# Generation of a *Leishmania* plasmid to express a mutated cytochrome c oxidase subunit in *Leishmania*


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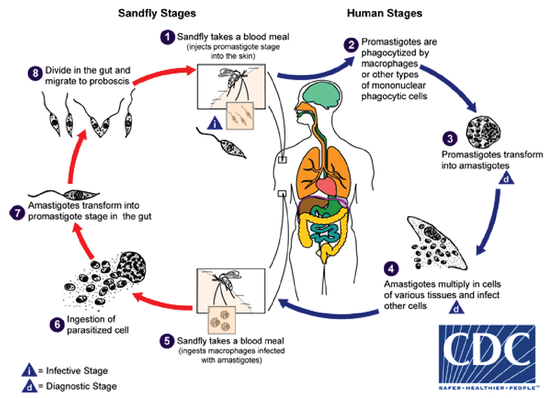
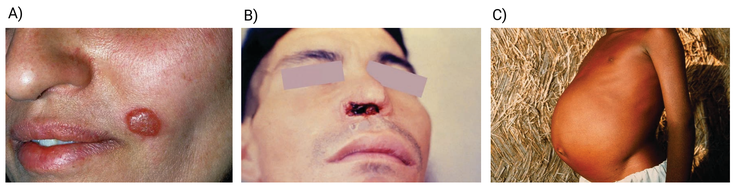
## INTRODUCTION



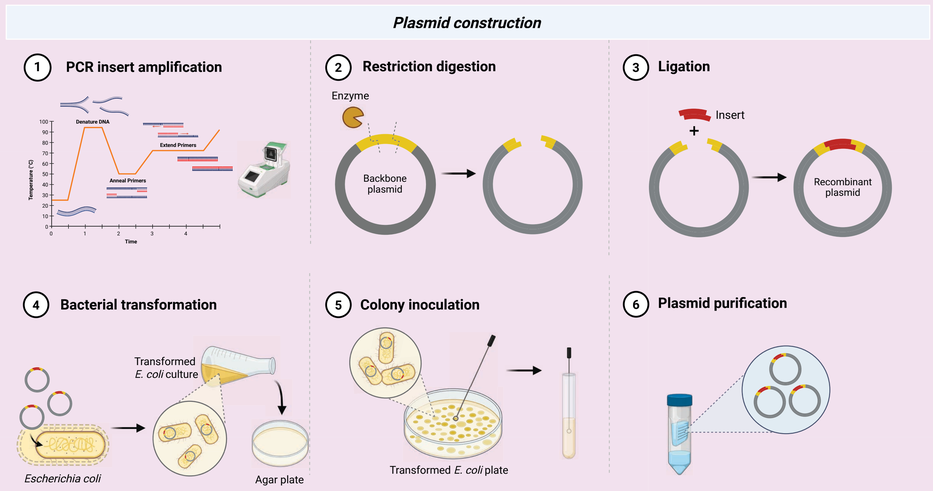

*Leishmania* life cycle. Centers for Disease Control (CDC)




A) Cutaneous leishmaniasis. B) Mucosal leishmaniasis. C) Visceral leishmaniasis.

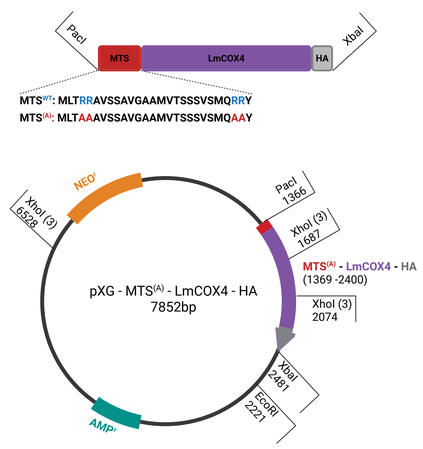
- *Leishmania* is a protozoan kinetoplastid parasite transmitted by phlebotomine sand flies and causes the parasitic disease leishmaniasis. The major forms of the disease are: cutaneous, mucosal, and visceral.
- Around 1 million people are infected annually in tropical and subtropical regions. Currently there is no vaccine and available drug therapies are ineffective due to toxicity and parasite drug resistance.
- We are examining the role of the positively charged arginine (R) residues in the N-terminal mitochondrial targeting signal (MTS) of the mitochondrial *Leishmania* protein LmCOX4 in the control of its localization and expression at insect (27°C) and host (33-37°C) temperatures. LmCOX4 is important for *Leishmania* mitochondrial function, hence represents a potential therapeutic target.
- The goal of this project is to generate a *Leishmania* expression construct encoding an HA-epitope tagged alanine (A) mutated LmCOX4 that lacks a positively charged MTS. This construct will then be introduced into *Leishmania* to determine the effect of MTS positive charge loss upon LmCOX4 expression.

## METHODS

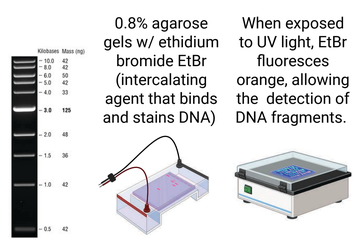



Schematic representation of steps required for cloning insert into an expression vector.

1. PCR amplification of the insert of interest.
2. Restriction digest of the backbone vector to create sticky ends compatible with insert.
3. Ligation of insert and backbone vector.
4. Bacterial transformation with insert containing vector.
5. Plasmid replication in bacterial system.
6. Plasmid purification.



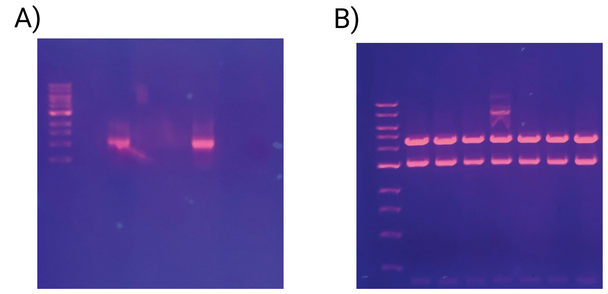

Schematic representation of the *Leishmania* gene expression plasmid, pXG, with the MTS$^{(A)}$ mutant variant of LmCOX4-HA inserted at the Pac I and Xba I sites of pXG, as indicated.




Schematic representation of an agarose gel electrophoresis system.

1. PCR amplification of MTS$^{(A)}$-LmCOX4-HA insert with PacI and XbaI restriction sites.
2. Size confirmation through agarose gel electrophoresis.
3. DNA purification: 1kb band (MTS$^{(A)}$-LmCOX4-HA insert).
4. Ligation of insert into pXG (*Leishmania* expression plasmid).
5. Transformation of DH5α competent cells with pXG-MTS$^{(A)}$-LmCOX4-HA.
6. Colony inoculation for plasmid amplification.
7. Plasmid purification: Miniprep of pXG-MTS$^{(A)}$-COX4-HA.
8. Size confirmation by digestion with XhoI. There will be 3 XhoI sites,therefore we expect fragments of 4.5, 3.05 and 0.4 Kb which will be confirmed by agarose gel electrophoresis.

## RESULTS




A) PCR amplification of LmCOX4 from the WT LmCOX4 gene template, using MTS$^{(A)}$ mutant forward primer (encoding 4 alanines instead of the WT 4 positively-charged arginines). The expected 1 Kb band is observed by agarose gel electrophoresis (see methods).

B) pXG + MTS$^{(A)}$-LmCOX4-HA clones digested with XhoI. The expected 4.5, 3.0 and 0.4 Kb XhoI fragments are observed.

## CONCLUSIONS AND FUTURE DIRECTIONS

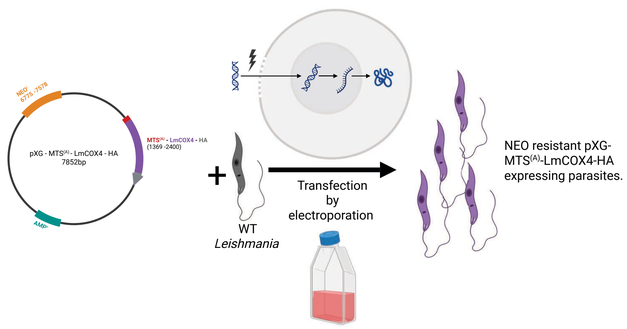



Fig. 9 Purified plasmids obtained will be transfected into *Leishmania major* parasites via electroporation. Expression of MTS$^{(A)}$-LCOX4-HA in transfected parasites is going to be confirmed through α-HA immunoblotting and fluorescent microscopy.

The mutated pXG-MTS$^{(A)}$-LCOX4-HA constructs will be transfected into *Leishmania* parasites.
Detection of HA-construct from the transfected parasites will provide insights into the role of the MTS in LmCOX4 cellular trafficking and mitochondrial import and its importance in mitochondrial energy metabolism.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