**Phage-free production of artificial ssDNA with *Escherichia coli***

Karl Behler1\*, Hendrik Dietz2, Dirk Weuster-Botz1

*1Technical University of Munich, Institute of Biochemical Engineering
Boltzmannstraße 15, 85748 Garching, Germany
2Technical University of Munich, Institute of Biomolecular Design
Am Coulombwall 4a, 85748 Garching, Germany
\*Corresponding author:* *k.behler@lrz.tum.de*

**Highlights**

* Phage-free ssDNA production
* Custom ssDNA sequence for DNA-origami
* Mass production of ssDNA becomes scalable

**1. Introduction**

Scaffolded-DNA-Origami is a nanotechnology where, upon hybridization of ssDNA strands, predefined nanostructures can fold in a one-pot self-assembling reaction. For every so produced nano-object one long ssDNA-strand (scaffold) and multiple short ssDNA-strands (staples) are needed. Up to now the staples are chemically synthesized oligonucleotides and the scaffold is a variant of the single-stranded DNA-genome of the M13-phage. [1]

M13-phages can be produced in high cell density cultivations in stirred-tank bioreactors by infecting *E. coli* cells during cultivation [2]. The phages can be obtained from the culture-supernatant and their ssDNA genome can be prepared in a few simple steps. However most of the sequence is set to be the genome of the M13-phage and synthesis of the staples is very costly. Thus it would be interesting to be able to biotechnologically produce a custom ssDNA where most of the sequence is free to choose.

This can actually be done using a phagemid, a phage-plasmid-hybrid, which by default contains only the packaging sequence and the ori of the phage. All additional bases in the phagemid can be chosen freely. Phagemid-ssDNA can be produced by infecting *E. coli* cells carrying one phagemid with a helperphage or by double-transformation of *E. coli* with a phagemid together with a helperplasmid [3, 4] The first technique however will yield a mixture of helperphages and phagemid-particles which then leads to a mixture of ssDNA-species. On top helperphages are virions which might remain in the bioreactor even after sterilization, which makes production by contract manufactures impossible. The second technique gives only very poor yields and cells transferred to fresh medium don’t produce at all which makes storing of those cells and scale-up even to the liter-scale more or less impossible.

**2. Methods**

M13 genome was purchased from New England Biolabs and phagemid vectors (pBluescript) where received from Addgene. For construction of plasmids PCR amplicons (from Plasmid and M13 genome) where assembled using Gibson assembly [5]. For all experiments *E. coli* NEB turbo (New England Biolabs) have been used. All cultivations have been performed in minimal medium [6]. Cultivation in shake flasks was held at 37°C and 250 rpm in a wise cube shaker (Witeg). High-cell density cultivations were performed in a 2.5 liter stirred tank bioreactor KLF2000 (Bioengineering) using a fed-batch operating mode. Execution was adopted from Kick et. al. 2015 [2].

**3. Results and discussion**

As production of artificial ssDNA using a helperplasmid gives very low yields, decoupling of biomass formation and production had to be achieved. For this purpose *E. coli* carrying a helperplasmid were grown to an optical density OD600 of 0.4 and afterwards infected with phagemid-particles. This however yielded no measurable new ssDNA product. The result is most likely to be attributed to the presence of M13 protein pIII which as described before [7, 8] mediates a kind of infection resistance.

To prove the assumption in an experiment, pIII was put under the expression control of a repressible promoter. *E. coli* carrying a plasmid with this gene-III-cassette could be infected with M13 phages when gene-III-expression was not induced but could not be infected, when gene-III-expression was induced. Upon this result we moved gene III from the helperplasmid to the phagemid enabling the infection of *E. coli* cells carrying a helperplasmid without gene III by phagemid-particles.

Using this system biomass formation could be separated from ssDNA production where cells only carrying the helperplasmid could also be stored at -80°C without loss of production capability. Also with this system the yield could be increased two-fold in shake flask cultivations. More importantly though, as production with the conventional method is not scalable, this new method makes biomass production at any scale possible. Using a stirred- tank bioreactor at the liter scale in a fed-batch operation mode, we were able to increase volumetric yields about 30-fold compared to the conventional method.

**4. Conclusions**

By moving M13 gene III from the helperplasmid to the phagemid a system could be established, where *E. coli* cells carrying only a helperplasmid could be grown without producing any phage-like-particles. Upon infection with phagemid-particles these cells started producing new phagemid-particles. Through this temporal decoupling of biomass formation and phagemid-particle production, *E. coli* cells could (i) be transferred to a bioreactor without losing their ability to produce phage-like-partikles and (ii) *E. coli* cells started producing phagemid-particles upon infection with the latter. This resulted in a 20-fold increase of volumetric ssDNA yield.

**References**

[1] P. W. K. Rothemund, Nature, vol. 440, no. 7082, pp. 297–302, 2006.

[2] B. Kick, F. Praetorius, H. Dietz, and D. Weuster-Botz, Nano Lett., vol. 15, pp. 4672–4676, 2015.

[3] L. Chasteen, J. Ayriss, P. Pavlik, and A. R. M. Bradbury, Nucleic Acids Res., vol. 34, no. 21, pp. 1–11, 2006.

[4] F. Praetorius, B. Kick, K. L. Behler, M. N. Honemann, D. Weuster-Botz, and H. Dietz, Nature, vol. 552, no. 7683, pp. 84–87, 2017.

[5] D. G. Gibson et al., Nat. Methods, vol. 6, no. 5, pp. 343–5, 2009.

[6] D. Riesenberg et al., J. Biotechnol., vol. 20, no. 1, pp. 17–27, Aug. 1991.

[7] J. D. Boeke, P. Model, and N. D. Zinder, vol. 16, pp. 185–192, 1982.

[8] A. K. Brödel, A. Jaramillo, and M. Isalan, Nat. Commun., vol. 7, p. 13858, 2016.