Electronic Supplementary Material (ESI) for Analytical Methods.
This journal is © The Royal Society of Chemistry 2020

Modification of Polyacrylate Sorbent Coatings with Carbodiimide Crosslinker Chemistry for Sequence-Selective DNA Extraction using Solid-Phase Microextraction

Marcelino Varona,[a] Olga I. Shimelis,[b] and Jared L. Anderson[a,*]

[a]Department of Chemistry, Iowa State University, Ames, Iowa 50011 United States
[b]MilliporeSigma, Bellefonte, Pennsylvania 16823 United States

Table of Contents

Table S1 S2
Table S2 S3
Table S3 S4
Figure S1 S5
Figure S2 S6
Figure S3 S7
Figure S4 S8
Materials and Methods S9
Selective extraction of DNA with oligo-functionalized PA fibers S10
Serial desorption experiment

Table S1. Sequences of oligonucleotides used in this study.

	Sequence
FAM labelled Oligo	5'-/5AmMC12/TTTTTTTTTTTTTTTTTTTT-36-FAM/-3'
Extraction Probe	5’-/5AmMC12/GAGGCCCACTCCCATAGGTT-3'
Extraction Probe with Blocker	5’-/5AmMC12/GAGGCCCACTCCCATAGGT/3SpC3-3'
Forward Primer	5'-AAAACGCCGCAGACACATCC -3'
Reverse Primer	5’-GAGGCCCACTCCCATAGGTT-3
Target	GGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTAT GCCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGCCC GTTTGTCCTCTAATTCCAGGATCATCAACAACCAGCACCGGACCAT GCAAAACCTGCACAACTCCTGCTCAAGGAACCTCTATGTTTCCCTCA TGTTGCTGTACAAAACCTACGGACGGAAACTGCACCTGTATTCCCA TCCCATCATCTTGGGCTTTCGCAAGTAACCTATGGGAGTGGGCCTC

Table S2. Fluorescence results of the washes following NHS/EDC coupling with FAM-labeled oligo

	Fluorescence Results			
Wash Number	Fiber 1	Fiber 2	Fiber 3	Fiber 4
1 (24 hr)	3500	1740	1362	3
2 (2 hr)	2828	1702	2246	8
3 (30 min)	1098	686	987	5
4 (1.5 hr)	120	58	81	2

Table S3. Results showing the relative ratio of DNA in the first to second desorption with and without Exonuclease III treatment. Ratios were obtained using the average DNA desorbed in the first and second desorption from 3 different extractions for each treatment.

	DNA first desorption:second desorption
Without Enzyme	12
With Enzyme	8810

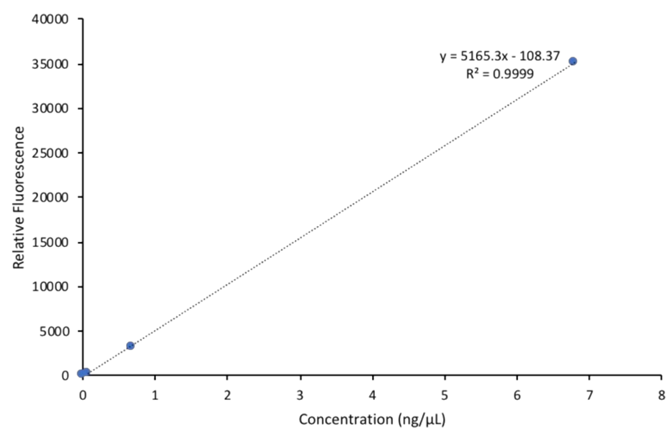

Figure S1. Four point calibration curve of dual-labeled (amine and FAM) oligonucleotide used to quantify loading following DNase treatment of the fibers. Triplicate measurements were recorded for each point.

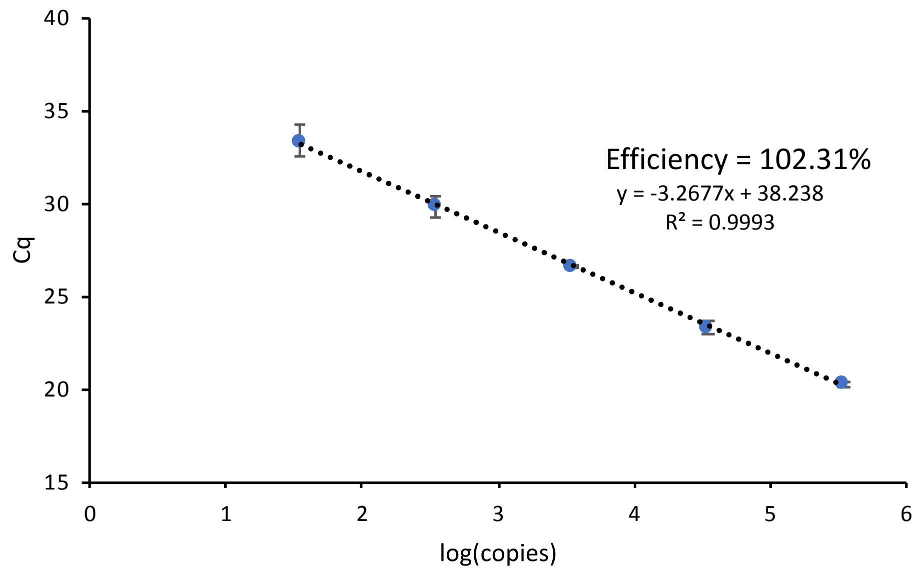

Figure S2. Five-point qPCR calibration curve of the target DNA sequence. Each point was measured in triplicate.

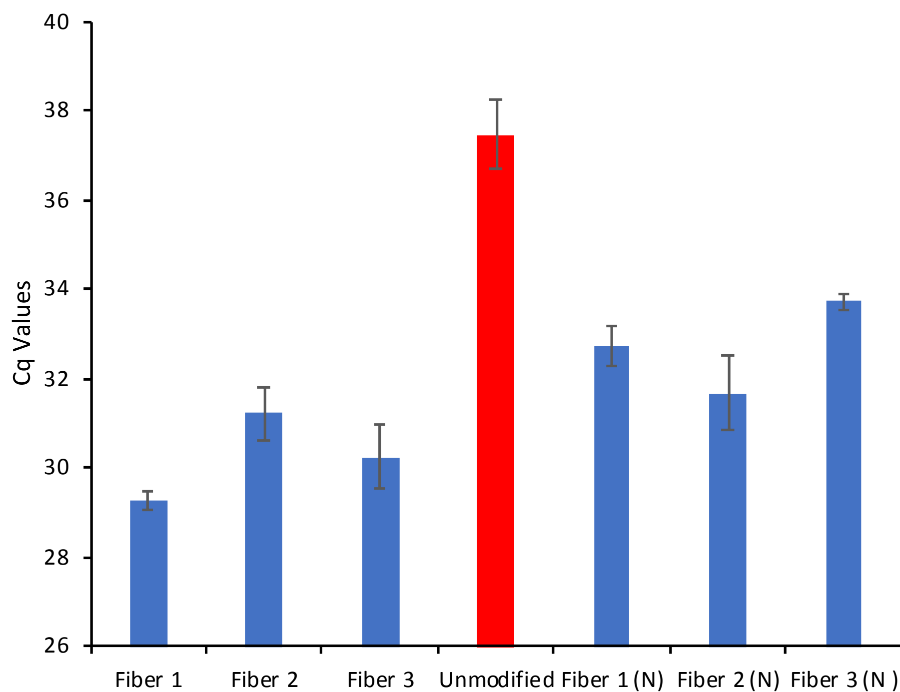

Figure S3. Extraction results from 6 different PA fibers following NHS/EDC modification chemistry. The modification and extraction performance is compared from two separate lots of fibers.

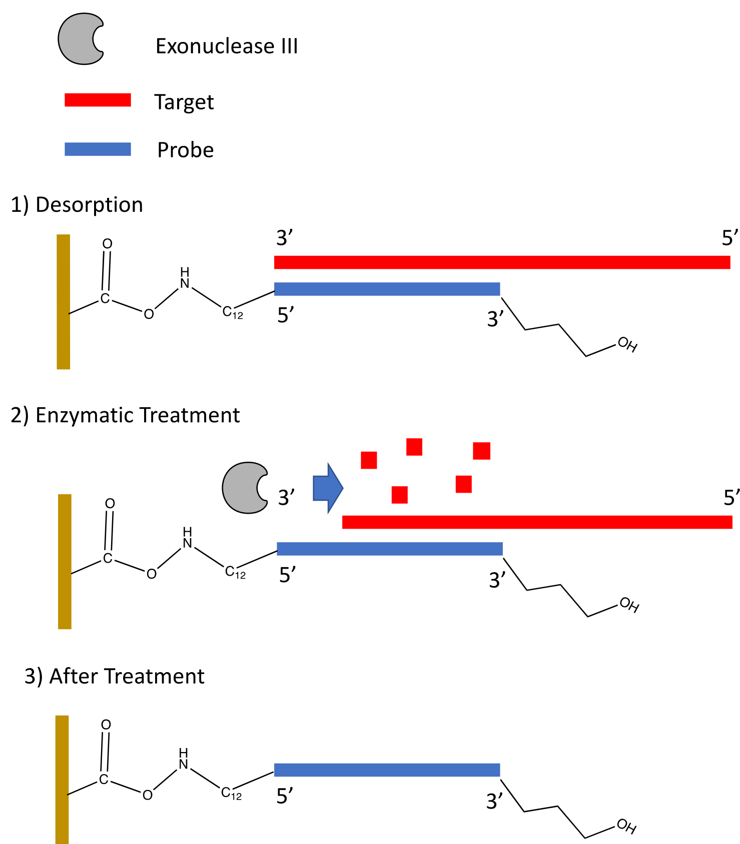

Figure S4. Representative schematic of the effect of using Exonuclease III to reduce carryover by hydrolyzing the target sequence after an initial desorption.

Reagents and Instrumentation

All primers and oligonucleotides used in this study were obtained from Integrated DNA Technologies (Coralville, IA, USA). The SPME fibers were obtained from Supelco (Bellefonte, PA, USA). For the coupling chemistry, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl, >97.0%), N-hydroxysuccinimide (NHS, +98%), citric acid >99.5%, salmon testes DNA, and 2-(N-morpholino)ethanesulfonic acid were obtained from Sigma Aldrich (St. Louis, MO, USA). Tris(hydroxymethyl)aminomethane (Tris) was obtained from P212121 (Ypsilanti, MI, USA). Sodium chloride 99%, disodium phosphate ≥99%, sodium carbonate ≥99.5%, and sodium bicarbonate ≥99.7% were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Exonuclease III was obtained from New England Biolabs (Ipswich, MA, USA). RQ1 RNase-Free DNase and 10X reaction buffer were obtained from Promega (Madison, WI, USA). Deionized water (18.2 MΩ cm) was used throughout the study and it was obtained from a Millipore Milli-Q water purification system. qPCR assays were performed on a CFX96 Touch qPCR instrument using SsoAdvanced Universal SYBR Green supermix from Bio-Rad Laboratories (Hercules, CA, USA). Fluorescence measurements were performed using a BioTek Synergy Hybrid H1 microplate reader using a black, flat bottom, Greiner Bio 384-well microplate. Fibers were conditioned using the inlet of an Agilent Technologies 6850 gas chromatograph (Santa Clara, CA, USA).

Selective extraction of DNA with oligo-functionalized PA fibers

A 1.5 mL, 10 pg mL^{-1} (34,800 copies mL^{-1}) DNA solution was prepared in a 2 mL Eppendorf LoBind tube. A small stir-bar was added into the tube to enable stirring. The cap was pierced to allow the PA fiber through the tube and into the extraction solution. The tubes with the fibers were immediately placed in a 90 °C water bath for 10 min. Following the heating process, the tubes were transferred to a room temperature water bath placed over a magnetic stir-plate. Following the hybridization process, the fiber was retracted and removed from the extraction solution. The fiber was subsequently washed with water to removed nonspecifically adsorbed DNA and subjected to a desorption in 10 µL of water at 90 °C for 5 min. After the desorption process, a 1 µL aliquot from the desorption solution was subjected to qPCR analysis.

Serial desorption experiment

The serial desorption experiments were performed as follows. Five desorption containers containing 10 μL of water were prepared and placed in a 90 °C water bath. After the sequence-selective extraction, the fiber was placed in a desorption container for 2 min and subsequently moved to another desorption container. This was done in intervals of 2, 3, 5, 5 and 5 min, giving a total of 20 min. A 1 μL aliquot was collected from each desorption container and subjected to qPCR. The percent desorbed was calculated in the following way. The amount recovered at each time interval was calculated and added to the amount recovered in any previous interval. This was divided by the total DNA recovered after the 5 intervals.

Let $t1 - t5$ be the amount of DNA recovered in each time interval.

$$\frac{t1}{(t1+t2+t3+t4+t5)} \times 100 = \text{\% recovered at 2 min}$$

$$\frac{t1+t2}{(t1+t2+t3+t4+t5)} \times 100 = \text{\% recovered at 5 min}$$

$$\frac{t1+t2+t3}{(t1+t2+t3+t4+t5)} \times 100 = \text{\% recovered at 10 min}$$

$$\frac{t1+t2+t3+t4}{(t1+t2+t3+t4+t5)} \times 100 = \text{\% recovered at 15 min}$$

$$\frac{t1+t2+t3+t4+t5}{(t1+t2+t3+t4+t5)} \times 100 = \text{\% recovered at 20 min}$$