Electronic Supplementary Information (ESI)

Nanobead handling on a centrifugal microfluidic LabDisk for automated extraction of cell-free circulating DNA with high recovery rates

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Table S1 *Sequences of the primers, mediator probes and universal reporters for the 2-plex mediator probe ddPCR and qPCR assay.*

Name	***Sequence 5'-3'***	***5' mod***	***3' mod***	***Internal***
Fwd_Primer_KRAS	GGCCTGCTGAAAATGACT	-	-	-
Rev_Primer_KRAS	ACAAAATGATTCTGAATTAGCTGTA	-	-	-
MP_UR05_G12D	ATGTCCCAGGTGCATGGCGTAGGCAAGAGTGCCTTGACGAT	-	MP Block	-
MP_UR02_WT	CTCCAGTTCGGTCCAGCTCCAACTACCACAAGTTTATATTCAG	-	MP Block	-
BRAF Fwd. Primer	GACCCACTCCATCGAGATTTC	-	-	-
BRAF Rev. Primer	GCTTGCTCTGATAGGAAAATGAG	-	-	-
MP BRAF WT_UR06	GGTAGGCTCACTGACTGTAGCTAGACCAAAATCACCTATTTTTACTGTGAG	-	MP Block	-
MP BRAF V600E_UR05	GGTAGGCTCACTGACTGTAGCTAGACCAAAATCACCTATTTTTACTGTGAG	-	MP Block	-
UR05_red	GACCGGCTAAGACGCGCCGGT7TGT*TGCACCTGGGACAT*CGACTAT	BHQ-2	UR Block	7=dC-Atto 647N
UR02_green	ATTGCGGGAGATGAGACCCGCAA8TGTTCACT*GACCGAACTGGAG*CA	BMN-Q535	UR Block	8=dT-FAM
UR06_green	ATTGCGGGAGATGAGACCCGCAA8TGTTCAGTGAGCCTACCTGCCTTC	BMN-Q-535	UR Block	8=dTFAM

Instruction for gBlock® resuspension (IDT manual)

The preparation of the synthetic gBlock® DNA follows the manufacturer's instruction.

1) Centrifugation at 3000 g
2) Add TE buffer to a final stock concentration of 10 ng/µl DNA
3) Vortex briefly
4) Tube incubation at 50°C for 20 min.
5) Vortex briefly and centrifuge

Instruction for HaeIII digestion (NEB manual)

The digestion of the human genomic DNA from Roche with HaeIII from NEB follows the manufacturer's instruction.

For 5 µg DNA:

1) 15 µl water
2) Add 5 µl NEB buffer (10 x)
3) Add 25 µl human genomic DNA (0.2 µg/µl) for a final amount of 5 µg DNA with a final concentration of 100 ng/µl
4) Add 5 µl enzyme HaeIII (concentration: 10 U/µl)
5) Pipet up and down (do not vortex)
6) Incubation for 1 h @ 37°C
7) Heat inactivation for 20 min @ 65°C

Table S2 Extraction protocol for cfDNA extraction with magnetic beads and QIAamp Circulating Nucleic Acid kit after optimization. *DNA gBlocks were spiked in the plasma sample, if no patient plasma was used. The concentration of the gBlock DNA is dependent on the experimental setup (10^5 DNA copies/ml plasma – 10^2 DNA copies/ml plasma).

Reagents	***Volume***	***Required handling steps***
Sample (plasma)	1000 µl	• *30s mixing by pulse vortexing* • *Incubate @ 60°C for 30 min*
DNA gBlock*	1 µl	
Proteinase K	100 µl	
ACL (lysis) buffer	800 µl	
ACB (binding) buffer	1800 µl	• *15-30s mixing by pulse vortexing* • *Incubate on ice for 5 min*
Magnetic silica beads ***Magtivio MD0200010002 LOT: NAF10702191C***	40 µl	• *Pipette the whole volume (3.7 ml) on beads in two steps (1.85 ml each)* • *Incubate after each step for 5 min on rotation wheel* • *Collect beads in magnet rack for 1 min* • *Discard supernatant*
ACW1 (washing) buffer	600 µl	• *Pipette 10x up and down* • *Collect beads in magnet rack for 1 min* • *Discard supernatant*
ACW2 (washing) buffer	750 µl	• *Pipette 10x up and down* • *Collect beads in magnet rack for 1 min* • *Discard supernatant*
96-100% ethanol non-denatured	750 µl	• *Pipette 10x up and down* • *Collect beads in magnet rack for 1 min* • *Discard supernatant* • *Open lid incubation @ 56°C for 10 min*
AVE (elution) buffer	100 µl	• *Pipette 10x up and down* • *Incubate 3 min on beads* • *Collect beads in magnet rack for 1 min* • *Store supernatant in eluate tube @ -20°C*

Table S3 Extraction protocol for cfDNA extraction with spin column and QIAamp Circulating Nucleic Acid kit. *DNA gBlocks were spiked in the plasma sample, if no patient plasma was used. The concentration of the gBlock DNA is dependent on the experimental setup (10^5 DNA copies/ml plasma – 10^2 DMA copies/ml plasma).

Reagents	***Volume***	***Required handling steps***
Sample (Plasma)	1000 µl	• *30s mixing by pulse vortexing* • *Incubate @ 60°C for 30 min*
DNA gBlock*	1 µl	
Proteinase K	100 µl	
ACL (lysis) Buffer	800 µl	
ACB (binding) Buffer	1800 µl	• *15-30s mixing by pulse vortexing* • *Incubate on ice for 5 min* • *Pipette the whole volume (3.7 ml) on membrane in six steps (0.7 ml each)* • *Centrifuge @ 5000 rpm for 0.5 min after each step* • *Discard liquid in collection tube after each step*
ACW1 (washing) Buffer	600 µl	• *Pass directly through membrane* • *Centrifuge @ 5000 rpm for 0.5 min* • *Discard liquid in collection tube*
ACW2 (washing) buffer	750 µl	• *Pass directly through membrane* • *Centrifuge @ 5000 rpm for 0.5 min* • *Discard liquid in collection tube*
96-100% ethanol non-denatured	750 µl	• *Pass directly through membrane* • *Centrifuge @ 5000 rpm for 0.5 min* • *Place spin column in new tube* • *Centrifuge @ 10,000 rpm for 3 min* • *Place spin column in new tube* • *Open lid incubation @ 56°C for 10 min*
AVE (elution) Buffer	100 µl	• *Incubate 3 min on membrane* • *Centrifuge @ 10,000 rpm for 1 min* • *Store eluate in tube @ -20°C*

Table S4 Cycling parameters for ddPCR 2-plex assay in Stilla Naica system.

Step	***Time***	***Temperature***
Partitioning	Default parameters from Stilla	
Hotstart	300 s	95°C
Cycling	45x	
Denaturation	15 s	95°C
Annealing	60 s	54°C
Pressure release	Default parameters from Stilla	

Table S5 Cycling parameters for qPCR 2-plex assay in RotorGene Q.

Step	***Time***	***Temperature***
Hotstart	300 s	95°C
Cycling	45x	
Denaturation	15 s	95°C
Annealing + Readout	40 s	56°C Readout channel gain: Red: 8; Green 10

Table S6 Final concentration of components in reaction mix for qPCR and ddPCR.

Component	***Final concentration in qPCR in nM***	***Final concentration in ddPCR in nM***
Forward primer	300	500
Reverse primer	300	1200
Mediator probe	200	1200
Universal reporter	100	240
Fluorescein	n.a.	100
PerfeCTa Multiplex qPCR Toughmix 5x	1x	1x

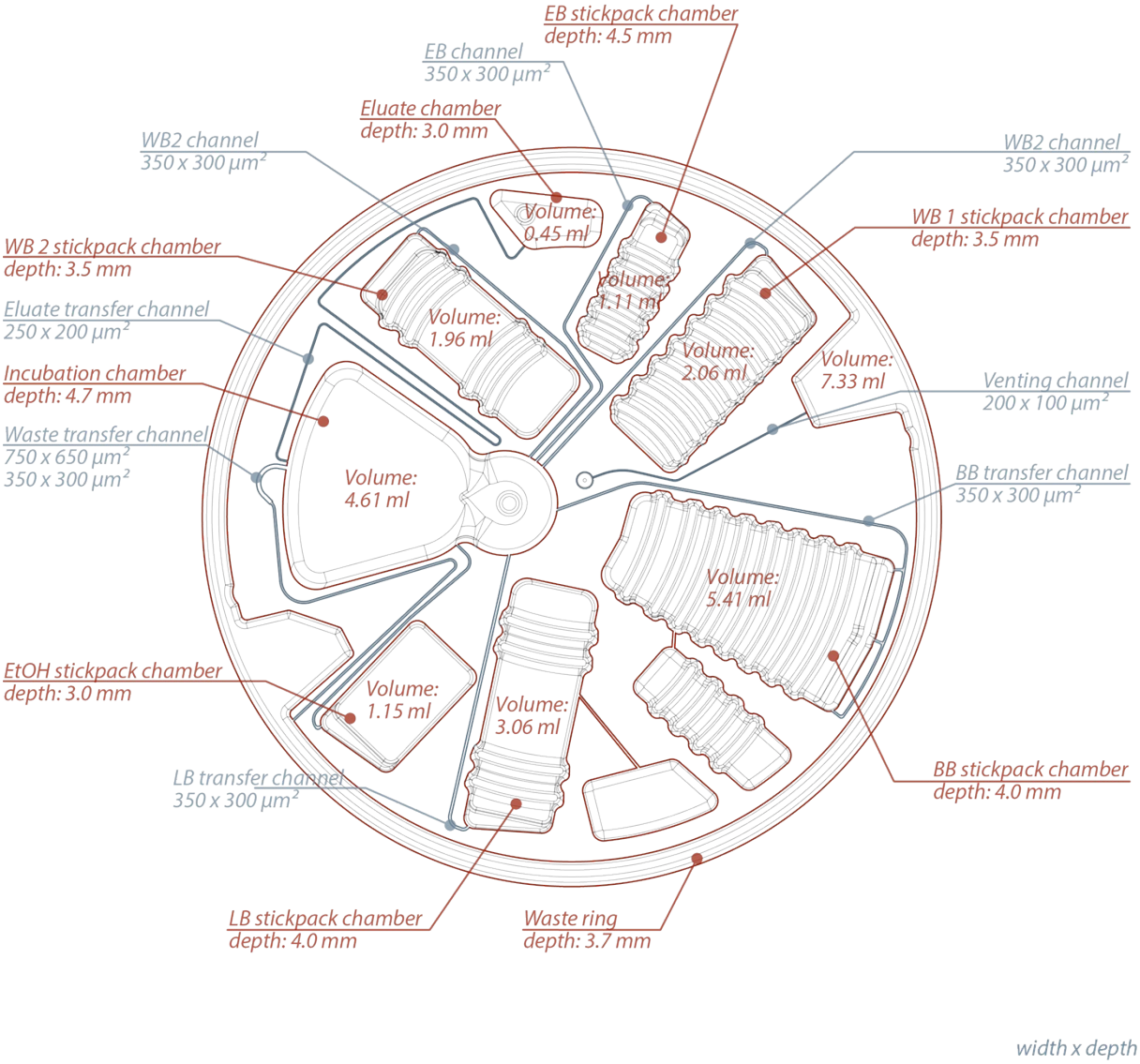


Figure S1 Channel and chamber dimensions of LabDisk for cfDNA extraction. The outer diameter of the LabDisk is 140 mm, the outer diameter of the ring structure for the waste is 134 mm.

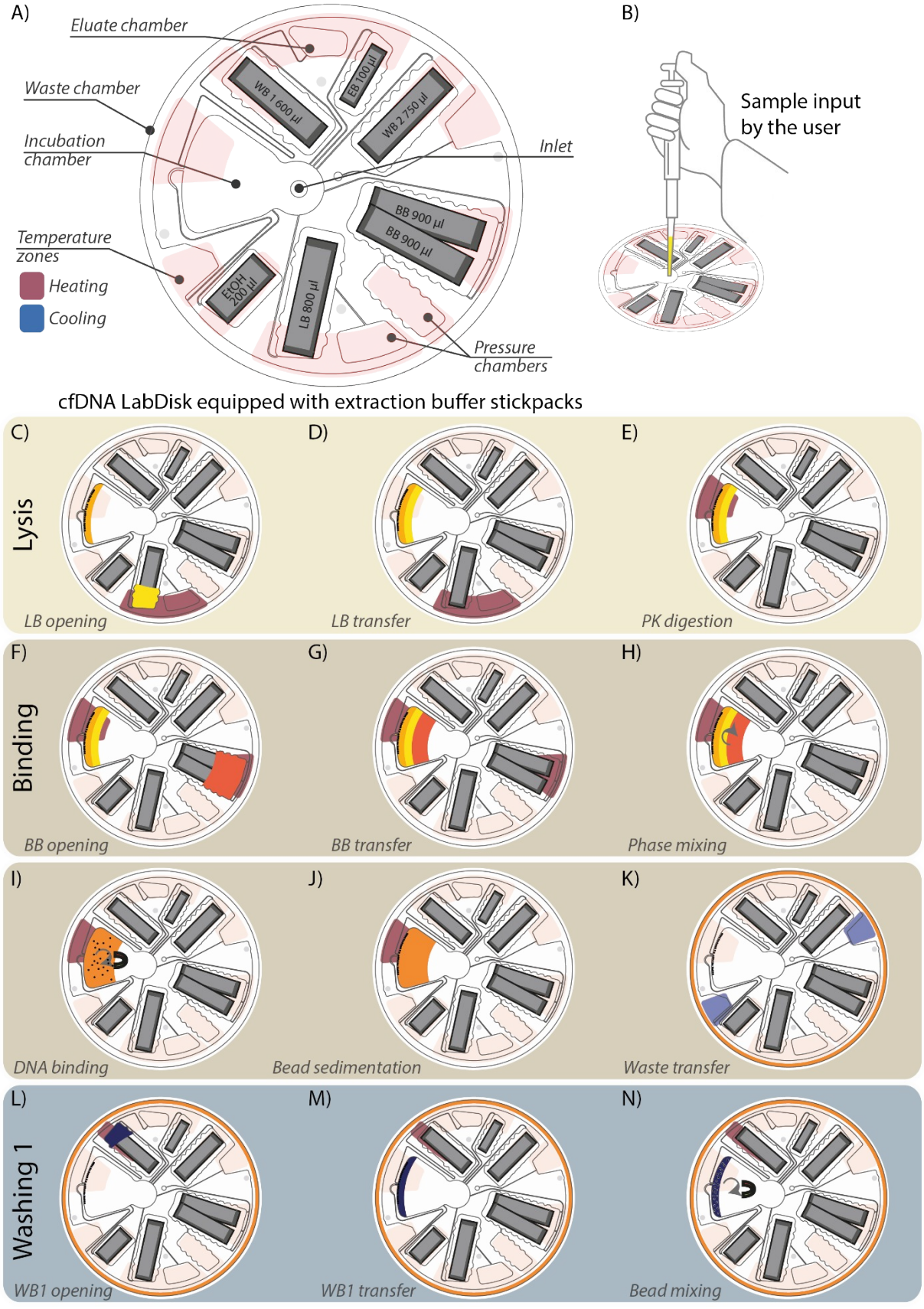


Figure is continued on the next page

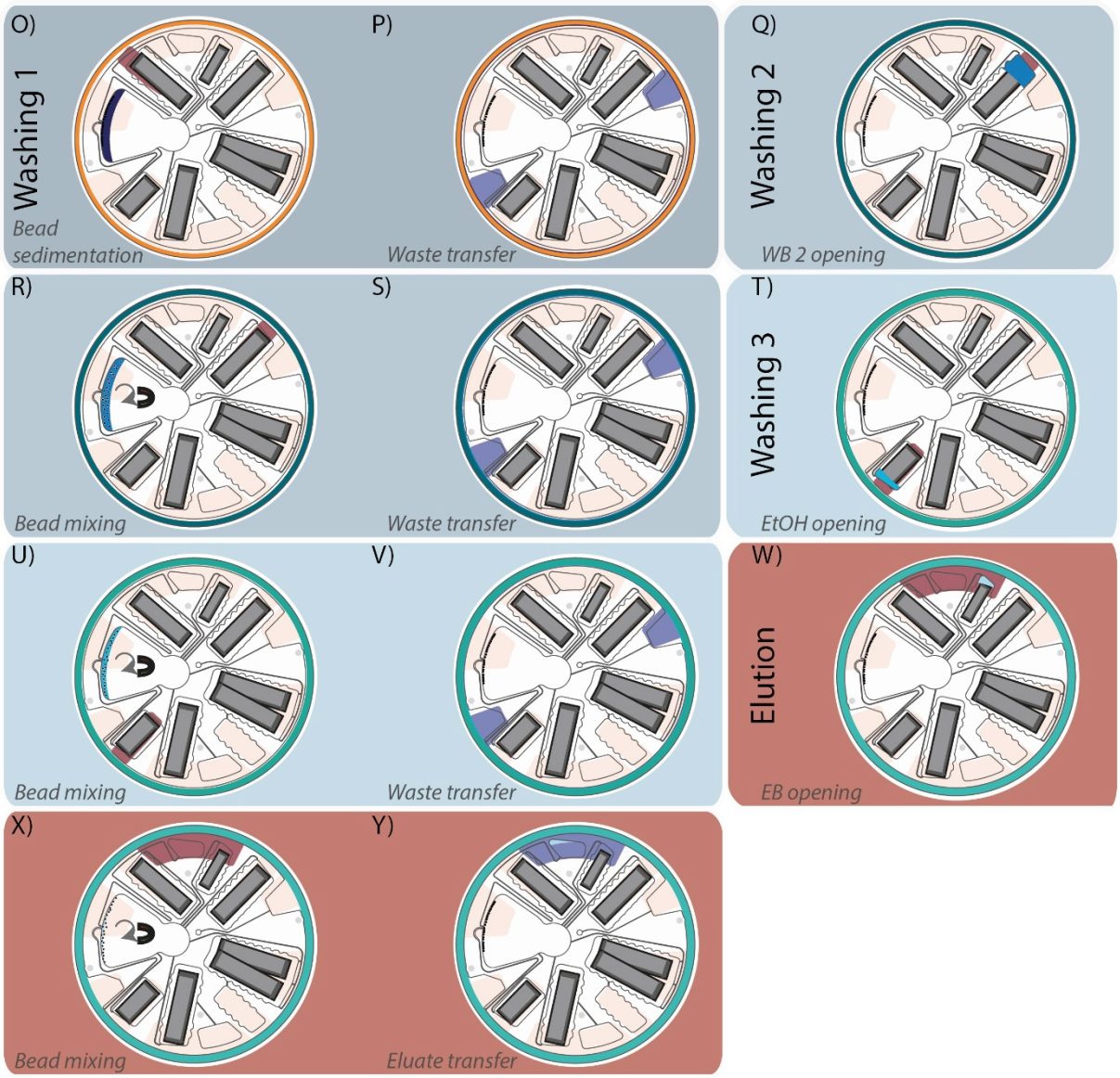


Figure S2 Microfluidic workflow for cfDNA extraction on an integrated LabDisk.

Evaluation of nanobead manipulation concept. To evaluate the three different designs for (Figure S3) retaining the nanobeads in the incubation chamber, three concepts were tested with spiking experiments. A synthetic DNA fragment of the tropical crawl frog (*Xenopus tropicalis*) with a length of 131 bp and a concentration of 10^4 DNA copies was spiked into 300 µl of plasma sample. Figure S4 shows that Design 3 had the best performance in respect to both the Ct-value (the lower, the higher the recovery rate). Moreover, Design 2 was prone to errors related to clogging of the channel by nanobeads (no SD could be calculated, because only one run out of 3 was successful). The higher Ct-values of Design 1 can be attributed to the 8 µl residual volume left in the chamber after transfer. This means that buffers are carried over into each subsequent step and the eluate is very likely contaminated by washing buffer. Consequently, Design 3 was chosen for the integration into the cfDNA extraction workflow on the LabDisk. Furthermore, we demonstrated that even beads with a diameter down to several hundred nanometres can be successfully retained in a single centrifugal microfluidic chamber while changing buffers. This is advantageous in comparison to microfluidic approaches where the beads need to be transferred from chamber to chamber to change buffers, as the space requirement on the LabDisk is significantly reduced.

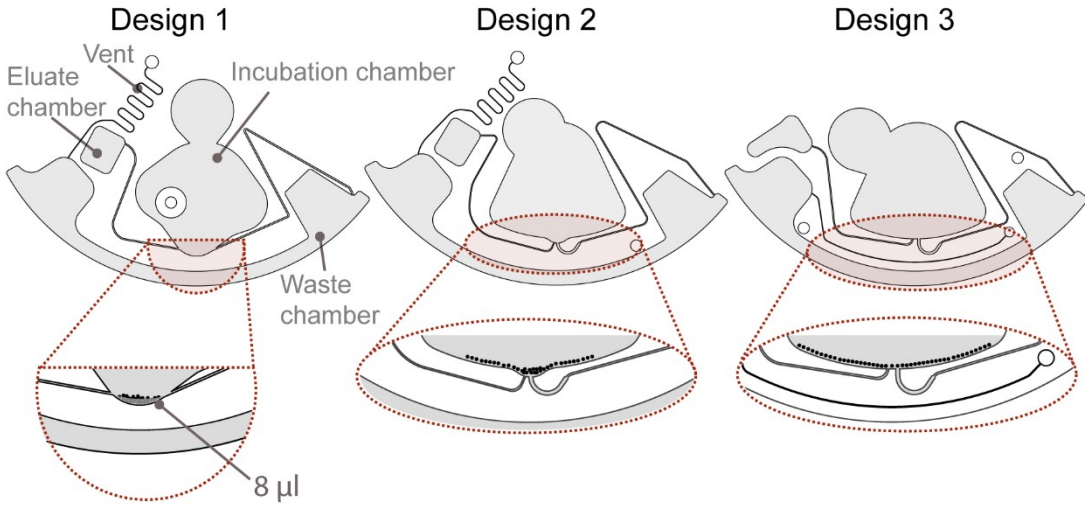


Figure S3 Evaluated concepts of a chamber geometry to retain nanobeads in the incubation chamber. Each design includes an incubation chamber, a waste chamber connected by a channel to a vent and an eluate collection chamber. Design 1: The transfer channels for both the eluate and waste transfers all but 8 µl of the liquid out of the chamber. The nanobeads are collected in this 8 µl cavity. Design 2: Here, the nanobeads are collected on the outer rim with a slope of 15° to efficiently transport the liquids out of the chamber. The chamber has an additional cavity in the centre of the rim to establish a more defined spot for elution. Design 3: Same principle as in Design 2, but without the additional cavity. For direct comparison, the three design variants were included on one single LabDisk and their volume reduced by a factor of three. Each incubation chamber is connected to a waste and to an eluate chamber. A resistance channel vents the waste. The sample and buffers are pipetted manually into the incubation chamber during the fluidic protocol, which makes it necessary to stop the processing device at the appropriate time points. In each of the three design variants, a different concept to retain the nanobeads is evaluated. Design 1 is characterized by a nanobead collection structure, which was designed in such a way that the downstream transfer channel is radially inward to a residual volume of 8 µl in the chamber. In this volume, the nanobeads can be sedimented. This guarantees liquid transfer from the chamber without affecting the sedimented nanobeads. Designs 2 and 3 collect the nanobeads on their radially outward rim (15° slope to guarantee the drainage of the liquid). Design 2 has an additional

cavity at the outlet to collect the nanobeads and potentially improve elution, which is carried out with comparably low liquid volumes (100 µl). Design 3 has no additional elements to collect the nanobeads.

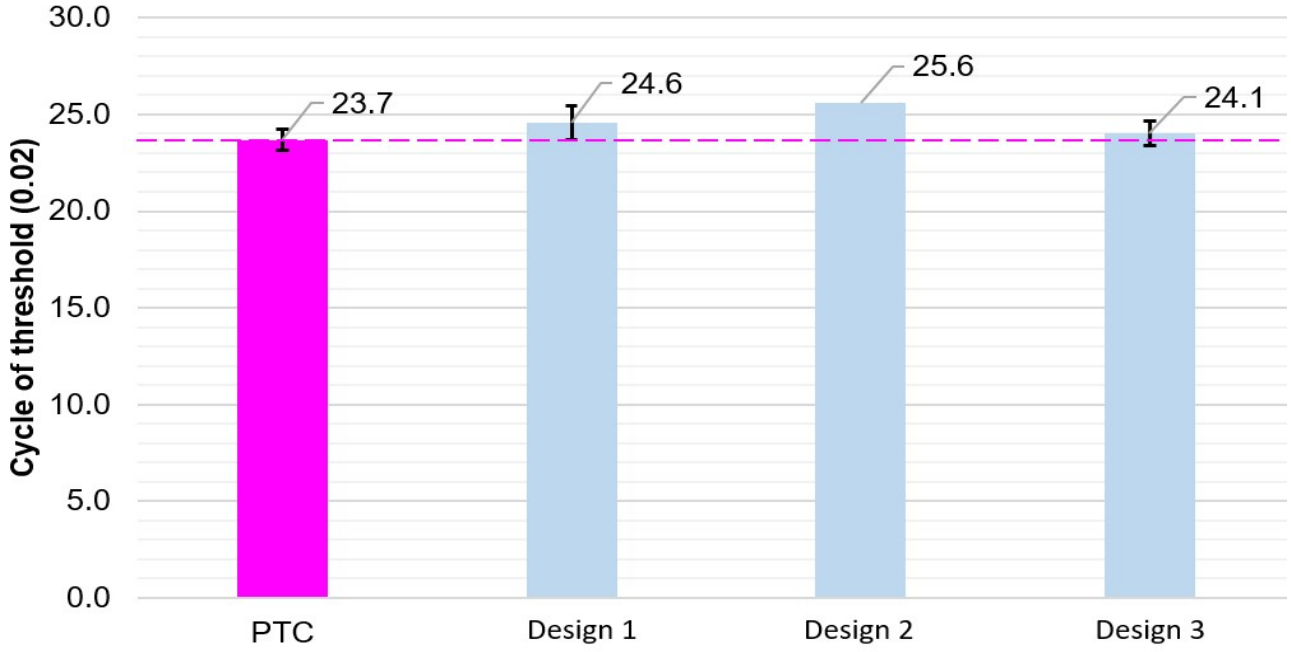


Figure S4 Evaluation of bead manipulation concept in three different incubation chambers (n=3). The DNA concentration in the PTC was defined to an extraction recovery of 100%. Design 1 showed a cycle of threshold (Ct) of 24.6 ± 0.9, Design 2 of 25.6 and Design 3 of 24.1 ± 0.6.

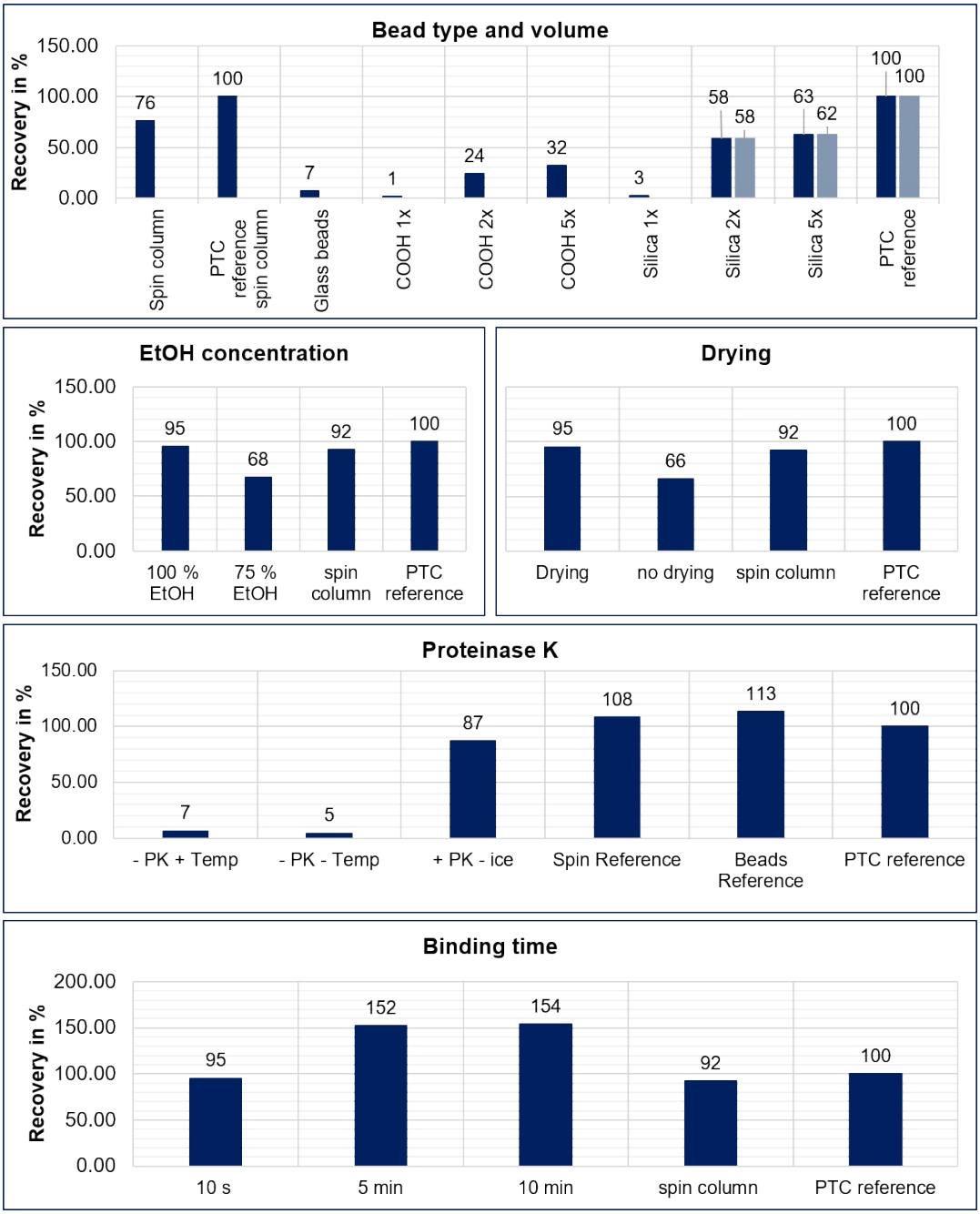
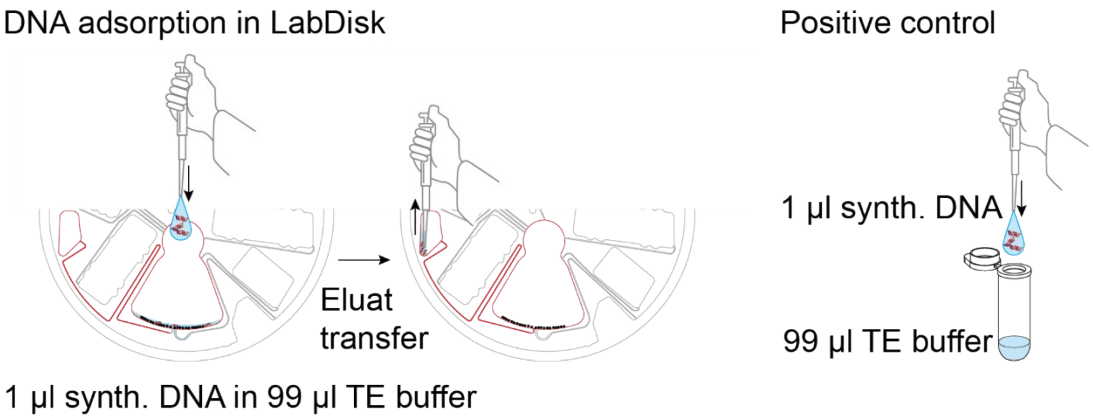


Figure S5 Optimization results for the bead-based approach in the manual workflow. The main impact for the optimization was found to be the EtOH concentration, bead drying and binding time. Moreover, modified beads with silica groups on the surface were the most suitable beads to establish the binding of the DNA to their surface with the buffer system used.



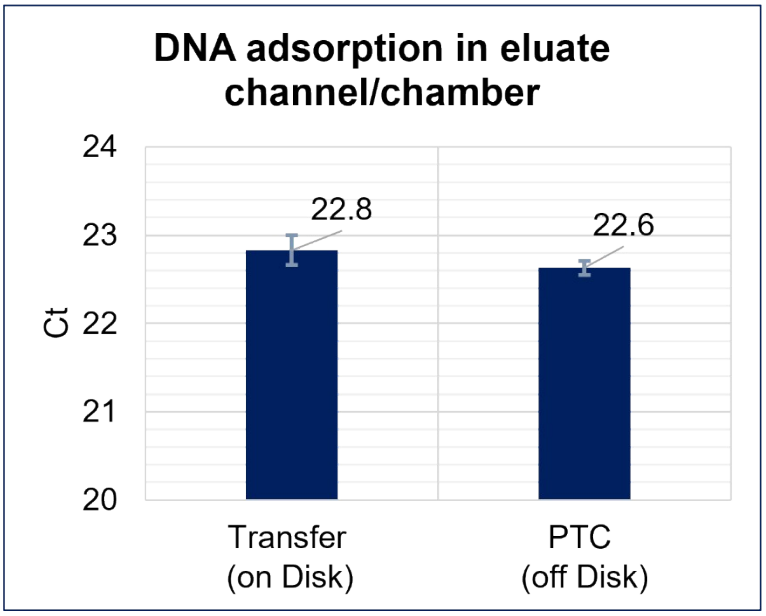


Figure S6 DNA adsorption in eluate channel and incubation chamber. Standard deviation on Disk: 0.3 and off Disk 0.2. DNA adsorption in LabDisk is found to be neglectable.

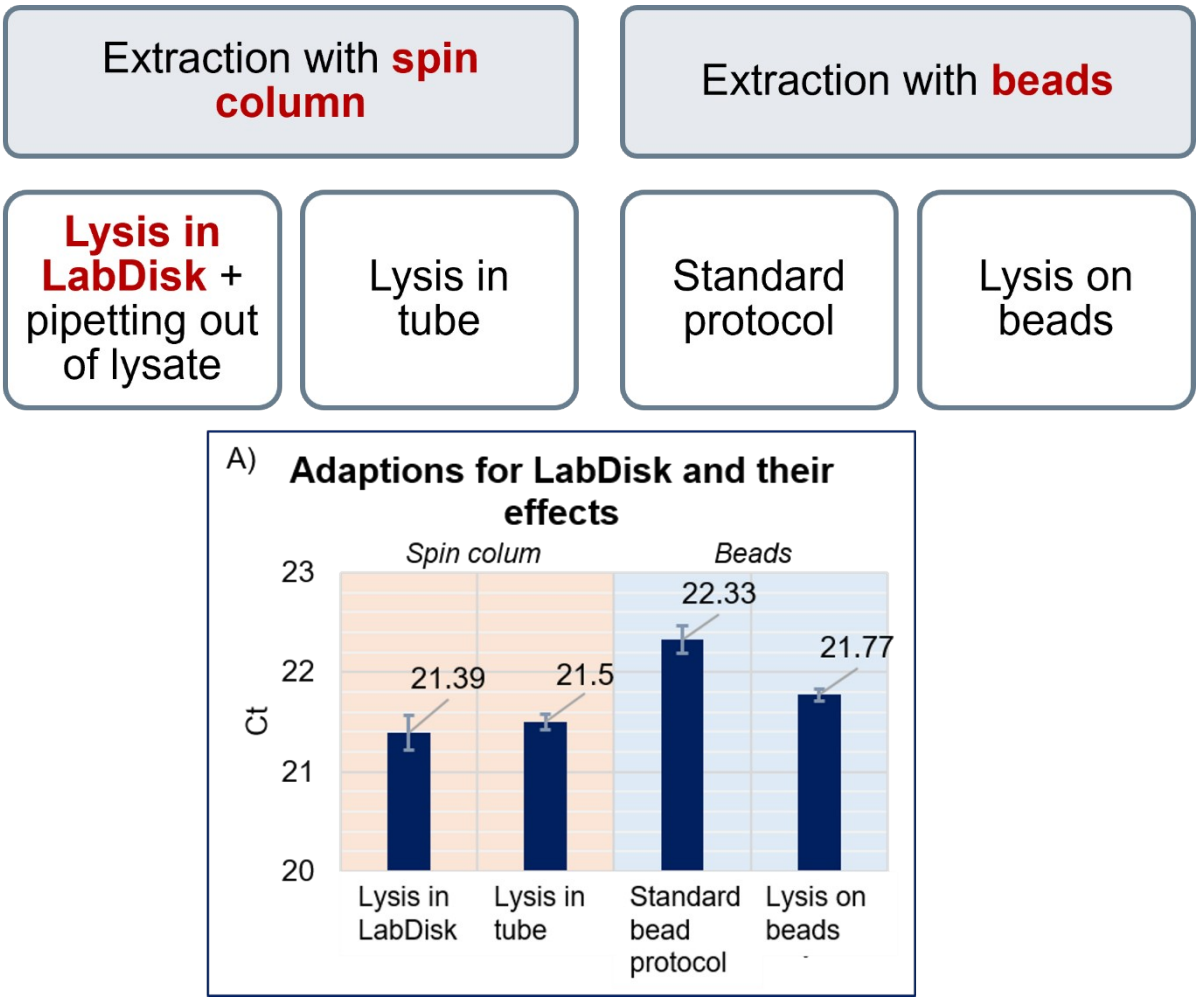


Figure S7 Influencing parameters for lysis efficiency (lysis in LabDisk and lysis with beads present). There is no negative effect for performing the lysis in the LabDisk and in presence of beads.

Table S7 Temperature and frequency for cfDNA LabDisk processing.

STEP	*DESCRIPTION*		*PARAMETER 1*	*PARAMETER 2*		*PARAMETER 3*		*PARAMETER 4*	
1	***OpMode***	Vaccum pump error when pressure > 670 mbar			3300				
2	***CoolingFan***	Cooling fan on							
3	***OpMode***	Define rotor height, here 270							
4		Temperature zone	64	Temperature	25°C	Time out	30s	Heating power	75%
5		Temperature zone	32	Temperature	80°C	Time out	30s	Heating power	75%
6	***LB stickpack opening***	Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
7		Wait	10 s						
8		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
9		Wait	30 s						
10		Temperature zone	14	Temperature	100°C	Time out	30s	Heating power	75%
11		Wait	60 s						
12	***LB transfer***	Loop start							
13		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
14		Temperature zone	14	Temperature	95°C	Time out	300	Heating power	75%
15		Wait	60s						
16		Frequency	3 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
17		Wait	60s						
18		Temperature zone	14	Temperature	25°C	Time out	30s	Heating power	75%
19		Wait	60s						
20		Loop end		# of loops	6				
21		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
22		Temperature zone	14	Temperature	95°C	Time out	30s	Heating power	75%
23		Wait	60s						

STEP	*Description*		*Parameter 1*	*Parameter 2*		*Parameter 3*		*Parameter 4*	
24		Frequency	3 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
25		Wait	60s						
26		Stop temperature zone	14						
27		Loop start	1						
28	***Mixing***	Frequency	30 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
29		Wait	6 s						
30		Frequency	3 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
31		Wait	6 s						
32		Loop end		# of loops	20				
33	***Lysis 30 min***	Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
34		Temperature zone	64	Temperature	60°C	Time out	60s	Heating power	75%
35		Loop start	1						
36		Wait	60s						
37		Loop end		# of loops	30				
38	***BB opening***	Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
39		Wait	30s						
40		Temperature zone	8193	Temperature	100°C	Time out	30s	Heating power	75%
41		Wait	60 s						
42	***BB transfer***	Loop start	1						
43		Frequency	30 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
44		Temperature zone	8193	Temperature	95°C	Time out	30s	Heating power	75%
45		Wait	60 s						
46		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
47		Wait	60 s						
48		Temperature zone	8193	Temperature	25°C	Time out	30s	Heating power	75%
49		Wait	60 s						
50		Loop end		# of loops	6				

51		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
52		Temperature zone	8193	Temperature	100°C	Time out	30s	Heating power	75%
STEP	*Description*		*Parameter 1*	*Parameter 2*		*Parameter 3*		*Parameter 4*	
53		Wait	60 s						
54		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
55		Wait	60 s						
56		Stop temperature zone	8193						
57	***Buffer mixing***	Loop start	1						
58		Frequency	30 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
59		Wait	6 s						
60		Frequency	3 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
61		Wait	6 s						
62		Loop end		# of loops	15				
63		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
64	***Cooling***	Loop start	1						
65		Temperature zone	64	Temperature	15°C	Time out	600	Heating power	75%
66		Wait	60s						
67		Loop end		# of loops	5				
68	***Binding 10 min***	Start timed loop	2						
69		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
70		Wait	5 s						
71		Frequency	10 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
72		Wait	5 s						
73		End timed loop	2	Duration	10 min				
74		Stop temperature zone	64						
75	***Magnet movement***	Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
76		Wait	20 s						
77	***Waste 1***	Frequency	30 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		

78		Wait	5 s						
79		Temperature zone	4128	Temperature	90°C	Time out	60 s	Heating power	75%
80		Wait	50s						
STEP	*Description*		*Parameter 1*	*Parameter 2*		*Parameter 3*		*Parameter 4*	
81		Frequency	3 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
82		Wait	10s						
83		Temperature zone	4128	Temperature	20°C	Time out	60 s	Heating power	75%
84		Wait	3 s						
85		Frequency	25 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
86		Loop start	1						
87		Wait	60 s						
88		Loop end		# of loops	5				
89		Stop temperature zone	4128						
90	***WB1 stickpack opening***	Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
91		Wait	30 s						
92		Temperature zone	2048	Temperature	100°C	Time out	30 s	Heating power	75%
93		Wait	60 s						
94	***WB1 transfer***	Loop start	1						
95		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
96		Temperature zone	2048	Temperature	95°C	Time out	30 s	Heating power	75%
97		Wait	20 s						
98		Frequency	10 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
99		Wait	20 s						
100		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
101		Wait	20 s						
102		Temperature zone	2048	Temperature	25°C	Time out	30 s	Heating power	75%
103		Wait	30 s						
104		Loop end		# of loops	2				

105		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
106		Temperature zone	2048	Temperature	95°C	Time out	30 s	Heating power	75%
107		Wait	10 s						
108		Frequency	10 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
STEP	*Description*		*Parameter 1*	*Parameter 2*		*Parameter 3*		*Parameter 4*	
109		Wait	20 s						
110		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
111		Wait	10 s						
112		Stop temperature zone	2048						
113	***Washing 1***	Loop start	1						
114		Frequency	8 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
115		Wait	5 s						
116		Frequency	15 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
117		Wait	5 s						
118		Loop end		# of loops	2				
119	***Magnet movement***	Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
120		Wait	20 s						
121	***Waste2***	Frequency	30 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
122		Wait	5 s						
123		Temperature zone	4128	Temperature	90°C	Time out	60 s	Heating power	75%
124		Wait	50 s						
125		Frequency	3 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
126		Wait	10 s						
127		Temperature zone	4128	Temperature	20°C	Time out	60 s	Heating power	75%
128		Wait	3 s						
129		Frequency	25 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
130		Wait	40 s						
131		Stop temperature zone	4128						

132	***WB2 stickpack opening***	Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
133		Wait	30 s						
134		Temperature zone	128	Temperature	100°C	Time out	30 s	Heating power	75%
135		Wait	60 s						
136	***WB2 transfer***	Loop start	1						
STEP	*Description*		*Parameter 1*	*Parameter 2*		*Parameter 3*		*Parameter 4*	
137		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
138		Temperature zone	128	Temperature	100°C	Time out	30 s	Heating power	75%
139		Wait	10 s						
140		Frequency	10 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
141		Wait	20 s						
142		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
143		Wait	20 s						
144		Temperature zone	128	Temperature	25°C	Time out	30 s	Heating power	75%
145		Wait	30 s						
146		Loop end		# of loops	2				
147		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
148		Temperature zone	128	Temperature	95°C	Time out	30 s	Heating power	75%
149		Wait	10 s						
150		Frequency	10 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
151		Wait	20 s						
152		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
153		Wait	10 s						
154		Stop temperature zone	128						
155	***WB2 washing***	Loop start	1						
156		Frequency	8 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
157		Wait	5 s						
158		Frequency	15 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		

159		Wait	5 s						
160		Loop end		# of loops	2				
161	***Magnet movement***	Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
162		Wait	20 S						
163	***Waste 3***	Frequency	30 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
164		Wait	5 s						
STEP	*Description*		*Parameter 1*	*Parameter 2*		*Parameter 3*		*Parameter 4*	
165		Temperature zone	4128	Temperature	90°C	Time out	60 s	Heating power	75%
166		Wait	50 s						
167		Frequency	3 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
168		Wait	10 s						
169		Temperature zone	4128	Temperature	20°C	Time out	60 s	Heating power	75%
170		Wait	3 s						
171		Frequency	25 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
172		Wait	40 s						
173		Stop temperature zone	4128						
174	***EtOH stickpack opening***	Frequency	40 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
175		Wait	30 s						
176		Temperature zone	16	Temperature	110°C	Time out	30 s	Heating power	75%
177		Wait	60 s						
178	***EtOH transfer***	Frequency	30 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
179		Temperature zone	16	Temperature	95°C	Time out	30 s	Heating power	75%
180		Wait	10 s						
181		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
182		Wait	30 s						
183		Temperature zone	16	Temperature	25°C	Time out	30 s	Heating power	75%
184		Wait	30 s						
185		Frequency	35 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		

186		Temperature zone	16	Temperature	95°C	Time out	30 s	Heating power	75%
187		Wait	20 s						
188		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
189		Wait	30 s						
190		Stop temperature zone	16						
191	***EtOH washing***	Loop start	1						
192		Frequency	8 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
STEP	*Description*		*Parameter 1*	*Parameter 2*		*Parameter 3*		*Parameter 4*	
193		Wait	5 s						
194		Frequency	15 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
195		Wait	5 s						
196		Loop end		# of loops	2				
197	***Magnet movement***	Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
198		Wait	20 s						
199	***Waste4***	Frequency	30 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
200		Wait	5 s						
201		Temperature zone	4128	Temperature	90°C	Time out	60 s	Heating power	75%
202		Wait	50 s						
203		Frequency	3 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
204		Wait	10 s						
205		Temperature zone	4128	Temperature	20°C	Time out	60 s	Heating power	75%
206		Wait	3 s						
207		Frequency	25 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
208		Wait	40 s						
209		Stop temperature zone	4128						
210		Frequency	5 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
211	***EtOH evaporation***	Loop start	1						
212	***10 min***	Temperature zone	64	Temperature	56°C	Time out	60 s	Heating power	75%

213		Wait	60 s						
214		Loop end		# of loops	10				
215		Stop temperature zone	64						
216	***EB stickpack opening and transfer***	Loop start	1						
217		Frequency	40 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
218		Wait	30 s						
219		Temperature zone	1792	Temperature	100°C	Time out	30 s	Heating power	75%
220		Wait	60 s						
STEP	*Description*		*Parameter 1*	*Parameter 2*		*Parameter 3*		*Parameter 4*	
221		Wait	60 s						
222		Frequency	5.5 Hz	Acceleration	10 Hz/s	Deceleration	10 Hz/s		
223		Wait	30 s						
224		Frequency	15 Hz	Acceleration	10 Hz/s	Deceleration	10 Hz/s		
225		Wait	10 s						
226		Temperature zone	1792	Temperature	40°C	Time out	30 s	Heating power	75%
227		Wait	60 s						
228		Loop end		# of loops	4				
229	***Elution***	Start timed loop	2						
230		Frequency	8 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
231		Wait	5 s						
232		Frequency	20 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
233		Wait	5 s						
234		End timed loop	2	Duration	4 min				
235	***Magnet movement***	Wait	20s						
236	***Eluate transfer***	Frequency	15 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
237		Temperature zone	1792	Temperature	40°C	Time out	30 s	Heating power	75%
238		Wait	60 s						
239		Loop start	1						

240		Frequency	15 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
241		Temperature zone	1792	Temperature	100°C	Time out	30 s	Heating power	75%
242		Wait	60 s						
243		Temperature zone	1792	Temperature	20°C	Time out	30 s	Heating power	75%
244		Frequency	8 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
245		Wait	10 s						
246		Frequency	6 Hz	Acceleration	5 Hz/s	Deceleration	5 Hz/s		
247		Wait	30 s						
248		Loop end		# of loops	2				