

# Molecular genetic variation in the Galway sheep breed

Dawn J. Howard Ph.D.<sup>1,2</sup>

<sup>1</sup> Teagasc, Athenry, Galway.

<sup>2</sup> Animal Genomics Laboratory, UCD.



# Context

- **Worldwide 1,000 breeds have become extinct during the last 100 years (FAO)**
- **Extinct Irish breeds include;**
  - Irish Hobby horse
  - White-tailed eagle
  - Kerry Mountain and Cladagh sheep (lost in 1970s)
- **Endangered Irish breeds include:**
  - Kerry, Dexter and Irish moiled cattle
  - Connemara pony and Irish Draught horse
  - Galway sheep breed

# Galway Breed

- The Galway breed is the only native Irish sheep breed.
- It is listed by FAO (DADIS) as a breed in danger of extinction
- Due to decrease in numbers in 1980s and 1990s



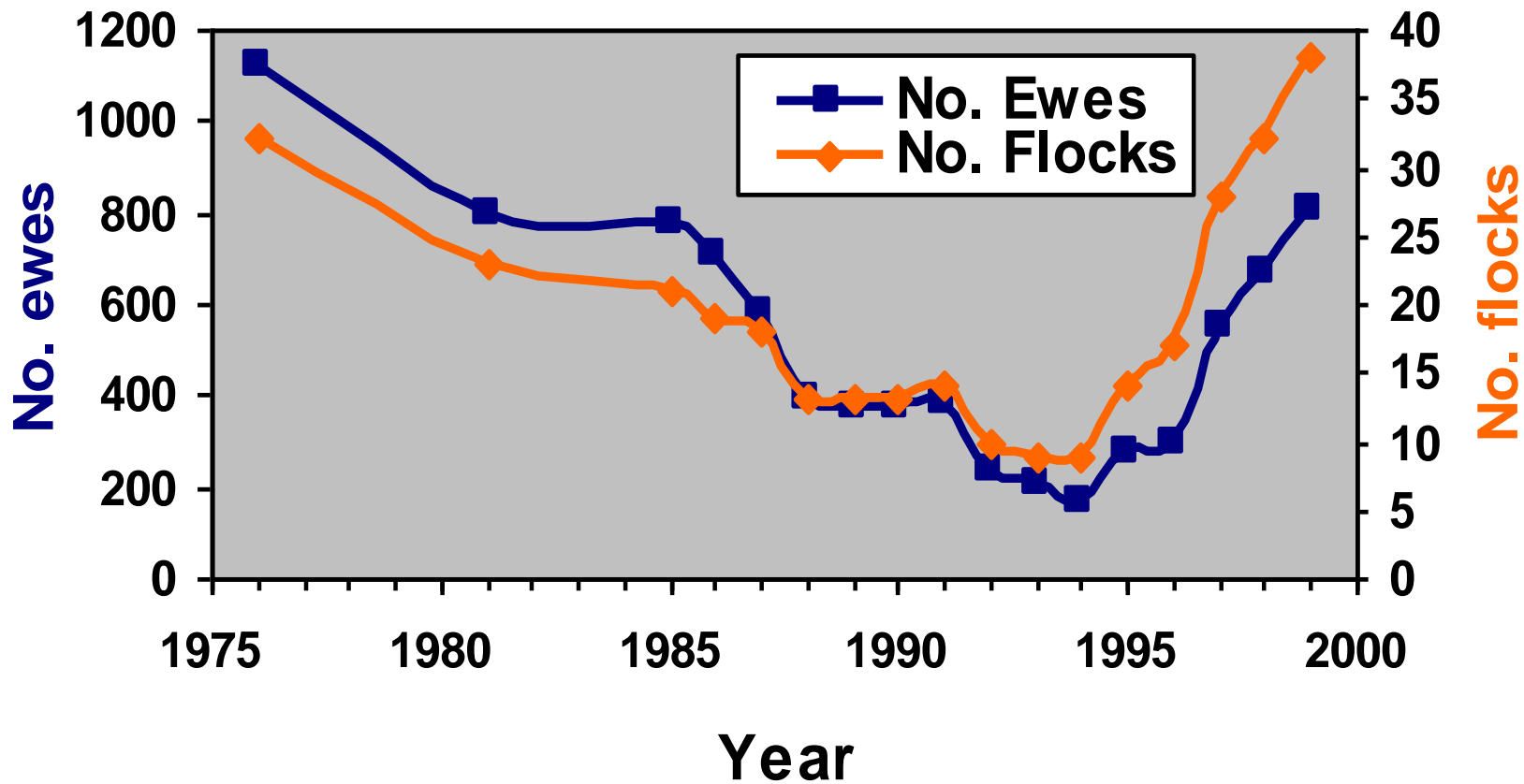
# Historical Background

- A long-woolled sheep breed being developed in Ireland up to mid 1800s
- Breeds used in its development include;
  - New Leicester
  - Southdown
  - Cotswold
  - Merino sheep
- Connaught was the principal breeding area
- At first the breed known as Roscommon breed
- 1870 recognised by Royal Dublin Society (RDS)
- 1895 Roscommon Breeders Society was formed

# Historical Background contd.

- By mid 1920s Roscommon Breeders Society became defunct
- 1923 the Galway Sheep Breeders Society was formed
- For a period the RDS listed both the Galway and Roscommon breeds
- From 1937 the Roscommon breed no longer listed in RDS catalogues
- Galway breed was the predominant breed until the mid 1970s when Suffolk cross lambs became more popular

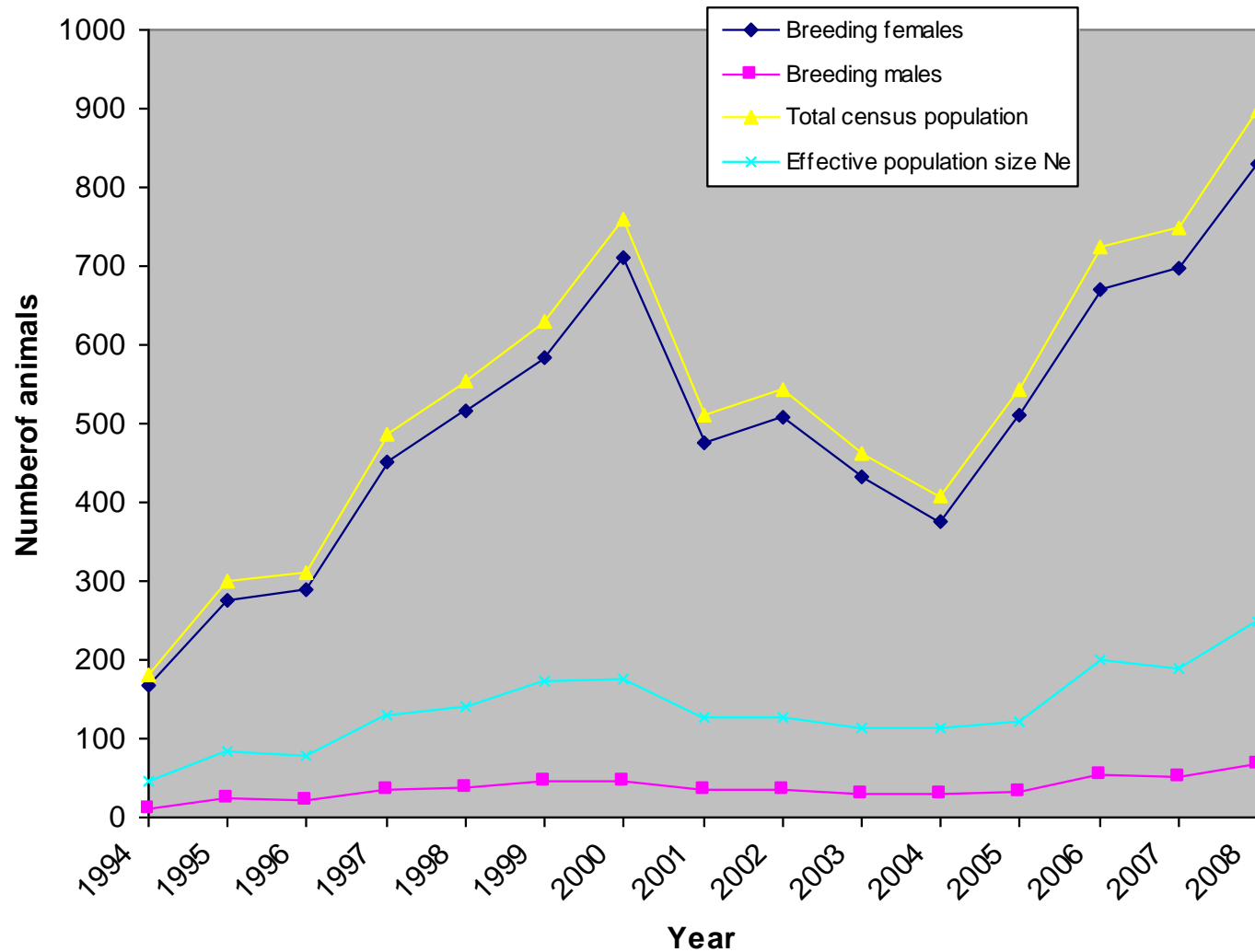
## Number of pedigree Galway ewes and flocks



# Population in 1994

- 9 flocks
- 169 ewes
- 11 rams of which 9 came from 8 core breeders

## Population history of Galway breed





# Overall Objectives

## ■ Objective 1:

Establish a DNA bank for pedigree Galway population.

## ■ Objective 2:

Establish the level of genetic variation in the Galway breed,

and compare this to other populations that have not experienced a bottleneck

# Methodology

## ■ Methodology 1:

The DNA bank – collection of blood, extraction of DNA , long-term storage

## ■ Methodology 2:

The genetic variation was evaluated from three perspectives:

1. 16 microsatellite loci markers
2. Control region of mitochondrial genome
3. Genetic markers on Y chromosome

# Background for microsatellite variation study

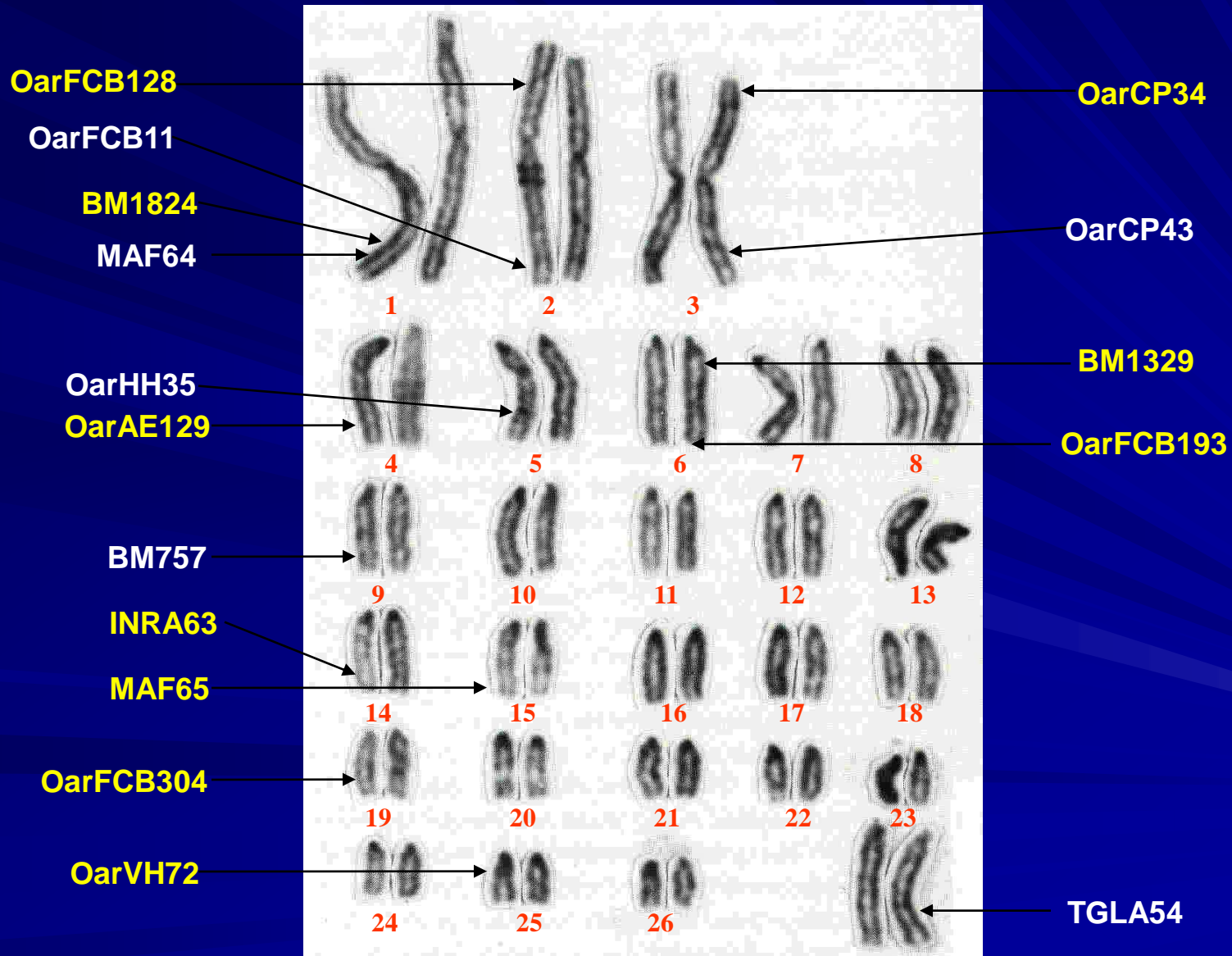
- **Microsatellites are repetitive stretches of DNA, usually di-, tri- or tetra-nucleotide repeats**
- **Microsatellite loci generally have many alleles**  
**and are considered to be neutral with respect to fitness**

# **Selection of microsatellite markers for study**

## **Considerations:**

- **Good coverage across the sheep genome**
- **Selected microsatellites that had been used in other studies – Econogene project and on FAO list**
- **Achievable workload – 16 markers chosen**

# Chromosomal location of microsatellite markers



# Selection of animals for study

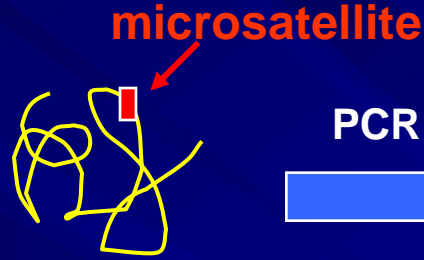
- Total of 94 Galways chosen from bank
  - All sires represented as equally as possible
  - Only one offspring per dam
- 70 Suffolk & 58 Texel
  - Both large populations and no known bottleneck
  - provide direct comparison of the Galway with non-endangered Irish populations.

# Methods



Sheep blood sample

EXTRACTION

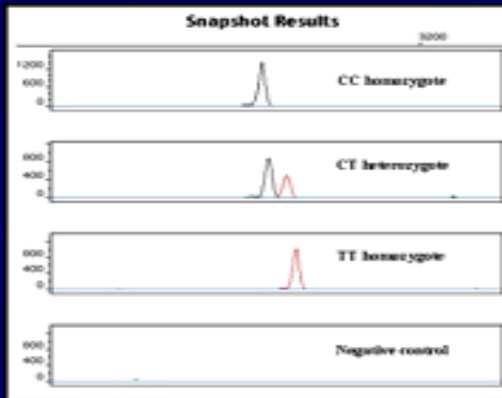


Sheep DNA

PCR



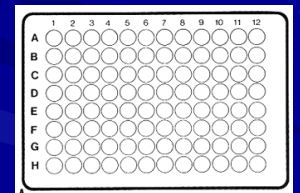
Fluorescently labelled  
microsatellite DNA



Output = microsatellite allele  
length in base pairs



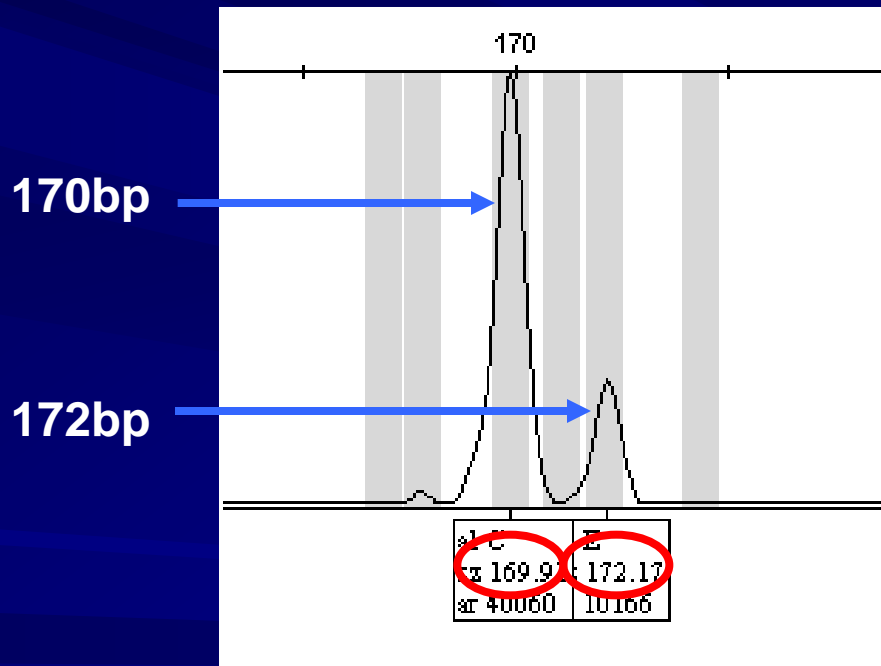
ABI 3100 Genetic Analyser



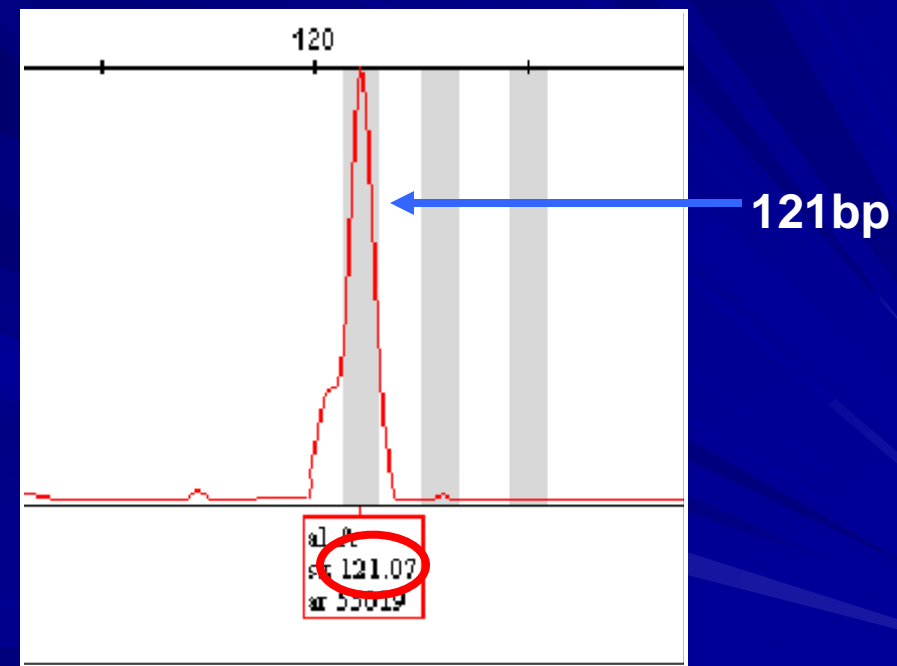
96 well microtitre  
plate

# ABI 3100 genetic analyser output

Heterozygous individual



Homozygous individual





# Genetic variation results

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Mean Diversity measure	Galway	Suffolk	Texel
No. of alleles	4.81	3.50	4.44
Effective no. of alleles	3.31	2.53	3.02
Allelic richness	4.91	3.92	4.33
Observed heterozygosity	0.60	0.60	0.52
Expected heterozygosity	0.69	0.59	0.58
PIC	0.62	0.51	0.60

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# Analysis of genetic differentiation

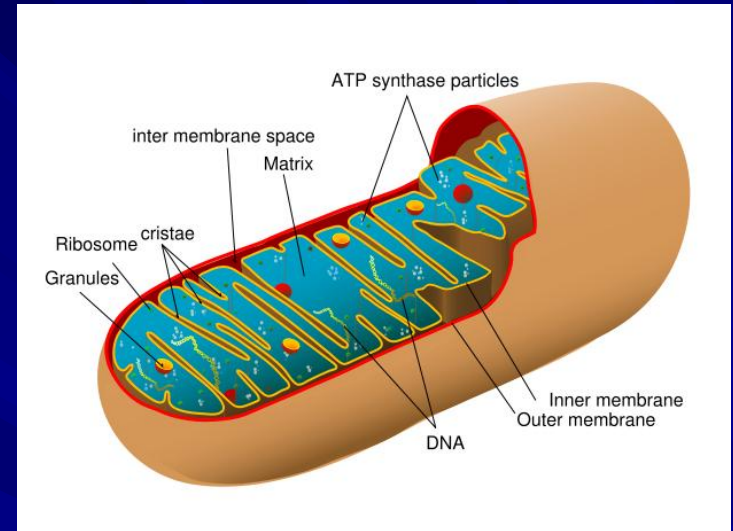
- Genetic structure - Sewell Wrights F-statistics
  - Measures deviations of genotype frequencies in subdivided populations in terms of  $F_{IS}$ ,  $F_{IT}$ ,  $F_{ST}$
  - High differentiation observed among the three Irish breeds ( $F_{ST} = 0.15$ )
- Galway, Suffolk and Texel - heterozygote deficiency

# Phylogenetic analysis

- Neighbour-joining tree constructed from allele sharing matrix data for three Irish breeds plus 57 populations from Econogene project
- Grouped Galway and Suffolk closer together than either is to Texel

# Mitochondrial variation study

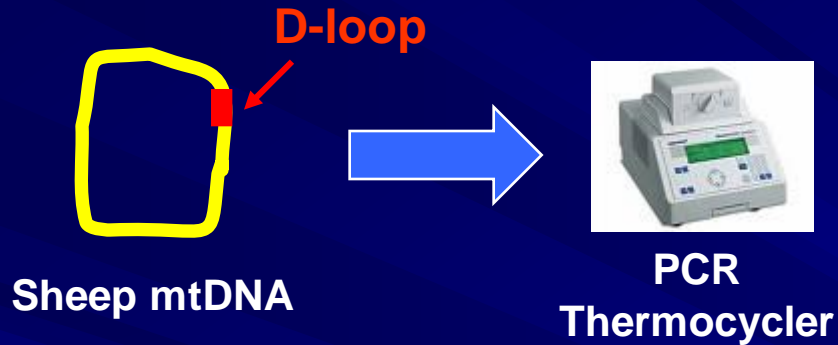
- **Maternally inherited**
- **No recombination**
- **High mutation rate**
- **Genome is 16 kb with 1.2 kb hypervariable D-loop.**
- **Mitochondrial DNA used to investigate:**
  - Maternal lineages
  - Genetic variation



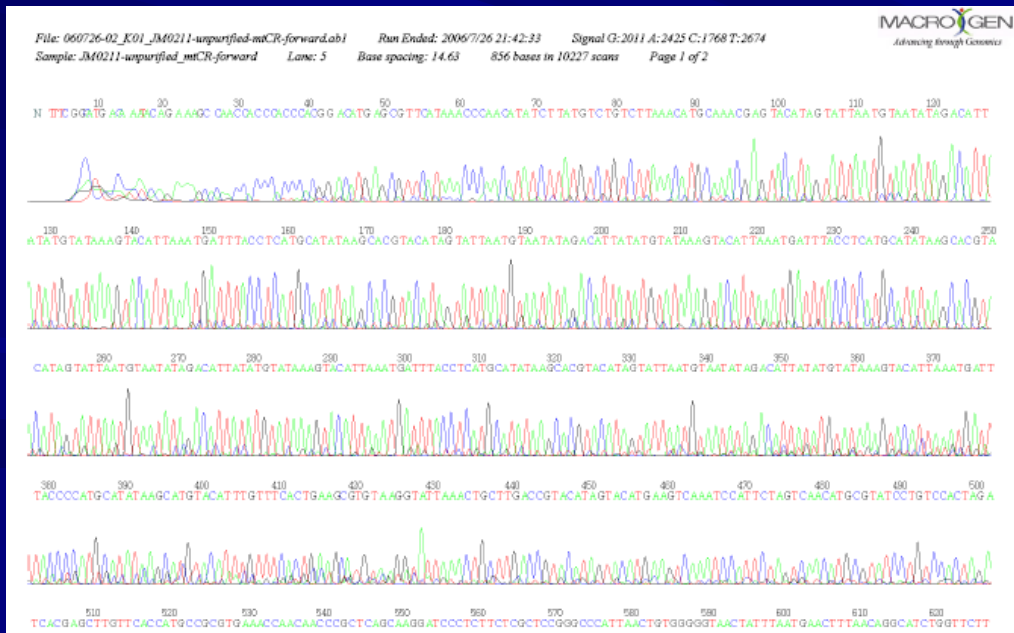
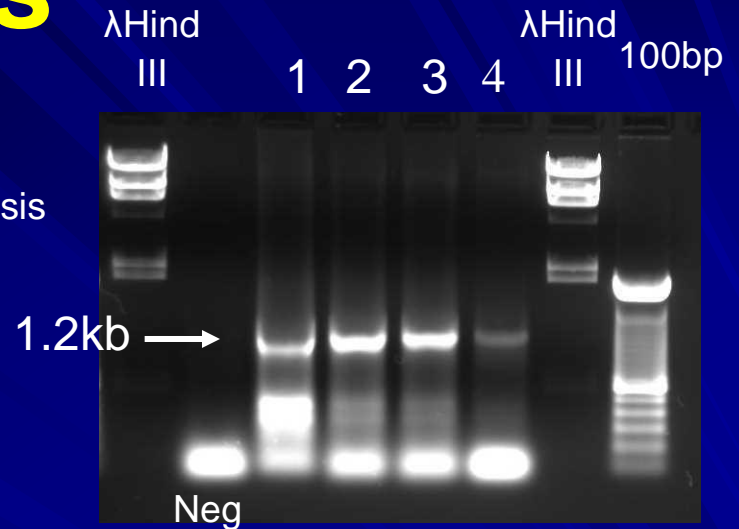
# Material used for study

- Total of 30 Galway sheep chosen from the DNA bank.
- DNA from 12 Suffolk and 10 Texel animals
- Selected 188 sheep mitochondrial DNA sequences from GenBank.

# Methods



Electrophoresis



electropherogram



ABI 3700 sequencer

# Phylogenetic analysis

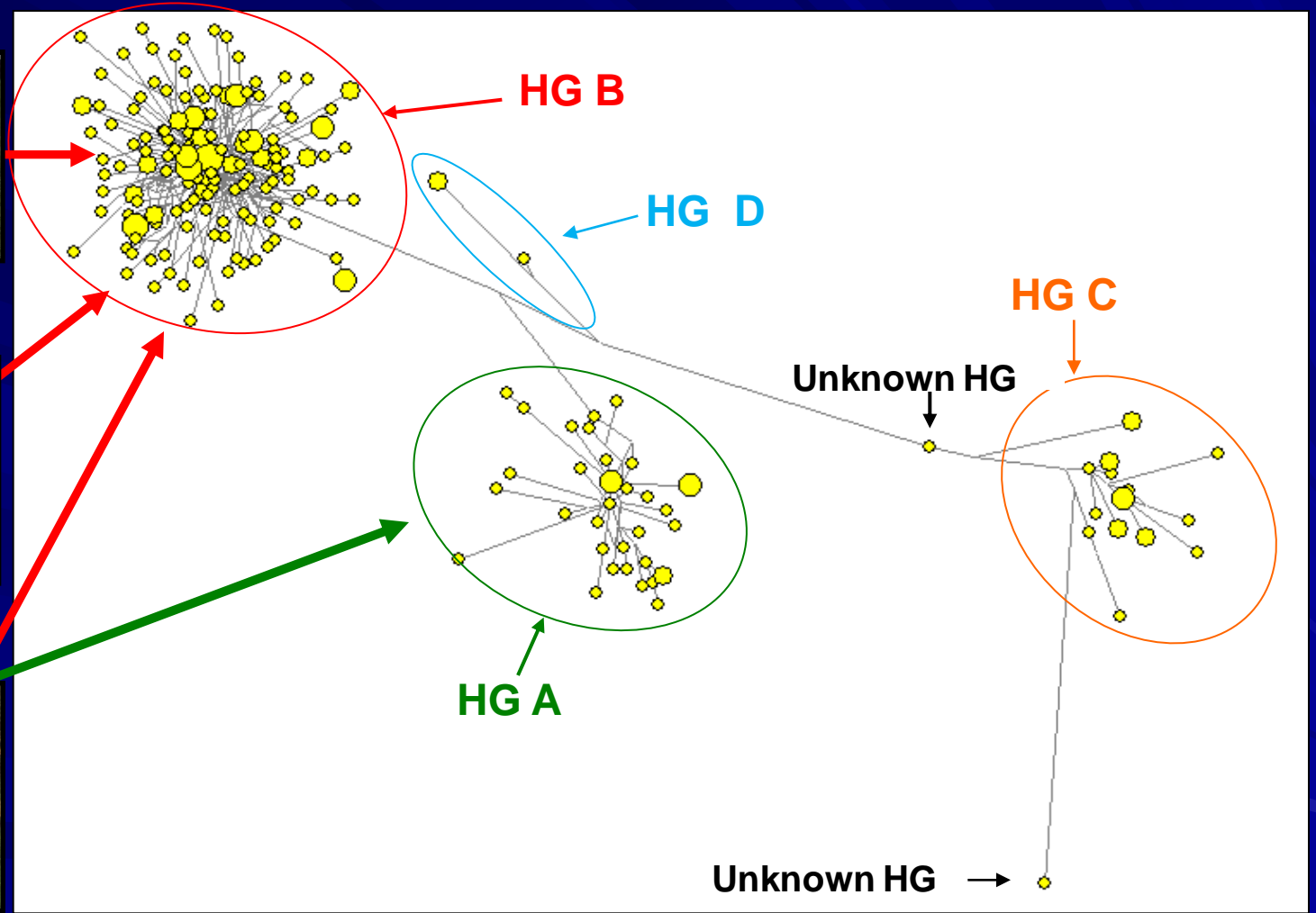
Galway



Suffolk



Texel



Haplogroup B	Haplogroup A	Haplogroup C	Haplogroup D
Karachas	Karachas	Karachas	Karachas
Mongolian	Mongolian	Mongolian	Mongolian
Karachul	Karachul	Karachul	
Small Tailed Han	Small Tailed Han	Small Tailed Han	
Churra Algarvia	Churra Algarvia	Churra Algarvia	
Tushin	Tushin	Tushin	
Hu	Hu	Hu	
Karachai	Karachai		Karachai
Texel	Texel		
Finnsneep	Finnsneep		
Speal Sheep	Speal sheep		
Olkusz	Olkusz		
Polled Dorset	Polled Dorset		
Garole	Garole		
Saloia	Saloia		
Badana		Badana	
Tibetan		Tibetan	
Galway			
Suffolk			
Oxford Down			
Romanov			
Swiniarka			
Russian Tsigai			
Merionpreto			
Old Speal Sheep			
Total no. breeds = 25 Total no. sequences = 179	Total no. breeds = 15 Total no. sequences = 36	Total no. breeds = 9 Total no. sequences = 18	Total no. breeds = 3 Total no. sequences = 3

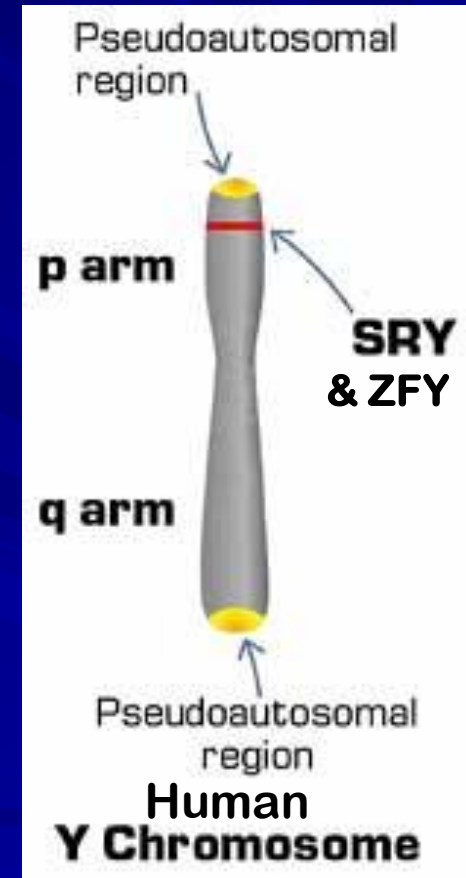


# Mitochondrial DNA variation

- Global and Irish mtDNA sequences
- Nucleotide diversity ( $\pi$ ) of 4 haplogroups (HG) – HG B most diverse
- Greatest divergence ( $k$ ) – between HG A and C
- $\pi$  for three Irish breeds – Texel most diverse
- Greatest divergence ( $k$ ) was observed when Texel was compared with either of the other two breeds

# Background for Y chromosome study

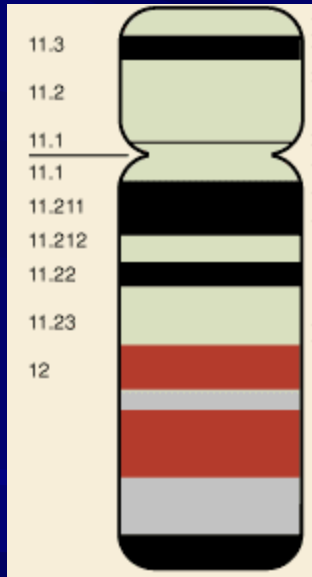
- Pseudoautosomal region - recombines with X
- Male Specific Y (MSY) region - no recombination.
- MSY genes are paternally inherited.
- Two known genes in sheep MSY region, ZFY & SRY



# Methods



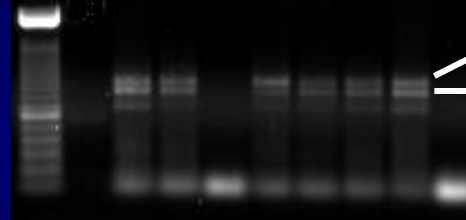
DNA from males



Y chromosome

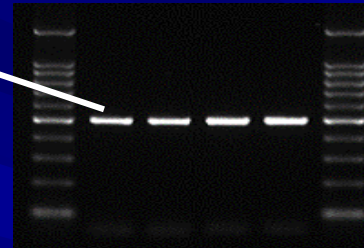
PCR

• ZFY intron



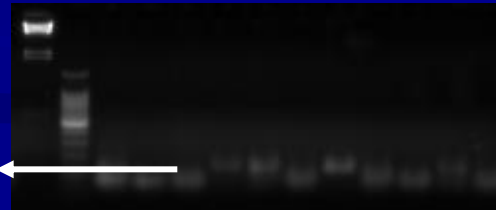
Lane 1: 100bp ladder, Lane 2: Empty,  
Lane 2 – 7: samples, Lane 8: Neg control

• SRY SNP



Lane 1: 100bp ladder, Lane 6: 100bp ladder,  
Lane 2 – 5: samples

• SRY microsatellite



~140bp

Lane 1:  $\lambda$  Hind III, Lane 2: 100bp ladder,  
Lane 3 – 12: samples Lane 13: Neg control



ABI 3700  
sequencer

# Methods

- 10 Galway males, 11 Suffolk males, 9 Texel males.
- Haplotypes were based on:
  - Single nucleotide polymorphism (SNP) called oY1 in SRY promoter region
  - SRY microsatellite called SRYM18
- Analysis of ZFY intron diversity

# Results

- There was no variation in the ZFY intron
- SRY SNP + SRY microsatellite = haplotype H5 in Irish breeds

Haplotype	[TTTTG] <sub>m</sub>	Indel G/-	[TG] <sub>n</sub>	SRY SNP	Breed
H5	3	G	16	G	Galway
H5	3	G	16	G	Suffolk
H5	3	G	16	G	Texel

- H5 is present in sheep from Asia and Europe, absent from African sheep

# Conclusions from microsatellites

- **The genetic variation in the Galway breed is within the range reported for other breeds.**
  - No evidence that the Galway breed has lost genetic diversity.
  - So bottleneck in 1994 did not have any major consequences
- **Galway phylogenetically closer to the Suffolk than to Texel**

# Conclusions from mtDNA

- Galway and Suffolk have one maternal lineage – HG B
- Texel has two maternal lineages – HG B and A
- Greatest divergence between HG A and C
- The divergence was least between Galway and Suffolk
- Of the three Irish breeds – Texel most diverse

# Conclusions from Y chromosome

- One paternal haplotype identified in the Galway, Suffolk and Texel breeds
- There was no variation in ZFY intron



# Overall conclusion

- **Current number of pedigree Galway sheep (800 ewes in 50 registered flocks) is sufficient to maintain the existing genetic diversity.**

# Acknowledgements

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