2a- Salting Out

Supplies: Sample; 5M NaCl; Vortex; 95% Ethanol; 70% Ethanol; Centrifuge; DNA-Free Water; 1.5 ml Tubes

Procedure:

1– Add 100 µl Of 5M NaCl & Vortex For 15 Seconds

2– Spin 7 Minutes @ 10,000 rpm

3*– Decant Supernatant Into A New, Clear 1.5 ml Tube

4– Add 1-Volume 95% EtOH — Invert Tube Several Times To Mix (slowly)

5– DNA Should Rise & Be Visible In The Alcohol Portion

6– Spin 15 Minutes @ 10,000 rpm

7*– Pour Off EtOH (waste)

8– Wash Pellet With 500 µl Of 70% EtOH- (Put Alcohol In Tube)

9– Spin 10 Minutes @ 10,000 rpm

10– Carefully Pipet Off EtOH

11– Dry Sample Overnight Or For One Hour In An Incubator

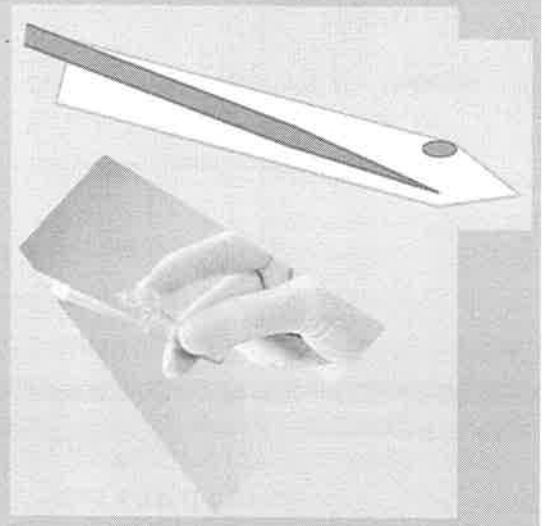
12— Add 100 µl Of Sterile Water & Incubate For 20 Minutes At 30° C

*In Step #3 Keep The Liquid & In Step #7 Keep The Solid !!

2b- Pipetting Off The EtOH

In Step 11 The DNA Pellet Should Be Stuck To The Side Of The Tube. The Pellet Should Not Be Disturbed. The Pellet Should Be On The Hinge Side Of The 1.5 ml Tube If You Spun The Tube Hinge -Side Out In The Centrifuge

Hold The Tube Horizontally & Use The Pipette To Carefully Remove The EtOH. The Trick Is To Not Touch The Pellet.



After Step 12 In 2a — Your Sample May Be Used Or Stored In The Freezer

3a- PCR Mastermix

Purpose: The Purpose Of PCR Is To Amplify A Specific Section Of DNA. The Process Involves Specialized Chemicals & A Series Of Heating & Cooling Reactions

Materials:

1– Buffer Solution To Stabilize The Reaction

2- DNTP's — These Are The DNA Bases A,T,C, & G

3– Taq Polymerase Is The Protein That Copies The DNA

4– MgCl Helps The Taq Bond Correctly To The DNA Molecule

5– BSA* Is A Serum Albumin Protein Derived From Cows— It Is Used To Stabilize The Reaction*

6– Sterile Water Is Used To Suspend The Reactants Together

7– Primers Serve As A Starting Point For The DNA Polymerases, Which Can Only Add Nucleotides To An Existing Strand Of DNA

*BSA Is The 'Secret' Ingredient In PCR It's Purpose Is Still A Point Of Discussion And May Be Excluded From Some Protocols

3b-Making A Mastermix

A Mastermix Is Simply A Recipe For PCR— To Account For Small Errors Add 10% To Your Sample Number In The Recipe-

Recipe: Following A Simple Recipe Is The Best Way To Start— As You Become Proficient You May Wish To Modify Your Ingredients -

Add The Ingredients In This Order For One Simple Reason– The Most Expensive Ingredients Are Put In Last So If You Make A Mistake It Will Not Be An Expensive One !

You Can Use This Recipe To Start:

Substance	Per Sample
Water	6.0 µl
PCR Buffer	0.65 µl
dNTP's	1.5 µl
MgCl	(In Buffer)
BSA	1.0 µl
For Primer	0.6 µl
Rev Primer	0.6 µl
Taq	1.0 µl

(Use With 1 µl Of Genomic Sample)

*Taq Has Many Variants– When Using 'Store-Bought' Taq Use Only 0.2 µl And Make Up The Volume With Pure Water

4-Thermocycler

Here Is A Sample PCR Protocol

Cycles 35 (steps 1, 2, & 3 Only)

First Step 94° C / 4 Minutes

Step 1 94° C / 15 sec

Step 2 50° C / 15 sec

Step 3 72° C /60 Sec

Step 4 72° C / 10 Minutes

Refrigerate Until Use With Gel Electrophoresis

These Are Questions That You Should Be Able To Answer If You Want To Demonstrate Mastery Of The Material

1– What Does Pro-K Actually Do ?

2– What Does Cell Lysis Solution Actually Do ?

3– What Is The Origin Of Taq Polymerase & Why Is That Significant ?

4– What Does The Cycling Of Temps In A Thermocycler Do To The DNA ?

Develop An Excel Document For PCR Protocols– It Makes The Math & Organization Much Easier !

1-DNA Extraction

Supplies: Weigh-Boat; Razor Blade; 10% Bleach Solution; Forceps; 1.5 ml Clear Tubes; Extraction Buffer; Pro-K; Tissue Sample

Set-Up: Clean Prep–Area: Three Beakers– Two With Water, One With Bleach: Clean Forceps Before Each Use; Place Sample In Weigh-Boat

Procedure:

1- Using The Forceps & The Razor Blade, Finely Chop The Tissue

2– Add 300 µl Extraction Buffer To A 1.5 ml Clear Micro-Tube

3– Add 10 µl Pro-K To The Tube

4– Move The Fragments To The Tube

5– Agitate Tubes & Place Into An Incubator (55-60 °C) If Possible Agitate Continuously Until Digested (Overnight)

6– Clean-Up Materials

Modified From Feldheim et.al Salting Out Procedure