Modified Chromosomal DNA Isolation
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Rec DNA
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1. (Done by TA) inoculate 125 ml of LBS broth with a single colonie of *P. leogunathi* from a plate and grow shaking at 37C overnight
2. Transfer 100 ml of the culture to a centrifuge bottle.
3. Spin at 6000g for 10 min at 4 C for 10 min and remove supernatant.
4. Add 1 ml of cold TES and mix cells with a p1000 pipette. When cells are well mixed add 9 more ml of TES.
5. Transfer cell suspension to an Oakridge tube (the opaque plastic tubes with a round bottom). Rinse bottle with 2 more mls of TES to remove the last of the cells and transfer this to the Oakridge tube as well.
6. Add 1 ml of freshly made lysosyme solution (10 mg/ml) along with 10 µl of RNaseA (10 mg/ml). Place on ice for 10 min to lyse cells.
7. Add Protinase K to lysed cells so that the final concentration is 50 ug/ml. Incubate for 10 min at 55C.
8. Add SDS to a final concentration of 1% and incubate for 10 min at 55C.
9. Add 1 volume of Phenol Chloroform Isoamyl (24:24:1) to the tube. Mix by gentle rocking for 5-10min. Mixture should turn a milky white color.
10. Spin at 17,000g for 10 minutes. When the spin is done quickly remove the upper layer taking care not to remove the white interface (this is the proteins and cellular debris) Use an inverted pipette to do this.
11. Repeat steps 9-10. The final time transfer the liquid into a clear 50 ml falcon tube.
12. Add one tenth volume of 3M sodium acetate (pH 5.2).
13. Add 0.7 volumes of Isopropanol The DNA should precipitate at this time and show up as whitish strings in the liquid.
14. Hook and wind the DNA on a pasture pipette (TA will demonstrate) and allow to dry for 5 minutes.
15. Rinse the DNA with 70% ethanol taking care not to dislodge the DNA from the rod. Allow it to dry for 10 minutes. The DNA should not be dry at this point, but should no longer smell of alcohol.
16. Break the end of the pipette off into a 1.5 ml tube. Fill the tube with 0.9 ml of TE, 0.1ml XbaI buffer and 10 µl XbaI restriction enzyme (10U/µl). Incubate at 37C overnight to allow the DNA to dissolve into the liquid and digest with the enzyme.