# CosSeq screening identifies amastigote fitness factors enhancing *Leishmania donovani* mammalian infection

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*Leishmania donovani* is the causative agent of fatal visceral leishmaniasis. These parasites have evolved strategies to survive inside phagocytic cells by subverting host antimicrobial activities. Mechanisms that govern intracellular *Leishmania* survival remain widely unknown and only few fitness factors enhancing parasite infectivity in the mammalian host have been identified to date. Here, to reveal new factors associated with infectivity of the mammalian amastigote stage, we conducted a genetic screen *in vivo* in infected hamsters using attenuated *L. donovani* parasites transfected with a hygromycin B-selectable cosmid library derived from virulent parasites, containing genomic inserts of 20–40 kb. As no hygromycin selection was applied during hamster infection, only parasites harboring cosmids conferring a fitness advantage in vivo were expected to persist during infection.

Four hamsters were infected with attenuated parasites transfected either with the cosmid library or with the empty clHYG vector as control (mock). After 20 weeks of infection, first clinical signs of illness and increased parasite burden were only observed in animals infected with cosmid-transfected parasites but not the mock control. Parasites were recovered from liver and spleen tissues, and cosmids were purified for high-throughput sequencing. To eliminate kDNA contamination, recovered cosmids were first transformed into bacteria prior to purification and high-throughput sequencing. Mapping of sequencing reads onto the Ld1S reference genome identified 16 enriched genomic regions located on different chromosomes. The most consistently

enriched cosmids contained fragments from chromosomes 1, 8, 25, 33, and 36 (termed cos1, cos8, cos 25, cos33, and cos36), detected in at least three of four hamsters and in both liver and spleen samples, suggesting a role in parasite fitness in the mammalian host. Notably, cos8, carrying a genomic fragment from chromsome 8 includes the gene encoding for superoxide dismutase A, a known virulence factor, thereby validating our screening strategy.

All selected cosmids were re-transfected individually into attenuated parasites to confirm their role in infectivity. While these cosmids did not impact metacyclogenesis, increased parasite burden in infected macrophages compared to mock controls was observed for cos8, cos33, and cos36, suggesting that the corresponding genomic fragments enhance amastigote differentiation, survival or proliferation. Mining our previous RNA-seq and proteomics data allowed us to prioritize a set of genes for down-stream analysis, including a gene on chr 33 encoding a putative NF-κB–activating domain, potentially involved in modulation of host immune responses. We are currently assessing the fitness function of this and other genes using over-expression and gene deletion strategies to validate their role in parasite infectivity in hamster bone marrow–derived macrophages.

In conclusion, the CosSeq-based strategy provides a powerful experimental framework beyond *Leishmania* for identifying pathogen fitness factors required for mammalian infection. Elucidating the genes within the selected cosmids driving amastigote infection will advance our understanding of host–parasite interactions and may inform future vaccine and drug development strategies.