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Colony Express - E. Coli colony screening

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Aime Jaskolowski[1]

[1]CRAG

Aime Jaskolowski
CRAG

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Protocol status: Working
We use this protocol and it's working

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Abstract

The “Colony Express” screening method is based on selecting positive clones according to the plasmid size contained in the colonies that grow under selection pressure (antibiotic). This method is useful as a preliminary screening tool because it is simple and inexpensive; however, **colony PCR** is always the preferred method for selecting positive clones.

Materials

Colony Express Buffer

- NaOH 0.2N
- Sucrose 20% w/v
- SDS 0.5% w/v
- 0.2% w/v Bromophenol Blue
- 30% v/v Glycerol
- Distilled water up to final volume

Agarose

TAE 1X Buffer

- 4.92 g Tris-Base
- 1.14 ml Glacial Acetic Acid
- 2 ml EDTA 0.5M pH8

Procedure

1 **Replica plate** the individual colonies obtained after the transformation process onto a new **LB + antibiotic** plate to obtain more starting material. Include a replica of the empty vector used for cloning as a control.

2 In a **96-well plate**, add **40 µl of Colony Express buffer** to each well. Using a sterile toothpick, transfer a small sample from each individual colony into the corresponding well. **Mix well to homogenize.**

3 Incubate for **10 minutes at room temperature.**

4 Load the samples onto a **0.6% agarose gel** (**be careful, as the gel will be very fragile!**) and run at mid-low voltage (for a large size 15cm x 20cm gel use 100 V)

5 Analyze the size of the clones by comparing them with the **empty vector control.**