.0	-	800	0.5 s (2×)
b	Bubble mixing	7	2.0	-	400	5.0 s (2×)
c	Temperature cycling: Fragmentation	-	-	37°C	400	8 min
	Temperature cycling: Enzyme inactivation			65°C		30 min
	Temperature cycling: Sample cooling			25°C		5 min
2	**Adaptor ligation**					
a	Transfer	6	2.0	25°C	800	1.0 s (2×)
b	Bubble mixing	7	2.0	25°C	800	0.5 s (10×)
c	Incubation	-	-	20°C	800	15 min
3	**USER cleavage**					
a	Manual loading of reagent	-	-	20°C	0	Manual
b	Bubble mixing	7	2.0	20°C	800	0.5 s (10×)
c	Incubation	-	-	37°C	800	15 min
				25°C		1 min
4	**First DNA cleanup**					
a	SPRI bead transfer	1	3.0	25°C	400	5.37 s
b	Bubble mixing	7	0.5	25°C	400	10 s
c	Incubation	-	-	-	200	10 min
d	Sedimentation	-	-	-	1000	15 min
e	Transfer supernatant to waste	7	-0.6 to -1.7	-	400	1 min
f1	First wash transfer	8	1.5	-	400	2.80 s
g1	First wash mixing and bead sedimentation	7	0.6	-	400	4.0 s
		-	-		1000	12 min
h1	Transfer first wash to waste	7	-0.6 to -1.7	-	400	1 min
f2	Second wash transfer	8	1.5	-	400	3.20 s
g2	Second wash mixing and bead sedimentation	7	0.5	-	400	4.0 s
		-	-		1000	12 min
h2	Transfer second wash to waste	7	-0.6 to -1.7	-	400	1 min
i	Air drying	7	0.5	-	1000	3 min
5	**PCR amplification**					
a	Transfer	2	1.5	-	400	5.0 s
b	Bubble mixing	7	2.5	-	400	5.0 s (2×)
c	Thermal cycling: Denaturation (initial)	-	-	95°C	400	60 s
	Thermal cycling: 6× Denaturation			95°C		20 s
	Thermal cycling: 6× Annealing			55°C		30 s
	Thermal cycling: 6× Extension			65°C		120 s
	Thermal cycling: Extension (final)			65°C		300 s
6	**Second DNA cleanup**					
a	SPRI bead transfer	1	3.0	25°C	400	2.84 s
b	Bubble mixing	7	0.5	-	400	10 s
c	Incubation	-	-	-	200	10 min
d	Sedimentation	-	-	-	1000	15 min
e	Transfer supernatant to waste	7	-0.6 to -1.7	-	400	1 min
f1	First wash transfer	8	1.5	-	400	3.65 s
g1	First wash mixing and bead sedimentation	7	0.6	-	400	4.0 s
		-	-		1000	12 min
h1	Transfer first wash to waste	7	-0.6 to -1.7	-	400	1 min
f2	Second wash transfer	8	1.5	-	400	4.10 s
g2	Second wash mixing and bead sedimentation	7	0.5	-	400	4.0 s
		-	-		1000	12 min
h2	Transfer second wash to waste	7	-0.6 to -1.7	-	400	1 min
i	Air drying	7	0.5	-	1000	3 min
7	**Elution**					
a	Transfer	5	1.5	-	800	1.0 s (2×)
b	Bubble mixing	7	2.5	-	800	5.0 s (2×)
Other	**SPRI bead mixing**					
a	Bubble mixing in reservoir R5 (repeated every 2 min until step 6a)	1	-1.5	Variable	Variable	5.0 s (repeated)