

Appendix I

Questionnaire on DNA extraction

NO.

Name:

Age:

Residence:

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Disease:

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Others:

Laboratory investigation:

TWBCs:

DNA concentration:

DNA ratio:

Appendix II

Extraction Protocol: Chelex

1. Remove premade tubes filled with 300uL 10% Chelex from refrigerator. 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 ddH₂O 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
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 beginning

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.

Appendix III

1X Phosphate Buffered Saline (PBS Buffer)

Dissolve the following in 800ml distilled H₂O.

8g of NaCl

0.2g of KCl

1.44g of Na₂HPO₄

0.24g of KH₂PO₄

1. Adjust pH to 7.4.

2. Adjust volume to 1L with additional distilled H₂O.

3. Sterilize by autoclaving

Red Blood Cell (RBC) Lysis Buffer

1. Dissolve the following in 800ml distilled H₂O

8.3g NH₄Cl

1.0g KHCO₃

1.8ml of 5% EDTA

1. Filter sterilize through 0.2um filter

2. Qs to 1000ml with distilled H₂O

Chelex 5% (100 ml)

5 g chelex

Fill to 100 ml with sterile H₂O, shake the bottle
bottom



Appendix IV



Vortex

