Transfection of *rab6* gene from *Leishmania donovani* using Biontex K2 transfection system

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Materials and Methods

Cell culture

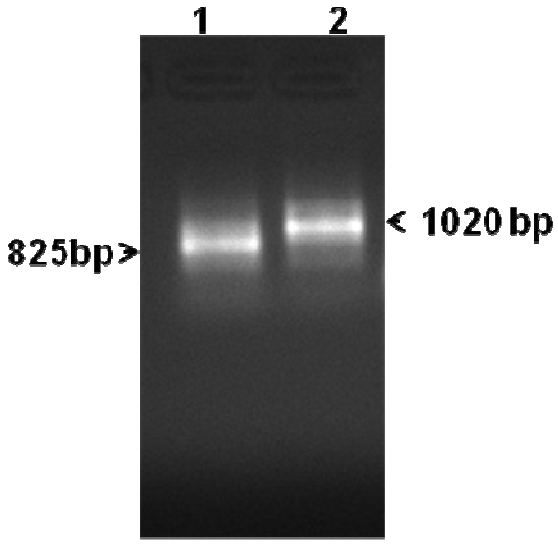
Promastigotes of *Leishmania donovani* strain (MHOM/IN/80/DD8) were maintained in Medium 199 (pH 7.2) (Sigma, St Louis, MO, USA) 2.05 mM L-glutamine, 12 mM HEPES buffer (Sigma), 10% (v/v) HIFBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Promastigotes were grown in vented T25 tissue culture flasks and maintained at 25°C. Promastigote cultures were initiated at 10^6 parasites/mL and subcultured every 3–4 day.

Cell transfection:

Late logarithmic phase of *Leishmania donovani* promastigotes ($1x10^7$cell/ml) were harvested by centrifugation at 4500 rpm for 5 min at room temperature. Promastigotes were treated with K2 multiplier and incubated for 2 hr at 25^{o}C before transfection. For this K2 Multiplier (5 µL) was added slowly onto the culture media (250 µL) and mixed by gently. Transfection solution (lipoplex) was prepared in serum-free cell culture medium. 20 µg of plasmid DNA (pET28a+ and *Ldrab6* gene) was added to a 9 µL of K2 transfection solution and incubated for 20 min at room temperature. Transfection solution (lipoplex) was added to the promastigotes cells and incubated for 24 hr at 25^{o}C. On the genomic level, transfection efficiency was monitored by amplified PCR product of *Ldrab6* gene encoded by the transfected plasmids.

Result:

L. donovani promastigotes were transfected with plasmid DNA (pET28a+ and *Ldrab6* gene) using Biontex K2 transfection system. Transfection was confirmed by PCR using T7 promotor of pET28a^{+} vector and *rab6* gene primer. Wild type promastigotes have 825 bp of *rab6* gene and transfected promastigotes have 1020 bp of expressed *rab6 gene* which was subcloned in pET28a+ expression vector.



Transfection of *Ldrab6* gene in *Leishmania donovani* by using K2 transfection system (Biontex). After transfection, pellet was used for DNA isolation by Qiagen kit (Cat. No.51104). Lane 1; PCR product of *rab6* gene from *L. donovani* (using *rab6* forward and reverse primer) was used as control, Lane 2; PCR product of transfected *L. donovan*i with *Ldrab6* gene (using T7 promoter, Lot# 38530153 as forward primer and *rab6* reverse as reverse primer).

Conclusions:

53 K2 transfection system was used by us for the first time in *Leishmania donovani.* It is based on
54 cationic liposome formulation which is used to increase transfection efficiency of plasmid DNA.
55 Transfected parasites show a band of the expected size (~1020 bp) when compare with wild type
56 parasites (825 bp). The transfection rate was increased approximately 80% when compare with
57 other transfection reagents.