

Supplementary Material

Title: Quantitative Analysis of Sequential Nucleic Acid Elution from Silica Paramagnetic Beads

Authors: Asher Varon^a, Justin Schares^b, Kuangwen Hsieh^a, Bradley Downs^c, Tza-Huei Wang^{*a,c,d}

^aDepartment of Mechanical Engineering, Johns Hopkins University, Baltimore, MD 21218, USA

^bCorteva Agriscience, Johnston, IA 50131, USA

^cInstitute for NanoBiotechnology, Johns Hopkins University, Baltimore, MD 21218, USA

^dDepartment of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21218, USA

* To whom the correspondence should be addressed.

T.H.W.

3400 N. Charles Street, 200A, Shaffer Hall, Baltimore, MD 21218

Telephone: (410) 516-7086

Fax: (410) 516-7254

Email: thwang@jhu.edu

Table of Contents

Figure S1. Training dataset for the soybean lysate - trait 1 large oligonucleotide.

Figure S2. Training dataset for the soybean lysate - trait 2 large oligonucleotide.

Figure S3. Training dataset for the small synthetic, “gBlock” oligonucleotide.

Figure S4. Statistical analysis of oligonucleotide size elution decay constants.

Figure S5. Standard curves for singleplex PCR oligonucleotide samples.

Figure S6. Standard curves for duplex PCR of large oligonucleotides in presence of the small oligonucleotide.

Training Set: Large Soybean Genome - Trait 1

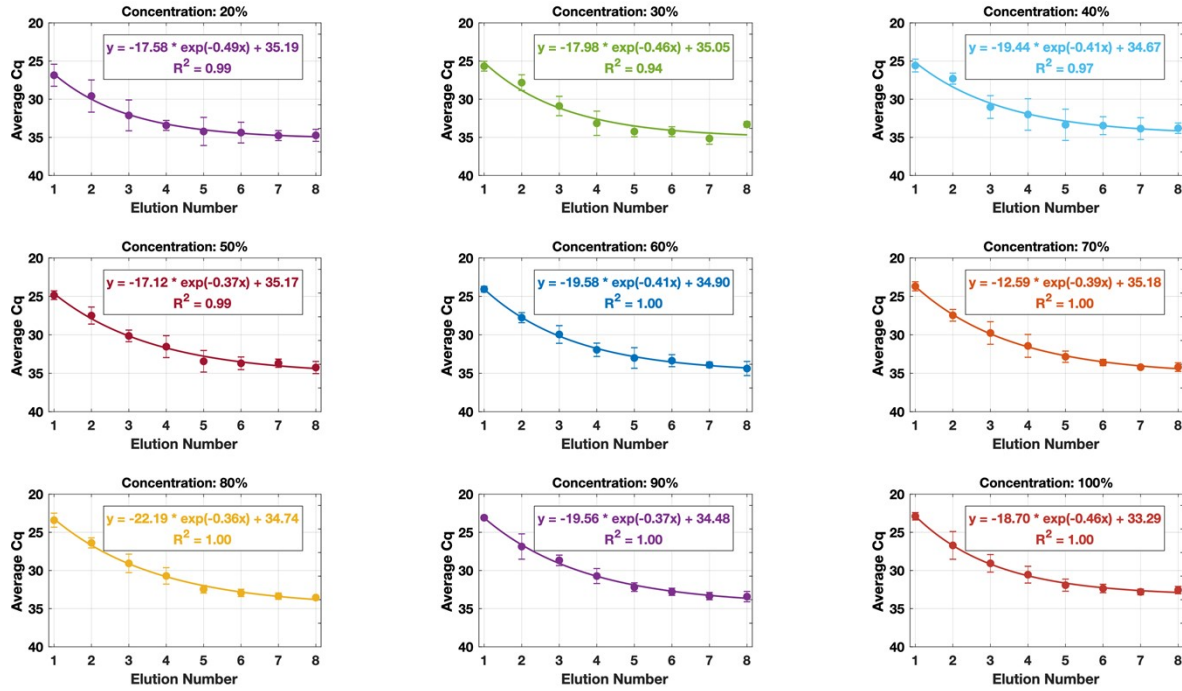


Figure S1: Training dataset for the trait 1 soybean genome. Trait 1 starting sample concentrations from 20%-100%, in 10% increments fitted to our hypothesized generalized model, as described in Equation 3, without fixed decay constants. Data represent mean Cq \pm SD from triplicate experiments (n = 3).

Training Set: Large Soybean Genome - Trait 2

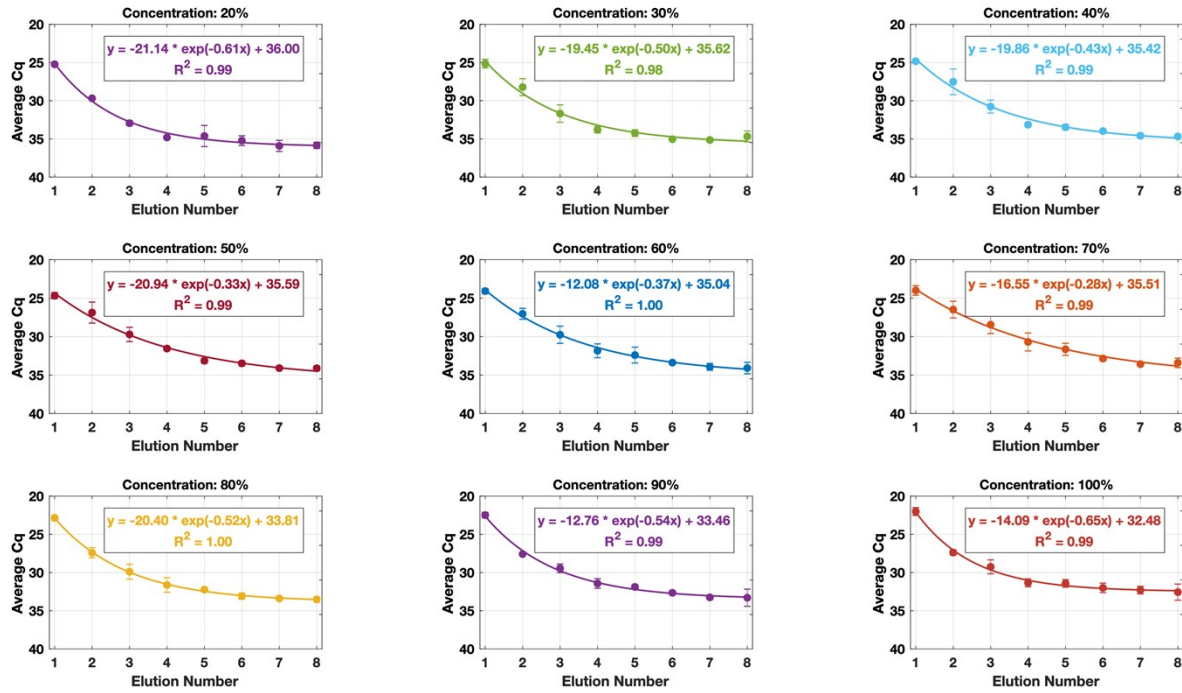


Figure S2: Training dataset for the trait 2 soybean genome. Trait 2 starting sample concentrations from 20%-100%, in 10% increments fitted to our hypothesized generalized model, as described in Equation 3, without fixed decay constants. Data represent mean Cq \pm SD from triplicate experiments (n = 3).

Training Set: Small Synthetic Oligonucleotide

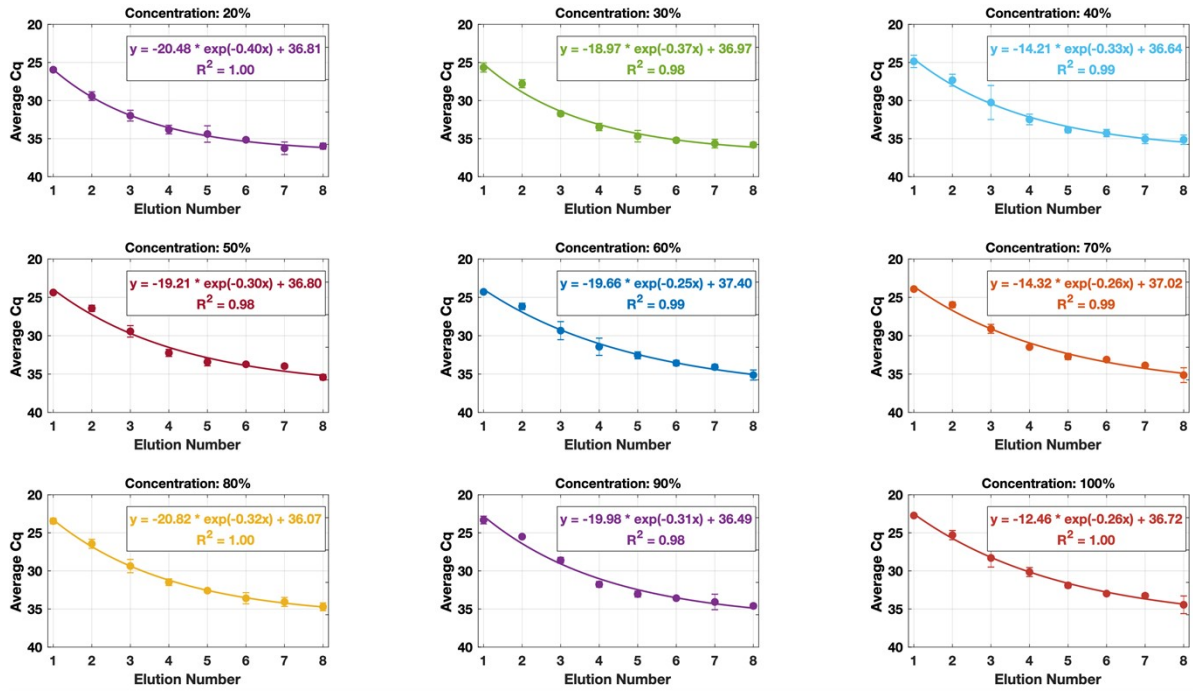


Figure S3: Training dataset for the double-stranded DNA fragment (gBlock). gBlock starting sample concentrations from 20%-100%, in 10% increments fitted to our hypothesized generalized model, as described in Equation 3, without fixed decay constants. Data represent mean Cq \pm SD from triplicate experiments (n = 3).

Comparison of Elution Rate

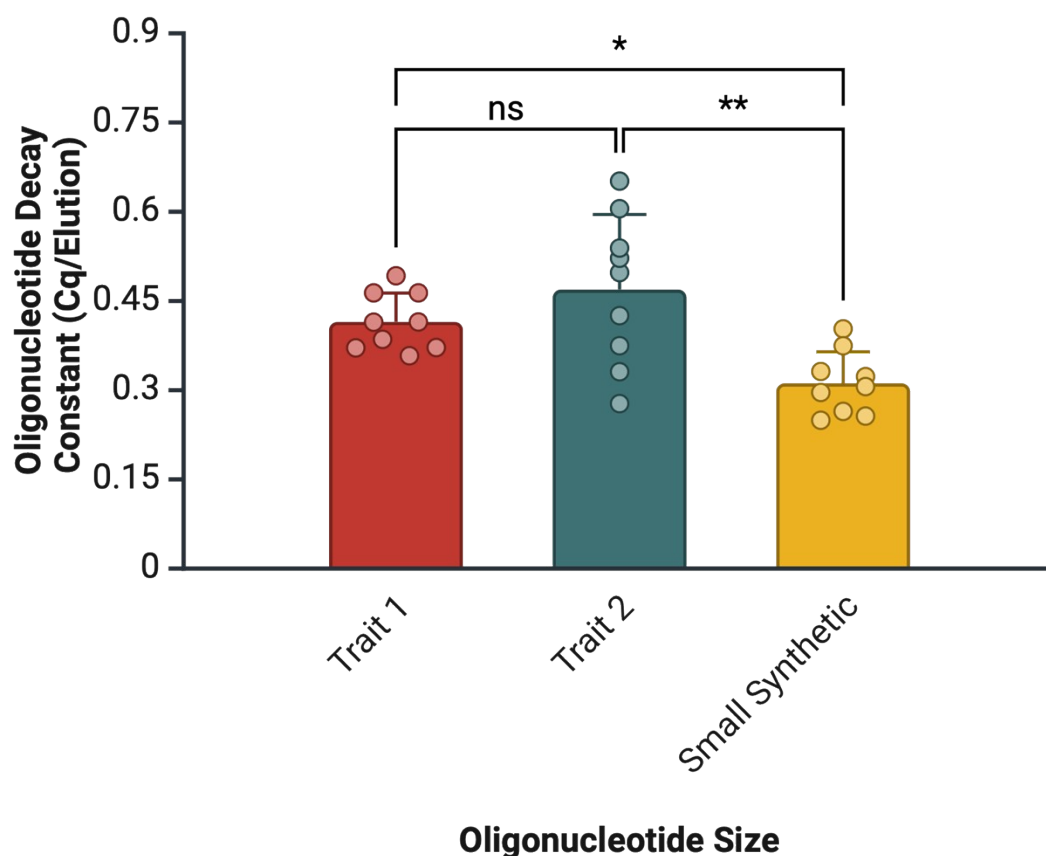


Figure S4: Statistical analysis of oligonucleotide size elution decay constant. Decay constants derived from the generalized models (Figs. S1–S3) were averaged for each target, and the resulting means were compared using the nonparametric Kruskal–Wallis test followed by Dunn’s multiple-comparisons post hoc analysis. Because each target comprised a small sample size ($n = 9$) and exhibited substantial variability in Cq measurements, normality was not assumed. Comparisons between the two soybean genome traits yielded an adjusted p-value of 1.0000, indicating no statistically significant difference. In contrast, comparisons between the double-stranded DNA fragment (gBlock) and the soybean genome targets yielded adjusted p-values of 0.03785 (trait 1) and 0.004022 (trait 2), respectively. The mean decay constant for each target was subsequently used for model validation.

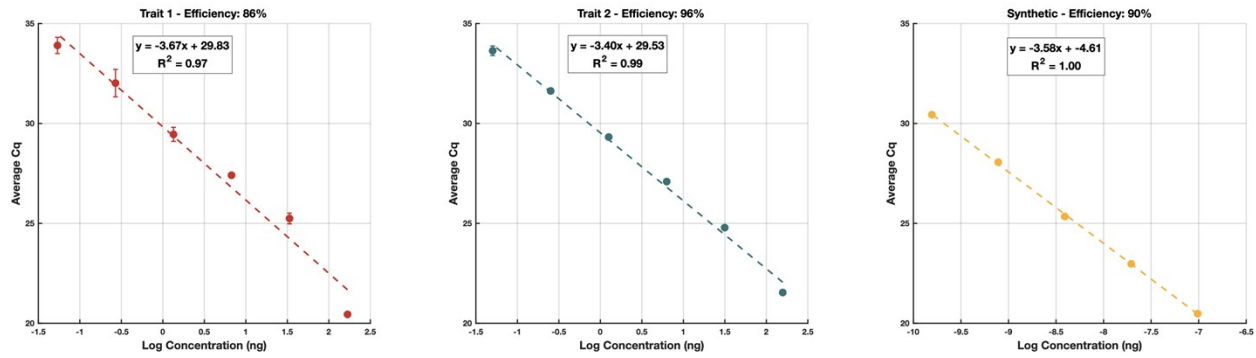


Figure S5: Standard curves for singleplex PCR targets. Subsequent five-point dilutions were performed for each target, and standard curves were fitted via linear regression. Soybean genomic DNA for trait 1 yielded a PCR efficiency of 86%, while trait 2 yielded an efficiency of 96%. The double-stranded DNA fragment (gBlock) yielded a PCR efficiency of 90%. Data represent mean \pm SD from triplicate experiments ($n = 3$).

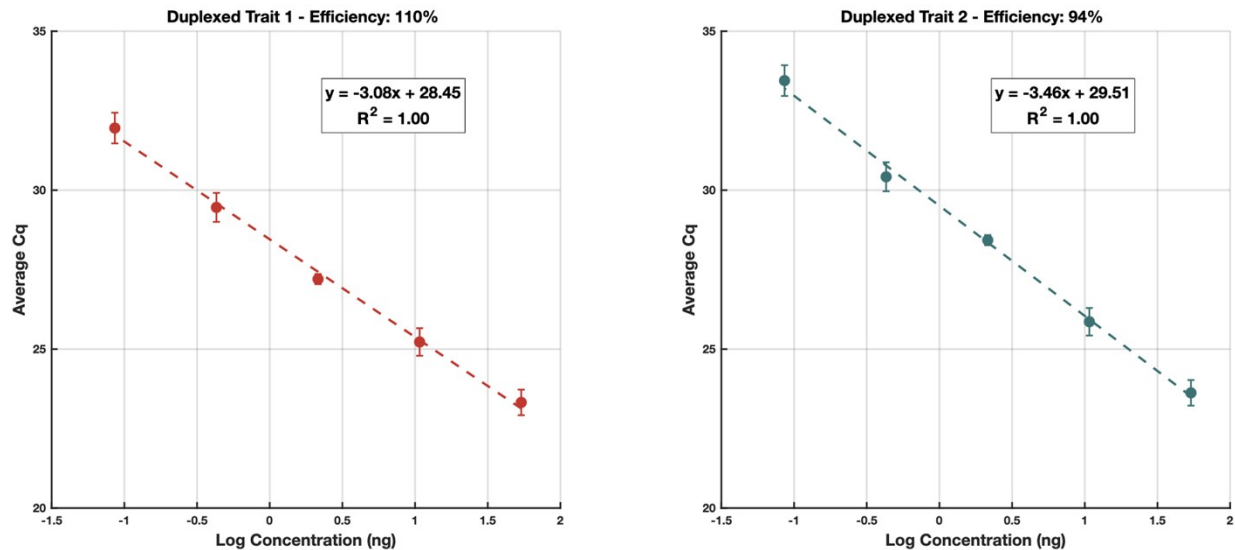


Figure S6: Standard curves for duplexed PCR of genomic DNA targets in the presence of the double-stranded DNA fragment (gBlock). Subsequent five-point dilutions were performed for each target in a duplex PCR format, and standard curves were fitted via linear regression. Trait 1 yielded a duplex PCR efficiency of 110%, while trait 2 yielded an efficiency of 94%. Data represent mean \pm SD from triplicate experiments ($n = 3$).