Assembling : bistable-> pSB1A2 & pSB6A1

Resource:
Bistable, pSB1A2 and pSB6A1 form Min Lin.

October 1st
Plasmid mini prep:
Bistable, pSB1A2, pSB6A1;

Double digest:
Bistable, pSB1A2, pSB6A1: Pst1 1uL, Xba1 1uL, plasmid 10uL, Buffer 2uL, water 6uL
37 ℃ 4 hour

Gel electrophoresis:
Products of double digest.
Separate the inserts and vectors.

DNA Gel purification:
Insert of bistable;
Vectors of pSB1A2 and pSB6A1;

DNA ligation:
System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ 2 hour

Transformation:
Products of ligation (bistable-pSB1A2, bistable-pSB6A1), competent cells 50uL each, Smear to LB plate with Amp

October 2nd
Result:
There are more than 100 colonies on each plate. I picked 6 of them to liquid LB to cultivate overnight to confirm the clonings.

October 3rd
Plasmid mini prep:
Bistable on pSB1A2 (6 different clonings);
Bistable on pSB6A1 (6 different clonings);

Double digest: to check the results
Bistable on pSB1A2 & pSB6A1: Pst1 1uL, Xba1 1uL, plasmid 4uL, Buffer 2uL, water 12uL

37 ℃ 2 hour

Gel electrophoresis: to confirm
Products of double digest.
Separate the inserts and vectors.

Function:
Most of colonies on the cloning plates became green on Oct. 3rd, which means the insert is Bistable, because the Bistable is on the CI434 side is green. This phenomenon proved that the clonings: transfer Bistable to pSB1A2 and pSB6A1 are successful.

By Shuke Wu

Assembling (three plasmids strategy):

Resource:
Bistable on pSB1A2 and pSB6A1 form me;
AND gate: the best AND gate us had constructed (pBad-SupD, pSal-T7ptag, RBS B0033);
CI on pSB3T3: from Haoqian Zhang

October 4th
Transformation: put bistable into E.coli
Plasmids: Bistable on pSB1A2 and pSB6A1,
Plate on Amp LB plates.

October 5th
Prepare competent cell
E.coli contained bistable plasmid,

Transformation: put AND gate into E.coli (contained bistable)
Plasmid: AND gate,
Plate on Amp & Kan LB plates;

October 6th
Prepare competent cell
E.coli contained bistable & AND gate plasmids,

Transformation: put AND gate into E.coli (contained bistable & AND gate)
Plasmids: six different T7-CI,
Plate on Amp, Kan & Tet LB plates;

October 7th-10th

Inducing test:

Then the colonies contained three plasmids were picked into 3 mL liquid LB and incubated until the OD600 value reached 0.4~0.6. At this time, we used different combinations of different concentration of arabinose and salicylate to induce. Every two hours we discarded 2mL LB culture, and added 2mL new LB into it, and the concentration of inducers should be kept. Repeating this step for 3 times, and then cultivated the culture overnight to ensure saturated induction and interaction between different modules. The results were tested by flowcytometry.

By Shuke Wu