2018-7-5

●Transformation and plate coating for bacteria

1. Purpose of the experiment: to introduce the constructed plasmid into DH5αto verify the success of the construction.

2. Experimental equipment

2.1. Reagent: one step cloning product, DH5α, LB liquid medium (37 ℃ preheating), LB solid medium (containing Amp).

2.2. Instruments: liquid transfer guns and gun heads, EP tubes, ice box, metal incubator, glass bead, 37℃ shaking bed incubator, 37℃ constant temperature incubator, centrifuge, alcohol lamp, etc.

3. Experimental steps

10 μl one-step cloning product (experimental group and two negative control groups) and 50 μl DH5α receptive bacteria solution were mixed into EP tubes. After 30 minutes in the ice box, 90 s in the metal incubator at 42℃, then 2 to 4 minutes in the ice box, 940 μl 37℃ pre-heated LB liquid medium was added to the EP tubes, then incubated in 37℃ shaking bed incubator for 1 hour, then 12000rpm centrifuged for 1 minute. The liquid transfer gun was used to remove 800 μl supernatant next to the alcohol lamp, the remaining culture medium was mixed with the bacteria, and then 50 μl bacterial solution was added to the LB solid medium (containing Amp), pour a certain amount of glass beads and shook constantly to smear the liquid evenly on the surface of the medium, then the glass beads were poured out and labeled on the petri dish and cultured overnight in a constant temperature incubator at 37℃.

2018-7-7

●Colony PCR

1. Purpose of the experiment: to detect whether the selected transformant is a target transformant.

2. Experimental equipment 2.1. Reagent: template DNA (1~8), ddH_2O, 10mM primer-F, 10mM primer-R, Green Taq Mix.

2.2. Instrument: ice box, PCR tubes, gradient PCR instrument, liquid transfer guns and gun heads, etc.

3.Experimental steps

The alcohol lamp was ignited. Next to the lamp, 20 μl ddH_2O was added to the PCR tubes with the liquid transfer gun. Then the single colony was mixed with the ddH2O in the PCR tubes from the experimental plate of the DH5α+LacI-P_{lac}-pGLO bacteria.Each solution was added to the PCR tube with the liquid transfer guns according to the following requirements. After shaking up, the solution was put into the gradient PCR instrument to carry out the PCR.

PCR dosage/μl	DH5α+LacI-P_{lac}-pGLO
Templet DNA	1
10mM primer-F	0.4
10mM primer-R	0.4
Green Taq Mix	5
ddH_2O	3.2

total volume	10

PCR condition setting		DH5α+LacI-P_{lac}-pGLO
Step one		95℃ 120s
30 cycle	Step two	95℃ 30s
	Step three	51℃ 30s
	Step four	72℃ 90s
Step five		72℃ 600s

●Detection of PCR by the electrophoresis gel

1. Purpose of the experiment: to detect whether the selected transformant is a target transformant.

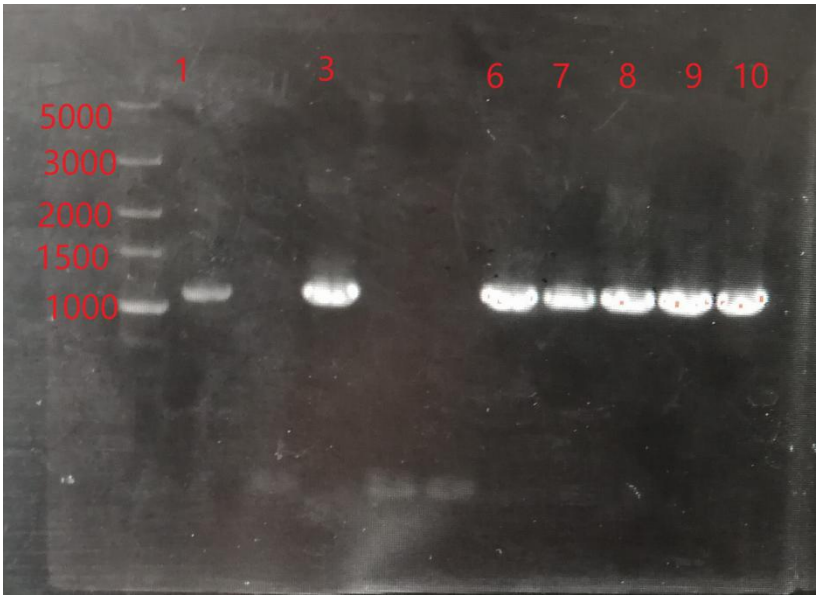
2. Experimental equipment

2.1. Reagent: the colony sample solution after PCR, 1×TAE, agarose, 4S Red Plus Nucleic Acid, 5000bp DNA Marker.

2.2. Instrument: liquid transfer guns and gun heads, conical bottle, cylinder, electronic balance, microwave oven, gel plate, electrophoresis apparatus, ultraviolet analyzer, etc.

3. Experimental steps 20ml 1×TAE was poured into the conical bottle, 0.2g agarose was added to the conical bottle, then heated in the microwave oven to dissolve. When the temperature was reduced to could be touched by hand, 1μl 4S Red Plus Nucleic Acid was added and shake well. Then pour it into the gel plate to be cooled and solidified, then put it into the electrophoresis apparatus, 4μl the liquid to be tested was added to the corresponding hole in the gel plate. After all the samples had been added, added 4μl 5000bp DNA Marker in the hole without the sample. Then turned on the power, and when the samples ran to the back half of the block and was close to the end, turned off the power, put the block into the ultraviolet analyzer, and observe the gel electrophoresis results.

4. Experimental result



2018-7-8

We selected ideal colonies and began to test the effectiveness of the repression system.