CURRICULUM

(1) Animal Development
(2) Chromosomes and Genes
(3) Genetic Polymorphisms
(4) Genetic Material
(5) Isolating a Gene
(6) Restriction Enzymes
(7) Protein Structure
(8) Blood groups
(9) Genetics markers
(10) Quantitative Trait Loci

(1) Practical DNA Extraction
(2) Electrophoresis
(3) PCR

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Student Grading:	Lecture	60%
	Practical	20%
	Tests	20%

WEEK ONE

Safety Precautions

- Lab coats must be worn in the lab at all times.
- Precautions must be taken on the laboratory to protect from chemicals and bio-hazards.
- Avoid microbial contaminants
- Avoid enzymes on skin
- Safety glasses must be worn in the lab
- Impermeable safety glasses to UV light must be worn to shield from UV light
- Use of gloves is compulsory
- No food or drinks are allowed in the lab
- All open flames must be extinguished when finished
- Never leave an open flame unattended
- Biohazardous materials must be disposed off carefully using biohazard containers
- Please notify instructor if you are pregnant
- Take care not to contaminate reagents. Use only fresh, sterile pipettes or pipette tips to remove reagents.
- At the end of each lab session, clean your glassware, leave to dry in dish drier.
- Replace all items to their normal place after use.
- Sanitize bench top before and after working

An up-to-date lab notebook is an essential part of a good research. Keep your notebook up to date. This will be collected and graded at the end for:

- purpose and date of experiment
- experimental design
- all calculations
- observations

Lecture : Introduction to course and project

Lab
- Aseptic Technique
- Pipetting demonstration
- Standard chemical and Reagent preparation
- Blood collection
- Semen collection
- Gel preparation
  - Agarose
  - SDS-PAGE
- DNA Extraction
- DNA Quantification
- Agarose gel Electrophoresis
- SDS Page Electrophoresis

(1) Primer on proper pipetting

(2) Experiments will not work properly if reaction conditions are not appropriate. The easiest errors are pipetting errors. If you do not use your pipette correctly you will add incorrect volumes to your reaction mixtures and you will waste a lot of time and expensive reagents, particularly enzymes. The Rainin Pipetman pipettes are pretty rugged. The numbers on the top of the pipette plunger (P20, P 100, P 200 or P 1000) are the maximum volume pipettable in microi-litres (µls). DO NOT wind the gear above the maximum volume, the pipette may be damaged. Use your index finger to pipette, it is easier than the thumb and more accurate. Do not let the plunger snap up when you are drawing up a sample. This will contaminate the pipette bore and proper amount will not be dispensed. Withdraw reagents slowly, smoothly and accurately

Do not stick pipette into the solution you draw up. Dipping into solution will cause some solutions which are viscous, such as enzymes, which contain glycerol to adhere to the outside of the pipette tip. Withdraw your sample from the top of the stock solution

WEEK TWO
CALCULATING DNA CONCENTRATION

(3)

(1) **DNA Extraction Protocol**
(from Blood)

Reagents

Use mix

8M Urea	-	480 gm
10m M TRIS	-	10mls (17 TRIS Hcl)
0.1% SDS	-	10mls (10% SDS)
1m M EDTA	-	2mls (0.5 M EDTA)
sd H_2O to	-	1000mls

Isotonic saline = 0.9%

Nacl	-	9gm
Sd H_2O	-	1000mls

1. Add equal volume of isotonic saline to blood samples
2. Mix, spin at 1600g for 12 mins (5000rpm)
3. Remove buffy coat with Pasteur pipette into cryotube
4. Add equa; volume of UREA/TRIS/EDTA
5. Mix and store at ambient temperature
6. Add 10ml TRIS EDTA (TE) to 5ml of buffy-urea mix
7. Shake on spiral mix for 30 mins to mix thoroughly
8. Add 10 mls liquefied phenol (in TRIS buffer) mix gently by hand for 10 mins.
9. Centrifuge at 5k for 5 mins.
10. Remove phenol layer and discard
11. Add 5 ml phenol + 5ml chloroform (in isoamyl alcohol)
12. Mix thoroughly (gentle mix by hand) 5 mins
13. Spin at 5k for 5 mins
14. Discard phenol layer
15. Add a second 5ml phenol + 5ml chloroform (in isoamyl alcohol)
16. Mix for another 5 mins
17. Spin at 5k for 5 mins
18. Add 10ml chloroform
19. Mix for 5 mins (gentle shake by hand)
20. Spin at 5k for 5 mins

21. Take acqueous phase into beaker containing 15ml cooled absolute ethanol

22. Hook out DAN with glass hook
23. Dry out or hook (upturn hook into alcohol beaker)
24. Break glass hook end into 500 µl
25. If no precipitate at stage 22, spin down tube and resuspend precipitate or start all over again.

NOTE: If DNA does not come up in strands, DNA in urea usually break up.

Therefore:

1. Put absolute alcohol into labeled Corex tubes
2. Pipette acqueous DNA phase into alcohol, seal with NESCO FILM
3. Cool in freezer for 30 mins
4. Spin down for 30 mins at 7k and 4^0C, i.e in refrigerated centrifuge
5. Discard supernatant
6. Leave precipitate to dry
7. Dissolve in 500 µl sd H_2O
8. Store in Eppendorf tube

WEEK THREE

(2) **DNA Quantification**

Supplies - dH_2O
Microfuge tubes
Quatz cuvettes
UV spectrophotometer

Procedure

(1) In a microfuge tube, make a 1:500 dilution of your DNA sample (499 µl d H_2O + 1 µl of DNA sample)

(2)Use a spectrophotometer to determine the absorbance of 260nm and 280nm. Use dH_2O as blank

(3) Clalculate DNA concentration if the original DNA sample in µl/ µl given that $1A_{260}$ unit = 50mg/ml of double stranded DNA

Calculate DNA concentration of the original DNA sample in µg/ µl given that 1 A_{260} unit = 50mg/µl of double-stranded DNA

	A_{260}	A_{280}	$A_{280/}$ A_{280}	Mg/ µl
PCR Product				

WEEK FOUR

(3) Agarose gl Electrophoresis of DNA samples

(Purpose:to confirm that DNA fragments are of expected size)

Supplies
10 x TBE buffer
Graduated cylinder
Electrophoresis unit
Power supply
Flask with stibar
Gel loading dye
Ethidium bromide solution
UV box
Molecular weight marker

(1) Prepare 0.8% (wt/vol) agarose gel in 1 x TBE
   (a) weigh agarose and place in a flask with stirbar.
   (b) Prepare 1 L of 1 X TBE by diluting 10X TBE with H_2O
   (c) Add approx. volume of 1 X TBE to agarose and melt in in microwave over a burner
   (d) Cool on stir plate until enough to touch with your bare hand take care not to create bubbles while stirring.
   (e) Pour molten agarose into sealed gel mold with comb. Allow get to sit still until it solidifies (30 mins)
   (f) Remove comb and put gel and tray into electrophoresis unit.
   The positive (red) electrode should be put at the end farthest from the wells. Poor 1X TBE into chamber until it covers gel.

(2) **Prepare sample to load**
   (a) Put samples into microfuge tubes and bring all samples to 20 µl with water as shown in the chart below.
   (b) Add 2 µl of 10X loading dye to each
   (c) Load gel as shown in table and run at 5 – 10 volts per cm (length of gel) usually about 80 volts
   (d) Stain gel in Ethidium Bromide (EtBr) solution according to manufacturer's protocol. (Aletrnatively EtBr is added to molten gel before pouring or to the running buffer.
   (e) View and photograph gel under UV light.

WEEK FIVE AND SIX

Reagents – Preparation.

10X TBE

108g Trisbase
55g Boric acid
40ml 0.5M EDTA (pH 8.0)
Bring to 1 L with dH_2O

10X Loading dye

0.25% bromophenol blue
0.25% Xylene Cyanol FF
50% glycerol (5ml)
Bring to 10ml with dH_2O

SDS – PAGE Reagent

4X Lower TRIS pH 8.8

TrisBase	18.17gm	OR	90.85gm
10% SDS	4,0ml		20.0ml or 2.0gm
q.s with H2O to	100ml		500ml

4X UPPER TRIS Ph 6.8

TrisBase	6.06gm	OR	30.3gm
10% SDS	4,0ml		20.0ml or 2.0gm
q.s with H2O to	100ml		500ml

Running Buffer (10X)

TRISBASE	60gm
Glycine	288gm
SDS (add last)	20gm
q.s with dH_2O to	2.0 litres

Reducing Buffer (2X)

10% SDS	3.0ml
Upper Tris 4X	1.25ml
β-mercaptoethanol	500 µl
Glycerol	1.0ml
Bromophenol Blue	Pinch
q.s with H_2O	10.0m

Reducing buffer 5X

SDS	750gm
2M Tris pH 6.8	1.25ml
β-mercaptoethanol	1.3 µl
Glycerol	2.5ml
Bromophenol Blue	Pinch
q.s with dH_20	10ml

Coomasie Brilliant R-250 Gel stain

Coomasie Brilliant Blue R-250	400mgs
Methanol	400ml (Add dye and methanol and stir for 3hrs)
Glacial Acetic acid	50ml (Add the acetic and stir for an additional 3 hrs
q.s with dH_2O to	1000ml (Add the dH_2O and stir overnight)

Filter the solution through a No 1 Whatman paper before using.

Destaining Solution

	1000ml	**5000ml**
Methanol	400ml	1600ml
Glacia acetic acid	250ml	1000ml
q.s with dH_2O to	1000ml	4000ml

10% Ammonium Persulfate (APS)

APS	1gm
dH_2O	10ml

RESOLVING GEL PREPARATION

5% gel	**1 gel**	**2 gels**	**3 gels**	**4 gels**	**1 large Gel**
dH_2O(ml)	4.0	8.0	12.0	16.0	20.0
4X Lower Tris (mL)	1.7	3.4	5.1	6.8	8.5
Protogel (ml)	1.2	2.4	3.6	4.8	6.0
TEMED (µl)	20 µl	20 µl	30 µl	30 µl	60 µl
10% APS (µl)	60 µl	90 µl	80 µl	75 µl	75 µl
10% gel					
dH_2O (ml)	2.8	5.6	8.4	11.2	14
4X Lower Tris (ml)	1.7	3.4	5.1	6.8	8.5
Protogel (ml)	2.3	4.6	6.9	9.2	11.5
TEMED (µl)	20 µl	20 µl	30 µl	30 µl	60 µl
10% APS (µl)	60 µl	80 µl	80 µl	75 µl	75 µl
12,5% gel					
dH_2O (ml)	2.2	4,4	6.6	8.8	11
4X Lower Tris (ml)	1.7	3.4	5.1	6.8	8.5
Protogel (ml)	2.9	5.8	8.7	11.6	14.5
TEMED (µl)	20 µl	20 µl	30 µl	30 µl	60 µl
10% APS (µl)	60 µl	80 µl	80 µl	75 µl	75 µl

3% STACKING GEL PREPARATION

dH_2O (ml)	1.6	4,4	6.6	8.8	11
4X Lower Tris (ml)	0.62	3.4	5.1	6.8	8.5
Protogel (ml)	0.3	5.8	8.7	11.6	14.5
TEMED (µl)	10	10	20	20	20
10% APS (µl)	30	40	46	40	40

Agarose

1.5gm in 100ml - small
3.5gm in 250ml - large

1 X TBE = 5ml 10X TBE q.s tp 50ml dH_2O
10mls 10X TBE q.s to 100mls dH_2O
200mls 10X TBE q.s to 2000mls

EtBr = 0.5µl in 50ml gel
1.0µl in 100ml gel
2.0µl in 250 ml gel

10% APS = 0.1g into 1ml sdH_2O
25% APS = 1.25gm into 1ml sdH_2O

WEEK SEVEN

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction is used to amplify DNA sequence encoding a DNA fragment

Precautions

Don't contaminate your sample with extraneous DNA
If extraneous DNA is introduced it will act as template for the primers and hence you will amplify unwanted sequences.

- Wear gloves
- Use clean pipetter tips
- Always use negative control with no template DNA added
- Use ultra pure water (sterile distilled)
- Use new disposable plastic
- Keep reagents on **ICE** at all times especially the DNA polymerase

Supplies BMC 1013 BTA gene in cattle. Ta 55^{o}C, Mg 1.5 µl, Product 218-244

Primer 1 5' AAAAATGATGCCAACCAAATT 3'
Primer 2 3' TAAGGTAGTGTTCCTTATTTCTCTGG 5'

Template DNA
dNTP mixture (10mM each of dATP, dTTP, dGTP, dCTP)
50mM $mgcl_2$
10X PCR buffer
thin-walled microfuge tubes
Pipette tips
Taq DNA Polymerase
Control PBR^{322} in HAE digest
Sterile distilled water

In thin-walled microfuge tubes set up three reactions each with a different template.

(1) Label reaction tubes and add the following reagents;

Compound	**Final concentration**	**Volume to add per PCR each**
dH_2O	-	80 µl
dNFP mix (10m M each)	0.2m M each	2 µl
10X Taq buffer	1X	10 µl
$MgCl_2$ (50mM)	1.5mM	3 µl
Total per PCR reaction tube		**95 µl**

2. To each reaction tube add the appropriate primers and template (remember that each tube will have different template)

Compound	Final concentration	Volume to add per PCR each
Template 0.1 µg/ µl	1ng/ µl	2 µl
Primer 1 (F) 10p mol/ µl	1 µM	1 µl
Primer 2(R)100pmol/ µl	1 µM	1 µl

(3) Add Taq DNA polymerase after 'hot start' below. Pause thermal cycler while Taq DNA polymerase is added. Tag DNA polymerase (2.5 u/µl) 1.0/ µl = 5 units

(4) Thermal cycling conditions

1X	94°C	3 mins	
30X	94°C	45 seconds	'hot start' add Taq Polymerase
	52°C	45 seconds	
	72°C	1.5 mins	
1X	72°C	10 mins	
1X	4°C	Hold	

EQUIPMENT	CAT NO	QUANTITY	COST
Microtubes			
1.5ml Natural	T 3566		14.50
1.5ml assorted	T 3691		15.00
Balances:			
Ohaus portable	B2402	1	134.00
Model LS 5000			
220V Ac Adaptor	P6181	1	44.40
Acculab			
Model GS1 – 200	B5429	1	99.90
Multiwell plates	M4029	5/pkg	21.60
Rainin Pippette man	5000 µl		
	2000 µl		
	1000 µl		
	40 µl		
	10 µl		
	0.5 – 1 µl		
Pipette tips			

CHEMICALS

	Cat No	Qty	Cost ($)
Agarose	A4679	50g	82.70
Human serum control	S7775	1ml	17.50
Low Range Molecular wt marker	M3913	1 Vial	27.00
Brilliant Blue	B8522	1 bottle	27.70
EDTA	E5513	50gm	15.60
Acryl/Bisacryl Blue	A9926	5X 100ml	68.30
Bromophenol Blue	B8026	5g	16.40
TEMED	T.9281	22ml	18.00
Urea	U 6504	500gm	39.20
2-Mercatothanol	M7154	25ml	9.00
dNTP mix	D7295	0.5ml	48.20
Trizma Base	T6066	100gm	16.50
PCR Core Kit	R:36.37/38	1 kit	51.80
	S: 26 – 36		
Phosphosate buffered Saline	P7059	1 litre	48.10
Tris glycrine	T7777	1 litre	42.70
	SDS Buffer		

TUTORIAL QUESTIONS

WEEK ONE

(1) What do you understand by the term 'genetic material'?

(2) Write out the nucleoside and the nucleotide of the genetic bases

WEEK TWO

(3) Illustrate with diagram the polypeptide chain

(4) Name and draw the pyrimidine and purine nucleotides and nucleotides. What are the phosphodiester bonds?

(5) Diagramatically represent the cleavage of a phosphodiester bond

WEEK THREE

(6) Name and draw the nuclesic and structure
(7) Describe the process of base pairing in the DNA

WEEK FOUR **CAT**

WEEK FIVE

(8) With diagram, illustrate Frame shift in the genetic formation. Name and write out possible alterations in frame shift.

(9) Give the codons for the 4 codon, 3 codon and 2 codon amino acids structures

WEEK SIX

(9) In a tabular form, write out the genetic code

(10) Write out the 3 hypotheses in the genetic code analysis. There are 3 possible ways of translating a nucleotide sequence into protein. With examples show the reading frame of a DNA coding system region and the amino acid configuration.

WEEK SEVEN

(13) Discuss nuclei acid catabolism
(14) What do you understand by Point Mutation. Name two types of point mutations. What are the causes of transition.

WEEK EIGHT **CAT**

WEEK NINE

(15) Explain with examples the tem 'Multiple allelism'. Show the order of arrangement of multiple alleles. In genes controlled by 10, 15, 28, 45 alleles. Calculate the number of possible genotypes.

WEEK TEN

(16) Write an essay on the detection of quantitative trait loci in livestock species
(17) What are genetic markers. Compare and contrast their roles in the mapping of qualitative trait loci.

WEEK ELEVEN

(18) How would you compare molecular genetics and traditional selection method in the improvement of animals.

(19) With diagram discuss the behaviour of DNA in a semi-conservative replication.

WEEK TWELVE

(20) Define

Genetic code		Transition
Genetic information		Transversion
Genetic mutation		DNA replication
Mutagenes		Hot spots
Isoagglutinogens		

Write out the heredity transmission of the following principal genes with the possible expectations in the offspring.

0 X 0
0 X A
0 X AB
A X A
A X B
A X AB
B X AB
AB X AB

WEEK THIRTEEN