EvoStat (PACE) Protocol

Huxley Run #2 January 7-11 2019

January 2019

Su	***Mo***	***Tu***	***We***	***Th***	***Fr***	***Sa***
		1	***2***	***3***	***4***	***5***
6	***[7***	***8***	***9***	***10***	***11***	***12 ...]***
13	***14***	***15***	***16***	***17***	***18***	***19***
20	***21***	***22***	***23***	***24***	***25***	***26***
27	***28***	***29***	***30***	***31***		

[Notes]

7 L of LB (2X autoclave. Final cycle 45-min)

Kanamycin (water) **:** 30ug/mL → 30mg/L → 210mg/7 L
JW5856 requires Kanamycin for the TRXA- "deletion" which is an insertion of kanamycin resistance gene.
(30ug/mL is from Baba et al. 2006)
Tetracycline : 10ug/mL → 10mg/L → 70mg/7 L
(for F+ maintenance)
Chloramphenicol *(either, but ethanol better)***:** 25ug/mL → 25mg/L → 175mg/7 L
Carbenicillin (water) **:** ***60ug/mL → 60mg/L → 420mg/7 L***

Glucose 175mL 20% by weight = .45% in 7 L (27mM)

HG-Selection Run in EvoStat: repeat of the previous run but with better flow control.

Cells: JW5856:t3trxA:MP6

Phage: M13T7rnaP
Ref: Jiarui's lab report of Nov 13.

1) Grow JW5856+MP6 to 0.5 OD in chamber 1.
2) Transfer 40 ml from chamber 1 to lagoon
3) Add sufficient arabinose to bring the lagoon to 0.3% arabinose
4) ?? alternative here is to use ATc at 30 ng/ml
5) Add M13RNAP to the lagoon at an MOI of about 1.0 (previously used 0.5)
6) Start the run with flow through the lagoon at 30 ml per hour

Autoclave nutrient (LB) twice over the weekend, added Kanamycin, Tetracyline, Carbenicillin, Chlorophenicol, and 25mM Glucose (175mL of 20% by weight for 7L).

Inoculate with cells Sunday Evening
Infect with Phage Monday.

[Previous run had one day sterility test, room temperature, without antibiotics]