RECHERCHE: les pistes du futur

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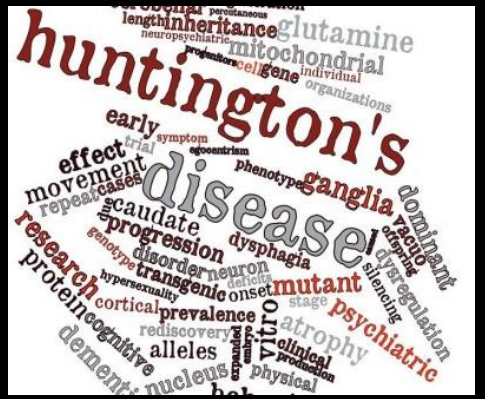
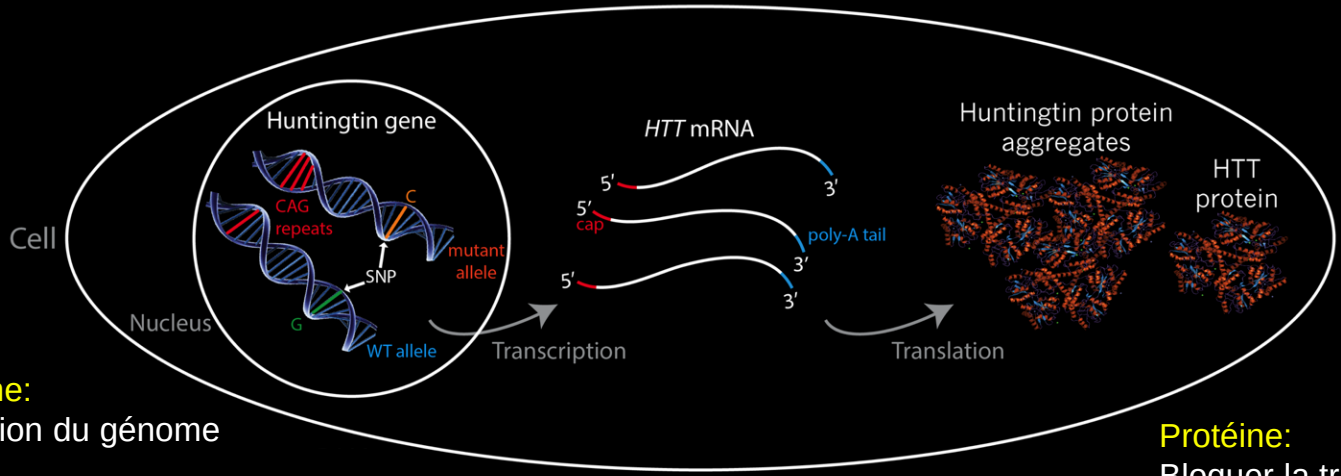
CHUV

DES CISEAUX MOLÉCULAIRES POUR LE TRAITEMENT DE MALADIES GÉNÉTIQUES?



HTT COMME CIBLE THÉRAPEUTIQUE

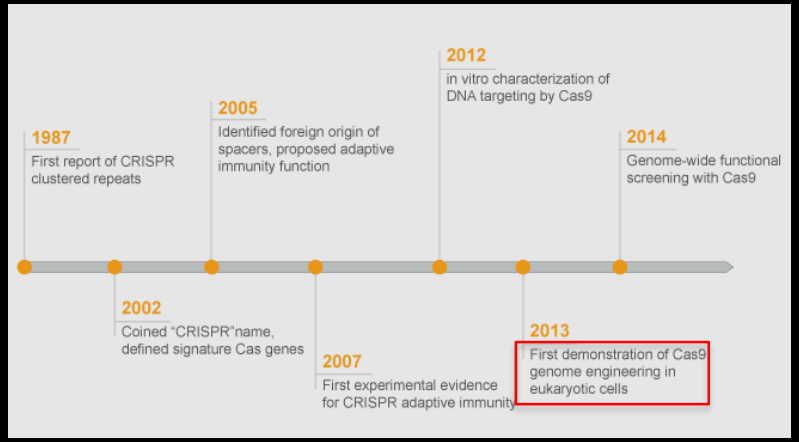


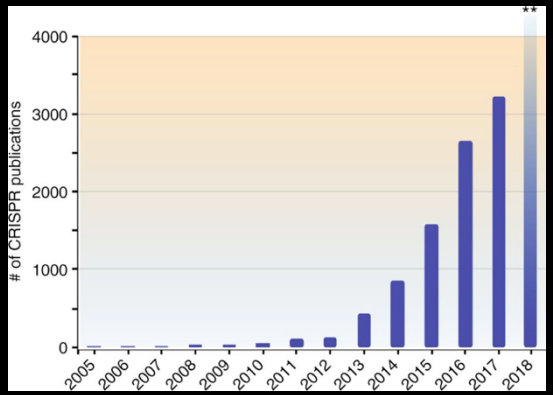
Gene:
Edition du génome

mRNA: dégradation du transcript

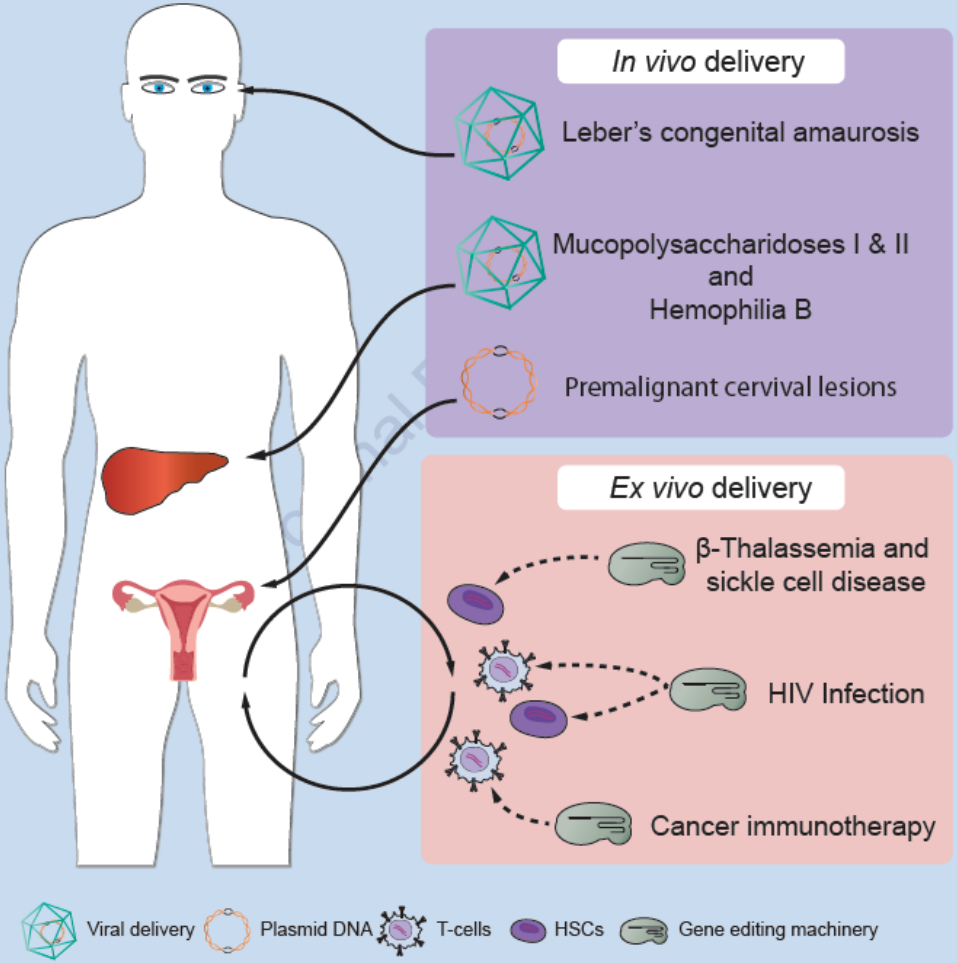
Protéine:
Bloquer la traduction
Améliorer l'élimination

LA REVOLUTION CRISPR-Cas9





Gene editing in clinical trials



ARTICLES (2020) nature medicine
https://doi.org/10.1038/s41591-020-0840-5

Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer

You Lu[1,3,4], Jianxin Xue[1,3,4], Tao Deng[2,3,4], Xiaojuan Zhou[1,3,4], Kun Yu[2,3,4], Lei Deng[2], Meijuan Huang[1], Xin Yi[4], Maozhi Liang[5], Yu Wang[6], Haige Shen[6], Ruizhan Tong[1], Wenbo Wang[7], Li Li[1], Jin Song[4], Jing Li[4], Xiaoxing Su[8], Zhenyu Ding[1], Youling Gong[1], Jiang Zhu[1], Yongsheng Wang[1,5], Bingwen Zou[1], Yan Zhang[1], Yanying Li[1], Lin Zhou[1], Yongmei Liu[1], Min Yu[1], Yuqi Wang[4], Xuanwei Zhang[1], Limei Yin[1], Xuefeng Xia[4], Yong Zeng[2], Qiao Zhou[9], Binwu Ying[10], Chong Chen[11], Yuquan Wei[11], Weimin Li[12] and Tony Mok[13]

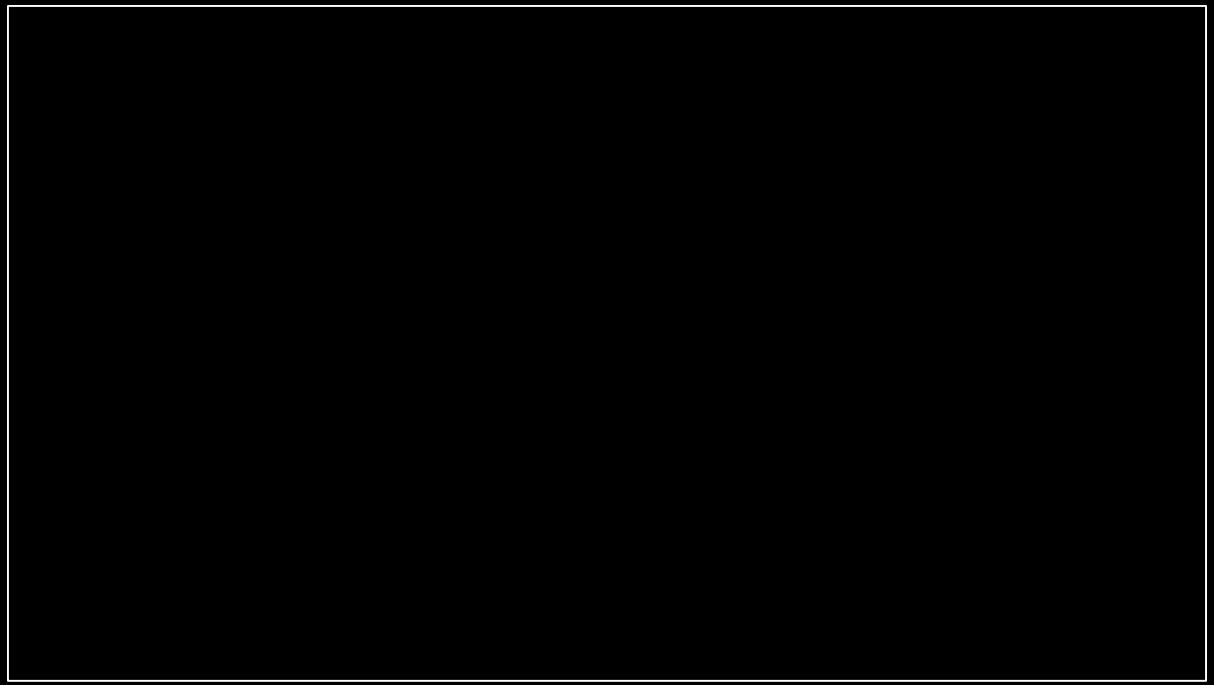
Science (2020) RESEARCH ARTICLES
Cite as: E. A. Stadtmauer et al., Science 10.1126/science.aba7365 (2020).

CRISPR-engineered T cells in patients with refractory cancer

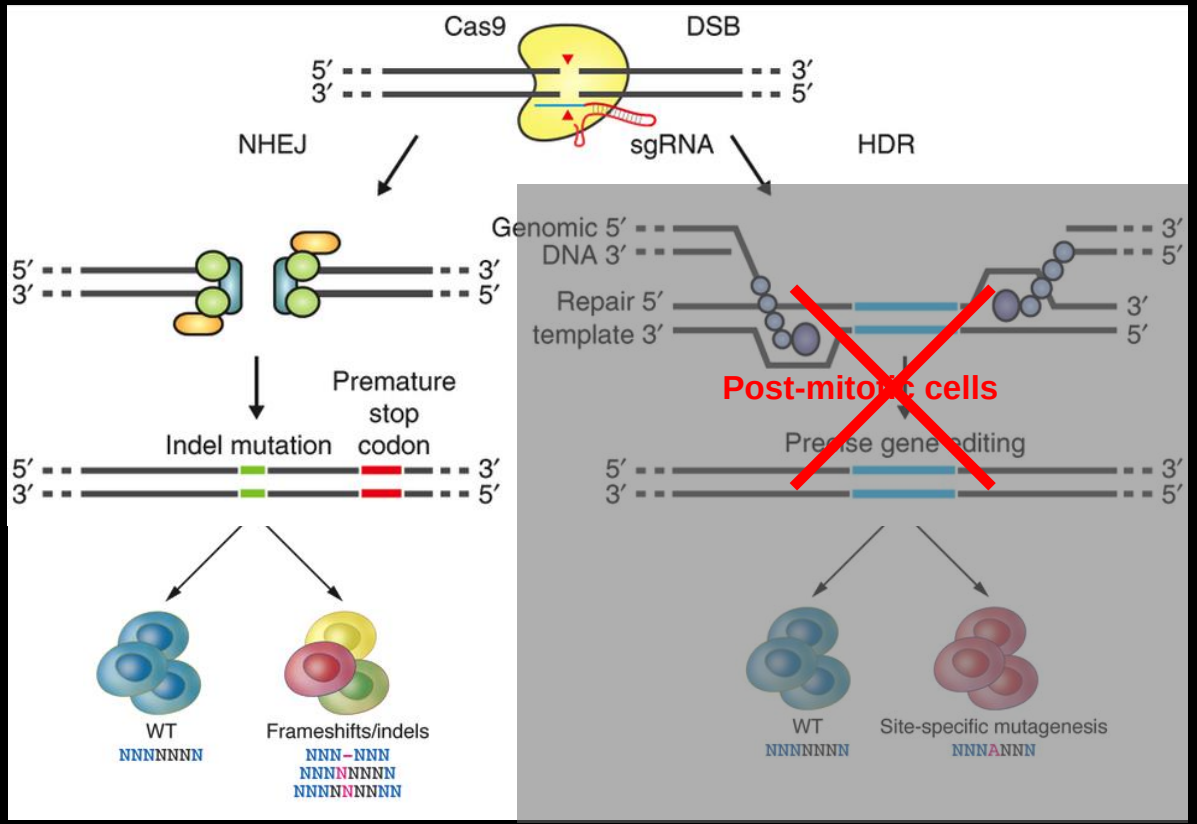
Edward A. Stadtmauer,[1,2*†] Joseph A. Fraietta,[2,3,4,5*] Megan M. Davis,[5,6] Adam D. Cohen,[1,2] Kristy L. Weber,[2,7] Eric Lancaster,[8] Patricia A. Mangan,[1] Irina Kulikovskaya,[2] Minnal Gupta,[2] Fang Chen,[2] Lifeng Tian,[2] Vanessa E. Gonzalez,[2] Jun Xu,[2] In-young Jung,[4,5] J. Joseph Melenhorst,[2,5,6] Gabriela Plesa,[2] Joanne Shea,[2] Tina Matlawski,[2] Amanda Cervini,[2] Avery L. Gaymon,[2] Stephanie Desjardins,[2] Anne Lamontagne,[2] January Salas-Mckee,[2] Andrew Fesnak,[5,6] Donald L. Siegel,[5,6] Bruce L. Levine,[5,6] Julie K. Jadlowsky,[2] Regina M. Young,[2] Anne Chew,[2] Wei-Ting Hwang,[9] Elizabeth O. Hexner,[1,2] Beatriz M. Carreno,[1,5,6] Christopher L. Nobles,[4] Frederic D. Bushman,[4] Kevin R. Parker,[10] Yanyan Qi,[11] Ansuman T. Satpathy,[10,11] Howard Y. Chang,[10,12] Yangbing Zhao,[5,6] Simon F. Lacey,[5,6*] Carl H. June[2,3,5,6*†]

Selon la base de données ClinicalTrial.gov du National Institute of Health, plus de 60 essais cliniques sur l'extraction de gènes sont actuellement en cours

CRISPR-Cas9: comment ça marche ?



EDITION D'UN GENE

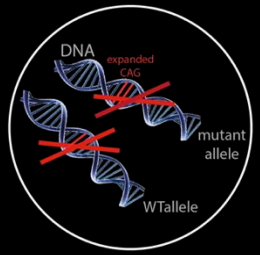


Inactivation du gène

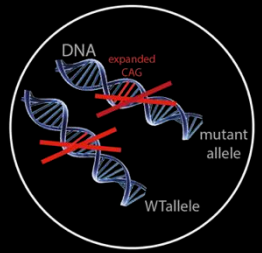
Réparation du gène

Ran et al. (2013) Nat. Prot. 8: 2281-1308

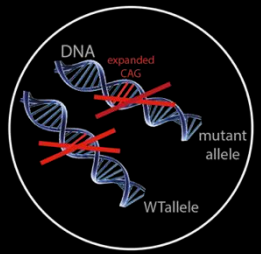
STRATÉGIES D'ÉDITION DU GÈNE HTT



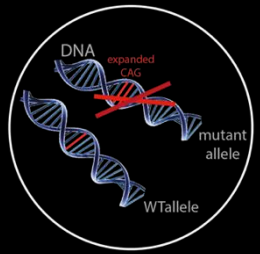
Merienne et al. (2017) Preuve de principe de l'inactivation in vivo de l'allèle HTT mutant et WT



Edition de gènes de deuxième génération

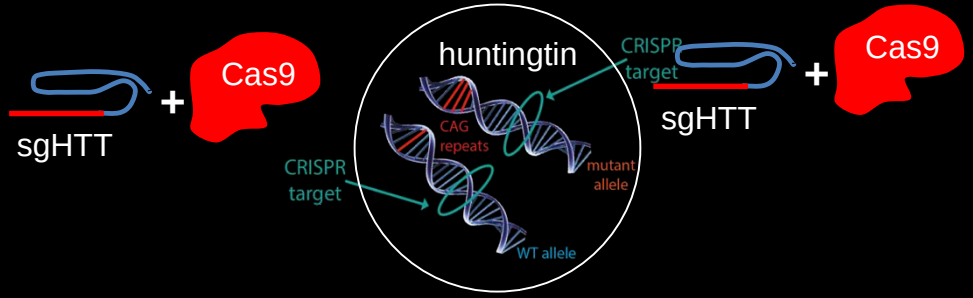


Revisiter les conséquences d'une inactivation de l'HTT sauvage chez l'adulte

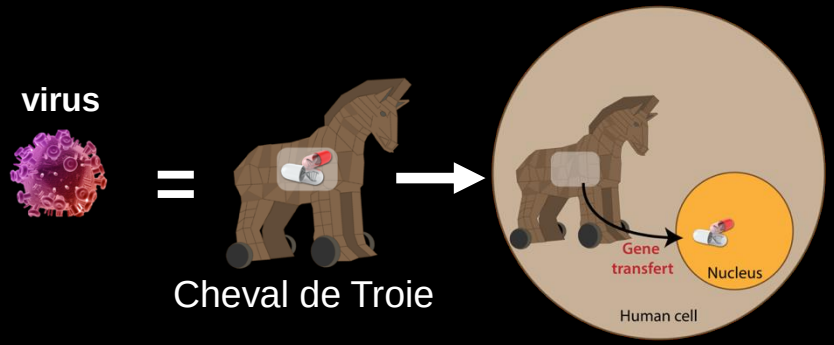


Inactivation de l'allèle HTT mutant et préservation de l'allèle HTT WT

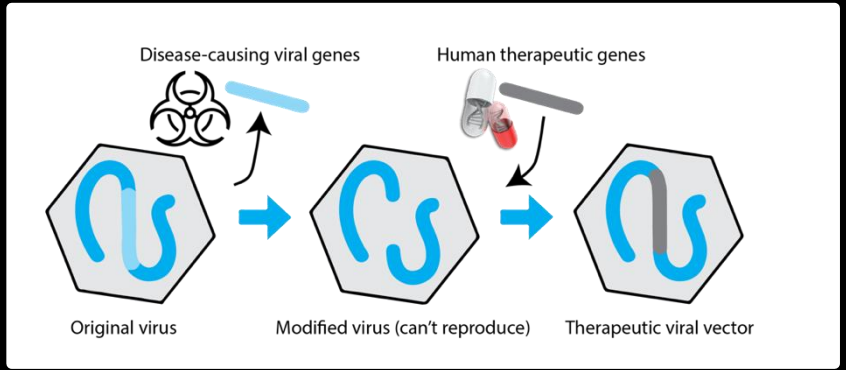
Comment inactiver l'HTT ?



THÉRAPIE GÉNIQUE : DU CONCEPT AUX SYSTÈMES D'ADMINISTRATION SOPHISTIQUÉS



Les virus sont conçus pour délivrer en toute sécurité n'importe quel gène thérapeutique

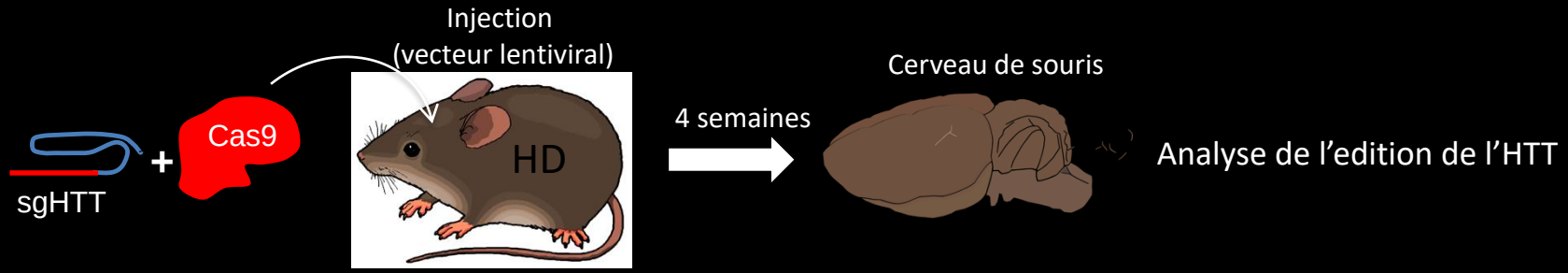


Strimvelis®, approved in May 2016:
Treat ADA-SCID by transplantation of autologous CD34+ cells transduced with a retro viral vector expressing Adenosine Deaminase

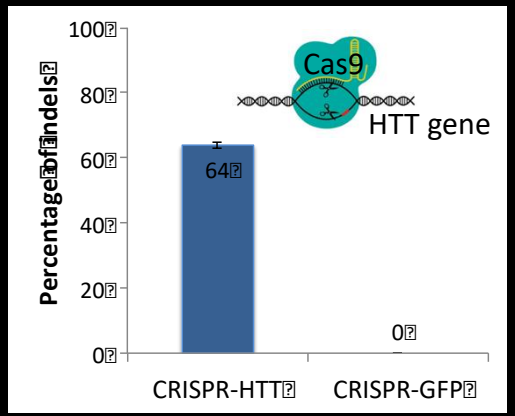
Luxturna®, approved Dec. 2017:
Restore vision in people with rare inherited retinal disease by a subretinal injection of AAV2 expressing working a copy of the *RPE65*.

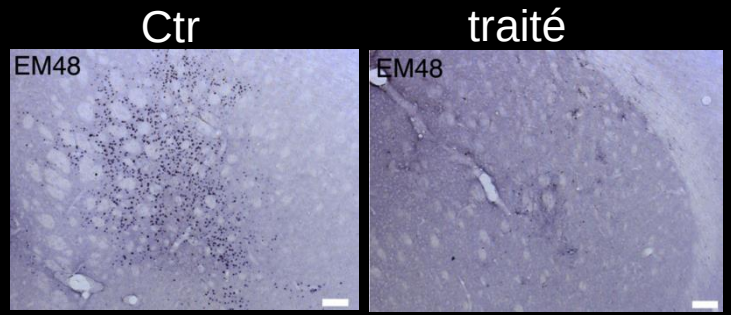
Les vecteurs AAV et lentiviraux sont les deux plus utilisés pour la thérapie génique

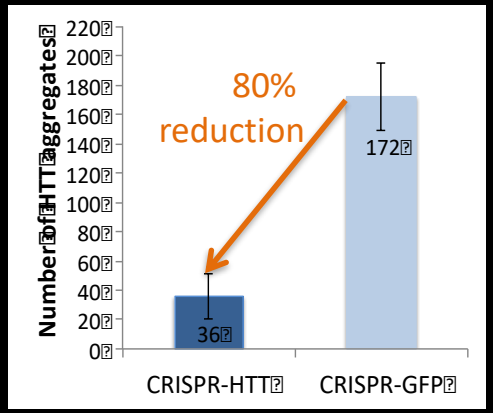
Inactivation du gène de la Huntingtine avec CRISPR-Cas9



Edition de l'HTT

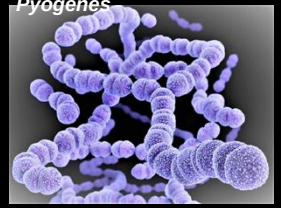






Merienne et al. (2017) Cell Reports 20: 2980-2991

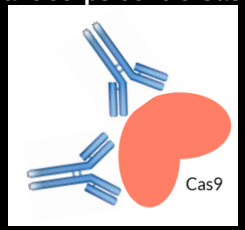
L'EXPRESSION CONSTANTE ET À LONG TERME DE CAS9 DANS LE CERVEAU?



Streptococcus Pyogenes

Staphylococcus Aureus

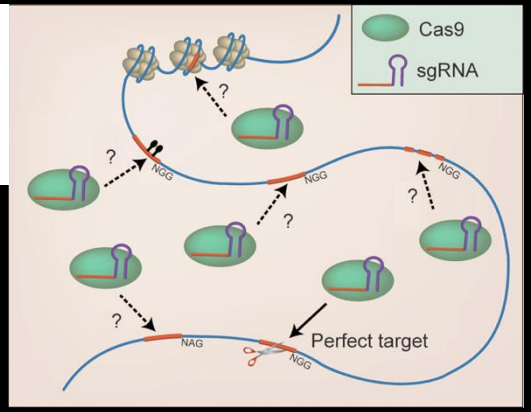
Cas9 bacterial nuclease



anticorps contre Cas9

Immunité préexistante

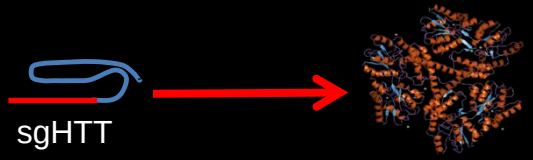
Clivage de gènes indésirables



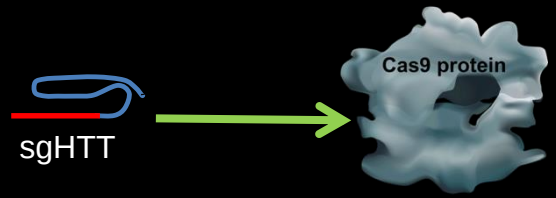
Développer un système d'édition transitoire du génome

Merienne et al. (2017) Cell Reports 20: 2980-2991

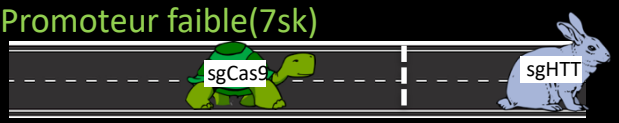
KamiCas9: système d'auto-inactivation de l'édition



H1/U6 = promoteurs forts



7sk = promoteur faible

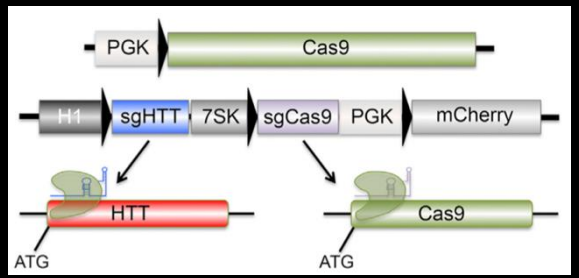


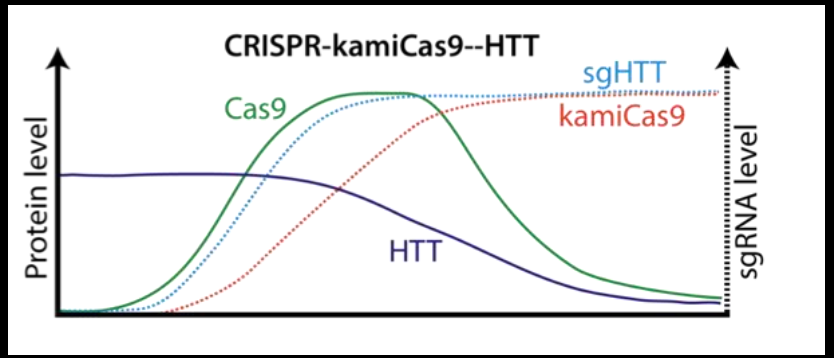
Promoteur fort

HTT inactivé

HTT gene inactivé

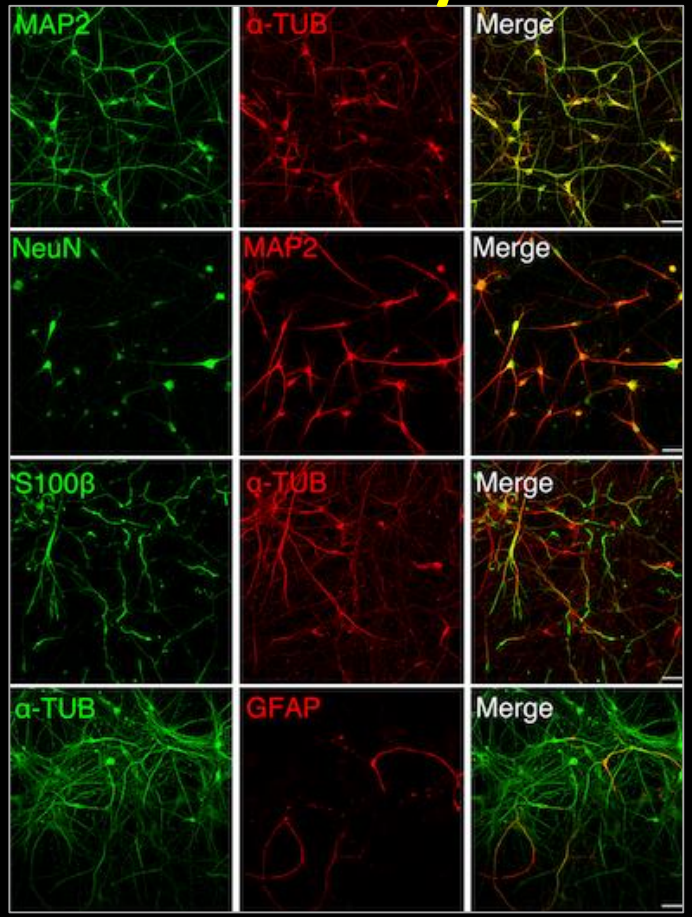
Cas9 inactivé



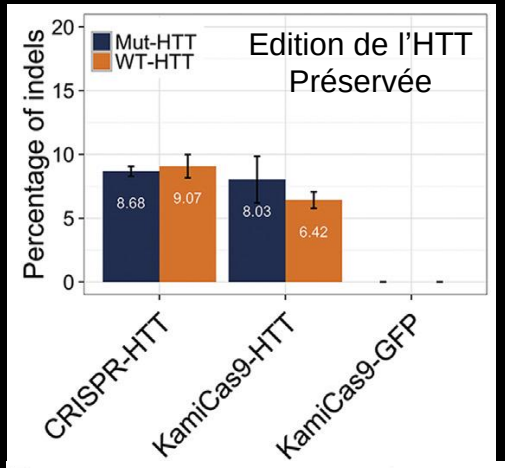


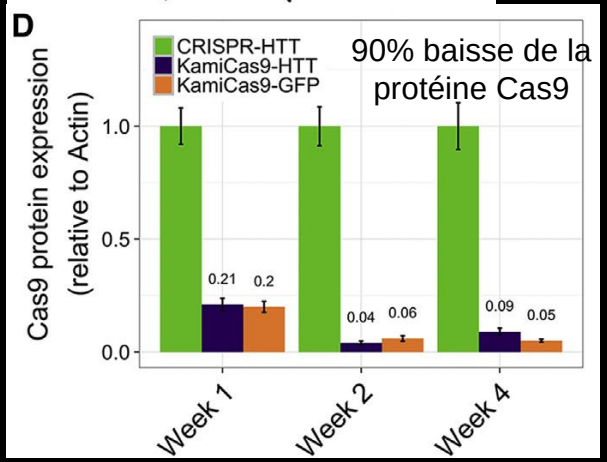
Merienne et al. (2017) Cell Reports 20: 2980-2991

KamiCas9: système d'auto-inactivation de l'édition



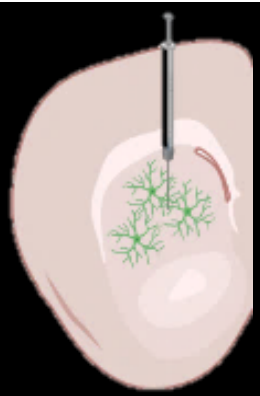
Cellules dérivées de cellules d'un patient MH

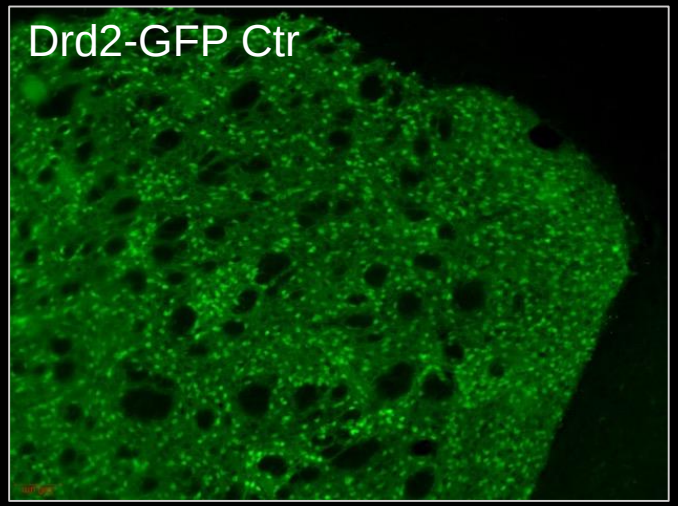


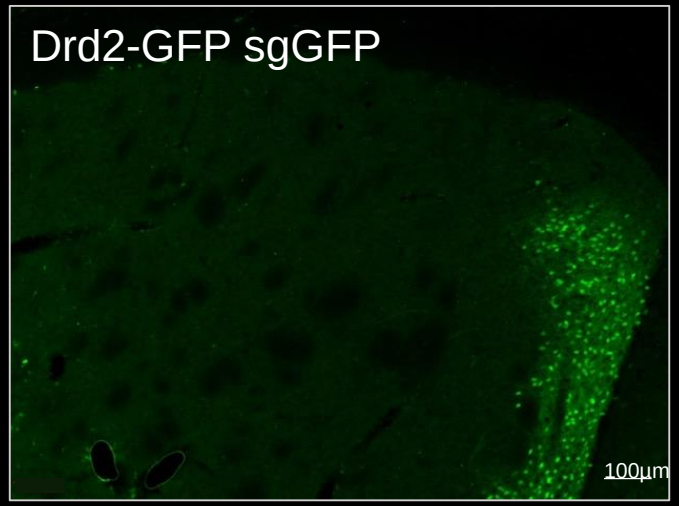


Merienne et al. (2017) Cell Reports 20: 2980-2991

ÉDITION GÉNIQUE OPTIMISÉE DE DEUXIÈME GÉNÉRATION POUR LE CNS



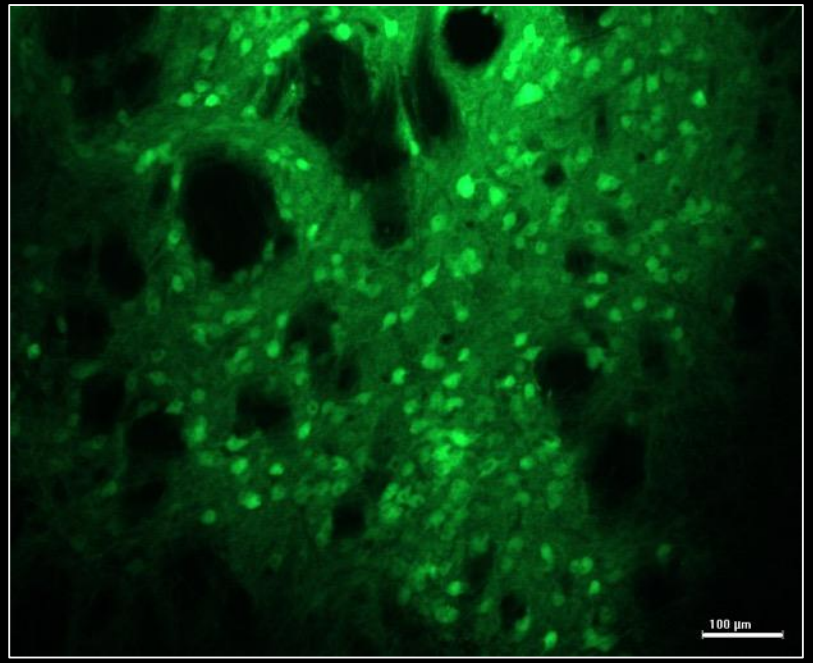


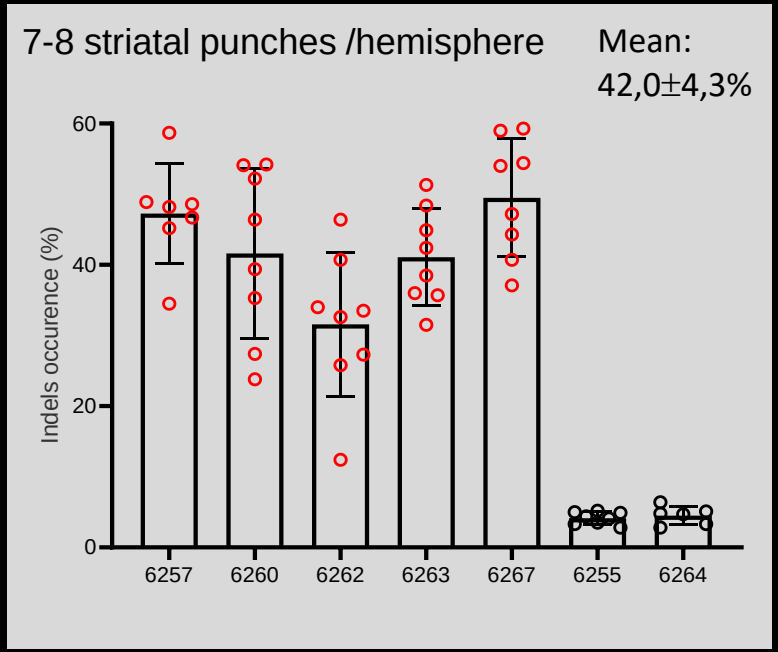


Souris hétérozygotes

Regio et al. unpublished

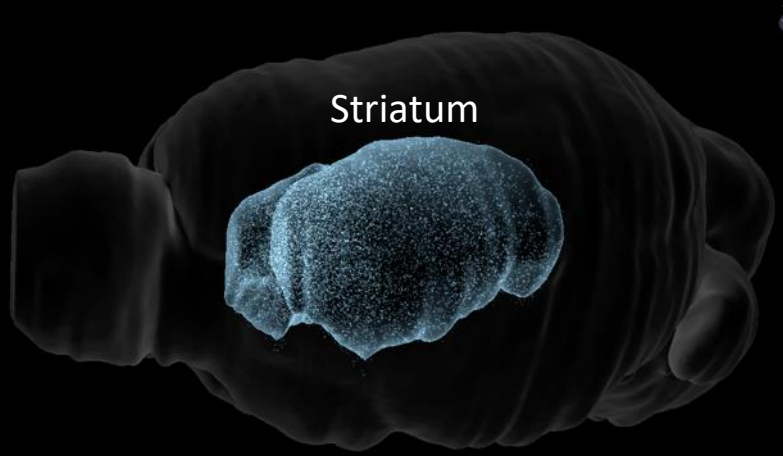
Edition de l'HTT dans le striatum: AAV2/1





TIDE analysis: 4 weeks post-injection
pAAV2ss-EFS-myc-nls-spCas9-nls-synA+
pAAV2ss-U6-sgHTT51 mouse-CMV-GFP

Efficacité d'édition dans le striatum



Neurones: 1,4 x 10^6 (50,8%)
Astrocytes: 2,38 x 10^5 (8,25%)
Toutes les cellules: 2,88 x 10^6

AAV2/1: neuronal
Cellules transduites(100% eficacy):
1,4 x 10^6 (50,8% of all cells)

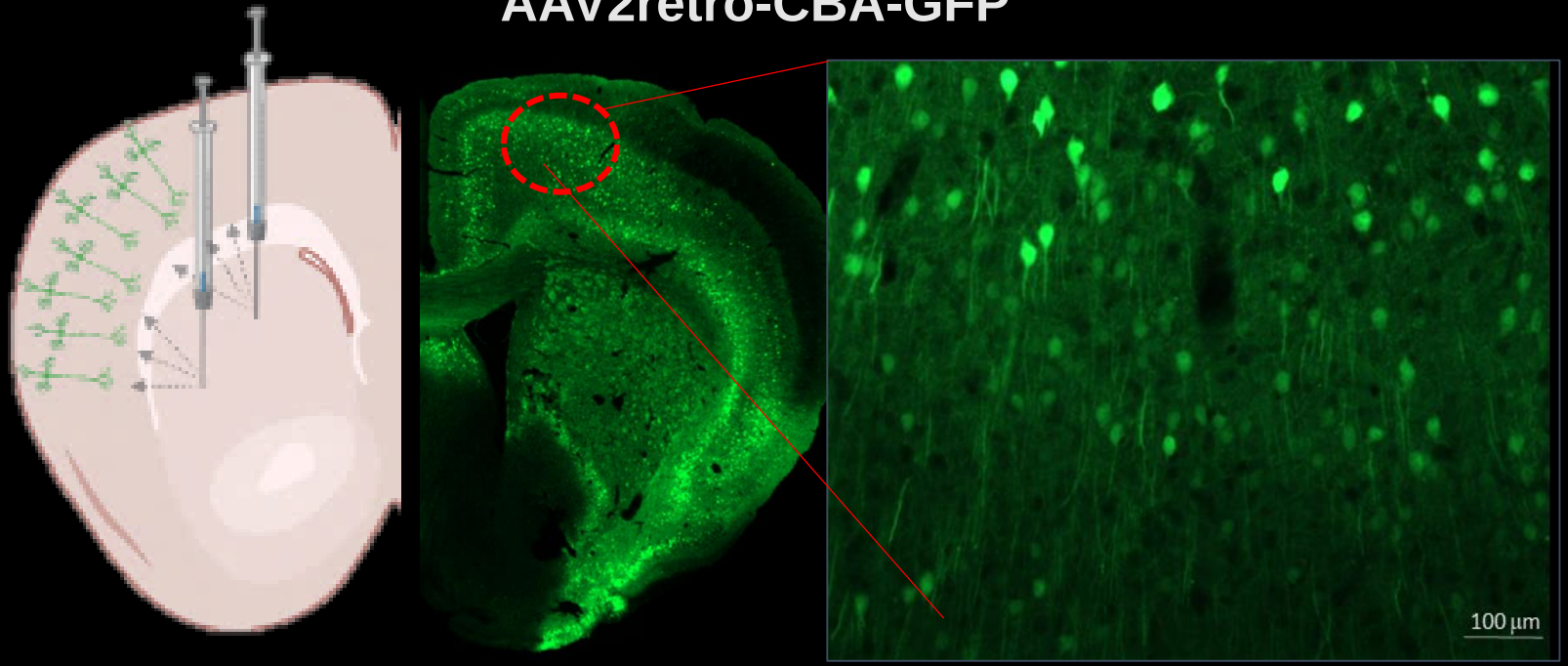
Edition de l'HTT: 42,0±4,3%

➡ correspond à une efficacité de 82,5% dans les cellules traitées

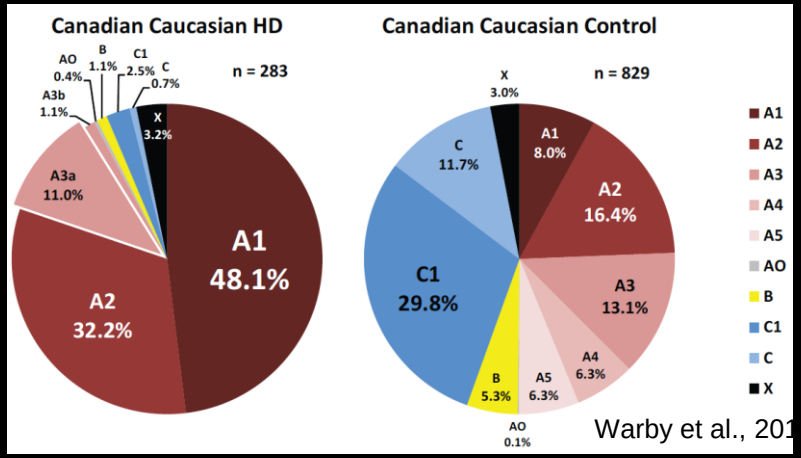
https://bbp.epfl.ch/nexus/cell-atlas/?v=v2&std=1

CIBLER LE CIRCUIT CÉRÉBRAL AFFECTÉ PAR LA MH

AAV2retro-CBA-GFP



VERS L'ÉDITION SPECIFIQUE DE L'HTT MUTEE: Polymorphismes d'ADN associés à la MH

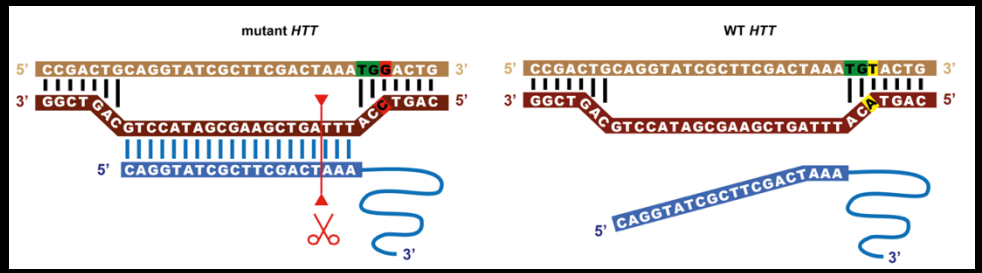


Warby et al., 201

Patients MH ont des ancêtres communs

⬇

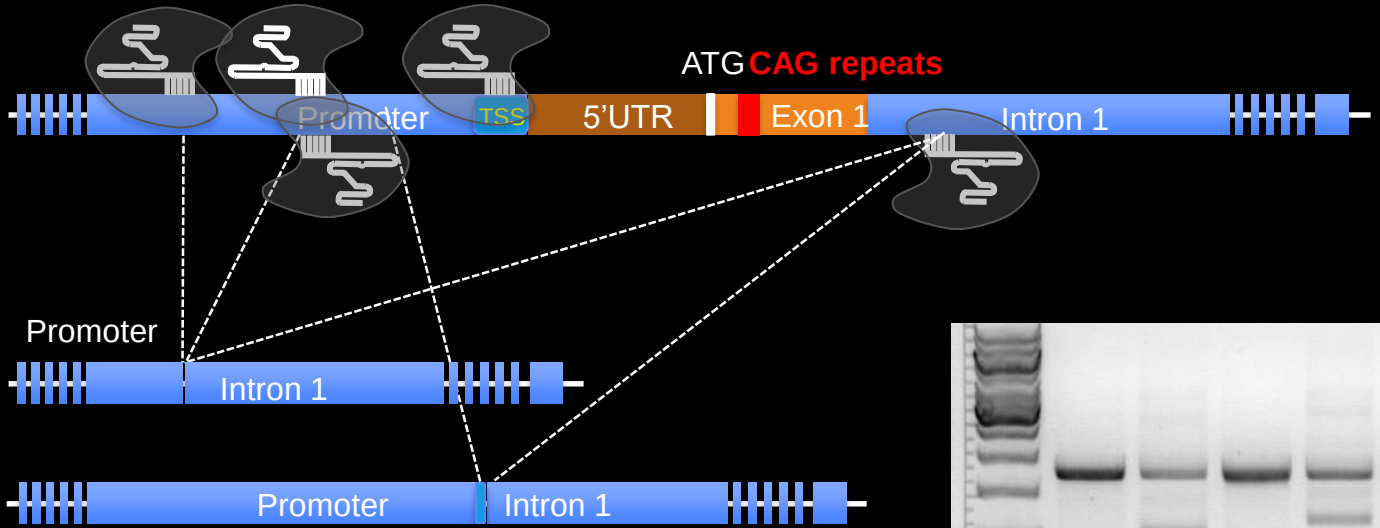
Des groupes de polymorphismes nucléotidiques simples (SNP) définissent différents haplotypes

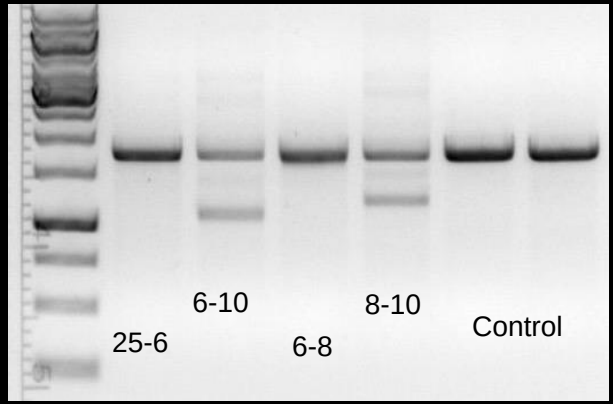


➡ Discrimination entre les allèles HTT WT et mutants à l'aide de SNP

Vachey et al. (2018)

Combiner deux sgRNAs pour induire une délétion du promoteur/exon 1 HTT





PCR: HTT deletion

FNSNF
Fonds national suisse de la recherche scientifique

SwissTransMed
Platforms for Translational Research in Medicine

AFMTÉLÉTHON
INNOVER POUR GUERIR

SEVENTH FRAMEWORK PROGRAMME

eurostars™

addgene
The nonprofit plasmid repository

KamiCas9 plasmids

University of Lausanne, Switzerland
Laboratory of Cellular and Molecular Neurotherapies

Nicole Déglon
Kevin Richetin
Nicolas Merienne
Virginie Zimmer
Daniel Blessing
Catherine Pythoud
Maria Rey
Alexia Boizot
Thomas Dequesne
Tim Beltraminelli
Lucas Herrgott
Sylvain Moser
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