Look What They've Done To My Shoes!

SCU_China Project 2016

Protocol

Knockout Team

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Cecropin XJ Expression TeamRED protocol

1. Primers designing.

2. Amplify the drug-resistant gene with homology arms.

PCR. Using primer A as primer and pKD3 plasmid as template to build the Biobrick of a chloramphenicol-resistant gene with a homology arm on each side. Gel extraction was performed to purify DNA fragment.

3. Transforming PKD46 plasmid into the competent cells

[Notice] (1) Screening with Amp for positive colonies. (2) All the cultures are performed at 30 degrees centigrade.

4. Formation of electro-transforming competent bacterial cells

Vaccinate the bacteria from step 3 at 1% concentration and culture for 1 hour at 30 degrees centigrade. Then add in L-arabinose (2% as final concentration) to induce for at least 90 minutes, forming electro-transforming competent bacterial cells. Add 10% amount of glycerol and make sure the total volume is under 100ml.

5 Electro-transformation

Chill down the electro-transformation cup in advance, and thaw the competent cells on the ice. Then add in the regenerant of gel extraction in step 2, mix up, move onto the ice in electro-transformation cup, stay for 10 minutes, and then start the electric shock.

[Notice] 1. The amount of the regenerant added into the cells depends on its concentration. Generally we add 2 to 6 microlitres, the higher the concentration is, the less we add. 2. Act rapidly when perform electrotransformation.

6. Screening for strains.

Spread bacterial fluid on chloramphenicol plate at 30 degrees centigrade, and pick single colonies into liquid culture medium, shake for 8 hours. Then adjust the temperature to 42 degrees centigrade. Continue to culture for 6 hours, and then perform PCR to figure out the possible aim strain, and use glycerol to conserve. Figure out the aim strain by sequencing.

7. Analyzing the concentration of indole and methylindole in the aim strain with HPLC