



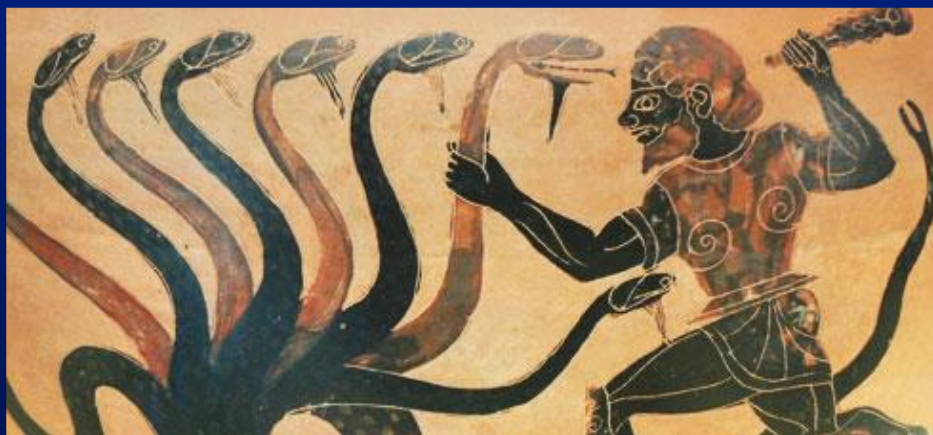
Metodi biologici applicati allo studio di SRLV

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Dipartimento di Scienze Veterinarie
Roma 5 Aprile 2022



Perché studiare la biologia di SRLV

- Non tutti sono uguali
- Metodi di diagnosi e controllo diversificati
- Alcuni (es. E1) modulano l'attività patogena di altri (es. B1)



Da Bertoni
IVV Berna

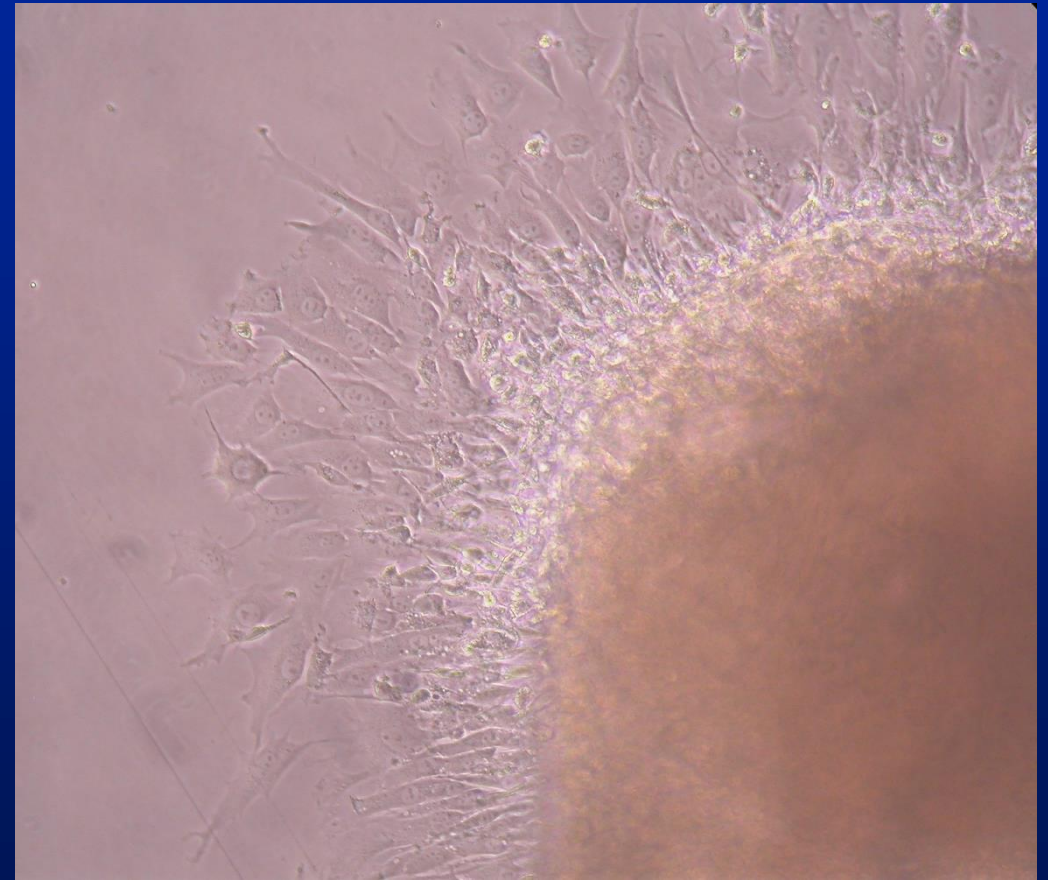
metodi

- In vitro
 - Isolamento
 - RT-activity
 - LTR activity
 - Entry assay
- In vivo
 - Indice clinico e istopatologico
 - Carica provirale
 - Genetica dell'ospite

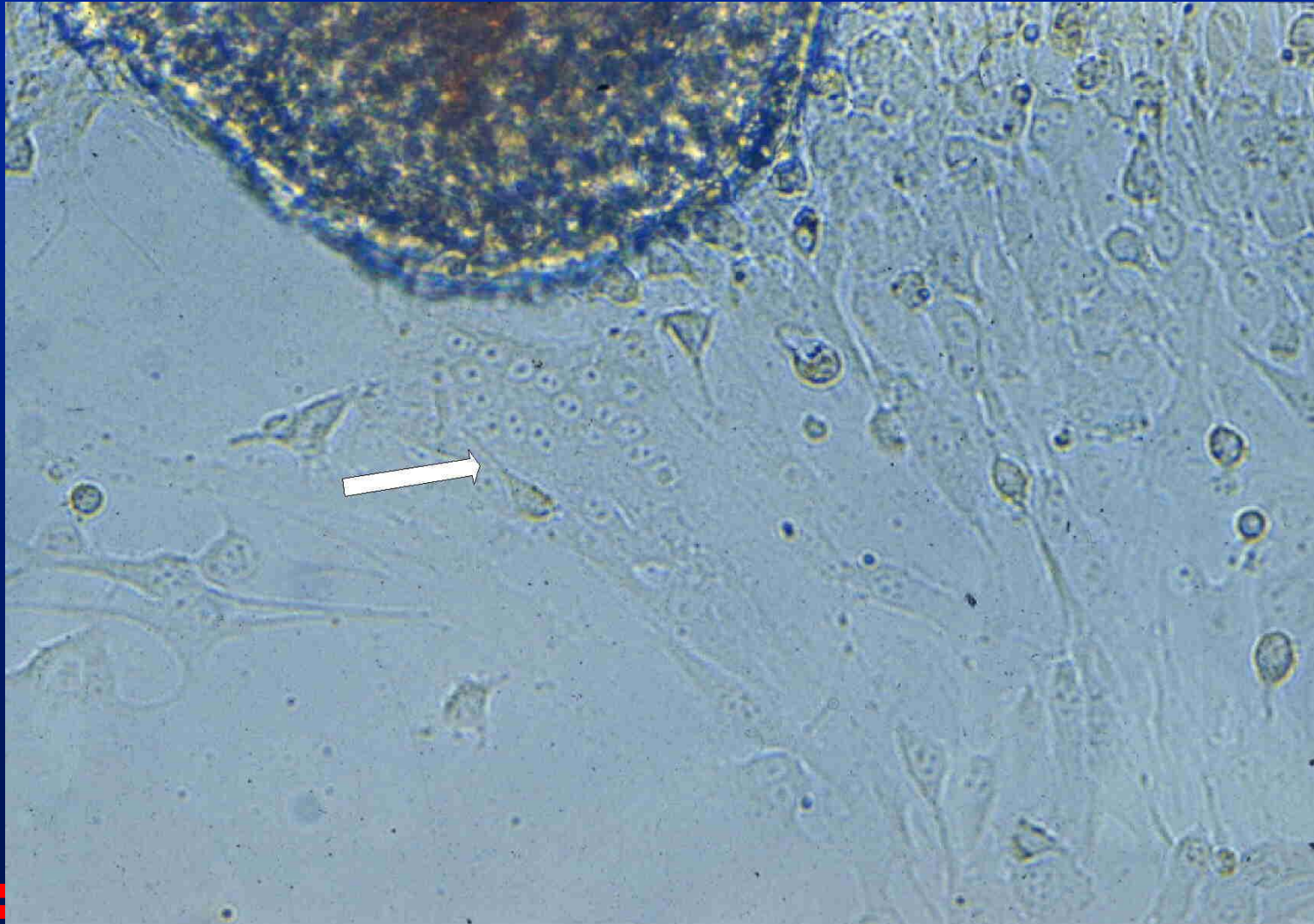


Isolamento virale: metodo 1 (animale sintomatico)

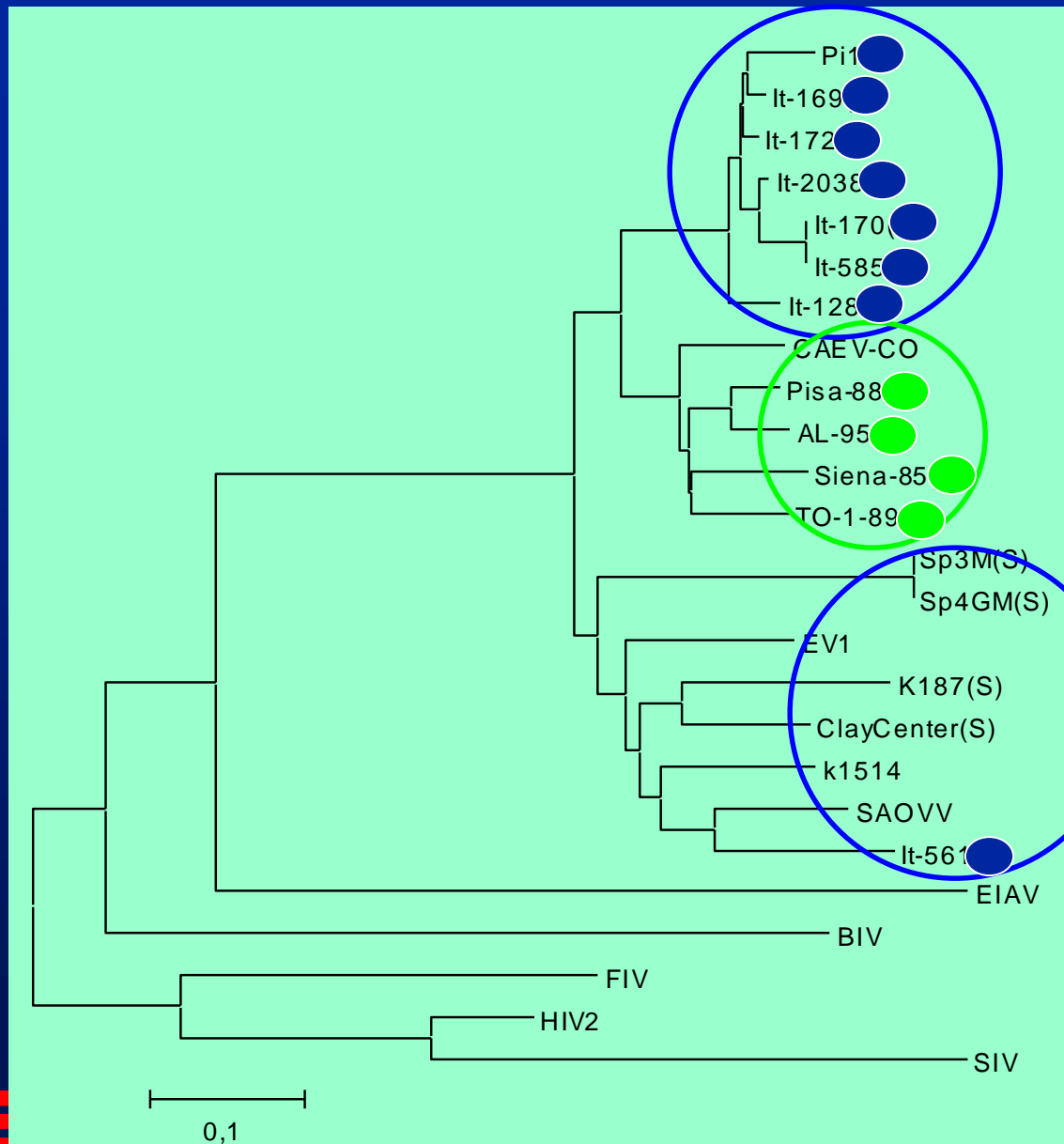
- Espianti di tessuto
 - Polmone (e LN Med)
 - Mammella (e LN Sm)
 - Membrana sinoviale
 - Plesso corioideo
 - Milza
- Passaggi settimanali
 - Colorazione giemsa x CPE



CAEV-TO 1/89: *espianto di sinovia da capra artritica*



Metodo 1: isolamenti 1985-1993



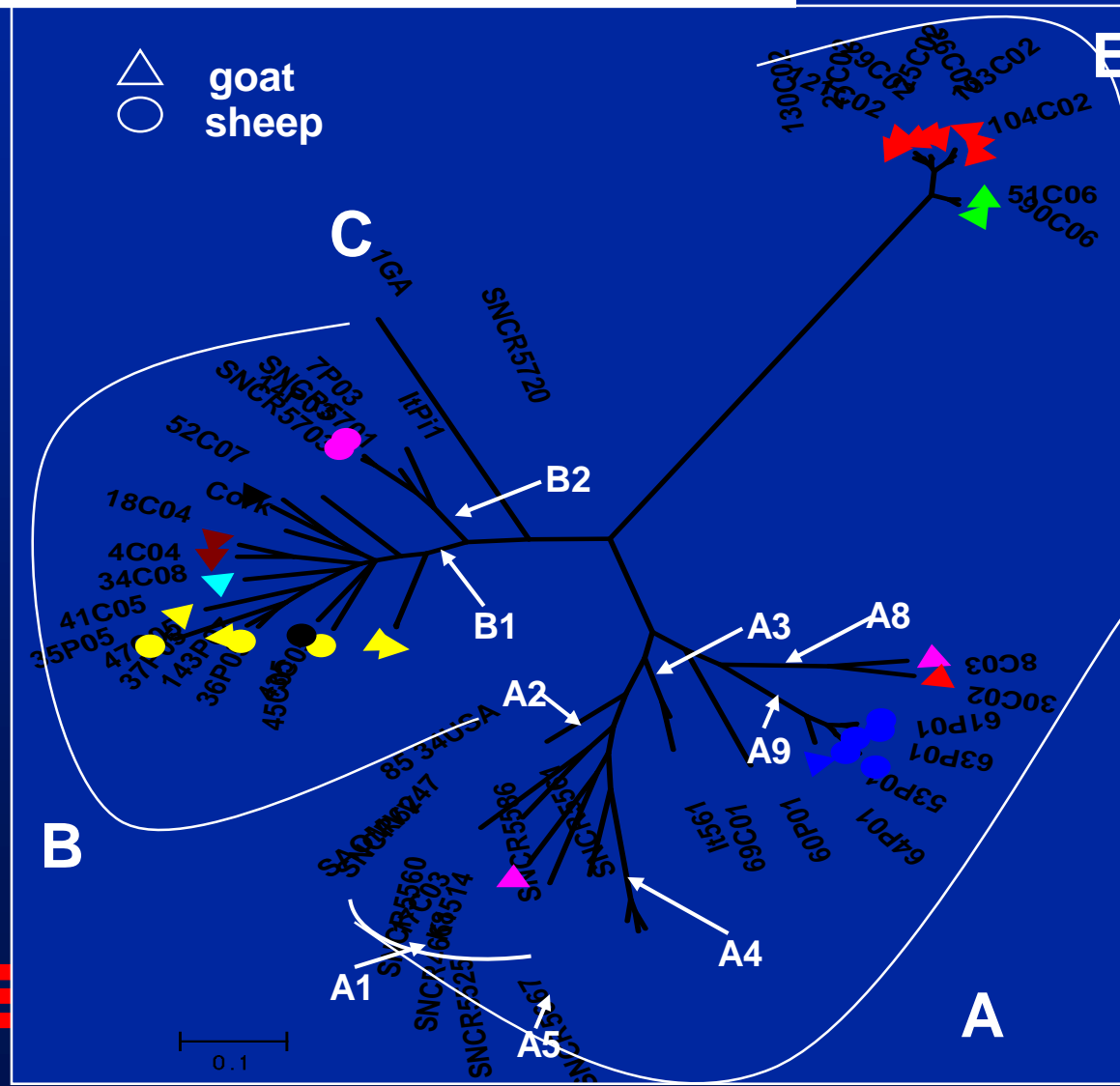
Italian sheep isolates (B2) ○

Italian goat isolates (B1) ●

Sheep isolates {
Europei
Nord Am
Africani

Short
CommunicationGenetic characterization of small ruminant lentivirus
in Italian mixed flocks: evidence for a novel
genotype circulating in a local goat population

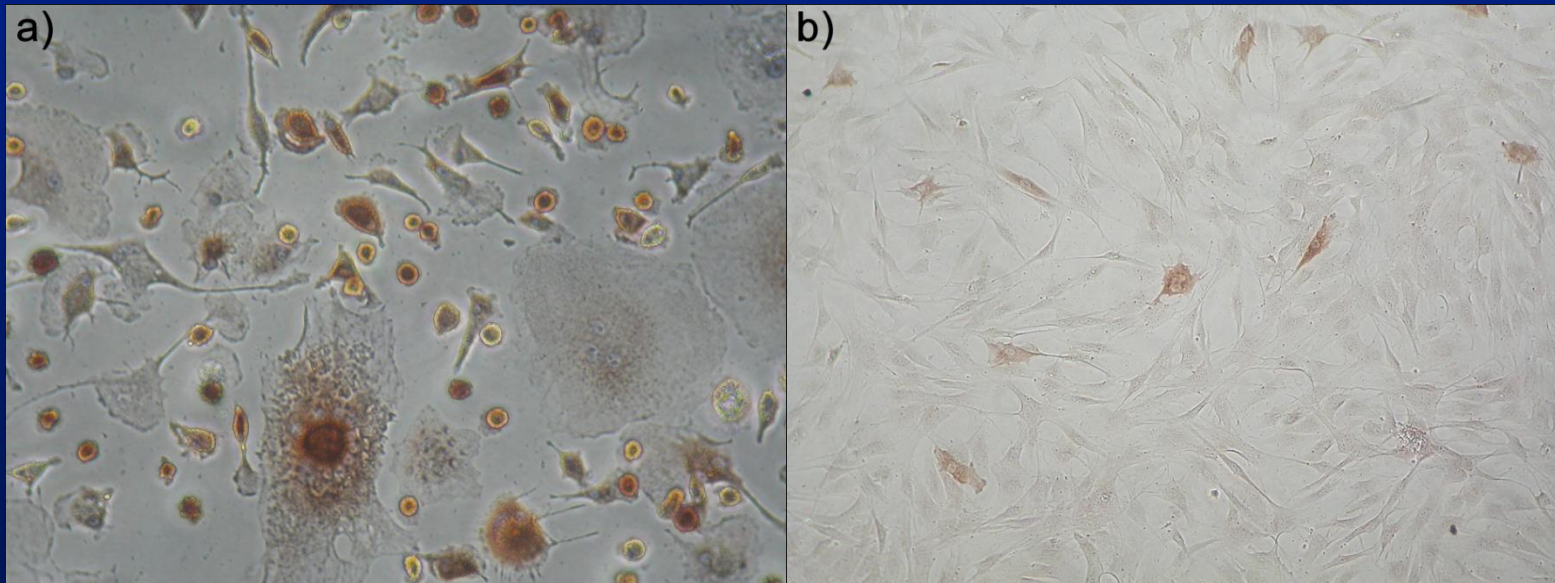
Elena Grego,¹ Luigi Bertolotti,¹ Antonio Quasso,² Margherita Profiti,¹
Daniela Lacerenza,¹ Dilek Muz³ and Sergio Rosati¹



Isolamento virale: metodo 2 (animale asintomatico PCR+)

- L'esempio di Roccaverano
 - Espianti da tutti i tessuti
 - CPE negativo
 - PCR positiva solo su milza

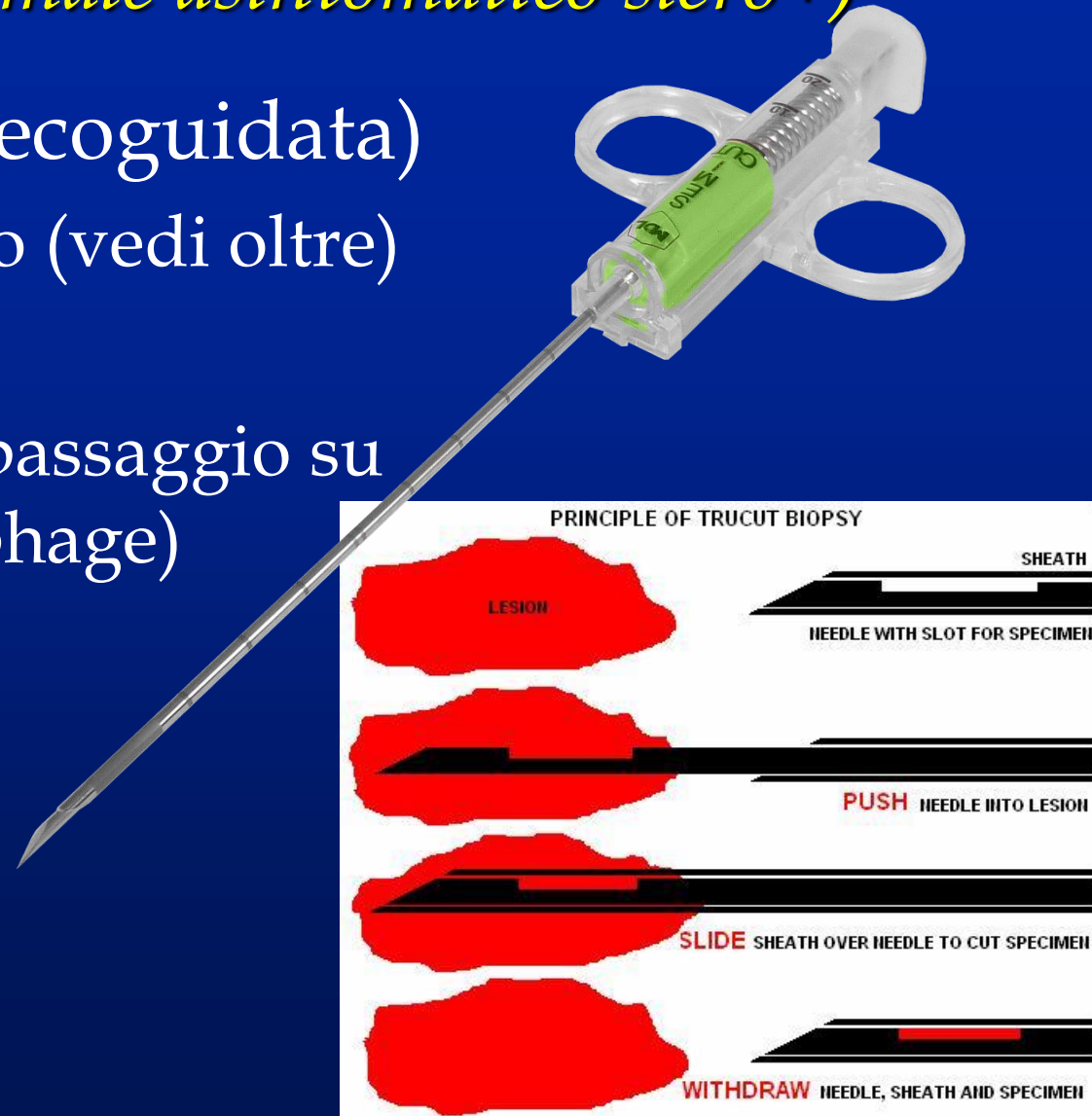
Macrofagi capra
5 giorni p.i.
ICC mouse anti rP25

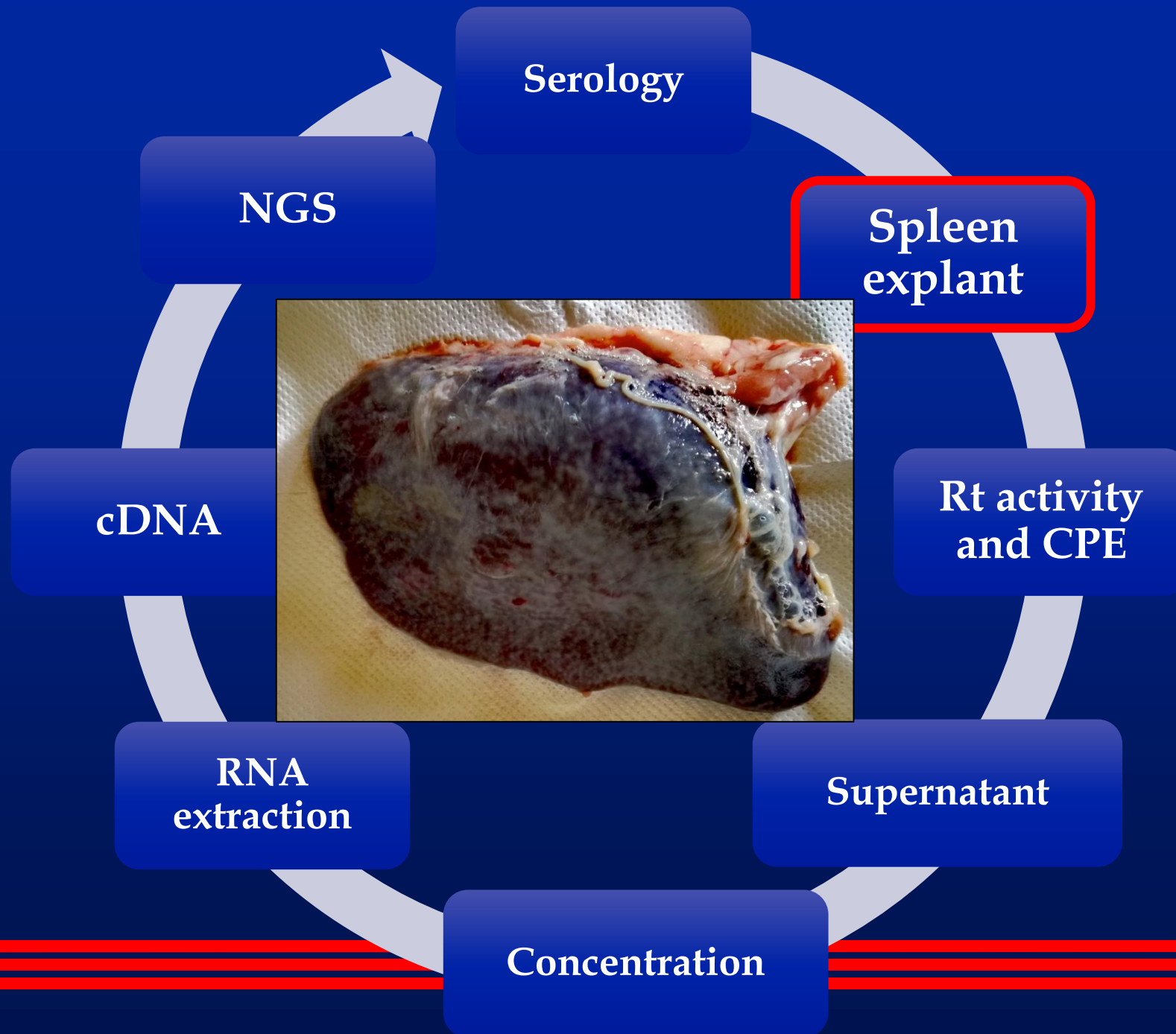


Fibroblasti feto capra
4 settimane p.i.
4 passaggi
ICC mouse anti rP25

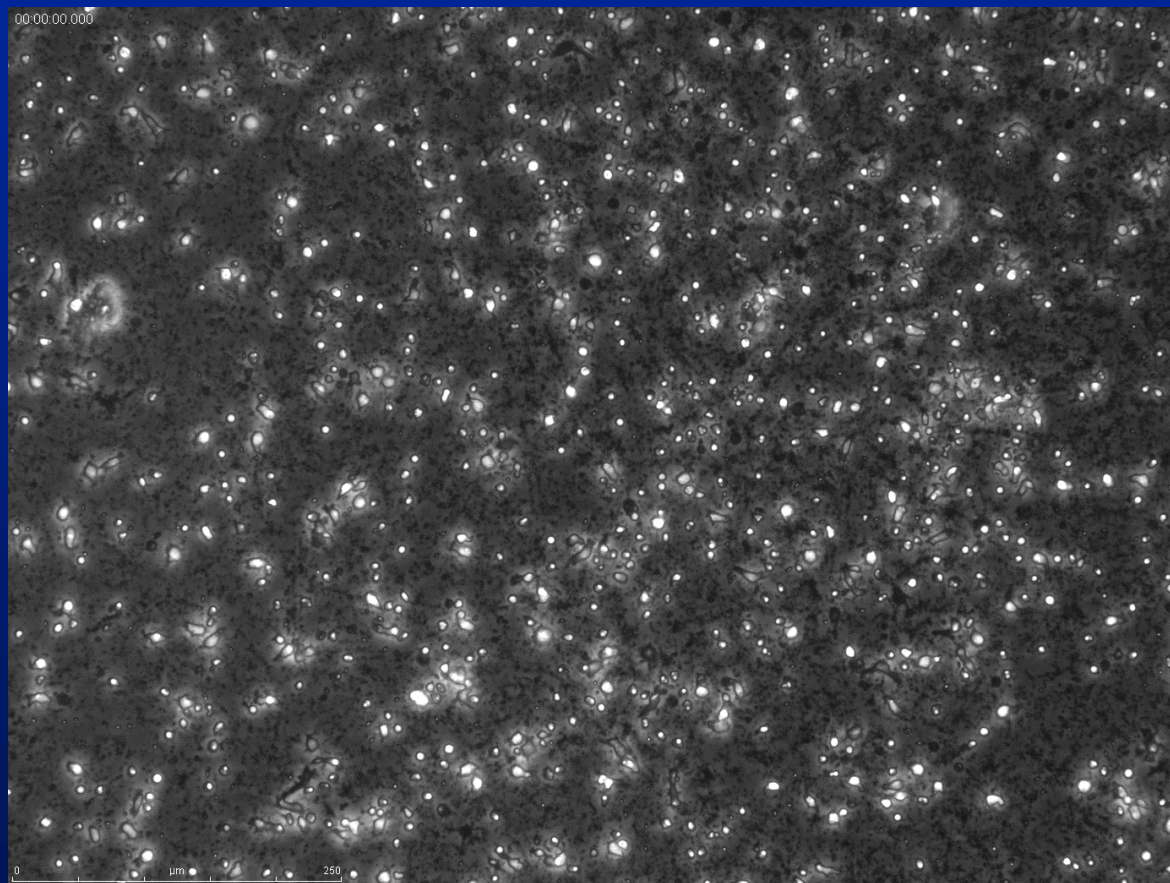
Isolamento virale: metodo 3 (animale asintomatico siero+)

- Espianto da milza (biopsia ecoguidata)
 - RT activity ad ogni passaggio (vedi oltre)
 - Passaggio nella stessa fiasca
 - Se il segnale RT diminuisce passaggio su BDM (blood derived macrophage)
 - Concentrazione su amicon
 - NGS

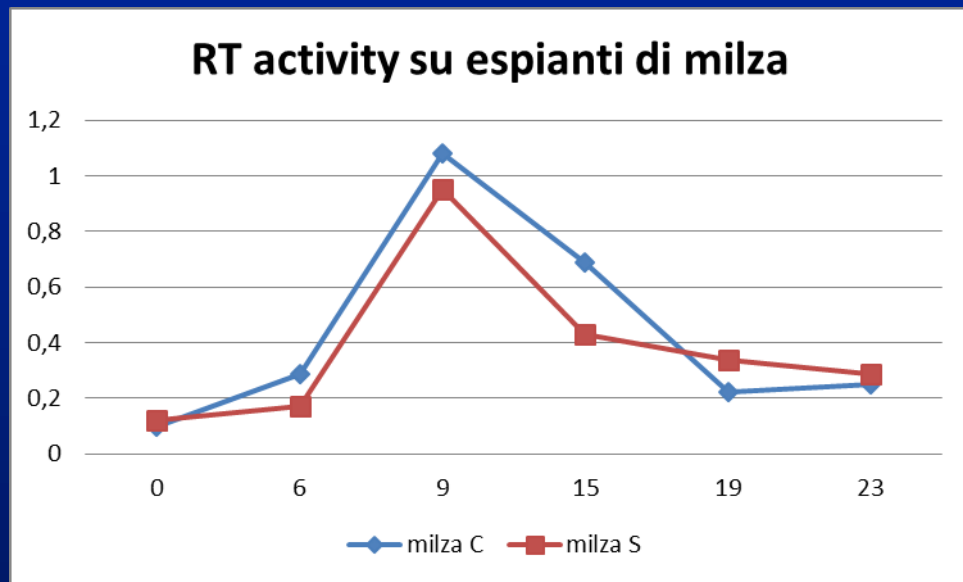




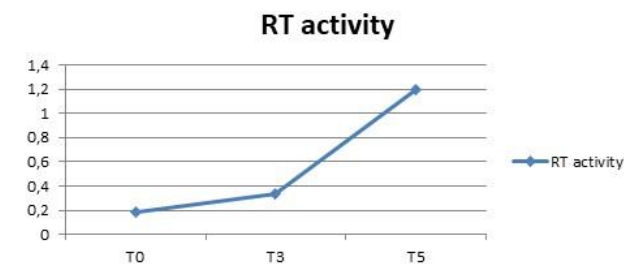
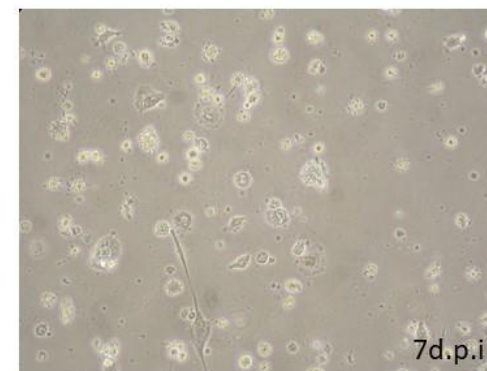
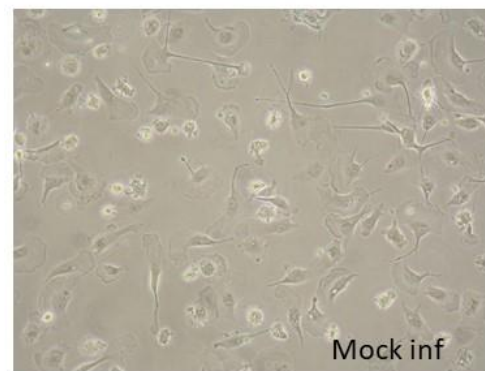
BDM time laps



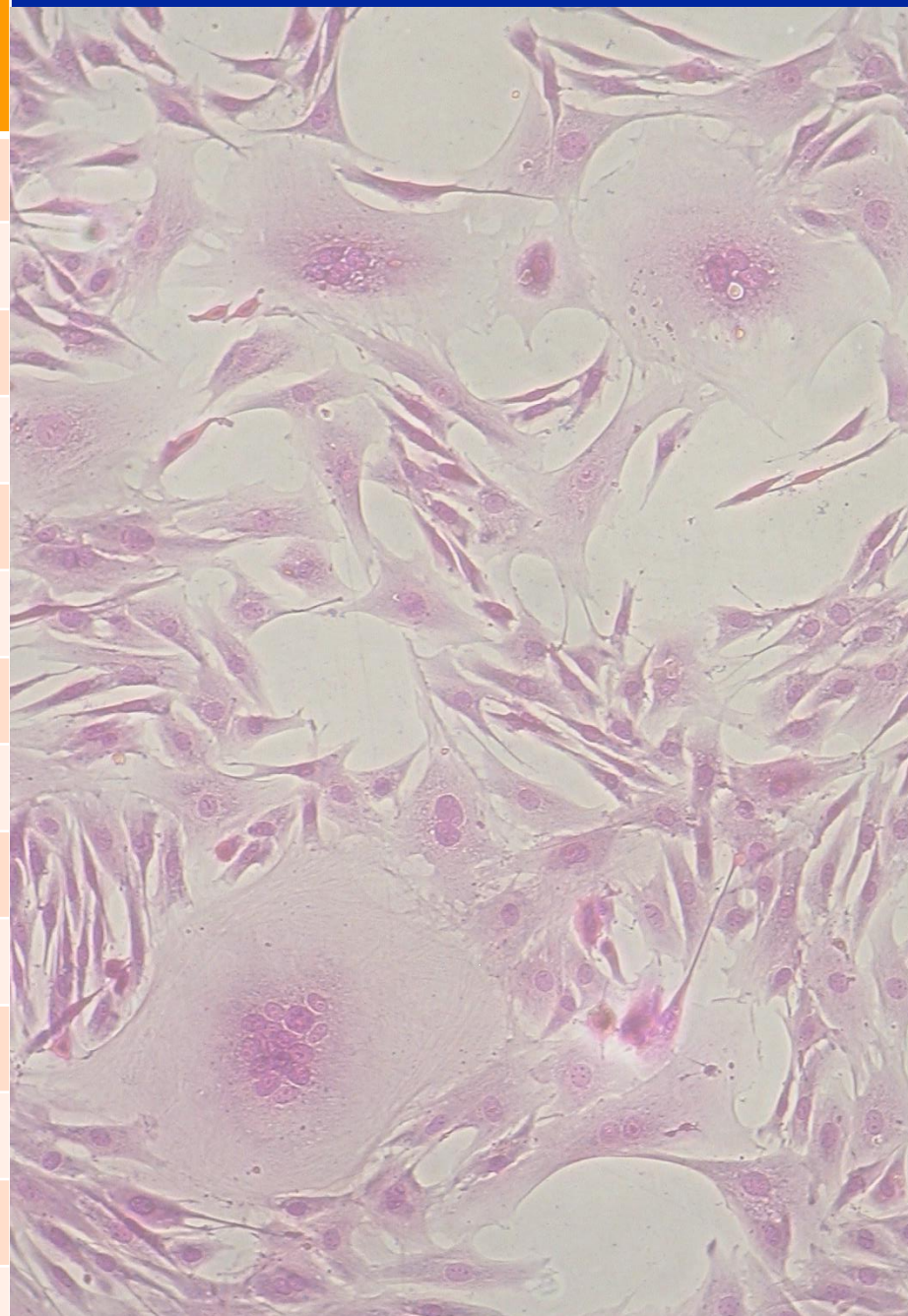
L'esempio di A8 (ceppo Elisabetta)



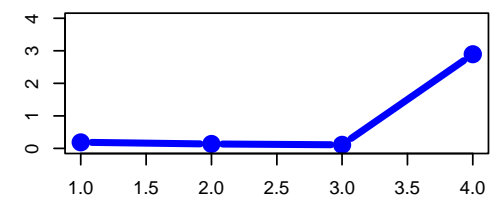
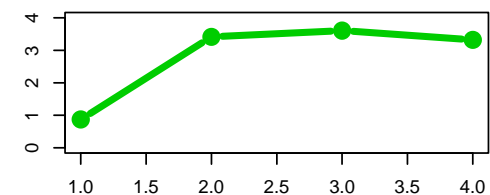
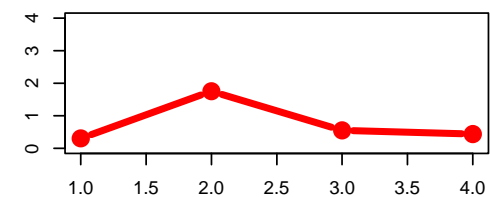
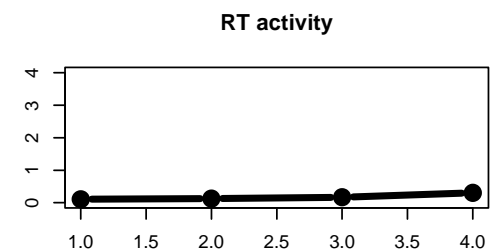
SRLV A8 macrophage culture



Sample	Serology genotyping	CPE
1	A/B	✓
2	A/B	✗
3	A	✗
4	A	✗
5	A/B	✗
6	E	✓
7	A/B	✗
9	A	✓
10	B	✓
14	A/B	✓
16	B	✓
17	B	✓
38	A	✓
VDA	A	✗



Sample	Serology genotyping	CPE	RT activity
1	A/B	✓	↗
2	A/B	✗	↘
3	A	✗	↗
4	A	✗	↗
5	A/B	✗	↗
6	E	✓	↗
7	A/B	✗	↗
9	A	✓	↗
10	B	✓	↗
14	A/B	✓	↘
16	B	✓	↗
17	B	✓	↗
38	A	✓	↗
VDA	A	✗	↘

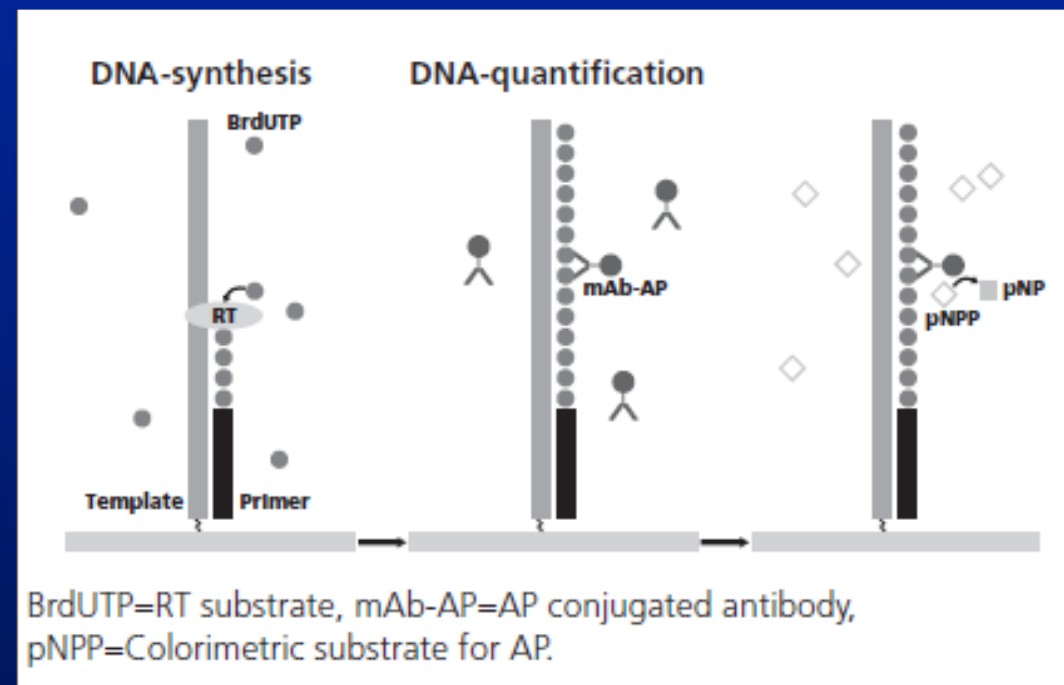


RT activity metodo colorimetrico

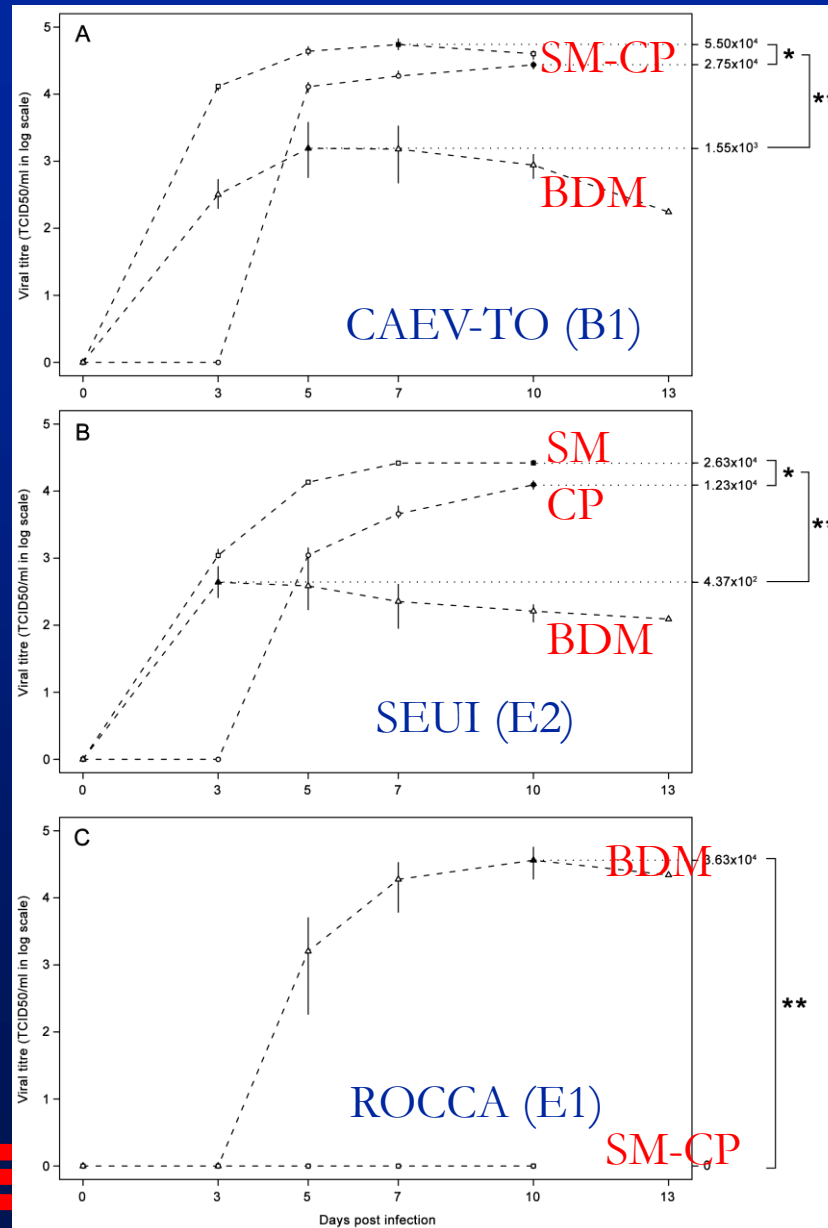
Misura l'attività enzimatica specifica della trascrittasi inversa, presente in ogni retrovirus

Utile in primo isolamento per verificare la presenza di particelle virali nel surnatante

Utile in prove di cinetica per valutare la permissività di diversi substrati cellulari

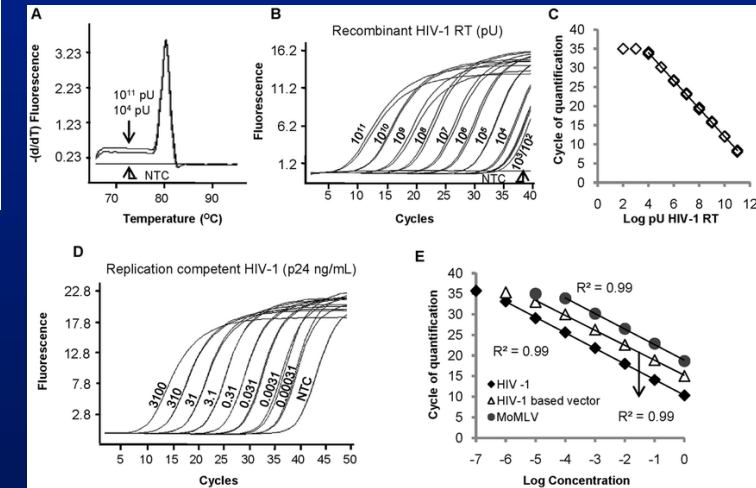
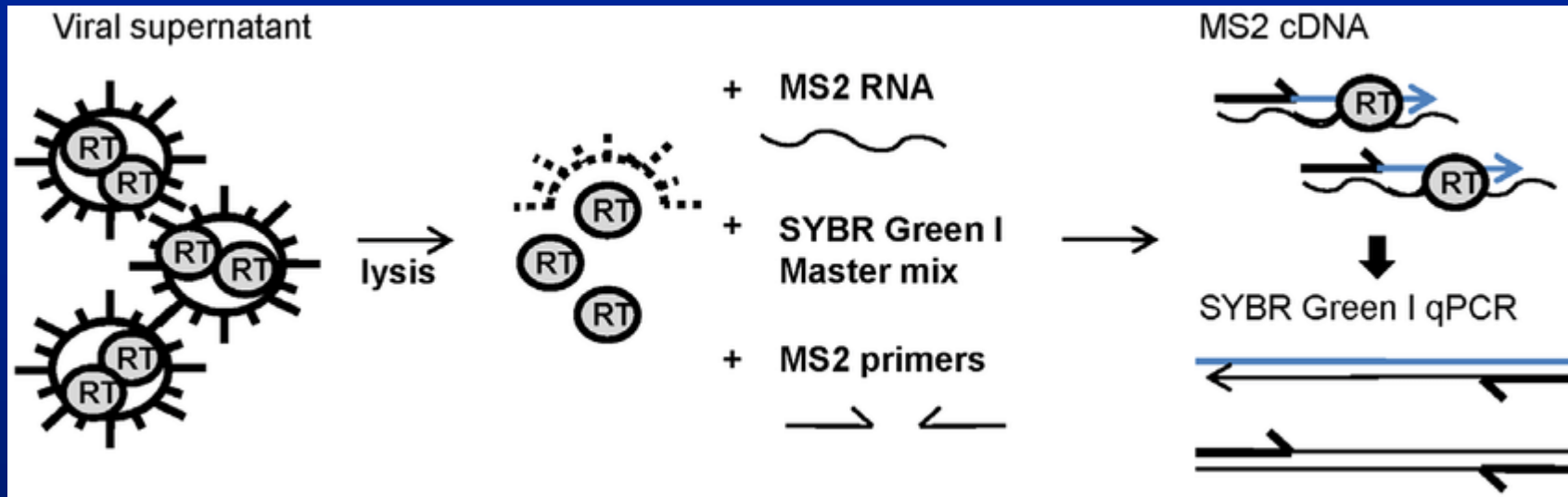


Growth Curve RT activity plotted against TCID50



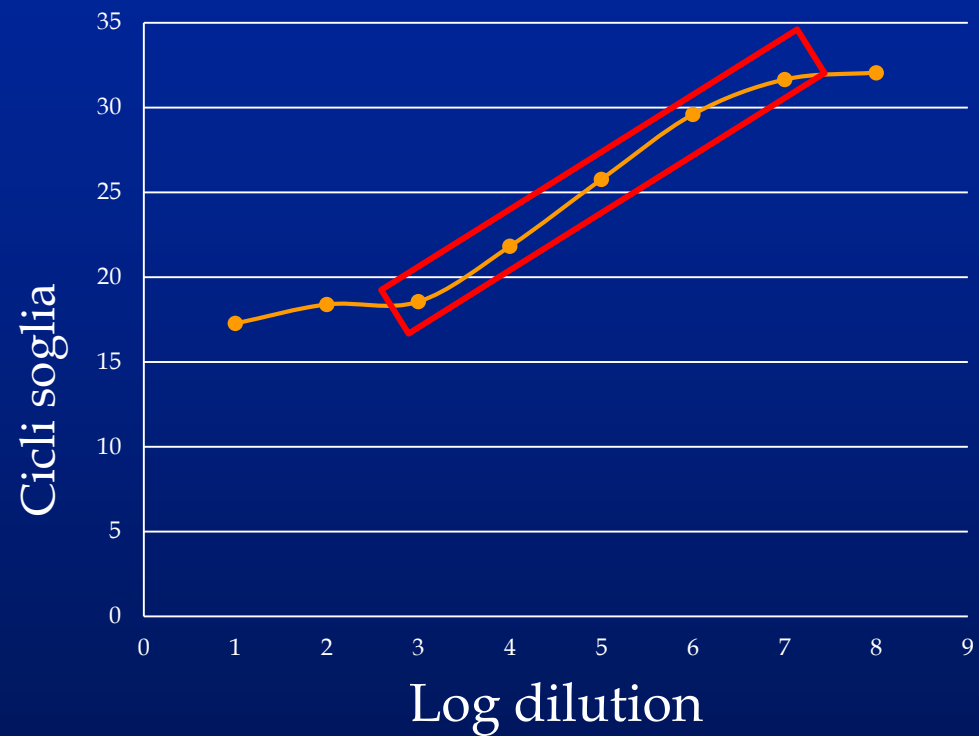
RT activity metodo molecolare

SG-PERT (SYBR Green Product Enhanced RT assay)





RT activity SRLV



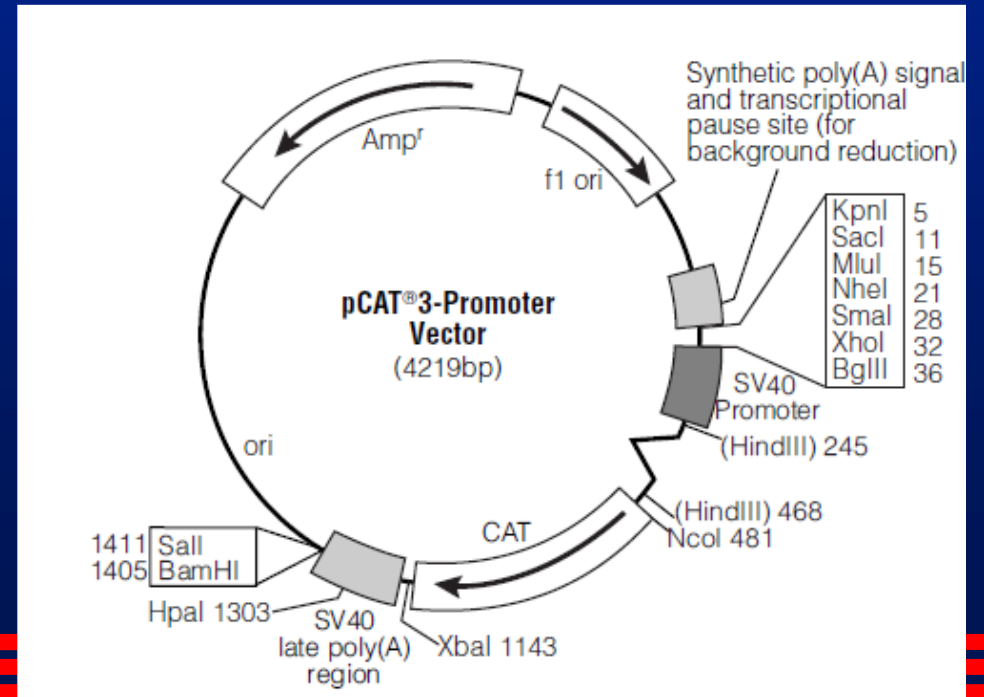
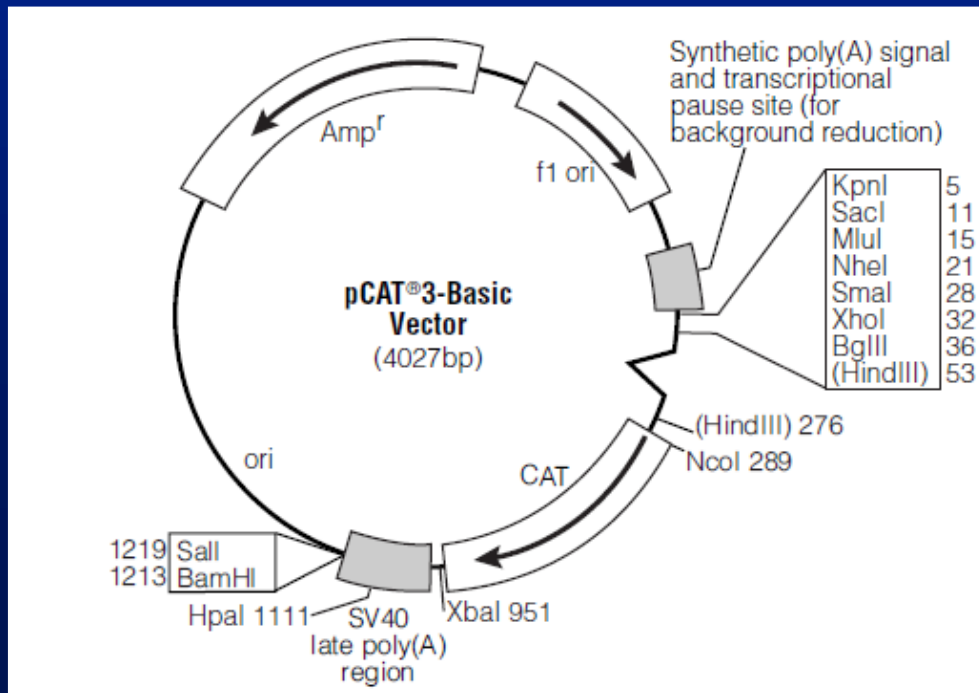
$R^2 = 0,998$

WLC-1



LTR attività del promotore virale

- Clonaggio del promotore virale (U3) nel vettore pCAT basic
- Trasfezione sulla linea cellulare
- Quantificazione CAT mediante ELISA
- Espressione relativa rispetto al promotore SV40

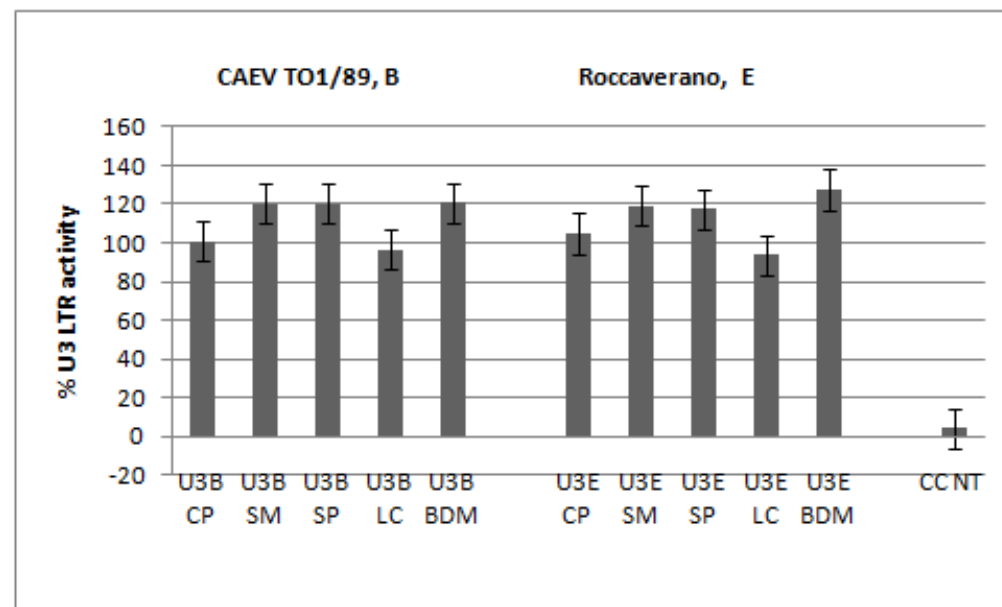


Promoter region U3

```

              10      20      30      40      50      60      70      80
U3Roccaverano  ....|....|....|....|....|....|....|....|....|....|....|....|
U3Seui         TGAAGCCCAAGAATCTAGGCCTCACTGGACTGAAACCTAAGACTGGCAATTCCGGGAAGGAGAGCATGGACTAAGGGGAA
U3CaevTo1/89   TGTGAGACATGGGTG-AGGAAGGACTG-AGAGCAGTCTAGGCCA--AATTCCTGTAAATCAGTTGGGGGGTTAAAGGAA
Consensus Caev TGTGAGACATGGGGA-AAAGAGGACTG-ATAACAACTAGGCCA--AATTCCTGTAAATCACTTGGGGGGTTATAGGAAA
Clustal Consensus **  *  *  *  *      *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
              AP1                                GAS                                TAS
              90      100     110     120     130     140     150     160
U3Roccaverano  -GGCAAACTCACCATGACATAGCAAA-TGTAACCACAAGTTCTGCTTTGCGAGCATAAGTCATTAAGCTGCTGAC-TGTC
U3Seui         AGGCAAAATCAGCATGACATAGCAAA-TGTAACCACAAGTTCTGCTTTGCTGGCTTAGGTCTGTAA-CAGCTGAC-C-TC
U3CaevTo1/89   -AGCAAGCTCACTATGACAAAGCAAAATGTAACCACAAGTGCTG-----ACAGTTGTAA-CAGCTGACACATC
Consensus Caev -AGCAAG-TCACTATGACAAAGCAAAATGTAACCGCAAGTGCTG-----ACAGATGTAA-CAGCTGACACATC
Clustal Consensus ****  ***  *****  *****  *****  *****  ***  *  *  *  *  *  *  *  *
              AML                                AP4  AP1
              170     180     190     200     210     220     230
U3Roccaverano  AGCTGATGCTTACTAATGCTGACTCGGGTACTA-ACACACTATATAAACCTGAGCTTG-TACTTGGGAGTCA
U3Seui         AGCTGATGCTTACTAATGCTGACTCGGGAATA-ACACACTATATAAGCCTTGAGATTG-TACTTGGGAGTCA
U3CaevTo1/89   AGCTGATGCTTGCTCATGCTGACATTGTAGCTTTGCACTGTATATAAGGAGAAGCTTTGCTGCTTGACGGCA
Consensus Caev AGCTGATGCTTGCTCATGCTGACACTGTAACCTCTGAGCTGTATATAAGGAGGAGCTTTGCTGCTTGCACTTCA
Clustal Consensus *****  *  *****  *  *  *  *  *  *  *  *  *  *  *  *
              AP4                                TATA-box
```

Viral promoter is active in all cell types tested

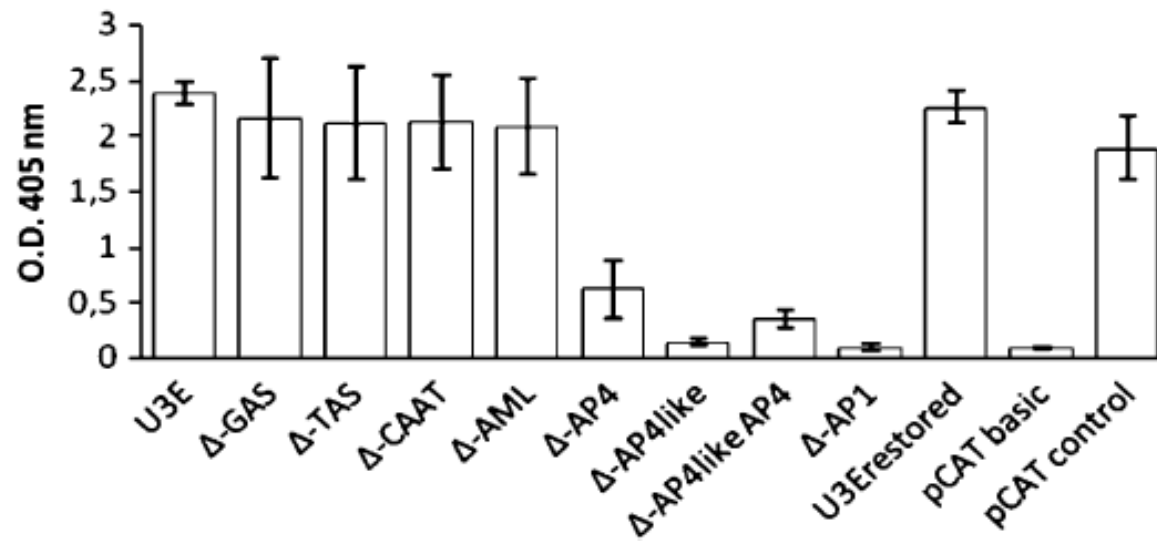
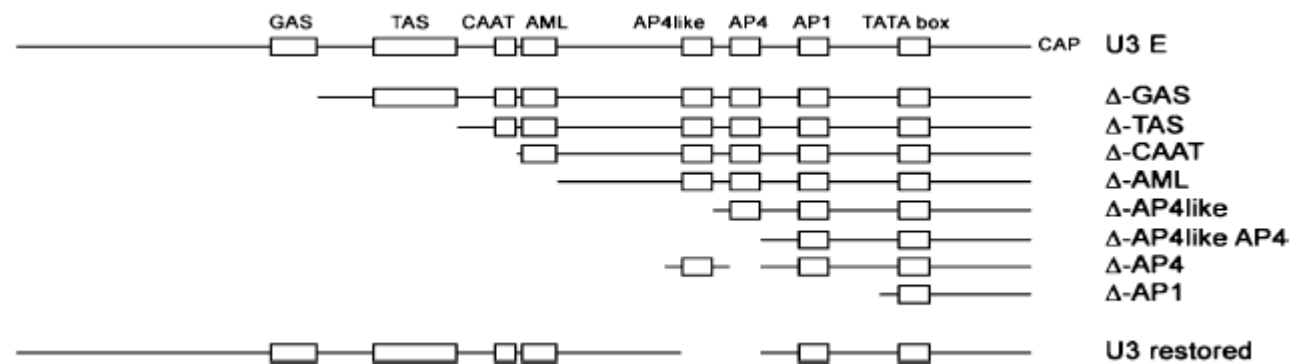


Vet Res Commun
DOI 10.1007/s11259-010-9390-5

EXTENDED ABSTRACT

LTR promoter activity of SRLV genotype E, strain Roccaverano


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S. Rosati



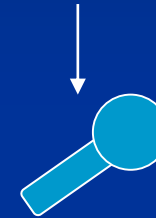
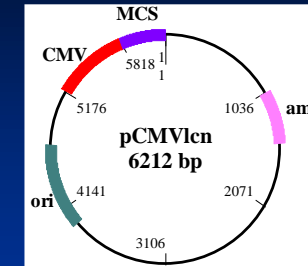
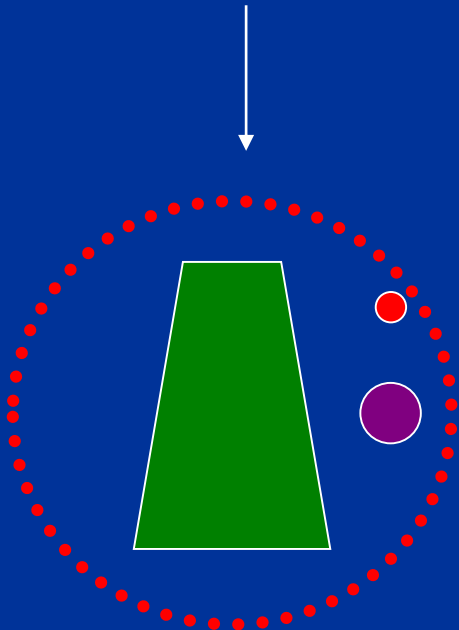
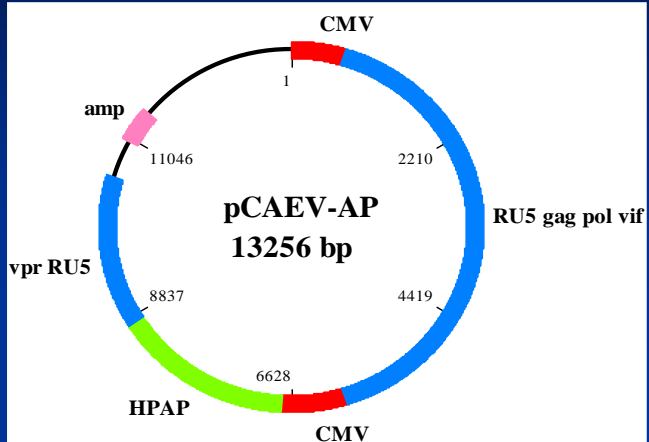
Promoter activity of the Roccaverano strain U3 region and other constructs after deletion of sites



Entry assay

- Produzione di particelle pseudovirali
 - Non competenti per la replicazione
 - Espressione intracellulare di un marcatore
 - Pseudotipi con differenti geni env
 - Consente di definire la presenza di idonei recettori per l'ingresso del virus
- 

Entry assay



Antigene capsidico



Fosfatasi alcalina termostabile



SU-TM (env)



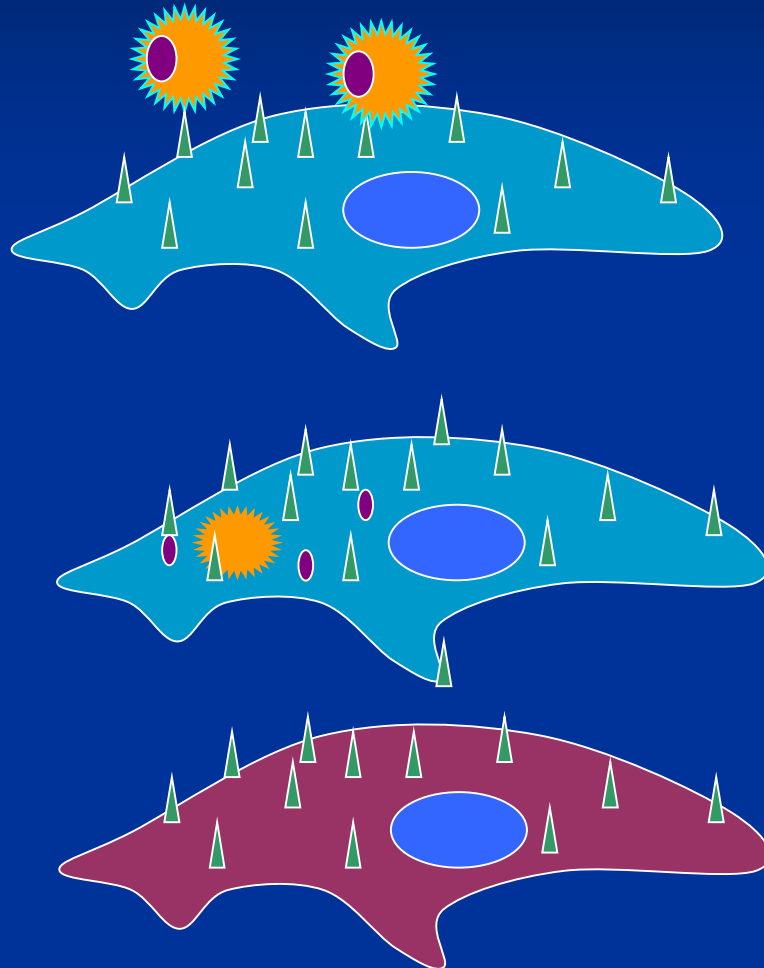
Trascrittasi inversa



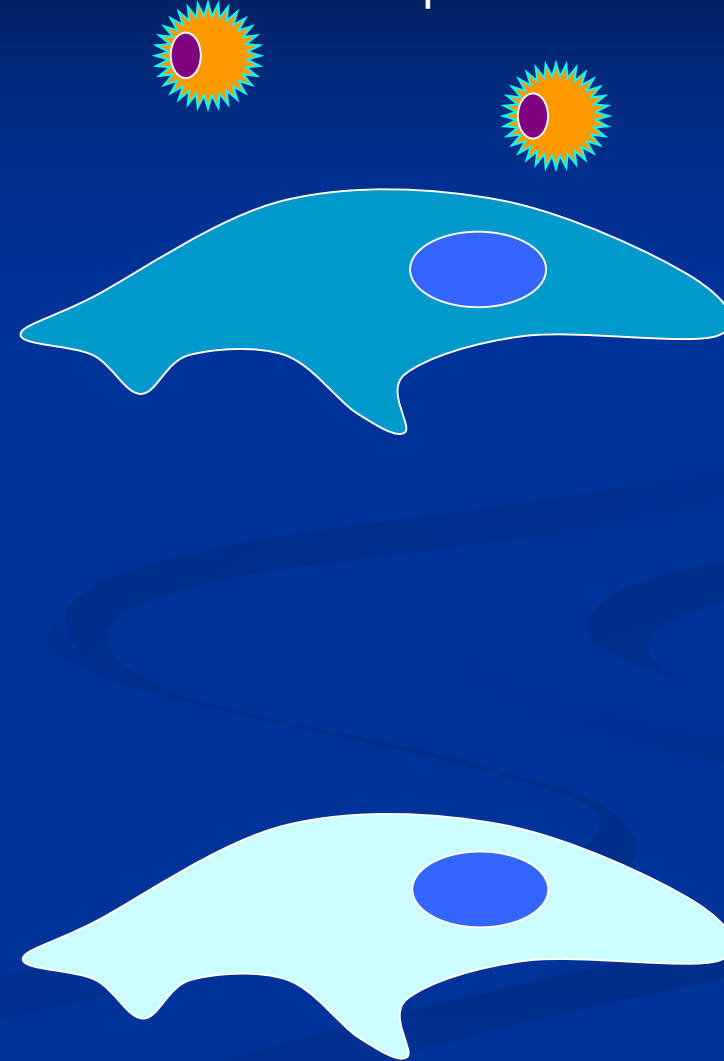
matrice

Entry assay

Cellula permissiva



Cellula non permissiva



infection

24h

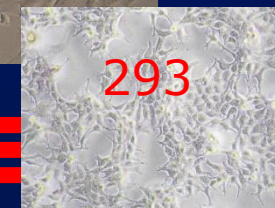
AP stain

Co-transfection
pCAEV gag/pol AP
pCMV-env (Roccaverano)
pCMV-env (CAEV)
pCMV-env (Seui)

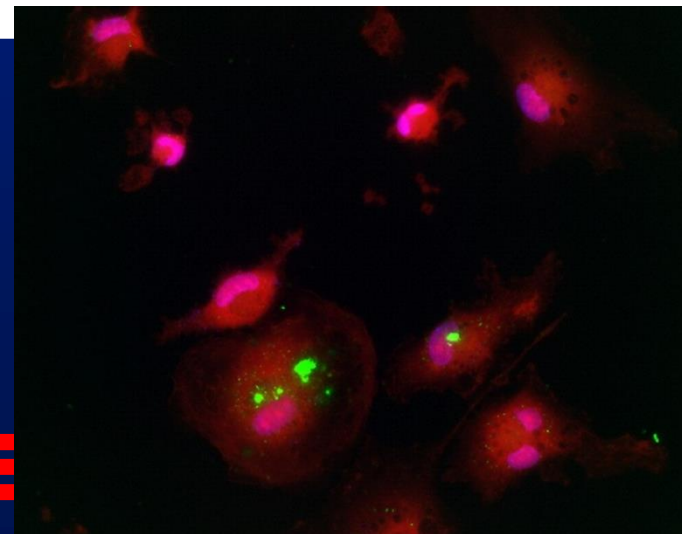
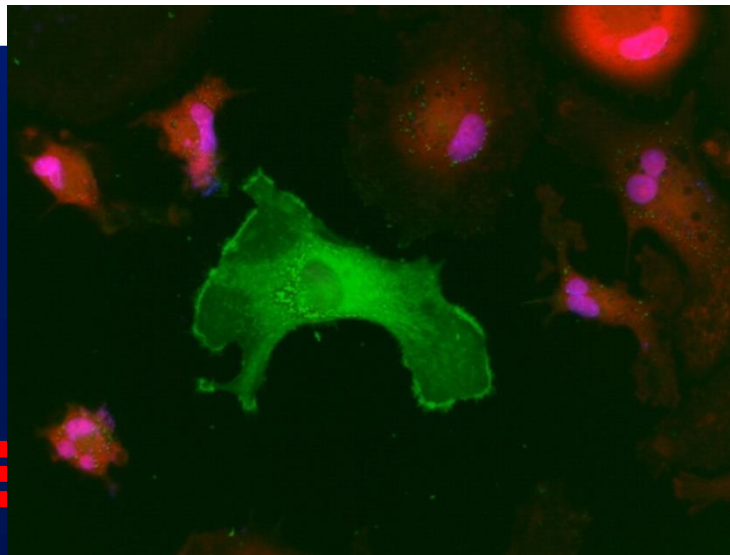
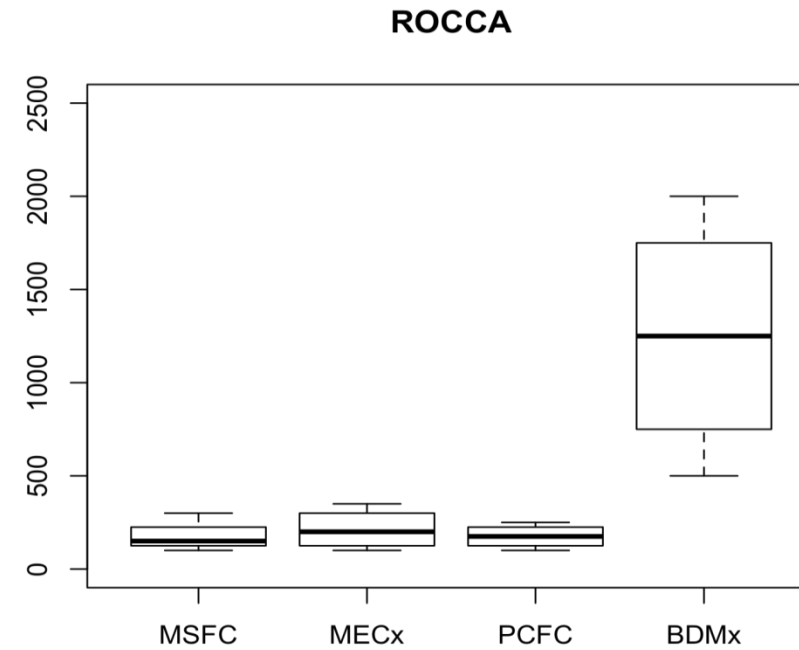
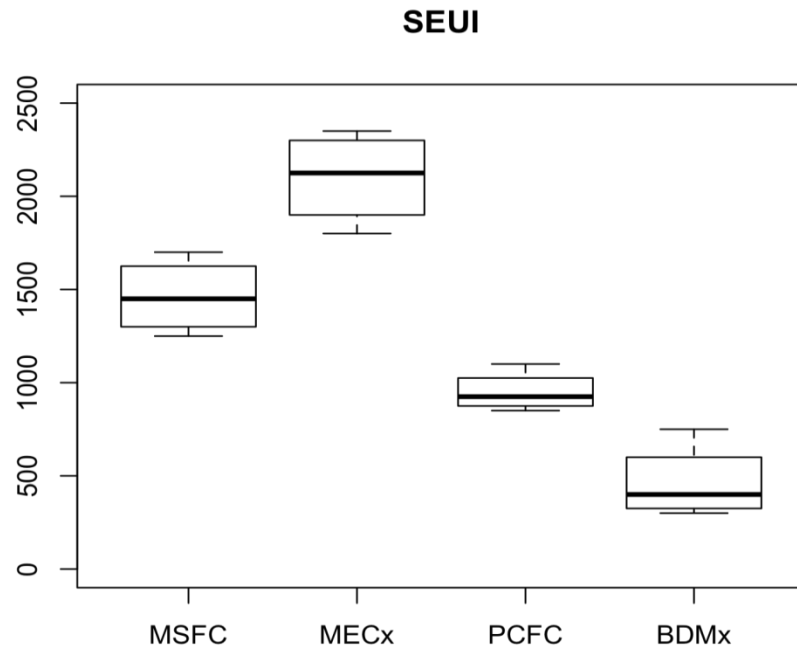


Fix and stain

AP foci



Titer of viral pseudotypes (focus-forming units/ml)

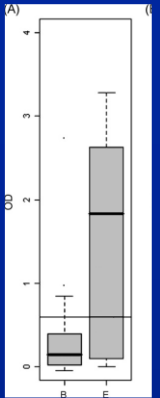


In vivo

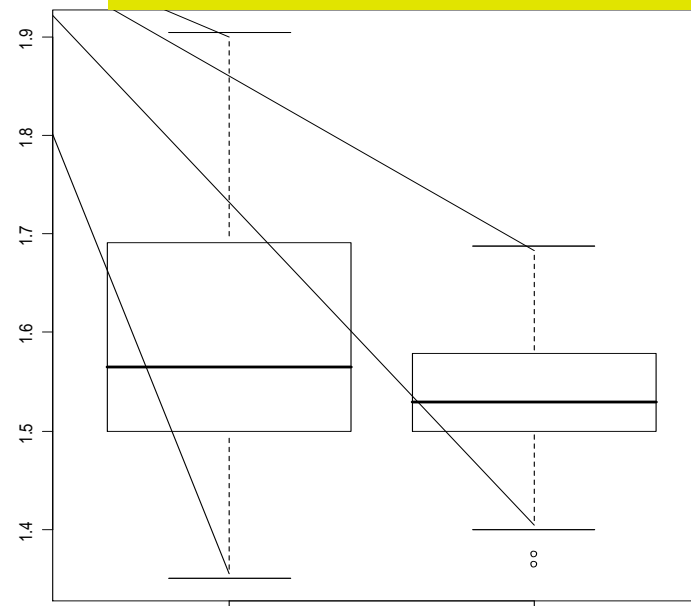
- Flock TA (strain B1)



- Flock BL (Strain E1)



Arthritic index TA vs BL



p-value = 0.04

mean of x mean of y
1.601115 1.532695

Indice istopatologico

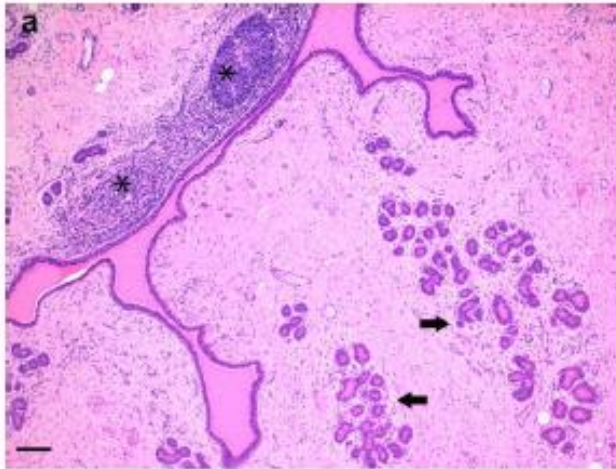
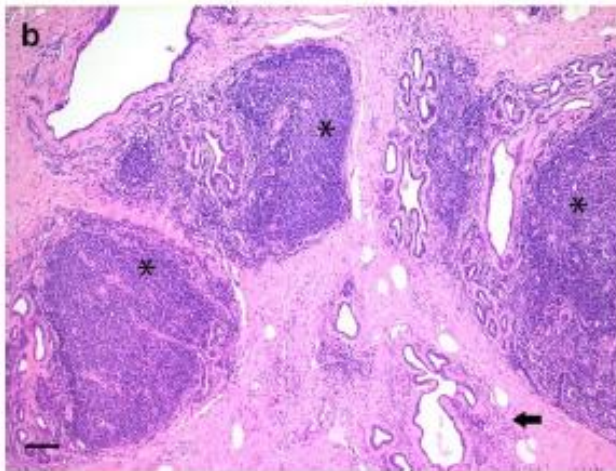


Fig. 6:

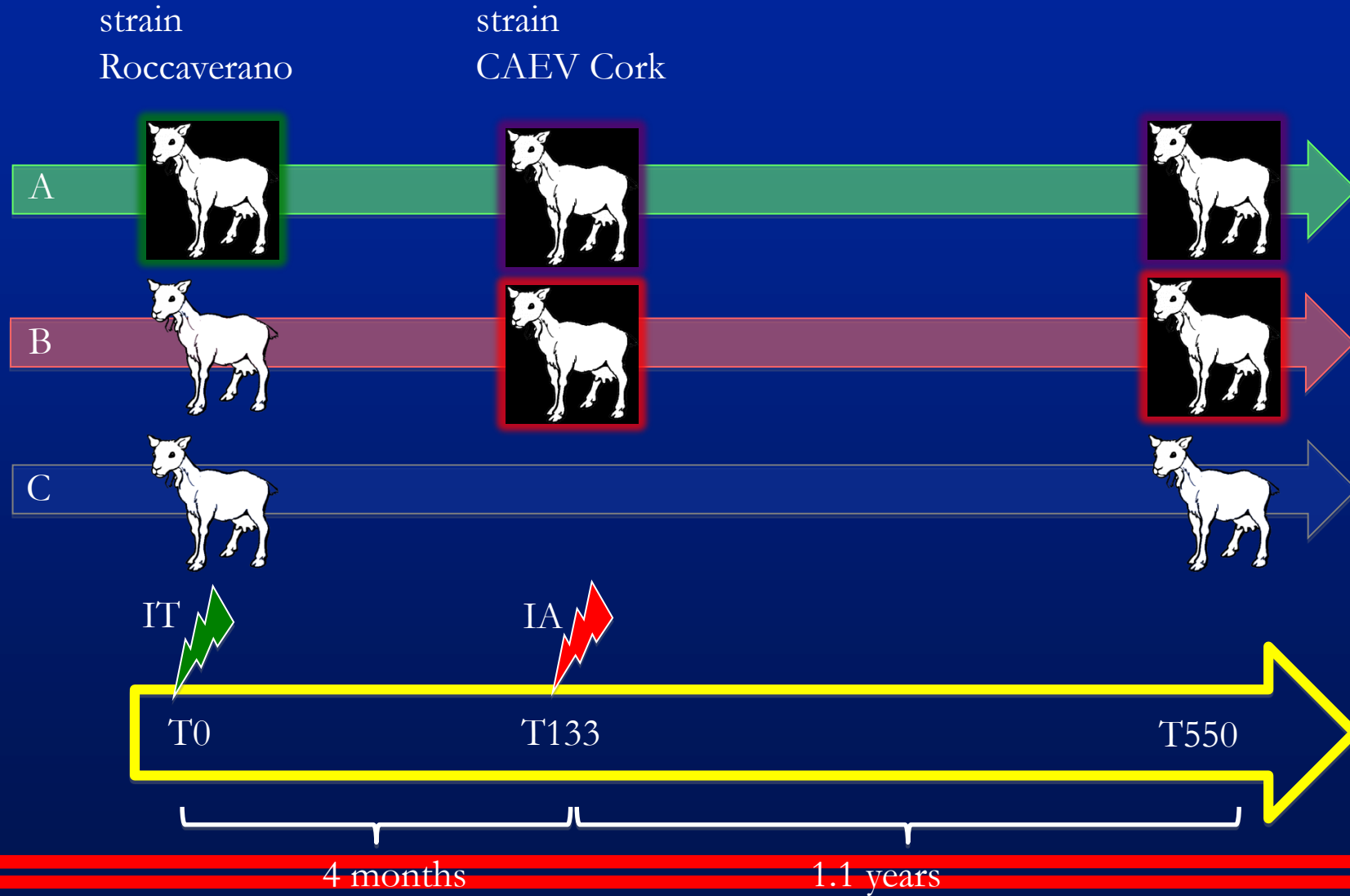
a: representative histopathological picture of a **grade 1** inflammatory reaction in goat #2 with small periductular lymphoid follicles (asterisks) and mild periacinar lymphocytic infiltrates (arrows).



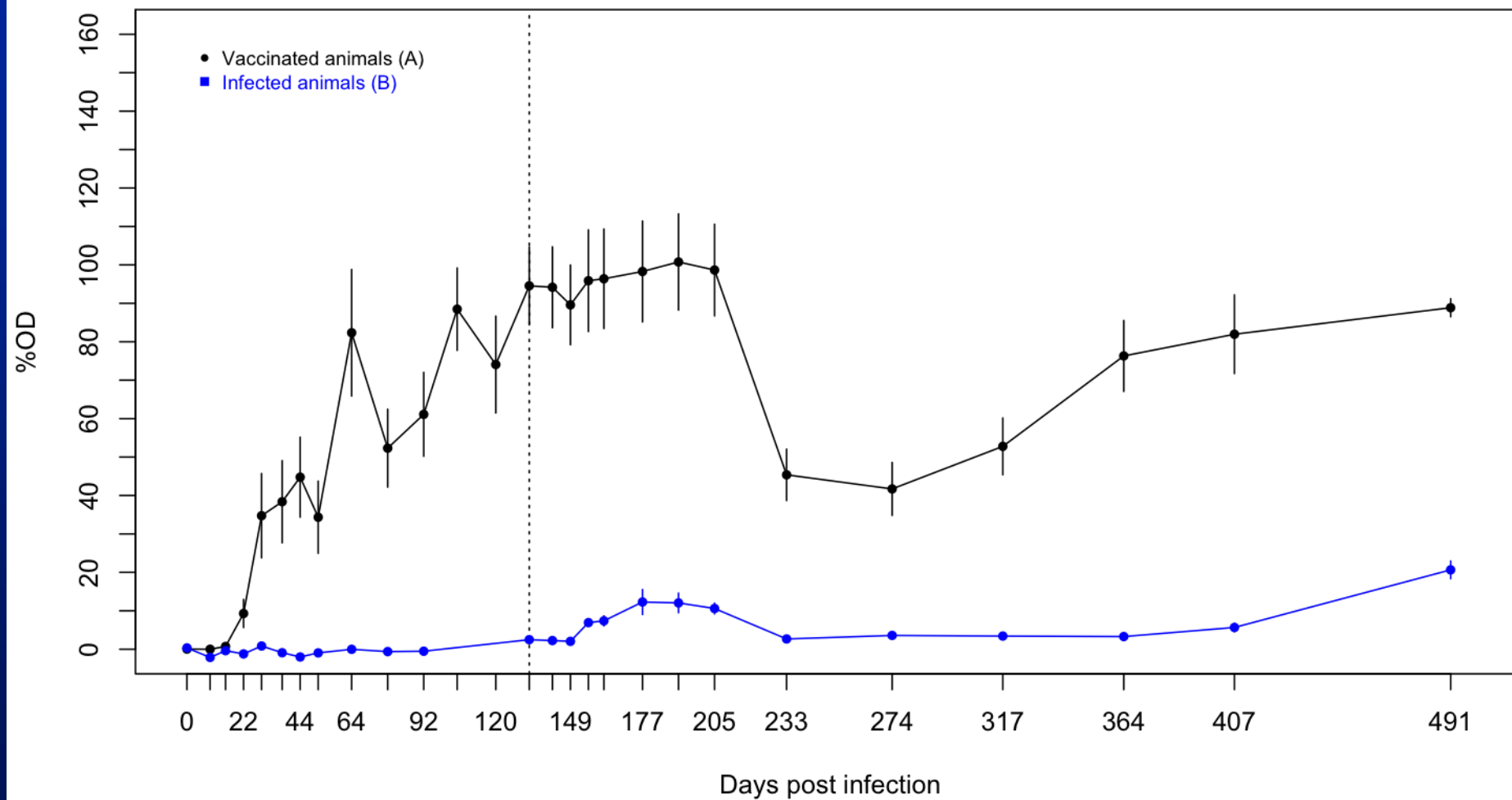
b: representative histopathological picture of a **grade 4** inflammatory reaction in goat #5 with many large lymphoid follicles (asterisks) and moderate lymphocytic periacinar infiltrates (arrow).

H&E stain, scale bar = 100 μ m

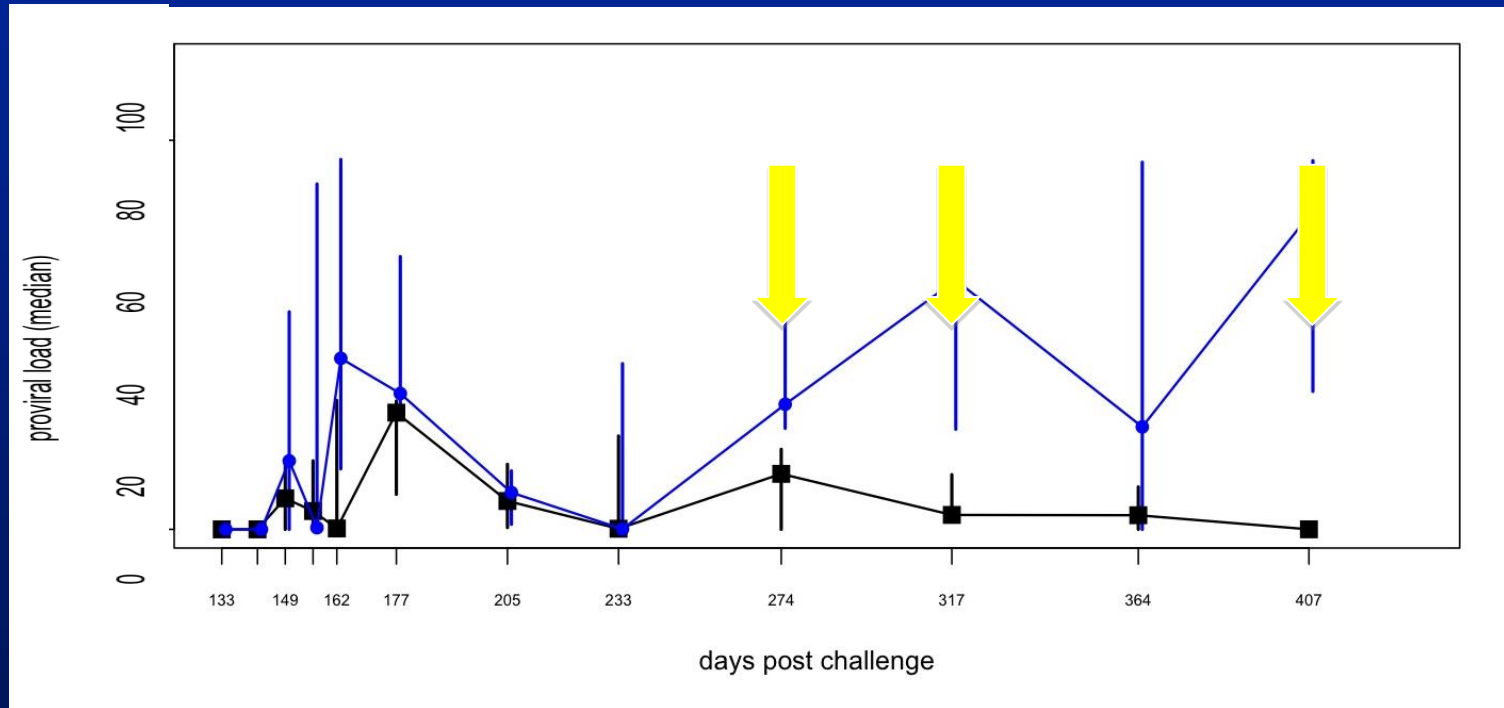
experimental infection



Seroconversion against E



- Challenge virus proviral load
 - TaqMan qRealTime *gag*



conclusioni

- Proprietà biologiche
 - Tipi cellulari (prevalentemente meccanismi di entrata)
- Studio delle proprietà biologiche non applicabile in routine (analisi al macello)
- Utile per programmare strategie eradicative
 - Es. Svizzera, Bolzano

