Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Evaluating Commercial Thermoplastic Materials in Fused Deposition Modeling 3D Printing for their Compatibility with DNA Storage and Analysis by Quantitative Polymerase Chain Reaction

Derek R. Eitzmann, and Jared L. Anderson*

Table of Contents

Table S1. Primers and DNA sequences used in this study.

BRAF 830	5'- TGGTTTCTCGACAACTGAACATTACAAGAAAATCTATCAGAAGTC TTTACAATAGTAGGAGTTTTTGATTGCTTGCTTACATTTTATCAGC ACTATAAAACTGATAGTTTTGTAGCTATCTATTAGTCCCTTTCAGA CCTCTGACCTTGCTCAGTGGTAGTTGAGATATAACTGAAGACTCT AAATTATATAACAATGAGGTGAGAAAAACATAATATTTCTCTTCC CTAAGTGCAGACTAAGATACTATCTGCAGCATCTTCATTCCAATG AAGAGCCTTTACTGCTCGCCCAGGAGTGCCAAGAGAATATCTGG GCCTACATTGCTAAAATCTAATGGGAAAGTTTTAGGTTCTCCTAT AAACTTAGGAAAGCATCTCACCTCATCCTAACACATTTCAAGCCC CAAAAATCTTAAAAGCAGGTTATATAGGCTAAATAGAACTAATC ATTGTTTTAGACATACTTATTGACTCTAAGAGGAAAGATGAAGTA CTATGTTTTAAAGAATATTATATTACAGAATTATAGAAATTAGAT CTCTTACCTAAACTCTTCATAATGCTTGCTCTGATAGGAAAATGA GATCTACTGTTTTCCTTTACTTACTACACCTCAGATATATTTCTTC ATGAAGACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTACAG TGAAATCTCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGTCTG GATCCATTTTGTGGATGGTAAGAATTGAGGCTATTTTTCCACTGA TTAAATTTTTGGCCCTGAGATGCTGCTGAGTTACTAGAAAGTCAT TGAAGGTCTCAACTATAGT-3'
BRAF 98	5'- TTCATGAAGACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTA CAGTGAAATCTCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGT CTGGATCC-3'
BRAF Forward	5'-TTCATGAAGACCTCACAGTAAA-3'
BRAF Reverse	5'-GGATCCAGACAACTGTTCAA-3'

*Portions of the DNA sequences are underlined to show the amplicon of the qPCR assay.

Table S2. Optimal print conditions used for each filament in the construction of all 3D printed devices.

Filament[a]	Printing Temp (°C)	Print Bed Temp (°C)	Fan Speed
Polylactic Acid (PLA)	200	60	100 %
Acrylonitrile Butadiene Styrene (ABS)	245	85	10 %
Co-polyester (CPE)	245	60	50 %
Nylon	245	70	20 %
Polycarbonate (PC)	275	110	0 %
Polypropylene (PP)	210	85	20 %

[a]Values in the table yielded 3D printed vessels with high surface quality and no visible aberrations for layer heights of 0.20 mm.

Table S3. DNA adsorption (percentage) calculated from the adsorption-time profiles of each storage vessel used in this study.

(A) Container Material	Percent DNA Adsorbed (%)[a]					
	1 hr	3 hr	6 Hr	12 hr	18 hr	24 hr
Fisherbrand™	12.1 ± 12.3 %	55.4 ± 10.2 %	84.3 ± 4.0 %	91.8 ± 4.6 %	97.4 ± 0.3 %	97.5 ± 0.1 %
Eppendorf DNA Lobind®	16.5 ± 9.4 %	57.6 ± 11.0 %	62.9 ± 4.9 %	90.0 ± 0.9 %	94.8 ± 1.2 %	96.8 ± 0.4 %
PLA	13.2 ± 3.8 %	-3.0 ± 8.6 %	13.9 ± 15.0 %	8.2 ± 9.2 %	38.9 ± 9.7 %	12.1 ± 0.7 %
Nylon	-21.8 ± 15.5 %	24.4 ± 8.1 %	-14.8 ± 9.5 %	33.7 ± 7.5 %	-8.9 ± 9.5 %	-46.1 ± 23.3 %
PC	58.8 ± 11.1 %	91.4 ± 0.5 %	97.2 ± 0.3 %	97.5 ± 0.2 %	97.2 ± 0.3 %	97.6 ± 0.1 %
CPE	22.5 ± 10.1 %	31.3 ± 15.8 %	67.0 ± 18.6 %	68.1 ± 20.2 %	74.9 ± 10.0 %	68.7 ± 12.5 %
ABS	47.4 ± 6.7 %	83.6 ± 3.6 %	96.1 ± 0.7 %	97.2 ± 0.2 %	97.6 ± 0.1%	97.6 ± 0.1 %
PP	55.4 ± 2.3%	85.3 ± 5.0 %	94.4 ± 1.5 %	97.2 ± 0.2 %	96.9 ± 0.6 %	97.1 ± 0.2 %

(B) Container Material	Percent DNA Adsorbed (%)[a]					
	1 hr	3 hr	6 hr	12 hr	18 hr	24 hr
Fisherbrand™	8.4 ± 10.0 %	35.6 ± 1.8 %	60.3 ± 13.4 %	85.3 ± 4.4 %	89.5 ± 5.5 %	86.3 ± 2.0 %
Eppendorf DNA LoBind®	1.1 ± 6.6 %	27.2 ± 1.7 %	49.9 ± 6.3 %	70.5 ± 12.8 %	76.1 ± 1.8 %	59.3 ± 13.0 %
PLA	8.4 ± 31.6 %	29.9 ± 14.2 %	35.7 ± 0.9 %	31.2 ± 16.0 %	28.3 ± 35.6 %	25.1 ± 15.2 %
Nylon	16.7 ± 25.7 %	38.3 ± 6.6 %	55.2 ± 6.8 %	26.2 ± 8.9 %	11.3 ± 34.0 %	29.8 ± 12.8 %
PC	45.3 ± 4.4 %	46.5 ± 7.8 %	72.4 ± 9.3 %	93.6 ± 1.3 %	95.2 ± 1.0 %	98.0 ± 1.1 %
CPE	45.3 ± 7.5 %	51.9 ± 5.7 %	56.5 ± 8.2 %	83.7 ± 7.7 %	95.4 ± 1.5 %	87.8 ± 4.7 %
ABS	-0.4 ± 33.2 %	57.0 ± 7.9 %	61.8 ± 17.4 %	72.7 ± 8.9 %	85.7 ± 4.8 %	94.2 ± 3.4 %
PP	13.9 ± 4.9%	52.0 ± 6.9 %	70.9 ± 5.9 %	78.4 ± 5.2 %	92.5 ± 5.5 %	98.0 ± 2.5 %

(C) Container Material	Percent DNA Adsorbed (%)[a]					
	1 hr	6 hr	12 hr	24 hr	48 hr	96 hr
Fisherbrand™	56.6 ± 8.7 %	79.2 ± 11.2 %	94.6 ± 1.0 %	99.9 ± 0.1 %	94.5 ± 8.7 %	94.7 ± 8.4 %
Eppendorf DNA LoBind®	44.3 ± 3.8 %	63.7 ± 8.0 %	83.6 ± 2.6 %	80.6 ± 14.2 %	96.0 ± 1.3 %	95.7 ± 1.4 %
PLA	18.9 ± 8.0 %	18.0 ± 7.8 %	22.1 ± 9.3 %	14.1 ± 19.4 %	20.5 ± 5.1 %	10.2 ± 13.6 %
Nylon	8.5 ± 5.7 %	-3.5 ± 16.6 %	11.5 ± 7.0 %	-12.4 ± 41.0 %	-32.8 ± 12.7 %	-19.6 ± 15.5 %
PC	61.8 ± 10.3 %	90.8 ± 4.5 %	64.4 ± 46.8 %	93.7 ± 4.7 %	88.3 ± 14.9 %	89.0 ± 13.7 %
CPE	-8.6 ± 34.0 %	18.6 ± 12.1 %	14.3 ± 5.8 %	-18.6 ± 28.7 %	-6.4 ± 9.6 %	-4.6 ± 6.6 %
ABS	9.8 ± 17.0 %	29.6 ± 24.2 %	13.7 ± 12.1 %	19.8 ± 25.9 %	1.1 ± 25.1 %	-0.8 ± 23.1 %
PP	23.1 ± 24.5 %	43.3 ±11.4 %	12.2 ± 17.1 %	26.6 ± 4.5 %	13.8 ± 19.2 %	13.2 ± 18.3 %

[a]The percent DNA adsorption was calculated by converting the obtained Cq values to remaining DNA mass and then compared with the initial DNA standard for each time-course. Table (A) was obtained using the adsorption-time profiles of 102 pg/mL 98 bp DNA and 2.5 M NaCl (Figure 2A), (B) was calculated from the 100 pg/mL 830 bp DNA and 2.5 M NaCl (Figure 2B), and (C) contains converted data from the 102 pg/mL 98 bp DNA and 200 mM NaCl adsorption time-course (Figure 2C).

Table S4. Sessile drop water contact angle measurements of white ABS filaments produced by Dynamism and Ultimaker. See Figure S1 for schematic representation of wall and flat surfaces from 3D printed model.

Sample[a]	Contact Angle (θ)	±
Dynamism - wall	69.8°	6.9
Ultimaker 1 - wall	73.7°	12.4
Dynamism - flat	114.3°	4.1
Ultimaker 1 - flat	112.0°	5.5

[a]Contact angles are reported as an average of triplicates for each sample surface. Measurements were obtained following video analysis and manual fitting of the static droplet.

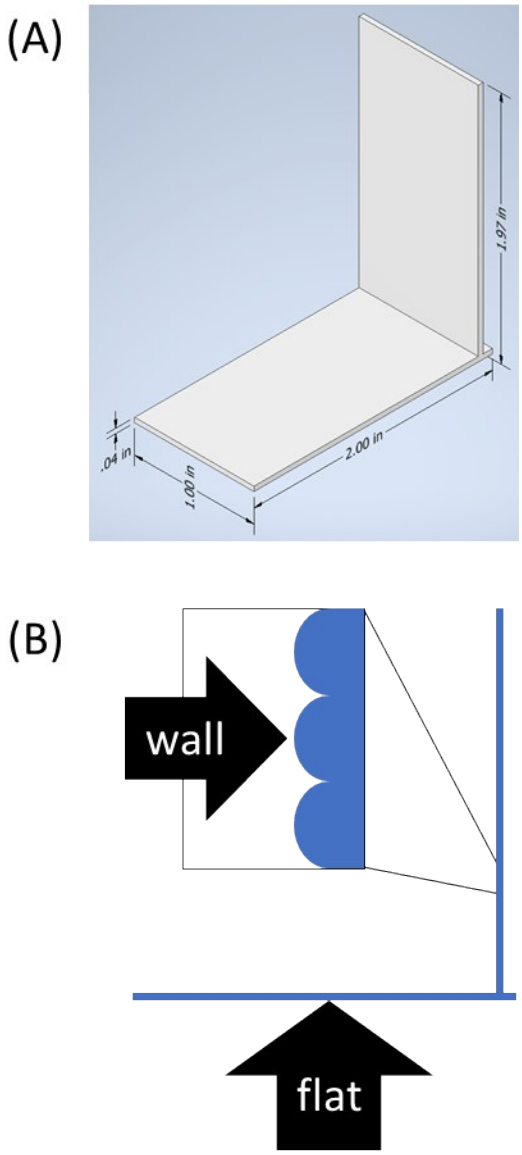

Figure S1. Schematic of 3D model designed for sessile drop contact angle measurements. Image (A) shows the 3D model featuring dimensions from Inventor software and (B) describes the expected surface morphologies following printing that were probed by contact angle measurements.

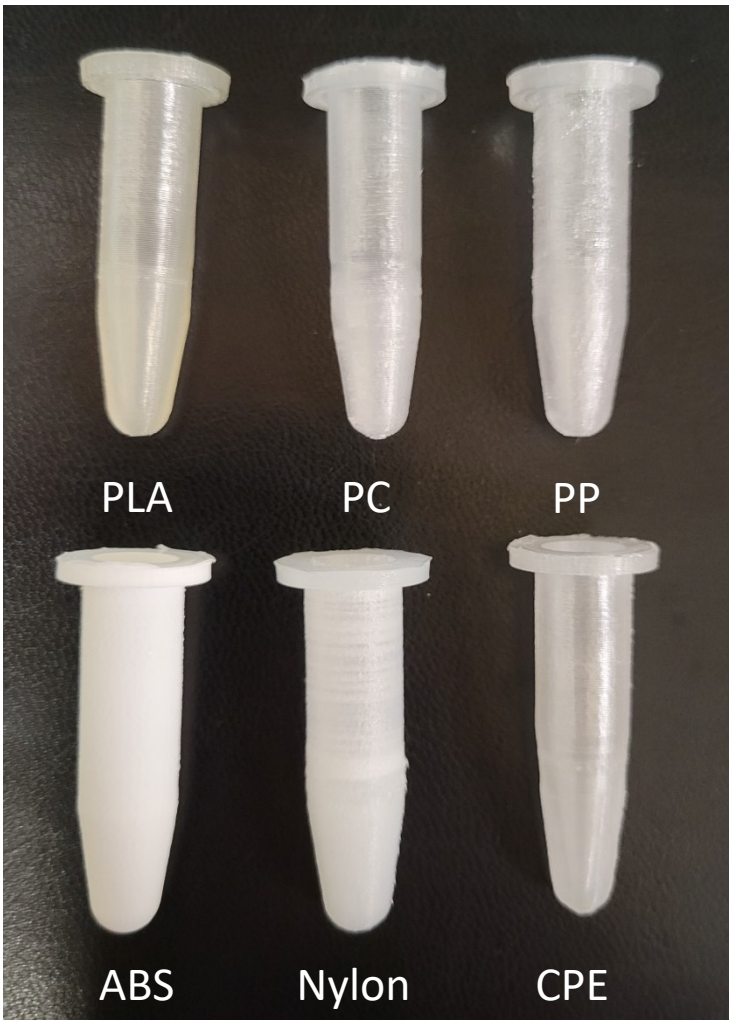

Figure S2. Images of 3D printed DNA storage devices used in the study. The thermoplastic filaments featured in the image are: polylactic acid (PLA), polycarbonate (PC), polypropylene (PP), acrylonitrile butadiene styrene (ABS), nylon, and co-polyester (CPE).

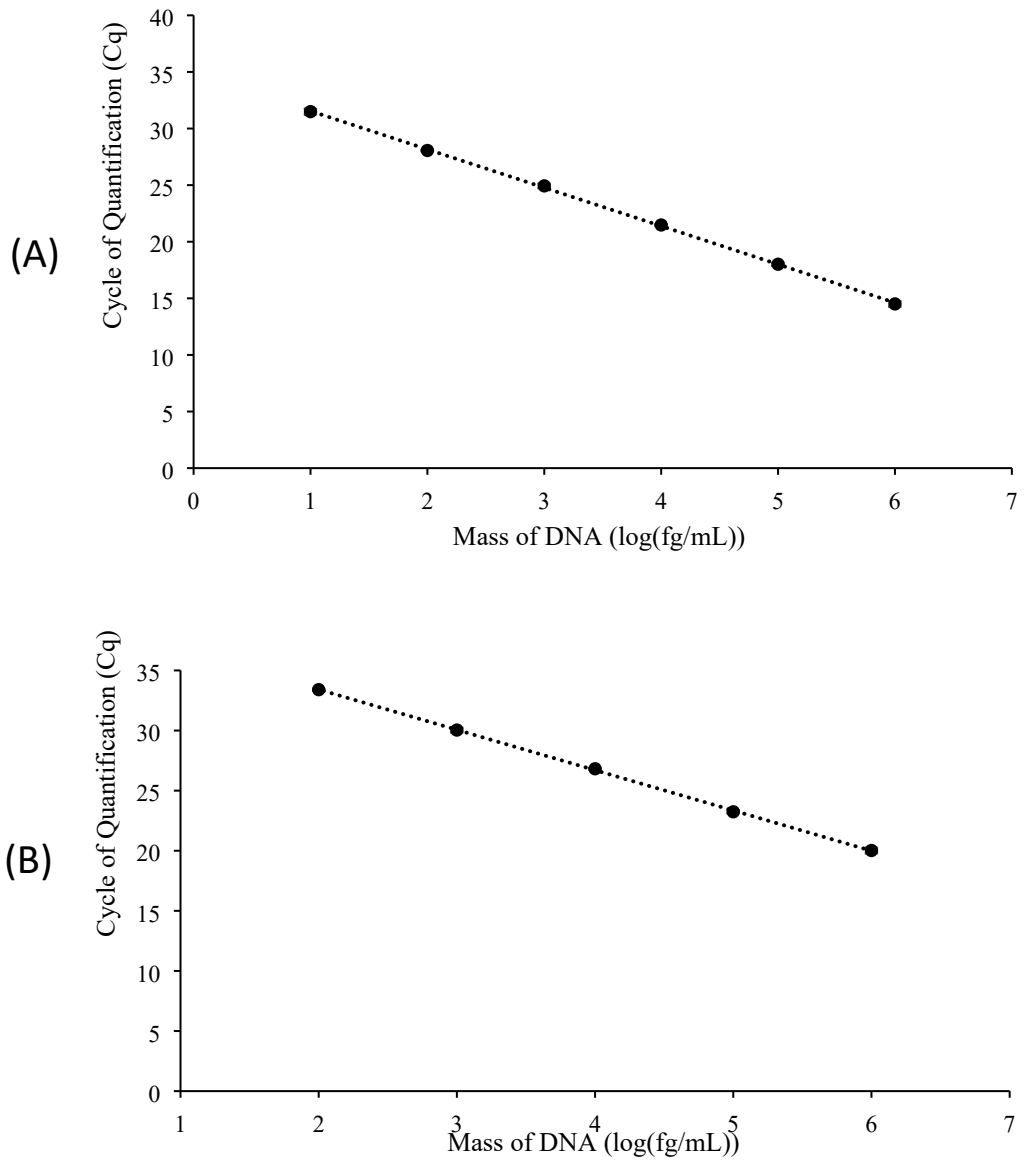

Figure S3. Calibration curves constructed for the quantification of two BRAF DNA templates in a 200 mM NaCl TE buffer (pH 7.50). A series of 10-fold dilutions (A) were constructed with purified 98 bp BRAF DNA stock solutions, and (B) a 830 bp BRAF DNA stock solution. The x-axis represents the concentration of DNA that is added into each qPCR reaction. The curves were used to convert experimental Cq values obtained by qPCR to mass concentration of DNA.