

Welcome!

The dynamics of rRNA pseudouridylation across *Leishmania donovani* life cycle and adaptation

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Bar-Ilan University, May 2021**

Introduction

- Background
- SnoRNA Modifications
- How Can We Detect Modifications



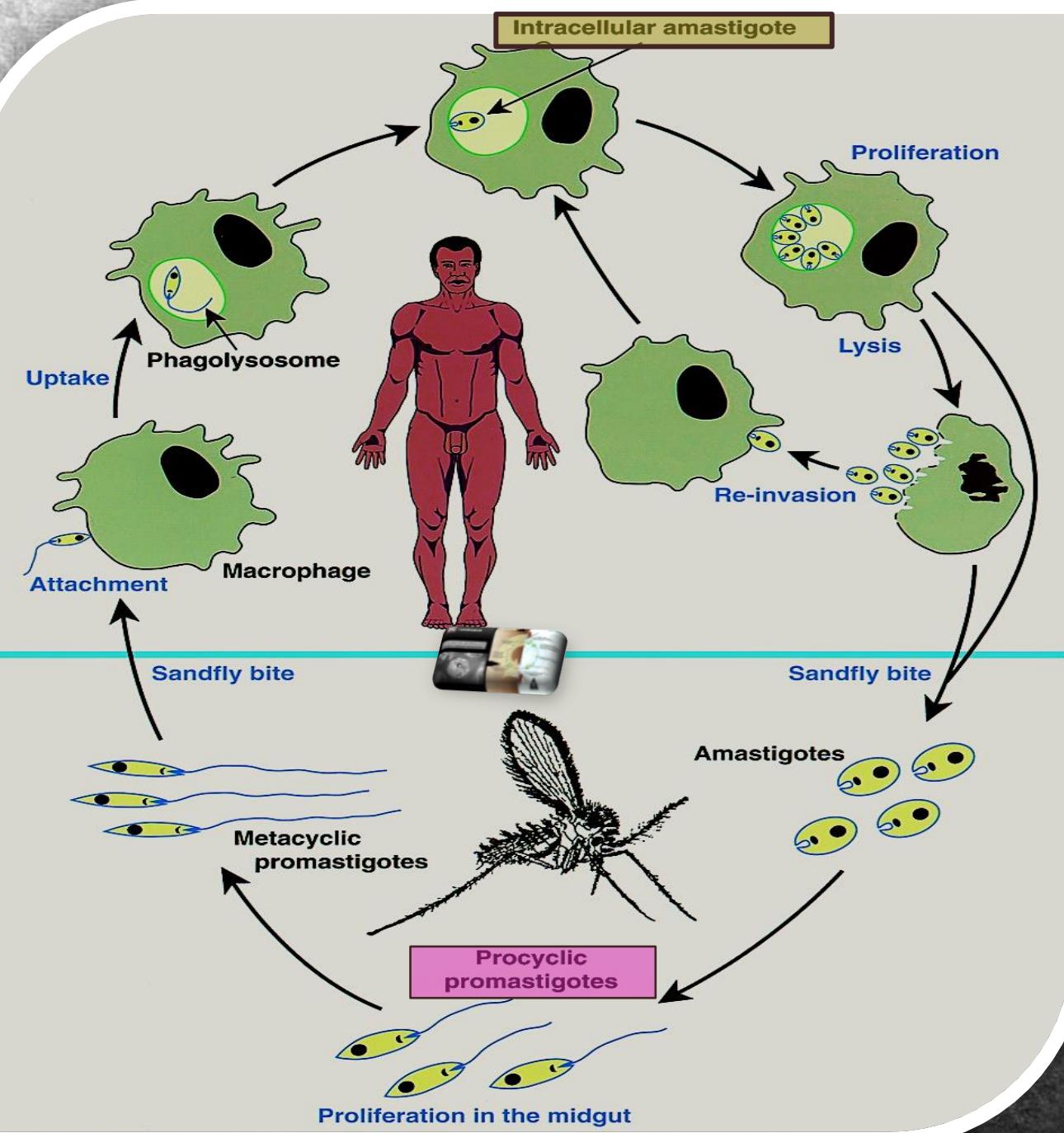
Background

- Trypanosomatids are *protozoan parasites* that cause infectious diseases:
 - Sleeping sickness (T.b)
 - Leishmaniasis (*L. donovani*; *L.d*, *L. major*; *L.m*)
- Leishmaniasis is a serious parasitic diseases worldwide:
 - Two million cases annually
 - 500,000 cases of visceral *Leishmaniasis* (VL) - caused by *L. donovani* (*L.d*)



Leishmania & Leishmaniasis





Leishmania & Leishmaniasis



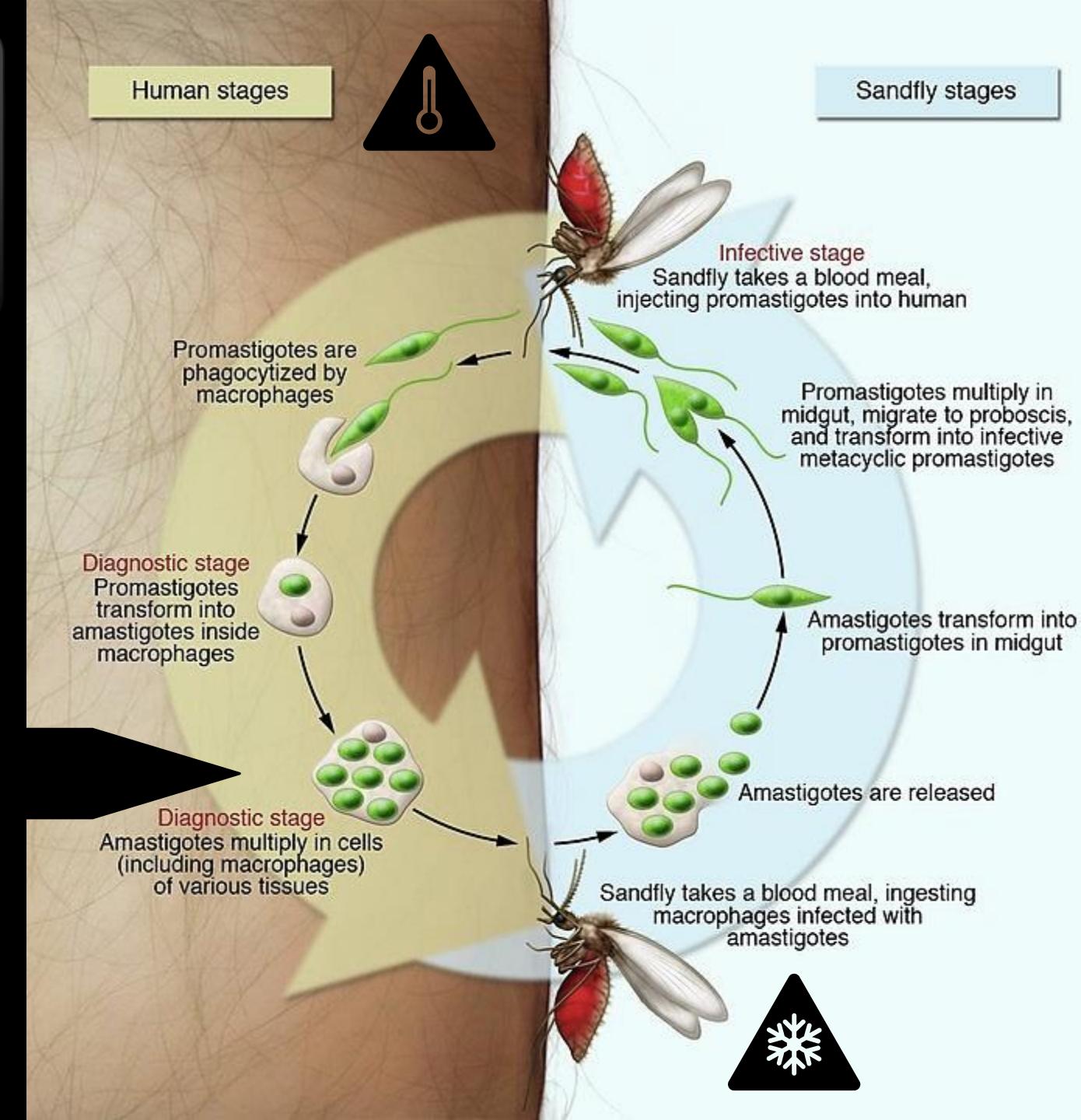
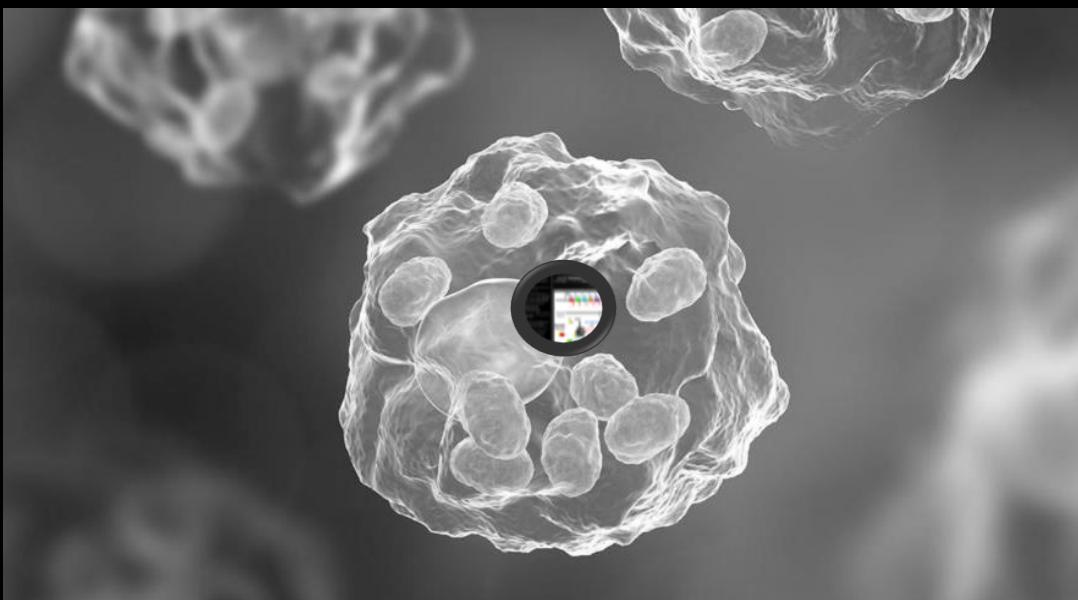
Leishmania cycles between 2 hosts:

1. The phagolysosomes of mammalian macrophages
2. The mid-gut of sand flies



Leishmania

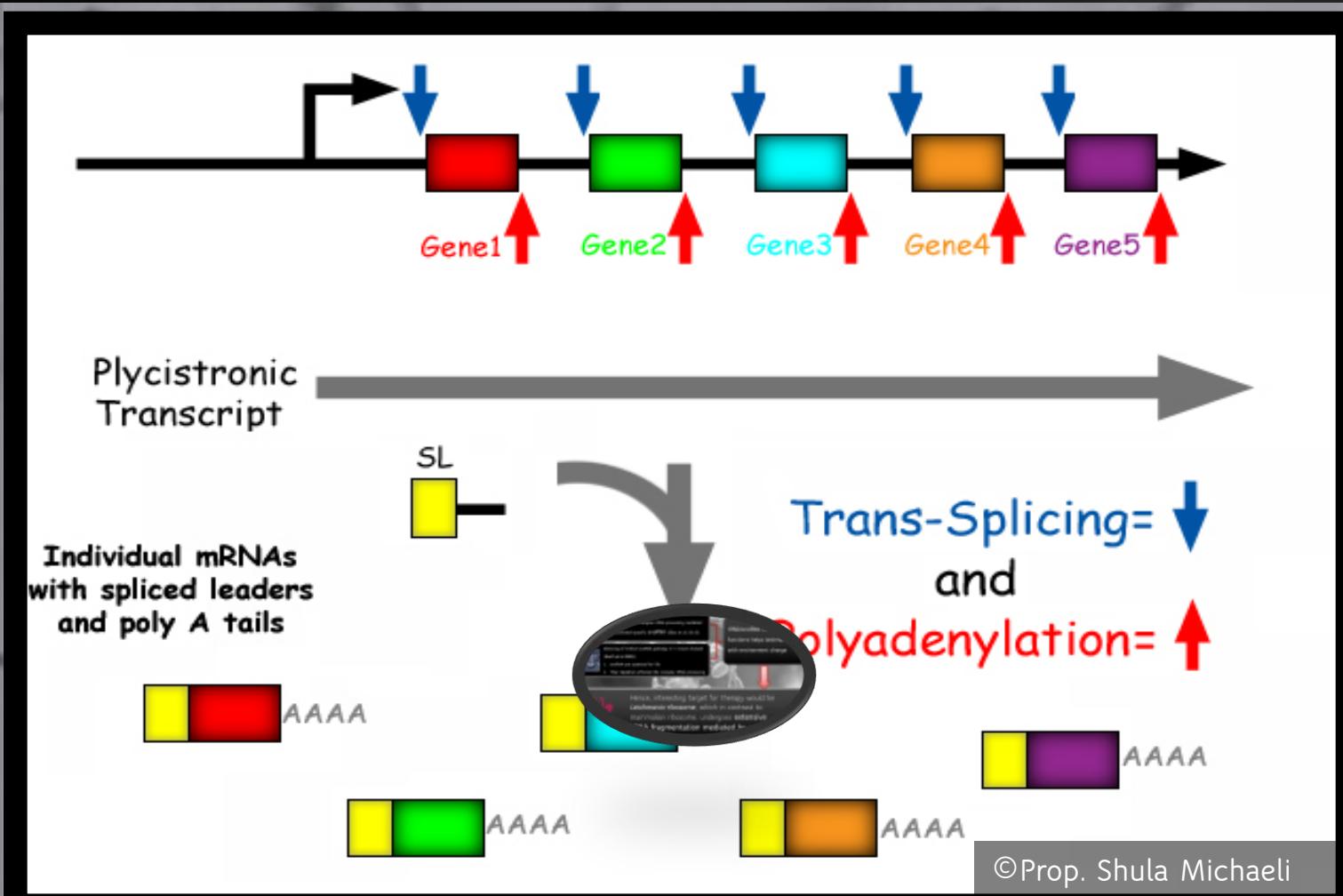
During their life, Leishmania cycle between 2 distinct environments



Leishmania:

- An important model to study post-transcriptional regulation, RNA splicing and RNA editing
- Genes arranged in polycistronic transcription units
- Mature mRNAs generated from precursors via a highly **parasite-specific trans-splicing process**
- Regulates gene expression mainly at the post-transcriptional level
- Possess special RNA processing mechanisms
- In contrast to most eukaryotes, Leishmania's LSU rRNA is **fragmented** into several pieces
 - This unique processing requires trypanosome-specific factors

Unique Processing Mechanisms



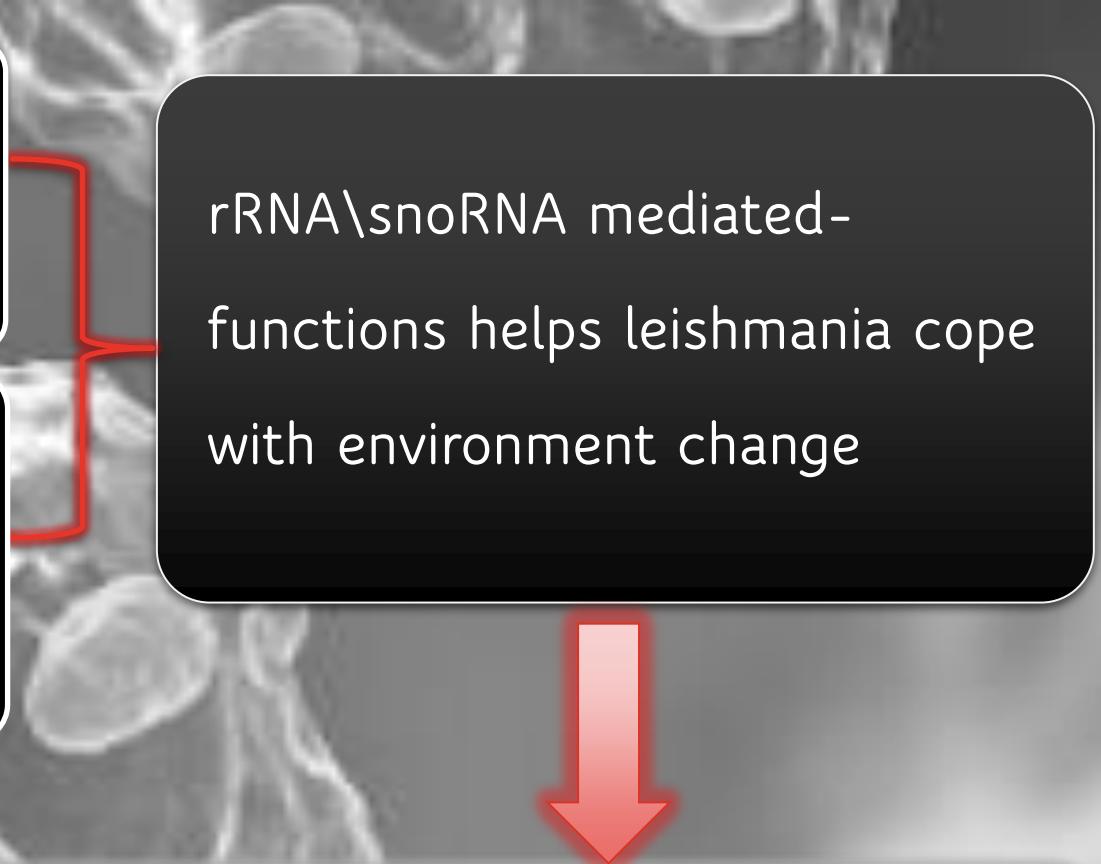


Leishmania ribosome undergoes rRNA processing mediated by trypanosomatid-specific snoRNA (Eliaz et al 2015)



Silencing of H/ACA snoRNA pathway in *T. brucei* showed (Barth et al 2005) :

1. snoRNA are essential for life
2. Their depletion affected the complex rRNA processing



Hence, interesting target for therapy would be *Leishmania* ribosome, which in contrast to mammalian ribosome, undergoes **extensive rRNA fragmentation mediated by snoRNAs**

SnoRNA Modifications



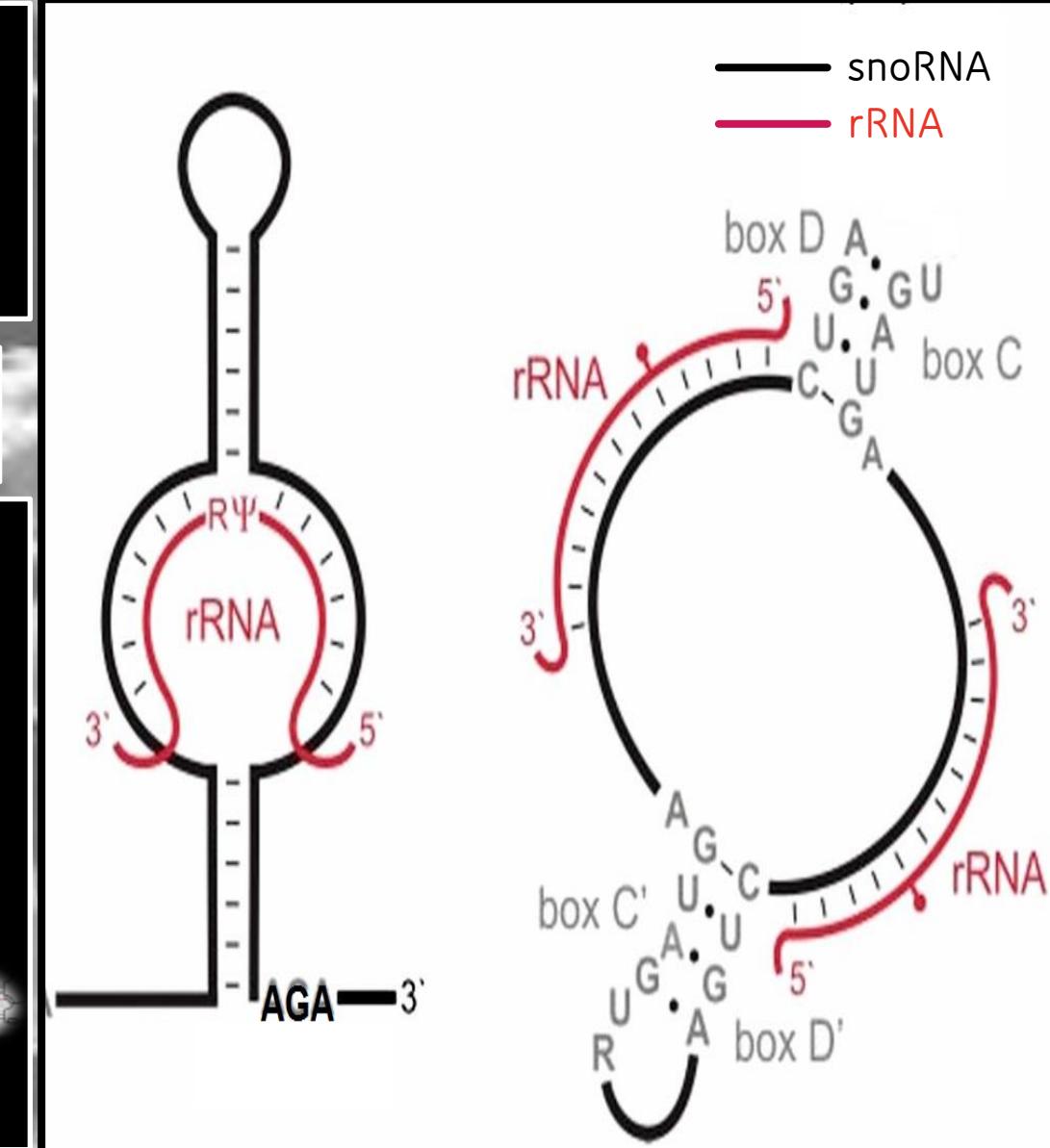
SnoRNA

Small nucleolar RNAs:

- A class of small RNA molecules
- Guide chemical modifications of other RNAs (mainly rRNA, tRNA, snRNA)

Two main classes of snoRNA:

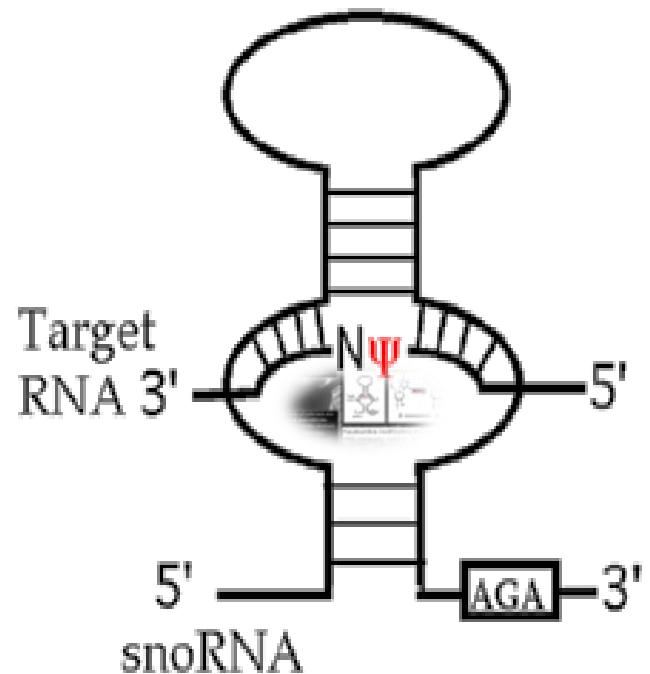
- C/D box snoRNA:
 - Methylation
 - NOP1
- H/ACA box:
 - Pseudouridylation
 - CBF5



SnoRNA H/ACA Modification

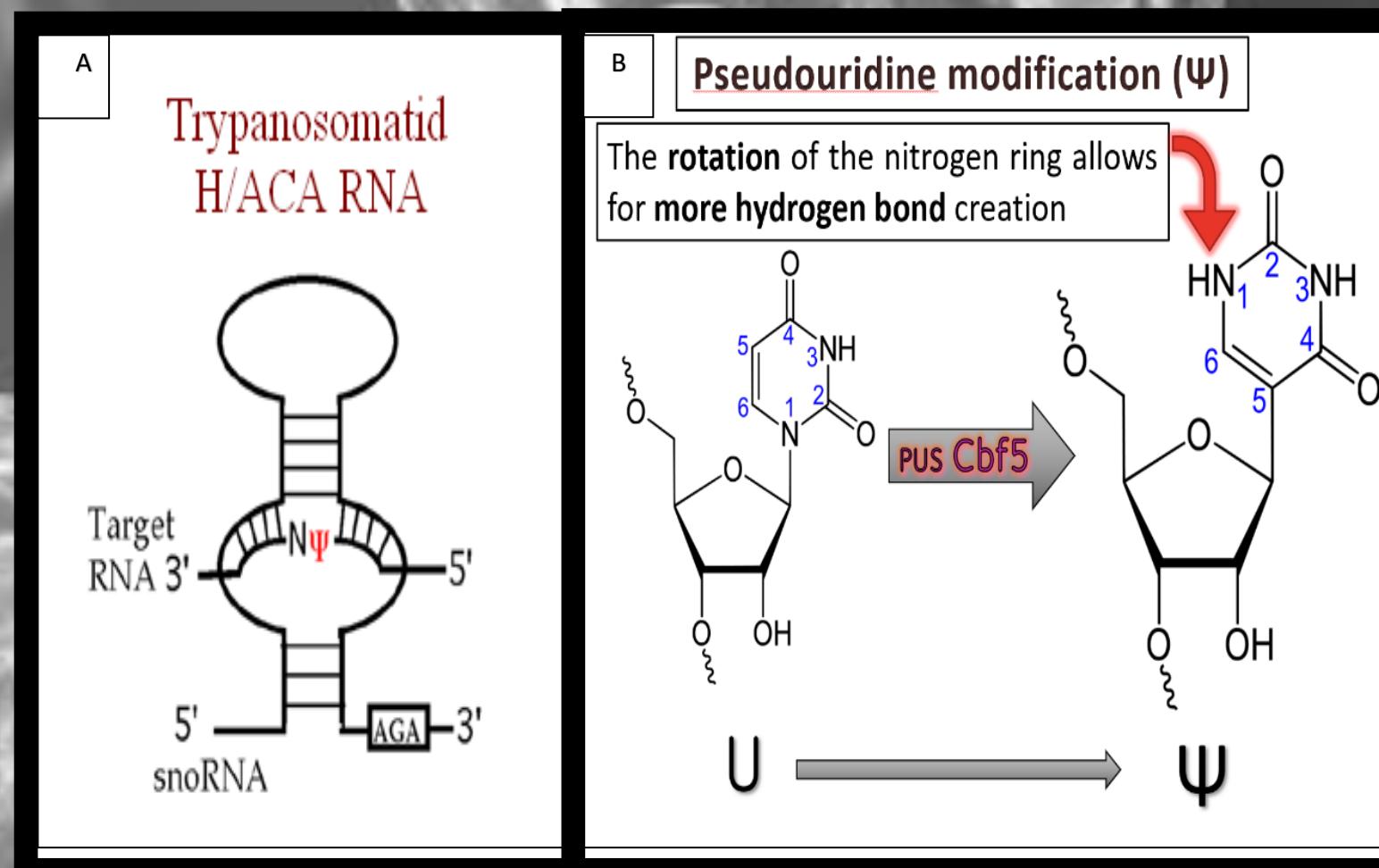
- Involved in converting **uridine to pseudouridine** on rRNA
 - Marking complimentary rRNA sequence for enzyme recognition
- Trypanosome H/ACA molecules (termed “H/ACA-like”) lack features that characterize H/ACA molecules in most other organisms (e.g., one stem and loop, AGA)

Trypanosomatid H/ACA RNA



Pseudouridine Modification

- A. Illustration of leishmania one-stem-and-loop H/ACA like snoRNA (secondary structure)
- B. Chemical transformation applied by PUS Cbf5 enzyme on its target (the marked in red Uridine in A)



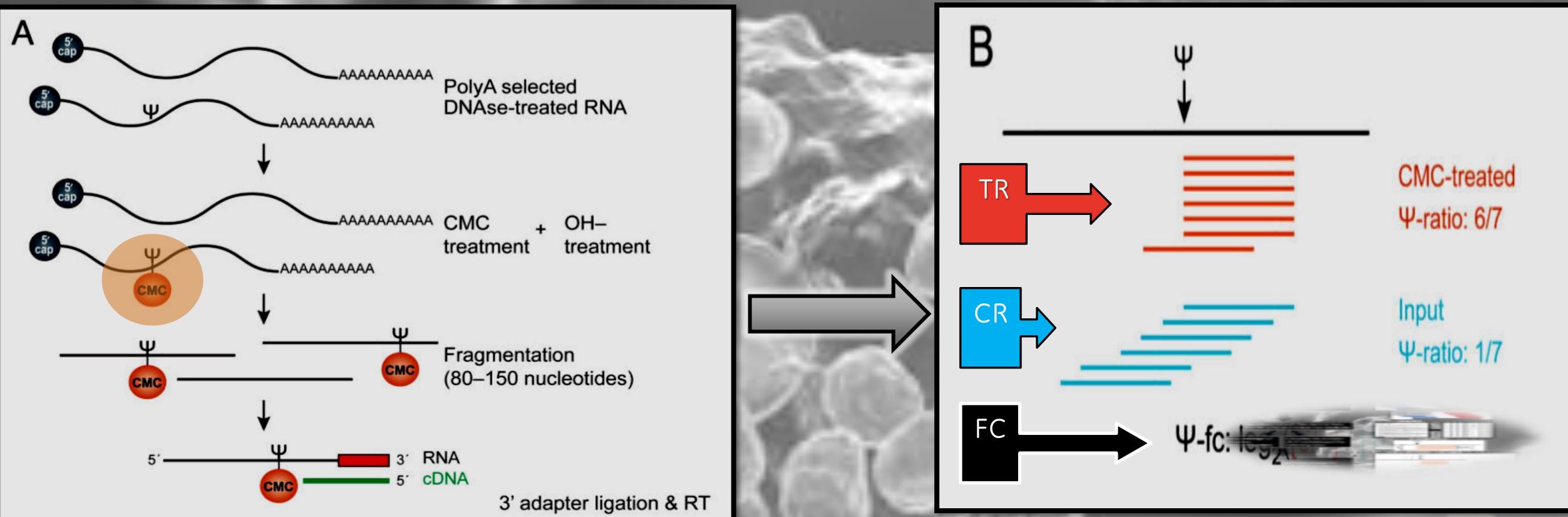
Pseudouridine modifications are crucial for rRNA processing, stability, and maturation



How Can We Detect Modifications

Method to Detect Modifications

Ψ -seq quantitatively measured transcriptome-wide pseudouridylation profiles



TR = Ψ -ratio of the CMC-treated sample (red, top) between reads terminating at a site & reads overlapping it

FC = Ψ -fc (black, bottom), the log 2-fold change of Ψ -ratio in the CMC-treated sample over non-treated control

Alignment: Align to paired-end sequences to genome or to rRNA using **smalt**



Data configuration: Convert files, Sam to Bam, then into a bed file using bamToBed from **bedtools**



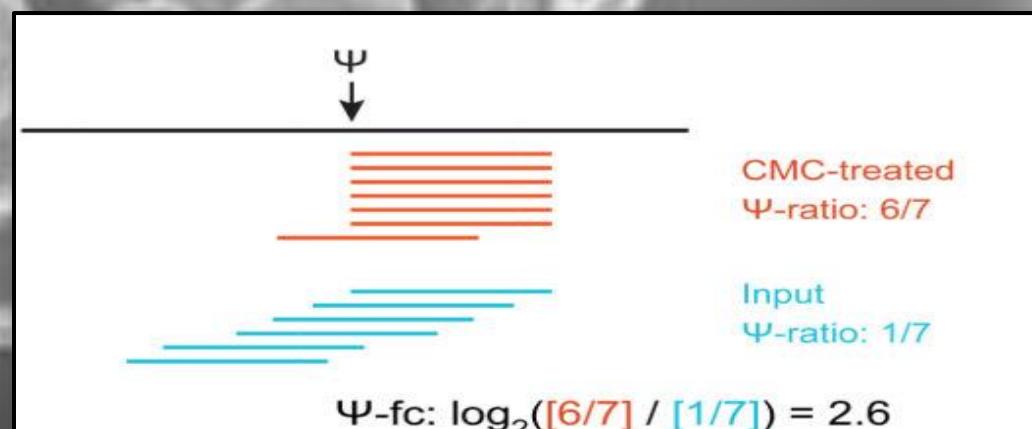
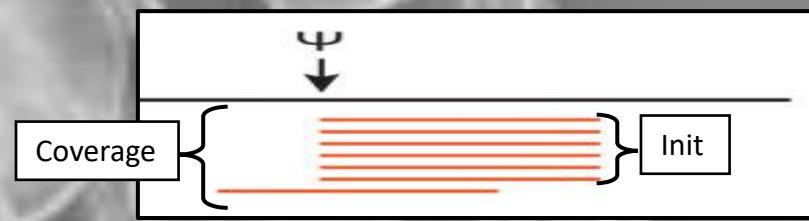
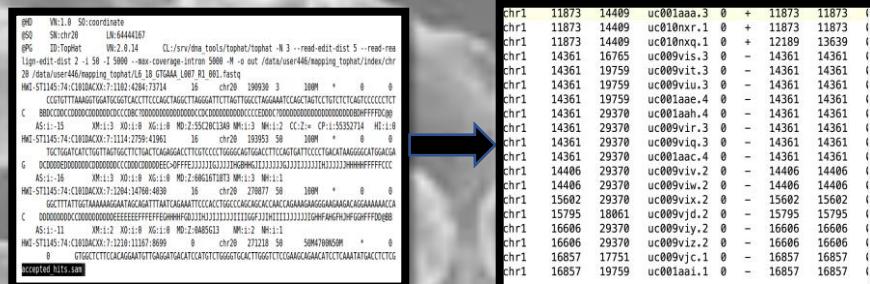
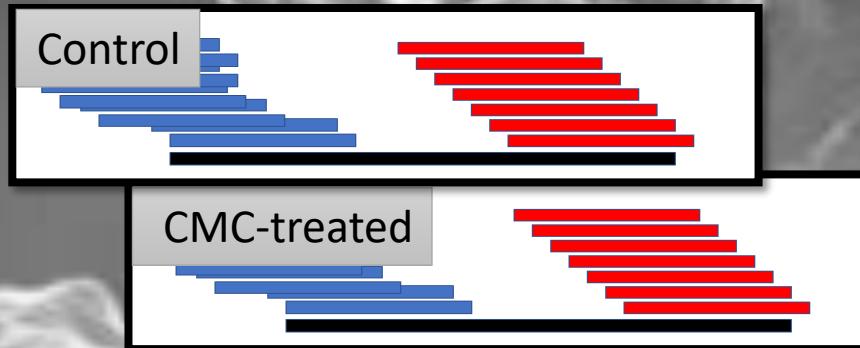
Calculate **coverage** of each nt using **genomeCoverageBed**

Calculate the number of nt's initiating at the following base



Ψ -fc calculation:

1. Calculate **pseudo ratio** and **fold-change** for each nt (= All the reads terminating at that nt / coverage number of reads of that nt).
2. Base 2 Log of the ratio (CMC-treated / control).



Research

- Research Goals
- Results
 - 1. Identification and Comparison of *L. major* snoRNAs in *L. donovani*
 - 2. Detection of Modifications
 - 3. The Dynamics of rRNA Pseudouridylation Across *L. donovani* Life Cycle and Adaptation

Research Goals

Main Goals

- A great deal of research and bioinformatical work was done to study a Leishmanial sub-specie; L. major.
- Relying on previous knowledge, we aimed to:



1. Identification of *L. donovani* snoRNAs (comparison with L. Major)
2. Identification of H/ACA-snoRNA modification sites (Ψ) in *L. donovani* - based on analysis of the results of CMC experiments and comparison with *L. major* sites
3. The Dynamics of rRNA Pseudouridylation Across *L. donovani* Life Cycle and Adaptation
 - Identification of differential modifications between the two life stages
 - Identification of differential modifications during artificial evolution of *L. donovani*
 - Testing the validity of Axenic form of amastigote-life-stage as a research model

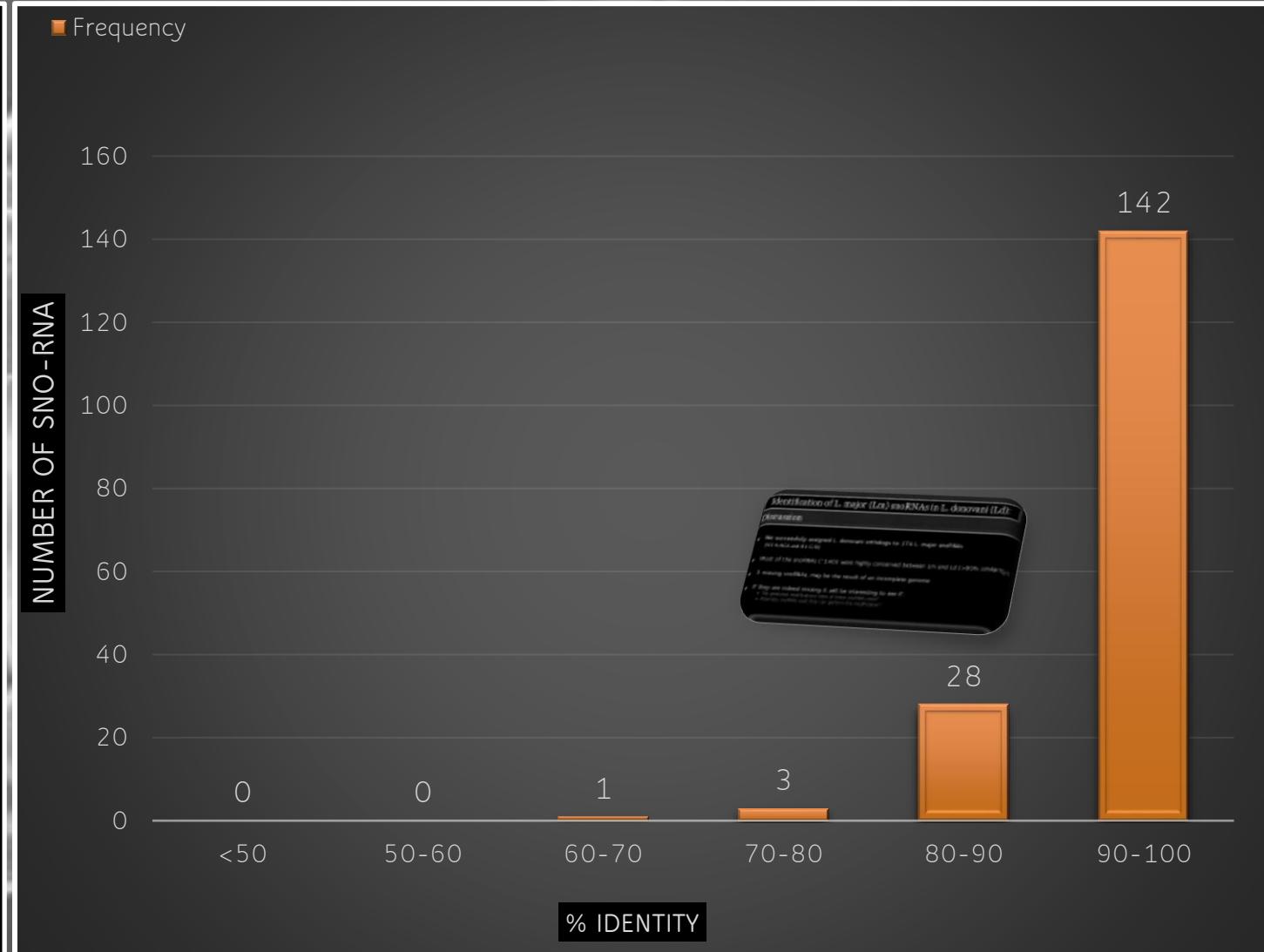
Results

Results

1. Identification and Comparison of
L. major snoRNAs in *L. donovani*

Identification of L. major (Lm) snoRNAs in L. donovani (Ld)

- * 177 known snoRNAs in L. major, 95 H/ACA and 82 C/D (Eliaz et al 2015)
- * L. donovani genome: LdBPK282 reference genome (Dumetz et al., 2017)
- * BLASTn to compare L. major snoRNAs sequences with L. donovani genome sequence
 - * Requiring:
 - * 95% query coverage
 - * e-value <= to 1e-10



Identification of *L. major* (Lm) snoRNAs in *L. donovani* (Ld):

Discussion

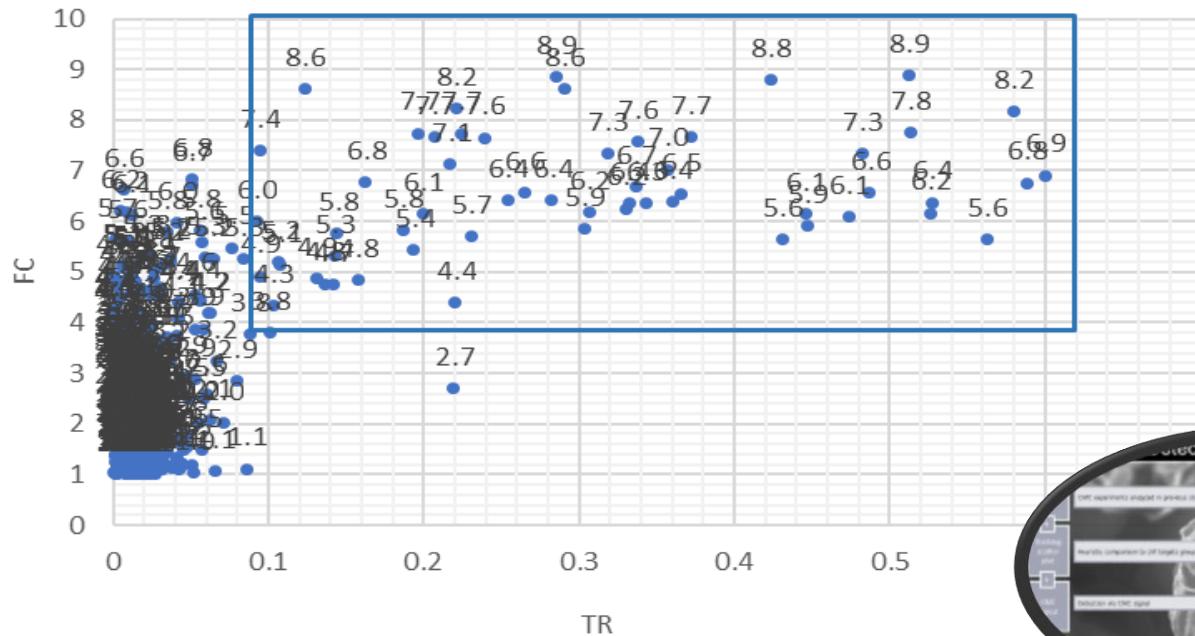
- * We successfully assigned *L. donovani* orthologs to 174 *L. major* snoRNAs (93 H/ACA and 81 C/D)
- * Most of the snoRNAs (~140) were highly conserved between Lm and Ld (>90% similarity)
- * 3 missing snoRNAs, may be the result of an incomplete genome
- * If they are indeed missing it will be interesting to see:
 - The predicted modifications-sites of these snoRNAs exist?
 - Alternate snoRNAs exist that can perform the modification?



Results

2. Detection of Modifications

FC vs TR



TR = Ψ -ratio of the CMC-treated sample (red, top) between reads terminating at a site & reads overlapping it

FC = Ψ -fc (black, bottom), the log 2-fold change of Ψ -ratio in the CMC-treated sample over non-treated control

Detection of Modifications

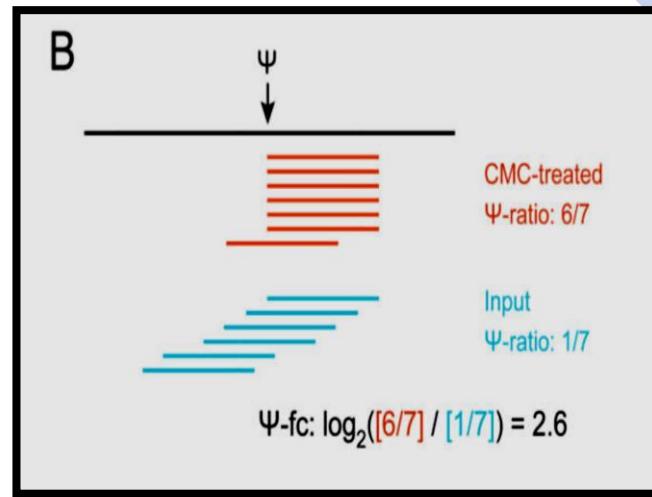
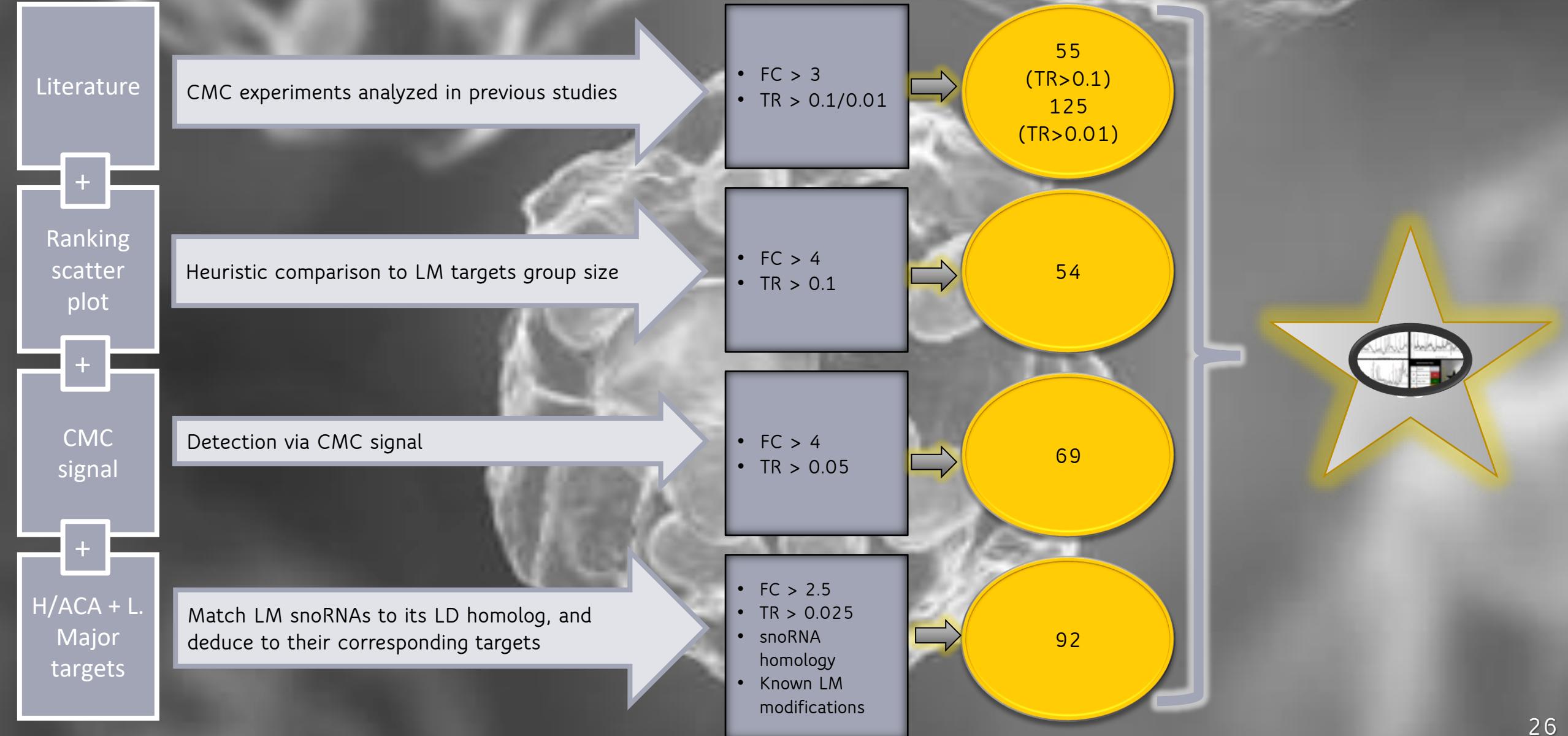
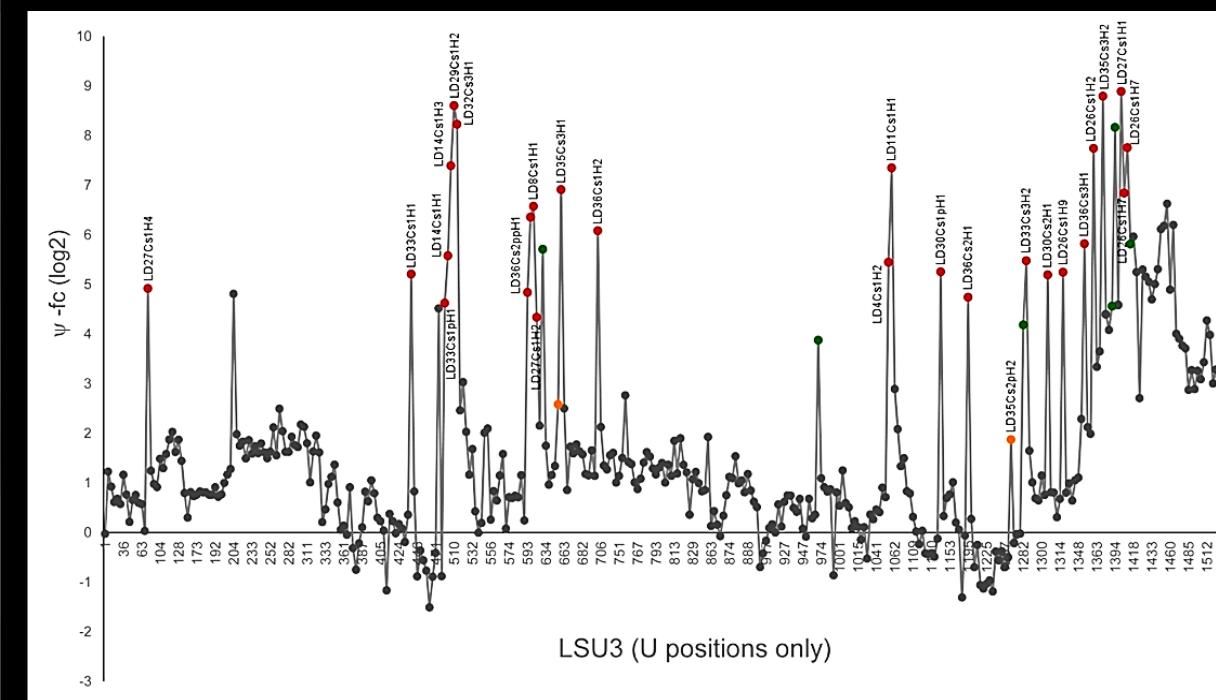
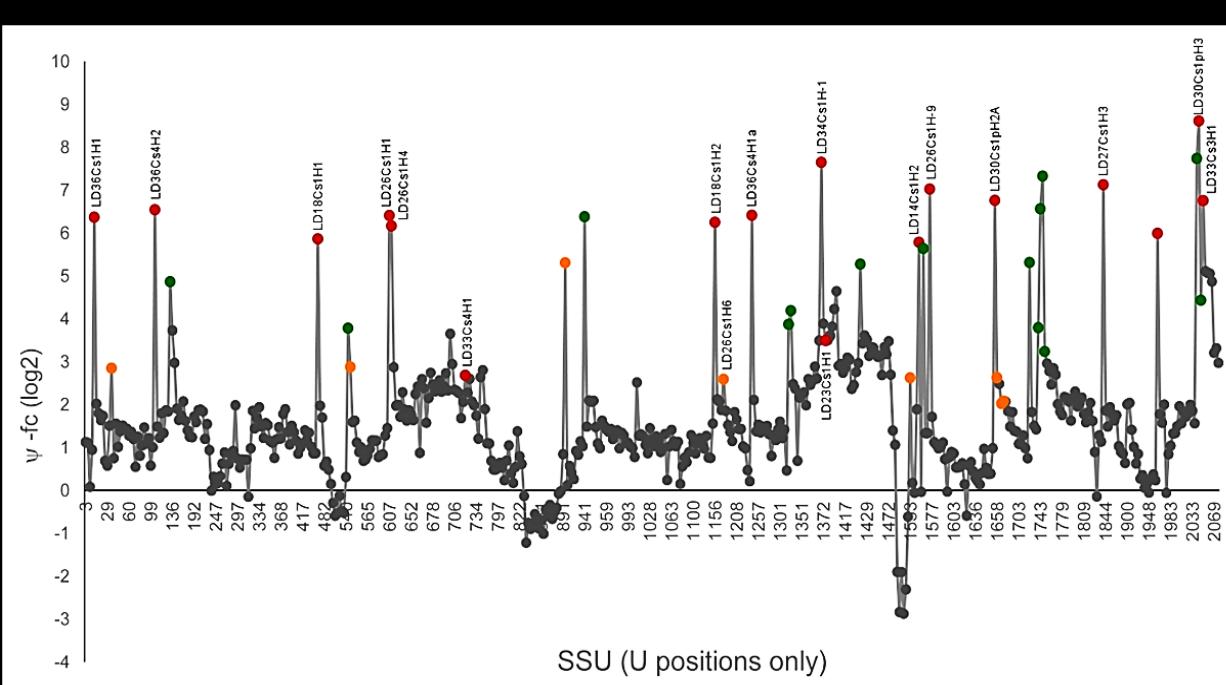
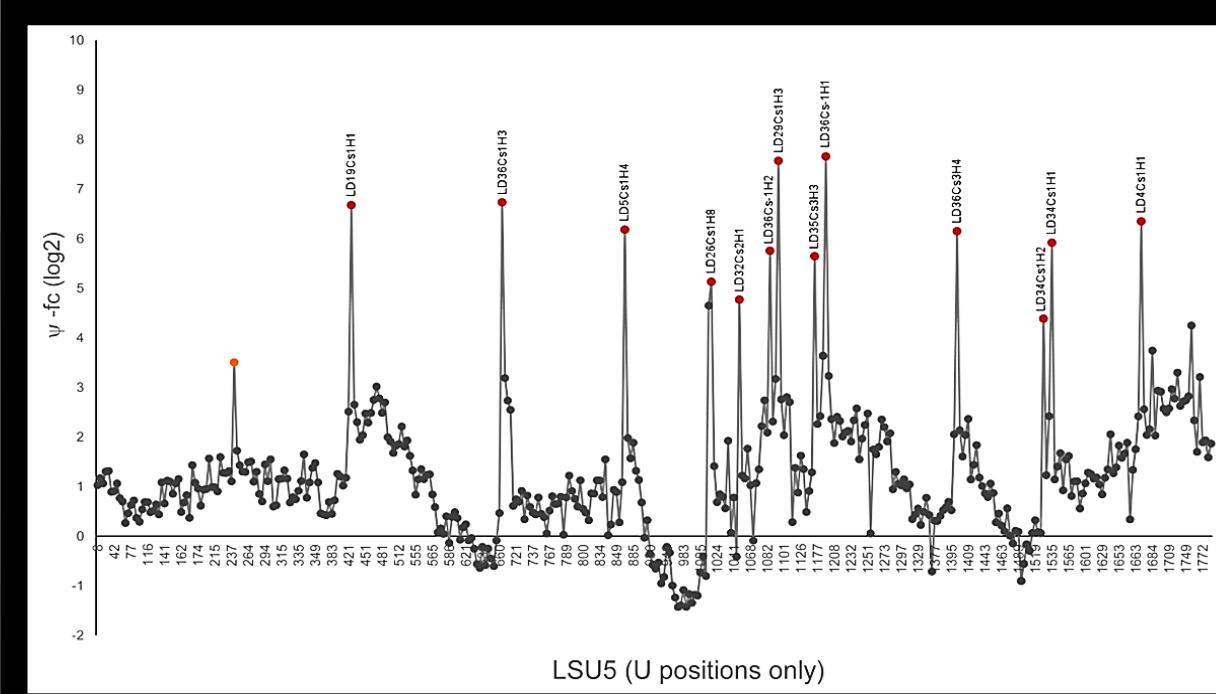


Table 2. The results for the CMC psi-seq analysis

TR	N (Ψ)	L. major known Ψ sites (out of 64)	3		N (Ψ)	L. major known Ψ sites (out of 64)	4	
			High indication list (out of 54)	High indication list (out of 54)			High indication list (out of 54)	High indication list (out of 54)
0.1	55	41	43	43	54	41	43	43
0.075	59	44	47	47	59	44	47	47
0.05	74	48	51	51	69	48	51	51
0.025	97	48	54	54	83	50	53	53
0.01	125	52	54	54	97	51	53	53

Detection of Modifications





MODIFICATION TABLE

	Decision	n
	Bone Fida Sites	58
	Likely Sites	21
	Possible Sites	11

**90
Sites**



Results

3. The Dynamics of rRNA Pseudouridylation Across *L. donovani* Life Cycle and Adaptation

We will examine 3 types of comparison

Comparison of developmental life stages

- We examined the relative difference in FC measures (value of the ratio: “population 1” FC/ “population 2” FC)
- A position with a ratio of 1.3 or higher is considered as a significant difference and is termed “Hyper-Modification”

Promastigote Vs Amastigote

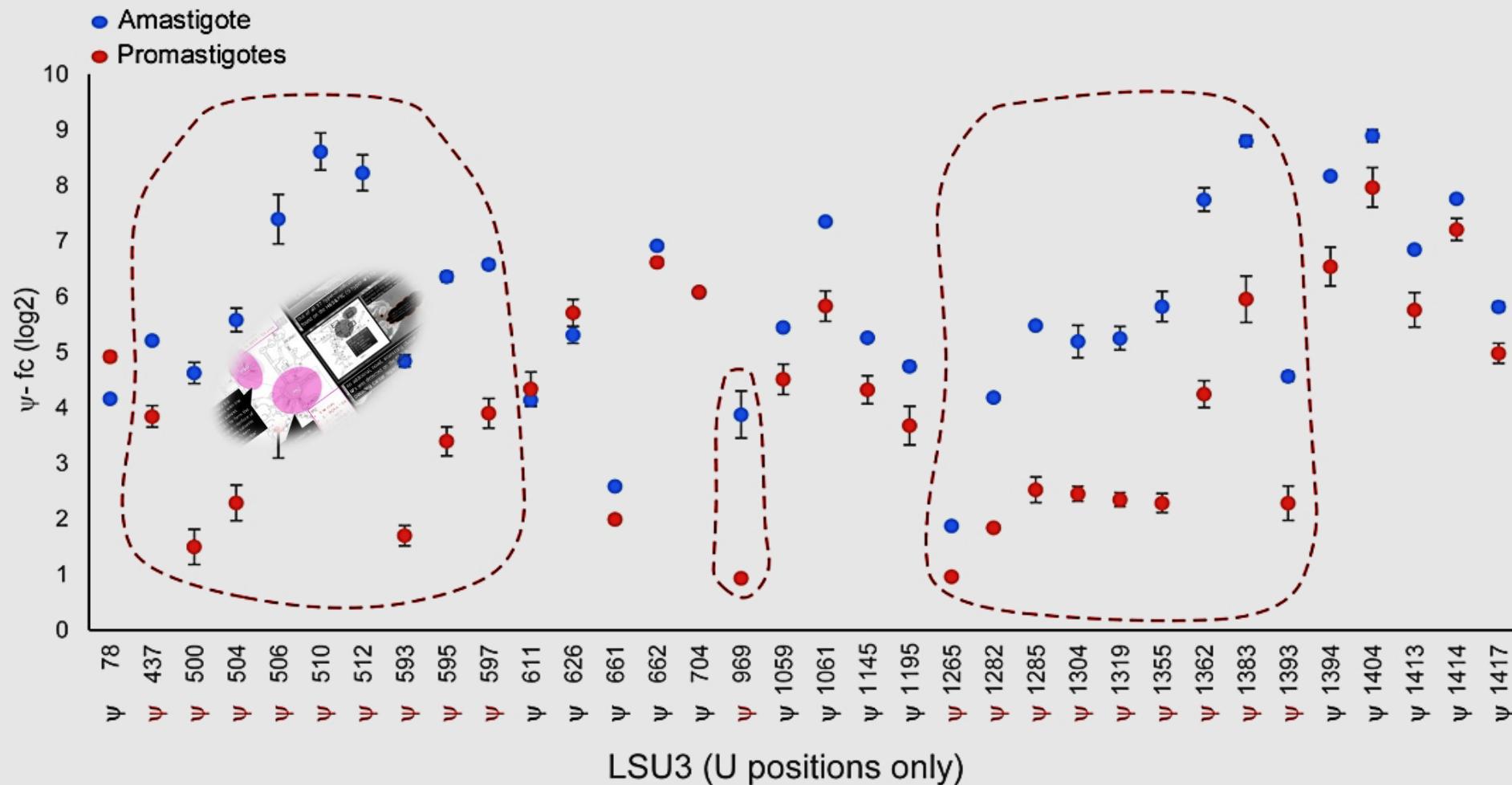
We examine the dynamics of pseudouridinylation during the life cycle of *L. donovani*:

- Ψ -seq mapping on rRNA enables inspection of detected Ψ -position for a difference in Ψ signal (FC) between *L. donovani* distinct life stages.
- Noticeable difference in specific positions, that are important functional ribosome loci, support the notion that in *Leishmania*, hyper Ψ 's on rRNA contribute to environmental adaptation.

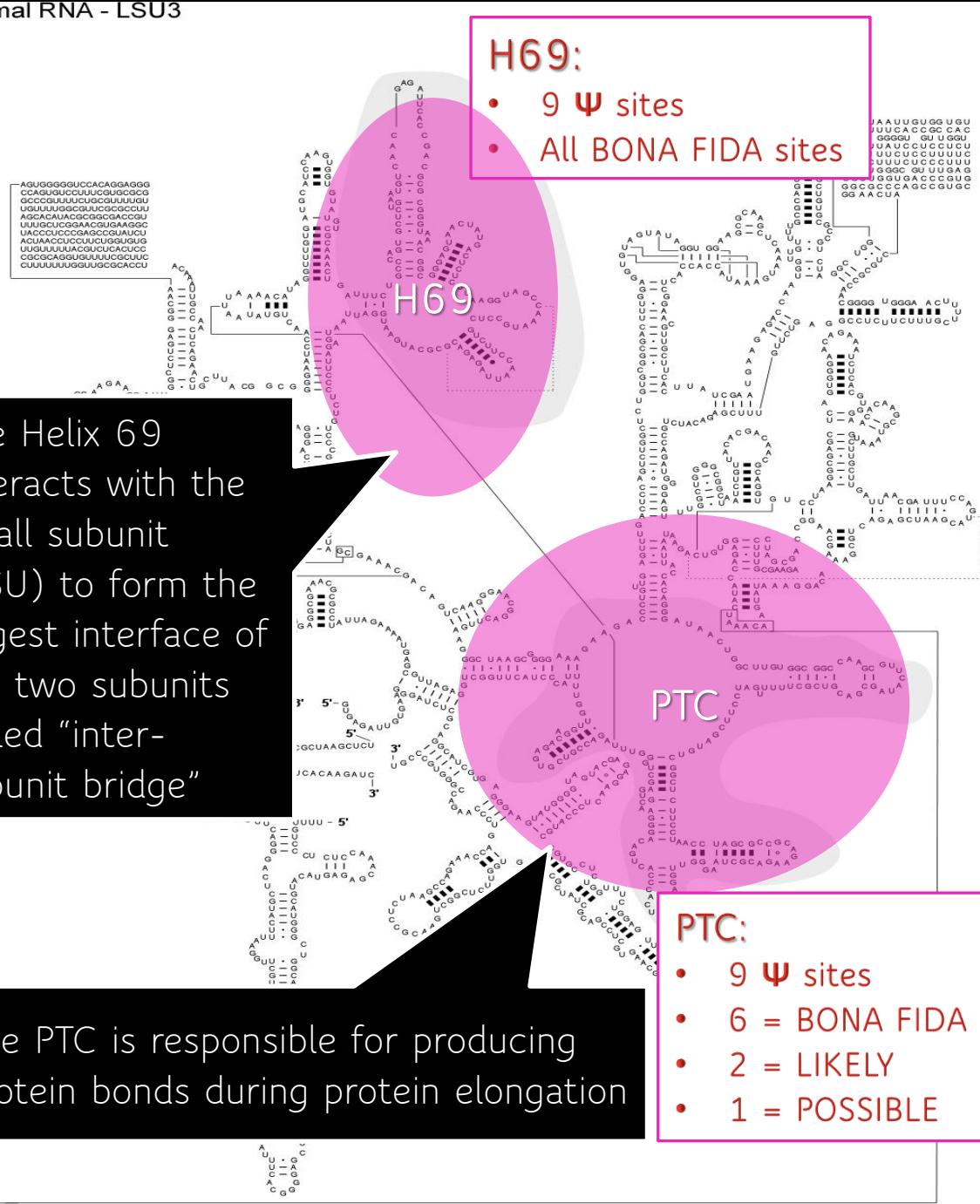
Comparison of developmental life stages

Developmental Life Stage	Developmental Life Stage	Description
Promastigote (P2/P3)	Amastigote	 A composite image showing the comparison of Leishmania life stages. On the left, a dark panel displays the text "Comparing Leishmania life stages". To its right is a grayscale electron micrograph of a promastigote cell, showing internal structures and rRNA distribution. Next is a color-coded diagram of an amastigote cell, where rRNA pseudouridylation is highlighted in red dots. A scale bar at the bottom indicates 1 μm.

Promastigote Vs Amastigote



- The graph presents the comparison of amastigote and Promastigote life form, as a repertoire of the 90 putative Ψ positions divided to its ribosomal subunits, in terms of the difference in FC.
- **37 of the sites** that are considered here to be modified in at least one of these life forms show this type of hyper-modified pattern

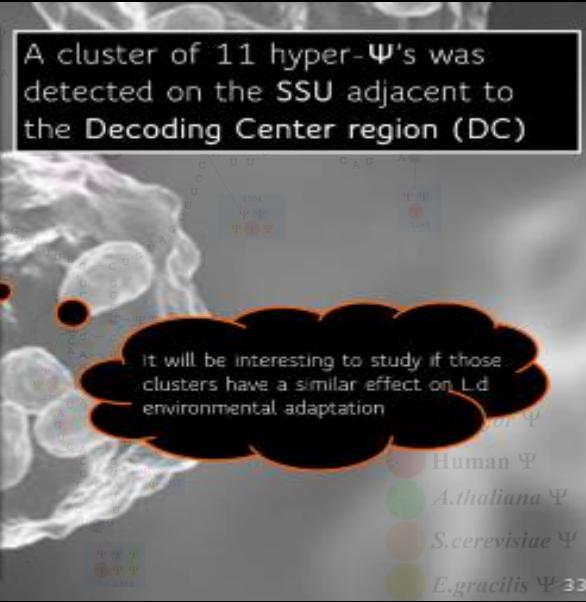
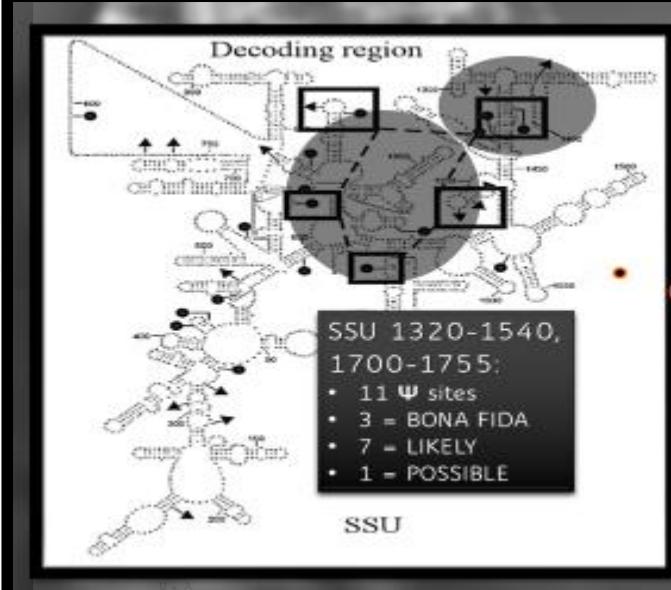


The Helix 69 interacts with the small subunit (SSU) to form the largest interface of the two subunits called "inter-subunit bridge"

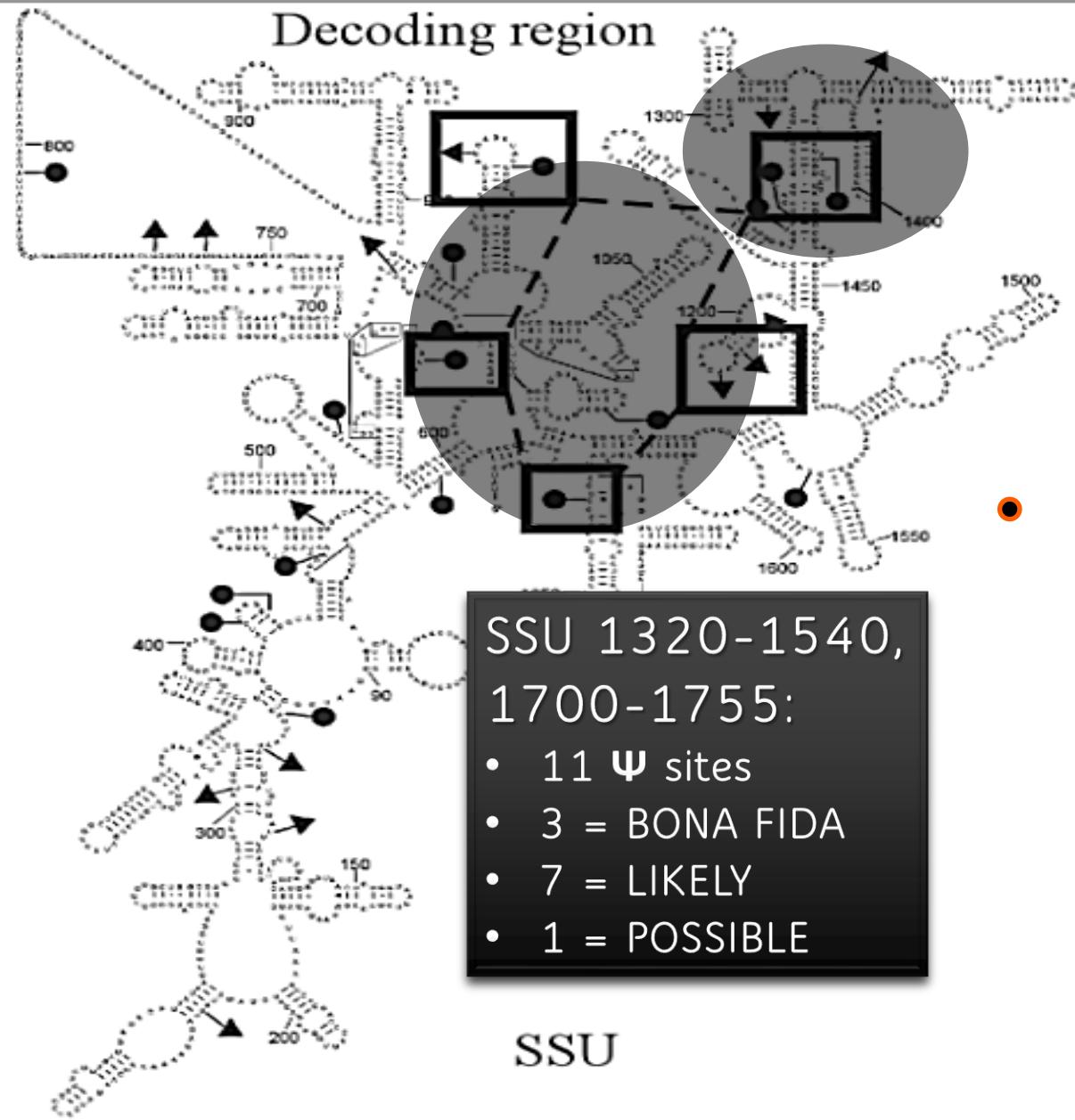
The PTC is responsible for producing protein bonds during protein elongation

Detection of Modifications

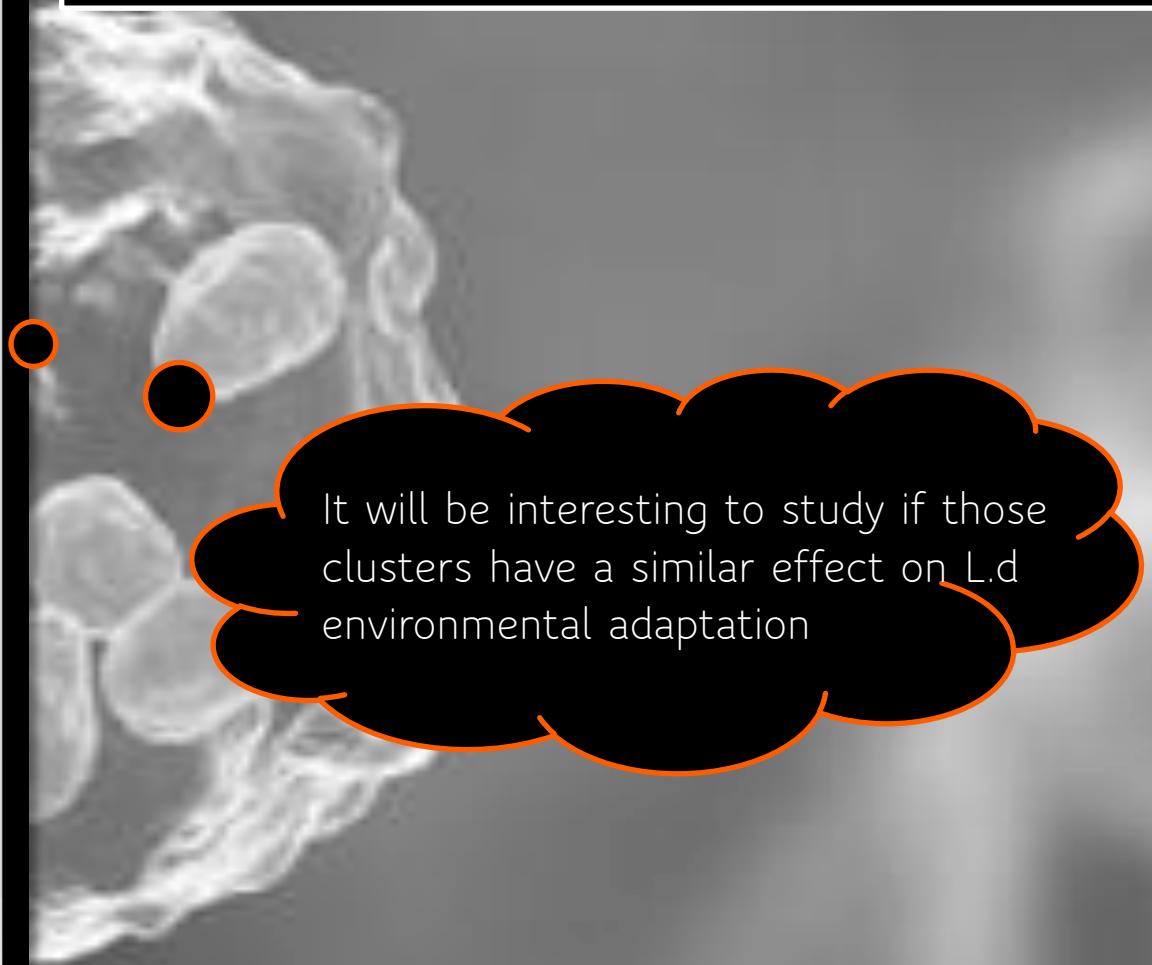
Out of all 37 hyper-modification detected, 18 were found on the H69&PTC (9 hyper- Ψ 's for each area)



An additional, novel, interesting cluster of 11 hyper- Ψ 's was detected on the SSU in close vicinity to the Decoding Center region (DC)



A cluster of 11 hyper- Ψ 's was detected on the SSU adjacent to the Decoding Center region (DC)



Promastigote Vs Promastigote p20 Vs Promastigote p150

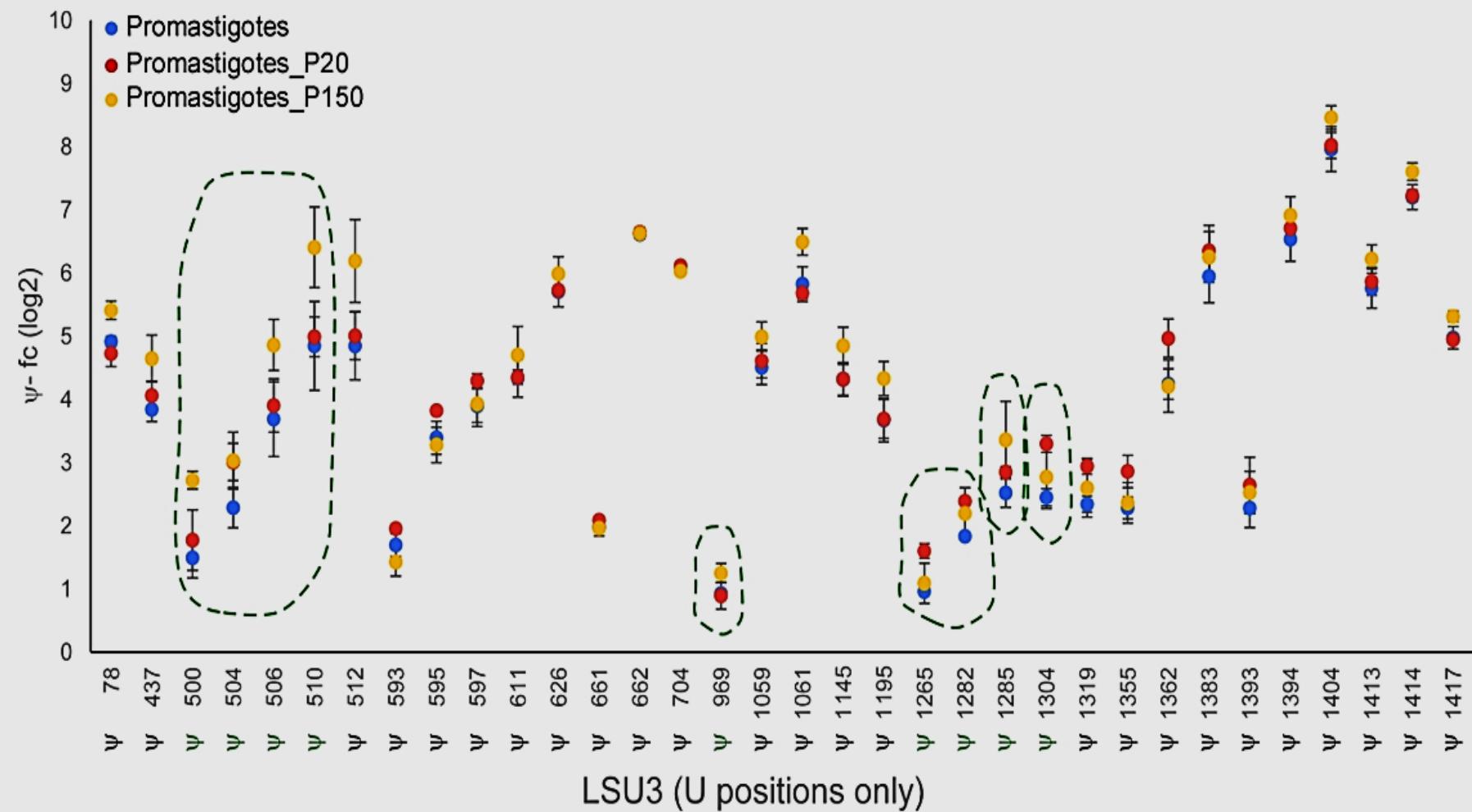
Ψ changes of promastigote stage were monitored during clonal adaptation over 150 passages:

1. The laboratory artificial evolution of L.d (repeating growth cycles) was analyzed to better understand the connection between Ψ levels and adaption of the parasite
2. Positions that are hyper-modified in both circumstances:
 - a) Between promastigote and amastigote life stages
 - b) Over repeating growth cycles in the labmight be important - thus needs to be regulated and modified by the parasite

Comparison of developmental life stages

Developmental Life Stage	Developmental Life Stage	Description
Promastigote (P2)	Promastigote (P20) Promastigote (P150+)	 The effect of the passages in culture on pseudouridylation

Promastigote Vs Promastigote p20 Vs Promastigote p150



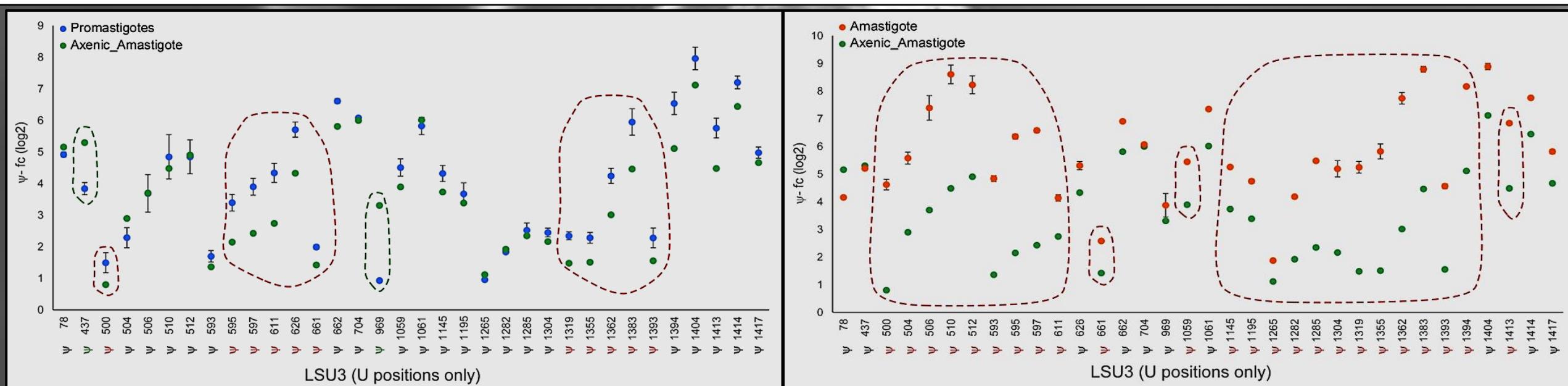
- The graph presents the comparison of the in vitro developing passages of the Promastigote life form, as a repertoire of the 90 putative Ψ positions divided to its ribosomal subunits, in terms of the difference in FC.
- **13 hyper-modified promastigote positions**, whose modification level changed during clonal adaptation.

Axenic-amastigote is a laboratory derived model of the amastigote stage.
I.e., a special form of the amastigote life stage that was passaged as promastigote, and through in-vitro manipulation proliferate as axenic formed amastigote.

Comparison of developmental life stages

Developmental Life Stage	Developmental Life Stage	Description
Promastigote (P2) \ Amastigote	Axenic Amastigote	 The validity of Axenic Amastigote as a research model

- Out of the 37 (aforementioned) hyper-modified sites that we have observed:
 - 29 were observed in the amastigotes vs. axenic comparison
 - 17 were observed in the promastigotes vs. axenic comparison
 - 12 appear in both.
 - Mostly, those results show a non-conclusive trend, as some instances demonstrate resemblance to one life form, as others demonstrate the opposite.
 - Moreover, some instances do not agree with either of the canonical life forms.



Discussion



Discussion

We have found 174, 98% compared to L. major 177 known snoRNA repertoire

We have suggested a standard by which to calibrate FC and TR measures

We have found 90 Ψ -sites, out of which 58 "BONA FIDA"

We have found 37 Hyper-modified Ψ -sites throughout L. donovani life cycle.

18 of the sites are centered in known LSU-ribosome functional loci.

13 out of the 37 Hyper-modified Ψ -sites are also Hyper-modified during clonal adaptation over 150 passages



It would be a good practice to customize FC and TR measures, that makes for a good modification signal in another organisms

As Axenic-amastigote results exhibited a non-conclusive trend, it would be advised to repeat the experiment

As 11 Ψ -sites are concentrated near an SSU-ribosome functional loci, it would be interesting to understand if those have influence on L. donovani fitness.

Thank you for listening

