**INTRODUCTION**

Plasmids are a key tool in synthetic biology, however there is still a lack of means to create precise and flexible plasmid systems. Proper control of plasmid copy number, compatible multiple plasmid groups, and the ability to keep the plasmids stable at minimum eagerly anticipated in current synthetic biology. Here we present a novel framework for easy, flexible and standardized work with plasmids.

**PLASMID COPY NUMBER CONTROL**

RNA I can be used to regulate plasmid copy number. Yet, RNA II secondary structure is important for replication, making modifying RNA I promoter difficult.

**PLASMID GROUPS**

Different recognition sequences were added to the end of RNA I and RNA II loops to engineer independently controlled plasmids. Only A or D are full interfering, even loop sequences were modified for each new plasmid group, to ensure the best plasmid copy number.

**GLOBAL COPY NUMBER CONTROL**

Modulating the copy number of all plasmid groups at once can act as an additional global control parameter, and CoIE1 replication gives a perfect hint on achieving this goal as it codes a protein called Rop. The Rop protein stops a handle of RNA I molecules from interfering. A loop such as RNA I-II binds to Rop.

**ACTIVE PARTITIONING SYSTEM**

Low copy number plasmids can be easily lost during division. Plasmids coded proteins ensure plasmid stability in low copy number plasmids. Rop binding to the stem-loop complex increases the RNA II interaction affinity, consequently lowering the plasmid replication initiation rate.

**SYNORI SELECTION SYSTEM**

In multiple plasmid systems, different antibiotics for each plasmid group are commonly used.

**METHODS**

**PLASMID COPY NUMBER DETERMINATION**

Copy number was estimated using Real-Time PCR. Two qPCR standard curves were generated—one for a plasmid copy number device and another for a plasmid-specific gene. By employing different standard curves we were able to evaluate total bacteria and plasmid number in the qPCR reaction. The plasmid copy number per cell is thus found by dividing the total plasmid number by the cell number.

**STABILITY ASSAY**

The plasmid loss rate was investigated by patching single colonies from different plates on LB agar plates with antibiotic. Only cells containing plasmids grew on the plated regions. Plated areas with no growth indicated plasmid loss. The percentage of cells that had lost their plasmids was estimated by using the ratio of total patches to patches that did not grow any cells.

**REFERENCES**


**ACHIEVEMENTS**

1. Engineered a synthetic origin of replication which allows:
   a. Plasmid copy number control (in a constitutive, inducible manner)
   b. Creation of specifically controllable synthetic plasmid groups.

2. Constructed a global (group-unspecific) plasmid copy number device.

3. Characterized and applied an active partitioning system for low copy number plasmid stabilization.

4. Designed and constructed a selection system that can hold up to five different plasmid groups in the cell using only one antibiotic.