

Identification of *Trypanosoma cruzi* discrete typing units through PCR-RFLP heat-shock protein 70.

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METHODOLOGY

DESIGN

hsp70 T. cruzi sequence alignment (Fraga et al., 2016)

PRIMERS

RESTRICTION ENZYMES

PCR-RFLP
hsp70 DTU

OPTIMIZATION

PCR *hsp70* DTU designed 545bp

MgCl₂, Primers, Taq DNA pol

Melting Temperature

ANALYTICAL EVALUATION

PCR analytical sensitivity

Panel of 6 *T. cruzi* each DTUs (Ten-fold DNA dilutions from 1 ng to 10 fg).

PCR analytical specificity

Panel of *Trypanosoma brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. congolense*, *T. vivax*, *T. equiperdum*, *T. theileri*, *T. evansi*, *Leishmania infantum* (chagasi), *L. mexicana*, *L. braziliensis*, *L. naiffi*, *L. peruviana*, *L. guyanensis*, *L. lainsoni*, *Plasmodium falciparum*. (10 ng)

REFERENCE PANEL EVALUATION

DNA from 25 *T. cruzi* isolates well-typed
Reference strains 10ng

CLINICAL EVALUATION

Clinical samples (blood): 348
Vector samples: 70
Animal Reservoir samples: 26

T. cruzi positive-PCR kDNA (Sturm et al., 1989) and PCR duplex *hsp70* (Fraga et al., 2014)

INTRODUCTION

Trypanosoma cruzi, the causative agent of Chagas disease, presents wide genetic diversity. Currently, six discrete typing units (DTUs), named TcI to TcVI, and a seventh one called TcBat are used for strain typing.

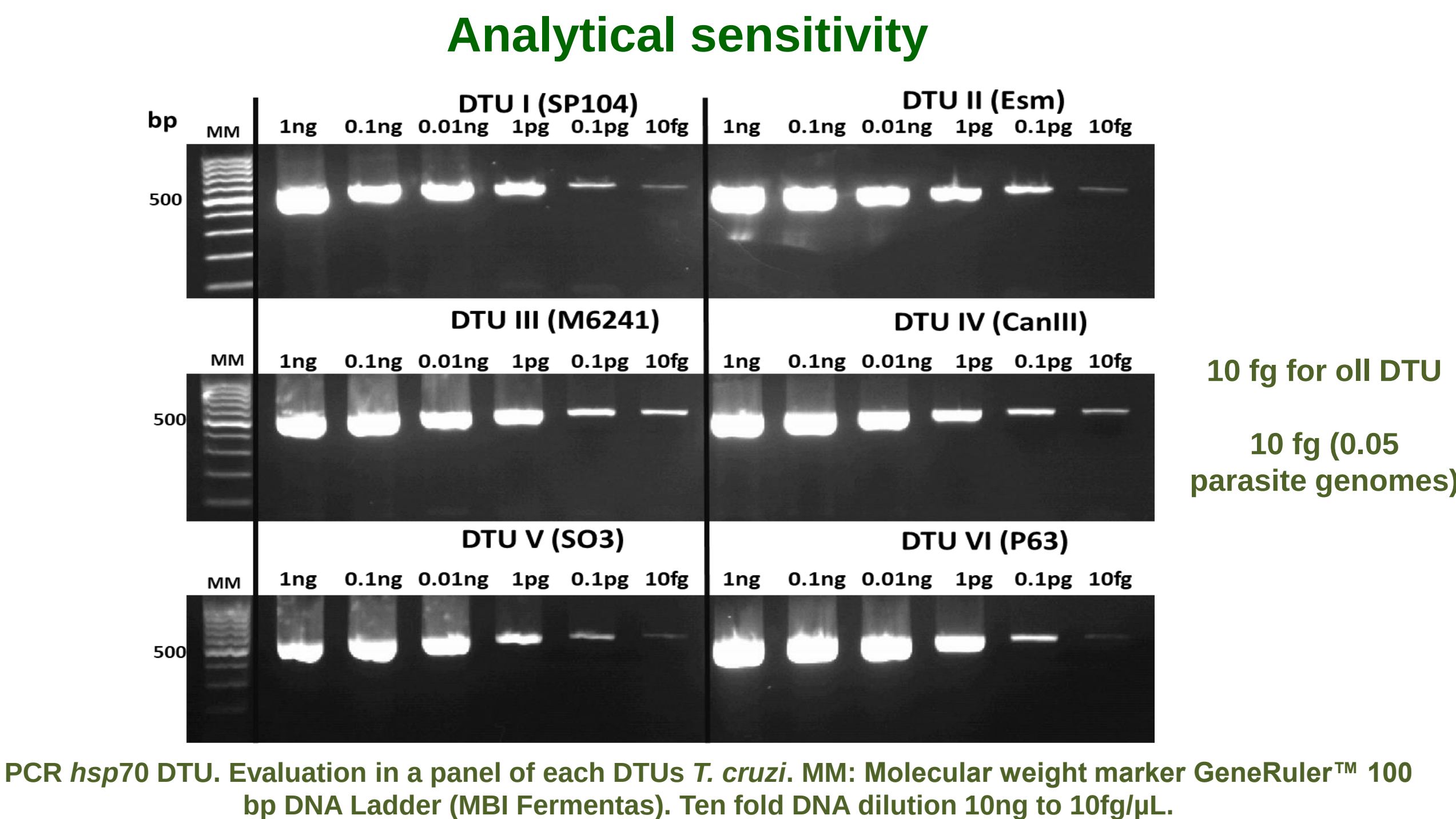
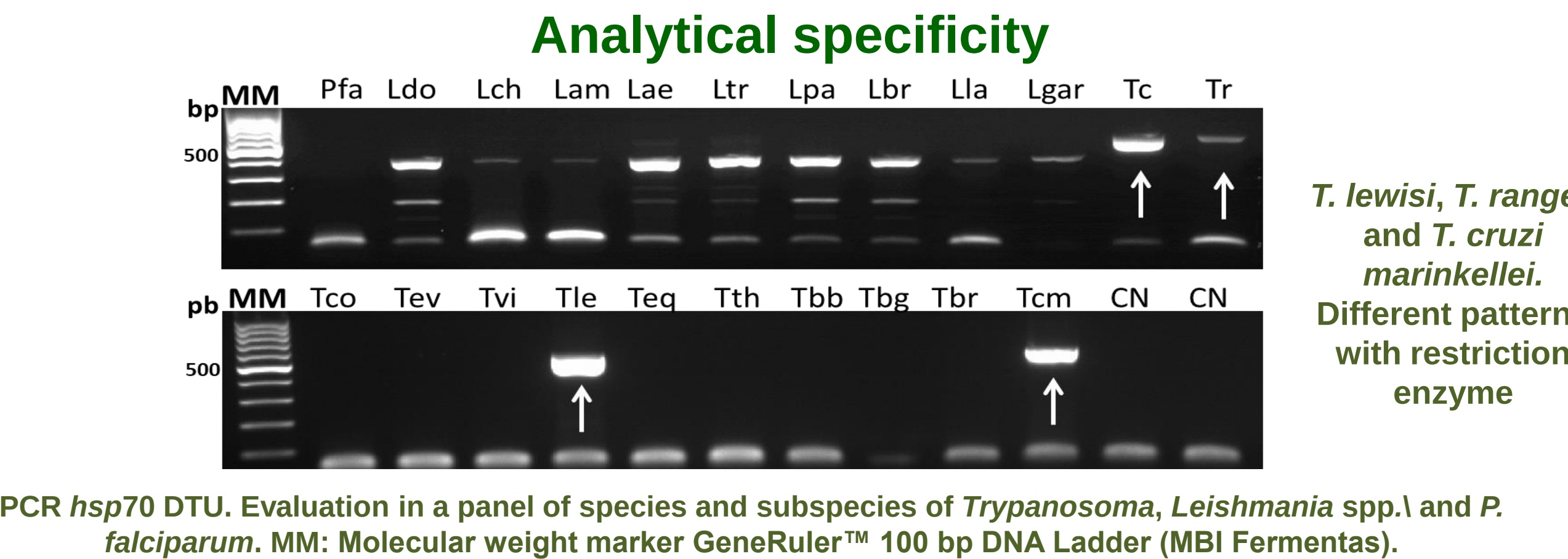
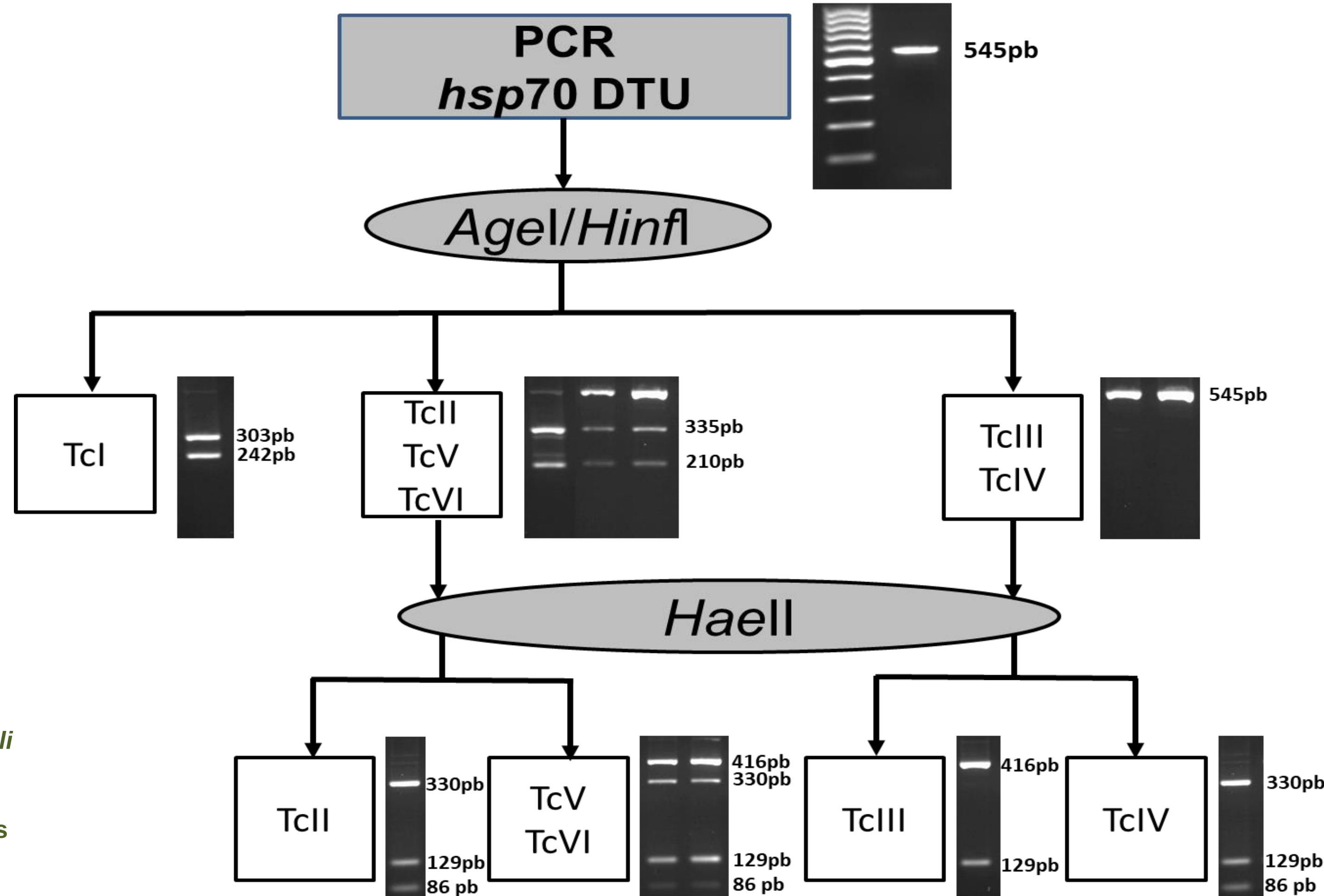
Various reports have shown that the parasite's genetic diversity has a profound impact on its epidemiological, biological, medical characteristics and diagnosis and treatment of the diseases. However, all are still under debate.

There is not consensus on the best method to identify the different DTUs. It is important to have simpler molecular methods and/or simpler and more reproducible strategies for the identification of parasite lineages, which are available for widespread use in endemic areas, manageable in any laboratory and can be applied directly to clinical and biological samples.

The 70 kDa heat-shock protein (HSP70) gene constitutes a promising evolutionary marker, for DTU differentiation. The phylogenetic analysis shown three groups of *T. cruzi* DTUs could be clearly distinguished, corresponding to TcI, TcIII, TcIV and TcII + V + VI.

OBJECTIVE: To exploit the cytoplasmic *hsp70* gene for detection and genotyping of *T. cruzi* DTUs through the design and evaluation of a PCR-RFLP.

RESULTS



PCR-RFLP *hsp70* DTU flowchart for identification of *Trypanosoma cruzi* DTUs

PCR-RFLP *hsp70* DTU genotyping algorithm validation with biological samples.

No samples	PCR-RFLP hsp70 DTU positive	Typing %	DTUs
Human clinical samples (blood) n=348			
Colombia n= 121	34	38,8%	25 TcI 2 TcII 2 TcIII 2 TcIV 2 TcV o VI 1 Mixed (TcI-TcIV)
Bolivia n=38	25		17 TcV-VI 5 TcI 2 TcII 1 Mixed(TcI-TcV-VI)
Belice n=1	1		1 TcI
Cuba n=1	1		1 TcI
Argentina n=2	1		1 TcV o VI
Mexico n= 185	73		64 TcI 2 Mixed TcI-TCII 3 MixedTcI- TcIV 1 TcIII 1TcIV 1 Mixed TcI-TcIII 1 Mixed TcI- TcV o TcVI
Triatomineos n= 70			
Mexico n=51	30	65,7%	24 TcI 6 Mixed (TcI-TcIV)
Colombia n=19	16		10 TcI 2 TcII/TcV/TcVI 1 TcIII 1 TcIV 2 Mixed (TcI-TcII/TcV/TcVI)
Reservoirs n=26			
Colombia n=26	12	46.2 %	10 TcI 1 TcIV 1 Mixed (TcI-TcIV)
Typing % n=444	55,5 %		

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• The cytoplasmic *hsp70* gene constitutes a genetic target that allows the differentiation of *T. cruzi* DTUs.

• The PCR *hsp70* DTU allows direct detection in clinical samples with high analytical sensitivity and specificity.

• The PCR-RFLP *hsp70* DTUs allows direct typing in clinical samples, triatomineos and animal reservoirs (around 50%) of the main DTUs of the parasite, although it requires combination with another molecular method to differentiate DTU V and VI.

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