



# 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

+++

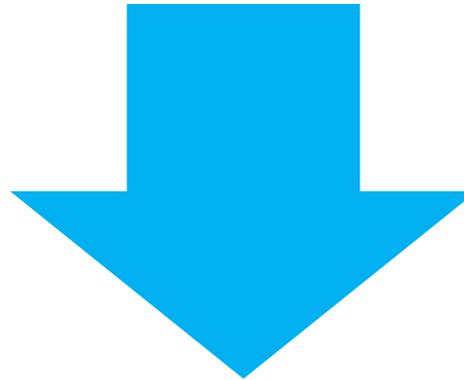
**ARNi**

Pas de machenerie de RNAi

long

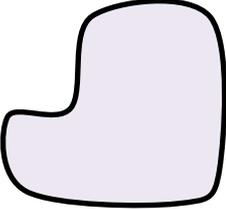
**KO**

long, plasticité génomique et  
aneuploïdie mosaïque



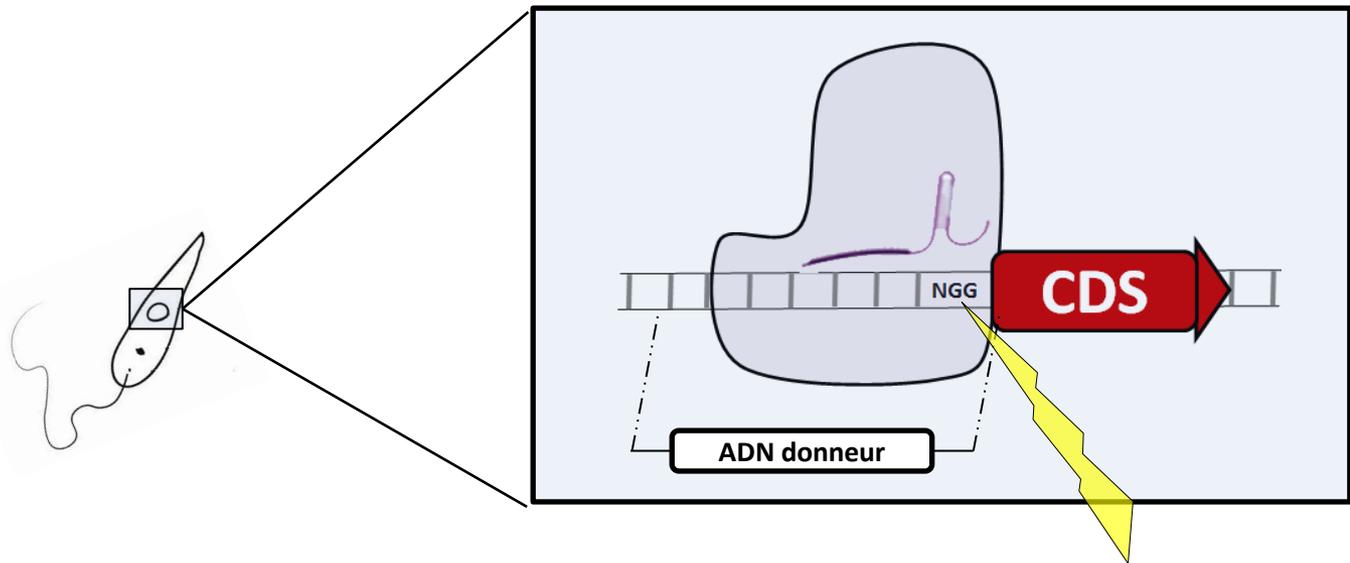
**CRISPR-Cas9**

# 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

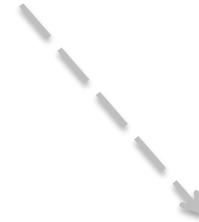
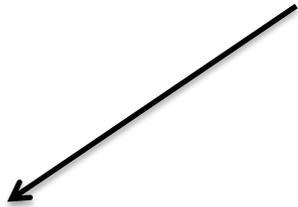
**KO Inductible**  
Stratégie épisomale ...En cours



PCR-based strategy  
Gene knockout



**CRISPR-Cas9**

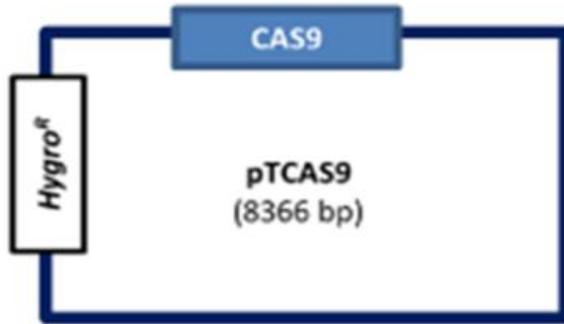


**Stratégie épisomale**  
Tagging *in situ* marker free

**Inducible Gene Knockout**  
Plasmid based Strategy ...On going

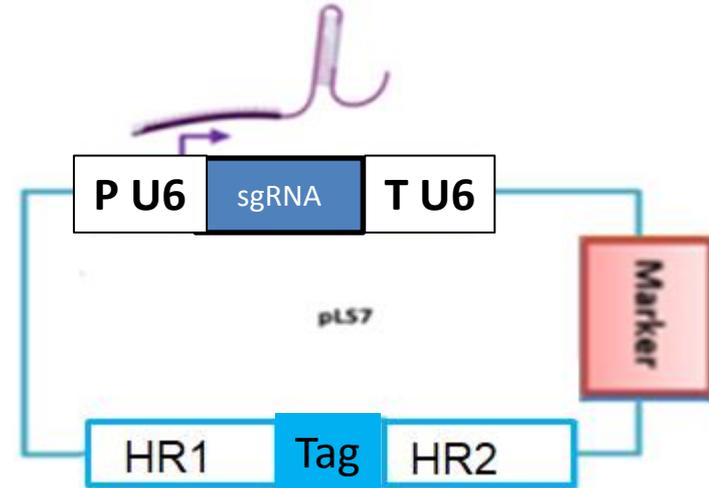
# CRISPR-Cas9: Tagging, une stratégie épisomale

1-Cas9



- ✓ **Endonuclease** expression

2-SgRNA

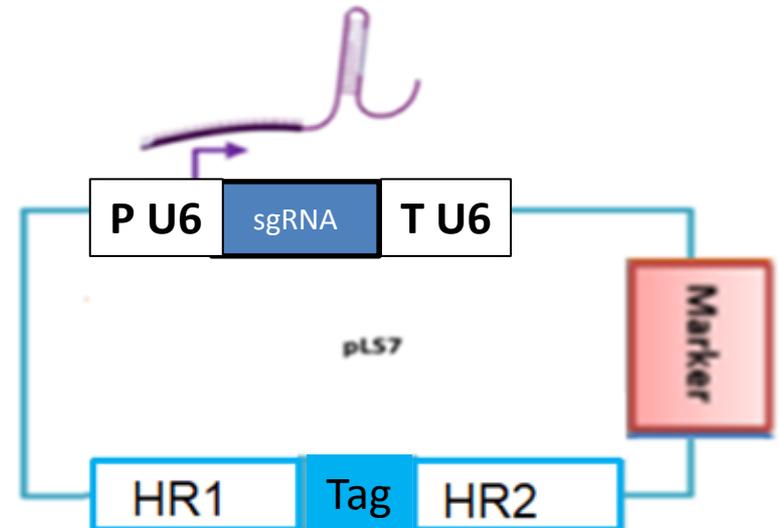
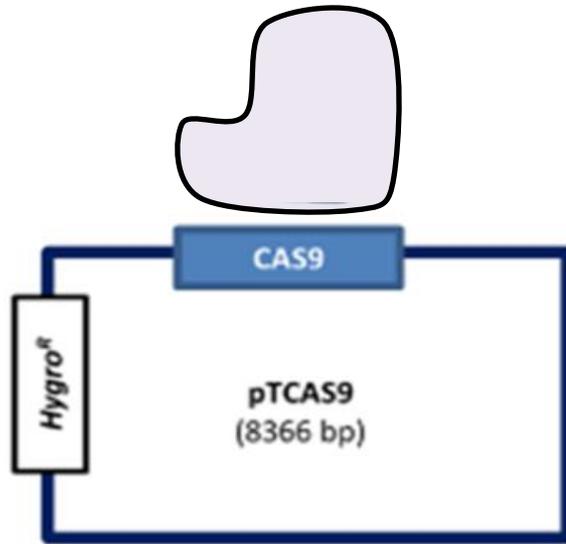


- ✓ **sgRNA** expression sous promoteur U6
- ✓ **ADN donneur** : RH + Tag

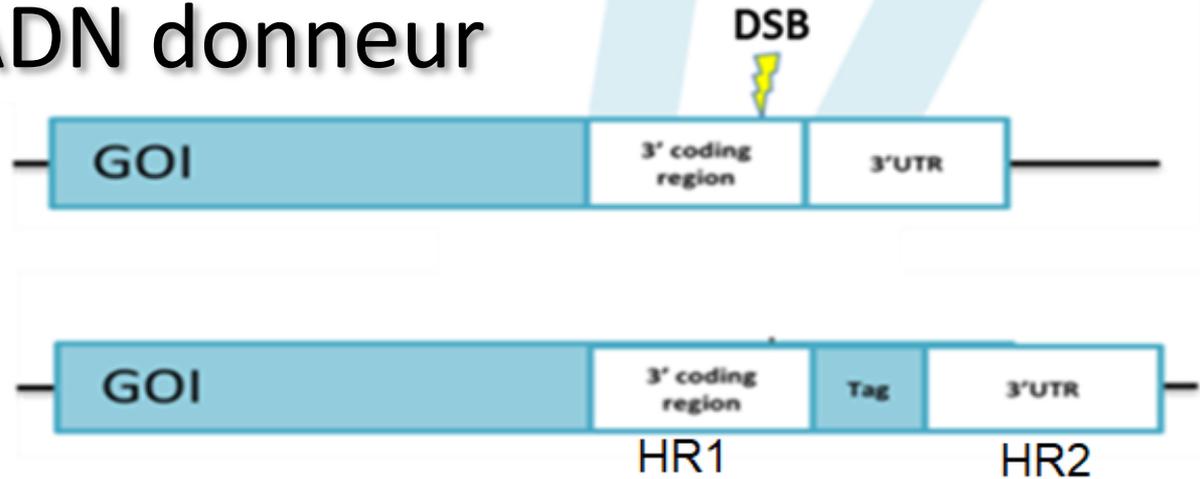
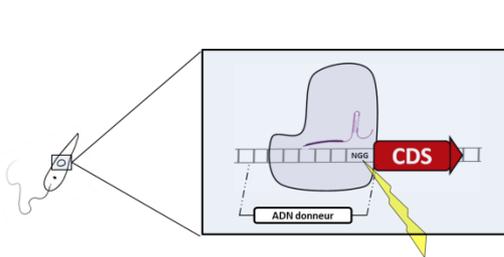
[In situ tagging marker free](#)

(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**

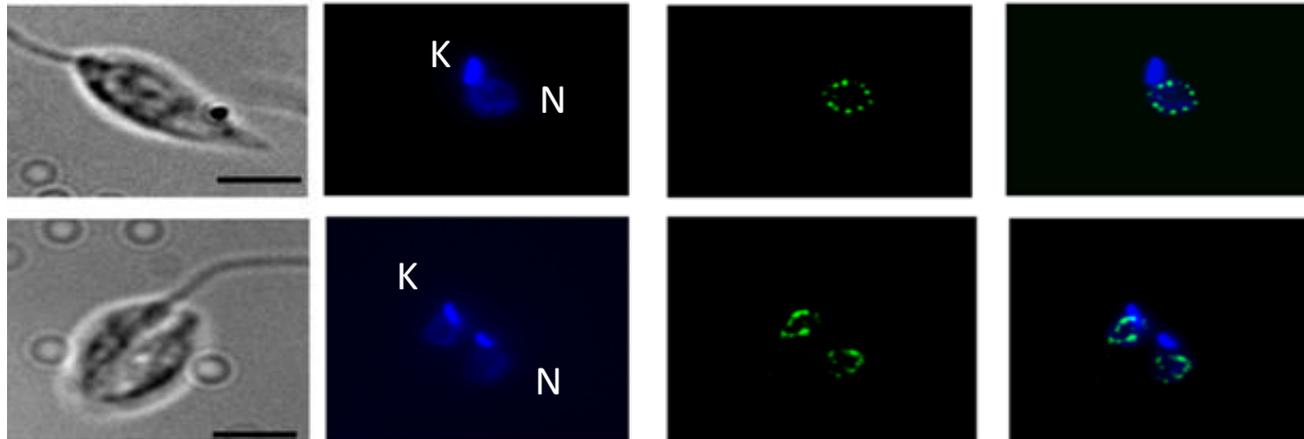
**Pas de modification des régions UTRs → prévient d'éventuel changement de localisation et préserve la régulation de l'expression du gène**

# Localization of MLP1 and MLP2 in *Leishmania* promastigote

MLP1 and MLP2: Myosin Like Protein 1 and 2

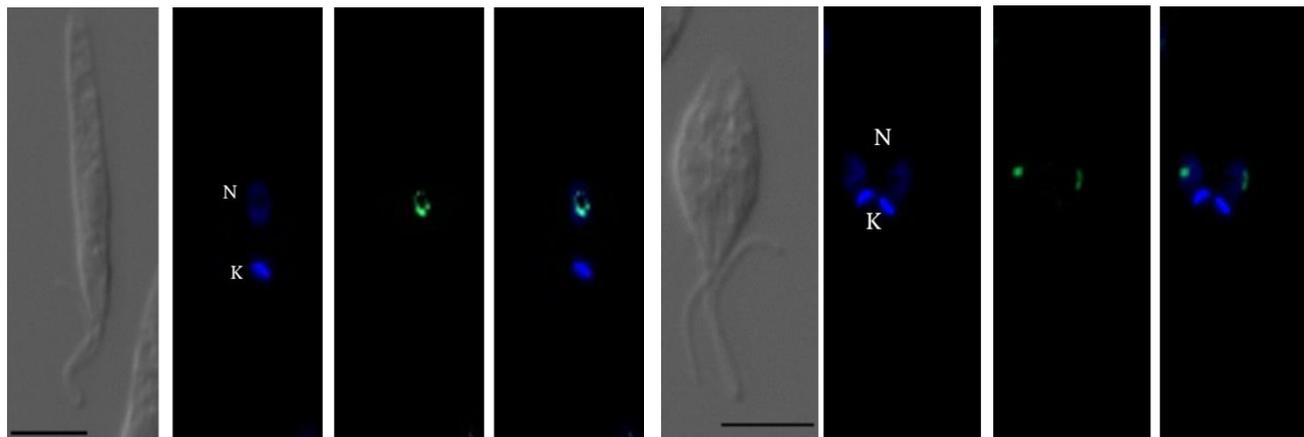
1N1K1F: cellule en interphase

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

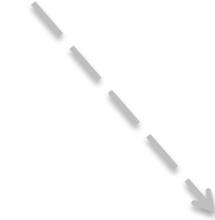
**Stratégie utilisant des  
produits PCR**

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*

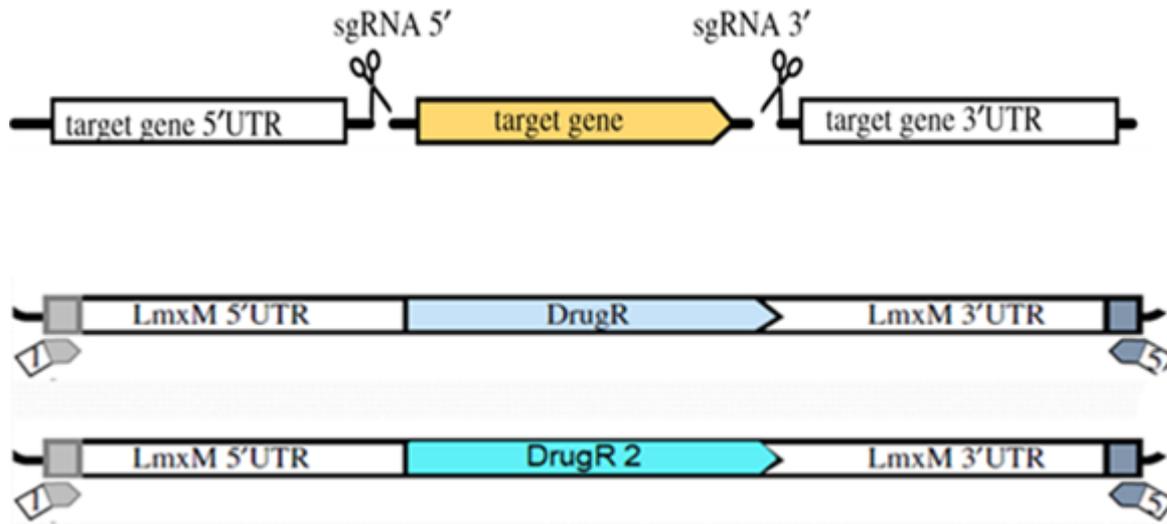
Tom Beneke *et al*, 2017

### Souches

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



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

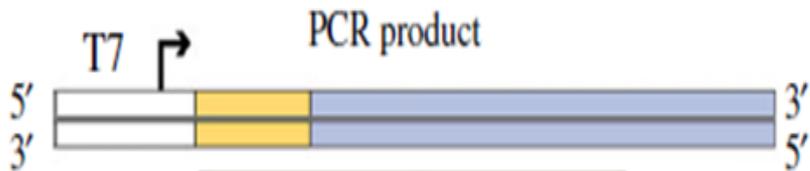
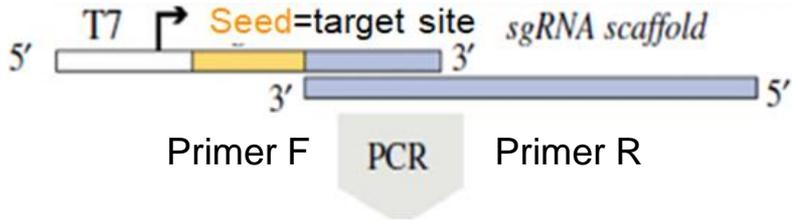


<http://www.leishgedit.net/Home.html>

Eva Gluenz lab,  
University of Oxford

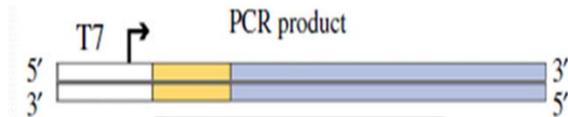
# CRISPR-Cas9 Knockout : PCR-based strategy

## PCR-generated sgRNA

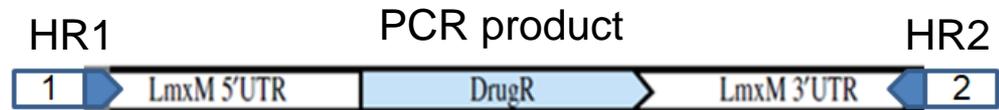
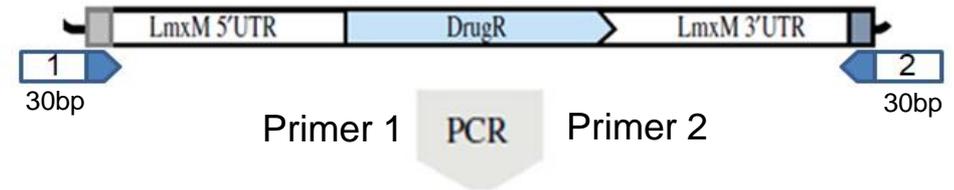


# CRISPR-Cas9 Knockout : PCR-based strategy

## PCR-generated sgRNA

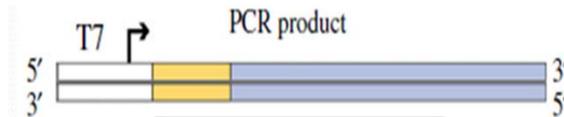


## PCR-generated Donor DNA

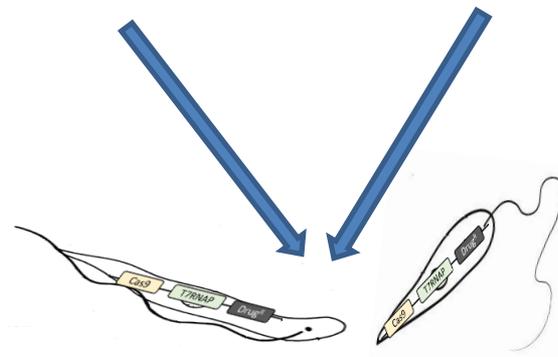
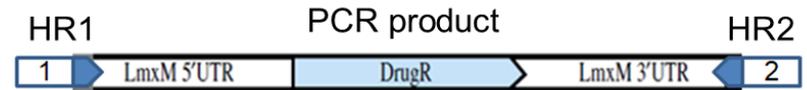
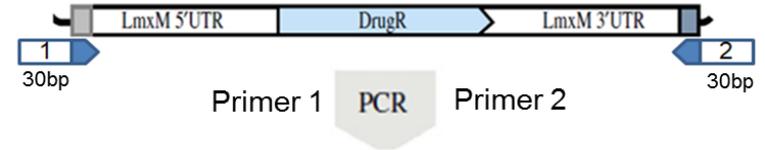


# CRISPR-Cas9 Knockout : PCR-based strategy

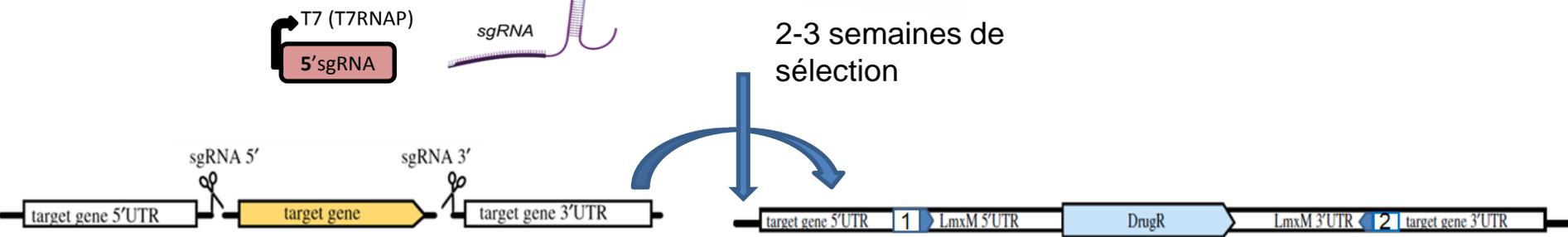
## PCR-generated sgRNA



## PCR-generated Donor DNA



2-3 semaines de sélection



# CRISPR-Cas9 Knockout : PCR-based strategy

## Knockout de Mlp2

- *Leishmania*

- *T. brucei*

PCR  
intégration

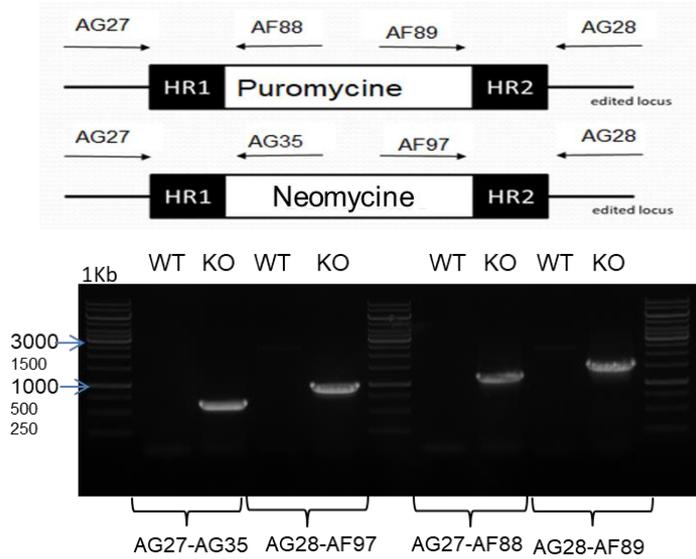
PCR allèle  
sauvage

# CRISPR-Cas9 Knockout : PCR-based strategy

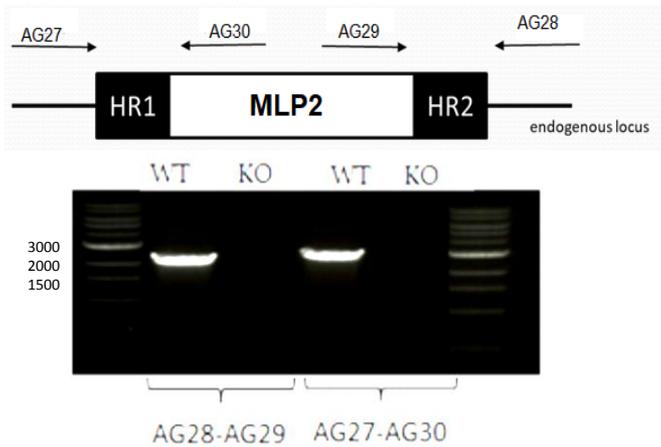
## Knockout de Mlp2

- Leishmania*

- T. brucei*



PCR  
intégration



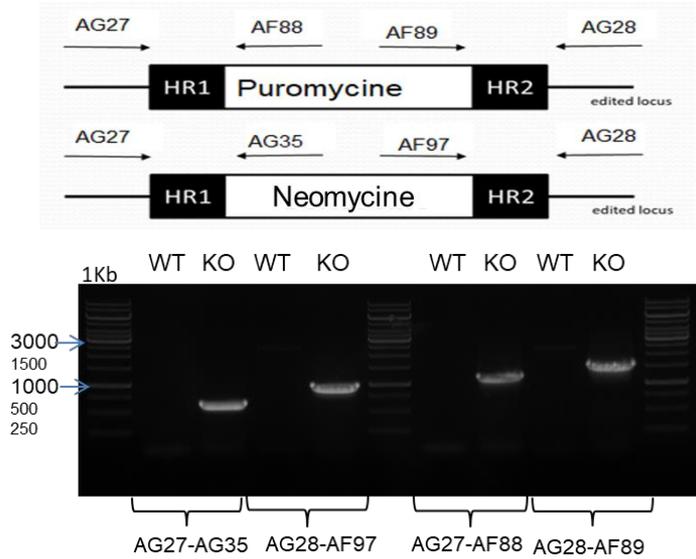
PCR allèle  
sauvage

# CRISPR-Cas9 Knockout : PCR-based strategy

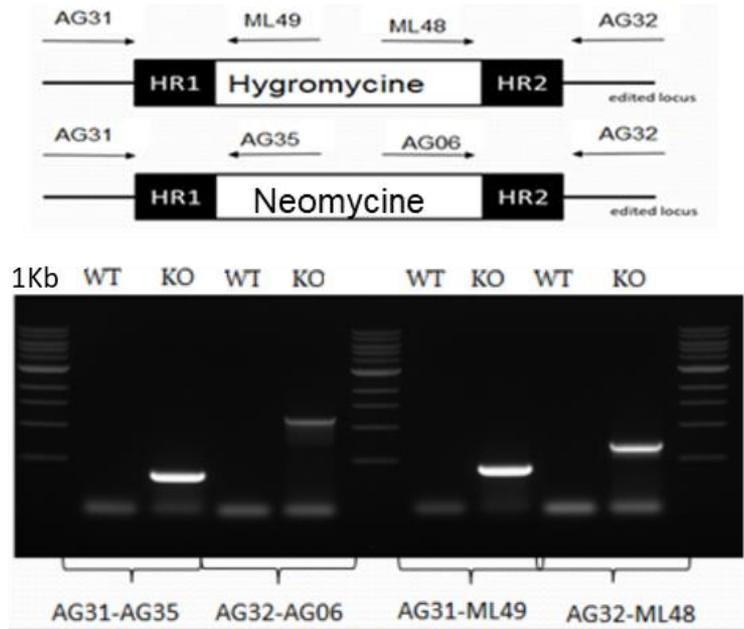
## Knockout de Mlp2

- Leishmania*

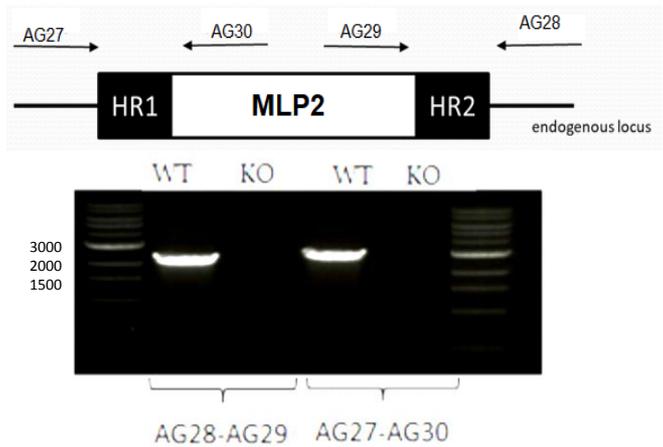
- T. brucei*



PCR  
intégration



KO-TbMLP2.....en cours



PCR allèle  
sauvage

# CRISPR-Cas9 Knockout

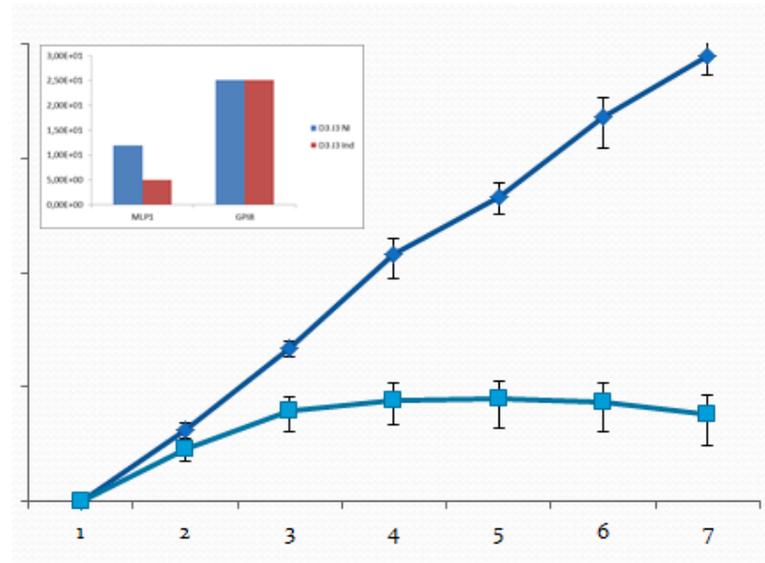
Knockout de Mlp1 chez *Leishmania* et *T. brucei*



Knockout de MLP1 →

Echecs

RNAi de TbMLP1 →



MLP1 est un gène essentiel

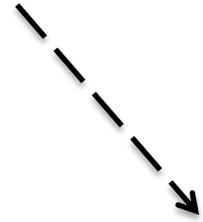
↩  
Système inductible

PCR-based strategy  
Gene knockout



# CRISPR-Cas9

Plasmid-based Strategy  
*In situ* tagging marker free



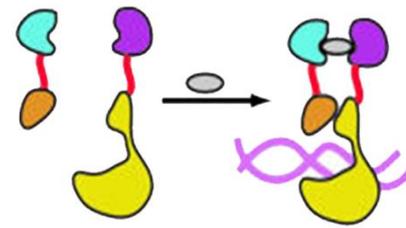
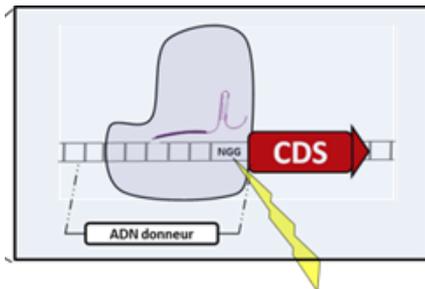
**KO Inductible**  
Stratégie épisomale ...

combinant

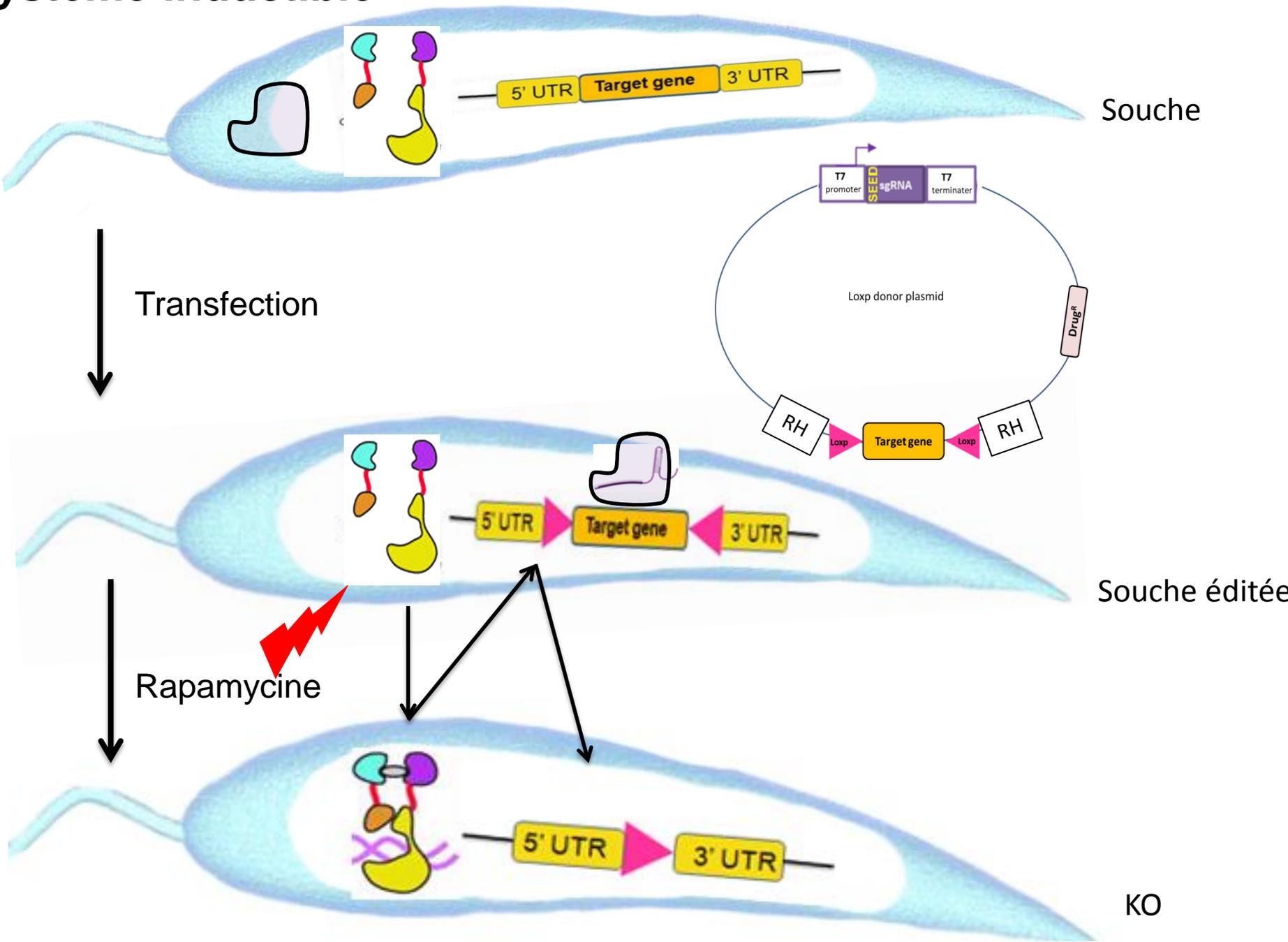
**Cas9**

et

**diCRE**



# Système inducible



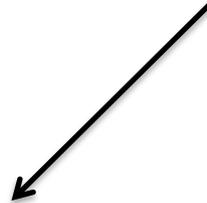
# Conclusion

## 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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