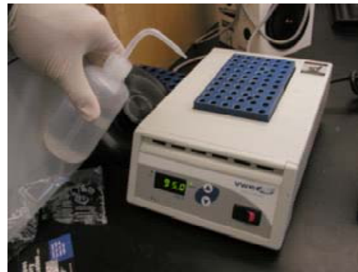
Extraction Protocol: Chelex

This is a fast, cheap, and effective method of DNA extraction. Because this is the first step towards PCR and amplifying your template DNA, you must maintain excellent sterile technique to prevent the contamination of your DNA extractions. Always use a negative chelex control (below) to evaluate your technique during the extraction phase.

Work in **LOW DNA** part of lab.

1. Remove premade tubes filled with 300uL 10% Chelex from refridgerator. You will need for each sample, plus 1 extra for a control. Handle the container with gloves and shake out the number you need into your gloved hand. **Do not** put extra tubes back into jar.

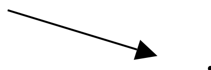
2. Label each Chelex tube to correspond to your sample listed on your Chelex worksheet. Label the tubes on the cap. Put as much information as you can manage, but keep labels legible. Adding initials and a date is always a good idea. Avoid labeling on the side of tube as this writing can be washed off during the incubation stage.



3. Turn on heating block. Set to 95°C. Fill holes with ddH_2O water.

4. Dip forceps into ethanol, then wave forceps through flame of an alcohol burner to ignite. When you are **certain** that the flame on the forceps has extinguished, repeat 2 times.

5. Using the sterile forceps, remove a small piece of tissue from your sample, uncap the tube of chelex, place sample in the appropriately labeled tube and close lid. The piece of tissue should be big enough to be visible, but not so big as to be easily visible. Imagine cutting a 0.2mm section of a standard staple. This is plenty big. Too much tissue may inhibit your reactions. The piece of tissue should be about as big as a period



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6. Repeat (step 5) with each sample in a new Chelex tube, being sure to **sterilize forceps 3 times between samples** (step 4)**.** When finished, make a negative Chelex control by

dipping your sterilized forceps into a tube of Chelex slurry. (It may be necessary to wipe excess tissue from forceps with a kimwipe prior to flame sterilization)

7. When finished with all tubes, vortex samples in chelex slurry for 10-15 seconds. Be sure lids are snapped on tightly before begining

8. Spin samples briefly (10-15 sec) at high speed in a microcentrifuge. This step is to ensure that the sample is inside the slurry of Chelex.

9. Incubate samples for 20 minutes at 95°C. The block temperature may drop slightly when doing this step. This drop is normal. Check tubes while incubating to ensure that lids have not popped off.

10. Vortex samples again for 10-15 seconds (Be careful as steam may pop lid off of centrifuge tube. Hold lids down).

11. Spin tubes again at high speed in microcentrifuge to ensure that all contents are in the bottom of the microcentrifuge tube.

12. Samples are ready to use (or not, see below). ONLY USE SUPERNATE FOR PCR REACTIONS. CHELEX BEAD WILL INACTIVATE TAQ!

Notes:
Chelex is notorious for being as fickle as it is cheap and easy. Here are some tips for good amplifications:

1. Sometimes samples work best if used immediately. Sometimes it is better to wait overnight before using them. Experiment and find what works for your species. Results can vary by taxa and by individuals.

2. When doing initial PCRs, do a serial dilution of template. The amount of a Chelex DNA extraction used in a PCR can be as high as half of the volume of the PCR or as low as 1 microliter of a 1:10,000 dilution. I find that 1 microliter of a 1:1 is good for most applications, but if your PCR doesn't work initially, vary template concentration.

3. If you don't get amplifications from your PCR the first time with a Chelex extract, repeat the vortex, spin, incubate, vortex, spin, sit overnight procedure described above. Often this will make a negative PCR work. Don’t ask me why…

Updated 1/15/04 HSF