

COLONY PICKING

Aim

To isolate colonies with antibiotic resistances (i.e. colonies for which the transformation was successful) and perform a PCR to confirm the presence of the BioBrick.

Procedure

1. Prepare PCR mix according to PCR protocol and pipette master mix into tubes or plate in which PCR will be performed.
2. Take agar plates from incubator and identify bacterial colonies.
3. Pick well-isolated and big colonies by taking a 200 μ l pipet tip and gently stabbing the colony.
4. Swirl pipet tip in tube / well with the appropriate PCR mix and then put tip into a tube or flask with 5-10 ml LB medium with relevant selection antibiotic.
5. Incubate tubes overnight on the shaker (37 °C, 180 rpm), wrap agar plates with parafilm and place in fridge in case more colonies need to be picked.
6. Perform PCR (choose the program according to your primers) and subsequent gel electrophoresis to identify colonies with the BioBrick.

Note!

When picking the colonies it is essential to avoid touching other colonies or agar not occupied by your colony.

Sources

This is a modified version of a protocol established by the Gunilla Karlsson Hedestam group at MTC, Karolinska Institutet.