# The LEXSY Technology - a valuable tool in *Kinetoplastida*

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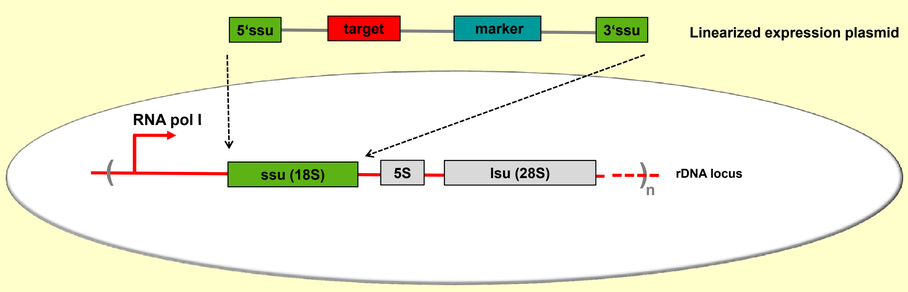
**ABSTRACT**

**We have engineered the non-pathogenic parasite of lizard *Leishmania tarentolae* into a flexible and efficient protein expression platform called LEXSY. This biosafety 1 host system can be used for expression of target proteins of other *kinetoplastida*, making use of the close relationship of cellular machineries including protein folding and modification.**
**On the other hand the LEXSY vectors are functionally in other *Leishmania* species and were used successfully in *L. amazonensis, L. donovani, L. infantum, L. major, L. mexicana* and also in the plant parasite *Phytomonas serpens.***
**Two principal LEXSY architectures yield constitutive or inducible expression. The constitutive version is based on integration of expression cassettes into the 18S rRNA gene (*ssu*) for transcription by the strong RNA polymerase I of the host.**
**Four alternative antibiotic selection markers permit integration of up to four expression cassettes into the *ssu* locus, useful e.g. for expression of multisubunit proteins.**
**The inducible version employs T7 RNA Polymerase and TET repressor, expressed from the *ssu* locus of an engineered host. Target gene cassettes under control of a T7 promoter TET operator assembly can be integrated into ß-tubulin (*ß-tub*) or ornithine decarboxylase (*odc*) loci. Alternatively, expression cassettes can be positioned on circular or linear episomes with telomeric ends. The use of a fusion of the *bleo* resistance and *cherry* fluorescence genes provides a tool for selection and screening.**
**The LEXSY toolbox has recently been complemented by a cell-free version for expression of recombinant proteins.**

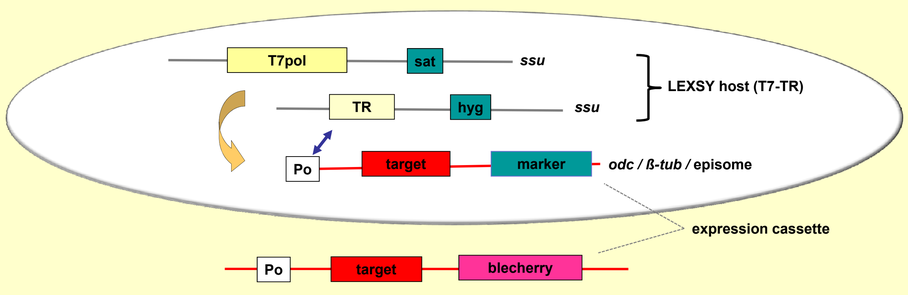
## Two configurations of *in vivo* LEXSY

### Constitutive expression of target genes




**Transcription by strong host RNA polymerase I. Target genes are flanked by host-specific calmodulin (*cam*) splicing signals. Due to high homology of *ssu* genes used for integration, LEXSY vectors are also functional in other *Leishmania* sp. as *L. amazonensis, L. donovani, L. infantum, L. major, L. mexicana*, and also in the plant parasite *Phytomonas serpens*. Four selection markers (LEXSY NTC, Hyg, Bleo and Neo) permit co-expression of multi-subunit proteins.**

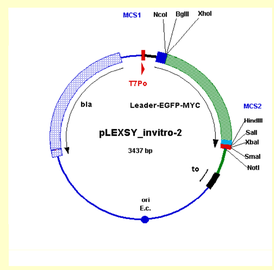
### Inducible expression of target genes




**Uncoupling from LEXSY host regulation due to transcription by heterologous T7 RNA polymerase controlled by TET repressor. Selection with LEXSY Bleo or Neo. Blecherry marker permits selection and online monitoring of induction.**

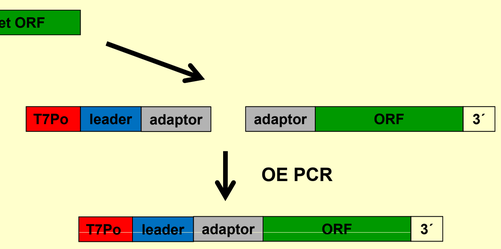
## Two configurations of *in vitro* LEXSY

### Plasmid based *in vitro* LEXSY
**- for high yields -**



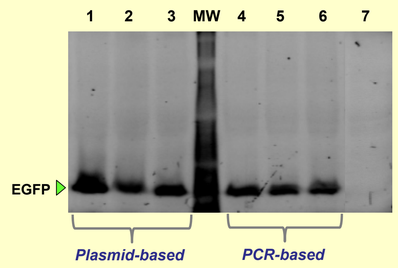

**Template generation by cloning of target genes into plasmid vector**

### PCR based *in vitro* LEXSY
**- for high throughput -**



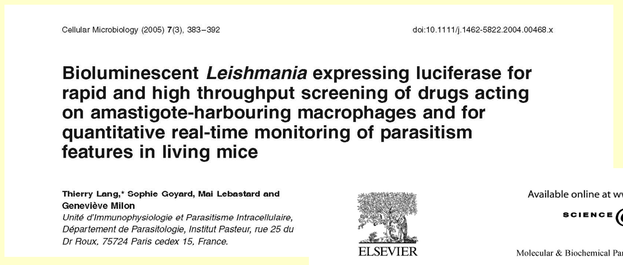

**Template generation by direct PCR amplification of target DNA by overlap extension (OE) PCR**

**Cell-free production of proteins in transcription-translation coupled LEXSY cell extracts**




**Cell-free production of EGFP reference protein with plasmid-based (lanes 1-3) and PCR-based (lanes 4-6) *in vitro* LEXSY. Lane 7 negative control without template, MW molecular size marker. The *in vitro* reactions were carried out for 2 h at 20°C, resolved on 12% SDS-PAGE and EGFP was *in situ* visualized on a UV transilluminator.**

## Selected applications of LEXSY in *Kinetoplastida* research



Cellular Microbiology (2005) 7(3), 383–392 doi:10.1111/j.1462-5822.2004.00468.x

**Bioluminescent *Leishmania* expressing luciferase for rapid and high throughput screening of drugs acting on amastigote-harbouring macrophages and for quantitative real-time monitoring of parasitism features in living mice**

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Differentiation of *Leishmania donovani* in host-free system: analysis of signal perception and response

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Functional characterization of LIT1, the *Leishmania amazonensis* ferrous iron transporter

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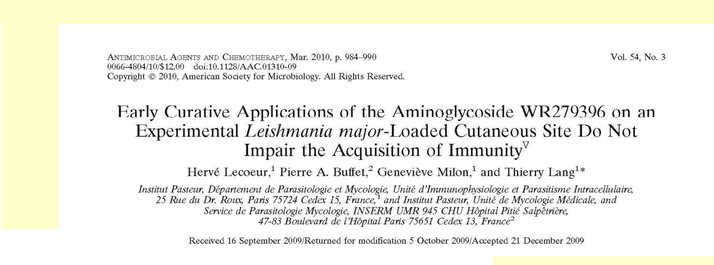

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Early Curative Applications of the Aminoglycoside WR279396 on an Experimental *Leishmania major*-Loaded Cutaneous Site Do Not Impair the Acquisition of Immunity▽

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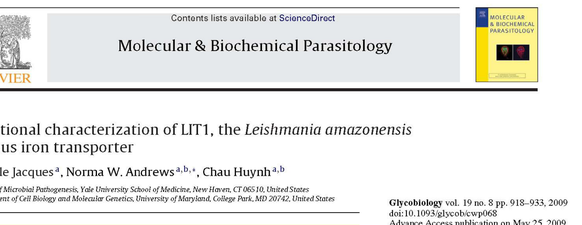

**Glycobiology** vol. 19 no. 8 pp. 918–933, 2009
doi:10.1093/glycob/cwp068
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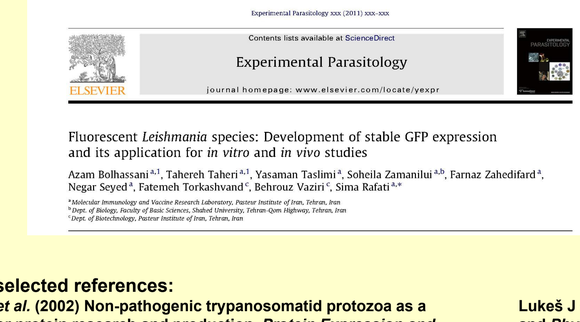
**Molecular analysis of a UDP-GlcNAc:polypeptide α-*N*-acetylglucosaminyltransferase implicated in the initiation of mucin-type *O*-glycosylation in *Trypanosoma cruzi***



Experimental Parasitology xxx (2011) xxx–xxx

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Fluorescent *Leishmania* species: Development of stable GFP expression and its application for *in vitro* and *in vivo* studies

Azam Bolhassani[a,1], Tahereh Taheri[a,1], Yasaman Taslimi[a], Soheila Zamanilui[a,b], Farnaz Zahedifard[a], Negar Seyed[a], Fatemeh Torkashvand[c], Behrouz Vaziri[c], Sima Rafati[a,*]

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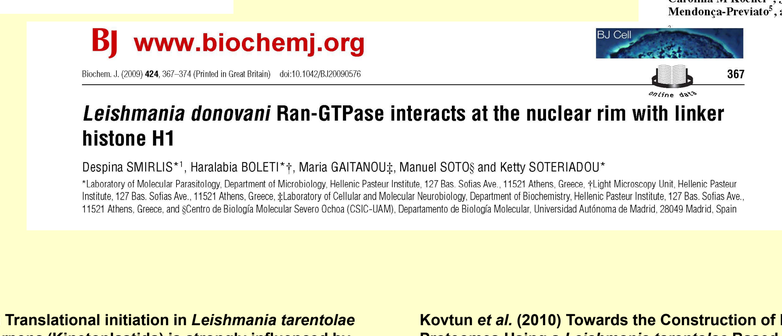

BJ www.biochemj.org

Biochem. J. (2009) **424**, 367–374 (Printed in Great Britain) doi:10.1042/BJ20090576

BJ Cell

367

**_Leishmania donovani_ Ran-GTPase interacts at the nuclear rim with linker histone H1**

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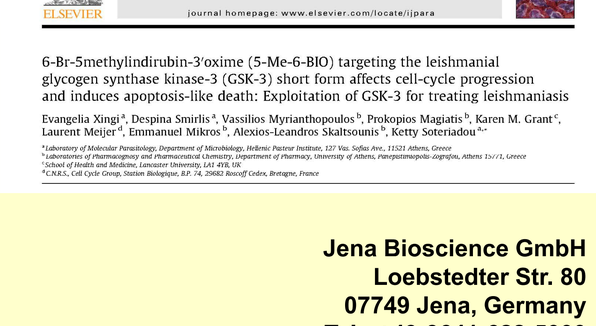

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6-Br-5methylindirubin-3′oxime (5-Me-6-BIO) targeting the leishmanial glycogen synthase kinase-3 (GSK-3) short form affects cell-cycle progression and induces apoptosis-like death: Exploitation of GSK-3 for treating leishmaniasis

Evangelia Xingi[a], Despina Smirlis[a], Vassilios Myrianthopoulos[b], Prokopios Magiatis[b], Karen M. Grant[c], Laurent Meijer[d], Emmanuel Mikros[b], Alexios-Leandros Skaltsounis[b], Ketty Soteriadou[a,*]

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