Overnight Cultures

- **LB broth** - nutrient media used to grow bacteria.
- **Ampicillin** - Antibiotic used to select for bacteria with the plasmid.
- The plasmid uses ampicillin resistance as its selectable marker
Overnight Cultures

- Each plate contains colonies of bacteria which contain random cDNA inserts from the *Wolffia* cDNA library.
- The plate’s nutrient media contains LB+ampicillin in order to select for bacteria which contain the plasmid.
- Each colony contains one piece of the cDNA library.

Overnight Cultures

- Many copies of the plasmid are necessary in order to analyze the cDNA insert.
- Therefore, we must grow each colony in a separate culture tube overnight at 37°C.
- The culture tubes must contain LB broth + Ampicillin. Why not just LB broth?
Overnight Culture Protocol

• Obtain 1 sterile culture tube and label it with your clone name. (Write on the tube not on the cap)

• Into each of sterile culture tubes, add 2 ml of LB +amp solution.

• Obtain a LB+ampicillin plate which contains the Wolffia cDNA library.

• Using a sterile toothpick, pick a single WHITE colony from the plate and inoculate the media of a single culture tube.

• Incubate with shaking (for aeration) at 37°C overnight.
Overnight Culture Protocol

• If the culture is successful it will become cloudy due to the large number of bacteria

Unsuccessful overnight culture
Successful overnight cultures