

# ***PCR- Restriction fragment length polymorphism (RFLP)***

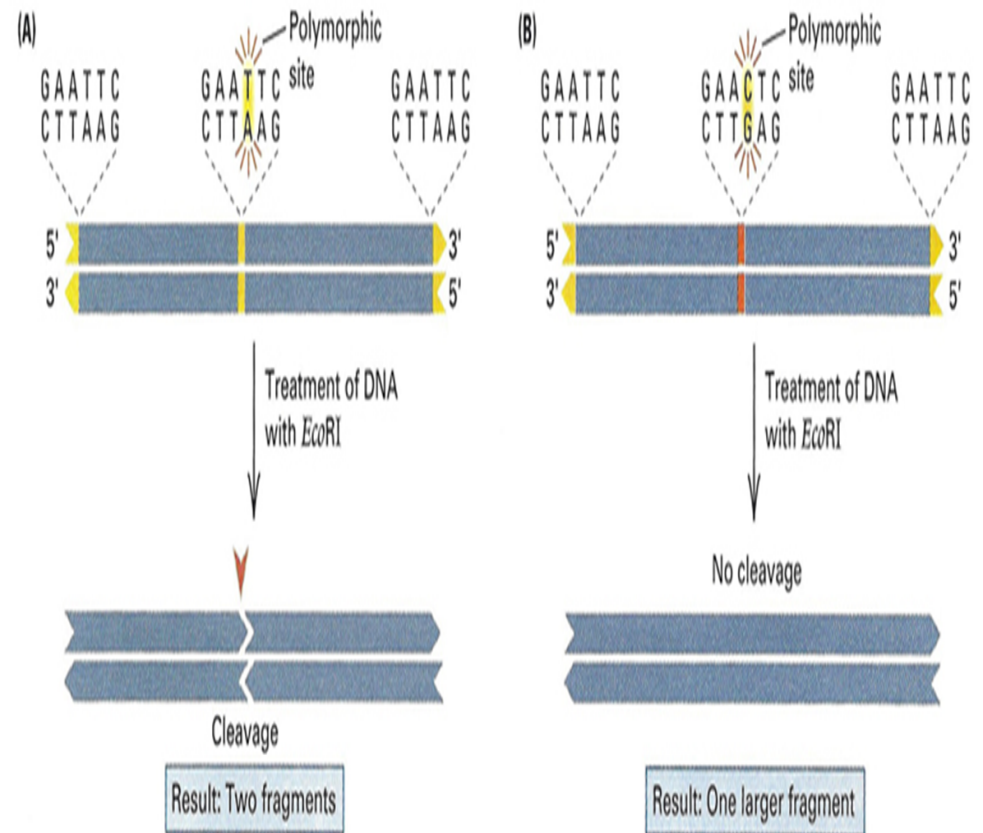
**Students' presentation 1**

**By**

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# Principle of the test

- **PCR-RFLP** is a technique that combines the **PCR** amplification with the **digestion** of PCR products by **restriction enzymes (RE)**.
- Cleaving DNA into **fragments of certain sizes**, whose analysis on **agarose gel** results in **different patterns** of fragment sizes, enabling the identification.

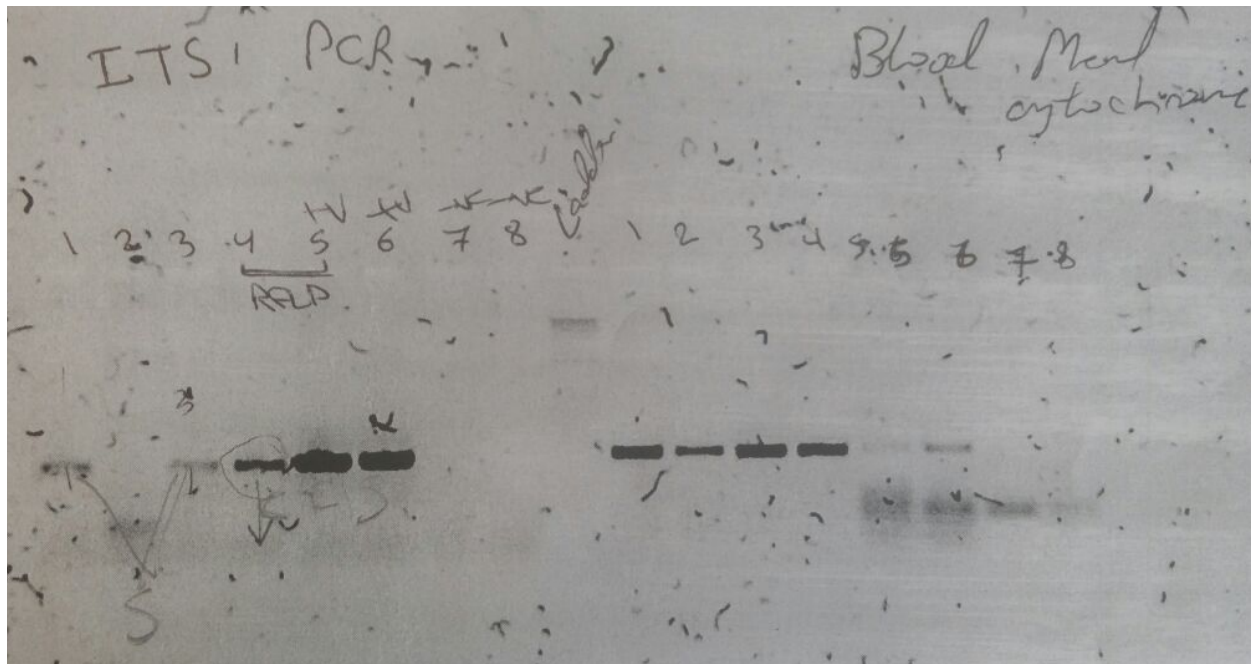
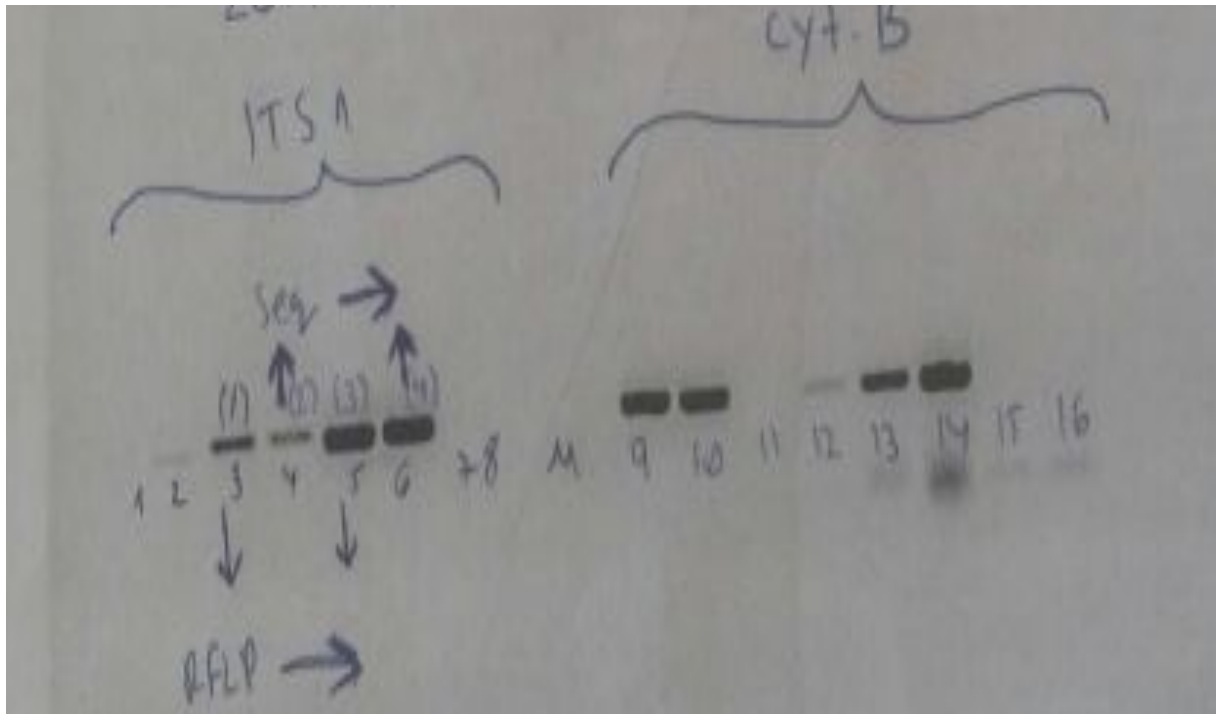


# First: *Leishmania* PCR analysis


DNA extraction  
from blood samples



ITS1 PCR assay



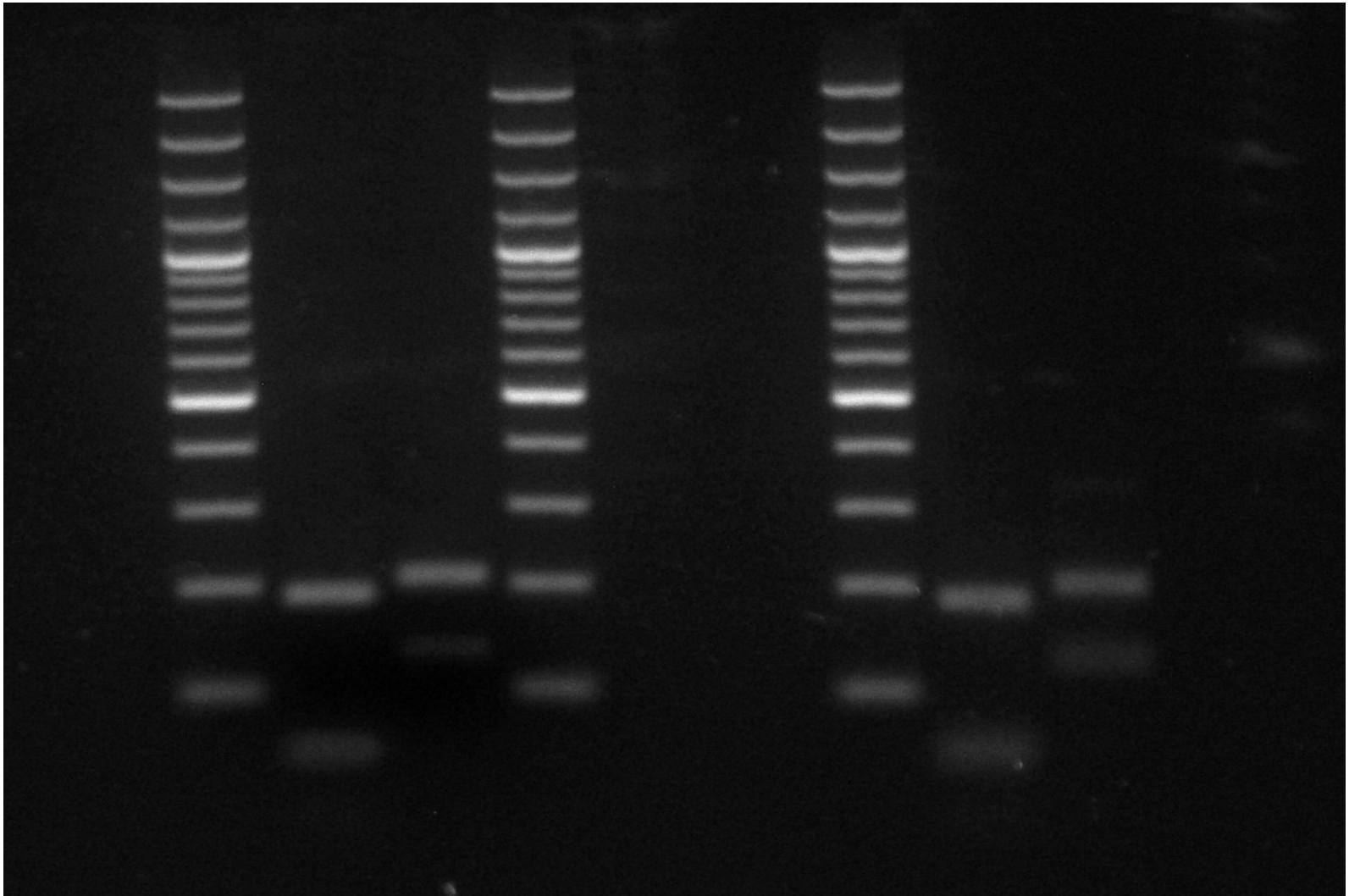
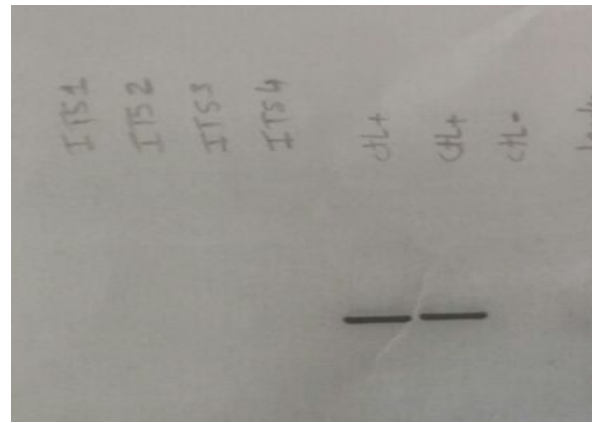
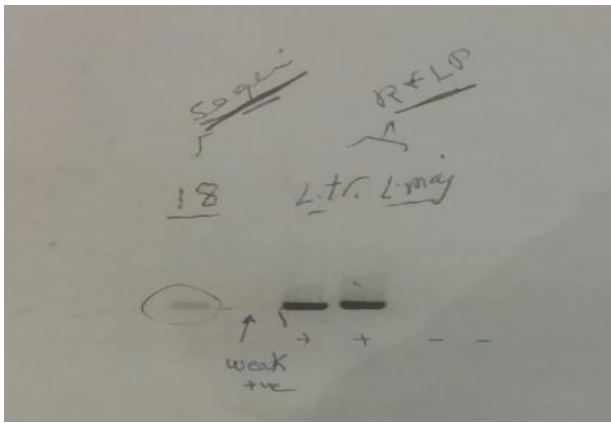
# For species identification

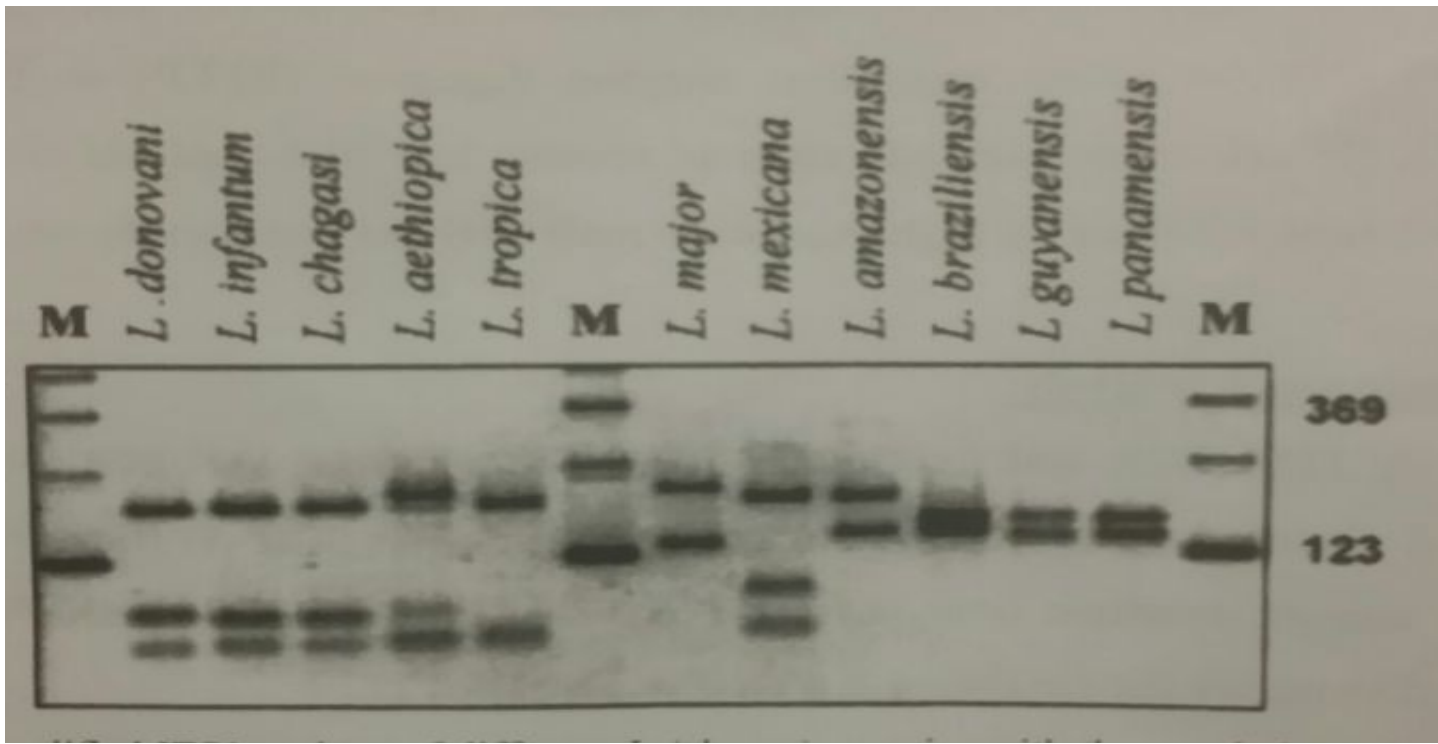


PCR product  
purification  
(Qiagene kit)

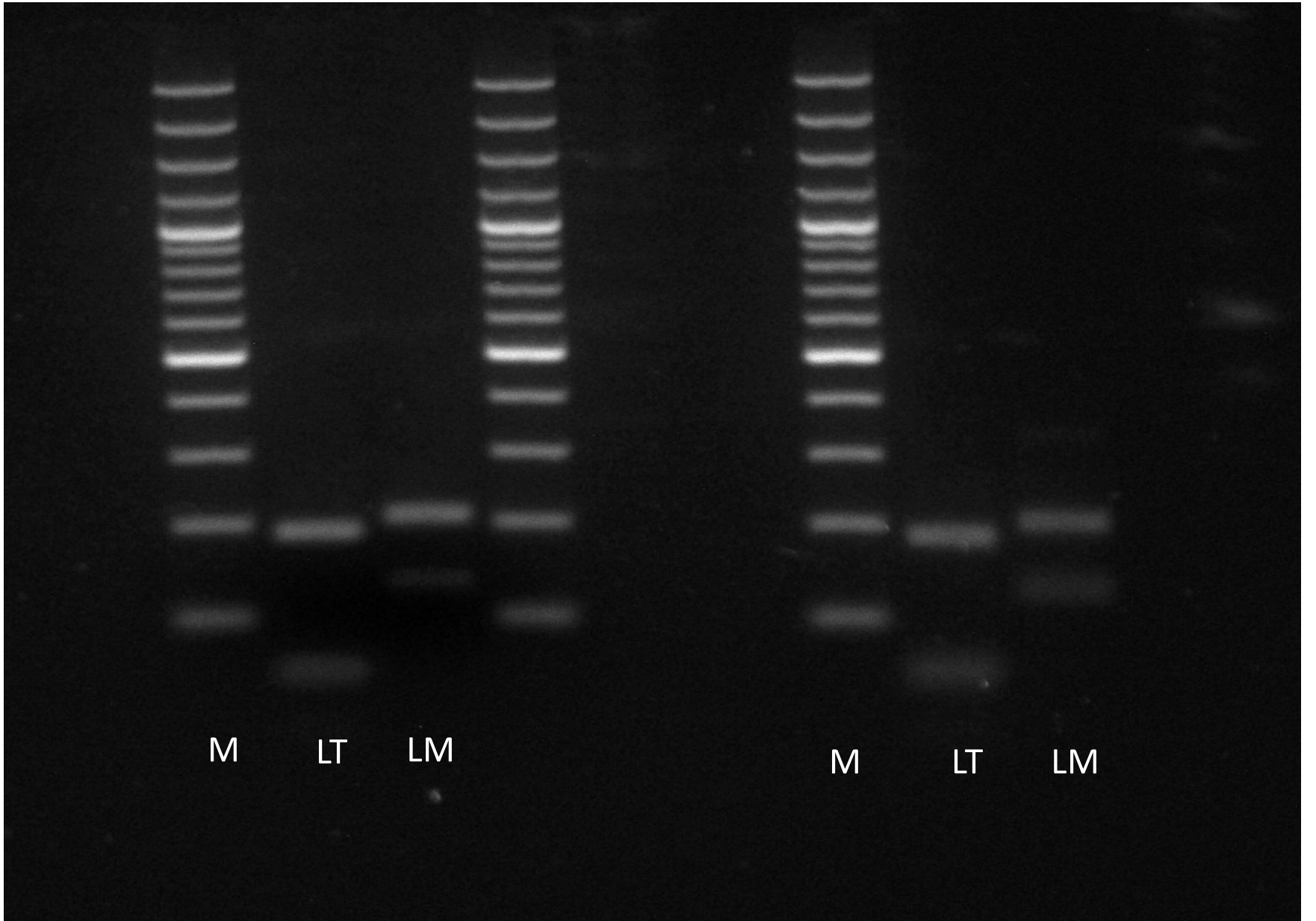


RFLP  
using  
Hae III enzyme





	<i>L. donovani</i>	<i>L. infantum</i>	<i>L. chagasi</i>	<i>L. aethiopia</i>	<i>L. tropica</i>	<i>L. major</i>	<i>L. turnica</i>	<i>L. mexicana</i>	<i>L. amazonensis</i>	<i>L. braziliensis</i>	<i>L. guyanensis</i>	<i>L. panamensis</i>
Band size (bp)				200	185	203	203					
obtained after	146	184	184	57	57	132	57	186	186	156	156	156
digestion with	75	72	72	54	53		53	88	142	143	137	139
<i>HaeIII</i> enzyme	54	55	55	23	24		24	59				





## But there are some disadvantages for this technique

- It does not detect all sequence and length variations within or among amplicons, since the **restriction enzymes** used **only recognise** a small number of potentially variable sites.
- Additionally, **it requires large amount of highly pure DNA**

Thank You!

