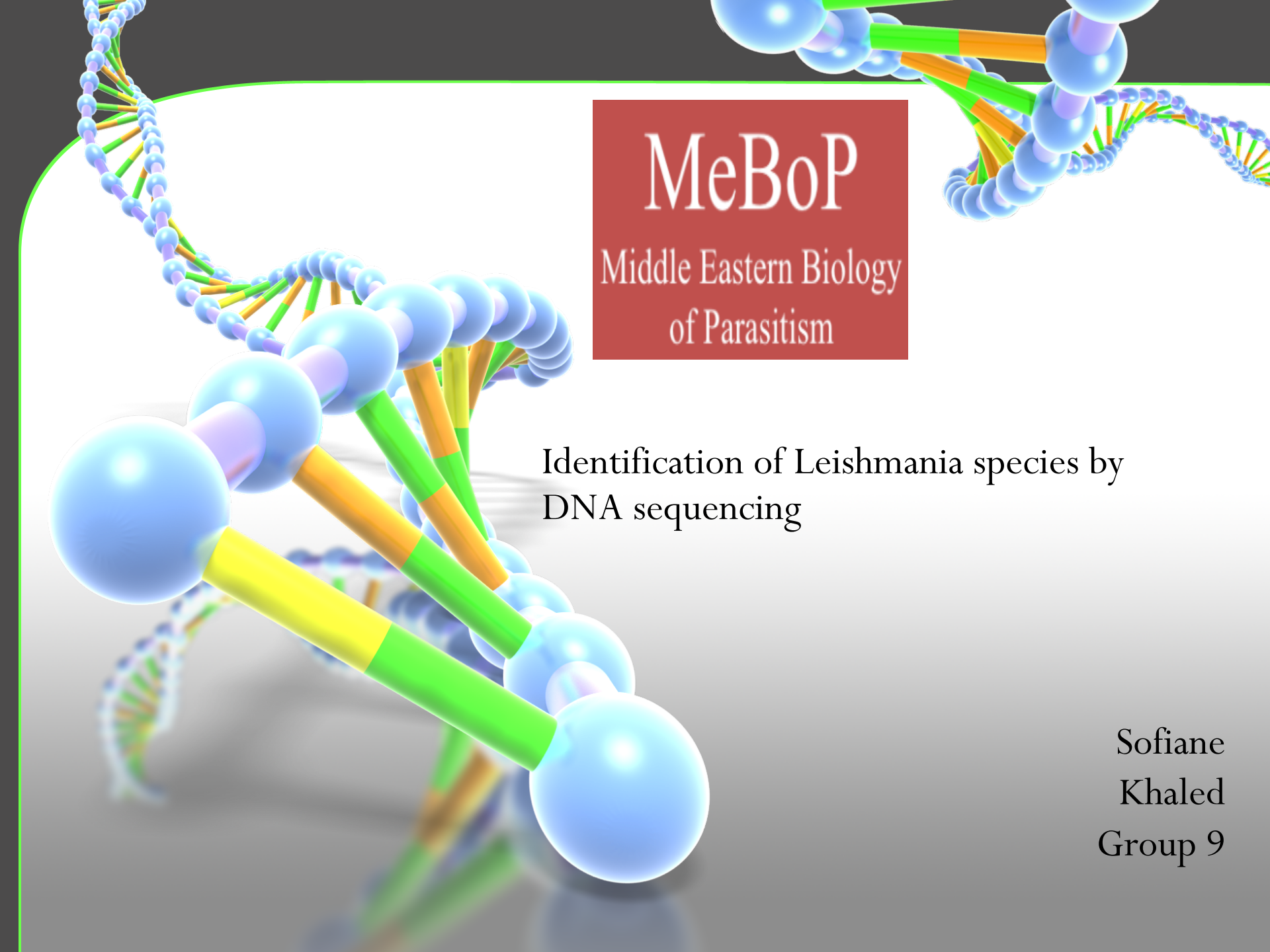


MeBoP

Middle Eastern Biology of Parasitism

Identification of Leishmania species by DNA sequencing

Sofiane
Khaled
Group 9

•Principle of DNA SEQUENCING

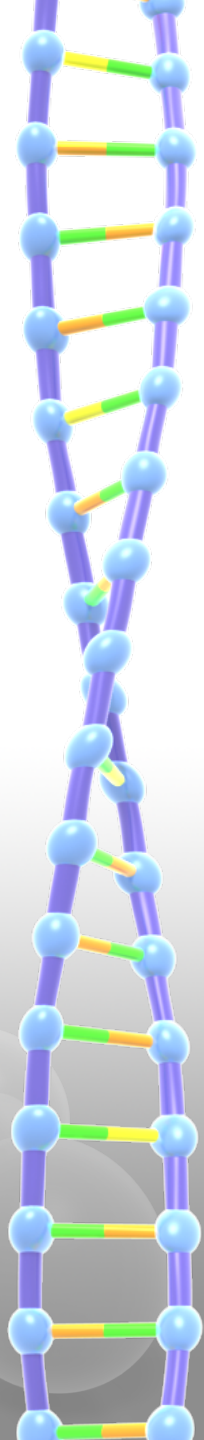
- Determining the order of bases in a section of DNA

Two separate methods for sequencing DNA were developed:

- To analyze gene structure and its relation to gene expression as well as protein conformation

1-chain termination method (Sanger)

2- chemical degradation method.



Leishmania genomics

There are a total of 8272 genes that codes for proteins in leishmania out Of these several genes could be choosen for sequencing :

- ribosomal internal transcribed spacer I(ITS I)
- Cytochrome b
- Heat schock protein 70 KD (HSP)
- Signal recognition protein SRP

Methods and materiel

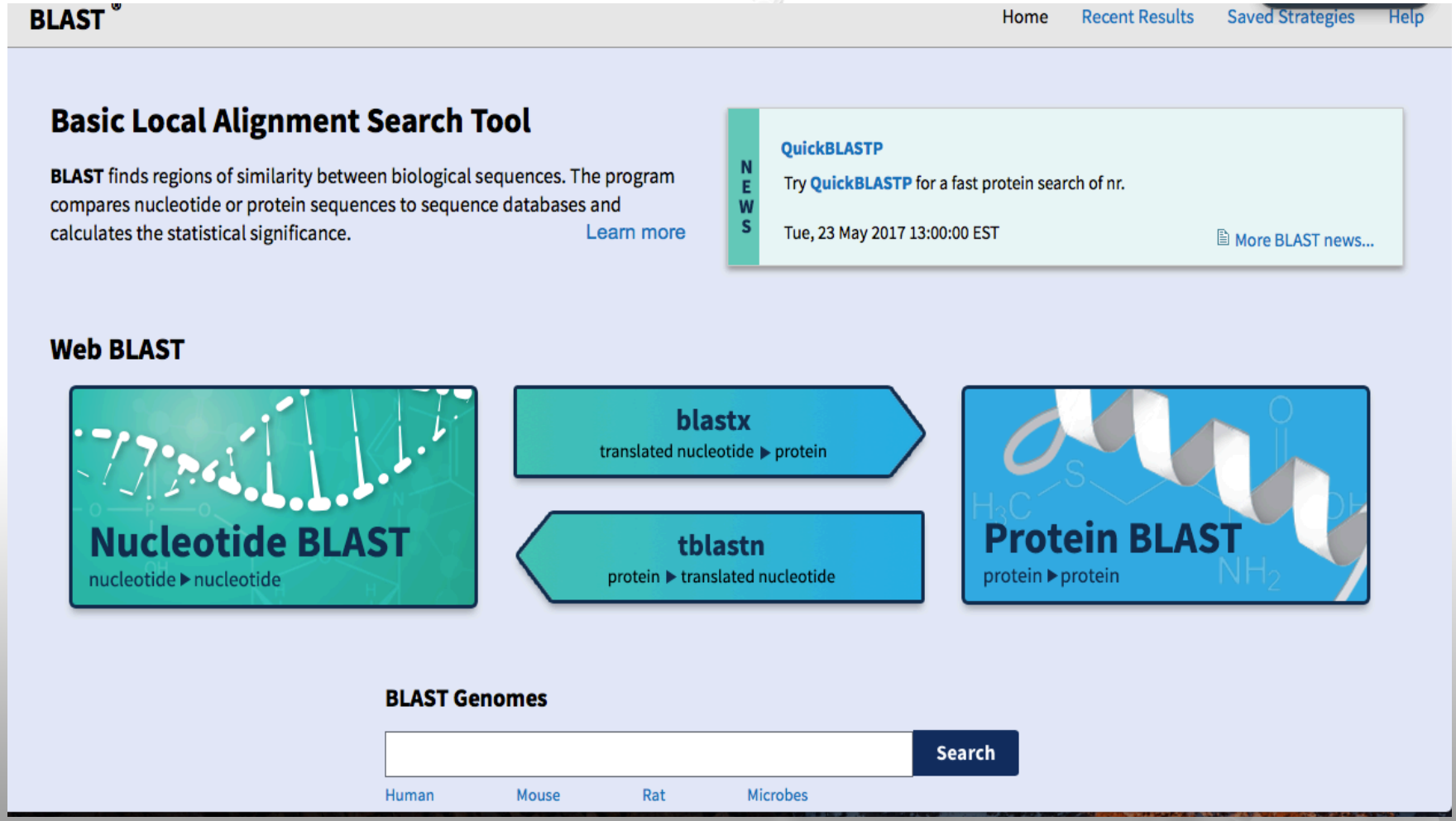
In our experiment We have conducted a PCR amplification of 5 samples including 3 blood samples and 2 biopsies

We selected and purified only positive clinical samples for Both **ITS1** and **cytochrome b** sequencing analysis using oligonucleotide primers :

- ITS I: ITS reverse primer / L 5.8 primer
- Cytochrom b : cyt 1 primer/ cyt 2 primer

And we sent PCR product for DNA sequencing

We carried out BLAST (basic Local alignement Search Tool)



Exemple of the results in BLAST software

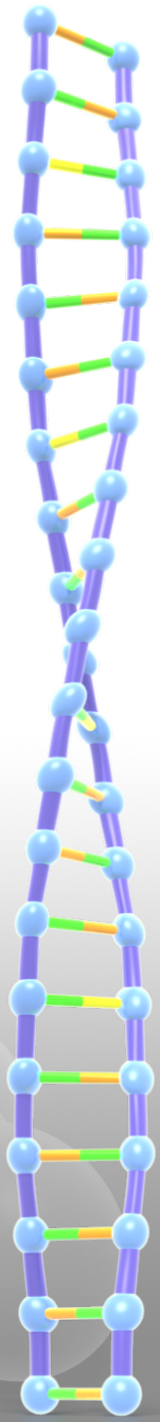
G9 /ITS-a

ATTATACATTATATATAGGCCTTTCCCACACATACACAGCAAACTTTTATACTCGAAGTTTGCAGTAAACA
AAAGGCCGATCGACGTTATAACGCACCGCCTATACACAAAAGCAAAAATGTCCGTTTATACAAATAT
ACGGCGTTTCGGTTTTGTTGGCGGGGGGGTGCGTGTGTGTGGATAACGGCTCACATAACGTGTCGCG
ATGGATGACTTGG CTTCCTATTTCGTTGAANAACGCAGTAAAGTGCGATAAGTGGTATCAAA

Leishmania tropica isolate 63P internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed sp	468	468	99%	1e-130	98%	KM454152.1
Leishmania tropica voucher IPM-MorES10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	468	468	99%	1e-130	98%	KC145161.1
Leishmania tropica voucher IPM-MorES-7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal trar	468	468	99%	1e-130	98%	KC145160.1
Leishmania tropica voucher IPM-MorES-5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal trar	468	468	99%	1e-130	98%	KC145159.1
Leishmania tropica voucher IPM-MorES-4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal trar	468	468	99%	1e-130	98%	KC145158.1
Leishmania tropica isolate 156 clone 4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	468	468	99%	1e-130	98%	FJ948456.1
Leishmania tropica isolate 156 clone 1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	468	468	99%	1e-130	98%	FJ948455.1
Leishmania tropica isolate 80 clone 5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra	468	468	99%	1e-130	98%	FJ948454.1
Leishmania tropica isolate 80 clone 4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra	468	468	99%	1e-130	98%	FJ948453.1
Leishmania tropica isolate 56 clone 3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra	468	468	99%	1e-130	98%	FJ948450.1

results of DNA sequencing were as follow

Group	ITS 1a	ITS 1 b	Cyt b
2	L. tropica	L. donovani complex	L.tropica
3	No Seq	L.tropica	No Seq
4	L.tropica	L.tropica	L.tropica
5	L.tropica	No Seq	L.tropica
6	?	L.tropica	L.tropica
7	L.tropica	L.tropica	L.tropica
8	L.tropica	L.tropica	L.tropica
9	L.tropica	L. donovani complex	L.tropica



Exemple of faulties in sequencing

G5 / ITS-b
NNNNN

No sequencing

G6 / ITS-a
CGCGATGGATGACTTGGCTTCCTATTTCNTTGAA

	score	score	cover	value		
Leishmania major strain Friedlin 5.8S ribosomal RNA (M3) RNA rRNA	60.2	60.2	100%	6e-07	97%	XR_002460819.1
Leishmania major strain Friedlin 5.8S ribosomal RNA (M3) RNA rRNA	60.2	60.2	100%	6e-07	97%	XR_002460818.1
Leishmania major strain Friedlin 5.8S ribosomal RNA (M3) RNA rRNA	60.2	60.2	100%	6e-07	97%	XR_002460817.1
Leishmania major strain Friedlin 5.8S ribosomal RNA (M3) RNA rRNA	60.2	60.2	100%	6e-07	97%	XR_002460816.1
Leishmania major strain Friedlin 5.8S ribosomal RNA (M3) RNA rRNA	60.2	60.2	100%	6e-07	97%	XR_002460815.1
Leishmania major strain Friedlin 5.8S ribosomal RNA (M3) RNA rRNA	60.2	60.2	100%	6e-07	97%	XR_002460814.1
Leishmania infantum internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence	60.2	60.2	100%	6e-07	97%	KX712139.1
Leishmania tropica isolate 9TZASI internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence	60.2	60.2	100%	6e-07	97%	KY974310.1
Leishmania infantum isolate HAM.15_Morocco internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence	60.2	60.2	100%	6e-07	97%	KY658235.1

Sequence showing similarity to more than one type of leishmania species

1. Heat schock protein 70 KD (HSP)
2. Signal recognition protein SRP

1. RFLP
2. HRM

Advantages

- High specificity
- Possibility to determine the phylogenetic tree.
- To get rapid results

Disadvantages

- Depending on PCR product
- Expensive