(1) Submission ID#1527360

A modular plasmid system for expression of genes in Neisseria gonorrhoeae

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Background

Neisseria gonorrhoeae is naturally transformable and there are a variety of genetic tools available for constructing mutations, gene deletions, and complementation. Plasmids are available for the introduction of new genes of interest and complementation, but replicating plasmids are often unstable and transformation efficiency is low. The pEG2 plasmid designed by Christodoulides et al. (2000) includes the gonococcal cryptic plasmid and shows increased transformation efficiency and stability. We have designed a new modular plasmid cloning system for the expression of genes of interest in the gonococcus, including fluorescent proteins. This modular system is based on Golden Gate cloning using TypeIIS restriction endonucleases, allowing the assembly of complex multipart plasmids from reusable DNA parts.

Aim/Methods

Using synthetic biology, we aimed to create a series of parts for the simple and rapid assembly of a gonococcal plasmid, that is also capable of replication within Escherichia coli. These parts include the linearised gonococcal cryptic plasmid, various promoters, genes encoding kanamycin and erythromycin resistance, and fluorescent proteins.

Results

We have designed modular parts and demonstrate a simple and effective method for the assembly of gonococcal plasmids. As proof of concept, we created a plasmid containing the linearised cryptic plasmid, the opa promoter, GFP and a kanamycin resistance gene. We also created the same construct with mCherry in place of GFP and an erythromycin resistance gene in place of the kanamycin resistance gene. Upon transformation into E. coli, both mCherry and GFP constructs expressed both fluorescent proteins. Transformation into N. gonorrhoeae was efficient, with many positive transformants recovered as demonstrated by PCR and expression of fluorescent proteins by microscopy. The plasmid is stable in N. gonorrhoeae in the absence of antibiotic selection.

Conclusions

Using Golden Gate cloning we have created a new modular plasmid system for expression of genes of interest in N. gonorrhoeae. We have designed a series of synthetic parts including the cryptic plasmid that confers plasmid stability and increased transformation efficiency. Proof of concept plasmids constructed using this system express fluorescent proteins in both E. coli and N. gonorrhoeae. Next steps include utilising this system for complementation of deletion strains.