

Supplementary information

A lab-on-a-chip system integrating DNA purification and
loop-mediated isothermal amplification for the
quantification of toxic diatom *Pseudo-nitzschia*
multistriata

Authors: Ahmed I. Alrefaey, Jonathan S. McQuillan, Allison Schaap, Fabrizio Siracusa,
Christopher L. Cardwell, John Walk, Daniel Rogers, Reuben Forrester, Matthew C. Mowlem
and Julie C. Robidart

Correspondence: Ocean Technology and Engineering, The National Oceanography Centre, European Way,
Southampton, UK, SO14 3ZH. Email: j.robidart@noc.ac.uk, Telephone: +443001312594

26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57

Table of Contents

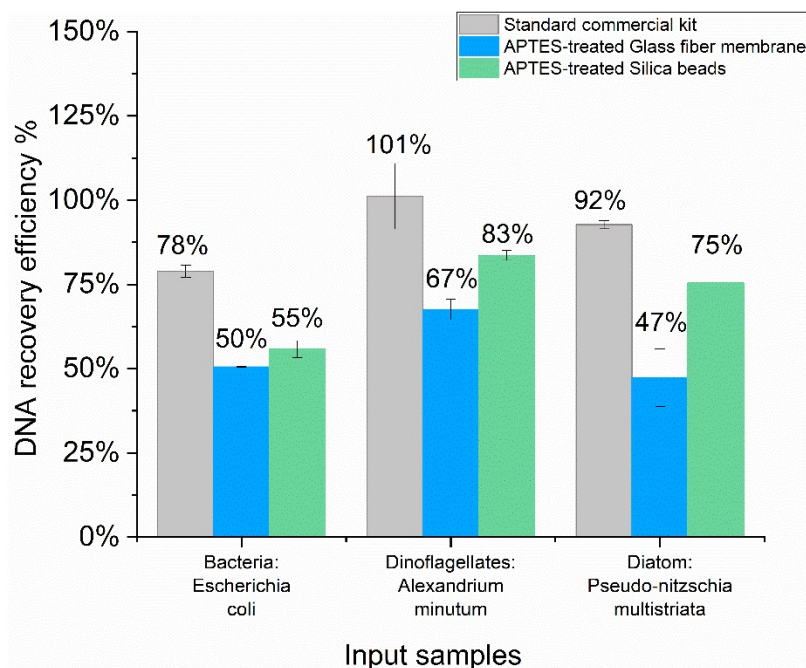
Part I: DNA Extraction Methods and Performance	3
S1. Quality verification of DMA-based extraction techniques	3
S2. Optimisation of the DNA extraction module	5
S3. Oxygen plasma treatment of silica beads	6
S4. Comparison of extraction reagent costs	7
Part II: LAMP Assay Development and Quantification	8
S5. Development of LAMP isothermal assay	8
S6. Quantification kinetics and standard curve generation	14
S7. Reproducibility of LAMPTRON modules	16
Part III: Microfluidic Hardware and Electronics	17
S8. Optimisation of the microfluidic design	17
S9. Autonomous pumping system	19
S10. LAMPTRON prototype	21
Part IV: Notes and references	21

Part I: DNA Extraction Methods and Performance

S1. Quality verification of DMA-based extraction techniques

The DMA extraction protocol was evaluated for the extraction of DNA from a variety of sources, including Eukaryotic cells (*Pseudo-nitzschia* diatom spp), Bacteria (*Escherichia coli*) and Dinoflagellates (*Alexandrium minutum*). The *Pseudo-nitzschia* cells were grown as previously described in section 2.1 of the main article. *A. minutum* CCAP 1119/15 was grown in L1 medium. ¹ *A. minutum* cells were enumerated and grown at the same incubation conditions as *Pseudo-nitzschia*, as detailed in section 2.1 of the main article. The bacterial strain used in this study was *Escherichia coli* NCTC9001 type strain (*E. coli*) that was obtained from the UK National Collection of Type Cultures (NCTC). *E. coli* cells were counted as described. ^{2, 3} Cells were harvested by centrifugation at 7,100 rpm for 5 minutes at 4°C and stored at -80°C until further processing, with the same protocol applied to samples for both the kit and DMA extractions to ensure any cell lysis effects were equivalent. Each replicate cell pellet was processed into the microfluidic chip to perform the same DMA extraction protocol as described in section 2.4.1 of the main article. DNeasy Mini Kit (Qiagen), DNeasy Plant Pro Kit (Qiagen), and GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) were used as standard methods to extract DNA from *P. multistriata*, *A. minutum*, and *E. coli* cells, respectively. The DNA recovery efficiencies from the commercial kits were averaged for comparison with the DMA-based techniques. For a bead-free control, glass fiber membranes (GF/F grade, Whatman™) were laser-cut to match the extraction-chamber layout using a CO₂-based cutter (Speedy 100, Trotec Laser GmbH, UK) and subsequently treated with ozone and APTES (as described in Section 2.3.2, the main text). We evaluate the extraction capabilities of two different silica substrates, incorporating beads and glass fibre membrane, against the

commercial extraction kits. The absolute extraction efficiency was calculated using the formula: Extraction Efficiency (%) = $(C \times V / \text{DNA input}) \times 100$, where C is the concentration of DNA in the extract (ng/L), V is the total volume in μL , and DNA input refers to starting DNA based on cell count and known DNA mass per cell.^{4, 5}



87

Supplementary Figure S1. Performance assessment of DMA-based method for DNA extraction from various samples. The DNA capture efficiency was evaluated by comparing the proposed DMA microfluidic method using silica beads and glass fibre paper with the reference extraction by widely used kits processing the same volume of sample.

The silica beads delivered higher DNA recovery compared to the membrane, irrespective of the sample type (Figure S1). For example, the recovery using silica beads was 55%±2.51 versus 50%±0.13 for *E. coli*, 83%±1.47 versus 67%±3.05 for *A. minutum*, and 75%±3.86 versus 47%±8.61 for *P. multistriata*. The capture of *P. multistriata* DNA on silica beads was approximately 75%±3.86, which is lower than recovery from the DNeasy Mini Kit (Qiagen) (92%±1.22). For the Dinoflagellate *A. minutum*, DNA recovery was 83%±1.47 using silica beads, lower than the DNA recovery of DNeasy Plant Pro Kit (Qiagen) (101%±9.71). Recovered DNA concentrations from *E. coli* were 55%±2.51 using silica beads vs. 78%±1.77 recovery

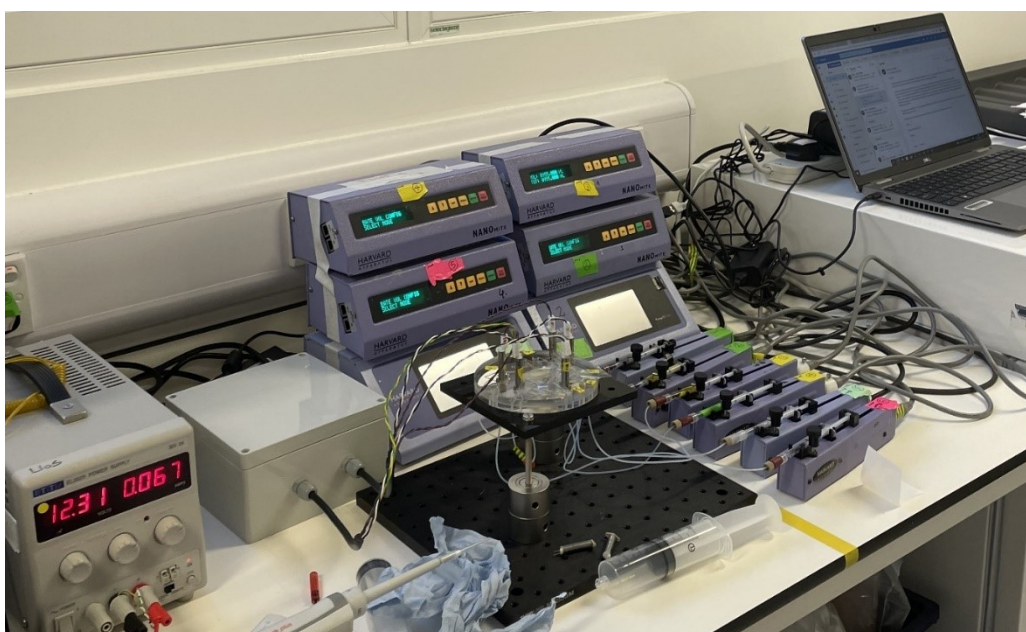
100 using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich). DNA extracts of *P. multistriata*
101 showed purity ratios (260/280) below the optimal value of approximately 1.8, as illustrated
102 in Figure 3A (main text). These purity values correspond with Qubit measurements of $7.38 \pm$
103 0.14 and 5.12 ± 0.33 ng/ μ L, indicating low DNA levels rather than contamination. When
104 extracting serial dilutions of *P. multistriata* from 10 to 10^5 cells/mL on-chip, low-purity DNA
105 samples did not inhibit LAMP amplification in independent experiments. The LAMP time-to-
106 threshold (Tt) was consistent, showing that sensitivity is unaffected by residual contaminants
107 in DNA elutes, as shown in Supplementary Figure S5.

108 **S2. Optimisation of the DNA extraction module.**

109

110 The optimisation of the DNA extraction module in LAMPTRON is described in Section 2.4.1 of
111 the main text, where reagent volumes and flow rates were optimised using fluid control via
112 solenoid valves (LFNA1250325H, The Lee Company, Connecticut, U.S.A.) and programmable
113 syringe pump (Harvard Apparatus, Holliston, United States).

114



115

Supplementary Figure S2. Optimisation of the DNA extraction module in LAMPTRON was conducted using a new transparent PMMA-built chip. Parameters such as reagent volumes, flow rates, and diffusion times were optimised within this experimental setup. Solenoid-operated valves were fixed on the test chip, which was connected to syringe pumps for controlled injection of samples and reagents, controlled via a Windows-based software.

S3. Oxygen plasma treatment of silica beads

The oxygen plasma treatment process used to activate the silica bead surfaces is described in Section 2.3.2 of the main text, and was carried out prior to packing the beads into the extraction microchamber of the microfluidic chip.



Supplementary Figure S3. Silica beads underwent oxygen plasma treatment to activate the surface silanol groups, employing a plasma activation system (Diener Electronic, Ebhausen, Germany). The effectiveness of the plasma treatment was assessed by observing the behaviour of water droplets on the beads. Resistance to wetting by beads indicated successful plasma treatment ⁶.

S4. Comparison of extraction reagent costs

147 This section presents a cost comparison of extraction reagents, demonstrating the economic
 148 advantage of the LAMPTRON method over conventional commercial kits.

149

150 **Supplementary Table S1.** Cost comparison for reagents between LAMPTRON extraction and
 151 Qiagen commercial methods.

152

153

Items Description	Component	Cost (£), inc. VAT per sample	Manufacturer	Product Number
Current extraction DMA-based method:	2% APES (aminopropyl triethoxysilane)	0.0061	Sigma-Aldrich	741442-100ML
	95% Ethanol	0.0045	Sigma-Aldrich	652261
	0.1 M Tris-HCl (pH 8.0)	0.0023	SERVA	39792.01
	10 mM EDTA	0.0085	Invitrogen	15575-038
	1% sodium dodecyl sulfate (SDS)	0.0054	Sigma-Aldrich	L4390-100G
	10% Triton X-100	0.0033	Sigma-Aldrich	93443-500ML
	0.1 mg/mL Proteinase K	0.000014	Qiagen	56304
	100 mg/mL Dimethyl adipimidate (DMA)	0.069	Alfa Aesar	L10515.09
	0.01 M Phosphate-buffered saline (PBS)	0.00035	Sigma-Aldrich	P4417-100TAB
	0.1 M Trisodium citrate		Alfa Aesar	45558
	10 mM sodium bicarbonate	0.00078	Sigma-Aldrich	S7277-250G
	Glass beads	0.0082	Sigma-Aldrich	G1152-100G
	DEPC-treated water	0.0054	Thermo Fischer	R0601
	Total cost per sample	£0.11		
Qiagen extraction method	DNeasy Plant Mini kit	4.18	Qiagen	69104
Total cost per sample	£4.34			

154

155

156

Part II: LAMP Assay Development and Quantification

S5. Development of LAMP isothermal assay

The gene of interest is a cytochrome P450 homologous to *dabD*, which is implicated in domoic acid (DA) biosynthesis in *Pseudo-nitzschia spp.*⁷⁻⁹ Cytochrome P450 enzymes are a superfamily of enzymes that are involved in the oxidative metabolism of a wide range of compounds, including drugs, xenobiotics, and endogenous substances such as fatty acids and steroids.¹⁰ Importantly, its role in DA biosynthesis was confirmed through transcriptomic^{11, 12} and sequence analysis of the cytochrome P450 *dabD* gene in toxin-producing *Pseudo-nitzschia spp*⁸, as well as functional assays indicating that cytochrome P450 DabD catalyses a key oxidative step in the DA biosynthetic pathway.^{7, 9}

BLAST analysis was performed using the published *dabD* gene sequence⁷ from *Pseudo-nitzschia multistriata* as the query. The sequence was searched against publicly available nucleotide (nt) database of the National Centre for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>) (accessed March 14, 2022) to identify homologous *dabD* sequences suitable for primer design.¹³ The search was performed using BLASTn with the following parameters: E-value cutoff = 0.05-0.1, match/mismatch scores = 2-3, word size = 7-28, gap existence cost = 5, extension cost = 2, and a threshold of 1-100 hits per query. This target sequence was then searched across available *P. multistriata* genomes in public databases such as the SZN institute database (<https://bioinfo.szn.it/>), the Earlham Institute's database (<http://apollo.tgac.ac.uk/>),^{14, 15} and the Ensemble database (<https://protists.ensembl.org/>) (accessed March 14, 2022). In total, 8 BLAST hits with ≥ 80% query coverage were retrieved when querying the *P. multistriata* genome and transcriptome databases using the *dabD* gene as input. These sequences were subsequently aligned using

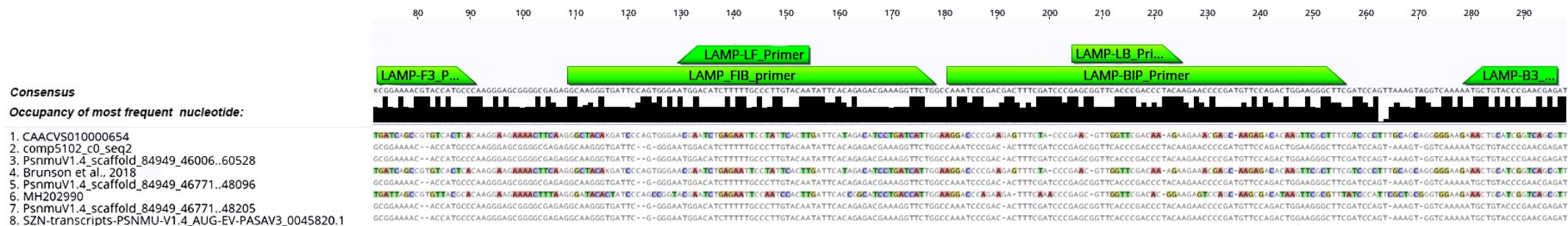
181 the ClustalW multiple sequence alignment tool (version 2.1) to identify conserved regions
182 suitable for oligonucleotide binding.¹⁶ To validate the accuracy of amplifying the target
183 sequence, additional verification measures were taken as follows: the consensus sequence
184 was subjected to comparison with the reference protein sequences available in the GenBank
185 database using BlastX.¹⁷ Furthermore, protein domains were analysed to examine the
186 translated protein sequence of the target conserved region.¹⁸ The bioinformatics analyses
187 were conducted using either the NCBI website's toolkit or Geneious Prime® v2019.2.3
188 software (Biomatters Ltd, Auckland, NZ).

189 The LAMP oligonucleotides flanked a consensus region of a total of 230 base pairs with a
190 pairwise identity of 76% (Figure S4). Table S1 contains the ID numbers and functional
191 annotations corresponding to the *Pseudo-nitzschia spp.* sequences that were employed to
192 determine the consensus target sequence. The consensus sequence obtained from the
193 alignment analysis was employed for the design of the LAMP assay. The designed
194 oligonucleotides share approximately 77.4% identity with all *P. multistriata* cytochrome P450
195 sequences (Figure S4). Among these, only two cytochrome P450 isoforms have been
196 confirmed in domoic acid biosynthesis: the *dabD* sequence from *P. multistriata*⁷, which
197 shares an average of 88% identity with our designed LAMP primers, and the MH202990
198 sequence from *P. multiseri* (NCBI), which shares 79% identity (Figure S4; Table S2). When
199 comparing these two confirmed isoforms (*CYP450 (dabD)* sequences of *Pseudo-nitzschia*
200 *multiseri* isolate 15091C3 (NCBI Gene ID: MH202990) with those of *Pseudo-nitzschia*
201 *multistriata*⁷, pairwise identity was 83.1%. Therefore, both sequences from *P. multistriata*
202 and *P. multiseri* were included in the alignment to generate a consensus sequence.

203 LAMP primers (Table 1) were designed to target six distinct regions of the consensus sequence
204 (Figure S4). Percentage identities for the LAMP primer binding sites were 77.9% for LAMP-

205 F3, 77.4% for LAMP-B3, 73% for LAMP-FIP, 77.8% for LAMP-BIP, 74.3% for LAMP-LF, and
206 81.3% for LAMP-LB (Figure S4). To evaluate potential secondary structures among LAMP
207 primers, the Oligo Analyser folding tool (<https://eu.idtdna.com/calc/analyzer>) and
208 PrimerExplorer™ V5 software (Eiken Chemical Co., Ltd., Tokyo, Japan) were employed with a
209 focus on the 3' end regions, as they are critical in promoting stable binding and elongation
210 during the amplification process, achieving specific LAMP amplification.¹⁹ The selection of
211 potential LAMP primer candidates was guided by a Gibbs free energy (ΔG) threshold of -7
212 kcal/mol. This criterion was employed to prevent mispriming among the six LAMP primers
213 during the amplification process.²⁰ Therefore, primer pairs with lower ΔG values, which could
214 result in thermodynamically stable heterodimers and non-specific LAMP products, were
215 excluded. This strategy effectively prevented the formation of heterodimers, thus ensuring
216 efficient and specific LAMP amplification (Figures 4A & 5A in the main article).

217 To evaluate the specificity of the designed LAMP primers, triplicate no-template control (NTC)
218 reactions were conducted. The assay's specificity was further assessed using genomic DNA
219 samples from a panel of 10 different *Pseudo-nitzschia* species and 8 non-target microalgal
220 strains, as detailed in Supplementary Table S4. All NTCs and non-*Pseudo-nitzschia* samples
221 consistently showed no amplification, indicating the absence of false positives and confirming
222 the high specificity of the LAMP assay. Positive amplification was observed exclusively from
223 *Pseudo-nitzschia* species, indicating selective detection by the LAMP method.



Supplementary Figure S4. Multiple Sequence Alignment of the *Cytochrome CYP450 DabD* gene sequences, revealing binding sites for the oligonucleotides used in the LAMP analysis of *P. multistriata* cells.

Supplementary Table S2. List of *Cytochrome CYP450 DabD* gene sequences obtained from *Pseudo-nitzschia* genomes and the nucleotide percent identification relative to *dabD* from ⁷.

Species	Strain	Accession Number	Functional annotation	% Identity <i>dabD</i> (relative to ⁷	Source
<i>P. multistriata</i>	B856	CAACVS010000654	Cytochrome CYP450	99.8	Ensemble Protists ^a
<i>P. multistriata</i>	B857	PSNMU-V1.4_AUG-EV-PASAV3_0045820.1	Cytochrome CYP450	52.6	SZN ^b
<i>P. multiseriis</i>	15091C3	MH202990	DabD, Cytochrome CYP450	83.1	NCBI ^c
<i>P. multistriata</i>	N/A	Brunson et al., 2018	DabD, Cytochrome CYP450	100	⁷
<i>P. multistriata</i>	B939	PsnmuV1.4_scaffold_84949_46006..60528	Cytochrome CYP450	55.1	14
<i>P. multistriata</i>	B936	PsnmuV1.4_scaffold_84949_46771..48096	Cytochrome CYP450	55.1	14
<i>P. multistriata</i>	B856	PsnmuV1.4_scaffold_84949_46771..48205	Cytochrome CYP450	55.1	EarlHam ^d
<i>P. multistriata</i>	B936	comp5102_c0_seq2	Cytochrome CYP450	52.6	15

^a Ensemble Protists; European Nucleotide Archive for Protist Genomes, ^b SZN; Genome database of the Stazione Zoologica Anton Dohrn, ^c NCBI; National Centre for Biotechnology Information GenBank, ^d EarlHam; The genome portal of the Earlham institute, UK.

232 **Supplementary Table S3.** The hits of BLAST analysis for LAMP amplicon.

BLASTN						
Species	Strain	Percent Identity	E- value	Score	Accession Number	Source
<i>P. multistriata</i>	B856	100%	6e-35	74	PSNMU_V1.4_AUG-EV-PASAV3_0045820	Ensemble Protists ^a
BLASTX						
<i>P. multistriata</i>	B856	96.4	2e-34	161	VEU37775	Ensemble Protists ^a
<i>P. multistriata</i>	B856	65.5	1.5e-11	109	VEU41686	Ensemble Protists ^a
<i>P. multistriata</i>	B856	34.2	0.00074	79	VEU45241	Ensemble Protists ^a
<i>P. multistriata</i>	B856	40.7	0.01	70	VEU44693	Ensemble Protists ^a

233 ^a Ensemble Protists; European Nucleotide Archive for Protist Genomes.

234 We compared the consensus fragment against ENSEMBL Protist genome repository using both
235 BLASTN and BLASTX analysis following default settings
236 (http://protists.ensembl.org/Pseudonitzschia_multistriata/Tools/Blast). The BLASTN results
237 revealed significant homologous matches with the PSNMU_V1.4_AUG-EV-PASAV3_0045820
238 gene of the Cytochrome P450 in *Pseudo-nitzschia multistriata* (Table S3). Furthermore,
239 BLASTX analysis of a 230 bp consensus sequence revealed that its protein product shares
240 40.7% with the complete cytochrome P450 DabD protein (Ensemble accession number:
241 VEU44693) from the ENSEMBL Protist genome database, validated as the DabD isoform
242 involved in domoic acid biosynthesis in *P. multistriata*. ⁸ Although the sequence similarity is
243 low, functional annotation and conserved domain features supported its selection as a
244 putative *dabD* homolog for primer design. Despite the limited genetic information available
245 on the species-specific DabD protein in the *P. multistriata* SZN-B954 strain, we extended our
246 investigation to identify the domain composition of the protein of isothermally amplified
247 products of the LAMP assay by using the NCBI tool of the Conserved Domain Database (CDD).
248 ¹⁸ The results revealed that the 230 bp consensus sequence displayed an identical domain

249 structure to the annotated Cytochrome P450 protein under NCBI accession number cl41757.
 250 Since cl41757 is one of many cytochrome P450 isoforms and lacks specific annotation linking
 251 it to domoic acid biosynthesis, further functional studies are needed to confirm its role in DA
 252 production. Nonetheless, domain structure supports classifying our amplified product within
 253 the cytochrome P450 family, which includes the DabD isoform previously implicated in DA
 254 biosynthesis in many *Pseudonitzschia* spp.^{7, 8, 11, 12}

255 **Supplementary Table S4.** Selectivity evaluation of the LAMP assay using *Pseudo-nitzschia*
 256 species and non-target microalgal strains.

Species	Isolate Accession	LAMP Amplification
<i>Pseudo-nitzschia multistriata</i>	SZN-B954	+
<i>Pseudo-nitzschia multistriata</i>	SZN-B955	+
<i>Pseudo-nitzschia pungens</i>	CCAP 1061/44	+
<i>Pseudo-nitzschia multiseri</i>	NWFSC 713	+
<i>Pseudo-nitzschia multiseri</i>	NWFSC 714	+
<i>Pseudo-nitzschia multiseri</i>	NWFSC 715	+
<i>Pseudo-nitzschia multiseri</i>	ML-54	+
<i>Pseudo-nitzschia multiseri</i>	ML-55	+
<i>Pseudo-nitzschia multiseri</i>	ML-56	+
<i>Pseudo-nitzschia multiseri</i>	ML-59	+
<i>Karenia brevis</i>	CCMP2228	-
<i>Karenia mikimotoi</i>	CCAP 1127/2	-
<i>Alexandrium tamarense</i>	CCAP 1119/25	-
<i>Synechococcus</i> sp	CCAP 1479/9	-
<i>Prorocentrum lima</i>	CCAP 1136/12	-
<i>Alexandrium minutum</i>	CCAP 1119/15	-
<i>Lingulodinium polyedra</i>	CCAP 1121/7	-
<i>Prorocentrum cordatum</i>	CCAP 1136/16	-

257

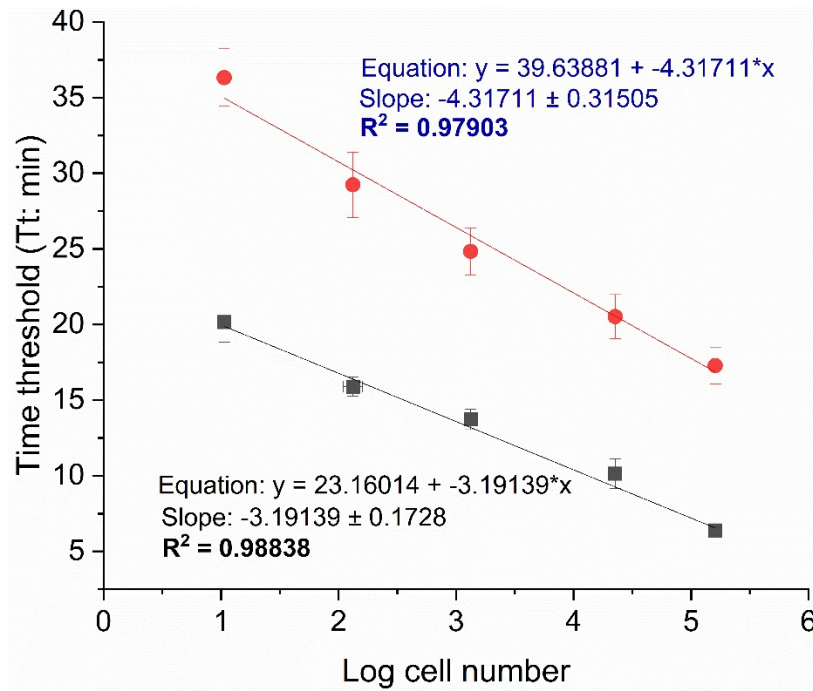
258

259

260 **S6. Quantification kinetics and standard curve generation**

261

262 To evaluate the quantitative performance of the LAMPTRON system, standard curves were
263 prepared by plotting threshold times (T_t , min) versus the logarithm of known input
264 concentrations of *P. multistriata* cells ranging from 1.6×10^5 to 1.07×10^1 cells/mL. DNA was
265 purified via on-chip extraction, followed by direct LAMP amplification on the LAMPTRON
266 (sections 2.4.1 and 2.4.2 of the main article). Each cell concentration was tested in triplicate.
267 Linear regression analysis was performed using Origin software (OriginLab, Northampton,
268 MA, USA), providing the correlation coefficients (R^2) to assess the linearity of the log-linear
269 relationship. Separate standard curves were constructed for the fresh and preserved reagent
270 conditions to compare performance. The resulting equations, slopes of the regression lines
271 and R^2 values are shown in Figure S5. The slope (m) of the resulting regression was used to
272 calculate amplification efficiencies, expressed as isothermal doubling time (IDT) using the
273 equation ($IDT = -0.301 \times m$), where m represents the slope of the graph of T_t value plotted
274 against the amount of target DNA/cells and 0.301 corresponds to a twofold change in
275 concentration at 2. ²¹ Gels were run using endpoint LAMP products, post-amplification (Figure
276 4B and 5B in the main article). Minor non-specific amplification products were observed in
277 endpoint gel electrophoresis after LAMP reactions due to the accumulation of looped DNA
278 structures and primer artefacts. Real-time fluorescence detection, rather than gel-based
279 confirmation, was used to determine threshold time (T_t) and quantification.



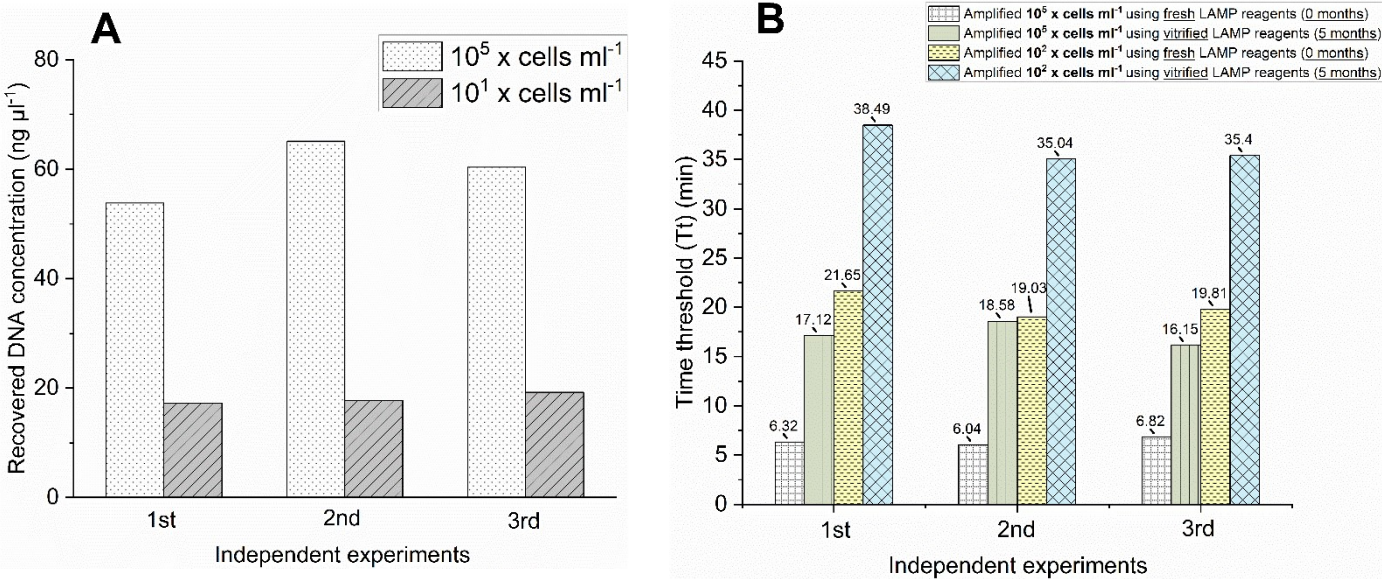
Supplementary Figure S5. Standard curve of the LAMPTRON system for quantifying *P. multistriata* cells in concentrations ranging from 1.6×10^5 to 1.07×10^1 cells/mL, as obtained using real-time fresh LAMP (black square) and vitrified preserved LAMP (red circle) methods. The data are expressed as the mean \pm SM (standard error of the mean) of at least 3 independent samples for each standard concentration. The insets show slopes, intercepts, and correlation coefficients (R^2) of linear regression of data for fresh LAMP (represented by the black text below the graph) and vitrified preserved reagents (represented by the blue text above the graph).

297 **S7. Reproducibility of LAMPTRON modules**

298

299 This section supports the reliability of the LAMPTRON analyser, including both extraction and
300 amplification modules, by demonstrating consistent DNA purification and amplification
301 results across independent experiments and reagent batches.

302



303

304 **Supplementary Figure S6.** Reproducibility of DNA purification and fluorescence
305 measurements by the LAMPTRON analyser. A) Comparison of the quantity of the DNA isolated
306 from 1.6×10^5 and 1.07×10^1 *P. multistriata* cells per mL from three independent experiments.
307 B) The changes of T_t values of both *P. multistriata* cell numbers using both freshly prepared
308 and pre-stored vitrified LAMP assays (5-month shelf-life) that were obtained by three
309 independent experiments on the LAMPTRON system.

310

311

312

313

314 **Part III: Microfluidic Hardware and Electronics**

315 **S8. Optimisation of the microfluidic design**

316

317 To optimise the functionality of the LAMPTRON chip, we evaluated various channel
318 dimensions and microchamber geometries to ensure laminar fluid flow and limited carryover
319 between solutions. To do this, we employed COMSOL Multiphysics 5.5 software to simulate
320 fluid flow in a 3D geometrical model of an extraction microchamber packed with silica beads.
321 Initially, we investigated the potential challenge of bead slurry aggregation in microchannels
322 which could block fluid flow and delay reagent flushing during the extraction process.²²
323 Simulation results using COMSOL demonstrated that the bead-packed reservoir remained
324 structurally stable, and steady laminar flow was observed under current microchannel
325 dimensions and operating conditions, as summarised in section 2.2 of the main article. The
326 resulting Reynolds number (Re) was below the threshold of 2300, allowing for steady laminar
327 flow through the bead-packed microchamber.²³ Another functional limitation we
328 investigated was the resistance to the fluid flow caused by packed glass beads in

329 microchannels, which could resist the fluid flow and lead to backpressure, as demonstrated

330 in similar bead-based systems.^{24, 25} This backflow could lead to a carryover of extraction

331 reagents, inhibiting amplification reactions and impeding the automated DNA extraction

332 process.²⁶ The simulation results shown in Figure S7-A indicate a steady velocity field that

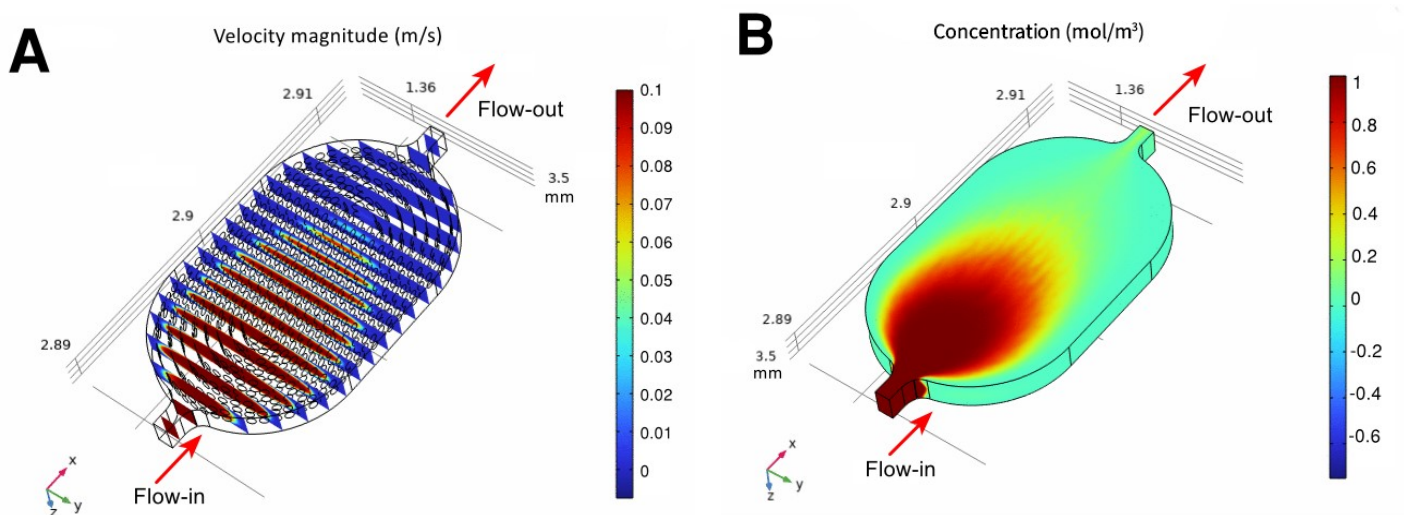
333 maintains the concentration profile through beads immobilised on the bed of the extraction

334 chamber, ensuring high DNA binding. In bench-top testing, sustained fluid retention was

335 achieved by positioning solenoid valves at both the inlet and outlet of the detection

336 microchamber, effectively preventing backflow that could lead to contamination or inhibit

the LAMP reaction. By opening these valves and operating the syringe pump, the eluted DNA was titrated to rehydrate the vitrified LAMP reagents that were previously manually loaded into the detection chamber. LAMP products were detected via a small and compact fluorescence unit (FluoSens, QIAGEN GmbH, Hilden, Germany) aligned directly beneath the reaction microchamber. The use of this miniaturised and compact fluorescence unit provides the advantages of simplicity, modest cost, and allows for quick and easy assembly with functional parts of the LAMPTRON prototype. As a result, the on-chip DNA extraction protocol and LAMP detection can be adapted into a semi-automated workflow.



345

346 **Supplementary Figure S7.** Surface concentration and velocity profiles of DMA-DNA
347 complexes (i.e., diluted species) within the extraction chamber packed with silica beads. The
348 simulation illustrates a single-phase flow interface model incorporating the transport of
349 diluted species. DMA-DNA complexes enter through the lower inlet, diffuse through the
350 packed silica bead matrix, and exit via the outlet, revealing transport obstructions and flow
351 heterogeneity within the microchamber.

352

353

354

355

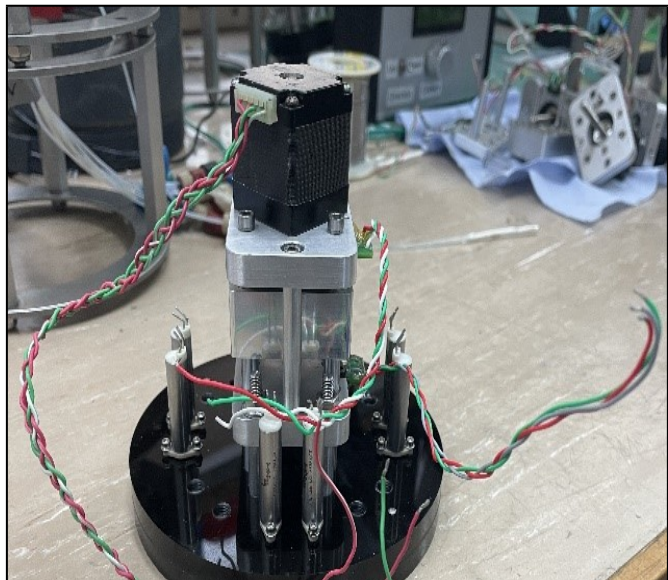
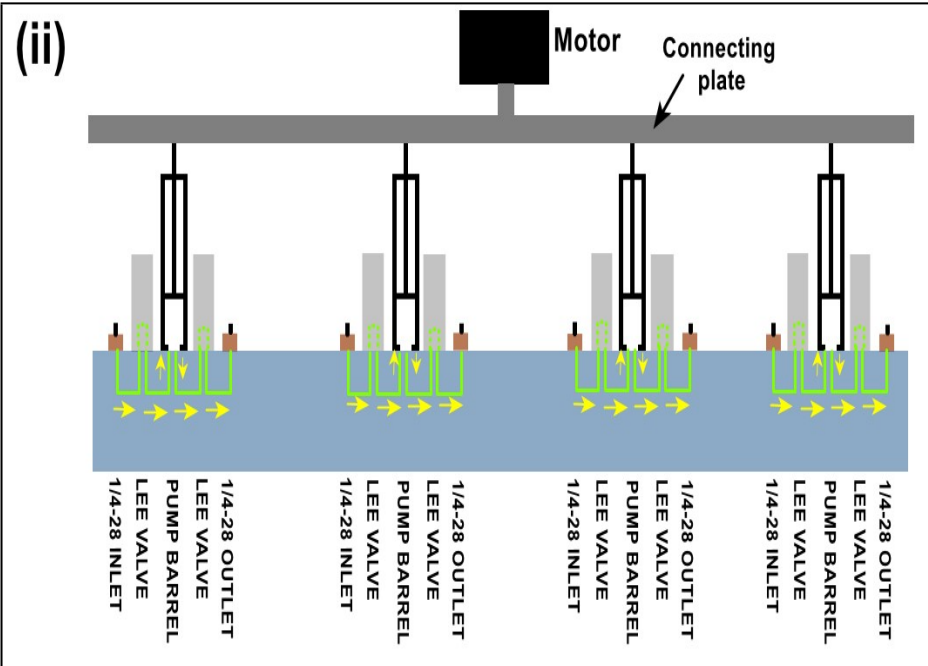
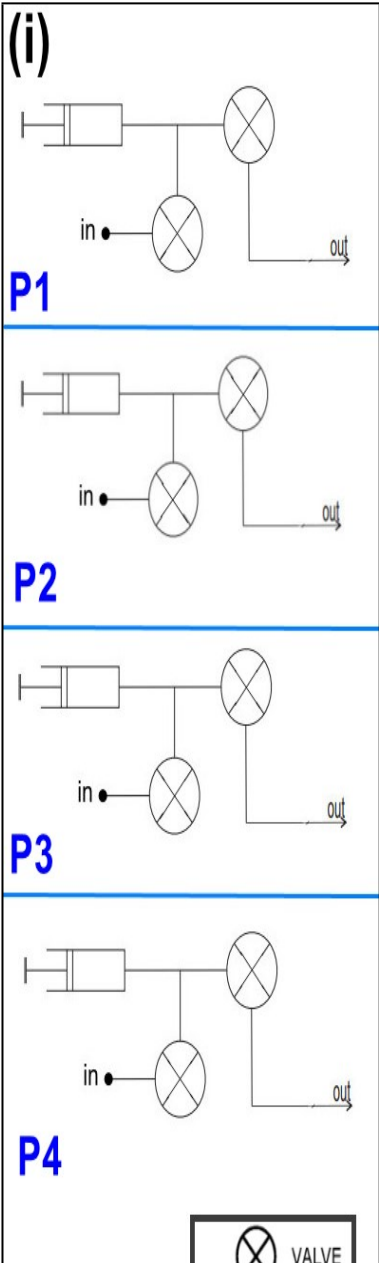
356 **S9. Autonomous pumping system**

357

358 The incorporation of the pumping system represents a step towards demonstrating the
359 autonomous operation capability of the LAMPTRON system. This pumping system has
360 previously proved its utility in conducting *in-situ* chemical analyses at depths of up to 6,000
361 metres, as deployed in multiple oceanic locations by the Ocean Technology and Engineering
362 Group (OTE) at the National Oceanography Centre.^{27, 28} For the first time, this system has
363 been adapted to process genomic samples, offering the potential for *in-situ* biological
364 analyses in a wide array of environmental settings.

365 To enable autonomous operation, a revised version of the existing pumping system was
366 developed, accommodating a configuration with four linked syringe pumps each equipped
367 with solenoid valves (LFNA1250125H, Lee Co., United States), and ¼"-28 threaded outlets, as
368 illustrated in Figure S8. This design allows for transitions between various pumping speeds
369 within a single pumping cycle. The term "pumping cycle" here refers to the sequence of steps

involved in moving fluids through microchannels when the valve changes from a closed to an open state.²⁹ Most importantly, this design supports a pumping sequence that involves the individual loading of each DNA extraction reagent in its respective container, followed by transportation into channels, mixing with different fluids or components such as microbeads, or disposal into a waste container. This flexible pumping system enables the free exchange among extraction reagents, facilitating an automated on-chip DNA extraction process.



390

391

392

393

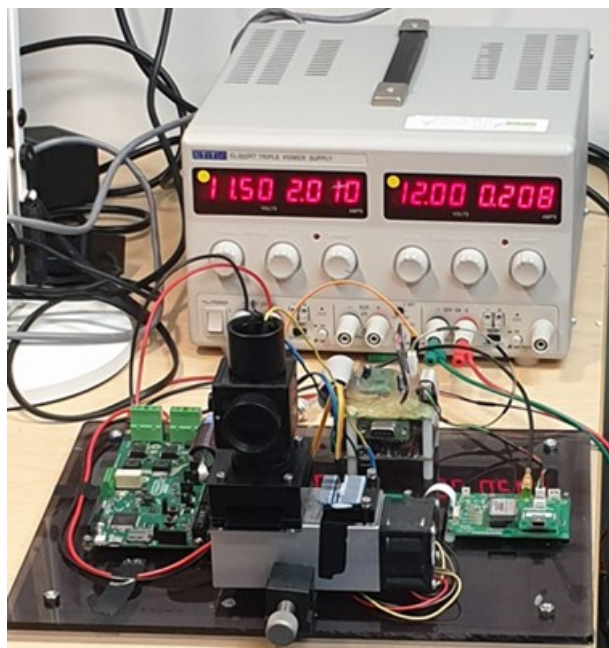
394 **Supplementary Figure S8.** Autonomous microfluidic pump system. i) A generic chip schematic
395 demonstrating multiple individual fluidic routes, each connected to one of the four parallel
396 syringe pumps (P1, P2, P3, and P4). ii) A schematic diagram shows the custom-made pumping
397 system, which includes four syringe pumps, valves, and ¼"-28 outlets for each pump, all
398 actuated by a Haydon Kerk Size 11 stepper motor linear actuator. iii) An image shows the
399 setup of the valve-controlled selective routing.

400 **S10. LAMPTRON prototype**

401

402 This section shows the LAMPTRON prototype, highlighting its Arduino-based electronic
403 control for heater regulation, temperature sensing, and data acquisition.

404



405

406 **Supplementary Figure S9.** The first prototype of LAMPTRON incorporated an Arduino circuit
407 for electronic control of the heater, temperature sensor and data capture.

408

409 Part IV: Notes and references

410

- 411 1. R. Andersen, J. Berges, P. Harrison and M. Watanabe, *Algal Culture Techniques*, 2005, 429-
412 539.
- 413 2. J. M. Newton, D. Schofield, J. Vlahopoulou and Y. H. Zhou, *Biotechnol Progr*, 2016, **32**, 1069-
414 1076.
- 415 3. D. I. Walker, J. McQuillan, M. Taiwo, R. Parks, C. A. Stenton, H. Morgan, M. C. Mowlem and
416 D. N. Lees, *Water Res*, 2019, **161**, 652-652.
- 417 4. K. Dilley, F. Pagan and B. Chapman, *Sci Justice*, 2021, **61**, 193-197.
- 418 5. H. Lee, C. Park, W. Na, K. H. Park and S. Shin, *Npj Precis Oncol*, 2020, **4**, 1-10.
- 419 6. B. Subeshan, A. Usta and R. Asmatulu, *Surf Interfaces*, 2020, **18**, 1-11.
- 420 7. J. K. Brunson, S. M. K. McKinnie, J. R. Chekan, J. P. McCrow, Z. D. Miles, E. M. Bertrand, V.
421 A. Bielinski, H. Luhavaya, M. Obornik, G. J. Smith, D. A. Hutchins, A. E. Allen and B. S.
422 Moore, *Science*, 2018, **361**, 1356-1358.
- 423 8. Z. Y. He, Q. Xu, Y. Chen, S. Y. Liu, H. Y. Song, H. Wang, C. P. Leaw and N. S. Chen, *Commun*
424 *Biol*, 2024, **7**, 1-12.
- 425 9. Z. Y. Nie, X. P. Long, N. E. Bouroubi, H. C. Liu, S. T. Cao, Y. X. Chen, X. F. Zheng and J. L.
426 Xia, *Curr Pharm Biotechno*, 2023, **24**, 599-610.
- 427 10. K. J. McLean and A. W. Munro, in *Encyclopedia of Signaling Molecules*, ed. S. Choi, Springer
428 New York, New York, NY, 2016, pp. 1-18.
- 429 11. S. Hardardottir, S. Wohlrab, D. M. Hjort, B. Krock, T. G. Nielsen, U. John and N. Lundholm,
430 *Bmc Mol Biol*, 2019, **20**, 1-14.
- 431 12. K. A. Lema, G. Metegnier, J. Quéré, M. Latimier, A. Youenou, C. Lambert, J. Fauchot and M.
432 Le Gac, *Genome Biol Evol*, 2019, **11**, 731-747.
- 433 13. S. F. Altschul, W. Gish, W. Miller, E. W. Myers and D. J. Lipman, *J Mol Biol*, 1990, **215**, 403-
434 410.
- 435 14. S. Basu, S. Patil, D. Mapleson, M. T. Russo, L. Vitale, C. Fevola, F. Maumus, R. Casotti, T.
436 Mock, M. Caccamo, M. Montresor, R. Sanges and M. I. Ferrante, *New Phytol*, 2017, **215**, 140-
437 156.
- 438 15. V. Di Dato, F. Musacchia, G. Petrosino, S. Patil, M. Montresor, R. Sanges and M. I. Ferrante,
439 *Sci Rep-Uk*, 2015, **5**, 1-14.
- 440 16. M. A. Larkin, G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F.
441 Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson and D. G. Higgins,
442 *Bioinformatics*, 2007, **23**, 2947-2948.
- 443 17. C. Camacho, G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer and T. L. Madden,
444 *Bmc Bioinformatics*, 2009, **10**, 1-9.
- 445 18. A. Marchler-Bauer, Y. Bo, L. Y. Han, J. E. He, C. J. Lanczycki, S. N. Lu, F. Chitsaz, M. K.
446 Derbyshire, R. C. Geer, N. R. Gonzales, M. Gwadz, D. I. Hurwitz, F. Lu, G. H. Marchler, J. S.
447 Song, N. Thanki, Z. X. Wang, R. A. Yamashita, D. C. Zhang, C. J. Zheng, L. Y. Geer and S.
448 H. Bryant, *Nucleic Acids Res*, 2017, **45**, 200-203.
- 449 19. F. V. Shirshikov and J. A. Bespyatykh, *Russ J Bioorg Chem+*, 2022, **48**, 1159-1174.
- 450 20. K. Malpartida-Cardenas, L. Miglietta, T. Peng, A. Moniri, A. Holmes, P. Georgiou and J.
451 Rodriguez-Manzano, *Sensors & Diagnostics*, 2022, **1**, 465-468.
- 452 21. G. J. Nixon, H. F. Svenstrup, C. E. Donald, C. Carder, J. M. Stephenson, S. Morris-Jones, J. F.
453 Huggett and C. A. Foy, *Biomol Detect Quantif*, 2014, **2**, 4-10.
- 454 22. R. Zhong, D. Liu, L. Yu, N. Ye, Z. Dai, J. Qin and B. Lin, *Electrophoresis*, 2007, **28**, 2920-
455 2926.
- 456 23. K. Benz, K. P. Jackel, K. J. Regenauer, J. Schiewe, K. Drese, W. Ehrfeld, V. Hessel and H.
457 Lowe, *Chem Eng Technol*, 2001, **24**, 11-17.
- 458 24. R. D. Oleschuk, L. L. Shultz-Lockyear, Y. B. Ning and D. J. Harrison, *Anal Chem*, 2000, **72**,
459 585-590.
- 460 25. K. A. Wolfe, M. C. Breadmore, J. P. Ferrance, M. E. Power, J. F. Conroy, P. M. Norris and J. P.
461 Landers, *Electrophoresis*, 2002, **23**, 727-733.

- 462 26. S. J. Reinholt and A. J. Baeumner, *Angew Chem Int Edit*, 2014, **53**, 13988-14001.
463 27. A. D. Beaton, A. M. Schaap, R. Pascal, R. Hanz, U. Martincic, C. L. Cardwell, A. Morris, G.
464 Clinton-Bailey, K. Saw, S. E. Hartman and M. C. Mowlem, *ACS Sens*, 2022, **7**, 89-98.
465 28. M. Mowlem, A. Beaton, R. Pascal, A. Schaap, S. Loucaides, S. Monk, A. Morris, C. L.
466 Cardwell, S. E. Fowell, M. D. Patey and P. Lopez-Garcia, *Front Mar Sci*, 2021, **8**, 1-15.
467 29. P. Woias, *Sensor Actuat B-Chem*, 2005, **105**, 28-38.

468