Ceiling for engineering complex biological systems?

Some explanations:
- Not enough parts?
- Insulation?
- Rules for composition?

Figure from *Nat Rev Mol Cell Biol* (2009) 10(6):410-422

Genetic screen

Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype
Evidence for the loose conservation of the X-region in E. coli 2CS sensor kinases

Site-Directed Mutagenesis

Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype

Genetic screen
Observation
7/7 groups got >100 colonies with 20ul EP mix
5/6 groups got no colonies, even with 200 ul EP mix !!

What could be the explanation?

10/26/11

Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype
Step 4: Study sequence change, phenotype

Dideoxy Sequencing: “Sanger” Method

Primer
Template
ATTAGACGTCCG
TAATCTGCAGGC

Cocktail:
+ dNTPs
+ ddNTP
+ polymerase
+ buffer
+ αP32-dATP
Automated “one pot” sequencing

| Primer | Template ATTAGACGTCCG TAATGTCAGGC | Cocktail: +dNTPs +ddNTPs* +polymerase +buffer |

Genetic screen

Step 1: Mutagenize gene of interest
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Possible mutant “phenotypes”

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Beyond the C-dog

protein activity?
protein stability?
cross-talk with other systems?


Summary