OVERNIGHT CULTURES AND RESTRICTION ANALYSIS

INTRODUCTION:

The aim of this method is to find right colony with right construction plasmid after successful cloning. This alternative method was practiced in our lab as most of the time colony PCR was inconclusive. This is a time consuming method but with high efficacy in our lab.

MATERIALS:

- Transformed colonies in plate
- LB media
- Antibiotic of choice
- Qiagen miniprep kit
- Restriction enzymes (In general, EcoRI and PstI)
- CutSmart Buffer
- MilliQ Water
- Qiagen gel extraction kit (if needed)

PROCEDURE:

- Pick 6-8 colonies from the plate after transformation and make individual overnight culture in LB media with right antibiotics.
- Next day, extract the DNA from all the cultures (2 ml approximately needed) using Qiagen miniprep kit, following their protocol and keep rest of the cultures in fridge. Label the DNA tubes properly corresponding to their cultures.
- Measure the DNA concentration in ng/µL using Nanodrop.
- Prepare enzyme master mix for restriction analysis (25 µL= approximately for 5 reactions) :

CutSmart Buffer	5 µL
EcorI	0.5 µL
PstI	0.5 µL
MilliQ Water	19 µL

EcoRI and PstI can be replaced by other specific enzymes depending on the DNA cut places.

- Digest DNA: Add 4 µL of this master mix into 4 µL of the extracted DNA (25 ng/µL for 100 ng in total) in PCR tubes and mix well with pipette. Perform this test for all the cultures.
- Digest these reactions (in three separate tubes) at 37 ^{o}C for 30 minutes and then heat kill at 80 ^{o}C for 20 minutes either manually or in the thermocycler.
- Run a gel electrophoresis in order to identify proper size of the insert and the vector.
- The sample that matches the correct size of the insert and the vector is grown in an agar plate (streaking from the culture) for further use.
- Throw the undesired cultures and DNA samples that do not match correct size of the cloned plasmid (insert and vector).