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Sequencing of PRNP in East Adriatic Sheep: New Non-synonymous Polymorphisms Linked to ARQ allele

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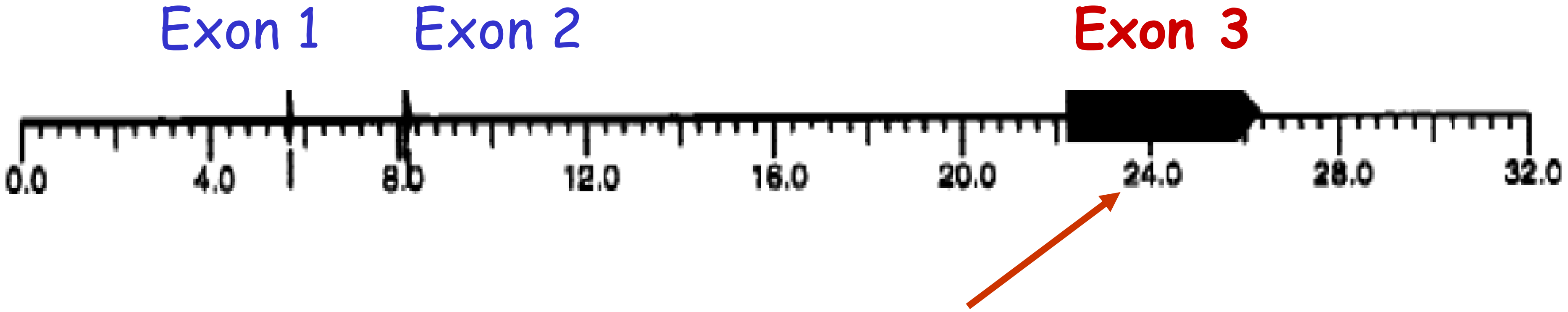
61th Annual meeting of the EAAP Heraklion-Crete Island, Greece,
23rd-27th Auguste 2010.

PRNP gene of sheep: 31 412 bp

- 3 exons (52, 98, i 4028 bp)
- 2 introns (2421 i 14 031 bp)



sequencing of 628 bp

Exon 3: codons 136 (A/V), 154 (R/H), 171 (Q/R/H)

Class	Genotype	Class of resistance
NSP1	ARR/ARR	Highly resistant to Scrapie
NSP2	ARR/**ARQ**	Resistant to Scrapie
	ARR/ARH	(genotype rare)
	ARR/AHQ	(genotype rare)
NSP3	**ARQ**/**ARQ**	Little resistant to Scrapie
	ARQ/AHQ	(genotype rare)
	AHQ/AHQ	(genotype rare)
	ARH/ARH	(genotype rare)
	AHQ/AHQ	(genotype rare)
	ARQ/ARH	(genotype rare)
NSP4	ARR/VRQ	Susceptible to Scrapie
NSP5	ARQ/ VRQ	Highly Susceptible to Scrapie
	VRQ/VRQ	
	VRQ/ARH	
	VRQ/AHQ	
	VRQ/ARR	

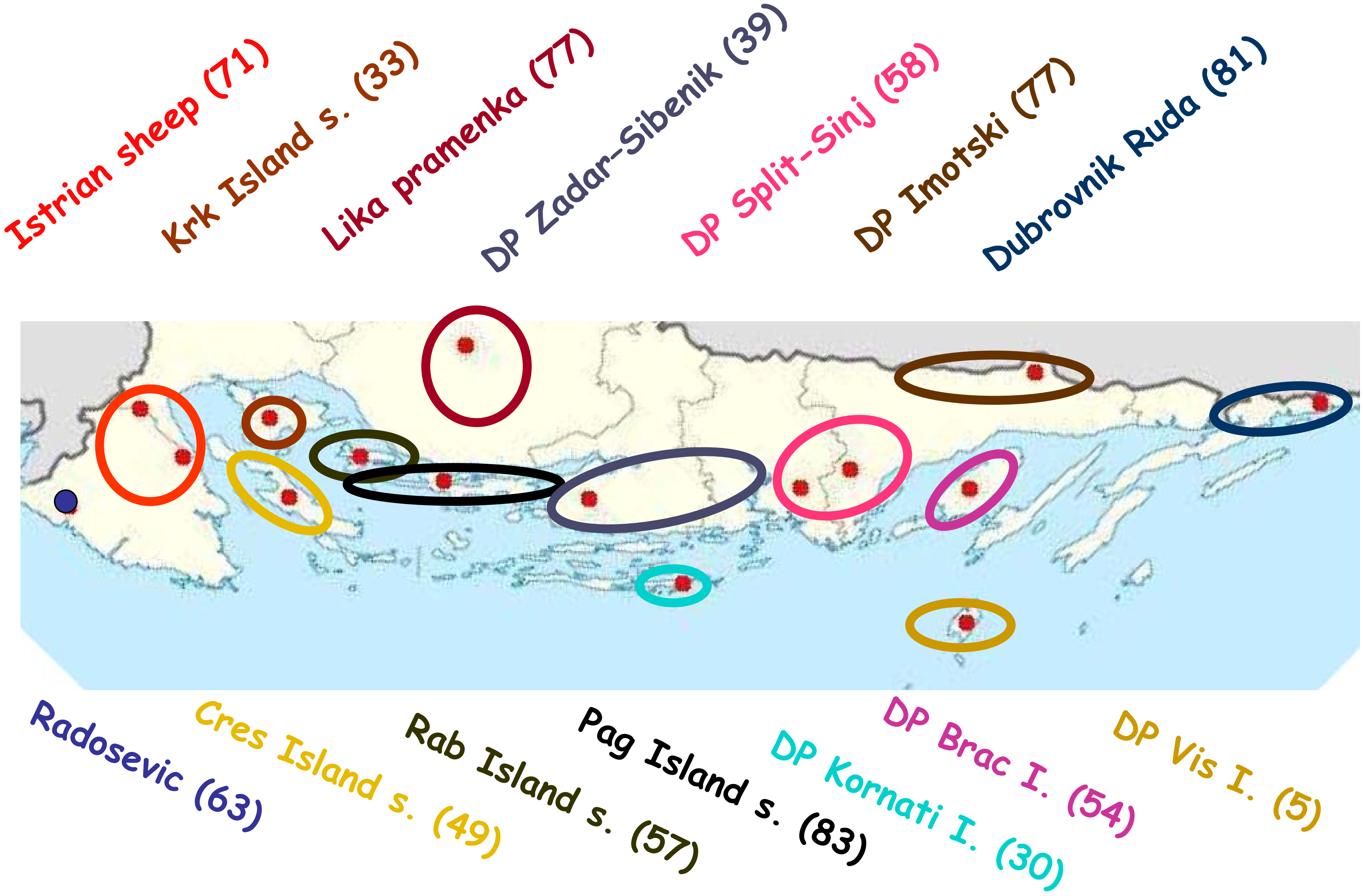
EU (2003) "Commission Decision of 13 February 2003 laying down minimum requirements for the establishment of breeding programmes for resistance to transmissible spongiform encephalopathies in sheep."

Official Journal of the European Union 41: 41-45

Compulsory MINIMUM requirements !

Risk of ARQ + additional polymorphisms (T112, T137, K176, etc…) ?!

- **Laegreid** et al. 2008.
- **Vaccari** et al. 2009.
- **Maestrale** et al. 2009.
- **Saunders** et al. 2009.


Istrian sheep (71)
Krk Island s. (33)
Lika pramenka (77)
DP Zadar-Sibenik (39)
DP Split-Sinj (58)
DP Imotski (77)
Dubrovnik Ruda (81)
Radosevic (63)
Cres Island s. (49)
Rab Island s. (57)
Pag Island s. (83)
DP Kornati I. (30)
DP Brac I. (54)
DP Vis I. (5)

Cres Island s.

Rab Island s.

Pag Island s.

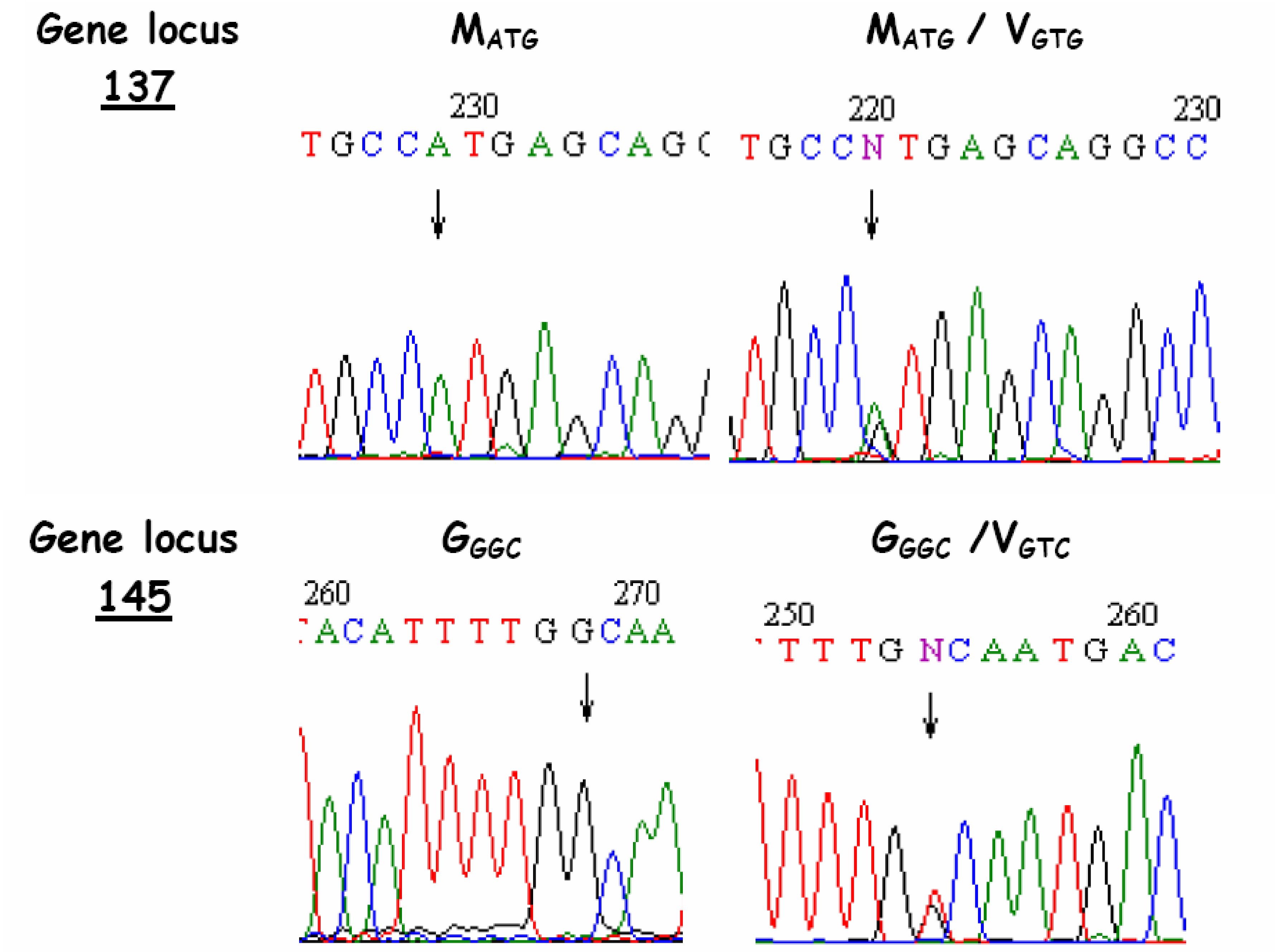
Krk Island s.

Lika Pramenka

Dalmatia Pramenka

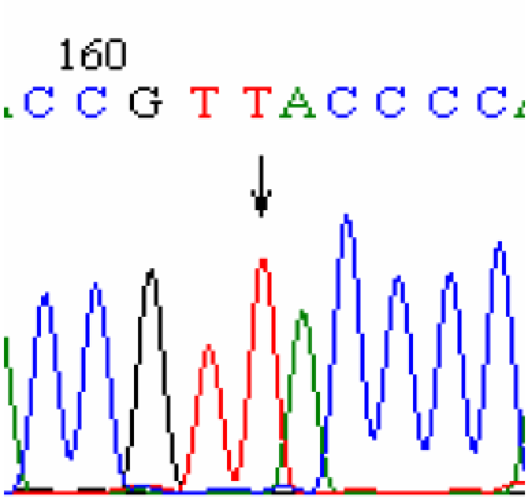
Istrian s.

Dubrovnik Ruda

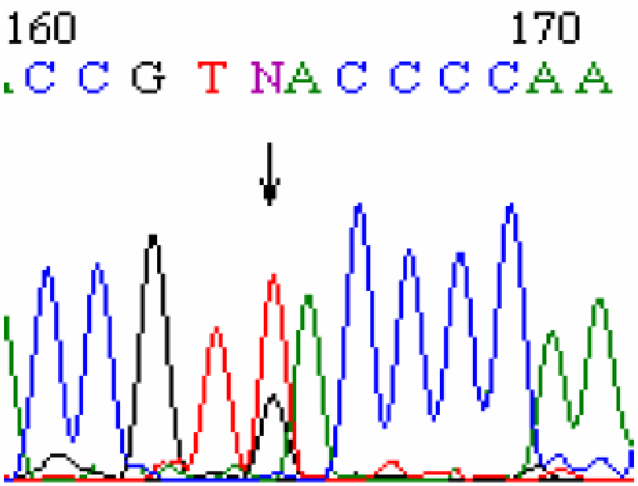

Gene locus
137
M_{ATG}
230
TGCCATGAGCAG
M_{ATG} / V_{GTG}
220
230
TGCCNTGAGCAGGCC
Gene locus
145
G_{GGC}
260
270
ACATTTTGGCAA
G_{GGC} /V_{GTC}
250
260
TTTGNCAATGAC

Gene locus
160

Y_{TAC}

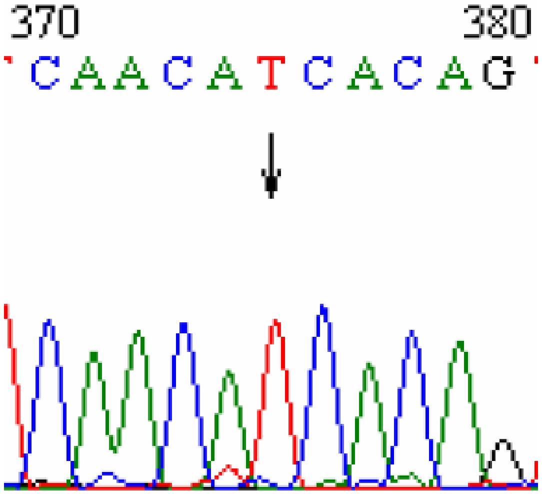


Y_{TAC} / D_{GAC}

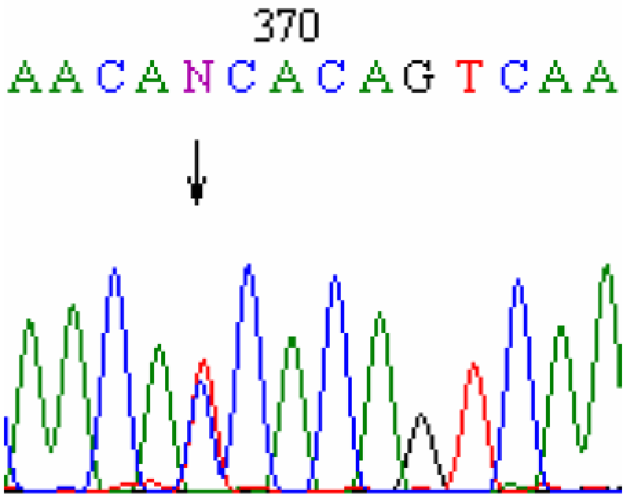


Gene locus
185

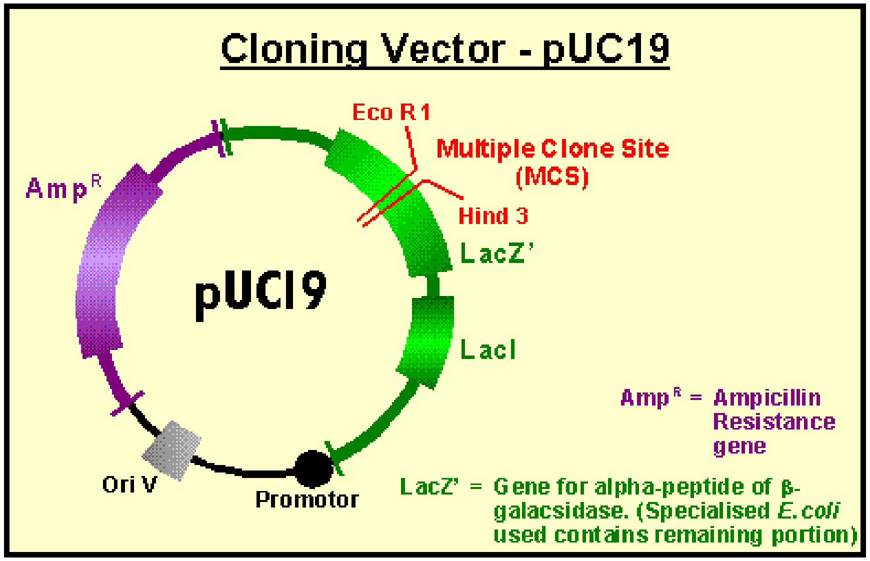
I_{ATC}



I_{ATC} / T_{ACC}



AT-cloning have confirmed new polymorphisms for 145, 160 and 185 locus while 137 still remain to be confirmed



SAS/Genetics 9.1.3.

```
proc allele data = RiskScra_2009c
      genocol delimiter ='/'
      haplo=est corrcoeff dprime
      exact=1000 boot=1000 seed=2503
      ;
      var H136_154_171
      g231 g237 g101 g112 g127 g137
      g141 g145 g160 g175 g180 g185
      ;
run;
```

PROC HAPLOTYPE

- Estimation of haplotype

Linkage disequilibrium

Locus	Rare allele	AHQ	ARH	ARK	ARQ	ARR	VRQ
101	$R_{101}CGG$	-	-	-	0.2	-	-
112	$T_{112}ACG$	-	-	-	3.8	-	-
127	$S_{127}AGC$	0.3	-	-	0.5	-	-
137	$V_{137}GTG$	-	-	-	0.1	-	-
141	$F_{141}TTT$	-	-	-	0.3	-	-
143	$R_{143}CGT$	-	-	-	0.3	-	-
145	$V_{145}GTC$	-	-	-	0.3	-	-
160	$D_{160}GAC$	-	-	-	0.1	-	-
175	$Q_{175}CAA$	-	-	-	0.1	-	-
180	$Y_{180}TAT$	-	-	-	0.4	-	-
185	$T_{185}ACC$	0.1	-	-	1.1	-	-
231	$R_{231}CGG$	6.9	0.8	-	9.6	-	0.1
237	$L_{237}CTG$	6.9	0.8	-	9.6	-	0.1

Conclusions

Analysis of 780 sequences revealed large number of rare alleles in PRNP (exon 3).

Linkage disequilibrium, composite genotypes and cloning strongly indicate:

additional non-synonymous polymorphisms are predominantly associated with ARQ allele.

Decrease of ARQ alleles → elimination of non-synonymous rare alleles at PRNP (exon 3) which potentially might be beneficial !

Dairy products in Mediterranean sheep population: quantification of scrapie risk

RISK

SCRA

FP6-2005-SME-COLL