DYS SEE

Colony PCR Protocol.

iGEM Thessaly

Protocols

Colony PCR Protocol

- Colony PCR is a method used to screen for plasmids containing a desired insert directly from bacterial colonies.

1. Mark the colonies on the plate and label them with numbers

   If you have a negative control plate, mark 1 to 3 colonies

2. Take out PCR tubes and label them as the colonies
3. Be sure that you have prepared replica plates and draw to the back of them distinct sections; one per colony. Label them as the above
4. Prepare the MasterMix

   Number of reactions: number of colonies, 1 NTC without colony,

   1 to 2 NTC with the colony/ies from the NTC plate, 1extra/10 colonies

5. Transfer 25 ul MM to the PCR tubes
6. Pick a colony with a tip
7. ''Poke" softly to the replica plate
8. Dissolve the rest in the PCR tube
9. Repeat for all the colonies
10. Quick spin down

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Reagents	**Volumes** (ul)
PCR water	19,9
10x kapa taq buffer A (Kapa Biosystems)	2,5
10μM dNTPs Mix	0,5
10μM Forward Primer VF2	1
10 μM Reverse Primer VR	1
Kapa Taq Polymerase (5u/μl) (Kapa Biosystems)	0,1

- Set the following protocol on a thermocycler

Temperature	Time	Cycles
95°C	3'	1
95°C	30''	35
Primer's Tm - 5°C	30''	
72 °C	0,06sec/bp	
72 °C	0,06sec/bp	1
4°C	∞	∞

Run the PCR product on an agarose gel and check the result

Pick the right colony/ies from the plate and prepare liquid cultures for minipreps

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