Look What They've Done To My Shoes!

SCU_China Project 2016

Protocol

Leucine Team

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Quantitative PCR

Materials

1 Tranzol up (transgene ET111)

2 TransScript All-in-One First-Strand cDNA Synthesis SuperMix for Qpcr (One-Step gDNA Removal)(Transgene AT341)

3 TransStart Top Green qPCR SuperMix

4 BL21 or DH5a containing overexpression vectors

5 qPCR machine

Procedures

1 inoculate microbe into 1.5 ml EP tubes and shaking overnight

2 8000g*4℃*2min centrifugation

3 discard supernatant and add 1 mL Tranzol up

4 then all procedure are done according to the protocol of Tranzol up product

http://www.transgen.com.cn/attached/down/ET111-01_2016090213.pdf

5 dissolve the RNA with 30 ul RNase free water

6 5ul of RNA are used in electrophoresis and 5ul are used to reverse transcription

7 reverse transcription

1 5ul RNA

2 4ul transcript buffer (Transgene AT341)

3 1ul gDNA remover

410ul RNase free water

Incubate in 42℃ for 15min and 85℃ for 5 sec

8 qPCR

1 2ul buffer from reverse transcription as substance for qPCR is added in tubes and other components are added according to protocol of the product.

http://www.transgen.com.cn/attached/down/AQ131-01_2016090115.pdf

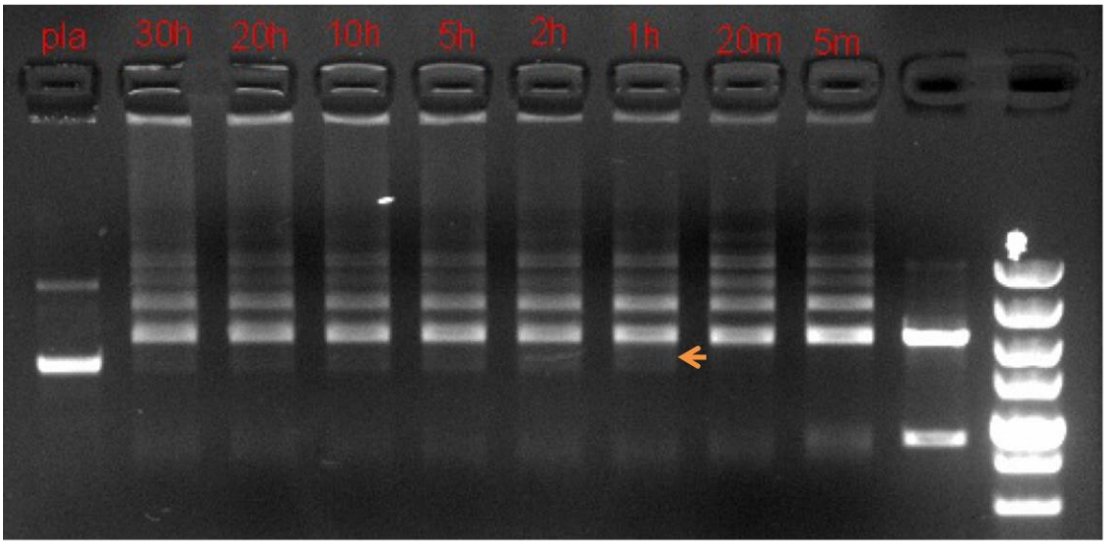
2 95℃ 30sec go into cycle of 95℃ 5sec and 55℃ 30sec for 45 cycles

Gibson assembly

1 Gibson assembly master reaction buffer 15ul (thaw the mixture and keep it on ice until ready to be used)

2 DNA fragments are added in equimolar amounts and add up to 20ul

3 incubation at 50℃ for 40 min



Time course of Gibson assembly

1h is optimum for Gibson assembly since we can see clear desired band have been produced. Pla in the figure refers to desired plasmid after Gibson assembly.

Leucine concentration in liquid LB medium detection

With the aim to detect the leucine in liquid medium, we artificially increase the concentration of leucine. However, the disturbance of other protein and peptide in medium can not be neglected and we design a novel method to process the medium so that the influence of other protein and peptide can be reduced.

Materials that should be prepared in advance

1 sulfosalicylic acid solution 20 g solid sulfosalicylic acid in dissolved in 100ml ddwater

2 ninhydrin solution 1 g ninhydrin is dissolved in 35ml ddwater

3 pH8.03 PBS A solution 4.5350g KH_2PO_4 is dissolved in 500ml ddwater

B solution 11.938g Na_2HPO_4 is dissolved in 500ml ddwater

pH8.03 PBS = 10ml A solution +190ml B solution

1 1ml sterilized LB medium is inoculated with microbe in 1.5 ml EP tube and shaking in 37 ℃ overnight

2 10000g*1min for centrifugation and then pipette 800ul supernatant to another new 1.5 ml EP tube

3 pipette 300 ml LB to other two new 1.5 ml tubes and add 100ul sulfosalicylic acid solution

4 shaking violently for 1 min

5 20000g*10min and then pipette 300ul supernatant to a new 1.5 EP tube

6 add 1ml pH8.4 PBS buffer and mix them by reversing the tube several times

7 all 0.15ml ninhydrin solution and mix them by reversing the tube several times

8 incubate the tube in room temperature for 1hr

9 detect the optical density via spectrophotometer