

DNA extraction from spores

PCR and other molecular techniques require DNA. We extract the DNA from *Dictyostelium* fruiting sori using Proteinase K. A 5% solution of chelex is used to make the DNA available. It causes complete removal of PCR inhibitors, such as metal ions that catalyze DNA digestion.

5% Chelex solution

- Combine 2.5 g BIO-RAD Chelex and 47.5 mL dH₂O in a 100 ml bottle with a stir bar. Keep refrigerated. The chelex will last approximately two weeks.

Preparing Samples

When chelexing, four samples for each clone are usually prepared. We use 8-tube strips so two clones can be done on one strip.

- Place the strips into an empty 200 ul tip box to hold them.
- Add 150 ul of chelex to each tube. The chelex should be kept stirring during this time so that the beads stay in solution and can be pipetted up. Use cut tips if chelex beads are not being pipetted up.
- Add 5-10 sori to each tube. Keeping an ento pin in each tube after adding the sori is a good way to keep from mixing up the tubes or forgetting which tube you last added sori to.
- After all of the tubes have sori in them, add 10 ul of 20mg/ml Proteinase K. The proteinase K is kept in the -20°C freezer in the DNA lab. Only take it out when you are ready to use it and put it back as soon as you are done with it.
- Close the strip tube lids and vortex for 10s.
- Place the strip tubes into the PCR machine. Use the strip roller to make sure the lids are down tightly.
- Choose the program CHELEX. Make sure that the program is set for 4 hours @ 56°C, 30 min @ 98°C, and 4°C for ever.