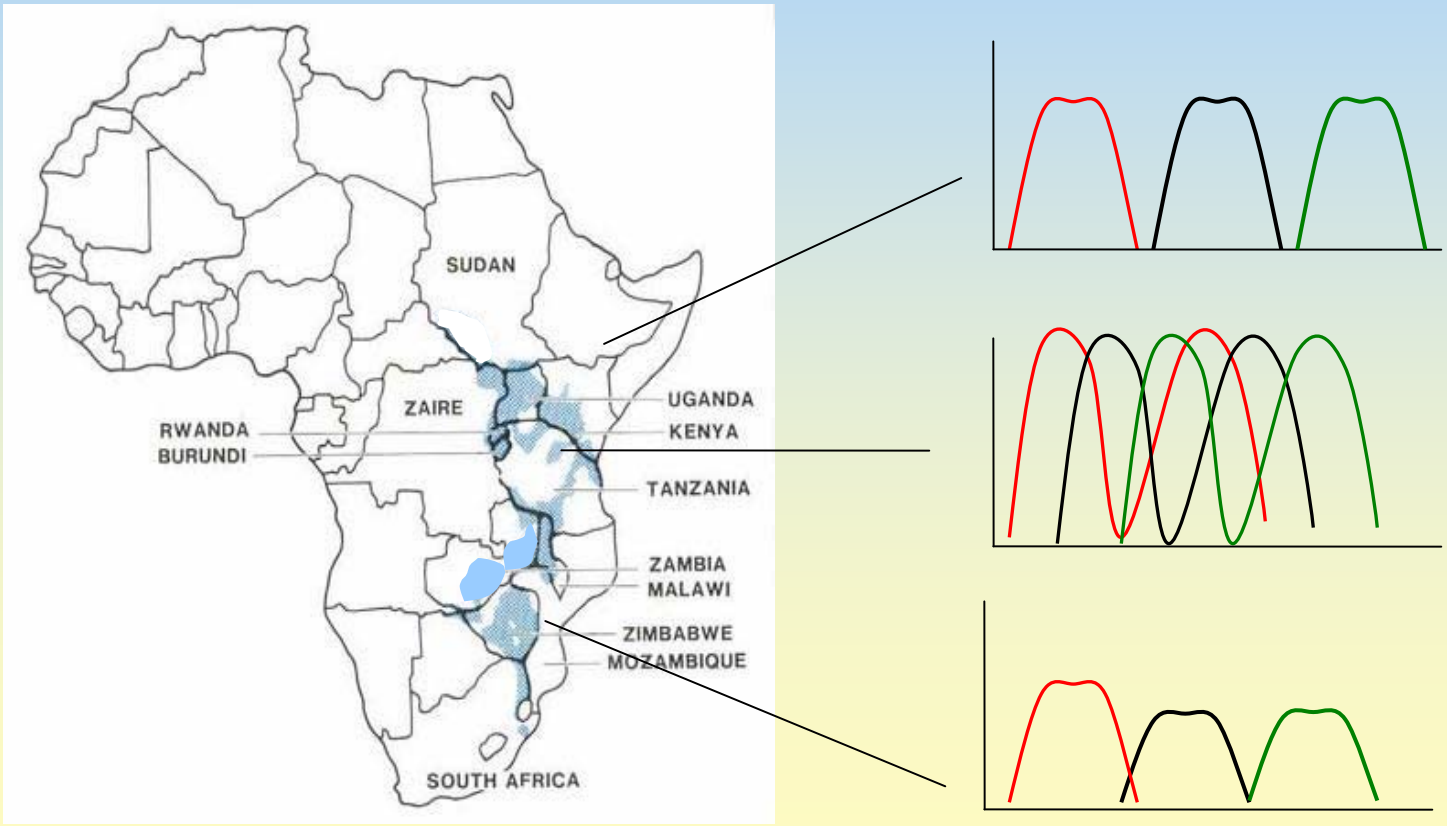
Molecular epidemiology in tick-borne diseases

Geysen Dirk
ITM, Antwerp

ECF epidemiology

Depending on tick ecology: year-round presence of all stages
seasonal presence of each stage



ECF molecular epidemiology

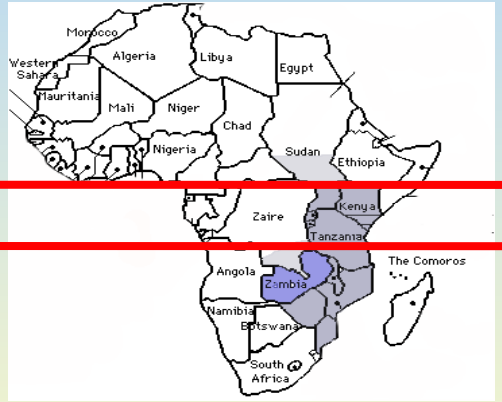
Parasite genotypes

T.parva distribution map

Homogeneous

Ticks

<2 generations/year



>2 generations/year

Heterogeneous

<2 generations/year

Homogeneous

Epidemiology of tick-borne diseases

Tick - host - parasite => transmission intensity

- Seasonal or year round presence of all tick stages
- Parasite infection rates in ticks
- Parasite infection rates in host
- Tick numbers on hosts
- Carrier status/ wildlife reservoir for parasite?

Molecular based tests

Many assays developed, often for specific needs
lack of field validation

By validation
=>> problems of aspecific reactions and sensitivity (carrier)

- Optimalisation of primerpairs is lenghty process

- Use of higher blood volumes and nested approach

Filterpaper bloodspots on Whatman Nr3: 0,17 - 1 microl blood in 1 PCR

EDTA bloodtubes with special extraction protocols 30-100 microl in 1 PCR

How to measure disease endemicity

- Transmission intensity

  ~ % carriers in population

  ~ Number of bites by infected ticks

  =>> Parameter: Mean age at first contact:
  only after using **longitudinal surveys**

⇒Approximation by EIR = entomological inoculation rate
meaning: nrs infective bites/animal/year

using tick **infestation** data +
using tick **infection** rate data

Epidemiology of tick-borne diseases

Alternative using a Cross sectional approach?

During/just after main transmission period
Collection of ticks from different animal age groups

mRNA extraction and PCR on cDNA

Animal infection rate: presence of **piroplasm** gene expression
Tick infection rate: presence of **sporoziete** gene expression
+
Tick infestation data (known for most regions)

Relation between EIR and prevalence

Epidemiology of tick-borne diseases

Advantages

Cross sectional

+

Increase in sensitivity of both:

Piroplasm detection through concentration of bloodmeal in tickgut
Maturation, thereby increasing nrs of **sporozoites** in salivary glands

Molecular epidemiological tools

1. Species identification using « pan » mDNA assay

2. Multi locus genotyping using nPCR-RFLP of important antigen genes

Epidemiology of vector-borne diseases

Advantage of pan species molecular tests

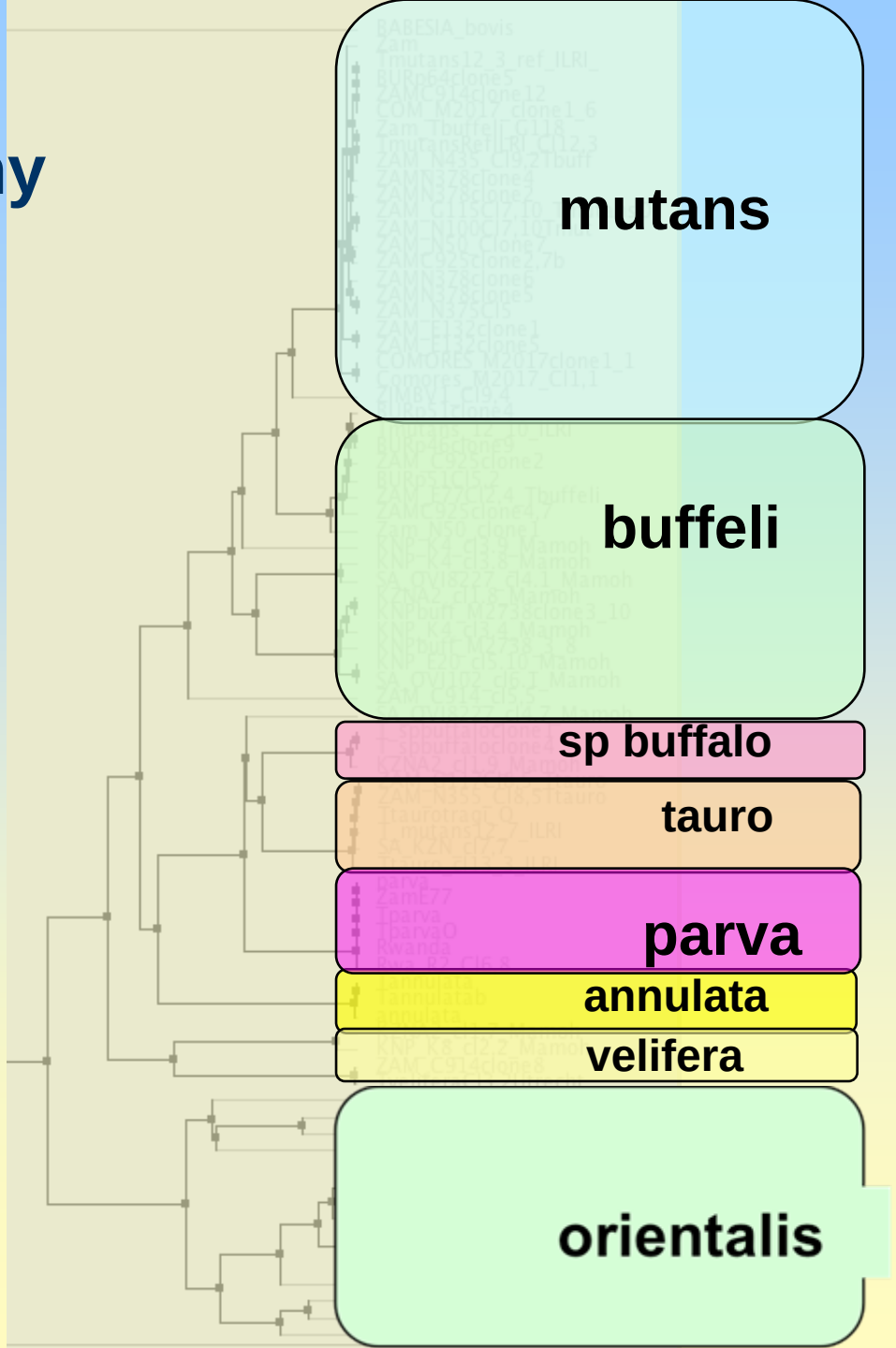
- Less sample transport restrictions
- Presence of different species within 1 PCR assay

=>> Cost equals multiple serology tests

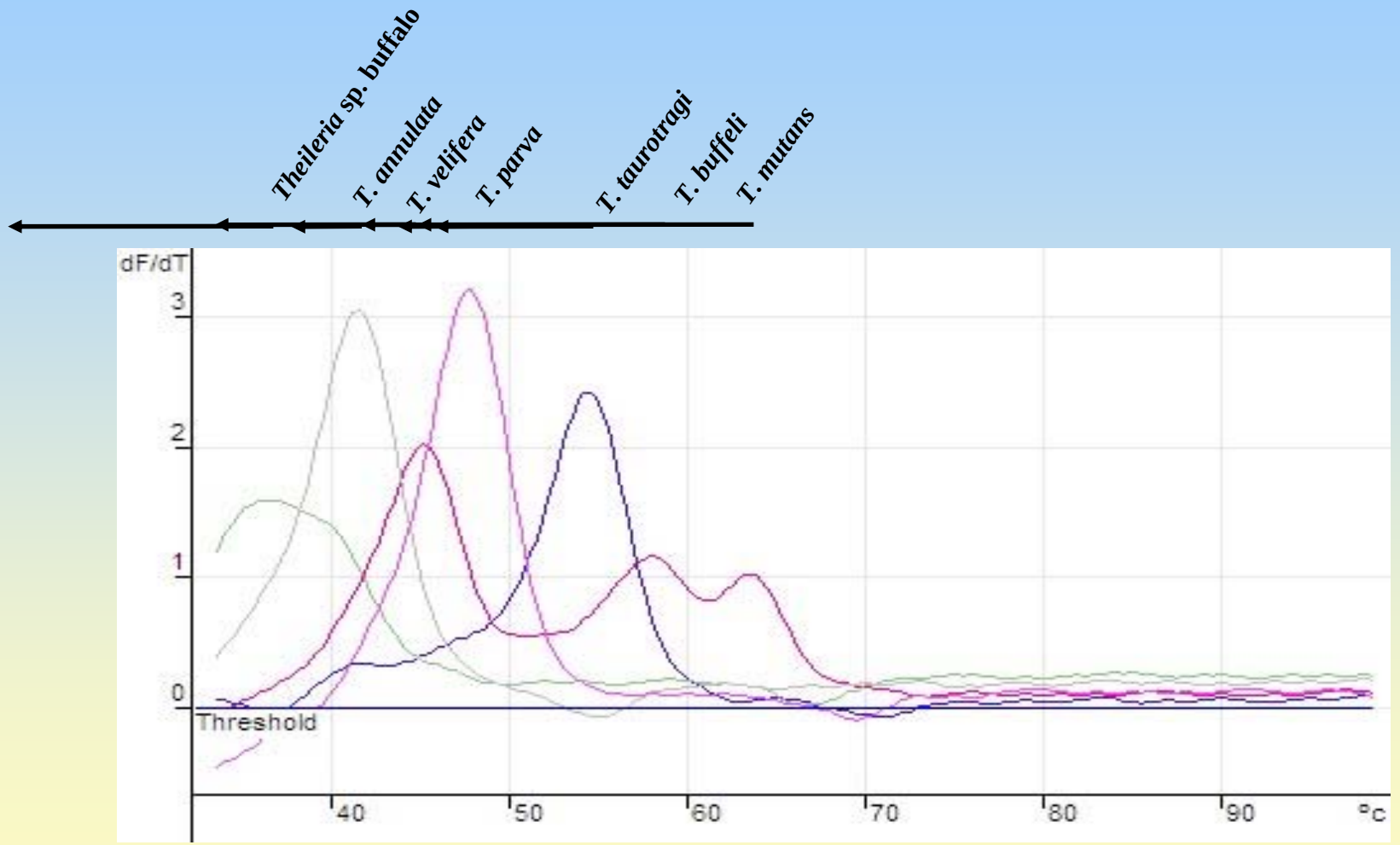
Species level: mitochondrial analysis

- Maternally inherited, no recombination history in sequences
- Tandemly repeated, multi copy => more sensitive than 18S
- FRET assay based on 45 Cox III sequences, and under validation.

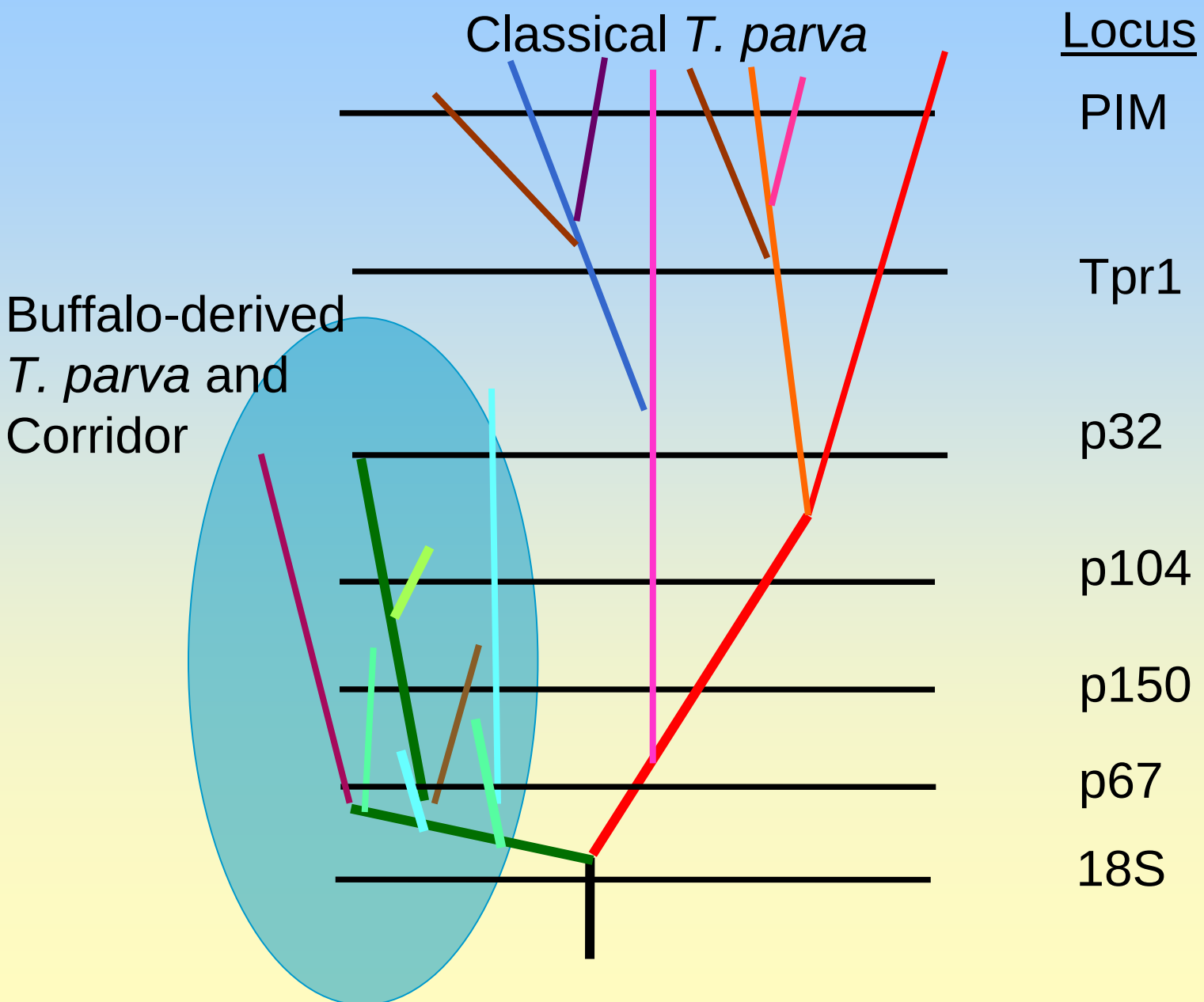
COXIII phylogeny



COXIII FRET results



Analysis of diversity



MLG: multilocus genotyping using polymorphic antigen genes as markers

Limited polymorphism

p67: conserved in bovine # buffalo

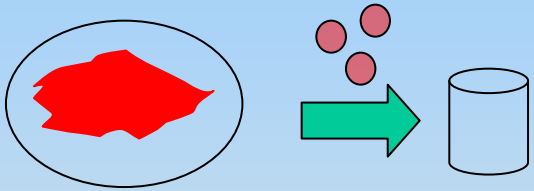
Polymorphic in both cattle and buffalo

p104: bovine alleles <> buffalo alleles

p150: bovine alleles <> buffalo alleles

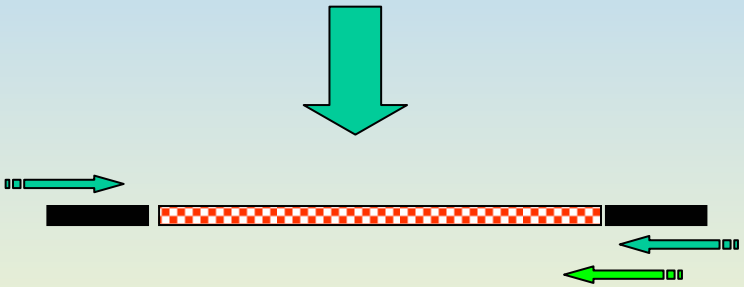
PIM: Polymorphic Immuno-dominant molecule

Methodology

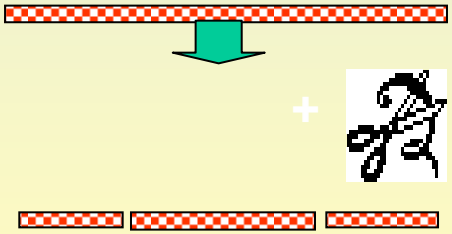


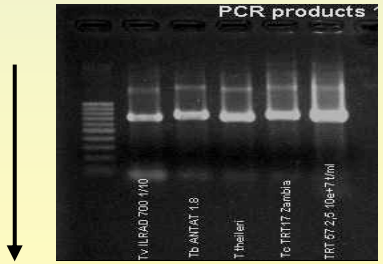
Filter paper blood spot

DNA extraction



Semi-nested PCR amplification



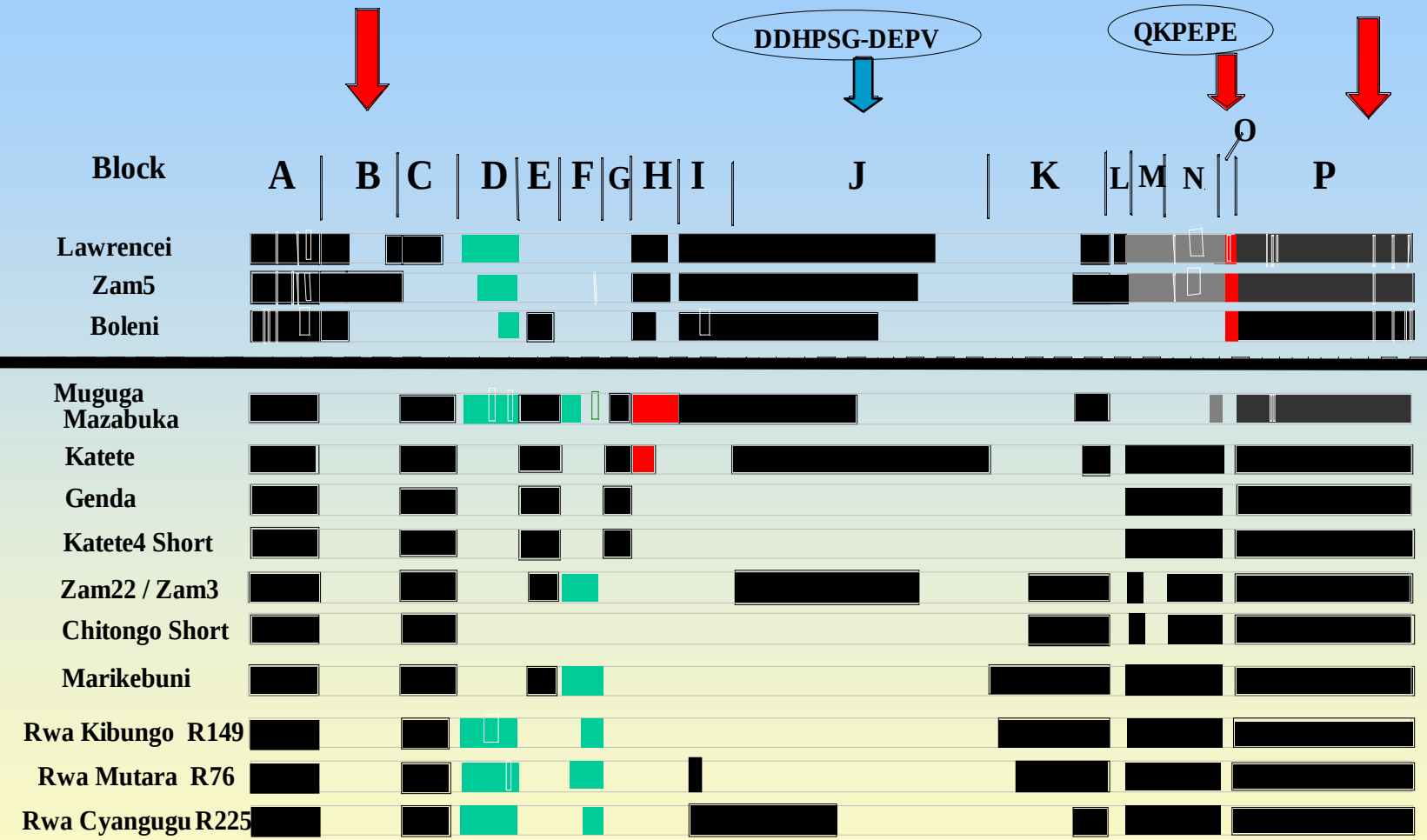


PCR product

Polymorphic immunodominant protein PIM

Single copy gene

Mosaic structure of PIM



useage

1. Can be used to genotype isolates and classify them in broad lineages, « *useful* » in identification of putative vaccine components in ITM.

2. Can be used in identification of possible recombinations between classical and buffalo-derived isolates.

p67 analysis

Conserved insert-negative sequence in classical cattle parasites

Very diverse sequences in buffalo parasites

Phylogeny:

2 clusters with different deletions: 2 speciation events?

- **Allele 1 cluster are known sequences in buffalo, with insert, and in bovines without insert**

- **Allele 2 cluster are new sequences in buffalo, also found in CD cases like Zam5 with and without insert**

p104 analysis

Buffalo majority: specific buffalo p104 allele 4

3 ladysmith carriers: Muguga allele 1

1 buffalo KNP: Uganda/Zambia/Marikebuni allele 2

No Boleni/Zam5 type: allele 3

New type:
1KNP buffalo, exp. cattle and Welgevonden

Conclusion

Results of genotyping SA buffalo isolates

- PIM sequence data reveal recombination events
- Large p67 buffalo polymorphism +
  indicative of 2 main parasite populations?
- Majority of buffalo EA buffalo p104 allele

Cattle genotypes present in buffalo (p104)

SA cattle (3) with bovine PIM and p104
but buffalo p67 in SA

Problem of role of T. sp buffalo, identical on 18S?