TARDIGARD

protocol

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Colony Picking and Colony PCR

Picking clones for further processing, may be colony PCR to verify if the plasmid is successfully transferred, or to further culture bacteria in shake incubator.

Colony PCR is to verify whether the desired plasmid is successfully transferred
Colony picking and colony PCR

1. Colony picking
   1.1 Materials
   Tips
   Pipettes
   Alcohol lamp
   Tweezers
   Shake incubator or pre-prepared PCR mix (depending on your next experiment plan)
   1.2 Set up and protocol
   a. Put the tip of the tweezer into alcohol lamp's flame for a few seconds
   b. Using the tweezer to pick up half/a small portion of the colony
   c. Mix it into PCR mix you have prepared or shake incubator with antibiotics and liquid medium
2. Colony PCR
   2.1 Material
   PCR machine
   1.5ul centrifuge tube
   Autoclaved ddH2O
   Ice in foam box
   Pipettes and tips
   Primer designed earlier
   2.2 Setup and protocol
   We are using product from takara, detail of product and protocol can be found in this linked website:
   http://r.search.yahoo.com/_ylt=A0SO80_TwKxZr3IARTVXNyoA;_ylu=X3oDMTEzOGdwa2xuBGNvbG8DZ3ExBHBvcwMyBHZ0aWQDVUkwMkM0XzEEc2VjA3Ny/RV=2/RE=1504522579/RO=10/RU=http%3a%2f%2fwww.clontech.com%2fxxclt_ibcGetAttachment.jsp%3fcItemId%3d14060%26minisite%3d10020%26secItmId%3d16633/RK=1/RS=P7D1q7uRnp5FwlPxVQAa_X7VeQE-

   a. First prepare an 200 ul system in 1.5ul centrifuge tube
   b. Prepare pcr mix
   template 1ul
   primer 1ul
   primer 1ul
   prime star premix= 100ul
   ddh2o 90ul
   add in order of largest volume to smallest

c. Set up the programme

The programme we are using is

	98C	3min
X30	98C	10sec
X30	55C	15sec
X30	72C	10sec
	72C	10min
	12C	--

d. Run gel electrophoresis

Analyse the result obtained, if target length of sequence is obtained