SporeNet #3 - Universal Landing Pads

Universal access to basic tools in order to participate in the future bioeconomy is an important goal to achieve for biotechnology to benefit all people and the planet. However, current methods to distribute large quantities of DNA are costly or require expensive freezers.

A solution to those problems is the shipping of DNA in spores, in particular, *Bacillus subtilis* spores. These spores are able to survive for decades at room temperature on paper, and can be easily revived by putting the paper containing the spores in an environment where they can grow, such as into LB broth. If used, we can lower the cost of shipping and storage of DNA parts by many orders of magnitude.

A major problem with the widespread adoption of Sporenet is that plasmid backbones must be modified in order for them to replicate in *Bacillus subtilis*. I propose that we use a method that does not require any changes to current plasmid backbones in order to implement Sporenet, taking advantage of 3 technologies available in *Bacillus subtilis*: inducible natural competence[7] for simple transformation, manP negative selection[5] for antibiotic-less selection, and genomic DNA recovery[1] for getting back out DNA from *Bacillus subtilis*.

A plasmid is first integrated into the *Bacillus subtilis* genome using natural competence. The recombination flanks for integration are the antibiotic resistance marker encoded on the plasmid itself - this means that **any plasmid with a standard antibiotic resistance marker can be directly integrated into the *Bacillus subtilis* genome from a simple *Escherichia coli* miniprep** (most origin of replications for *Escherichia coli*, such as ColE1, do not function in *Bacillus subtilis*). In order to select for integration into the genome, the manP negative selection marker is used, which only requires adding mannose to the media.

The resultant strain, **if intended to be used with PCR, can be directly distributed, otherwise, a simple, cheap, in-vivo genomic recovery step allows us to shuffle any integrated plasmid into any shuttle vector** that we want to recover with. This strain, with the integrated plasmid inside of a shuttle vector, can then be itself distributed.

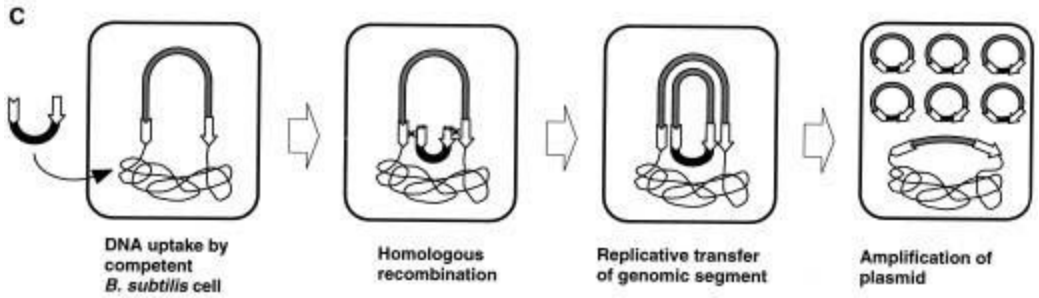

Fig 1C from “Recombinational Transfer of 100-Kilobase Genomic DNA to Plasmid in *Bacillus subtilis* 168”[1] demonstrating how genomic recovery works. Integrated plasmid DNA is colored grey, and an outside shuttle vector is colored black.

Theoretically, this system would allow for stable DNA storage and distribution without the use of antibiotics - however, we may want to keep at least one antibiotic resistance gene in our strains to prevent environmental contamination in less resourced laboratories. Optimally, this antibiotic should not be in the “WHO Model List of Essential Medicines” and the “WHO Model List of Essential Medicines for Children”[3] - for example, kanamycin[4].

I propose the synthesis of approximately 20 large DNA objects to test direct integration of *Escherichia coli* plasmids into the *Bacillus subtilis* genome using negative selection. We aim to answer the questions:

1. Can we directly integrate non-shuttle plasmids from *Escherichia coli* into the *Bacillus subtilis* genome with negative selection?
2. Can we recover those integrated plasmids using PCR or using genomic recovery?
3. Can we effectively distribute DNA in *Bacillus subtilis* without antibiotic selection?

Research Deadlines:
From the date of receiving DNA, we conservatively estimate the experiment will take 10 weeks:

Time	Experiment
2 weeks	Integration of constructs into *Bacillus subtilis*
2 weeks	Validation of integration of constructs into *Bacillus subtilis*
2 weeks	Integration of plasmid DNA into *Bacillus subtilis*
2 weeks	Validation of integration of plasmids into *Bacillus subtilis*
2 weeks	Test genomic recovery using PCR or a shuttle vector AND test distribution of spores with integrated plasmid DNA

The outcome of these experiments will be published after this period of time regardless of positive or negative results.

People:
Sporenet #3 was initiated by Keoni Gandall (EndyLab, Stanford).

IP Rights:
There are 3 technologies that enable this version of Sporenet: inducible natural competence[7], manP negative selection[5], and genomic DNA recovery[1]. Inducible natural competence is currently patented under US patent US8389283B2 [2], however, they only claim usage in *Bacillus licheniformis*, and so we would be free to operate in *Bacillus subtilis*. At the time of writing (March 11, 2020) I could find no patents covering mannose negative selection[5] or genomic DNA recovery.

I plan on using the REG120 *Bacillus subtilis* strain, which was received from the BGSC under permissive terms, so there should be no issues with material transfer[6].

References:

[1] “Recombinational Transfer of 100-Kilobase Genomic DNA to Plasmid in Bacillus subtilis 168” https://dx.doi.org/10.1128%2FJB.183.18.5453-5458.2001
[2] “Methods of obtaining genetic competence in Bacillus cells” https://patents.google.com/patent/US8389283B2/en
[3] https://www.who.int/medicines/publications/essentialmedicines/en/
[4] “Deletion of kanamycin and capreomycin” https://www.who.int/selection_medicines/committees/expert/22/applications/s6.2.4_kanamycin-capreomycin_MSF.pdf?ua=1
[5] “Development of a markerless gene deletion system for Bacillus subtilis based on the mannose phosphoenolpyruvate-dependent phosphotransferase system” https://doi.org/10.1099/mic.0.000150
[6] http://www.bgsc.org/getdetail.php?bgscid=1A1277
[7] “Construction of a Super-Competent Bacillus subtilis 168 Using the PmtlA-comKS Inducible Cassette” https://dx.doi.org/10.3389%2Ffmicb.2015.01431