Low-mutation-rate, reduced-genome *Escherichia coli*: an improved host for faithful maintenance of engineered genetic constructs

Csörgő B, Fehér T, Tímár, Blattner FR, Pósfai G.
Microbial Cell Factories (2012), 11:11.

Presented by Queenie Chan
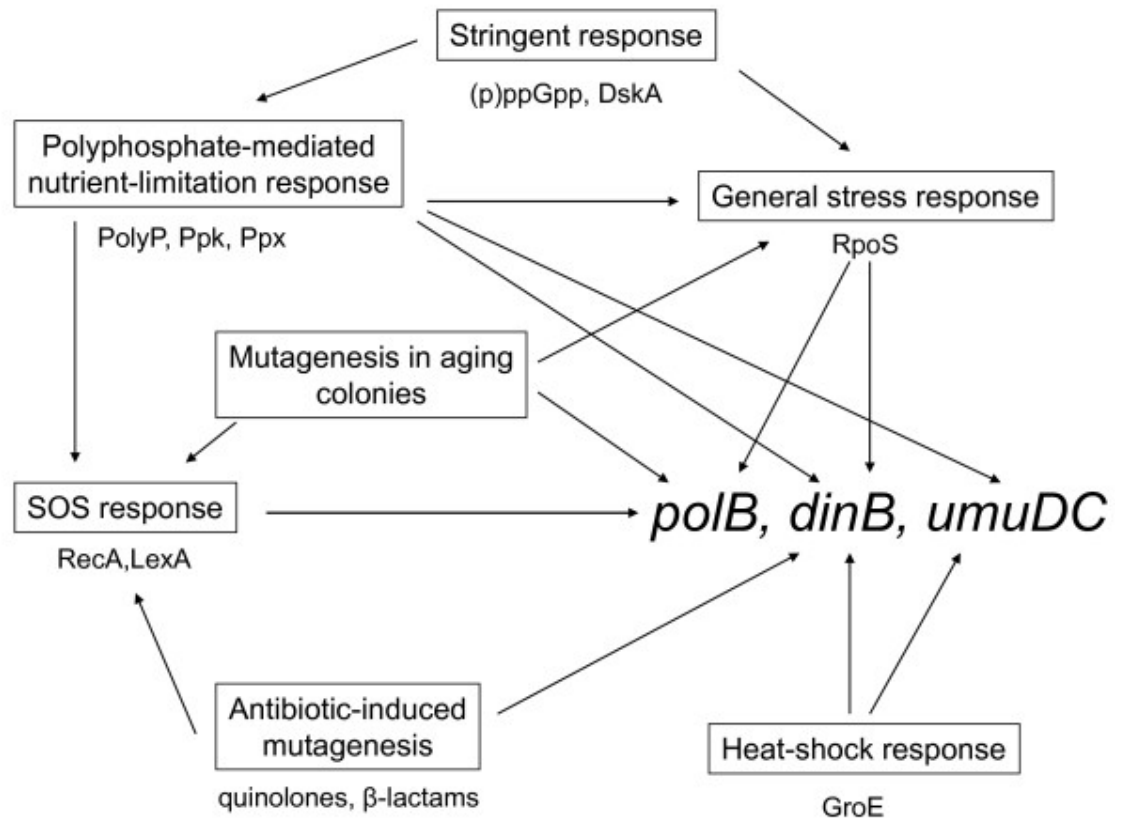
April 18, 2012

Background

Genetic variation

- In <u>nature</u>: leads to evolution & survival of the fittest

- In <u>lab conditions</u>: not favorable, can lead to unwanted genotypic and phenotypic alterations

How do mutations arise?

- Stress → SOS response
  - DNA polymerases: can bypass damaged sites and stalled replication forks; create mutations
    - Pol II
    - Pol IV
    - Pol V

Goal

- Create chassis that allows stable production of growth-inhibiting biomolecules for applications in synthetic and molecular bio

- Why growth-inhibiting biomolecules, specifically?
  - Mutants usually positively selected for

Project

- MG1655: wild-type *E. coli* strain

- MDS42: reduced genome *E. coli* strain
  - Genes irrelevant for lab applications (such as insertion sequences) deleted

I. Modifying MDS42 by creating variants that are more genetically stable

II. Evaluated variants under stressful conditions

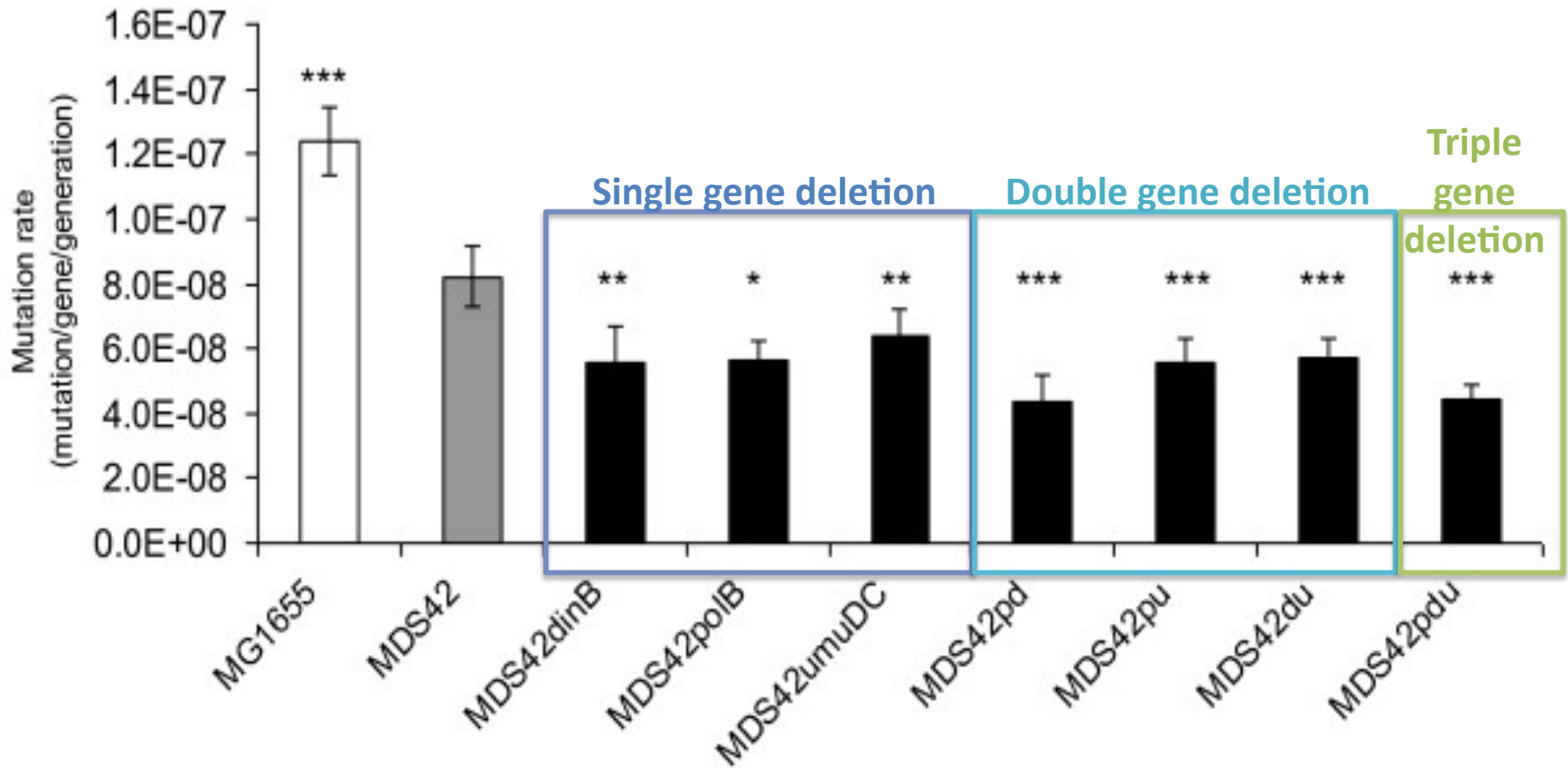III. Evaluated variants transformed with toxic-product-producing gene

Creating Variants

Measuring mutation rates of variants

Polymerase : *gene*
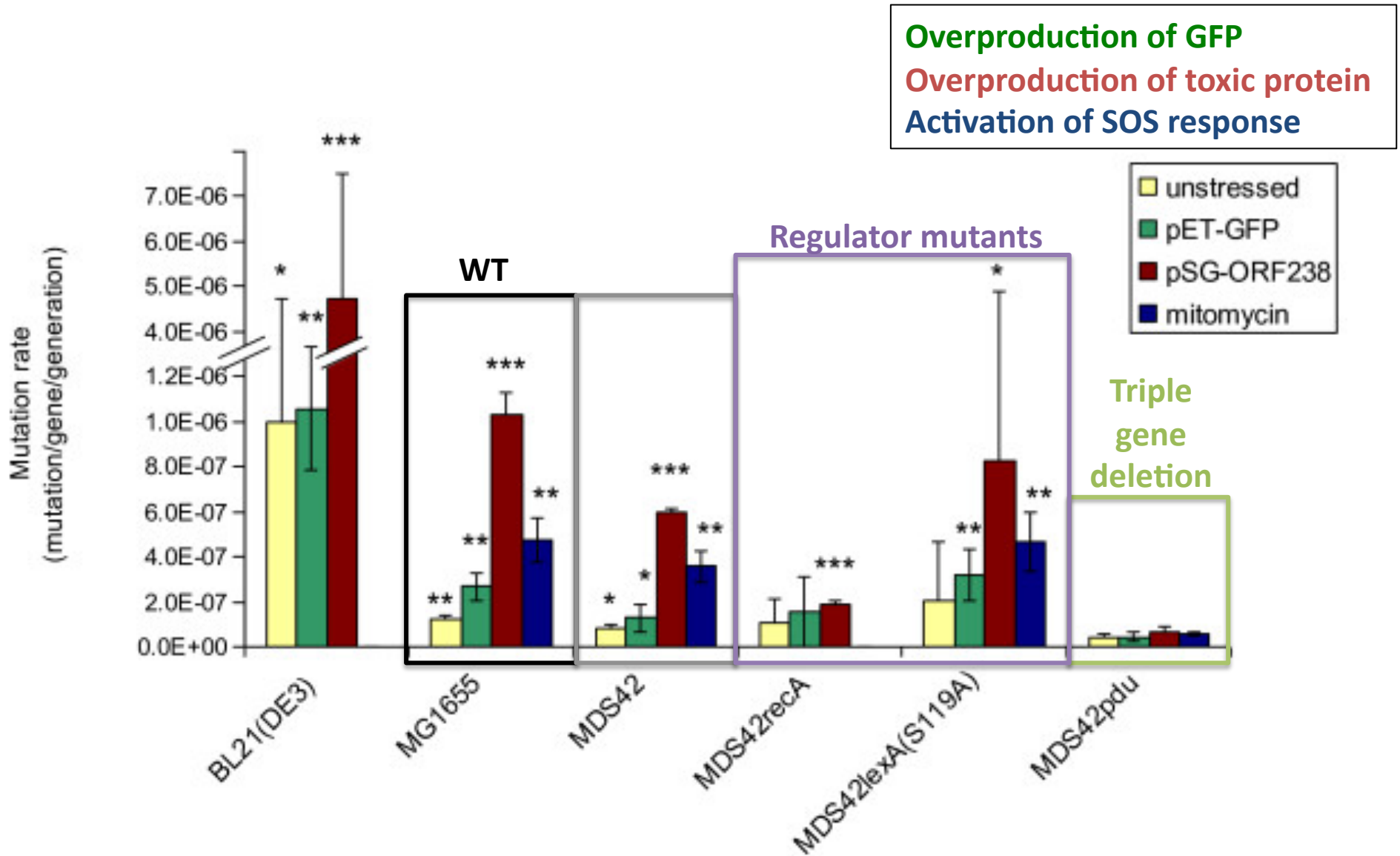Pol II : *polB*
Pol IV : *dinB*
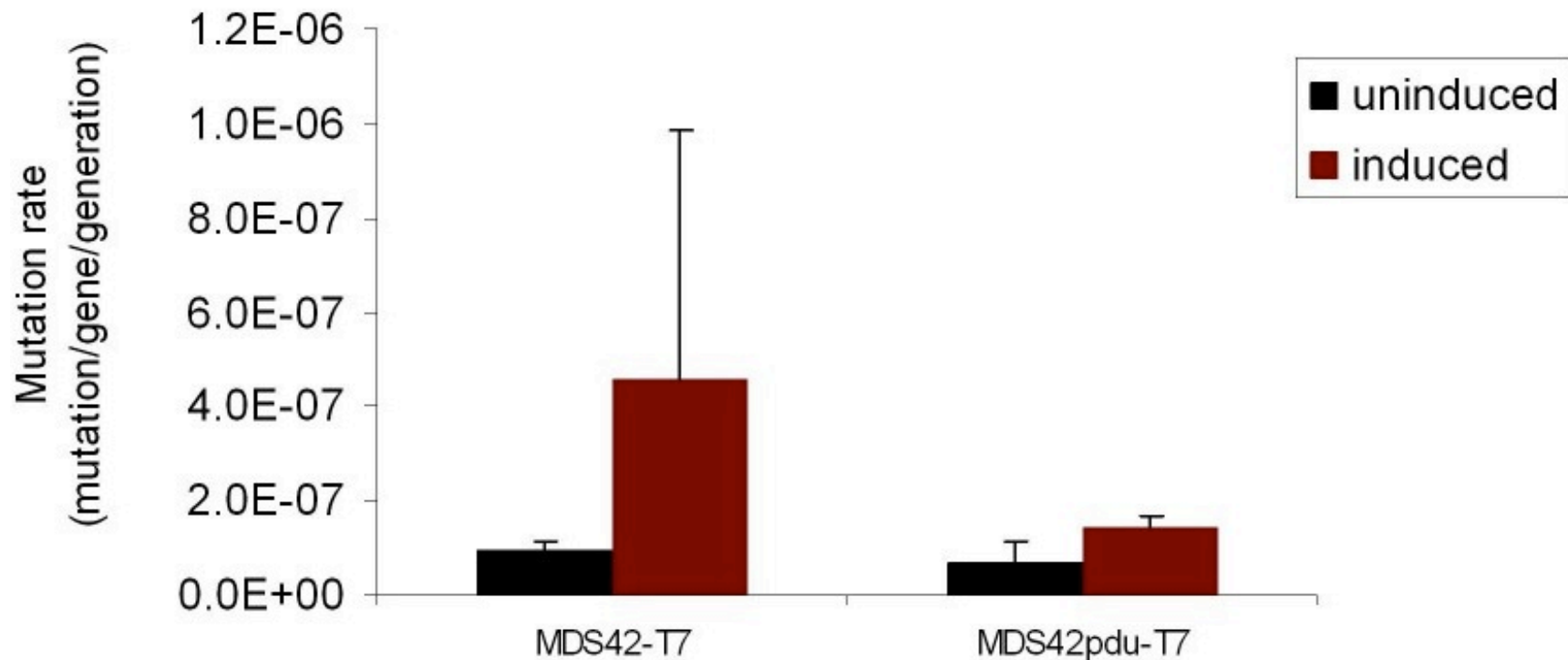Pol V : *umuDC*

* p < 0.05
0.001

** p < 0.01

*** p <

Evaluating under Stressful Conditions
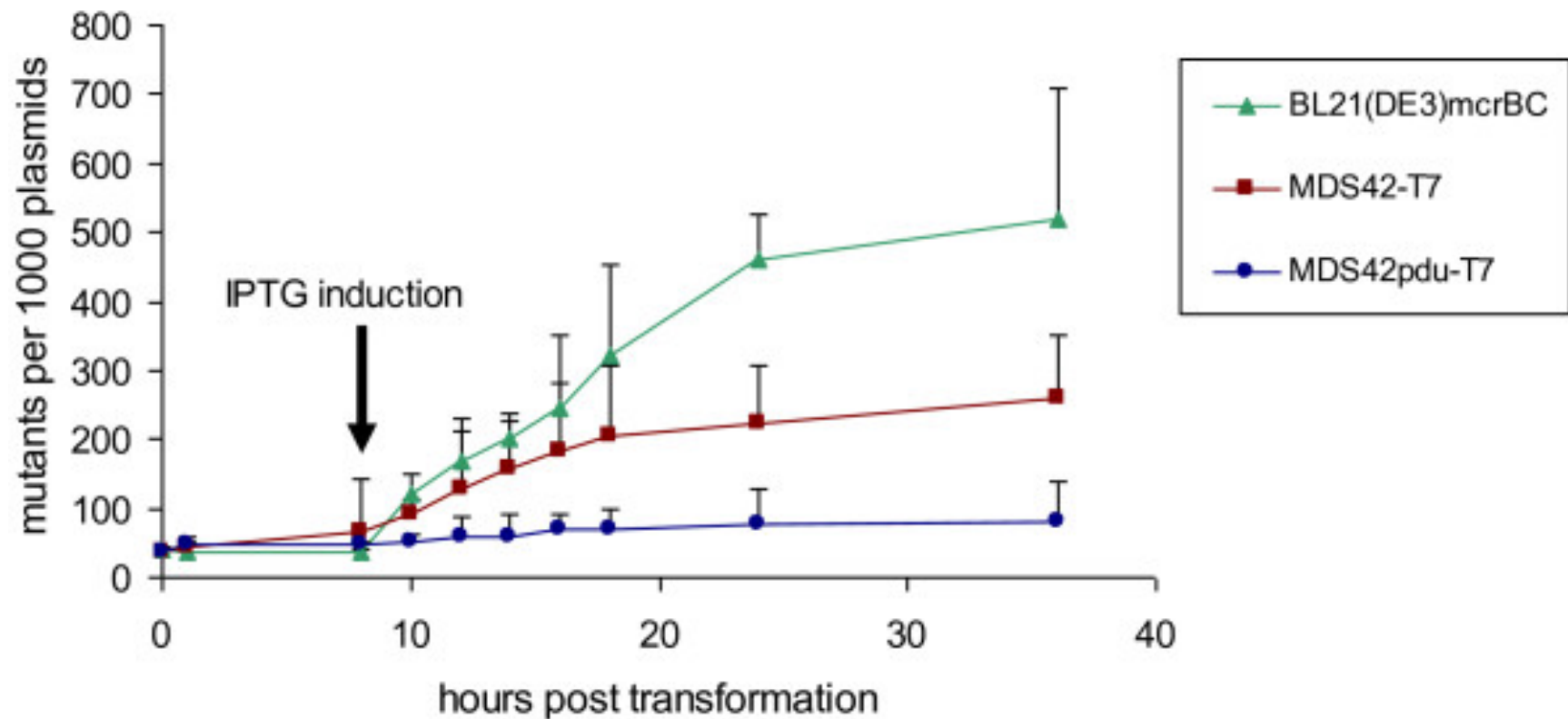
Evaluating with Toxic Protein Gene

pSin32   +   {
BL21(DE3)mcrBC
MDS42-T7
MDS42pdu-T7
}

Test with Toxic Protein Gene

Measuring number of mutants in different strains over time

Conclusion

- Eliminating 3 polymerases involved in SOS response significantly decreases mutation rates

- MDS42pdu most genetically stable variation of MDS42

  - Lowest rate of mutation in response to:

    - Stressful conditions
    - Production of toxic products

Significance

- Developed chassis that is able to stably produce growth-inhibiting biomolecules
- Applications:
  - Synthetic & molecular biology
  - Biotechnology
 - Cloning
  - DNA therapeutics

Concerns

- Explanation for MDS42pd's (double gene deletion) slightly lower mutation rate than MDS42pdu (triple gene deletion)

- Explanation for difference in MDS42lexA (S119A) and MDS42recA (regulator mutants)'s reaction to stress
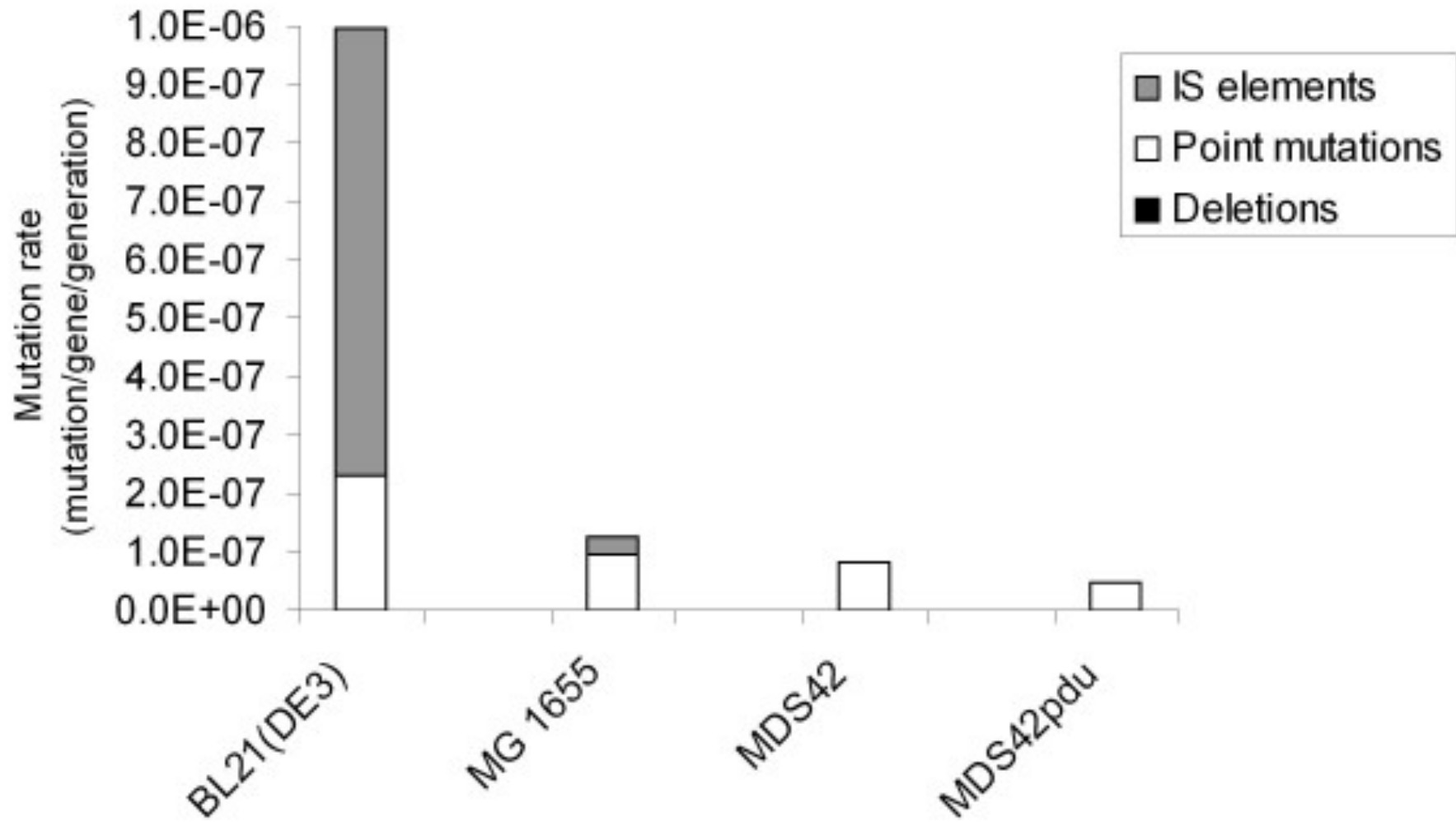
- Inclusion of BL21(DE3)

Questions?

Supplementary Slides:
Creating Variants

Verifying that variations do not affect normal cell growth
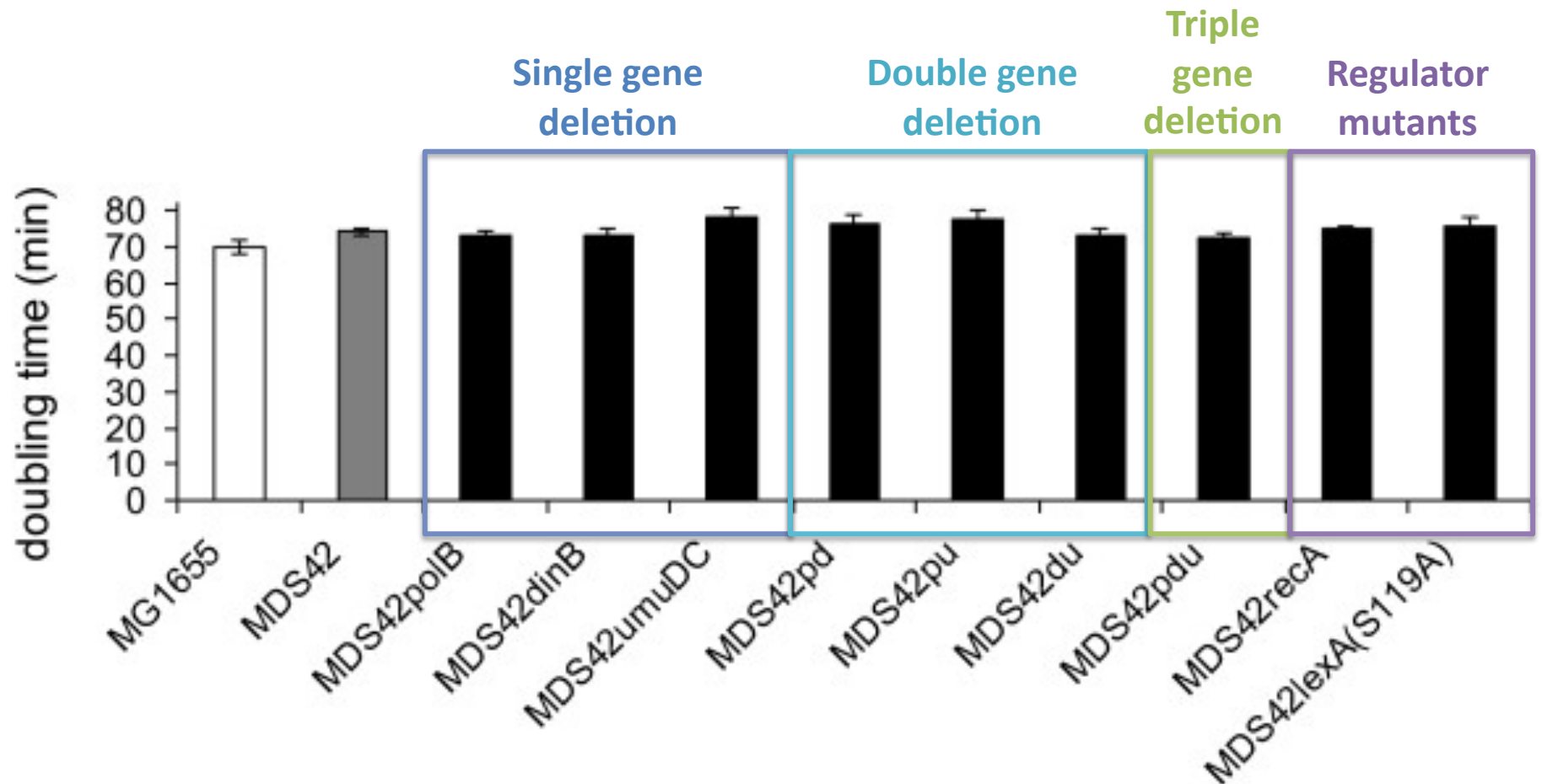
Supplementary Slides:
Characterizing Types of Mutations

Supplementary Slides:
Checking IPTG Inducibility of strains