PCR amplification of Leishmania ITS-rRNA gene and cyt. b followed by RFLP for species identification

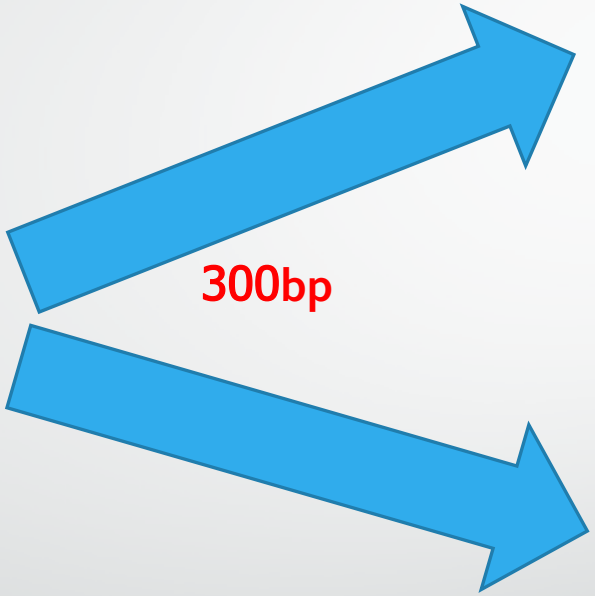
Shany Assaraf & Layla El Moussawi

Background

RFLP

Restriction fragments length polymorphism

A technique that used to distinguish between different species based on the different length of DNA fragments obtained after digestion with specific restriction enzymes



Cyt. B

Encodes cyt b. protein which is conserved among species.

Has a low gene copy number 1-2.

ITS rRNA

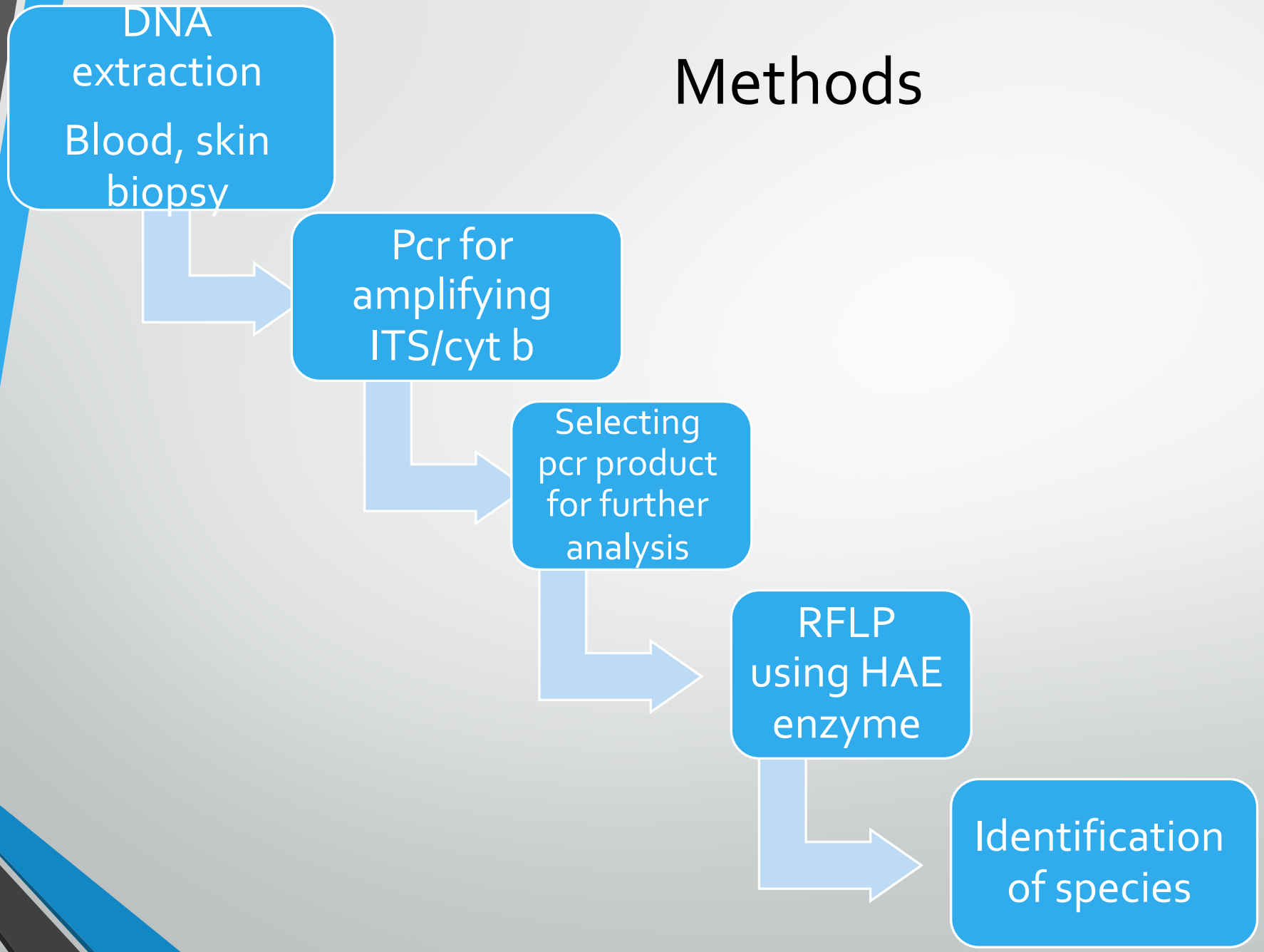
Internal transcribed spacer.

a. Spacer DNA within the rRNA.
b. Conserved among L. species.
c. Has 7-20 gene copy number.

Aim

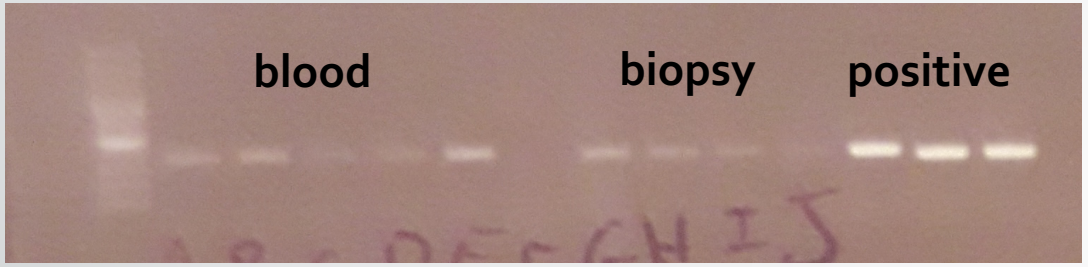
1. Amplifying ITS rRNA gene and cyt b gene
2. Restriction of ITS PCR product and identification of different species of Leishmania.

Methods


DNA extraction
Blood, skin biopsy
Pcr for amplifying ITS/cyt b
Selecting pcr product for further analysis
RFLP using HAE enzyme
Identification of species

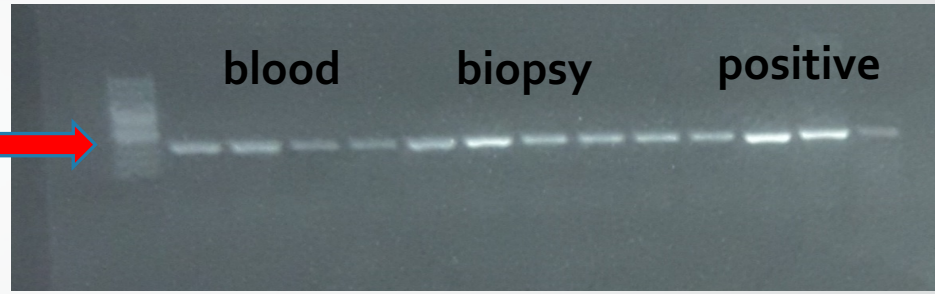
Results

Group 8- ITS



Group 7- ITS

300bp



Conclusions and Future directions

- **Ir**

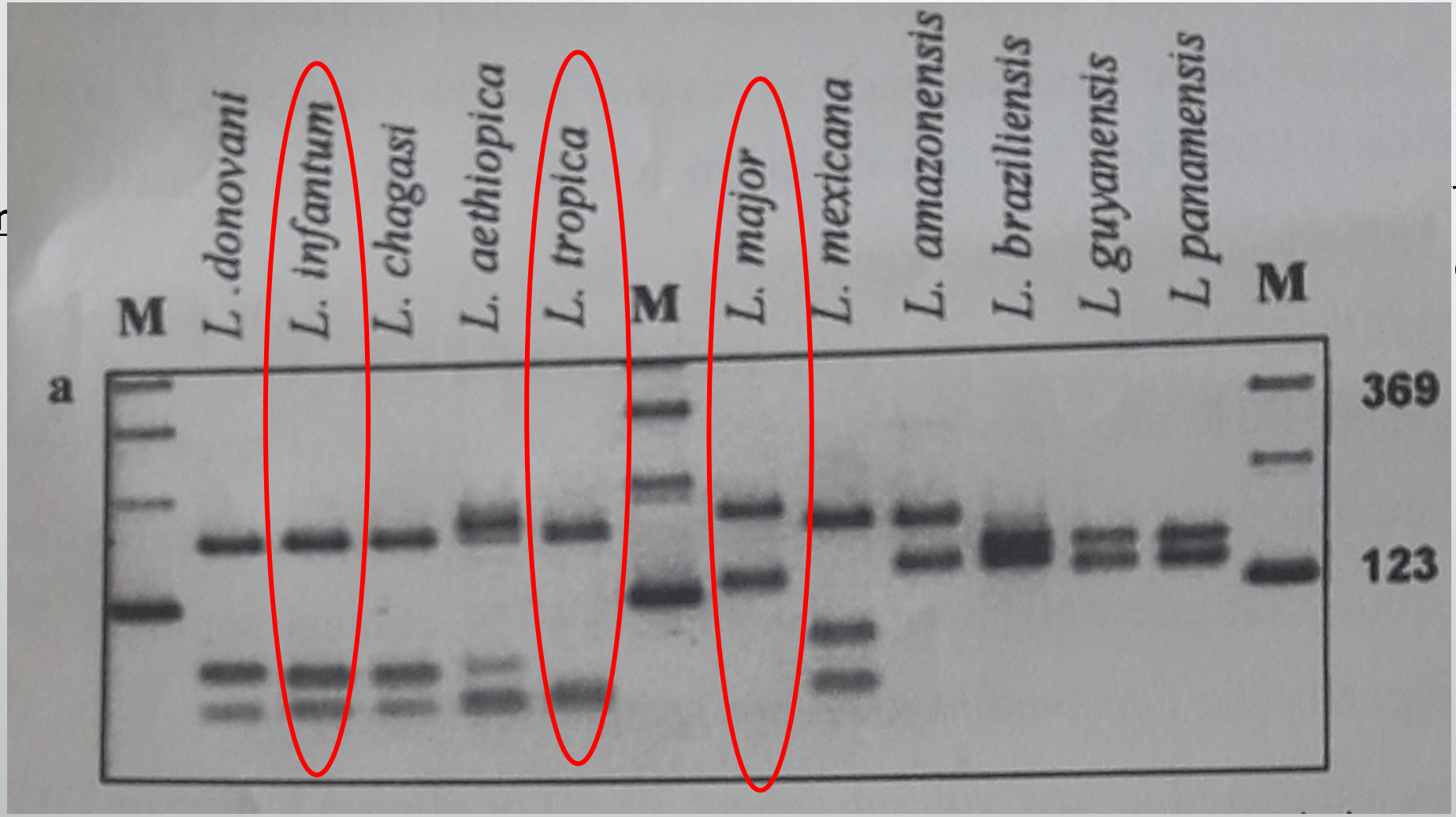
a.

b.

c.

t b.

ddle



Challenging questions:

- Primers' cross reactivity- designing primers against non homologous inter-species sequences.
- Quantification and differentiation between fresh and old blood meal- deep sequencing.
- Drug testing- using different kind of drugs depends on the life cycle of the parasite (adjust concentrations).

Thank you for listening
Questions?