



Edition des génomes des trypanosomatidés par le système CRISPR-Cas9

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Outils de génie génétiques chez les trypanosomatidés

Trypanosoma brucei

Leishmania sp.

Intégration dans le génome

Tagging

système épisomal

+++

ARNi

Pas de machenerie de RNAi

long

KO

long, plasticité génomique et
aneuploïdie mosaïque



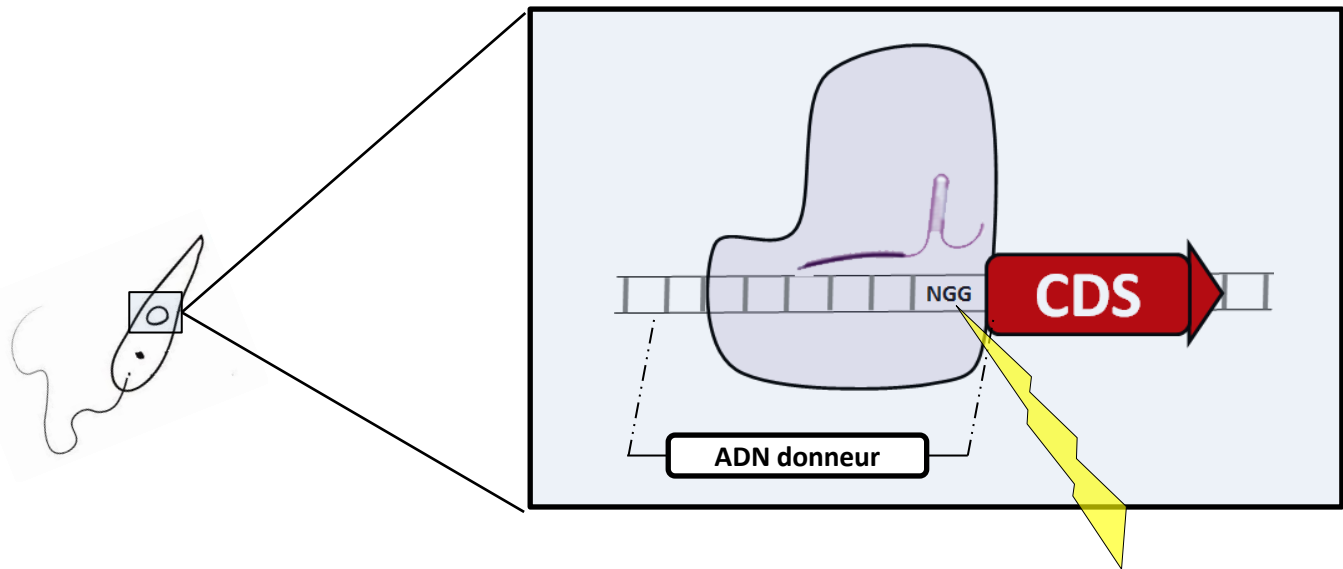
CRISPR-Cas9

CRISPR-Cas9: un puissant outil de génie génétique

1-Cas9 

2-SgRNA 

3- **ADN donneur**



Cassure double brin

Réparation par recombinaison homologue

**Stratégie utilisant des
produits PCR**

KO

CRISPR-Cas9

Stratégie épisomale
Tagging *in situ* marker free

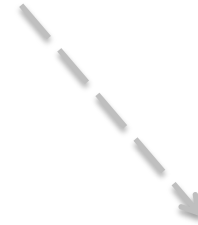
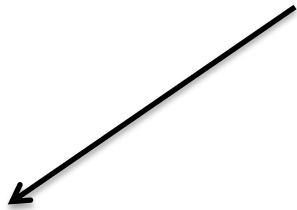
KO Inductible
Stratégie épisomale ...En cours



PCR-based strategy
Gene knockout



CRISPR-Cas9

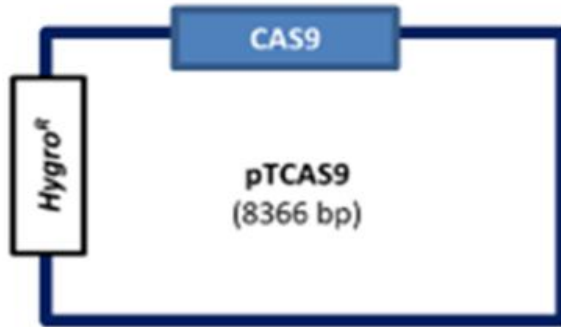


Stratégie épisomale
Tagging *in situ* marker free

Inducible Gene Knockout
Plasmid based Strategy ...On going

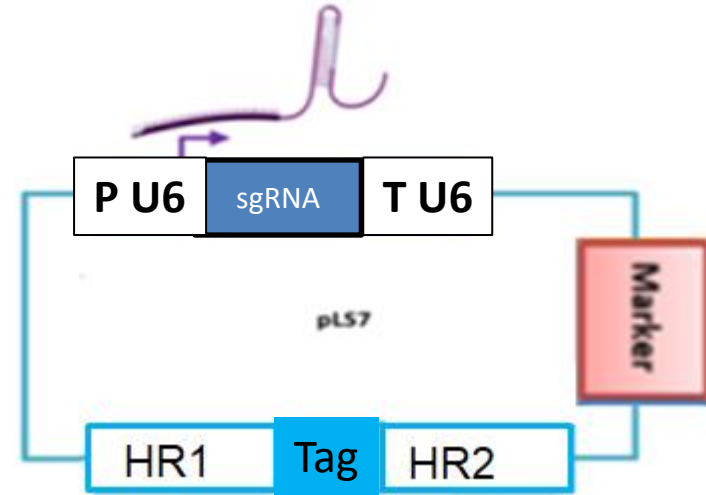
CRISPR-Cas9: Tagging, une stratégie épisomale

1-Cas9



- ✓ **Endonuclease** expression

2-SgRNA

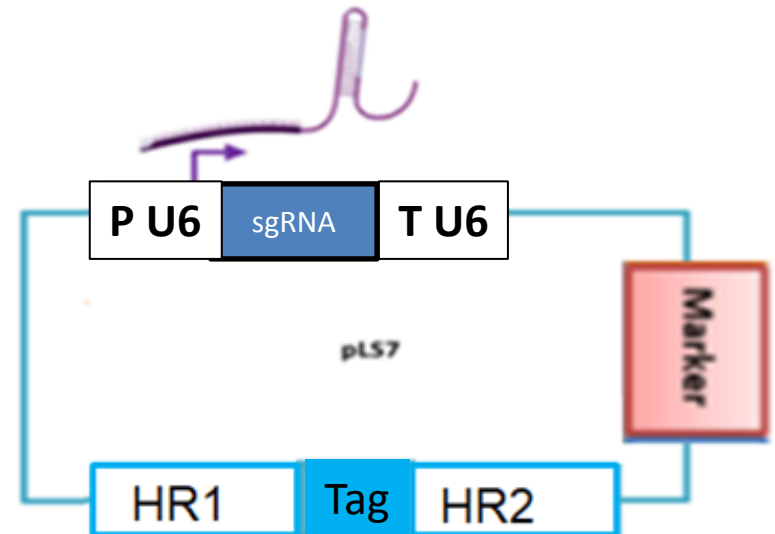
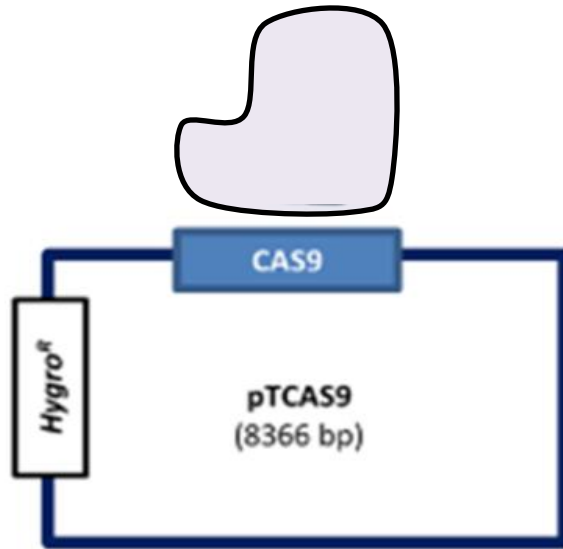


- ✓ **sgRNA** expression sous promoteur U6
- ✓ **ADN donneur** : RH + Tag

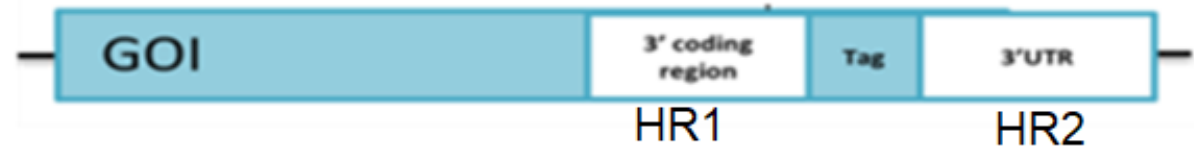
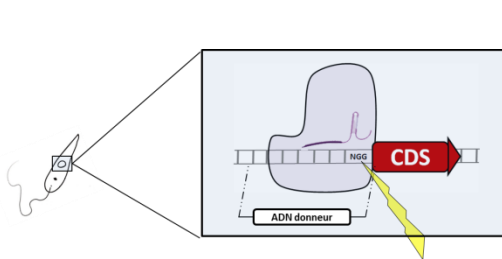
[In situ tagging marker free](#)

(Modifié de Sollelis *et al*, 2015)

CRISPR-Cas9: Tagging, une stratégie épisomale



3-ADN donneur



Insertion du Tag à l'extrémité N ou C-terminale

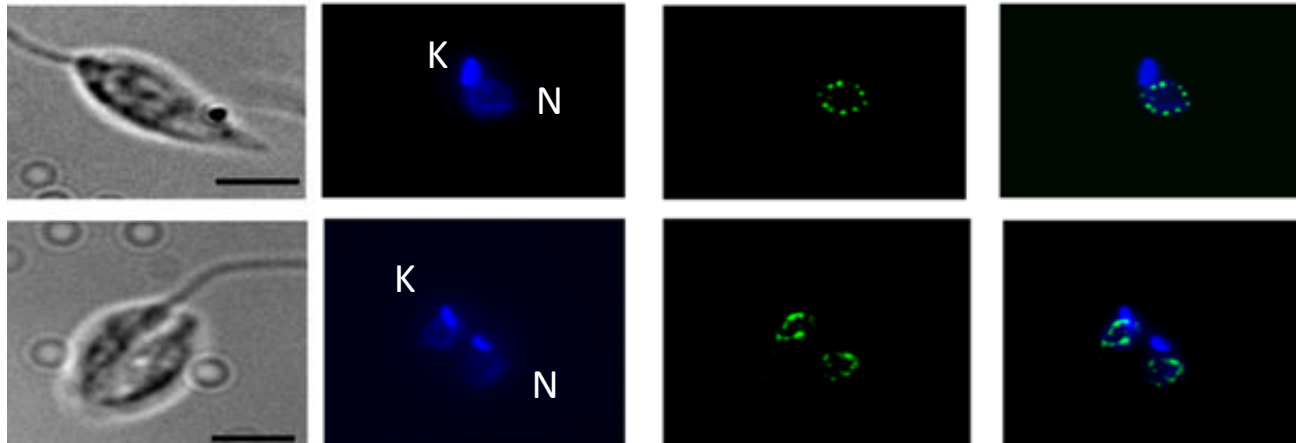
Pas de modification des régions UTRs → prévient d'éventuel changement de localisation et préserve la régulation de l'expression du gène

Localization of MLP1 and MLP2 in *Leishmania* promastigote

MLP1 and MLP2: Myosin Like Protein 1 and 2

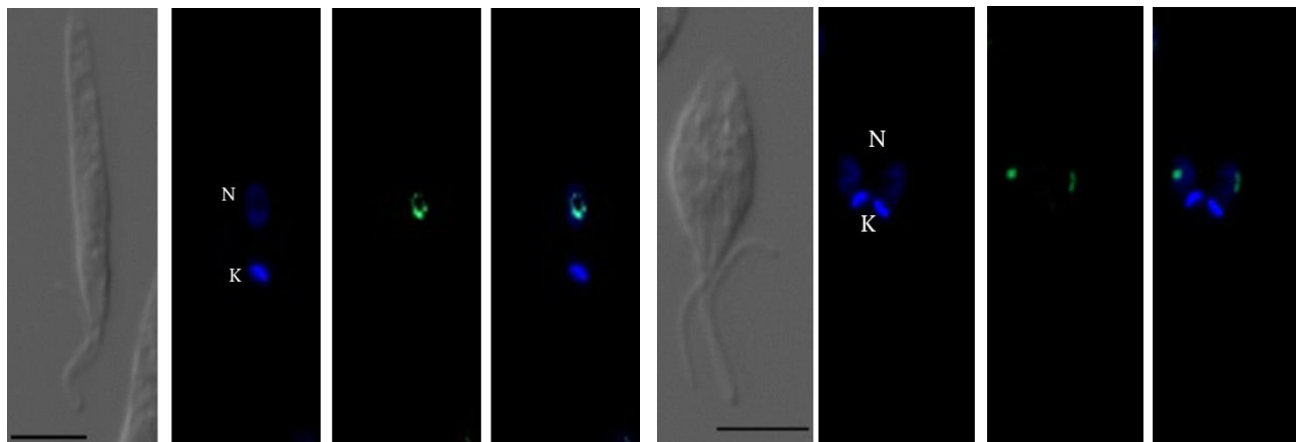
1N1K1F: cellule en interphase

2N2K2F: cellule en division



MLP1:
Enveloppe nucléaire
Tout le long du cycle

MLP2



MLp2:
Enveloppe nucléaire dans
les cellules en interphase

Relocalisation aux **pôles du fuseau** en mitose

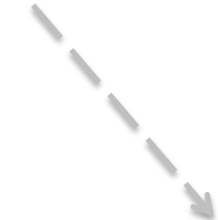
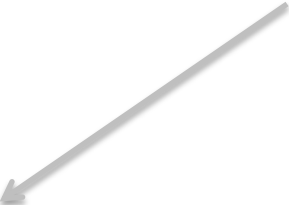
**Stratégie utilisant des
produits PCR**

KO



CRISPR-Cas9

Plasmid-based Strategy
in situ tagging marker free



Inducible Gene Knockout
Plasmid based Strategy ...On going

CRISPR-Cas9 Knockout : PCR-based strategy

The first high-throughput CRISPR Cas9 system in *T. brucei* and *Leishmania*

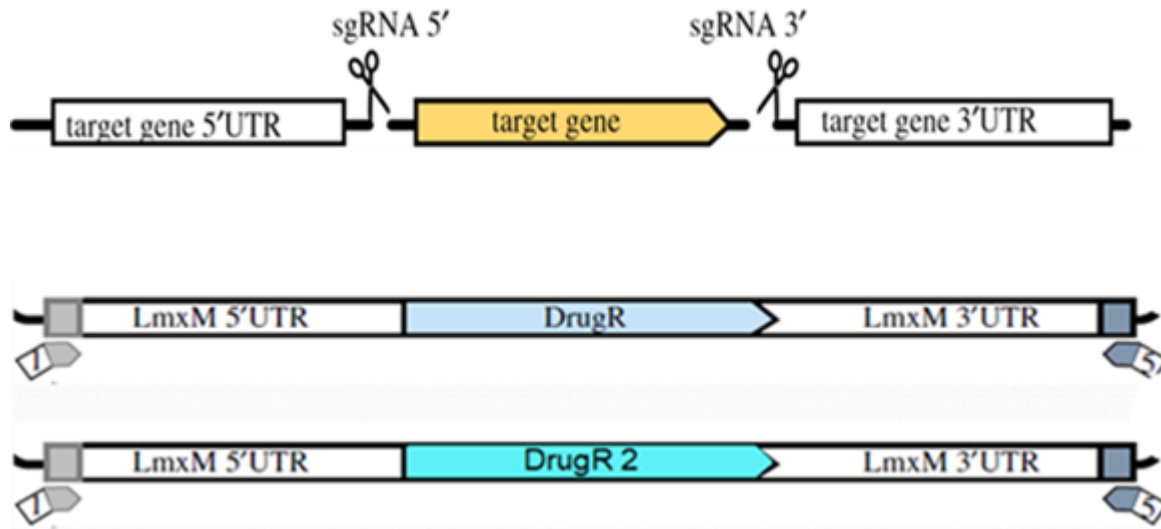
Tom Beneke *et al*, 2017

Souches

Trypanosoma. brucei et *Leishmania spp*
avec Cas9 et T7 pol intégrées



2 sgRNA et 2 cassettes de marqueurs de résistance → Améliore l'efficacité

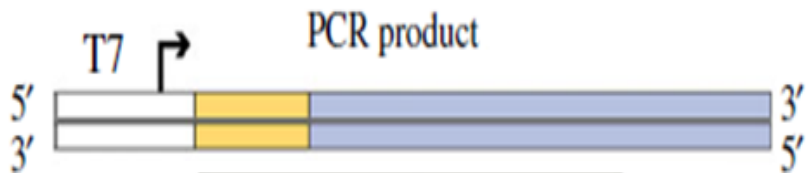
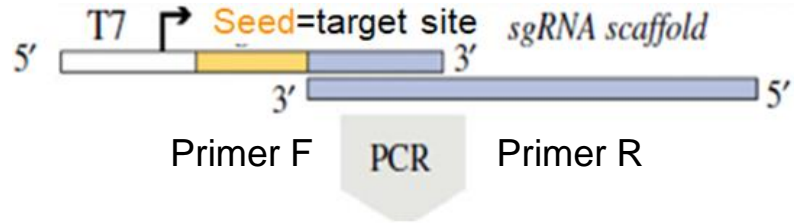


<http://www.leishgedit.net/Home.html>

Eva Gluenz lab,
University of Oxford

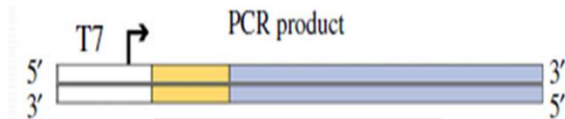
CRISPR-Cas9 Knockout : PCR-based strategy

PCR-generated sgRNA

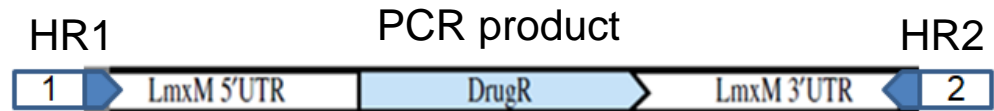
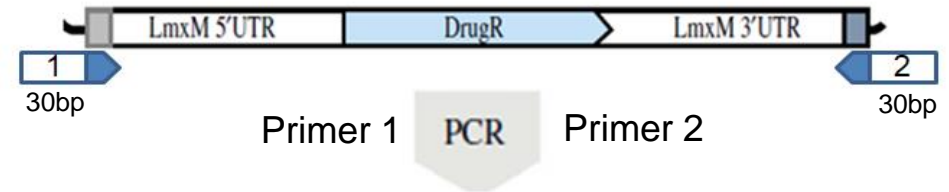


CRISPR-Cas9 Knockout : PCR-based strategy

PCR-generated sgRNA

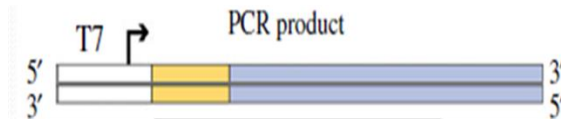


PCR-generated Donor DNA

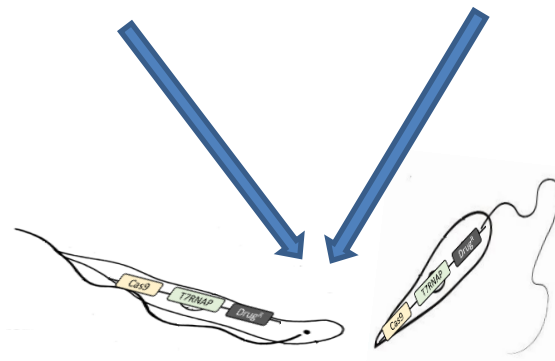
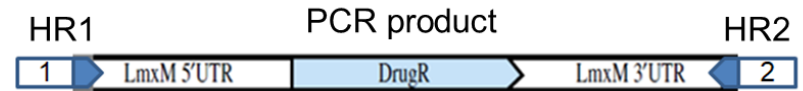
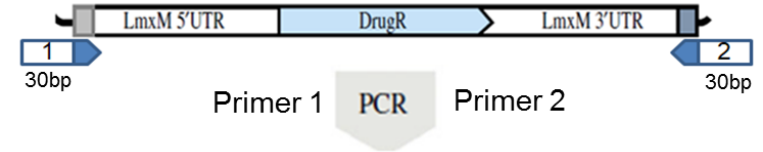


CRISPR-Cas9 Knockout : PCR-based strategy

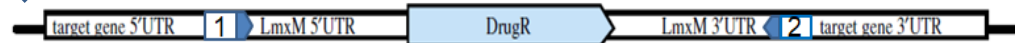
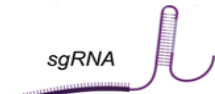
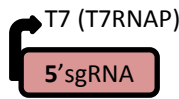
PCR-generated sgRNA



PCR-generated Donor DNA



2-3 semaines de sélection



CRISPR-Cas9 Knockout : PCR-based strategy

Knockout de Mlp2

- *Leishmania*

- *T. brucei*

PCR
intégration

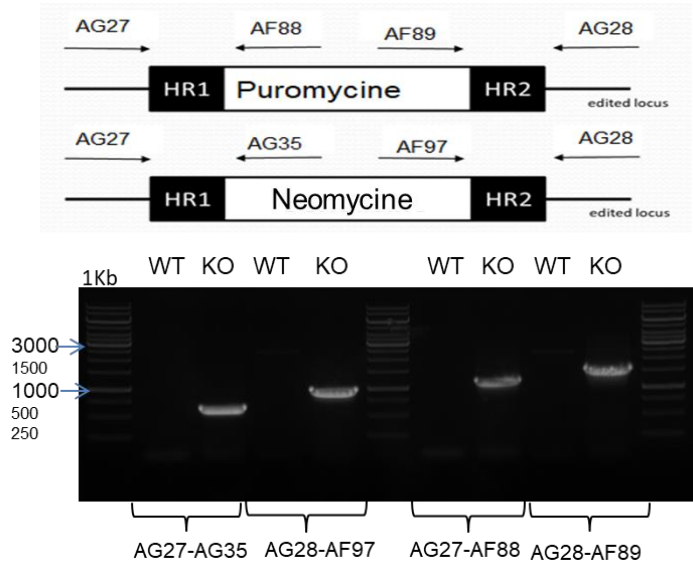
PCR allèle
sauvage

CRISPR-Cas9 Knockout : PCR-based strategy

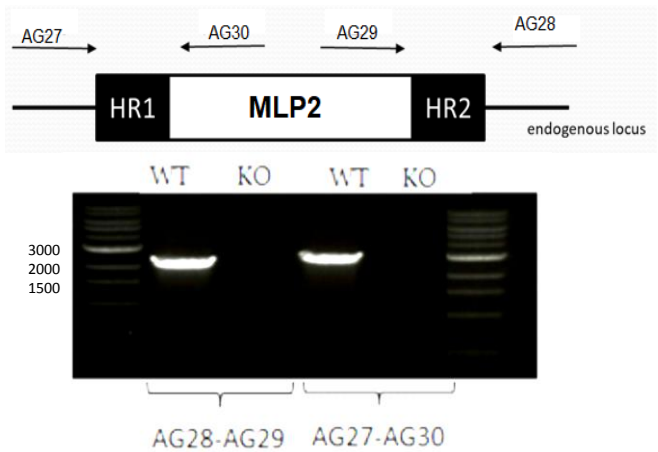
Knockout de Mlp2

- Leishmania*

- T. brucei*



PCR
intégration



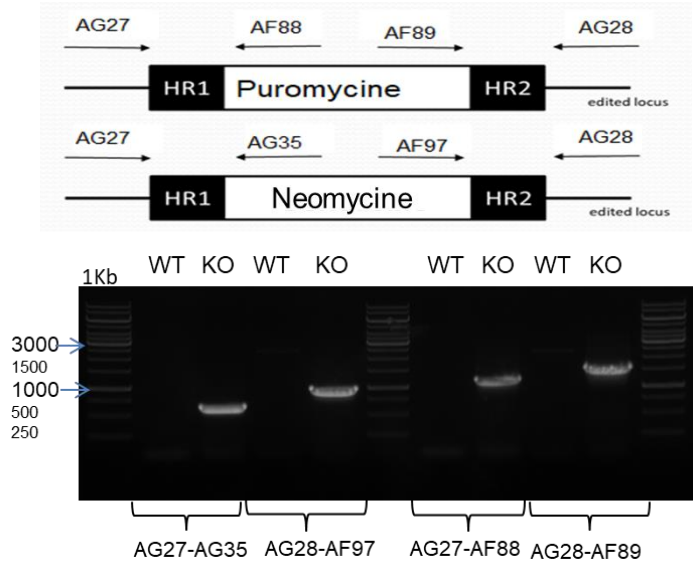
PCR allèle
sauvage

CRISPR-Cas9 Knockout : PCR-based strategy

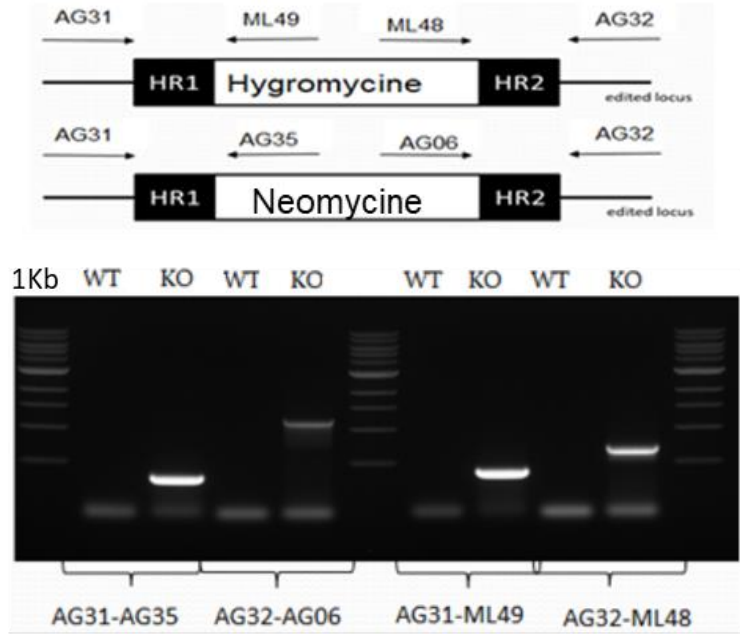
Knockout de Mlp2

- Leishmania*

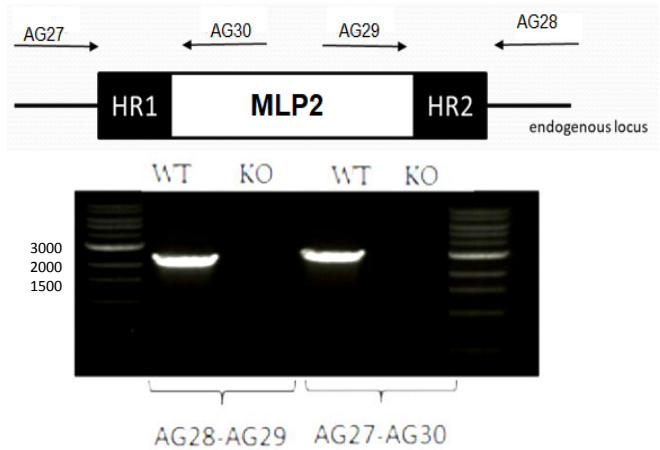
- T. brucei*



PCR
intégration



KO-TbMLP2.....en cours



PCR allèle
sauvage

CRISPR-Cas9 Knockout

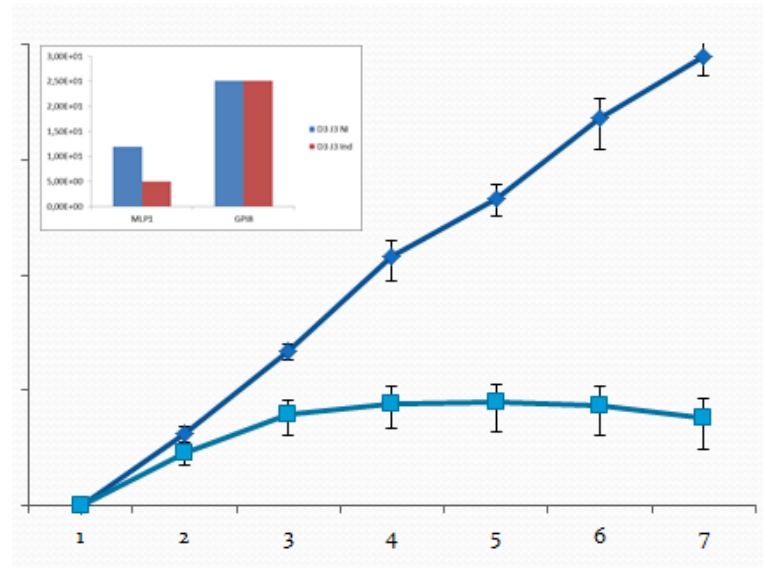
Knockout de Mlp1 chez *Leishmania* et *T. brucei*



Knockout de MLP1 →

Echecs

RNAi de TbMLP1 →



MLP1 est un gène essentiel

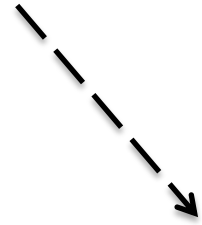
↩
Système inductible

PCR-based strategy
Gene knockout



CRISPR-Cas9

Plasmid-based Strategy
In situ tagging marker free



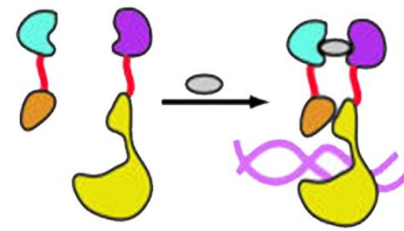
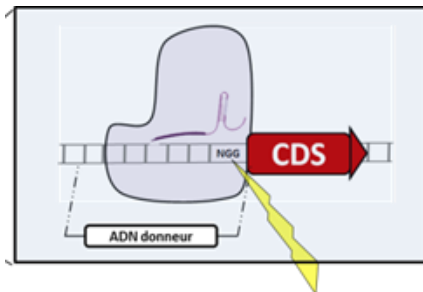
KO Inductible
Stratégie épisomale ...

combinant

Cas9

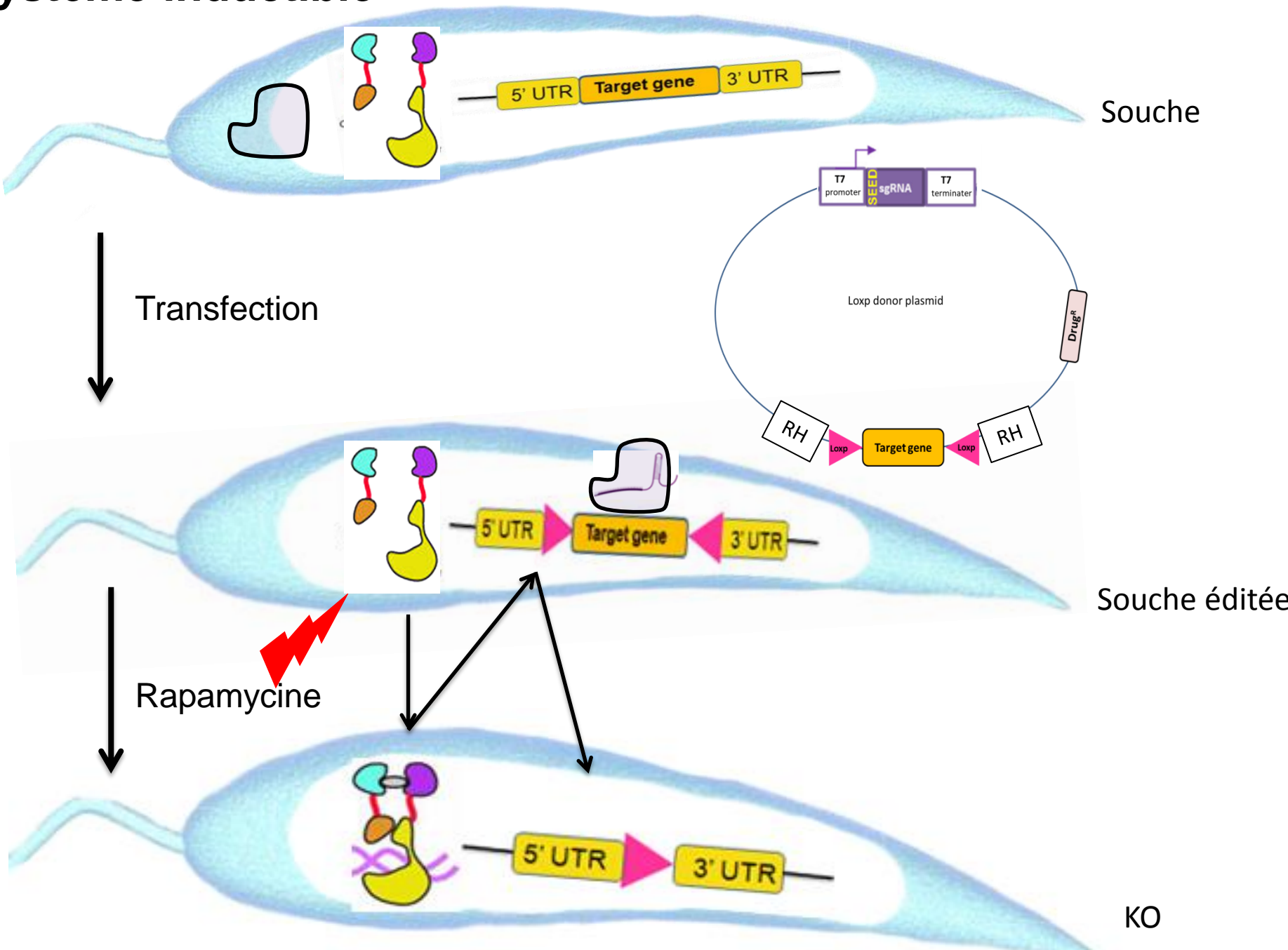
et

diCRE



Mottram's lab

Système inductible



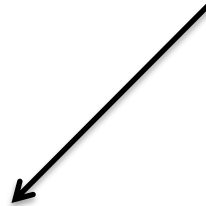
Conclusion

Pour les Knockout

Uniquement des produits de PCR, sans clonage moléculaire (Beneke et al , 2017)



CRISPR-Cas9



Pour le tagging

Stratégie épisomale permettant de réaliser l'édition sans marqueur de résistance, sans modifier les UTRs (Sollelis et al, 2015)

Pour le knockout des gènes essentiels

En développement, un système inductible combinant le CRISPR-Cas9 et le système DiCRE





Remerciements...



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