

# AN INTRODUCTION TO POPULATION GENETICS

# Fish stocks; methods for identification, delimitation, and characterization.

## ***Stock identification***

- Catch statistics (movements of fleet, landings)
- Tagging-recapture studies (Lea-tag, Floy-tag, PIT tags, eye-tags)
- Biological characterization (growth, age at first maturity etc)
- Genetic characterization (gene markers, allele frequencies)

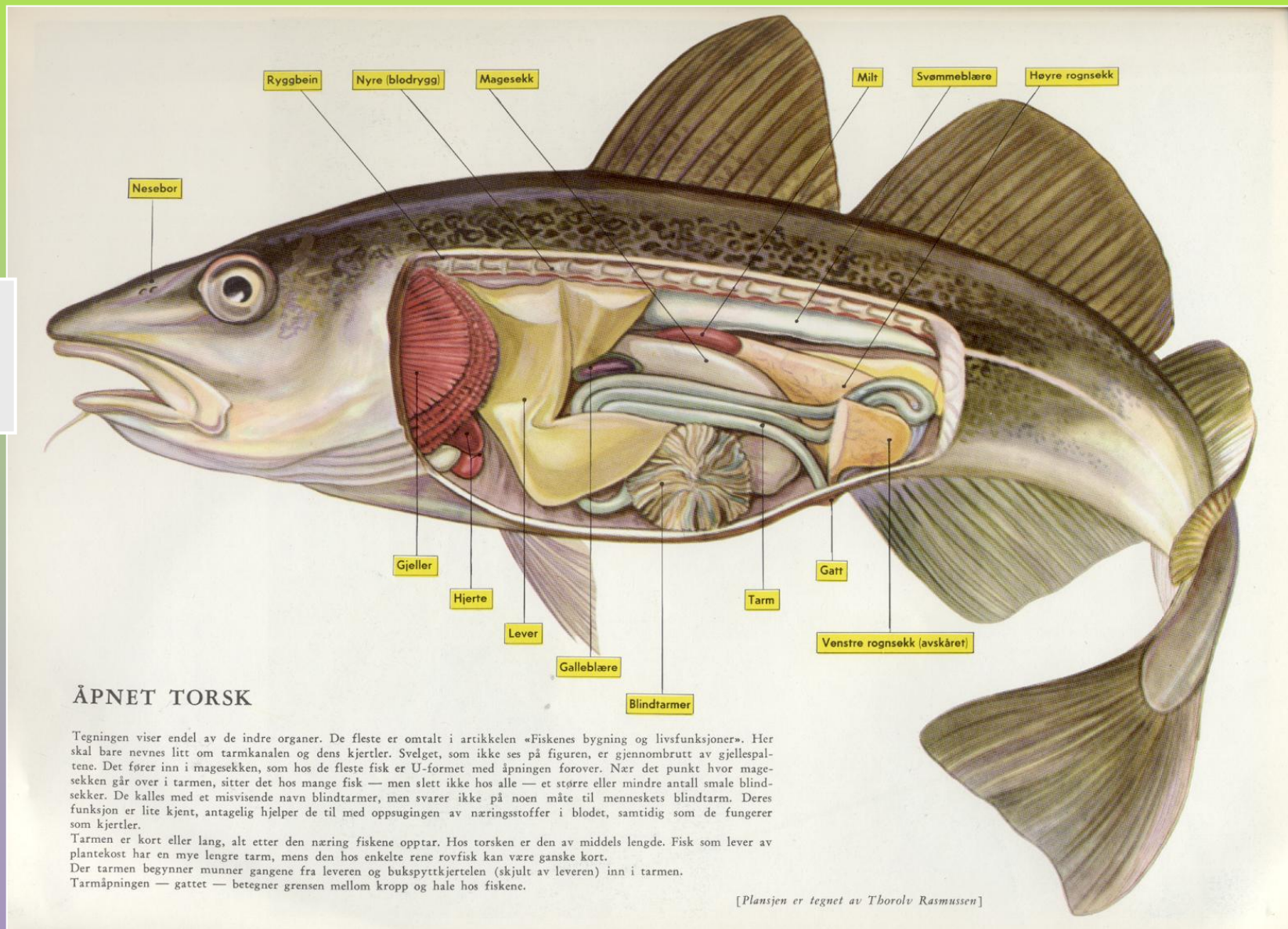
## ***Practical sampling***

- Length, weight, age, sex, gonadic stage, maturity age
- Fish scales and/or otoliths for ageing, tissue biopsy and autopsy
- Blood or tissue samples for genetic and clinical chemistry analyses
- Securing data and measurements in e.g. Excel spreadsheets

## ***Analyses***

- Age determination (scales, otoliths)
- Electrophoretic analyses (blood, isozymes, DNA markers)
- Relative yearclass strength from age composition in samples
- The term MLY (maximum long term yield) or MSY (...sustainable...)

## Methods in fish(eries) biology



The morphology of fishes is basis for species identifications, and clue to their habitats and way of life. The "bird" and three dorsal fins are, e.g. characteristic of gadoids. Internal anatomies and organs are more or less variations on the same theme for vertebrates.

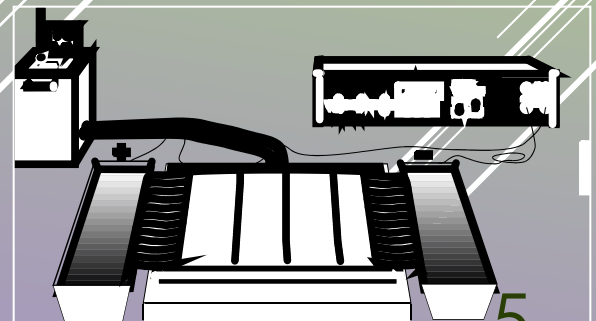
# ***Population genetics methods***

## *Population genetic methods*

Gained increased interest for the detection and delimitation of reproductive units, particularly for species which do not lend to tagging/recapture.

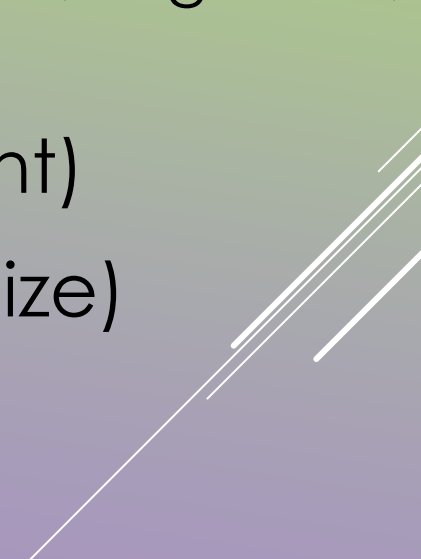
In order to apply genetic tools we need to know the individual genotype for specific heritable traits. Such information is readily obtained by various **electrophoretic methods**.

In this course we use the individual genetic signature for species identification, as well as for establishing genetic characteristics for populations.





# POPULATION GENETICS AND GENE FLOW: CONSERVATION RELEVANCE

- ▶ Determine units for management
  - ▶ Heterozygosity estimates
    - ▶ Population bottlenecks
    - ▶ Hardy-Weinberg assumptions (mutation, migration, selection, drift, inbreeding)
  - ▶ PVA (Population Viability Assessment)
  - ▶ MVP (Minimum Viable Population Size)
  - ▶ Effective population size
    - ▶ Number of breeding individuals
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When having collected samples and performed electrophoretic determination of individual genotype for some character, there are two basic types of statistical tests to be performed:

**First**, we want to test whether each of our samples are drawn from unit populations, i.e. from "pure" populations in so-called Hardy-Weinberg equilibrium.

This we do with the so-called **Chi-squared Goodness-of-fit test**.

**Secondly**, we want to test whether there are genetic differences between our samples, i.e. whether they may have been drawn from different populations with different genetic characteristics.

This is done with another chi-square test, the so-called **Chi-squared RxC contingency table test**, also called the chi-squared test of homogeneity.

## KEY CONCEPT

**Hardy-Weinberg equilibrium provides a framework for understanding how populations evolve.**



- ▶ Biologists use models to study populations.
- ▶ Hardy-Weinberg equilibrium is a type of model.



# HARDY-WEINBERG EQUILIBRIUM DESCRIBES POPULATIONS THAT ARE NOT EVOLVING.

- ▶ Genotype frequencies stay the same if five conditions are met.
  - ▶ very large population: no genetic drift
  - ▶ no emigration or immigration: no gene flow
  - ▶ no mutations: no new alleles added to gene pool
  - ▶ random mating:  
no sexual selection
  - ▶ no natural selection:  
all traits aid equally  
in survival



- ▶ Real populations rarely meet all five conditions.
  - ▶ Real population data is compared to a model.
  - ▶ Models are used to studying how populations evolve.




# The Hardy-Weinberg equation is used to predict genotype frequencies in a population.

- ▶ Predicted genotype frequencies are compared with actual frequencies.
  - ▶ used for traits in simple dominant-recessive systems
  - **must know frequency of recessive homozygotes**
  - **$p^2 + 2pq + q^2 = 1$**


	$p$	$q$
$p$	$p^2$	$pq$
$q$	$pq$	$q^2$


"The Hardy-Weinberg equation is based on Mendelian genetics. It is derived from a simple Punnett square in which  $p$  is the frequency of the dominant allele and  $q$  is the frequency of the recessive allele."


## VARIABLES

$p$  = frequency of allele  $T$   
(dominant allele) 

$q$  = frequency of allele  $t$   
(recessive allele) 

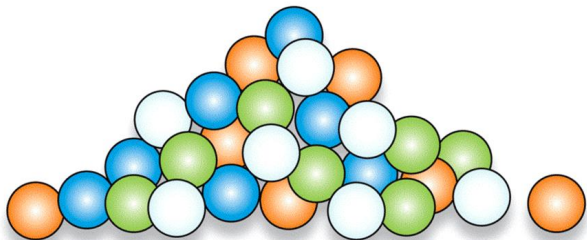
$p^2$  = frequency of fish with  $TT$   
(dominant homozygous genotype) 

$2pq$  = frequency of fish with  $Tt$   
(heterozygous genotype) 

$q^2$  = frequency of fish with  $tt$   
(recessive homozygous genotype) 

- Genetic drift changes allele frequencies due to chance alone.

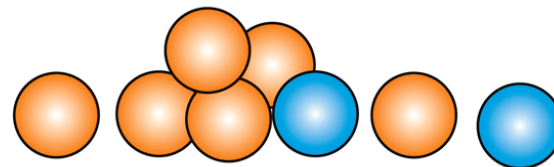
### INITIAL POPULATION



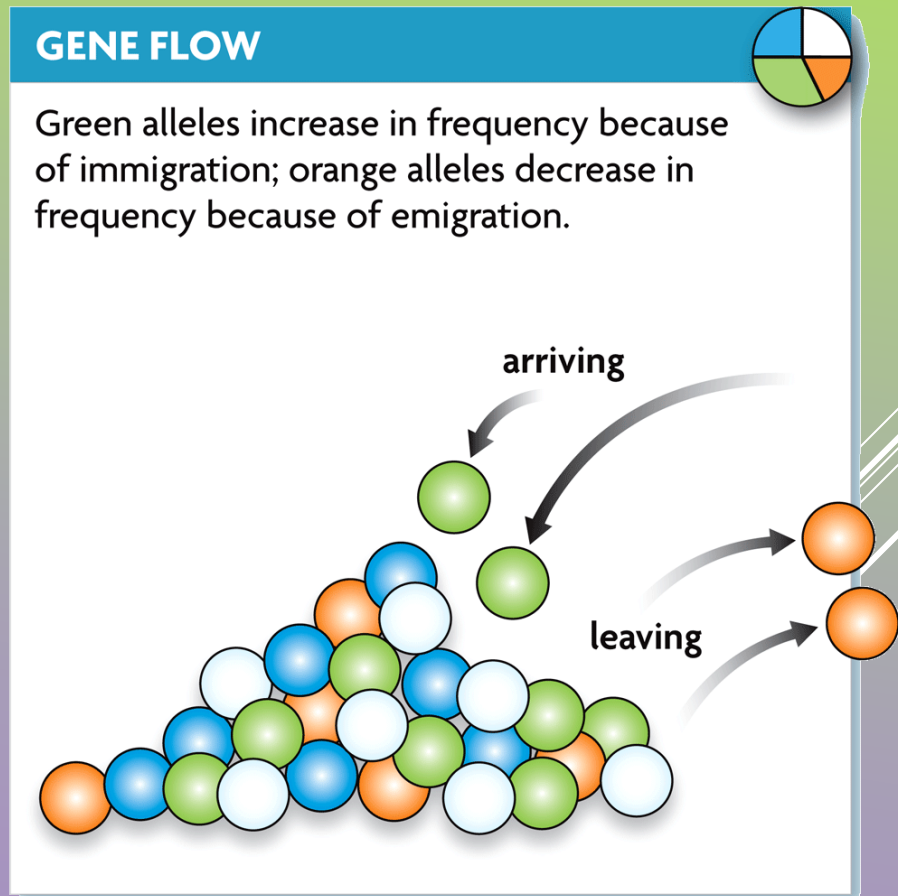
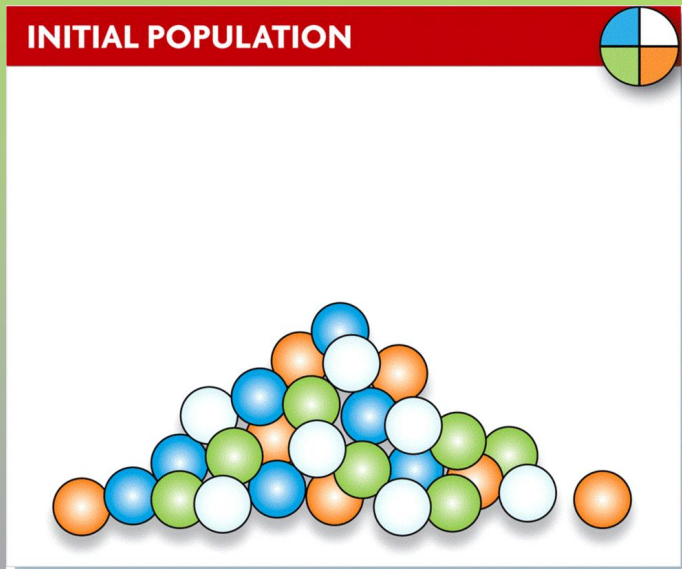
### GENETIC DRIFT



After a bottleneck event, only orange and blue alleles remained in the small population. Through genetic drift, orange alleles increase in frequency.

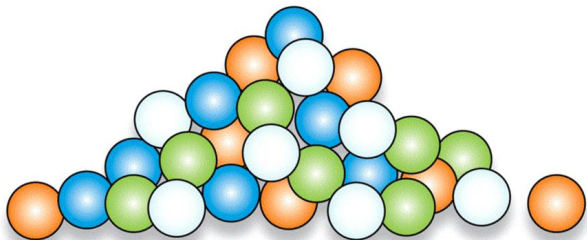


- ▶ **Gene flow moves alleles from one population to another.**



- **Mutations produce the genetic variation needed for evolution.**

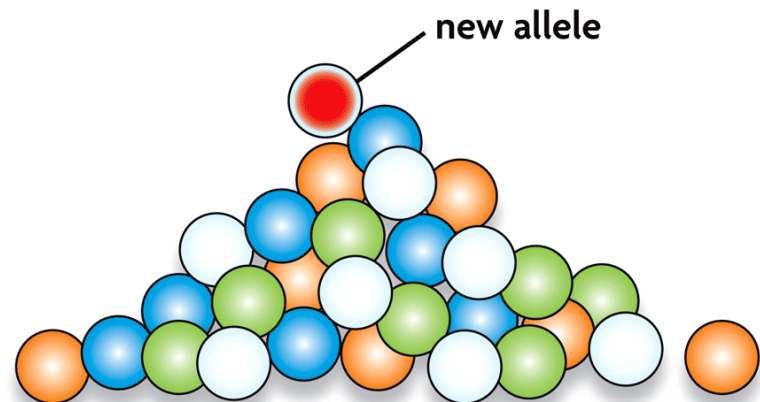
### INITIAL POPULATION



### MUTATION

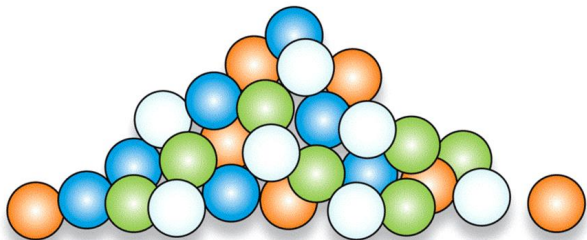


A new allele, associated with red body color, is formed through mutation. This could affect sexual selection if red body color improves mating success. It could affect natural selection if red body color increases the chance for survival.



- ▶ Sexual selection selects for traits that improve mating success.

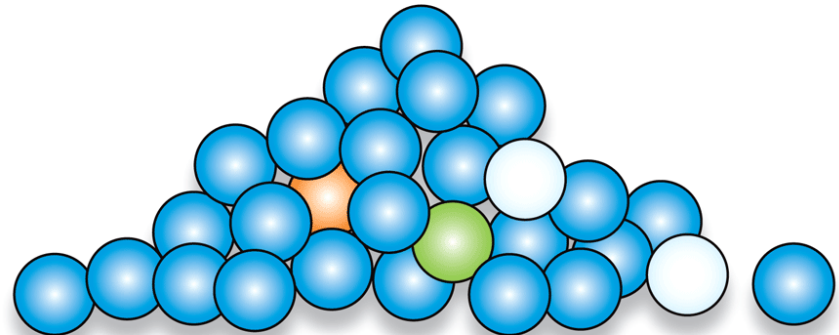
### INITIAL POPULATION



### SEXUAL SELECTION

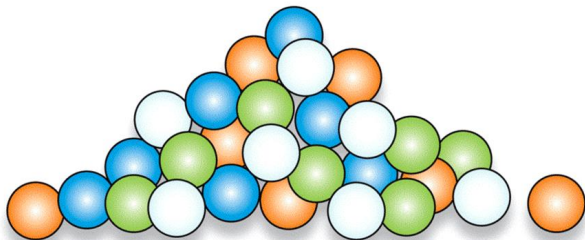


Blue alleles are associated with blue body color, which improves mating success. Blue alleles therefore increase in frequency.



- **Natural selection selects for traits advantageous for survival.**

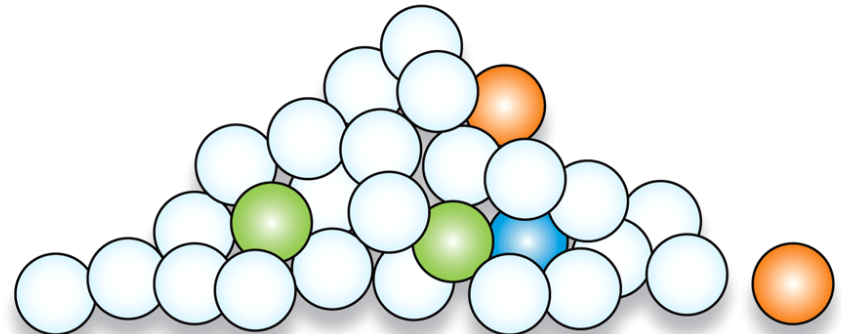
### INITIAL POPULATION



### NATURAL SELECTION

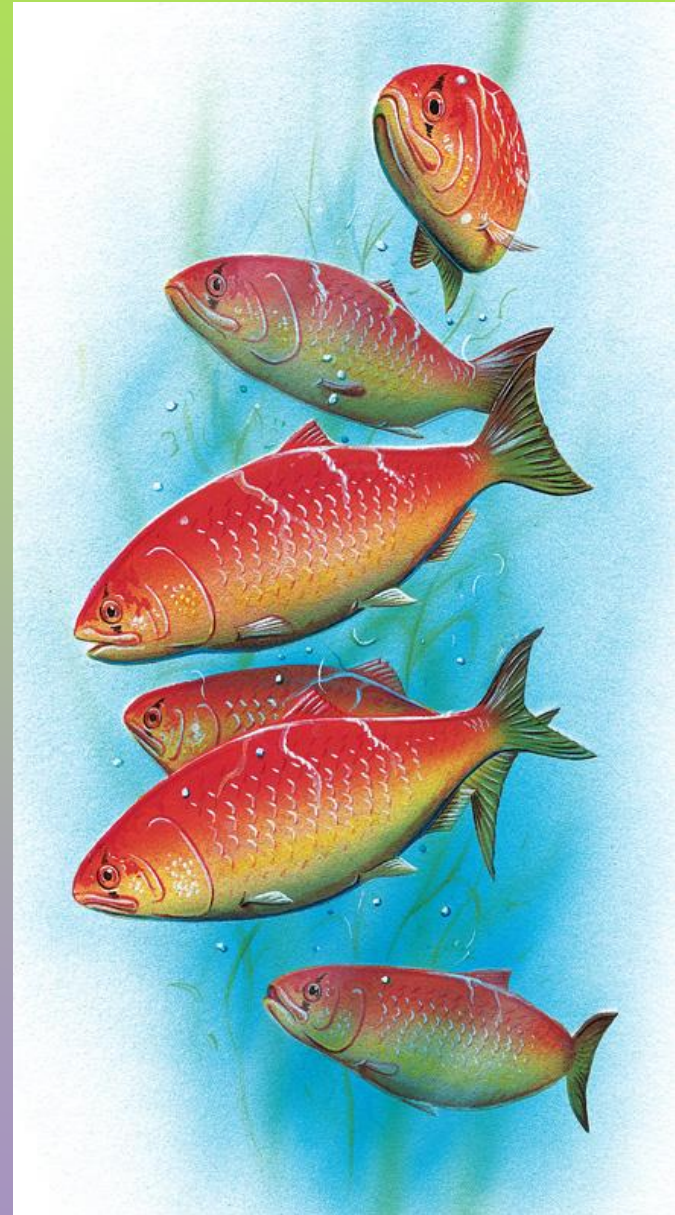


White alleles are associated with white body color, which allows individuals to blend in with their environment and avoid predation. White alleles therefore increase in frequency.



# ► In nature, populations evolve.

- expected in all populations most of the time
- respond to changing environments




# **SPECIATION**

## **KEY CONCEPT**

**New species can arise when populations are isolated.**

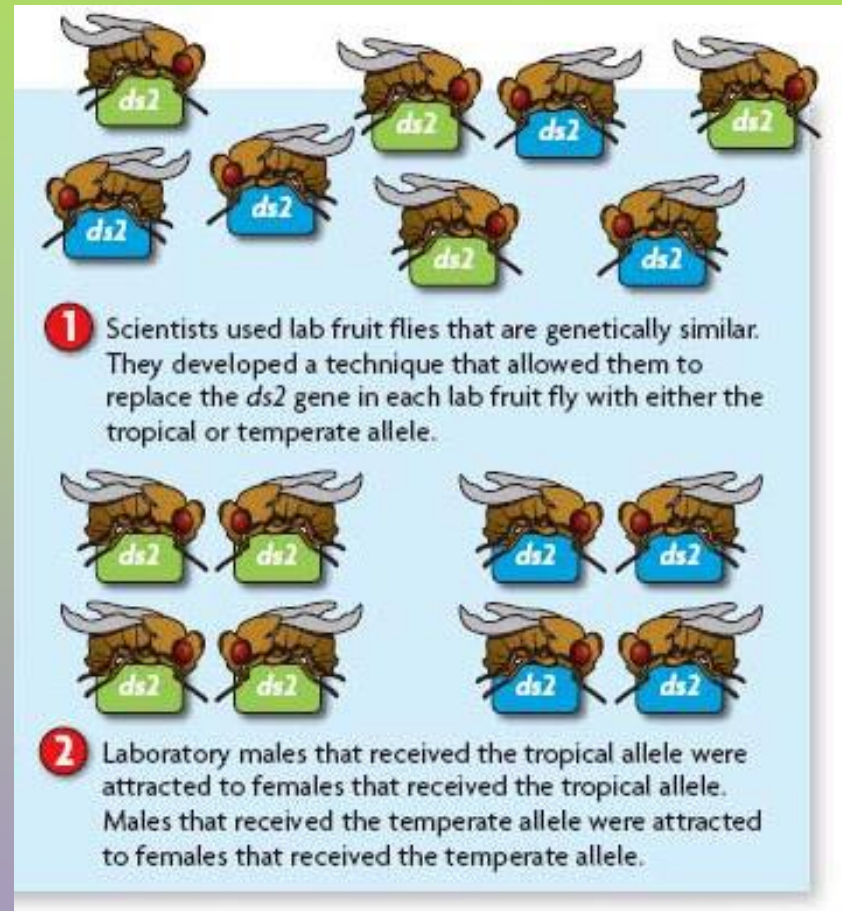


# THE ISOLATION OF POPULATIONS CAN LEAD TO SPECIATION.

- ▶ Populations become isolated when there is no gene flow.
    - ▶ Isolated populations adapt to their own environments.
    - ▶ Genetic differences can add up over generations.
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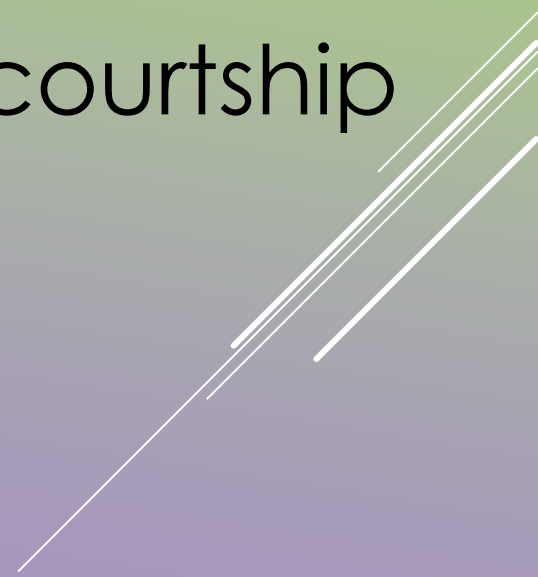
► Reproductive isolation can occur between isolated populations.

- members of different populations cannot mate successfully
- final step to becoming separate species



- Speciation is the rise of two or more species from one existing species.

# POPULATIONS CAN BECOME ISOLATED IN SEVERAL WAYS.

- ▶ Behavioral barriers can cause isolation.
    - ▶ called behavioral isolation
    - ▶ includes differences in courtship or mating behaviors
- 
- Several thin, parallel white lines of varying lengths and angles are positioned in the bottom right corner of the slide, creating a modern, abstract graphic element.

# Geographic barriers can cause isolation.

- called geographic isolation
- physical barriers divide population



## Temporal barriers can cause isolation.

- called temporal isolation
- timing of reproductive periods prevents mating

# Methods to characterize stocks by their frequencies of various individual Genotypes

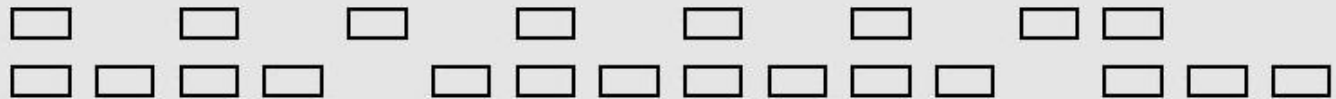
## Skjematisert elektroforese-gel for individuell genotyping

Anode (+)

Far Mor Avkom  
AA BB AB



Kontroll-  
krysning



Genotyper i stikkprøve (her 16 tilfeldige individ) tatt fra en naturlig populasjon der beregnet frekvens av genet for bånd A er lik 0,31, og for B 0,69.

Katode (-)

## Genetic nomenclature (jargon):

Synonyms: Gene frequency = allele frequency = allelic proportion

The frequency of an allele (e.g. *A*) is often abbreviated  $q_A$ ,  $q_B$ ,  $q_C$ , *etc*, or alternatively,  $p_A$ ,  $q_B$ ,  $r_C$  etc.

In general, the phenotypes (e.g. active isozymes, proteins) are written in normal font (e.g. AA phenotype) while genotype is written in *Italic* (AA genotype).

However, there is no general consistency between textbooks in how this is handled.

- Populations are the real evolutionary units.
- The raw material of evolution are mutations, which can accumulate with time.
- Evolution can thus be defined as "any change in allele frequencies".
- The frequencies of different alleles at a locus can be changed by the 4 evolutionary forces, which are:

- Mutations
- Random genetic drift
- Gene flow (immigration)
- Selection

If these forces are nullified, we have what is called a "Hardy-Weinberg population";

A panmictic (random mating), statistically ideal population where the allele frequencies, and genotype frequencies, are constant and do not change over generations (so called H-W equilibrium).

The population genetic approach is to assume a H-W equilibrium situation, and then study how the 4 evolutionary forces each, and in combinations, influence the allele- and genotypic frequencies within and between populations.

# Underlying assumption for the H-W law about genotypic proportions:

- Panmixi (random mating)
- No mutations
- No random genetic drift (i.e. infinitely large population)
- No gene flow between populations (i.e. no immigration/emmigration)
- No selection (same fitness of all genotypes)

## The Hardy-Weinbergs law:

"In a panmictic, statistically ideal population, the genotypic proportions are determined by the allele frequencies ( $p$  and  $q$ ) at the locus, according to the binomial formula. (multinomial if more than two alleles)":

$$(p+q)^2 = p^2 + 2pq + q^2 \text{ (if only two alleles)}$$

The allele frequencies will be constant over generations and restore the same genotypic proportions in each new generation.

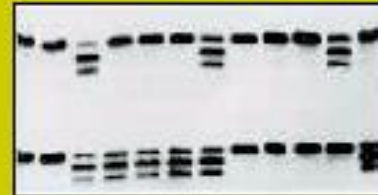
Allele- and genotype frequencies can thus serve as population characteristics.

# Methods for the study of frequencies of single genes

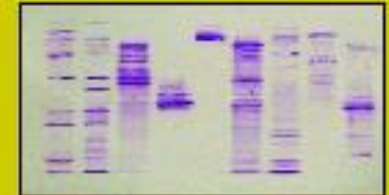
*Drosophila* sp.; anatomiske mutasjoner



Cellulose-acetat isozymer



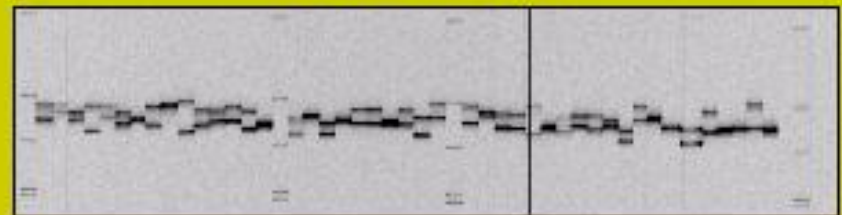
IFPAG proteiner



*Cepaea hortensis*; skallfarge mønster



PAGE mikrosatellitter



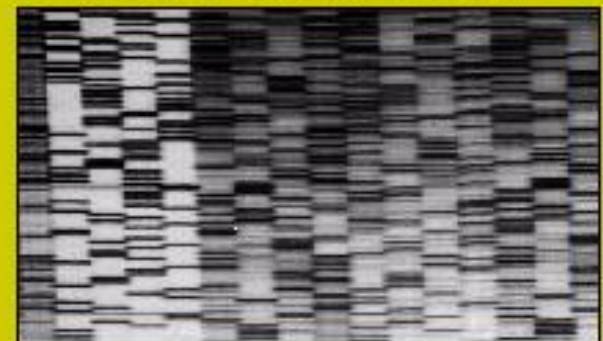
*Salmo trutta*; småprikking



*Homo sapiens*; rulle tunge på langs



PCR / PAGE direkte sekvensering



# Chi-squared test for goodness-of-fit to H-W proportions

Assume that we have studied a locus with two alleles by electrophoresis.

We have named the alleles *F* and *S* according to their electrophoretic migration distance (**F**ast and **S**low).

There are three possible genotypes (*FF*, *SF* og *SS*). In a sample of  $N=50$  individuals from a natural population we observed 10 with genotype *FF*, 28 with genotype *SF*, and 12 with genotype *SS*.

For this we use a so-called chi-square Goodness-of-fit test.

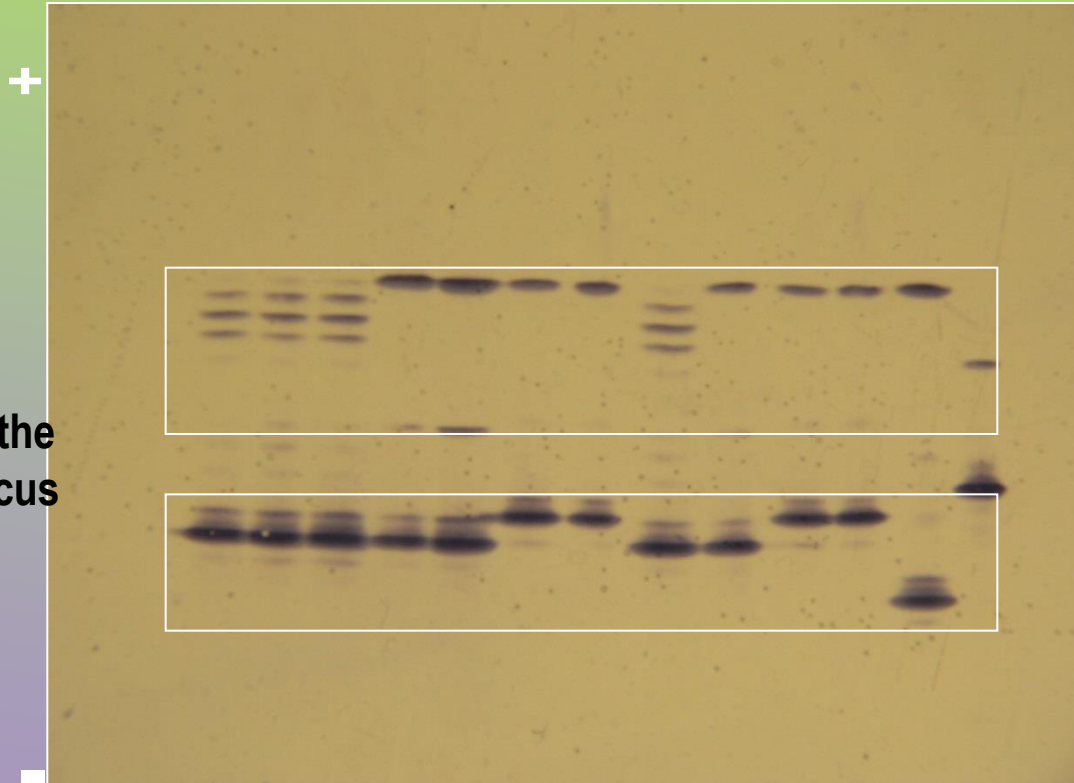
The degrees of freedom (df) is caclulated as the number of genotypes minus the number of alleles (i.e.  $df = 3-2 = 1$ ).

	<i>FF</i>	<i>FS</i>	<i>SS</i>	N	Freq. <i>F</i>	Freq. <i>S</i>
Observed	10	28	12	50	0.48	0.52
Expected	(11.5)	(25.0)	(13.5)	(50.0)		

Goodness-of-fit to Hardy-Weinberg expected distribution:  $\chi^2 = 0.742$ ,  $df=1$ ,  $P = 0.389$ ,  
i.e. not significant deviation between observed and expected genotype distribution.

# Genetic species identifications

Gel stained for 13 individual fish



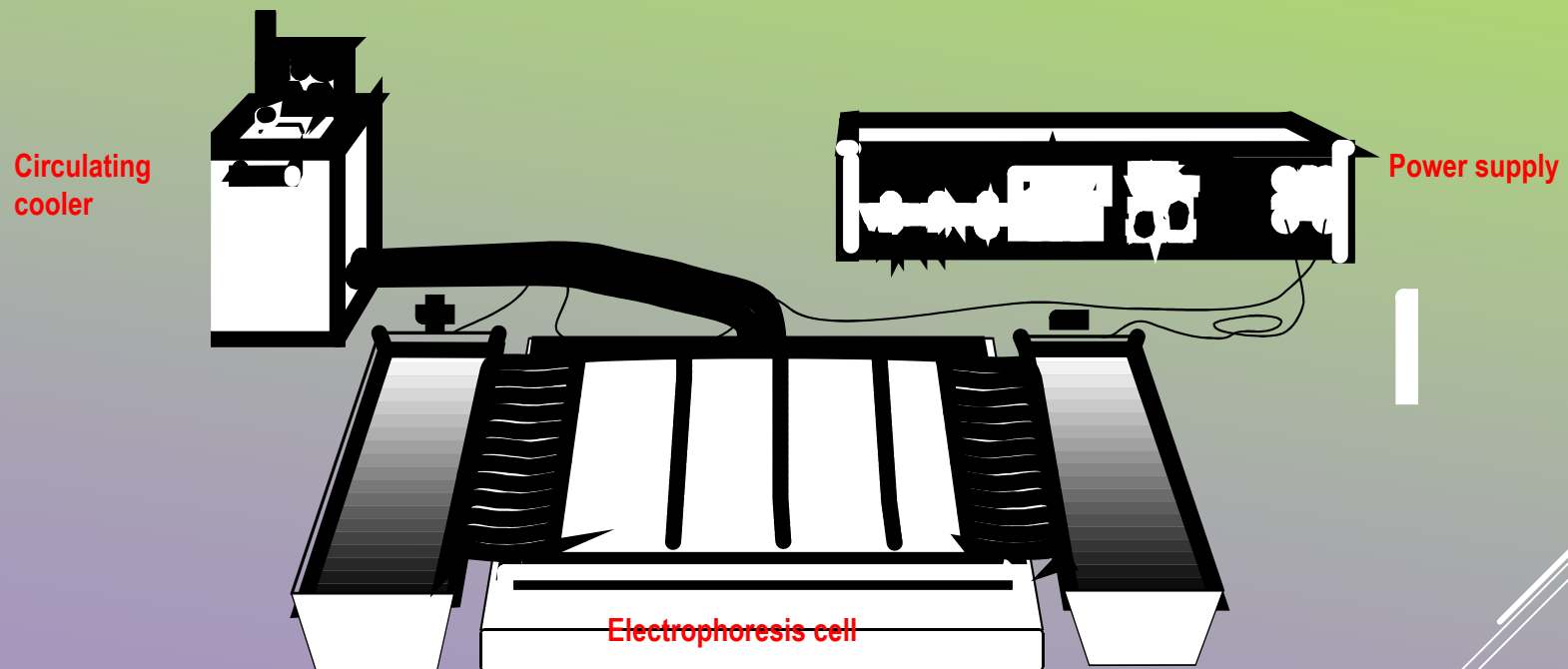
Upper frame shows non-diagnostic heart locus bands; cod heart locus polymorphism evident.

Lower frame shows muscle locus bands; these bands are diagnostic to species in Norwegian gadoids.

Bands in-between the frames are inter-locus hybrid molecules.

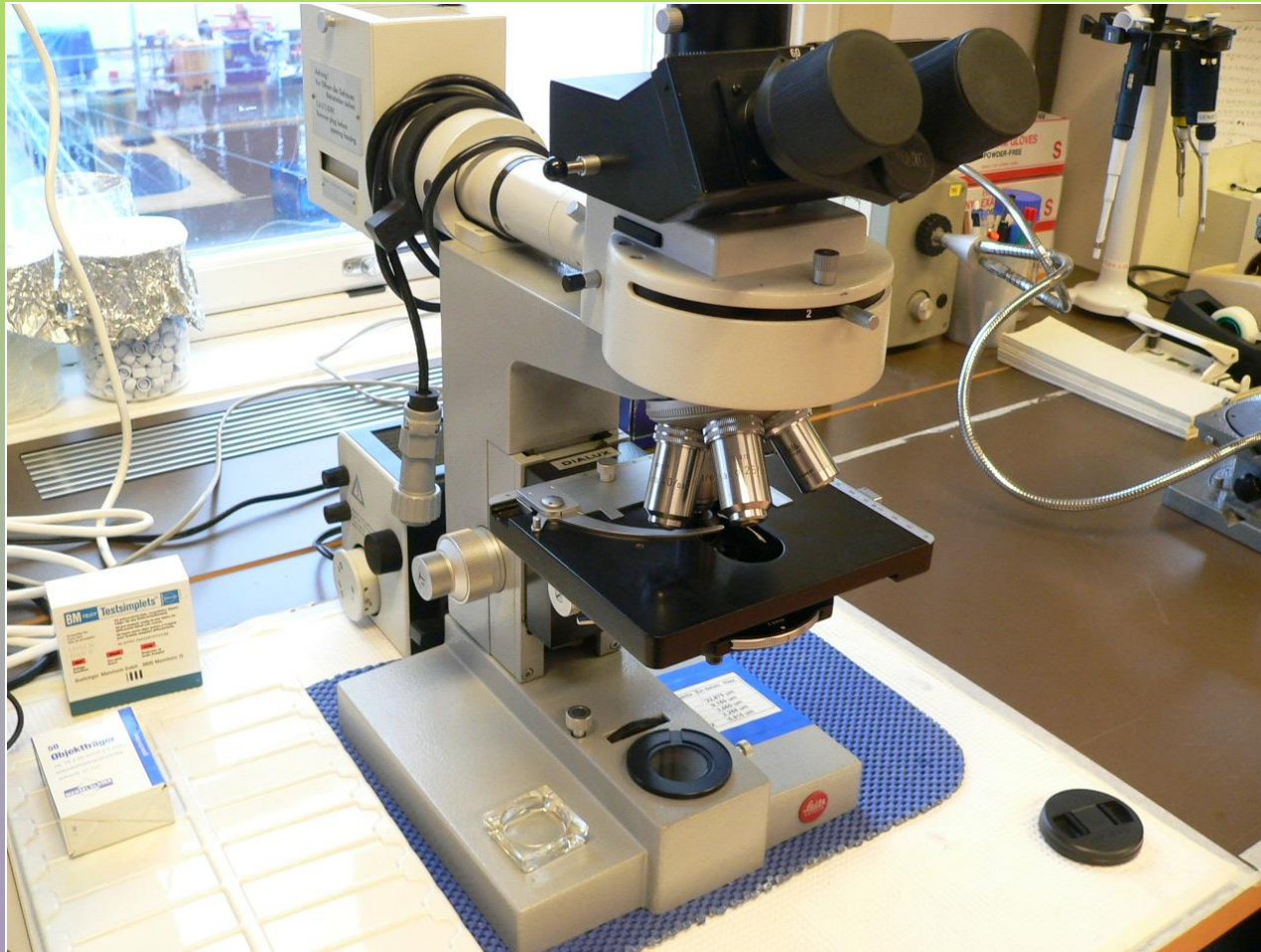
# SEPARATION SLIDE

# Starch gel electrophoresis setup





**Age determination by otoliths is done in a stereo-microscope with low magnification (10-16 X ocular, no objective).**



**DIALUX microscope used for study of blood smears.**



Instrument for clinical-chemical analyses (Reflotron "dry chemistry")



Instrument for Isoelectric focusing in polyacrylamide gel (IFPAG) 39



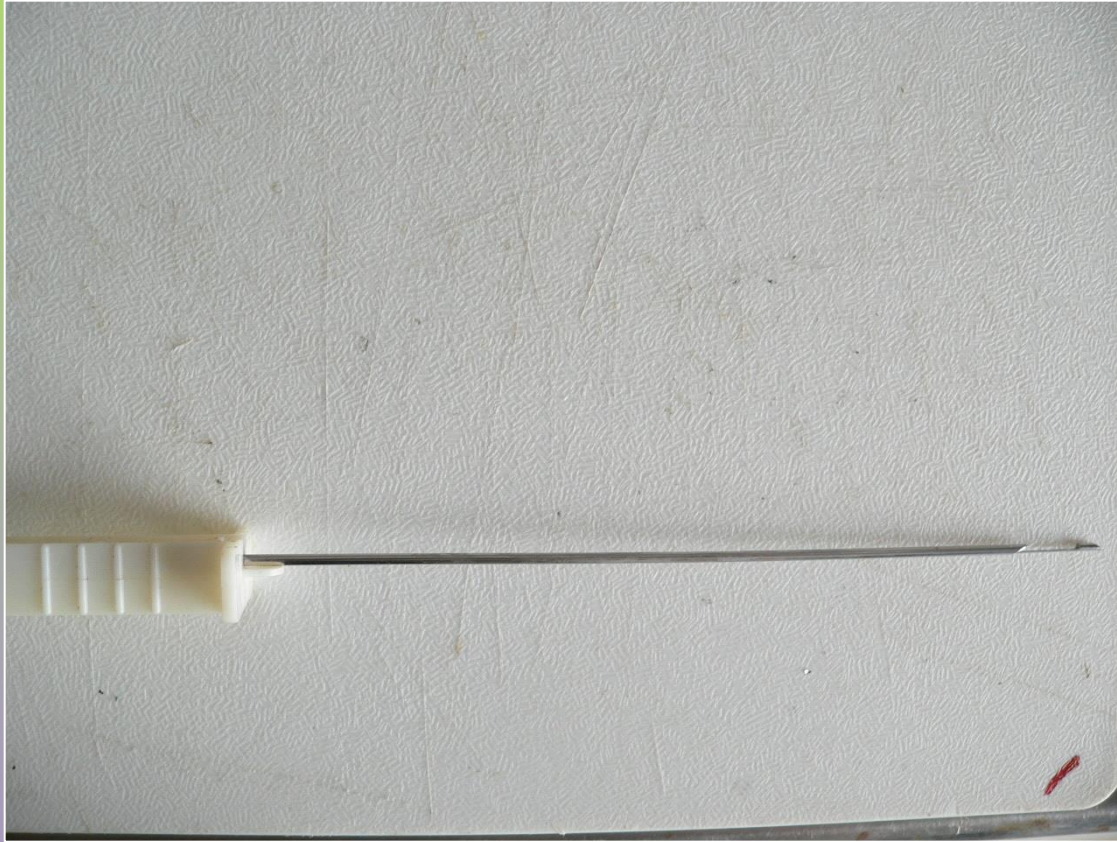
**Haematocrit centrifuge**



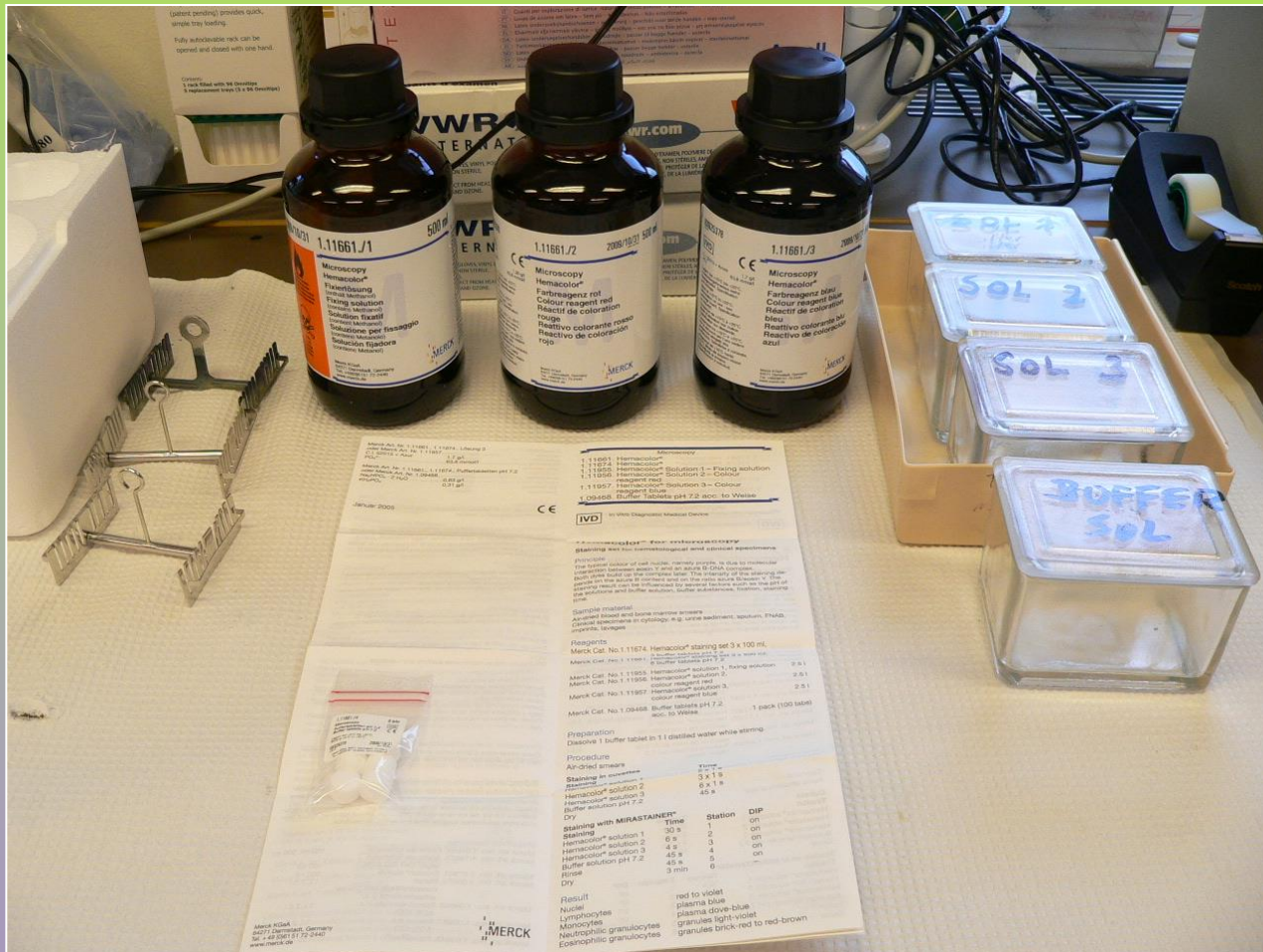
**Tagging needle and Lea hydrostatic tag**



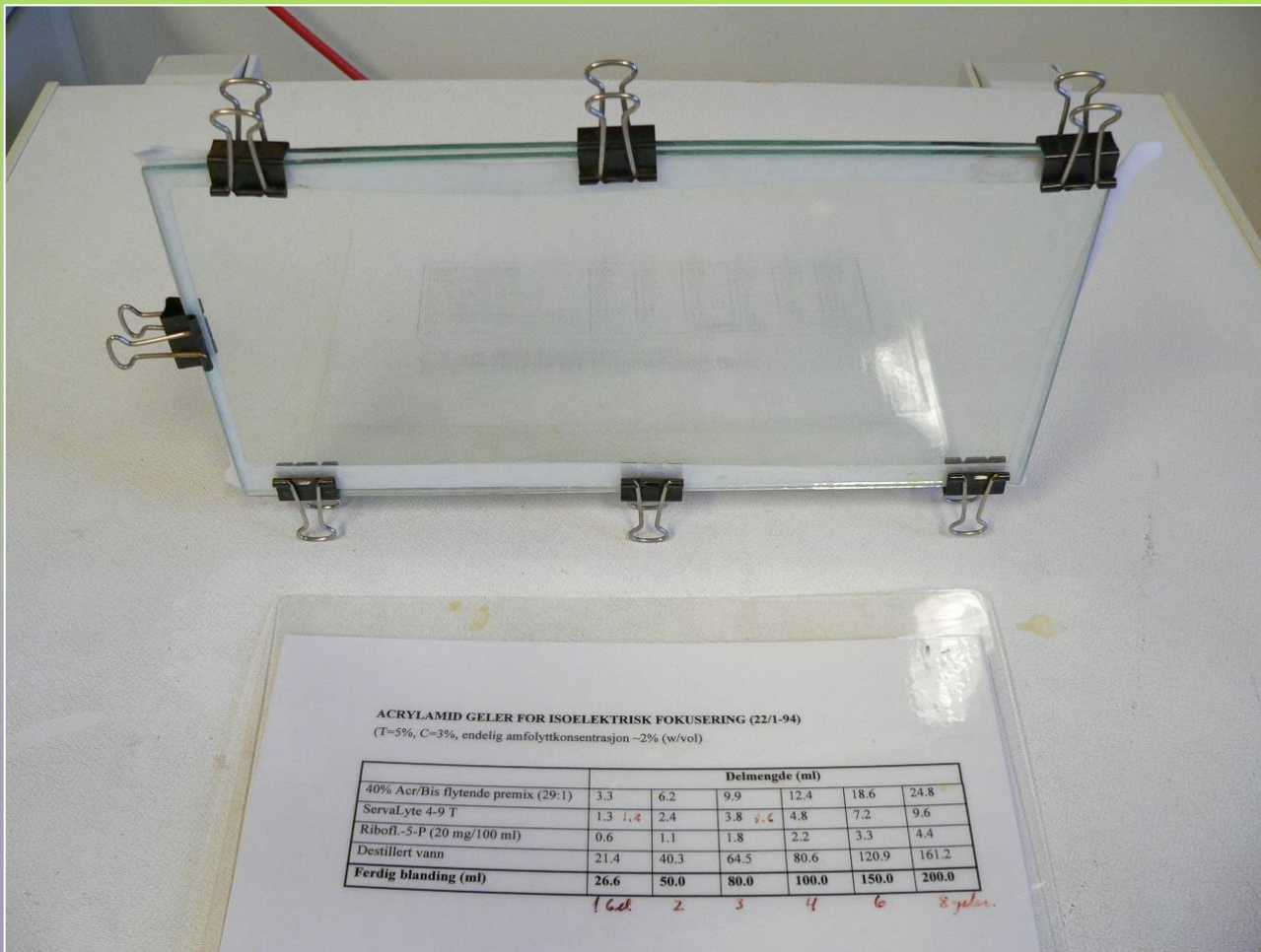
**Tagging gun and Floy anchor tags**



**Biopsy needle**



Merck "Hemocolor" quick staining kit for blood smears



Glass cassette and recipe for molding gel for IFPAG

## Gel pattern interpretation

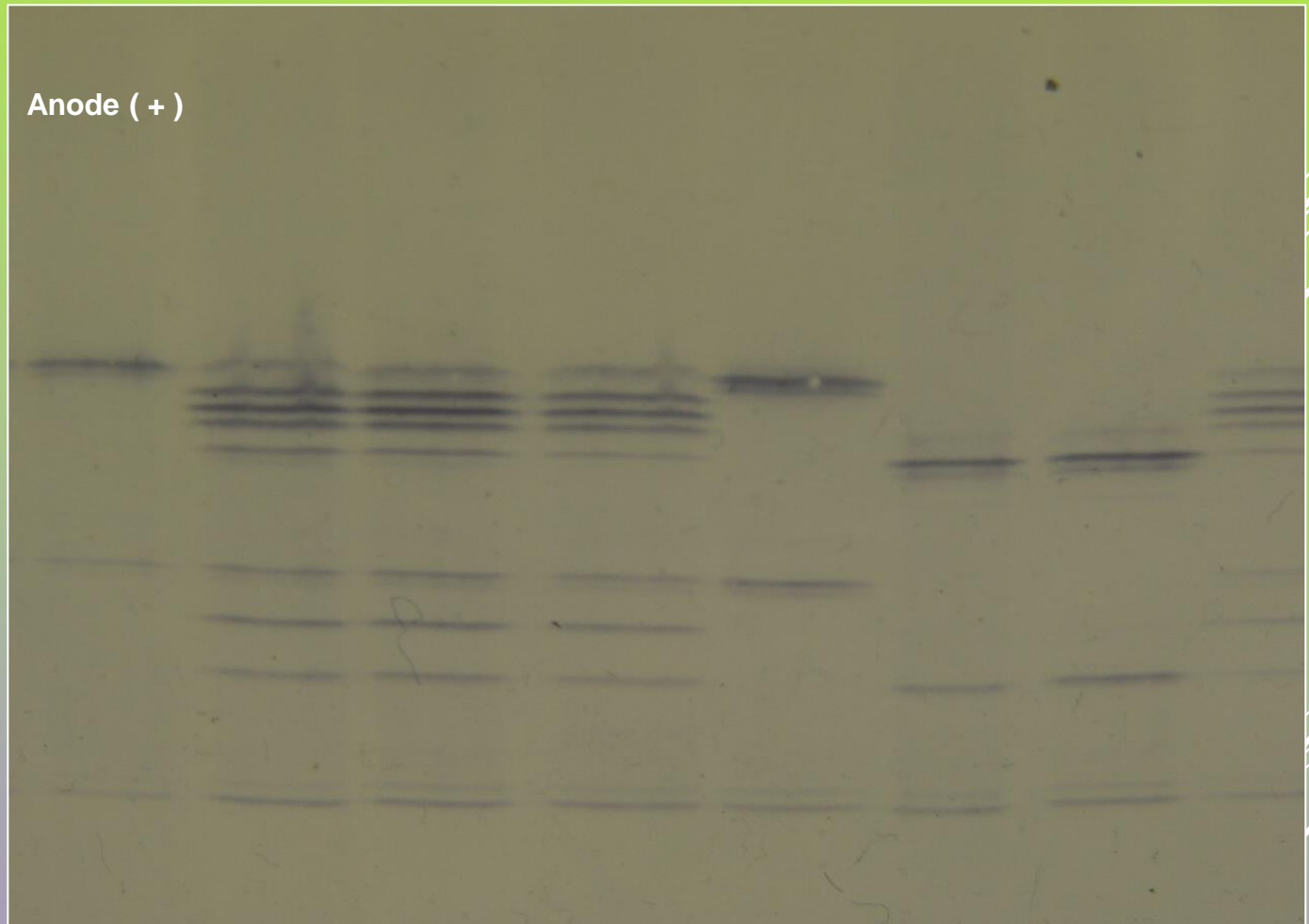
Heart locus  
(1 or 5 bands)



Muscle-heart  
hybrid bands  
(1 or 3 bands)



Muscle locus  
(1 band)



Cathode ( - )

**Close-up photo of IFPAG gel stained specifically for the enzyme LDH, lactate dehydrogenase (E.C. 1.1.1.27). The three common genotype patterns for the "heart locus" polymorphism are seen in the uppermost (anodic) part of the gel.**