

Lezione XXXVII-XXXVIII

martedì 10-I-2012

corso di genomica

laurea magistrale Biotecnologia
Industriale

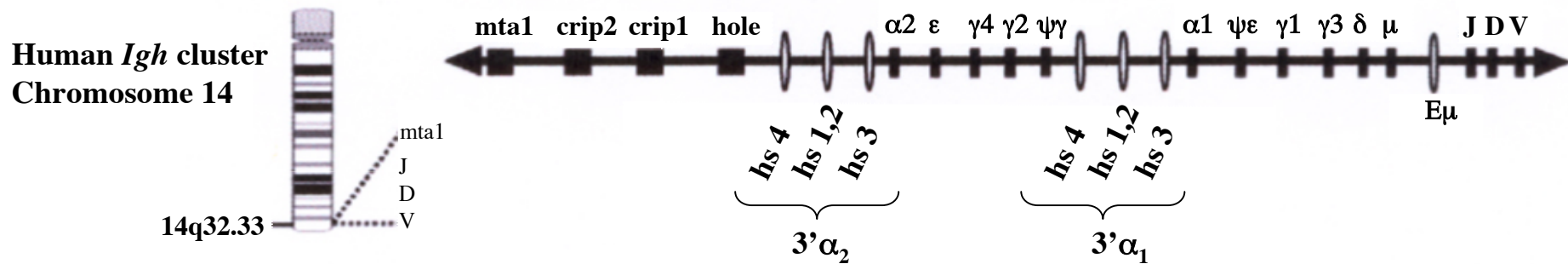
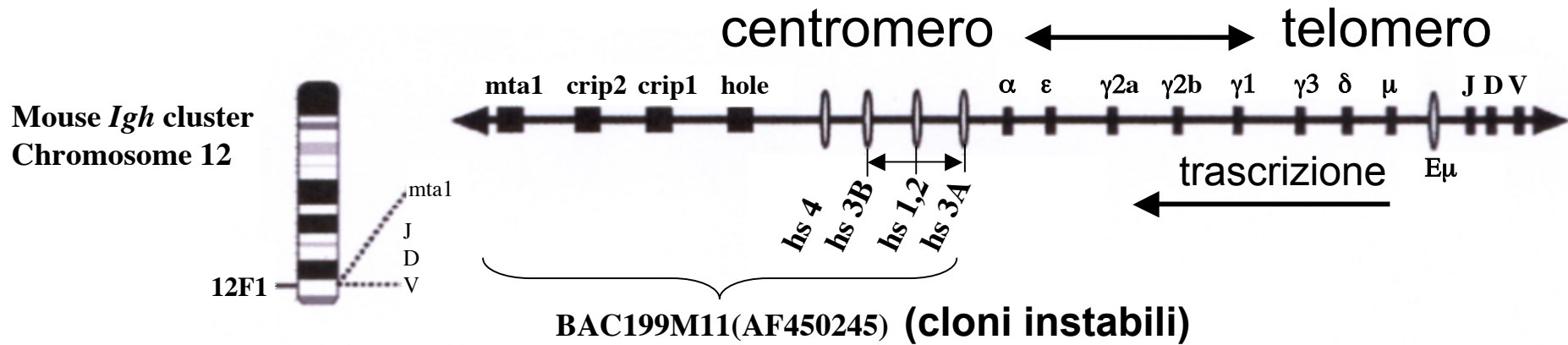
aula 8

orario : Martedì ore 14.00 - 16.00

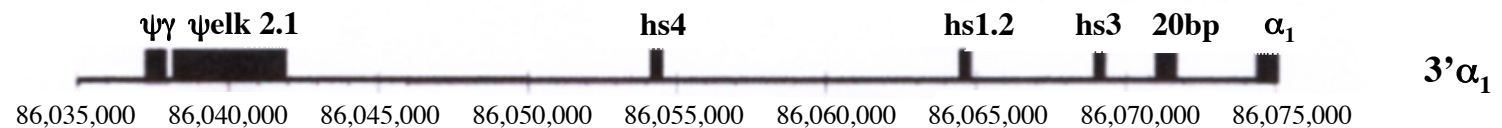
Giovedì ore 13.00 - 15.00

D. Frezza

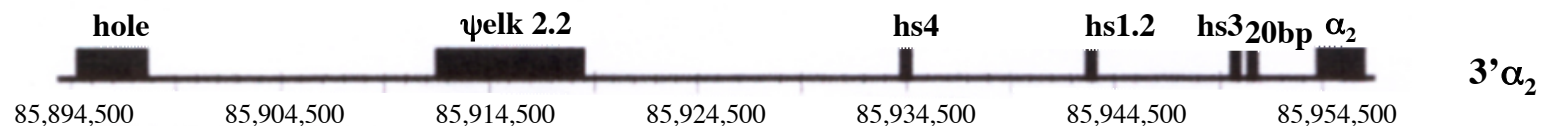
cluster Ig topo-uomo (duplicazione)



IgH3'RR-1

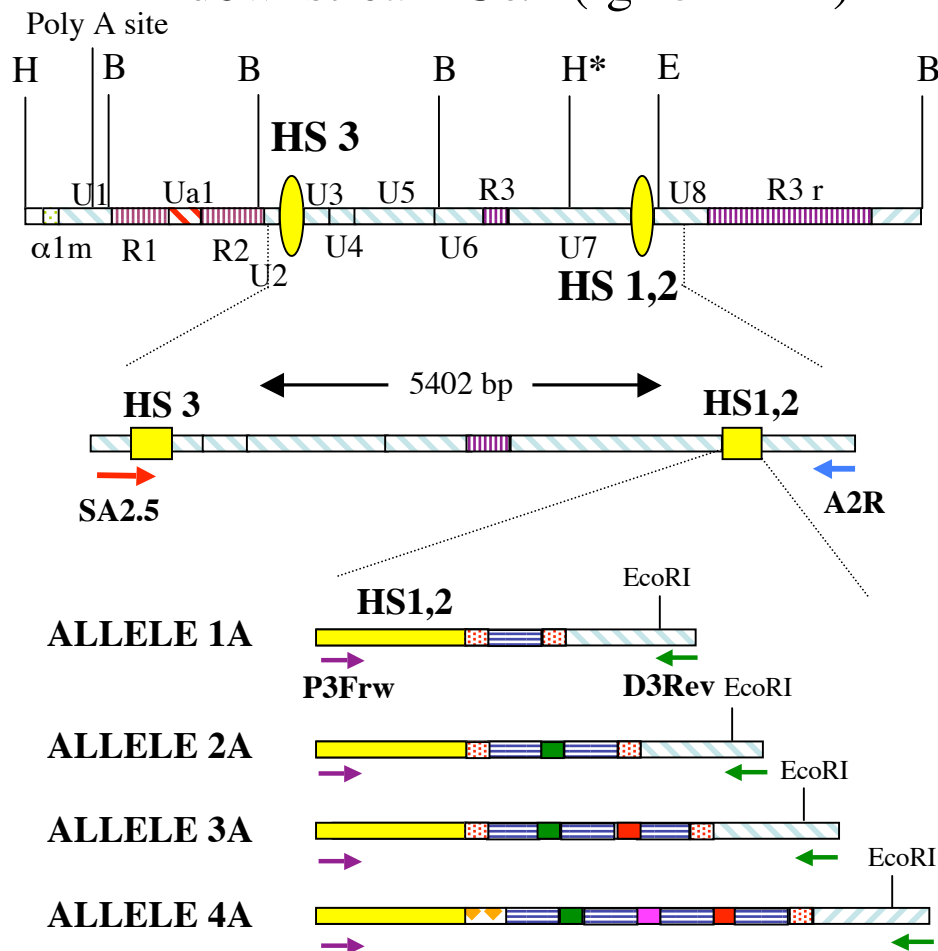


IgH3'RR-2



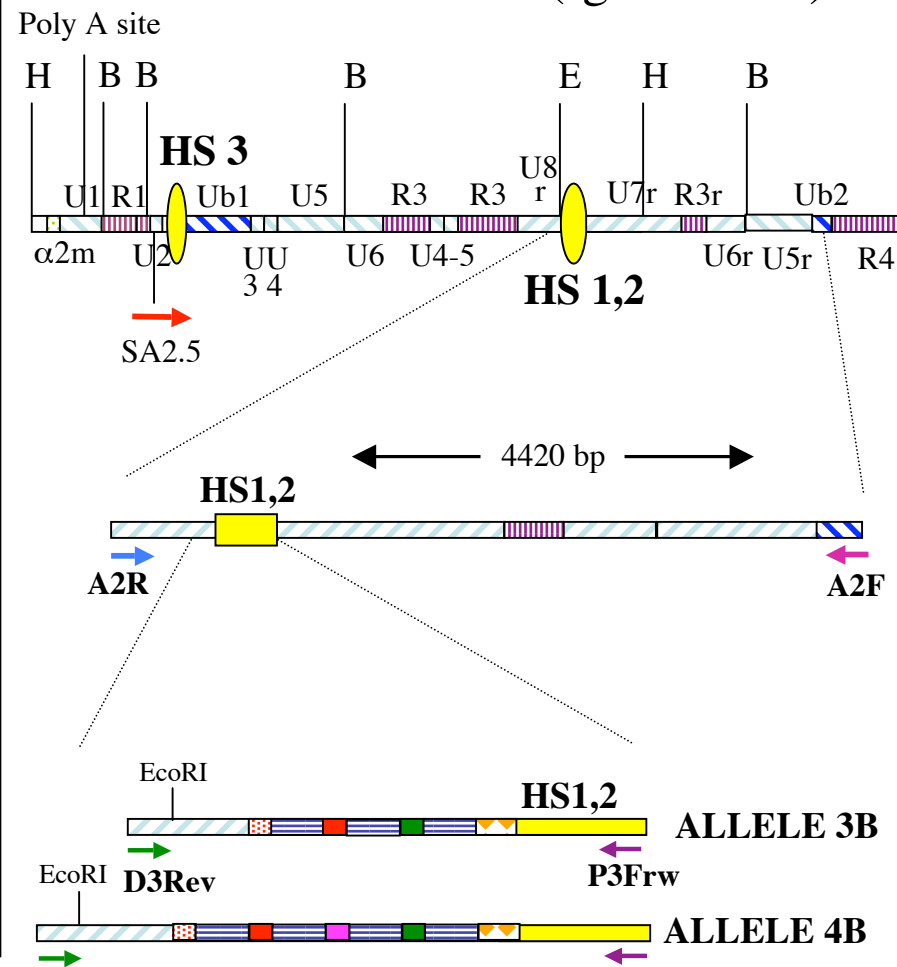
A

Selective amplification of HS1,2-A downstream Cα1 (IgH3'RR-1)

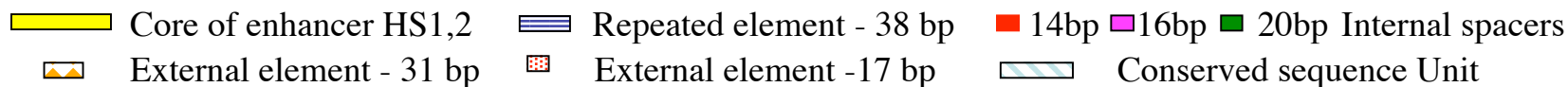


B

Selective amplification of HS1,2-B downstream Cα2 (IgH3'RR-2)



i polimorfismi

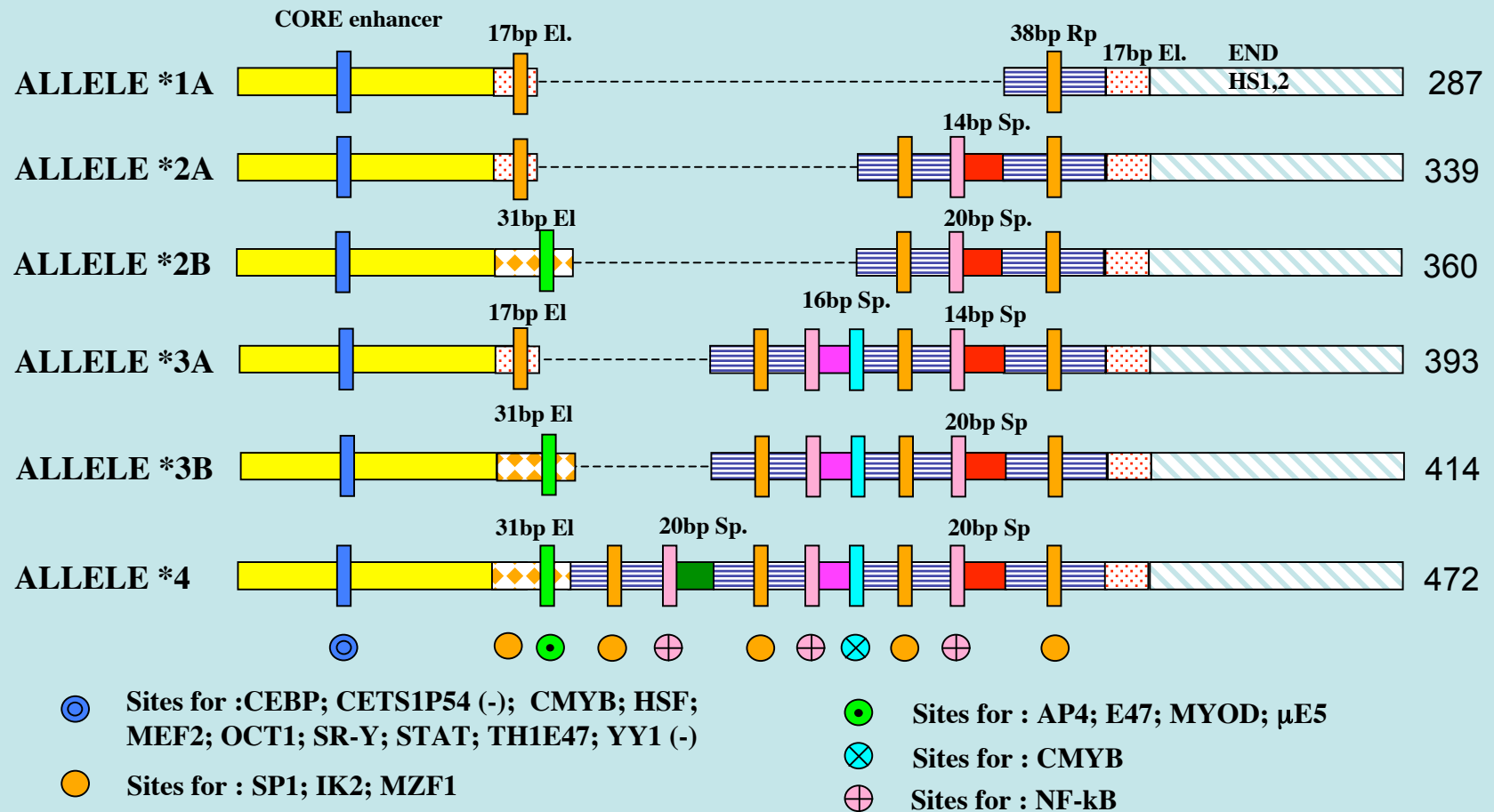




TOR VERGATA

6 alleli

Figure 3 polymorphism of HS1,2A



Polymorphism of the human $\alpha 1$ immunoglobulin gene 3' enhancer HS1,2 and its relation to gene expression

YVES DENIZOT, ERIC PINAUD, CORINNE AUPETIT, CAROLINE LE MORVAN, EMMANUELLE MAGNOUX, JEAN CLAUDE ALDIGIER & MICHEL COGNÉ *Laboratoire d'Immunologie-UMR CNRS 6101, Faculté de Médecine, Limoges, France*

Immunology 2001, 103: 35-40

Genotypes	AA	BB	AB	AC	BC
Total number: 513	71/513	177/513	255/513	7/513	3/513
%	13.8	34.5	49.7	1.3	0.58

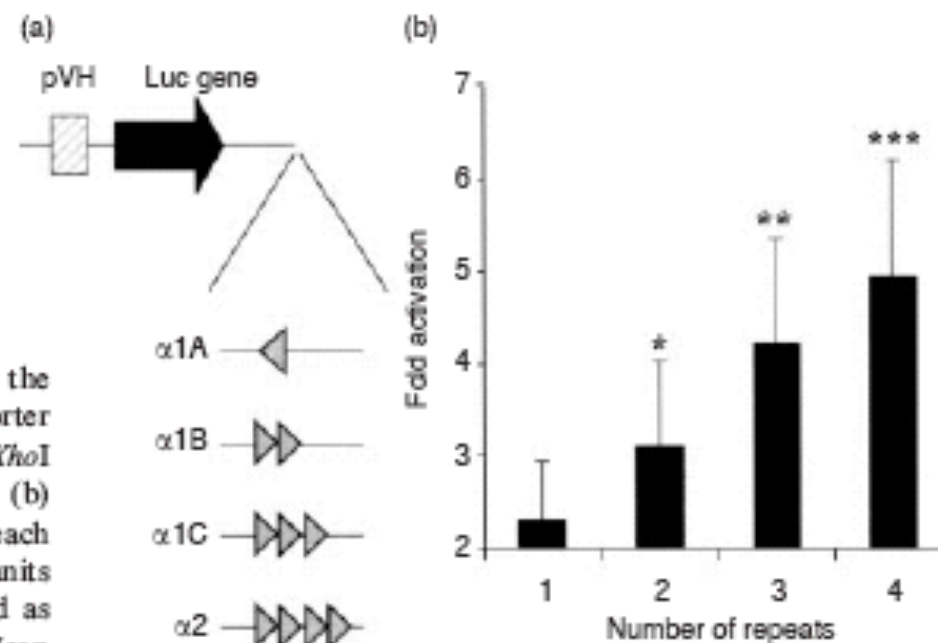
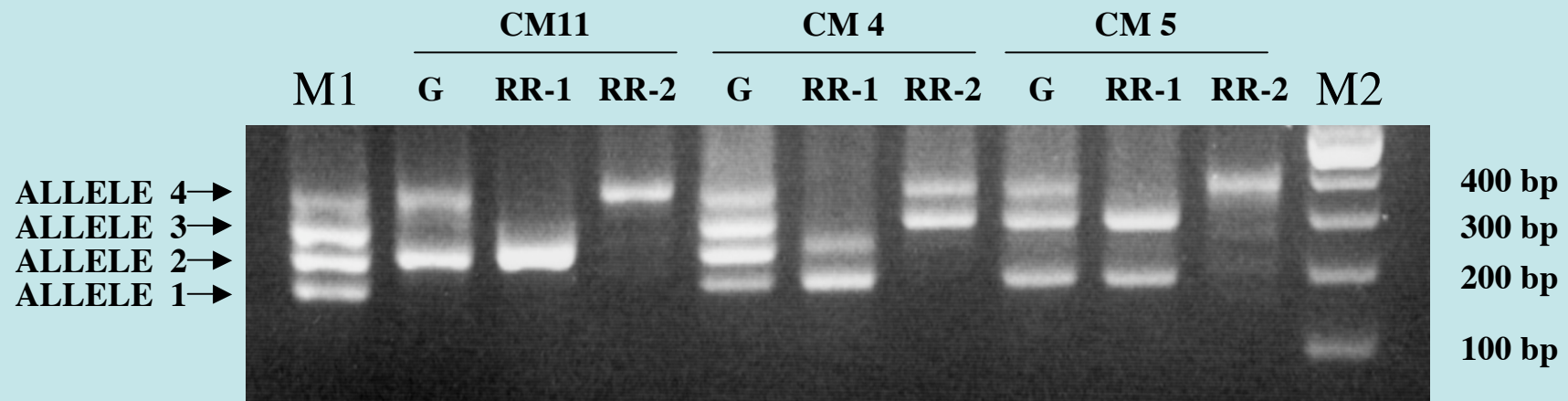


Figure 4. Transcriptional strength of the three PCR alleles of the $\alpha 1$ h1,2 enhancer and the $\alpha 2$ h1,2 allele in a luciferase gene reporter assay. (a) Construction: h1,2 elements were cloned as *Bam*HI-*Xho*I fragments in *Bam*HI-*Sal*I sites of the pGL3-pVH vector. (b) Estimation of luciferase activity for the four fragments. For each assay, luciferase light units were divided by β galactosidase light units to normalise the cell transfection efficiency. Results are reported as fold-activation as compared to the pVH promoter basal activity. Mean $\pm$ SEM of four independent experiments with six assays in parallel. * $P=0.005$, ** $P=0.024$ and *** $P=0.019$ as compared to $\alpha 1A$, $\alpha 1B$ and $\alpha 1C$, respectively (*t*-test for paired samples).

gel genomico e selettivo degli alleli

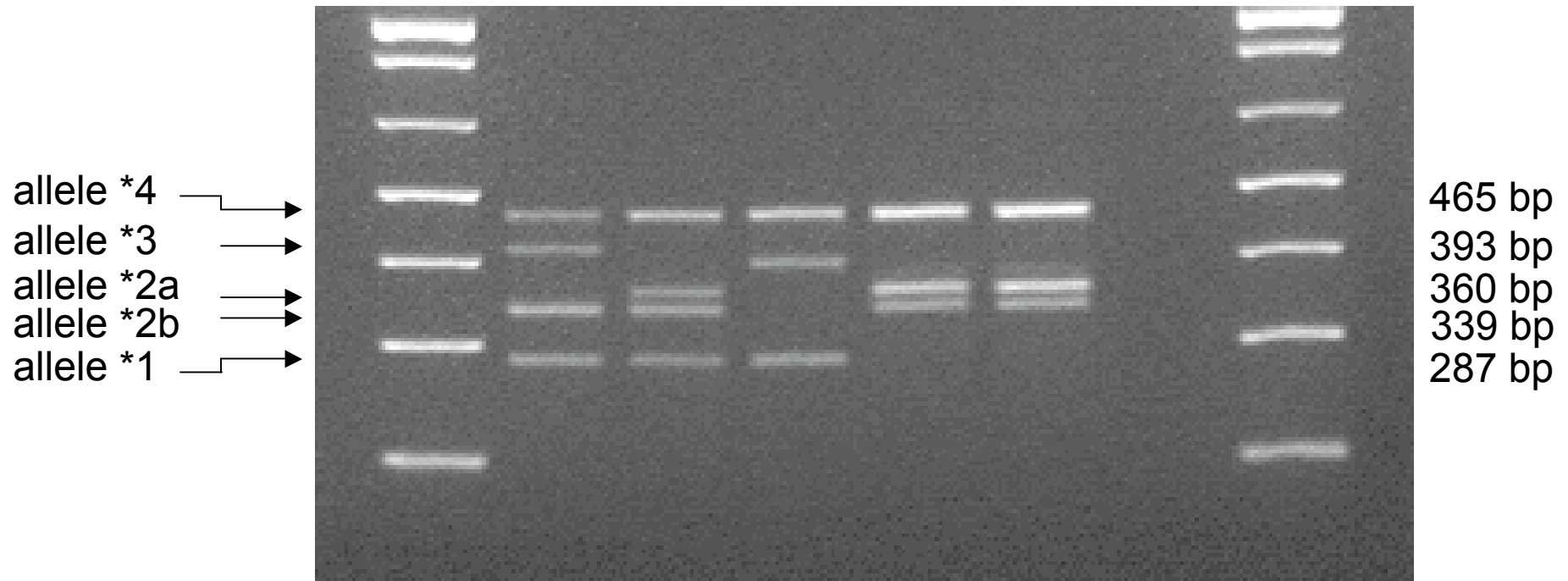


amplificazione G=genomica o selettiva RR-1; RR-2

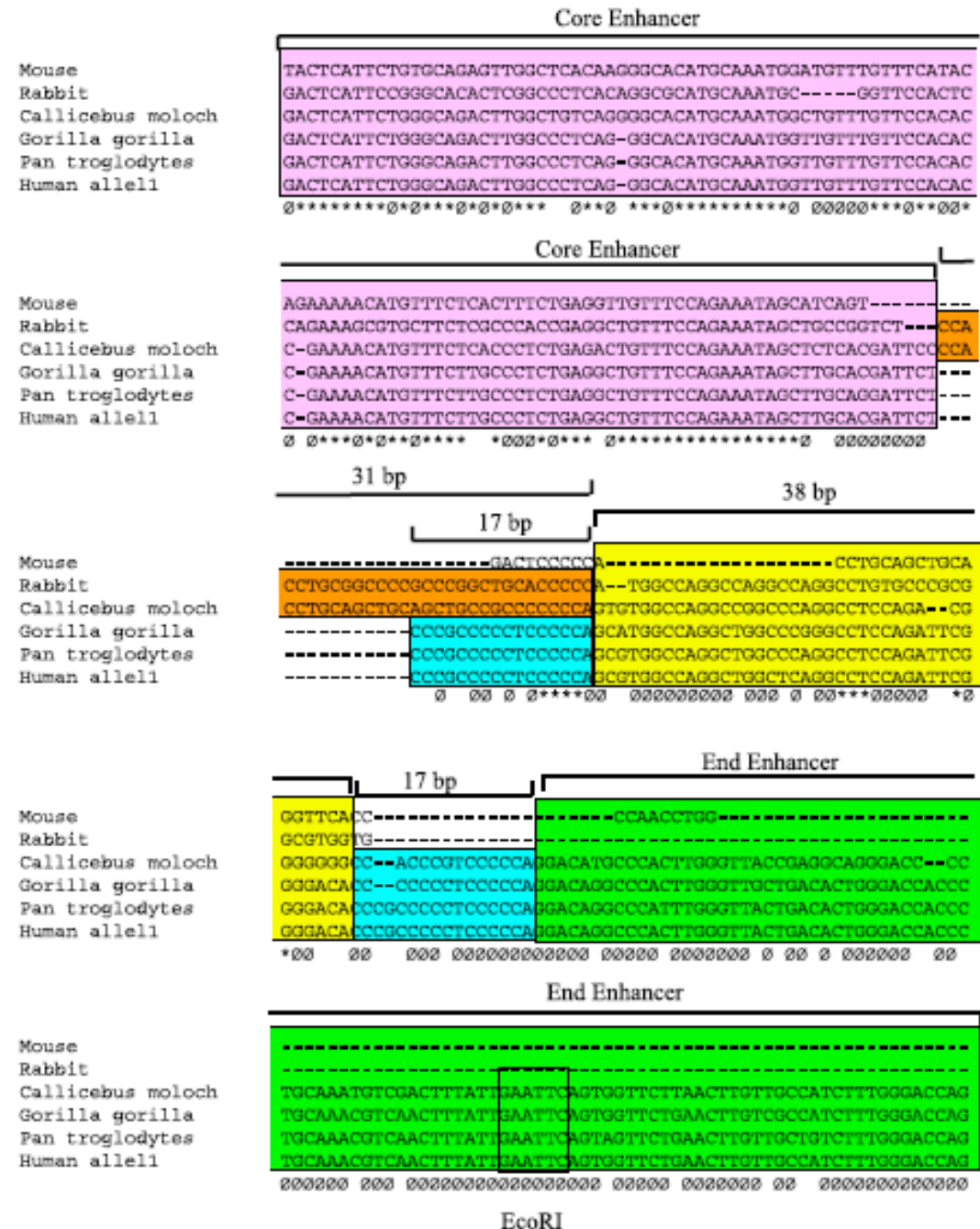
nell'amplificazione genomica si vedono gli alleli delle 2 regioni senza PCR selettiva applicata invece per amplificare selettivamente A o B.

i due alleli *2a, *2b e *3a, *3B

amplificazioni da DNA genomico dalle due 3'RR senza selezione



avevamo visto che nel locus 3'RR-B l'allele *3 aveva l'elemento 31mer rispetto al 17mer del locus 3'RR-A adesso abbiamo (ho) visto che anche l'allele *2 esiste nelle due forme con il 17mer ed il 31mer, cambiano le consensus!



conservazione in altre specie

Homo s.; Gorilla; Orango; Homo s. allele 4; Cavia; Macaca; Callitrix; Canis; Equus; Felis; Pteropus; Rattus; Mus; Bos; Sus; Callicebus; Monodelphis (opossum); Capra; Gallus.

Homo all	GAATCATTCTGGACAGCTTGG-CCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAA-CATGTTTCTGGCCCTGGAGGCTGTTCCAGAAATAGCTTGAC-AGTTCT-----CCCGCCCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-ACTCCGCGCTCTCCCGGATTCAG
Gorilla	GAATCATTCTGGACAACTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAA-CATGTTTCTGGCCCTGGAGGCTGTTCCAGAAATAGCTTGAC-AGTTCT-----CCCGCCCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-ACTCCGCGCTCTCCCGGATTCAG
Pan	GAATCATTCTGGACAACTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAA-CATGTTTCTGGCCCTGGAGGCTGTTCCAGAAATAGCTTGAC-AGTTCT-----CCCGCCCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-ACTCCGCGCTCTCCCGGATTCAG
human all4	GAATCATTCTGGACAGCTTGG-CCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAA-CATGTTTCTGGCCCTGGAGGCTGTTCCAGAAATAGCTTGAC-gatttccacgtgagctgacagcagcagcc-accgtgtgtggcaggtggcagagcctccagattggggagcc-----CCCGCCCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-ACTCCGCGCTCTCCCGGATTCAG
Cavia	GAATCATTCTGGACAACTTGGCTCTGAGGACATGCAAAATGATTTTGTTCACACGAAAA-CTAGCTT-PTCAGCTCTGGAGCTGTTCCAGAAATAGCTTGACG---CCCGACCTGACGTACAGAGCCCTCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
macaca	GAATCATTCTGGACAACTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAA-CATGTTTCTGGCCCTGGAGGCTGTTCCAGAAATAGCTTGACGAGTTCT-----CCCGACCTGACGTACAGAGCCCTCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
Callitrix	GAATCATTCTGGACAGCTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAA-CATGTTTCTCACCCCTGGAGCTGTTTCAGAAATAGCTTCAGAGTTCT-----CCCGACCTGACGTACAGAGCCCTCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
Canis	-ACTCATTCTGGACAGCTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAAACATGTTCTCACCCCTGGAGGCTGTTCCAGAAATAGCTTGACGAC-----CCCGACCTGACGTACAGAGCCCTCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
Equus	-ACTCATTCTGGACAACTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAAACATGTTCTCACCCCTGGAGGCTGTTCCAGAAATAGCTTGACGAC-----CCCGACCTGACGTACAGAGCCCTCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
Felis	-ACTCATTCTGGACAACTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAAACATGTTCTCACCCCTGGAGGCTGTTCCAGAAATAGCTTGACGAC-----CCCGACCTGACGTACAGAGCCCTCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
Pteropus	GAATCATTCTGGACAACTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACG-AAAAACATGTTCTCACCCCTGGAGGCTGTTCCAGAAATAGCTTGACGAGTGGGCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
Rattus	GAATCATTCTGGACAGCTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAAACATGTTCTCATTTCTGGAG-----CCAAAAATAGCATCATGATTCCTCCAGCTGACGTTCAGAGTTTCAGAGCC-AGCTCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
Mus	-ACTCATTCTGGACAGCTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACACGAAAAACATGTTCTCATTTCTGGAGGCTGTTCCAGAAATAGCTTGACGAGTGGCT-----AGTTGGCCAGG-TGGCCAGGCTTCAGATTTCAGAG-ACTCTGGCCAGTGG
Bos	---TCATTCTGGACAGCTTGGCCCTCAGAGGACATGCAAAATGATTTTGTTCACAC
Sus	-ACTCATTCTGGCTCTGGAGGCTTATTTCCAAAAACGAGCTCTATACCTC
Callicebu	-----TTGTTTGTTCAAAAACAAAA-CTGGATTCT
Monodelph	-----CTCCGCGCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-AGTTCT-----CCCGCCCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-ACTCCGCGCTCTCCCGGATTCAG
Capra	-----CTCCGCGCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-AGTTCT-----CCCGCCCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-ACTCCGCGCTCTCCCGGATTCAG
Gallus	-----GTTCCAAAAATAG--GACCTTTTTT-----CCCGCCCTCTCCCTCAAGTGGAGCTTCAGAGCTTCAGATTTCAGAG-ACTCCGCGCTCTCCCGGATTCAG

quali altre analisi ?

il polimorfismo si può studiare dal punto di vista funzionale

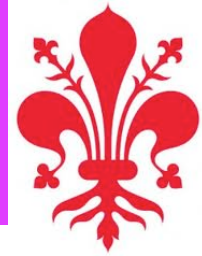
struttura e modelli in “silicio” (transcr. fact.; 3D models)

CHIP chromosome immuno-precipitation

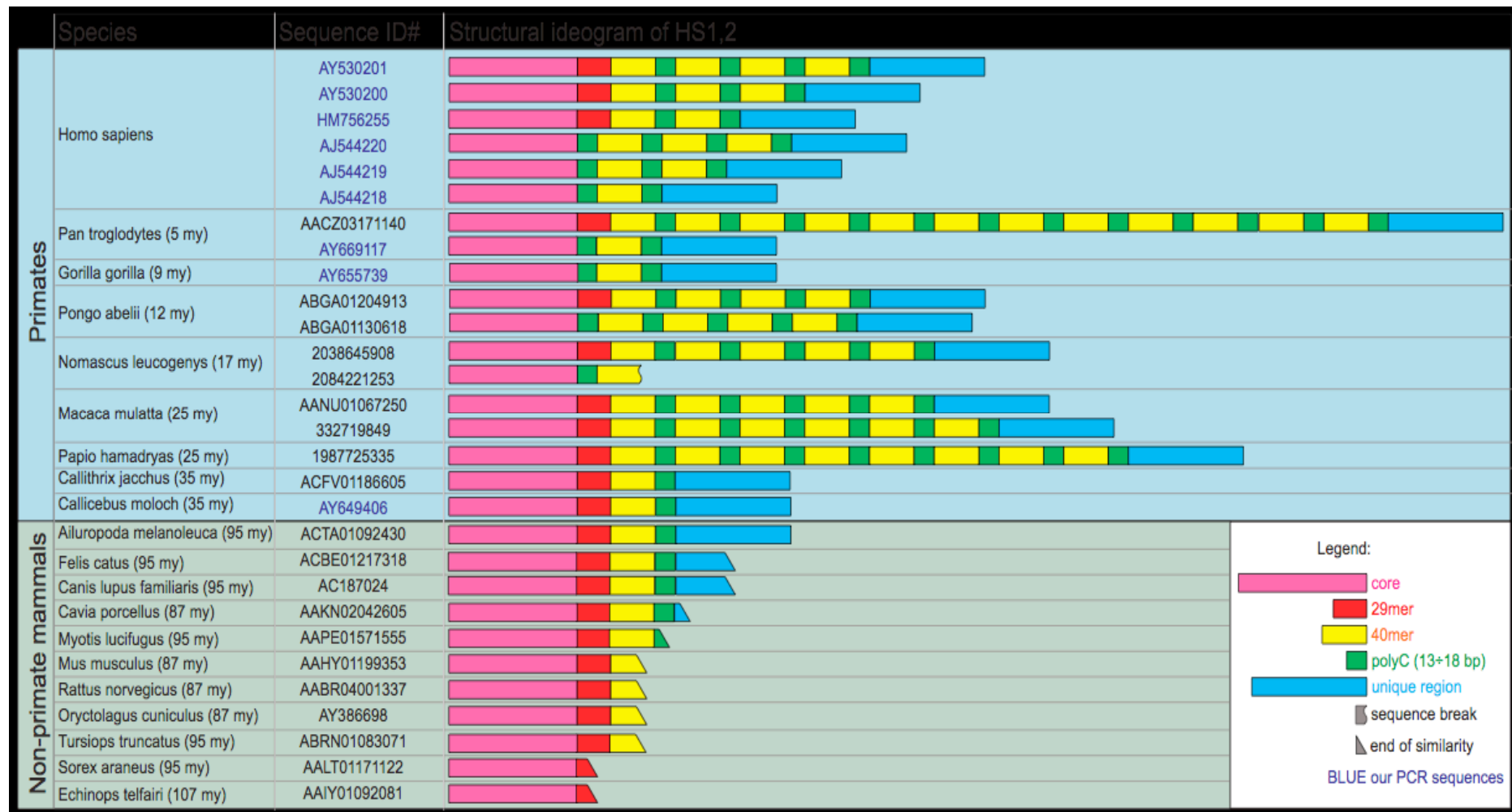
EMSA electrophoretic mobility shift assay:

- incubazione della sonda con le consensus con estratti nucleari
- corsa elettroforetica
- competizione con anticorpi o con consensus che sottraggono la formazione del complesso e fanno sparire il segnale

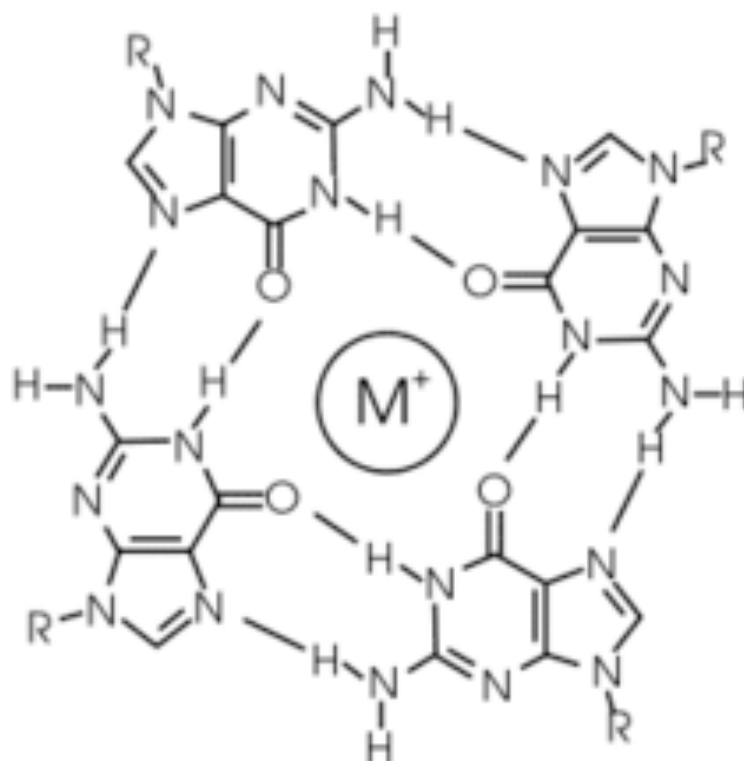
Il polimorfismo dell'enhancer hs1.2



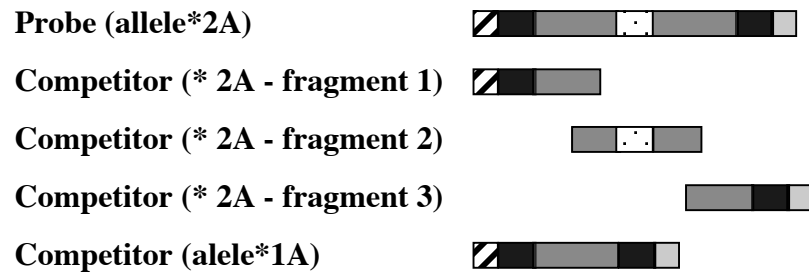
Dai genomi completi in bd: sono presenti a partire dai primati



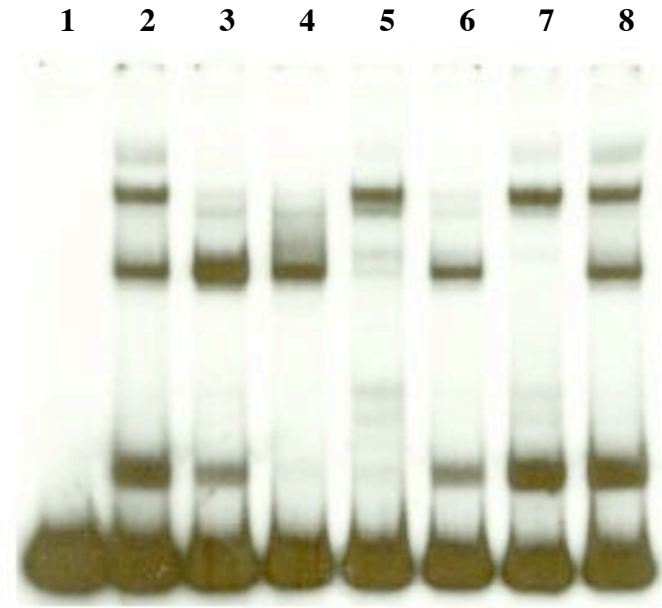
modello tetraplex



mappatura tramite competizione con “gel shift” EMSA



a →
b →
c →
d →



SP1 compete banda B *

NF-kB “ banda C *

frgm 1 ≈ allele *1A comp B (+D) *

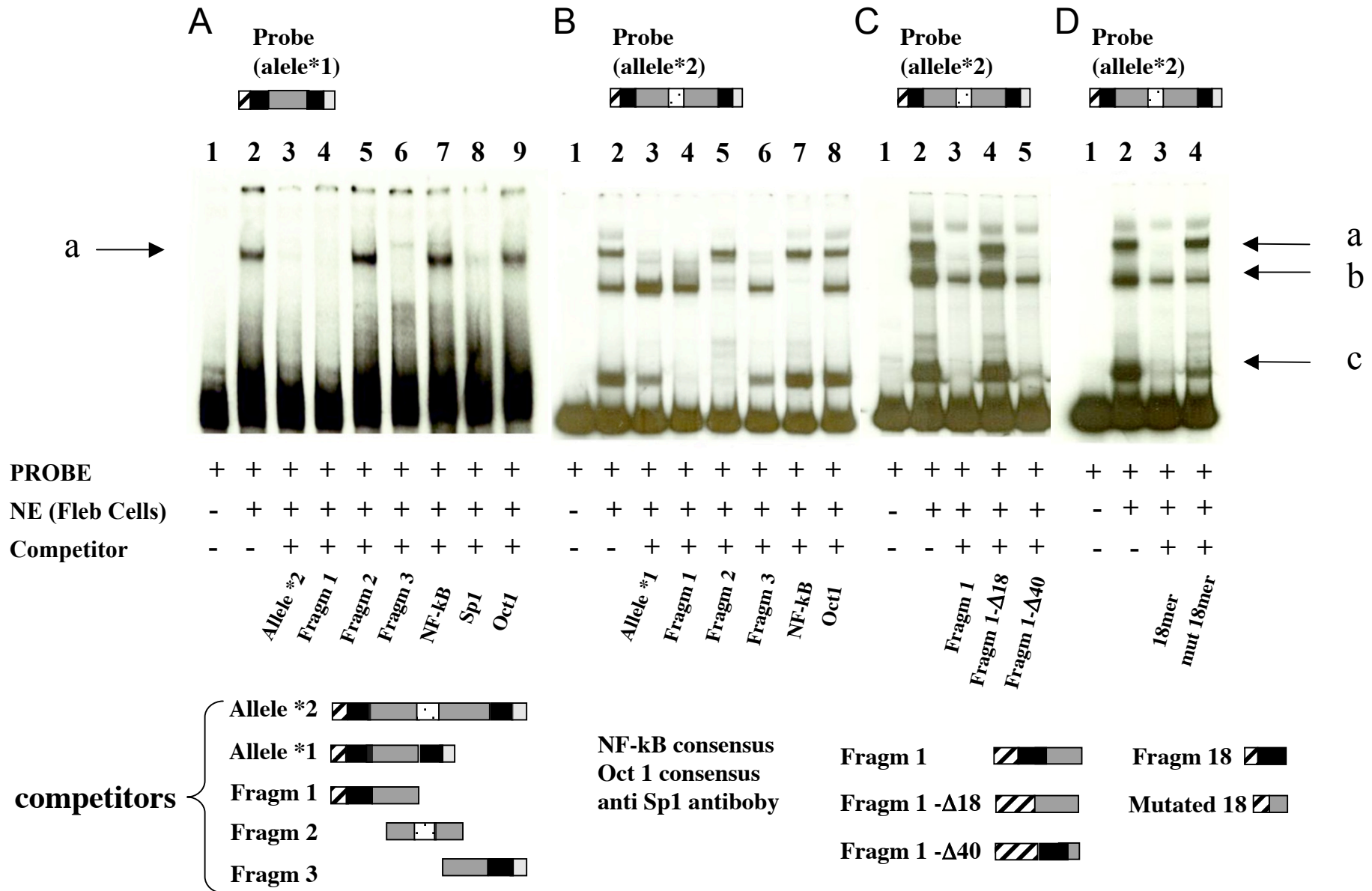
frgm 2 comp C (+D) ≈ NF-kB *

frgm 3 “ B (-D) *

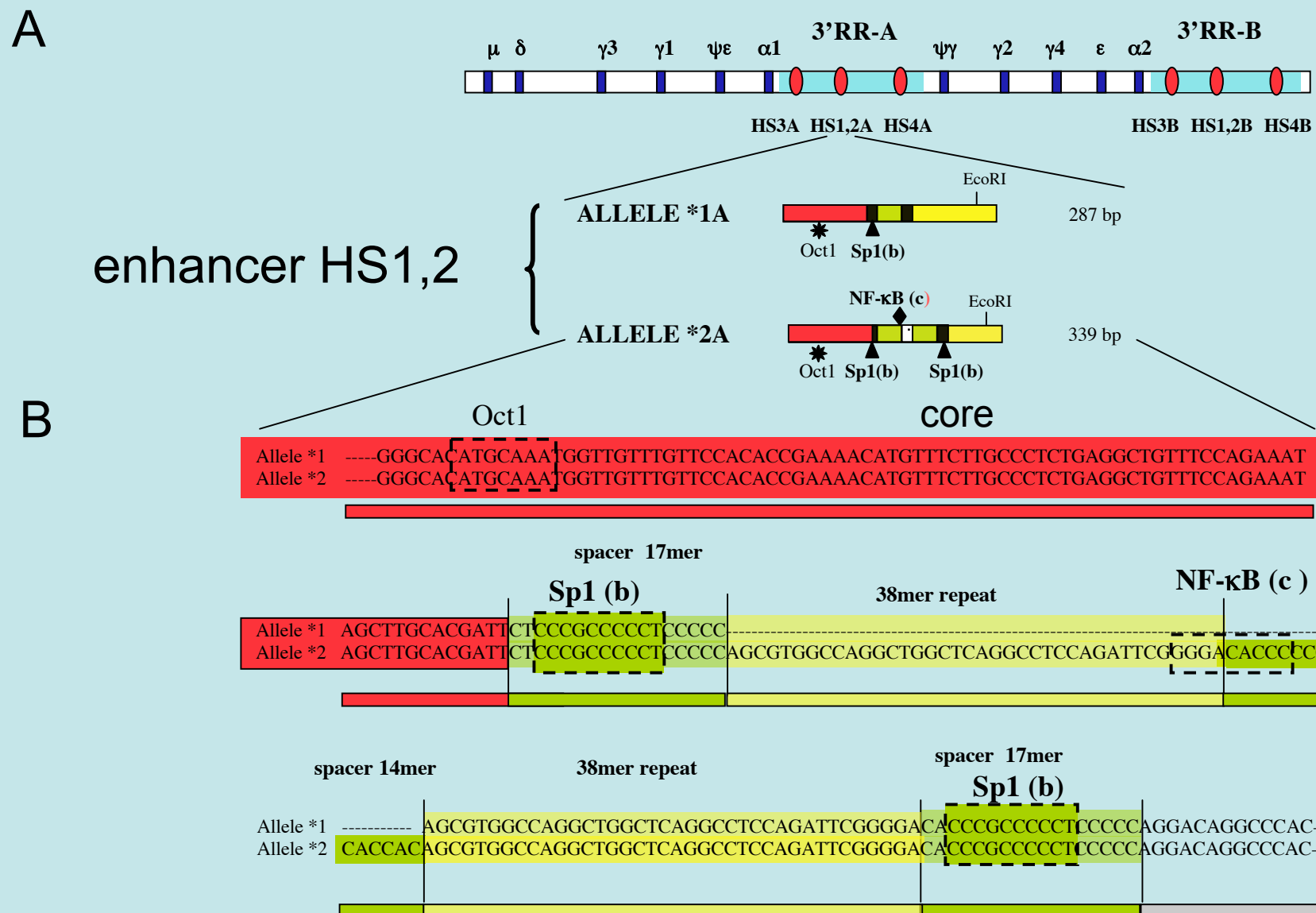
Probe (allele *2A)	+	+	+	+	+	+	+	+
NE (Fleb cells)	-	+	+	+	+	+	+	+
Competitor	-	-	+	+	+	+	+	+
			Allele *1A	*2A frg 1	*2A-frg2	*2A-frg3	NF-kB	Oct
			*	*	*	*	*	
					*	*	*	

EMSA electrophoresis mobility shift assay with allele *1 and *2

DNA binding experiment with nuclear proteic extracts



Consensus for Sp1 and NF-κB in HS1,2-A allele*1 and *2





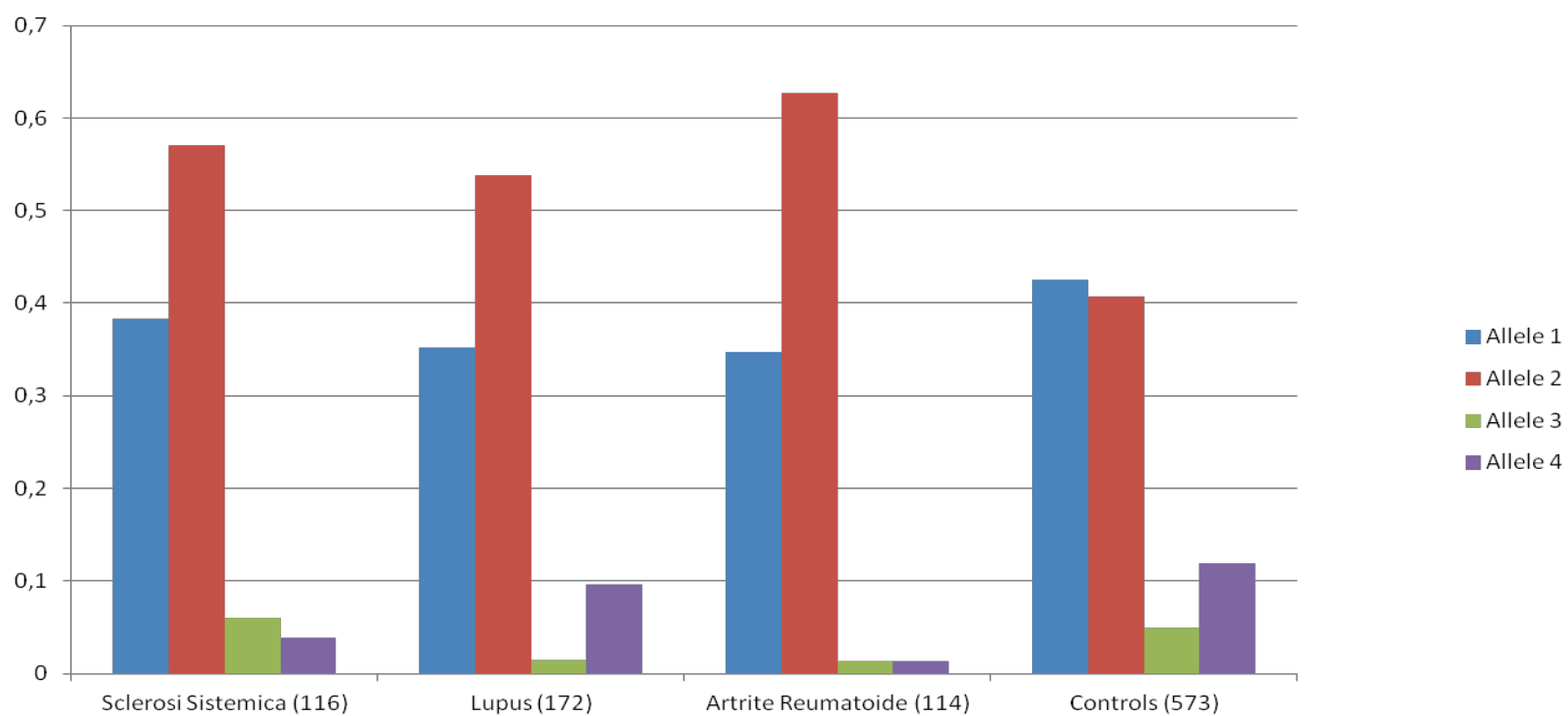
associazione con patologie autoimmuni dell'enhancer hs1.2

Analisi su pazienti con Sclerosi sistemica
Artrite reumatoide
Lupus eritematoso

I associazione con aumento della frequenza dell'allele *2
nei pazienti con queste patologie

Il associazione tra soggetti con livelli alti di IgM del siero e
l'allele *2

patologie autoimmuni



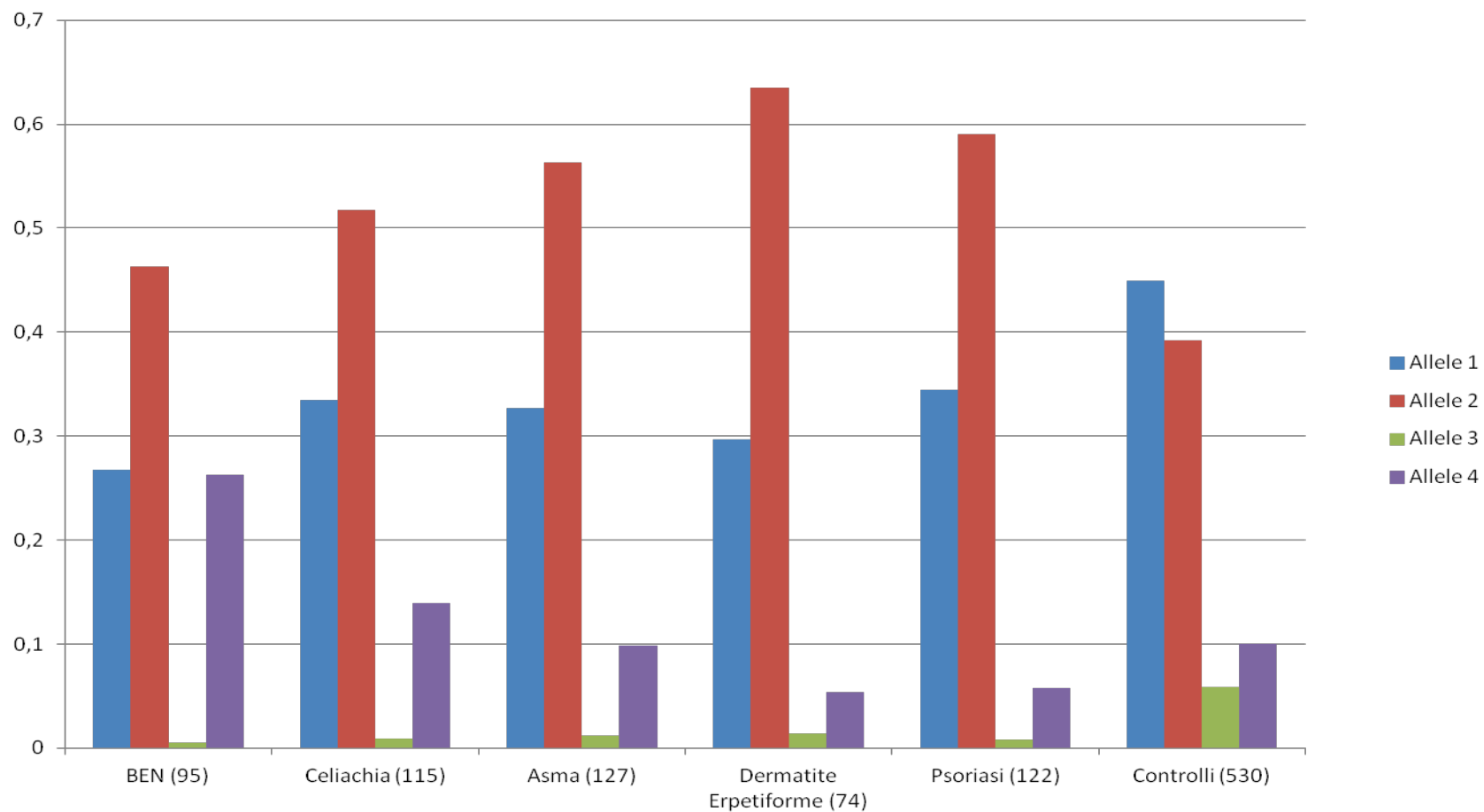
associazione con patologie immuno- infiammatorie

Analisi su pazienti con : celiachia
psoriasi
dermatite erpetiforme
nefropatia balcanica cronica
asma

L'infiammazione fa parte della cronicità di queste patologie

Asma e Psoriasi mostrano correlazione tra livelli di Ig del siero e frequenza dell'allele *2 dell'enhancer hs1.2

associazione con infiammazione



Vaccinazioni I



2 coorti indipendenti vaccinati con antigeni diversi

I coorte di 193 bambini di età sotto 5 anni

Vaccinati per antigene influenzale H1N1

H2N3

B

II coorte 392 studenti del PTV di età sopra 20 anni

Vaccinati contro HBV (virus epatite B)

Analisi della risposta: quantità di antigeni specifici

non correla con le frequenze alleliche di hs1.2

Vaccinazioni II

I soggetti con risposta alta media o bassa alla vaccinazione non correlano con le frequenze alleliche di hs1.2

I soggetti vaccinati con livelli totali di Ig del siero alti medi o bassi correlano come nelle patologie con hs1.2

Le frequenze alleliche nei soggetti con livelli di Ig alti medi o bassi si differenziano ulteriormente

Vaccinazioni III

<i>f</i>	H1N1		
	NR	R	HR
ALLELE *1	0,341 ± 0,05	0,371 ± 0,04	0,258 ± 0,03
ALLELE *2	0,523 ± 0,05	0,536 ± 0,04	0,617 ± 0,04
ALLELE *3	0	0	0
ALLELE *4	0,136 ± 0,03	0,093 ± 0,02	0,125 ± 0,03

<i>f</i>	IgM		
	LOW	MEDIUM	HIGH
ALLELE *1	0,478 ± 0,04	0,411 ± 0,04	0,018 ± 0,01
ALLELE *2	0,433 ± 0,04	0,478 ± 0,04	0,821 ± 0,04
ALLELE *3	0	0	0
ALLELE *4	0,089 ± 0,02	0,112 ± 0,03	0,161 ± 0,03
TOTAL	67	67	56

<i>f</i>	H3N2		
	NR	R	HR
ALLELE *1	0,297 ± 0,05	0,315 ± 0,04	0,347 ± 0,03
ALLELE *2	0,581 ± 0,05	0,585 ± 0,04	0,528 ± 0,04
ALLELE *3	0	0	0
ALLELE *4	0,122 ± 0,03	0,1 ± 0,02	0,125 ± 0,02

<i>f</i>	IgG		
	LOW	MEDIUM	HIGH
ALLELE *1	0,479 ± 0,04	0,373 ± 0,04	0,038 ± 0,02
ALLELE *2	0,443 ± 0,04	0,485 ± 0,04	0,821 ± 0,04
ALLELE *3	0	0	0
ALLELE *4	0,079 ± 0,02	0,142 ± 0,03	0,141 ± 0,03
TOTAL	70	67	53

<i>f</i>	B		
	NR	R	HR
ALLELE *1	0,312 ± 0,06	0,341 ± 0,03	0,308 ± 0,04
ALLELE *2	0,584 ± 0,07	0,541 ± 0,03	0,577 ± 0,04
ALLELE *3	0	0	0
ALLELE *4	0,104 ± 0,04	0,118 ± 0,02	0,115 ± 0,02

<i>f</i>	IgA		
	LOW	MEDIUM	HIGH
ALLELE *1	0,485 ± 0,04	0,366 ± 0,04	0,055 ± 0,02
ALLELE *2	0,404 ± 0,04	0,5 ± 0,04	0,836 ± 0,03
ALLELE *3	0	0	0
ALLELE *4	0,111 ± 0,03	0,134 ± 0,03	0,109 ± 0,03
TOTAL	68	67	55



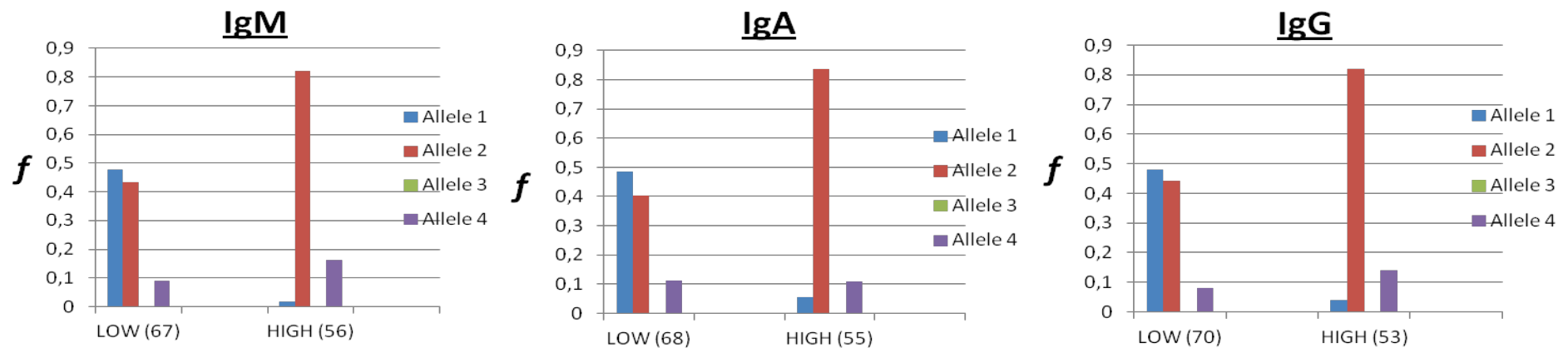
TOR VERGATA

Vaccinazioni IV



I soggetti con Ig alte medie e basse a confronto:
le frequenze alleliche di hs1.2 nei bambini e soggetti adulti

Età 3-5 anni maggiore copertura con allele *2 ?



Età 21-26 anni

