



Tehran University of Medical
Sciences Publication
<http://tums.ac.ir>

Iran J Parasitol

Open access Journal at
<http://ijpa.tums.ac.ir>



Iranian Society of Parasitology
<http://isp.tums.ac.ir>

Original Article

Preliminary Information of Iranian Lizard *Leishmania* Promastigote Transcriptome Sequencing by Next -Generation Sequencing (NGS) Method

Farveh Ehya ¹, Sima Kalantari ^{2,3}, Mojgan Bandehpour ^{4,5}, *Bahram Kazemi ^{4,5}

1. Department of Biotechnology, Tarbiat Modares University, Tehran, Iran
2. Digestive Oncology Research Center, Digestive Diseases Research Institute, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
3. Regenerative Medicine Group (REMEDI), Universal Scientific Education & Research Network (USERN), Tehran, Iran
4. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received 12 Mar 2023

Accepted 07 Jul 2023

Keywords:

Lizard *leishmania*;
RNA sequencing;
Transcriptome;
Next generating
sequencing

*Correspondence Email:

bahram_14@yahoo.com

Abstract

Background: A lizard *Leishmania* has been isolated from a lizard (*Agama agilis*) in Iran. Its genome sequence has not been determined, so far.

Methods: The study was done at Shahid Beheshti University of Medical Sciences, Tehran, Iran in 2017-2023. *Leishmania* promastigotes were cultured in RPMI₁₆₄₀ culture medium and collected at logarithmic phase by centrifugation. Parasite RNA was extracted by the Qiagene standard kit and its quantity and quality was determined and sequenced by NGS method with Illumina PE machine at BGI Company (China).

Results: The number of 8316 mRNA, 83 tRNA, 63 rRNA, 83 ncRNA, 5 snRNA, 1039 snoRNA, 36 region, and 3 repeat regions, 8343 CDS, 9597 Exon and 9292 Genes were identified in promastigote of Iranian lizard *Leishmania*.

Conclusion: Genomic elements of Iranian lizards *Leishmania* (with unique characteristics) were determined and identified by NGS system.



Introduction

Leishmaniasis is one of the most important skin and visceral infections in Iran. There is no vaccine for field prevention of this infection in the population at risk. The WHO recommended that vaccine research was not useful for field study and that modified traditional methods (such as leishmanization) should be used to control the disease in at-risk populations (1,2). A *Leishmania* was isolated from lizard (*Agama agillis*) in Iran, which is not pathogenic for humans (3). This parasite can be a candidate for leishmanization and because its post-translational modifications is similar to humans, it is useful for the expression of pharmaceutical proteins. For the use for leishmanization as well as the expression of recombinant proteins, there are not much information about its genomic sequence.

The Iranian lizard *Leishmania* has been used for leishmanization (in laboratory animals) and as a host for the expression of several eukaryotic recombinant proteins like Interleukin 12 (4), human

Coagulation factor VII (5), monoclonal antibody against interleukin-2 receptor (6), IL-29 [INF- γ] (7) and amelogenin - the protein used in ossification in dentistry (8).

Ivens et al. identified the genome of *L. major* (9) and Peacock et al. compared the genomes of three species of *Leishmania* (10). The haploid genomes of *Leishmania* species have 32816678 base pairs organized in 36 chromosomes (9) with 911 RNA Genes and 39 pseudogenes were identified (9). Overall, 8272 genes are known to encode proteins. Protein producing genes are long polycistronic genes without transcription factors (11, 12). With a new look at *Leishmania*, Myler et al. determined the role of genes in *L. major* (13, 14).

The genetic information of most organisms is in cDNA sequences known as EST (expressed sequence tags) (15). Most *Leishmania* genes do not have introns (16) and PCR

product of chromosomal DNA is used for gene cloning (17-21). Gene transcription for protein production in eukaryotes is done by RNA polymerase II, and transcription by RNA polymerase I is for the production of ribosomal RNA. Transcription in kinetoplasts includes RNA polymerase I and trans-splicing mechanism (22).

We aimed to sequence the transcriptome and miRNAome of the Iranian lizard *Leishmania*.

Materials and Methods

Sample collection

This study was done at Shahid Beheshti University of Medical Sciences, Tehran, Iran in 2017-2023. All glass containers were washed with DEPC treated water (water containing diethyl pyrocarbonate) and placed at 250 degrees Celsius for one hour to remove RNase. All required buffers were prepared with DEPC treated water. Glass containers were used for RNA extraction. Iranian lizard *Leishmania* promastigotes were cultured in RPMI₁₆₄₀ medium and collected by centrifugation at logarithmic phase Qiagen RNeasy Mini Kit was used for RNA extraction.

Quantitative and qualitative of extracted RNA

The quality, quantity and integrity of total RNA were evaluated using the spectrophotometer Nano Drop1000 and Bioanalyzer 2100 (Agilent Technologies, CA) and using the 28S/18S & 23S/16S test called the RIN test, the samples with the RNA quality score (RIN) > 7.0 were used for RNA sequencing.

Preparation RNA for Sequencing by NGS

The steps were done as protocol in <https://www.thermofisher.com/de/de/home/life-science/dna-rna-purification-analysis/rna-extraction/rna-applications/rna-sequencing-sample-preparation.html>

Sequencing by Next Generation Sequencing method

This protocol lasts for 3-5 days and includes several steps as follows. Library production and sequencing with Illumina PE device was performed as follows Day 1: RNA isolation, DNase addition and RNA fragmentation. Day 2: cDNA synthesis and integration of dUTP

into the second strand. Day 3: preparation of Illumina Paired End sequencing library and dUTP labeled strand destruction. Day 4: Amplification of the cDNA library (Fig. 1).

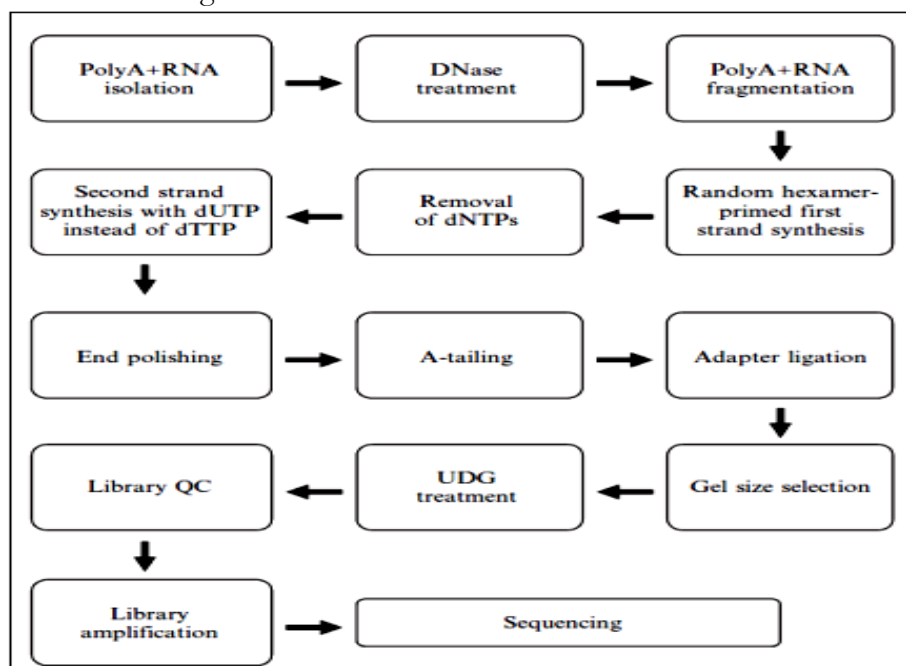


Fig. 1: The steps for sample preparing for NGS

Results

The steps of sample collection and preparation were successfully completed as Fig. 1, and then RNA extraction and purification was performed based on a specific protocol. RNA samples were quantitated and RIN > 7.0 were used for RNA sequencing (Table 1). The

RNA samples were sequenced by BGI Company, China, and the raw sequencing data were received and analyzed by experts. This report mentions the number of identified transcripts (Table 2). The results of NGS were classified based on *L. major* strain Friedlin complete genome as reference strain (supplementary data file).

Table 1: Test results of extracted RNA samples

No.	Sample Number	Tube No.	Concentration (ng/μl)	Volume (μl)	Total mass(μg)	RIN	Test Result
1	A: 8521803003922	1	36.18	20	0.5789	6.9	Qualified
2	B: 8521803003923	1	51.29	20	0.66	7.2	Qualified

3	C:	1	46	20	0.74	8.1	Qualified
8521803003924							

Table 2: Classification of genomic elements identified in the genomic organization of Iranian lizard *Leishmania* by NGS method

<i>Genomic elements</i>	<i>Number</i>	<i>Genomic elements</i>	<i>Number</i>
rRNA	63	mRNA	8316
snoRNA	1039	tRNA	83
snRNA	5	miRNA	83
Sequence Feature	84	Pseudogenes	96
Region	36	CDS	8343
Exon	9597	Repeat region	3
Gene	9292		

Discussion

Leishmania is a protozoan parasite that has similarities and differences compared to other eukaryotic cells. Some of its characteristics, such as polycistronic transcription, are shared with prokaryotic cells (23, 24). The unique characteristics of this parasite have attracted researchers. In recent years, *Leishmania* species have been used as hosts for the production of recombinant proteins (4-8). A suitable host is an essential factor in the production of recombinant proteins. Prokaryotes such as *Escherichia coli* grow in inexpensive culture media and have a short multiplication time, which provides high efficiency in the production of recombinant proteins. However, prokaryotes do not perform post-translational modifications such as glycosylation, phosphorylation, and carboxylation. Some eukaryotic proteins are non-functional after translation in *E. coli*, and some of them accumulate as inclusion bodies in the cytoplasm of the host cell. If these proteins are produced in a prokaryotic host, they will not fold properly, and their third and fourth structure are not formed (25). Proliferation of yeasts such as *Pichia pastoris*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* also require long culture time and post-translational modifications in these microorganisms are not complete processes (26). Culture media for eukaryotic cells are expensive

and require specialized culture conditions and laboratory equipment. Because *Leishmania* is easily and inexpensively maintained in NNN culture medium and can multiply rapidly; it is preferred over others. This parasite can also perform post-translational modifications (22,27). These cause *Leishmania* species to be considered as a suitable host for the production of recombinant pharmaceutical proteins (27-30). Attempts have been made to produce some therapeutic proteins in this parasite (31-34). However, more research is needed before they can be widely used as hosts for the production of recombinant proteins. Researchers in biochemistry, pharmacology and immunology, who are involved in the development of new drugs as well as the production and testing of vaccines, need appropriate cell models. *Leishmania* species are expected to be suitable candidates for these cases (35,36).

If *Leishmania* is used for protein expression, we need some information on its proteases, as may lysis recombinant protein. In this research, the transcriptome (mRNA and ncRNA) of Iranian lizard *Leishmania* was investigated and comprehensive and complete information was obtained about this native and non-pathogenic *Leishmania*. Considering the post-translational changes of this parasite, which is similar to humans, it is a good suggestion for use in the production of recombinant proteins. The *Leishmania* expression system is almost identi-

cal to the RBS method in prokaryotes, while the post-translational modifications of the protein produced through this system are almost identical to those of mammalian proteins. Multisubunit proteins have the unique three-dimensional structures of eukaryotic cells and are similar to mammalian proteins in terms of post-translational modifications (37). The production of multi-subunit proteins in *Leishmania* is done at a cheaper price and does not require extensive biological safety facilities, compared to mammalian cells, it has a high growth rate, its culture medium is cheap, and it grows at a temperature of 26 degrees Celsius, and does not require Incubator does not have CO2 (37-38).

Conclusion

For the first time, genomic elements of Iranian lizards *Leishmania* (with unique characteristics) were determined and identified by NGS system.

Acknowledgements

This article was extracted from project number 963473 granted by National Institute of Medical Sciences Research Development of Iran (NIMAD) and grant number 18369 supported by Shahid Beheshti University of Medical Sciences. Projects were carried out at the Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Moreira POL, Nogueira PM, Monte-Neto RL. Next-Generation Leishmanization: Revisiting Molecular Targets for Selecting Genetically Engineered Live-Attenuated *Leishmania*. *Microorganisms*. 2023; 11: 1043.
2. Mohebbali M, Nadim A, Khamesipour A. An overview of leishmanization experience: A successful control measure and a tool to evaluate candidate vaccines. *Acta Trop*. 2019; 200:105173. doi: 10.1016/j.actatropica.2019; 105173.
3. Kazemi B, Tahvidar- Bideroni Gh, Hashemi Feshareki R, Javadian E. Isolation a lizard *Leishmania* promastigote from natural host in Iran. *J Biol Sci*. 2004; 4(5): 620-623.
4. Donyavi T, Bandehpour M, Kazemi B. Preparation of transgenic Iranian lizard *Leishmania* coding HIL-12 .Iran J Microbiol. 2017; 9(5): 305-311.
5. Mirzaahmadi S, Asaadi -Tehrani G, Bandehpour M, et al. Expression of recombinant human coagulation factor VII by the Lizard *Leishmania* expression system. *J Biomed Biotechnol*. 2011; 2011: 873874.
6. Jalali A, Bandehpour M, Chegeni, R, et al . Expression, Purification, and Evaluation of Anti-IL-2R α Antibody Secreted by *Leishmania tarentolae*. *Int J Pept Res Ther*. 2021; 27(1): 301-307.
7. Taromchi AH, Kazemi B, Mahmazi S, Bandehpour M. Heterologous Expression of Human IL-29 (IFN- λ 1) in Iranian Lizard *Leishmania*. *Iran J Biotechnol*. 2013; 11(3): 168-174.
8. Yadegari Z, Bandehpour M, Kazemi B, Sharifi-Sarasiabi Kh. Expression of Recombinant Human Amelogenin in Iranian Lizard *Leishmania* and Its Biological Function Assay. *Iran J Public Health*. 2015; 44(7): 987-996.
9. Ivens AC, Peacock CS, Worthey EA, et al. The genome of the kinetoplastid parasite, *Leishmania major*. *Science*. 2005; 309 (5733): 436-42.
10. Peacock CS, Seeger K, Harris D, MurphyL, Ruiz JC, Quail MA. Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. *Nat Genet*. 2007; 39 (7): 839-47.
11. McDonagh PD, Peter J. Myler PJ, Stuart K. The unusual gene organization of *Leishmania major* chromosome 1 may reflect novel transcription processes. *Nucleic Acids Res*. 2000; 28(14): 2800-3.

12. Myler PJ, Audleman L, deVos T, et al. *Leishmania major* Friedlin chromosome 1 has an unusual distribution of protein-coding genes. Proc Natl Acad Sci U S A. 1999; 96(6): 2902-6.
13. Myler PJ, Sisk E, McDonagh PD, et al. Genomic organization and gene function in *Leishmania*. Bioche Soc Trans. 2000; 28(5): 527–31.
14. Myler PJ, Beverley SM, Cruz AK, et al. The *Leishmania* genome project: new insights into gene organization and function. Med Microbiol Immunol. 2001; 190 (1-2): 9-12.
15. Gentil LG, Lasakosvitsch F, Silveira JF, Santos MR, Barbiéri CL. Analysis and chromosomal mapping of *Leishmania (Leishmania) amazonensis* amastigote expressed sequence tags. Mem Inst Oswaldo Cruz. 2007; 102(6): 707-11.
16. Fong D, Lee B. *Beta tubulin* gene of the parasitic protozoan *Leishmania mexicana*. Mol Biochem Parasitol. 1988; 31(1): 97-106.
17. Hejazi SH, Zia-Jahromi N, Bandehpour M, et al. Gene Cloning of Iranian *Leishmania major* Mannose-1-Phosphate Guanyltransferase. Iran J Parasitol. 2009; 4(3): 1-9.
18. Kheirandish F, Bandehpour M, Haghghi A, Mohebbali M, Mahboudi F, Kazemi B. Molecular cloning, Expression and Enzymatic assay of Iranian *Leishmania major* pteridine reductase 1. Iran J Parasitol. 2008; 3 (2): 1-9.
19. Rasouli M, Zavarani Hoseini A, Kazemi B, Alborzi A, Kiany S. Expression of heat shock protein 70 of MCAN/IT/96/LON-49, a tool for diagnosis and future vaccine research. Iran J Immunol. 2009; 6(2): 75-86.
20. Kazemi B, Tohidi F, Bandehpour M, Yarian F. Molecular Cloning, Expression, and Enzymatic Assay of *Pteridine Reductase 1* from Iranian Lizard *Leishmania*. Iran Biomed J. 2010; 14(3): 97-102.
21. Shaddel M, Oormazdi H, Akhlaghi L, Kazemi B, Bandehpour M. Cloning of *Leishmania major* P4 Gene. Yakhkhteh Medical Journal. 2008; 10(3): 201-4.
22. Lee MG, Van der Ploeg LH. Transcription of protein-coding genes in trypanosomes by RNA polymerase I. Annu Rev Microbiol. 1997; 51: 463-89.
23. Worthey EA, Martinez-Calvillo S, Schnauffer A, et al. *Leishmania major* chromosome 3 contains two long convergent polycistronic gene clusters separated by a *tRNA* gene. Nucleic Acids Res. 2003; 31(14): 4201–10.
24. Flinn HM, Smith DF. Genomic organization and expression of a differentially regulated gene family from *Leishmania major*. Nucleic Acids Res. 1992; 20(4): 755-62.
25. Ma Y, Lee CJ, Park JS. Strategies for Optimizing the Production of Proteins and Peptides with Multiple Disulfide Bonds. Antibiotics (Basel). 2020; 9(9):541. doi:10.3390/antibiotics9090541
26. Rosano GL, Ceccarelli EA. Recombinant protein expression in *Escherichia coli*: advances and challenges. Front Microbiol. 2014; 5:172.
27. Rosenzweig D, Smith D, Myler PJ, Olafson RW, Zilberstein D. Posttranslational modification of cellular proteins during *Leishmania donovani* differentiation. Proteomics. 2008; 8(9): 1843-50.
28. Hem S, Gherardini PF, Osorio y Fortéa J, et al. Identification of *Leishmania*-specific protein phosphorylation sites by LCESI- MS/MS and comparative genomics analyses. Proteomics. 2010; 10(21):3868-83.
29. Kaur J, Sundar S, Singh N. Molecular docking, structure-activity relationship and biological evaluation of the anticancer drug monastrol as a *pteridine reductase* inhibitor in a clinical isolate of *Leishmania donovani*. J Antimicrob Chemother. 2010; 65(8):1742-8.
30. Kumar P, Kumar A, Verma SS, et al. *Leishmania donovani pteridine reductase 1*: biochemical properties and structure-modeling studies. Exp Parasitol. 2008; 120(1):73-9.
31. Basile G, Peticca M. Recombinant protein expression in *Leishmania tarentolae*. Mol Biotechnol. 2009; 43(3):273- 8.
32. Soleimani M, Mahboudi F, Davoudi N, et al. Expression of human tissue plasminogen activator in the trypanosomatid protozoan *Leishmania tarentolae*. Biotechnol Appl Biochem. 2007; 48(Pt 1): 55-61.
33. Hemayatkar M, Mahboudi F, Majidzadeh A K, et al. Increased expression of recombinant human tissue plasminogen activator in *Leishmania tarentolae*. Biotechnol J. 2010; 5(11):1198-206.
34. Dortay H, Roeber B. A highly efficient pipeline for protein expression in *Leishmania tarentolae* using infrared fluorescence protein as marker. Microbiol Cell Fact; 2010; 9: 29.

35. Sereno D, Lemesre JL. Axenically Cultured Amastigote Forms as an In Vitro Model for Investigation of Antileishmanial Agents. *Antimicrob Agents Chemother.* 1997; 41(5): 972–976.
36. Gupta S, Nishi. Visceral Leishmaniasis: Experimental models for drug discovery. *Indian J Med Res.* 2011; 133(1): 27-39.
37. Abdi Ghavidel A, Jajarmi V, Bandehpour M, Kazemi B. Polycistronic Expression of Multi-Subunit Complexes in the Eukaryotic Environment: A Narrative Review. *Iran J Parasitol.* 2022; 17(3): 286-295.
38. Abdi Ghavidel A, Aghamiri SH, Jajarmi V, Bandehpour M, Kazemi B. The Influence of Different Culture Media on the Growth and Recombinant Protein Production of Iranian Lizard *Leishmania* Promastigote. *Iran J Parasitol.* 2022; 17 (4)543-55.