Mitochondrial DNA Lab

Standard Operating Procedure (SOP)

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# DNA extraction

**Notes**

Use preferred method, below is an example of saline cheek cell DNA extraction

**Procedure**

Prepare 10ml of 0.9% saline and 100µL of 5% Chelex (Or instagene matrix) for each DNA extraction

Turn on 1.5/2.0ml dry block heater and set to 99OC

Ensure all tubes/consumables to be used are labelled with unique identifier for each sample (1x cup, 2x Eppendorf 1.5ml tubes and 1 x Chelex tube)

* Mix 10ml of saline solution in mouth for 1 min. (Ensure no food or fluids are consumed before)
* Spit contents into labelled cup
* Swirl contents and pipette out 1ml to put into a **labelled** 1.5ml Eppendorf tube
* Spin at 14,000rpm for 2 minutes to make a pellet at bottom of tube
* Remove only the top of the liquid (supernatant) by carefully pipetting out; ensure pellet is still present after removal of supernatant
* Add 100µL water, vortex briefly to re-suspend pellet
* Add 30µL of re-suspended pellet to new tube containing 100µL Chelex and vortex for 30 seconds
* Heat @ 99OC for 10 minutes
* Put in to ice to cool for 1 minute, vortex for 30 seconds after
* Spin at 14,000rpm for 2 minutes
* Transfer 30µL of the top of the liquid (supernatant) to a clean labelled 1.5ml Eppendorf tube, this is the final DNA extract

# PCR

**Notes**

Mastermix must not be colored as sequencing utilizes fluorescence for detection

Primers must be the sequence as shown below and at a concentration of 10µM

F-5’-TTA-ACT-CCA-CCA-TTA-GCA-CC-3’

R-5’-GAG-GAT-GGT-GGT-GGT-CAA-GGG-AC-3’

Due to the small volumes, pipetting must be carried out carefully under supervision

**Procedure**

Prepare the master mix for each reaction in a labelled, dome cap, 0.2µL PCR tube

|  |  |
| --- | --- |
| Reagent | Volume |
| Water | 9.0µL |
| Taq Master Mix | 12.5µL |
| Primers | 2.5µL(1.25uL each F/R) |
| **Total** | **24**µ**L** |

Add 2.0uL of extracted DNA to the corresponding PCR tube, also use a known positive control and water for negative control.

**!Important! Spin PCR tube briefly in minifuge to ensure DNA and PCR mix are at the bottom of tube**

Run final 26µL PCR reaction on a thermal cycler with the following conditions;

|  |  |  |  |
| --- | --- | --- | --- |
| Step | Temperature | Time | Cycles |
| Initial Denaturation | 95 OC | 10min | 1 |
| Denaturation | 95 OC | 30sec | 30 |
| Annealing | 56 OC | 30sec |
| Extension | 72 OC | 30sec |
| Final Extension | 72 OC | 2min | 1 |
| Hold | 4 OC | Until removed | ∞ |

Upon completion remove tubes and run an electrophoresis gel to check for amplification. Only samples with definitive bands should be sequenced therefore a gel **must** be run in order to save time and cost of sequencing.

Finally freeze at -20 OC until ready to be sequenced

# Post-Sequencing Processing results

**Notes**

There are various ways to accomplish the same goals and objectives and many analysis programs and websites in order to do this as such this is only a brief overview.

**Checking overall sequence**

The first and most important part is to check that the sequence is visible and has good separation in the peaks. Ideally the electropherogram should be around 400bp long and have clean sharp peaks that have a clear separation between them. If any samples deviate from this there can be many reasons the most common being contamination (By salt or leftover product from extract/PCR) or over/under load of DNA.

**General analysis**

There are 2 basic methods of analyzing the data produced by finding a similar sequence for comparison (BLAST) or by finding differences to a reference sequence (rCRS).

**Common single base repeat problem**

In this sequence it is quite common (Approx. 10% students) to encounter a repeating ‘c’ that causes the sequence to fail at that point. The way to compensate for this is to run the reverse sequence, this will stop after repeating ‘g’. Invert and flip this sequence and you will have the other half to the forward sequence, simply copy and paste them together.