WHITE PAPER

Comparison of bacteriophage derived single stranded DNA scaffolds for assembling DNA origami nanostructures

DNA origami was introduced by Paul Rothemund in 2006 when he described the programmed folding of a long single strand of DNA (scaffold) into defined 2D DNA shapes using small single-stranded DNA strands (staples) to hold the final shape[1]. Using these general design principles, researchers and scientists in the DNA origami field have designed intricate 2D and 3D shapes on the nanoscale with incredible precision.

Recent research in this field has generated multi-component devices capable of machine-like movement and 3D structures capable of delivering drugs and other biomolecules in living systems. As DNA origami designs grow more intricate and have applications in day-to-day activities, the integrity of individual building blocks will be as important as the final function.

As an active participant in DNA origami research, Guild BioSciences saw a need for quality, cost effective commercially available ssDNA scaffold. Having a rich history in bacteriophage based research and products, Guild BioSciences seized the opportunity to meld our phage expertise with our interest in DNA origami to fill a need within the DNA origami community through our bacteriophage derived FOUNDATION™ ssDNA scaffolds.

Here we present the performance of Guild BioSciences' FOUNDATION™ ssDNA scaffold in assembly of DNA origami and compare it against M13mp18 ssDNA products from other commercial vendors in the United States of America.

Advantages over our competitors:

- High quality at a low price point
- Convenience of ready to use (100 nM) stock tubes
- Every production lot is sequenced to confirm identity
- A sample from each production lot is folded into origami structures to ensure functionality by the end-user
- Accelerated stability testing provides long-term consistency

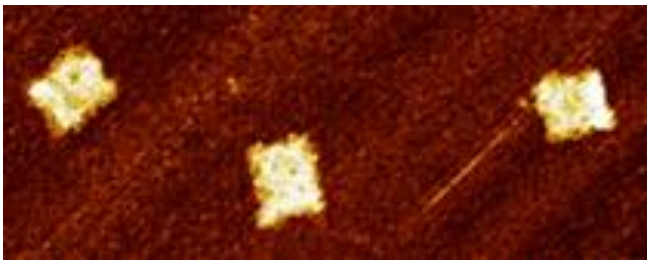


Figure 1. Atomic force micrograph of DNA origami rectangles folded using FOUNDATION™ ssDNA.

Results and Discussion

DNA origami folding reactions are commonly performed using a pH 8 buffer (Tris, EDTA, and $MgCl_2$) as well as scaffold and staple DNA in a 1:10 molar ratio. The original ssDNA scaffold, M13mp18, remains the most widely used scaffold across all published DNA origami research. In this study, three commercially available M13mp18 (7249 nucleotides in length) ssDNA products were analyzed (**Table 1**).

Table 1. Scaffold DNA used in this study

Vendor	Product Number
Guild BioSciences	D441-010-1mL100
New England Biolabs™	N4040S
Bayou Biolabs	P-107

A ready to use tube of scaffold DNA makes origami folding reactions quick and simple to assemble. Furthermore, it reduces the risk of erroneous concentrations due to fractional math required to set up reactions. One of the goals in the development of Guild BioSciences' FOUNDATION™ ssDNA scaffold was packaging at the ready to use concentration 100 nM. DNA tubes received directly from New England Biolabs™ or Bayou Biolabs were diluted to the ready to use concentration of 100 nM in 10 mM Tris, 1 mM EDTA pH 8 (TE) following the concentrations marked on the tubes and as indicated in product information.

One component of quality ssDNA scaffold is a pure DNA product at the indicated concentration. Figure 2 shows the UV absorbance spectrum of each ssDNA scaffold. The concentration of ssDNA can be calculated by absorbance at the 260 nm. DNA from Guild BioSciences and New England Biolabs™ had an absorbance at 260 nm (0.5 mm path) of at least 0.339, the expected value for a product at 100 nM. The 260 nm absorbance for Bayou Biolabs was 17% lower than expected based on the indicated concentration. On top of the 260 nm values, the spectrum allows for a qualitative analysis at purity of the product. In this spectral analysis one noticeable peak is visible centering at 310 nm in the New England Biolabs™ product. The identity of this contaminant is unknown.

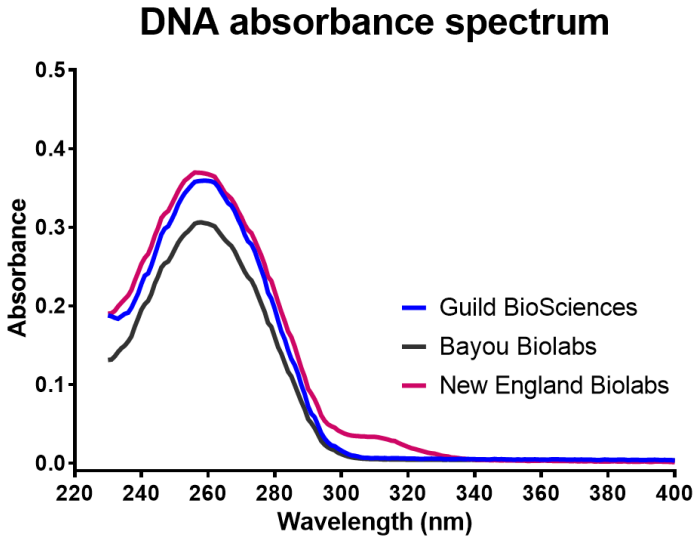


Figure 2. UV Absorbance spectrum of ssDNA products. The absorbance of ssDNA scaffolds was measured at 1 nm increments from 230 nm to 400 nm on a SpectraMax i3 reader using a low-volume (2 uL) slide and coverslip with a pathlength of 0.5 mm. DNA was standardized to 100 nM (224 ng/µL) using the manufacturers' indicated concentrations prior to analysis. Values displayed are after TE subtraction. Spectral traces are representatives of three replicates.

A second component of quality ssDNA scaffold is performance in a DNA folding reaction. This can be evaluated by assembling a simple structure with a proven set of staple strands in a thermocycler with known ramp parameters. The performance of each ssDNA product was assessed in TE buffer containing 12 mM Mg^{2+}, 20 nM scaffold DNA, and 200 nM staple strands to build a 2-dimensional rectangle. Detectable unassembled scaffold DNA may be indicative of contaminating DNA bearing a distinct nucleotide sequence or mutations within the M13mp18 sequence which cannot base pair with staple strands. Agarose gel electrophoresis is used to separate folded from unfolded products with scaffold DNA being a standard for comparison (Figure 3). All side-by-side reactions tested had no detectable unfolded scaffold DNA and Bayou Biolabs 2D rectangle origami displayed higher order structure up the top of the gel. In general, the FOUNDATION™ ssDNA product performs at least as well as New England Biolabs™ in this analysis. To examine the nanoscale differences in folding, atomic force and electron microscopy is used to visualize the structures. Two-dimensional rectangle origami structures were assembled under identical conditions using Guild BioSciences' FOUNDATION™ scaffold or Bayou Biolabs scaffold and extracted from the same agarose gel before analysis by atomic force microscopy (Figure 4). Rectangles assembled using FOUNDATION™ ssDNA appeared to have the most homogenous shape and size as well as smoother edges.

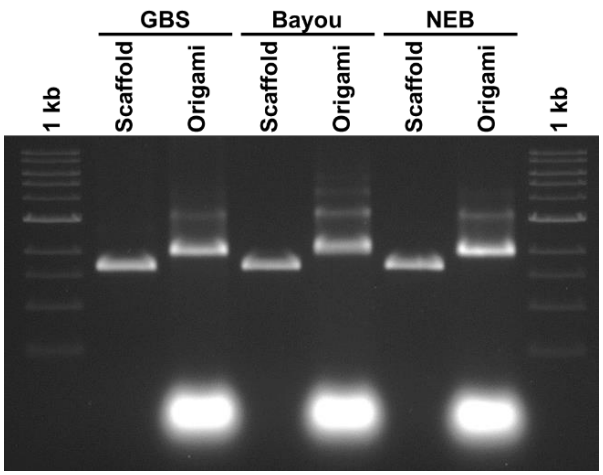


Figure 3. Agarose gel of assembled DNA origami nanostructures. Completed reactions to assemble rectangular DNA origami nanostructures were visualized on a 1% agarose gel with 11 mM Mg^{2+}. Origami rectangles are seen as bands migrating slower than scaffold. Free staples are bright, fast migrating bands. Scaffold lanes contain 0.2 pmol (448 ng) of product.

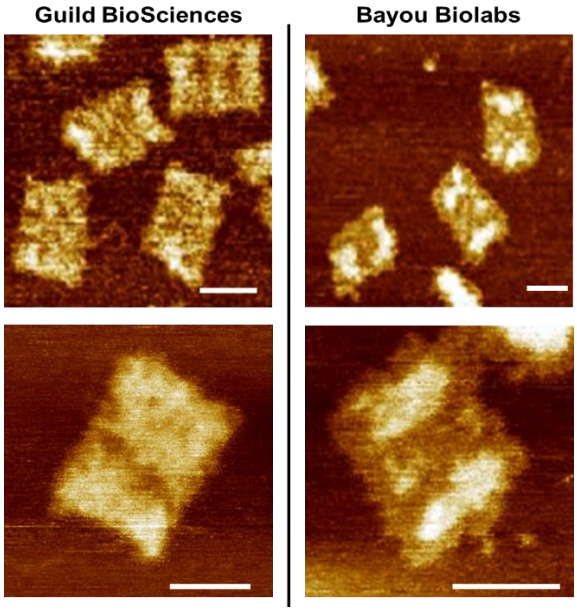
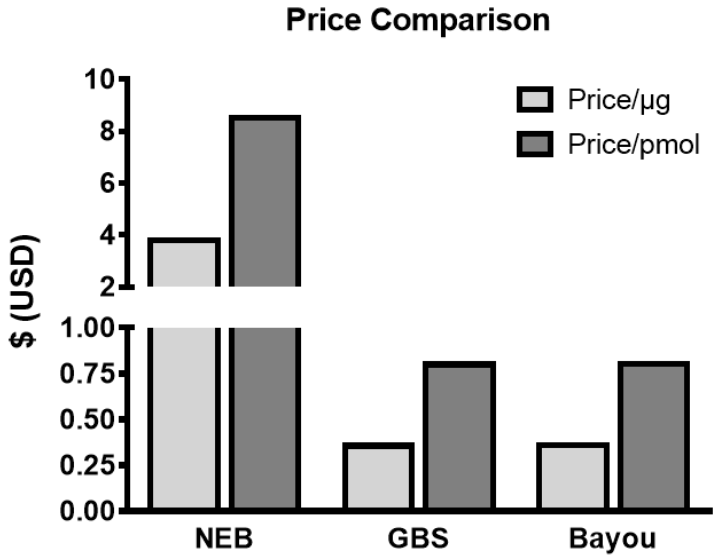


Figure 4. DNA origami folding comparison. Atomic force micrographs of rectangular DNA origami nanostructures assembled with Guild BioSciences' FOUNDATION™ ssDNA (left) or Bayou Biolabs M13mp18 scaffold (right). Reactions were assembled and processed simultaneously. Scale bar represents 50 nm.

Additional important criteria for a quality ssDNA scaffold are sequence identity, absence of both single and double stranded DNA nucleases, and proportion of ssDNA vs dsDNA in each preparation. All ssDNA scaffolds tested had complete sequence identity, exhibited no nuclease activity after 16 hours at 37 °C, and were completely digested by Mung bean nuclease, a ssDNA-specific enzyme.

On top of product quality, price is a major factor that drives consumer decisions. End users will be faced with the choice to make ssDNA scaffold in-house or purchase from a commercial source. While in-house production may appear to have a reduced cost, failed and inconsistent production may exceed the cost of a consistent commercial product. Furthermore, time spent by a technician, graduate student or post-doctoral scholar producing ssDNA is time that could be spent performing experiments and obtaining results. The price per microgram (µg) and pmol was calculated using each manufacturer's advertised price and the DNA concentration measured in the tubes used for this study (Figure 5). Pricing for Bayou Biolabs M13mp18 ssDNA product (P-107) reflects the reduced DNA concentration (15% less than stated) measured in two independent production lots.



Vendor	Price/µg	Price/pmol
New England Biolabs	$3.80	$8.51
Guild BioSciences	$0.36	$0.80
Bayou Biolabs	$0.36	$0.80

Figure 5. Price comparison by microgram and picomole quantities.

Conclusion

This study demonstrates that FOUNDATION™ ssDNA is a clean DNA product that meets all advertised criteria. It assembles into DNA origami nanostructures at least as well as competitors' products and costs over 1/10th of the price of New England Biolabs™ M13mp18 ssDNA product. To learn more or to discuss volume discount pricing, please contact Guild BioSciences at info@guildbiosciences.com or visit http://www.guildbiosciences.com/foundation_ssdna.

Methods

DNA Absorbance

All analysis was performed using one product lot purchased directly from each commercial supplier. Samples were stored at -20 °C upon receipt and thawed slowly at ambient temperature before performing experiments. Each ssDNA scaffold was diluted in 50 mM Tris, 1 mM EDTA, pH

8.0 to a concentration of 100 nM (224 ng/µL) using the manufacturers' indicated concentration. Absorbance was measured using a SpectraMax i3 (Molecular Devices) multi-mode plate reader. A low-volume slide and coverslip were loaded with 2 µL of TE or scaffold sample and absorbance was read in 1 nm increments from 230 nm to 400 nm. TE values were subtracted from scaffold values to give final absorbances.

DNA origami nanostructure assembly

Each M13mp18 ssDNA scaffold was mixed at 20 nM with 200 nM ssDNA staple strands in 50 mM Tris, 1 mM EDTA, 12 mM $MgCl_2$. Structures were formed through thermal annealing by heating to 65 °C for 10 minutes followed by slow cooling to 30 °C at -1 °C every 5 minutes. Structures were analyzed by 1% agarose gel electrophoresis in 1X TBE with 11 mM $MgCl_2$.

Atomic force microscopy

AFM images were collected at an independent laboratory on a Bruker AXS Dimension Icon atomic force microscope and ScanAsyst mode (Bruker Corporation). DNA nanostructures in excised agarose gels were purified before analysis using "freeze 'N squeeze" purification tubes (BioRad). A 5 µL drop of purified structure was deposited onto the surface of freshly cleaved mica and allowed to incubate for 2 min. The samples were then washed with 1 ml of ddH_2O and dried with compressed nitrogen. Images were collected using a ScanAsyst-Air silicon nitride cantilever with a measured spring constant of 0.79 N m^{-1} and nominal tip radius of 1 nm.

Reference:

1. Rothemund, P. 2006. *Nature* **440**, 297-302.

Ordering Information

Product	Cat. No.
7249 FOUNDATION™ ssDNA Scaffold (M13mp18)	D441-010-1mL100
7308 FOUNDATION™ ssDNA Scaffold	D441-020-1mL100
8064 FOUNDATION™ ssDNA Scaffold	D441-050-1mL100

Find out more at guildbiosciences.com/foundation_ssdna