Electronic Supplementary Material (ESI) for Lab on a Chip

Electronic Supplementary Information - ESI

Centrifugal gas-phase transition magnetophoresis GTM – a generic method for automation of magnetic bead based assays on the centrifugal microfluidic platform and application to DNA purification

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The supporting material includes:

(1) Fabrication of magnetic bead pellets

(2) Set-Up of LabDisk and Magnet on the processing device

(3) Composition of PCR reactions, Sequences of the used primers and probes

(4) Statistical PROBIT analysis of recovered DNA

(5) Single experiment: Yield of recovered lambda phage DNA

(6) DNA purification efficiency using bead pellets

(7) Movie File showing centrifugal gas-phase transition magnetophoresis according to Figure 1

1) Fabrication of magnetic bead pellets

Silica coated magnetic beads were used as a mobile solid phase for DNA binding and transport. For the manufacturing of the pellets, a suspension of 10 µL magnetic beads and 40 µL of a trehalose-water solution (D(+)-Trehalose Dehydrate, Carl Roth, Germany; concentration 50 mg/mL) was prepared. Beads and trehalose solution were mixed thoroughly, pipetted onto a scale pan and left for overnight drying. After evaporation of the water, pellets of beads remain where trehalose acts as a mechanical stabilizer. However, the bead pellets resuspend quickly when brought in contact with DNA extraction reagents.

2) Set-Up of LabDisk and Magnet on the processing device

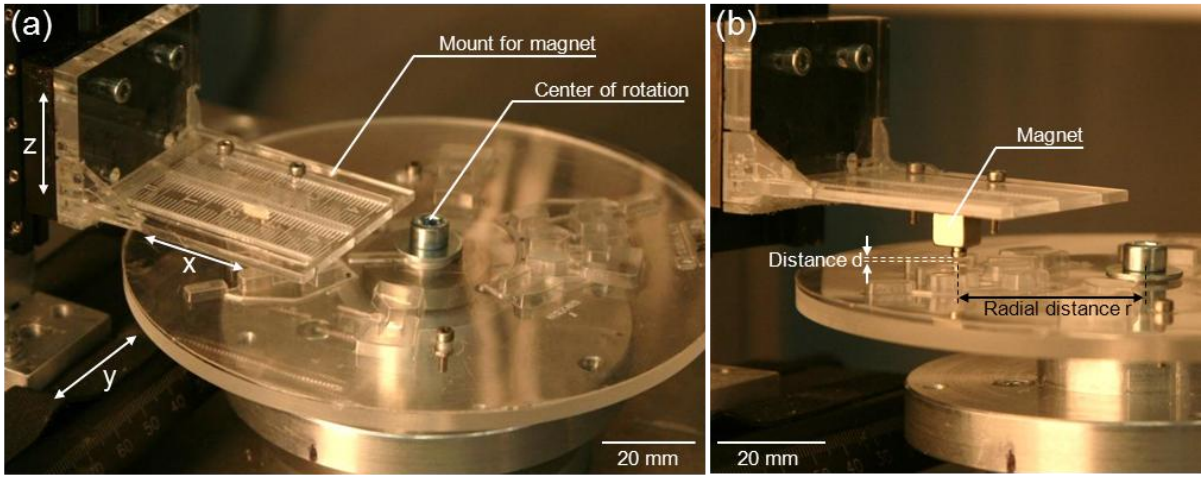

Fig. S1: Testrig for evaluation of GTM. Key elements are a microfluidic LabDisk and a disk spinning device equipped with a stack of permanet magnets. (a) LabDisk, fixed to rotor of spinning device. (b) The magnet is positioned at a distance *d* of about 0.5 mm above the microfluidic chambers and at a distance *r* of 36 mm to the centre of rotation.

3) Composition of PCR reaction and sequences of primers and probes

A single 10 µL PCR reaction was composed of a 2x concentrated ready to use PCR reaction mix (Roche Light Cycler 480 Probes Master, Roche Applied Sciences, Germany), forward and reverse primer, specific TaqMan probe, PCR grade water and 1 µL of DNA elute (Table S1).

Table S1: Composition of a single 10 µL PCR reaction

Volume [µL]	Reagent
5	PCR ready-to-use reaction mix
0.3	Forward Primer 10µM
0.2	Probe 10 µM
0.3	Reverse Primer 10µM
3.2	PCR grade water
1	Eluate containing the DNA

For quantification of *L. innocua* DNA via real time PCR, a 200 base pair fragment of the lin02483 gene was amplified using forward primer 5'-AAC CGG GCC GCT TAT GA-3', reverse primer 5'-CGA ACG CAA TTG GTC ACG-3' and probe 5'-FAM-TTC GAA TTG CTA GCG GCA CAC CAG T-DDQ1-3' (Rodriguez-Lazaro et al., *AEM*, 2004). Quantification of lambda phage DNA was conducted by amplification of a 95 base pair fragment using forward primer 5'- GGG ATC ATT GGG TAC TGT GG-3', reverse primer 5'- CAG ACT TGG GGG TGA TGA GT-3' and probe 5'-Cy5- TGT AAA AAC ACC TGA CCG CTA TCC CTG-BHQ2-3' (all ordered from Biomers, Germany). Thermocycling started with an initial 7 min hot start at 95°C followed by 50 cycles of 95°C / 15 seconds denaturation and 60°C / 30 seconds hybridization and extension.

4) PROBIT Analysis

Initially, the *L. innocua* DNA concentration was determined that can be amplified with 95% confidence. Therefore, a dilution series from *L. innocua* DNA according to Table S2 was prepared. Subsequently, PCR replicate testing was conducted for each dilution and the proportion of positive PCR's compared to the number of replicates was calculated. Based on these results, the mean DNA concentration that can be amplified with 95% confidence was calculated to 58.5 DNA copies μL^{-1}. If the maximum possible DNA concentration in the eluate is expected to be above 58.5 DNA copies μL^{-1} and all PCR reactions show positive amplification results, real-time PCR was used for DNA quantification. If the maximum possible DNA concentration is expected to be below 58.5 copies or if single PCR reactions fail in being amplified, quantification was done by comparing the proportion of positive PCR reactions to the PROBIT regression derived from the dilution series (Fig. S3).

Table S2: Number of PCR replicates, number of positive PCR reactions and ratio of positive PCR reaction versus number of replicates for each dilution.

# DNA copies [cop/µL]	# Replicates	# Positives	Probability [%]
400	7	7	100
120	7	7	100
40	7	5	71.4
12	7	2	28.6
4	10	2	20.0
1.2	10	1	10.0
0.4	15	0	0

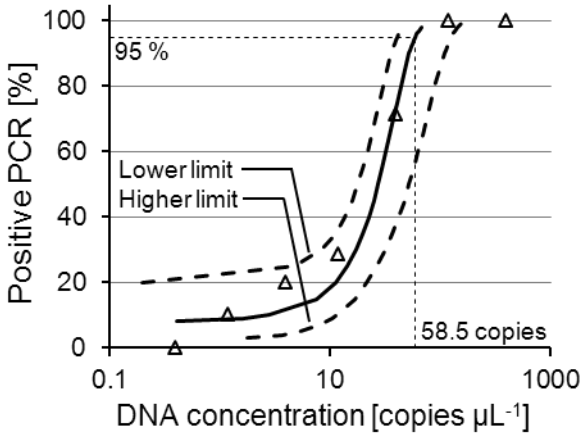

Fig. S2: Probability for a positive PCR reaction as function of DNA concentration. Triangles depict proportion of positive PCR reactions calculated from Table S 2. Black line represents PROBIT regression fit with higher and lower 95% confidence limit (dashed line). DNA concentrations of 58.5 copies can be amplified with 95% confidence.

5) Single experiment: Yield of recovered lambda phage DNA

Table S3: Number of recovered lambda phage DNA copies for LabDisk and reference purification quantified by real-time PCR. Each sample contained 17.5 • 10^3 DNA copies (theoretical maximum). Yield of LabDisk and reference purification are compared to the theoretical maximum.

LabDisk No.	Inserted DNA	On-LabDisk		Manual reference	
		Mean	Ratio LabDisk / inserted	Mean	Ratio manual reference / inserted
	[copies]	[copies]	[%]	[copies]	[%]
1	1.75×10^4	6.1×10^3	35	11.4×10^3	65
2	1.75×10^4	6.3×10^3	36	5.60×10^3	32
3	1.75×10^4	6.9×10^3	39	7.10×10^3	41
4	1.75×10^4	7.9×10^3	45	10.5×10^3	60
5	1.75×10^4	4.0×10^3	23	11.7×10^3	67
6	1.75×10^4	5.5×10^3	32	10.8×10^3	62
7	1.75×10^4	4.4×10^3	25	12.2×10^3	70
8	1.75×10^4	6.2×10^3	35	10.7×10^3	61
9	1.75×10^4	6.5×10^3	37	14.7×10^3	84
10	1.75×10^4	3.9×10^3	22	9.26×10^3	53
11	1.75×10^4	5.4×10^3	31	10.0×10^3	57
12	1.75×10^4	5.4×10^3	31	17.0×10^3	97
13	1.75×10^4	1.8×10^3	10	13.9×10^3	79
14	1.75×10^4	6.9×10^3	40	14.5×10^3	83
15	1.75×10^4	7.1×10^3	40	18.8×10^3	107
16	1.75×10^4	2.4×10^3	14	19.9×10^3	114
17	1.75×10^4	5.2×10^3	29	14.2×10^3	81
18	1.75×10^4	6.9×10^3	40	14.6×10^3	83
Mean		5.5×10^3	31	12.6×10^3	72
Std. Dev.		1.7×10^3	10	3.74×10^3	21

6) DNA purification efficiency using bead pellets

Since the magnetic beads used for DNA purification are pelleted for pre-storage, we evaluated, whether pelleting has an effect on the yield of DNA recovery after purification. Insufficient resuspension or agglutination of the bead pellet would result in less available surface for DNA binding. Therefore, DNA purifications from ten aliquots of *L. innocua* lysate ($8.5 \bullet 10^6$ lysed CFU / 200 µL) were conducted by the reference purification method (see method section). In the first case, dried magnetic bead pellets were added to five samples while in the second case the equivalent (10 µL) of standard bead suspension was added to another five samples. The purification with bead pellets and with bead suspension recovered 41% ± 20% (mean ± std. dev.) and 44% ± 17%, respectively, compared to the viable count of the *L. innocua* lysate (Fig. S3). Thus, pelleting of beads does not have a significant negative influence on the yield of recovered DNA when purification was conducted according to the reference protocol, in particular using a vortexer.

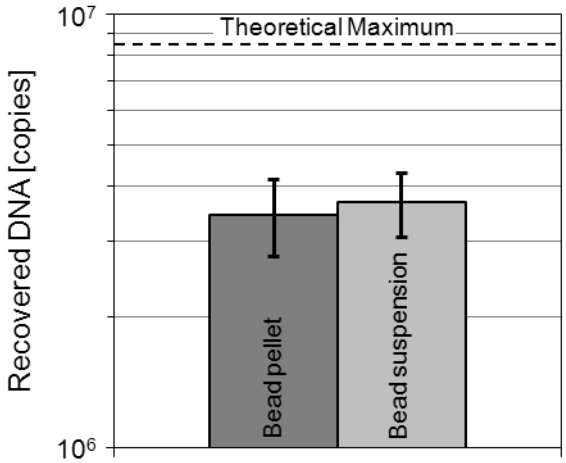

Fig. S3: Comparison of DNA recovery by the reference method performed either with dried magnetic bead pellets or with a magnetic bead suspension. Recovery determined by real-time PCR of the recovered DNA versus viable count of *L. innocua* lysate (theoretical maximum).