ARIA

# Colony PCR Protocol

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# Colony PCR

## Introduction

Colony PCR is convenient for determining the presence or absence of insert DNA in plasmid constructs. Individual transformants are lysed through heat to release the plasmid DNA from the cell, so it can serve as template for the amplification reaction.
The primers designed to specifically target the insert DNA can be used to determine if the construct contains the DNA fragment of interest.

## Materials

- Single Colonies
- Sterile water
- DreamTaq polymerase
- Taq Mix 2x
- Forward Primer
- Reverse Primer

## Procedure

### Colony PCR

1. **Prepare template**
    1. **Pick a single colony** (E. coli with gRNA plasmid) with a pipette tip and swirl in a small amount of sterile water. Repeat this for each colony selected (it is **important** to mark the selected colonies on the plate to avoid repetition).
2. **Save clones for later culture**
    1. In order to **store your clones longer term,** colonies are streaked on an LB plate.
    2. To do so, **divide the LB-agar/Kan plate into 8 sections** with a permanent marker. Each section corresponds to a particular well.
    3. It is important to leave **margins** between the sections so that the *E. coli* cells do not mix and avoid problems in case of contamination.
    4. In the case of **condensation** drops on the plates, look at their size. If they are very large and interfere with seeding, it may be worth discarding the plate. If they are small, do not sow over them.
    5. Once the plate is prepared, let the **colonies grow.**
3. **Lysing bacteria and setting up PCR reactions**
    1. The remaining bacteria-water suspension will serve as the **template for your PCR reaction.**
    2. The bacteria are **lysed** during the initial heating step of the PCR reaction to release the plasmid DNA by directly adding a small volume of the sample to the PCR reaction.
    3. This is done by using a **DreamTaq polymerase** (1 uL/primer).
    - Taq is cheaper than **Kapa** but has a higher error rate.
    - It already incorporates the **dye** needed for electroforesis.
4. **Mix preparation (for a single colony):** Final volume: 25uL
    1. 12,5uL of **Taq Mix 2x**
    2. 1uL of **Forward Primer**

3. 1uL of **Reverse Primer**
4. Colony (pick it directly, volume not considered)
5. 10,5uL of **H20 miQ** until 25uL [1].

## Bibliography

1. Kenkel, B. (n.d.). Plasmids 101: Colony PCR. Retrieved October 19, 2021, from Addgene.org website: https://blog.addgene.org/plasmids-101-colony-pcr?gclid=CjwKCAjw2bmLBhBREiwAZ6ugo-ehjqBAE9PgQhVhMltF3mnXzSuj_FbOpX1V8AgPIrU5SaKK0_cMgRoCvrQQAvD_BwE