Isolation of BAC DNA using Autogen 850

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Laura Bertani broth
Chloramphenicol
Sigma, Cat. C-0378
Autogen reagents

Procedure

1. Use one colony or a pure glycerol stock to inoculate Laura Bertani broth with 12.5 µg/ml chloramphenicol. Incubate at 37˚ C over night, in a shaking incubator.

2. Add 4.5 ml of the over night culture per well of the tube unit (the number of wells used depends on the desired amount of DNA).

3. Load the samples into the sample rack of the Autogen (located at the left). *Only even numbers of tube units can be loaded!* Set the same number of empty tubes for the final product into the DNA rack (located at the right). Use program number 2 (no DNA resuspension at the end of the cycle).

4. Remove tubes with the DNA pellet from the Autogen.

5. Add water to the tube wells and resuspend the DNA. The total volume of water depends on the number of units used, however it should not exceed 400 µl if the prep is to be processed in a 1.5 ml eppendorf tube.

6. Add RNaseA, (10 mg/ml stock) to a final concentration of 20 µg/ml and incubate at 37°C for 1h.

7. Do a phenol extraction (phenol to aqueous ratio 50:50); take the top layer.
   Do a phenol/chloroform extraction (phenol/chloroform to aqueous ratio 50:50); take top layer.
   Do a chloroform extraction (chloroform to aqueous ratio 50:50); take top layer.

8. Precipitate the DNA by adding 1/10 vol of 3 M NaOAc and 2 volumes of ethanol. Incubate over night at -20°C. Spin for 15 min at 14000 rpm. Discard supernatant and allow the pellet to dry.

9. Resuspend pellet in H_2O (vol. depends on the size of the pellet).