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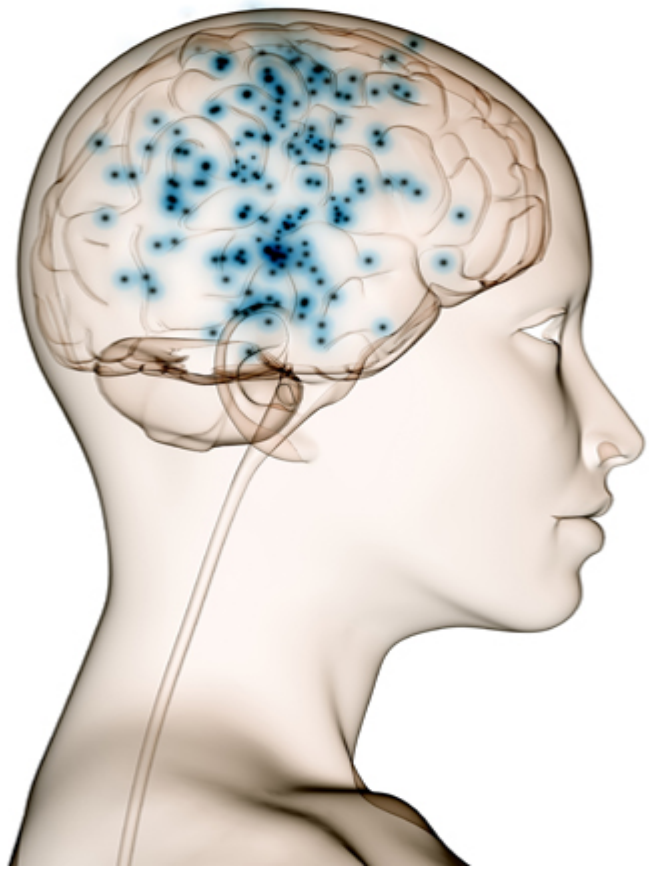

Allele-Specific Silencing Of Huntingtin using an adenovector

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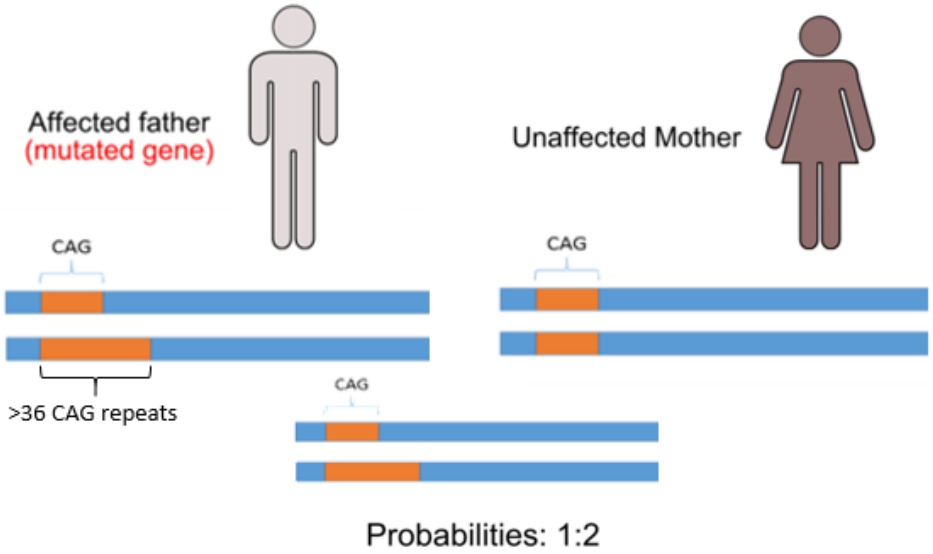
Huntington disease

Genetic disease

- Huntington disease is a dominant inherited neurodegenerative disorder
- Huntington is characterized by involuntary movements and psychiatric disturbance

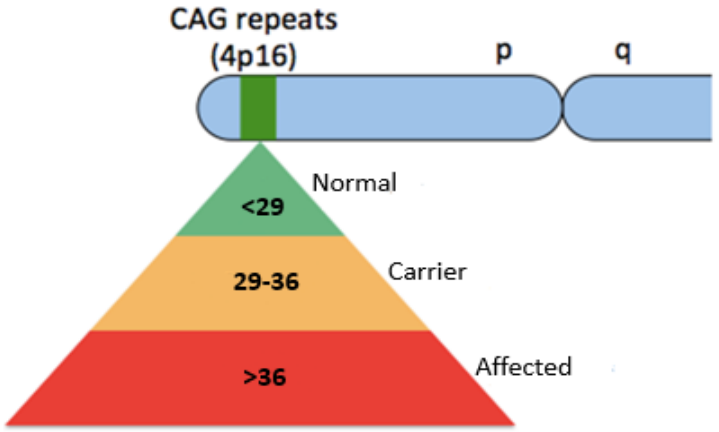


Probabilities: 1:2

Why using gene therapy for Huntington?

- The worldwide prevalence → 5–10 cases per 100,000 persons
- The life expectancy of a person with Huntington → 15 to 20 years from the onset of symptoms
- There are no available therapies that delay onset or slow progression

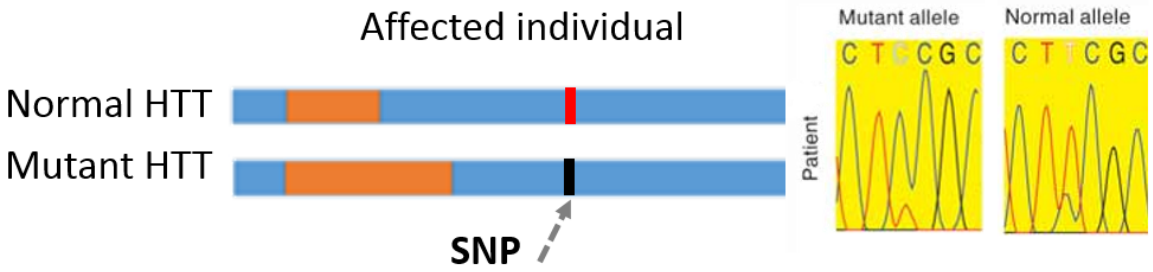
Huntington →caused by the expansion of a CAG repeat



SNPs and allele specific silencing

Strategy : remove mutant HTT allele and leave the normal one ➔ Allele specific silencing

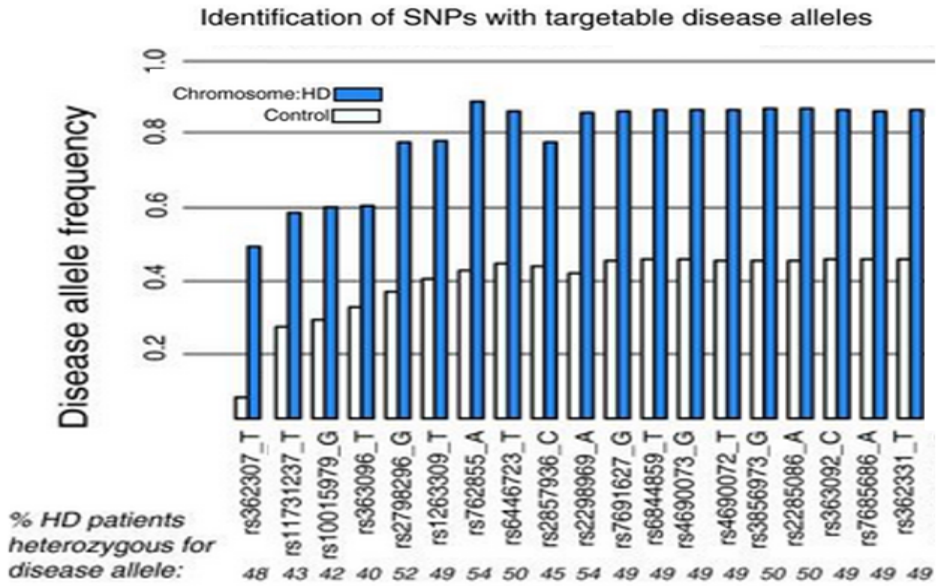
Example : SNP allele specific



- Target the CAG expansion ➔ non-selective reduction
- Allele specific silencing by targeting **SNP associated with the mutated allele**

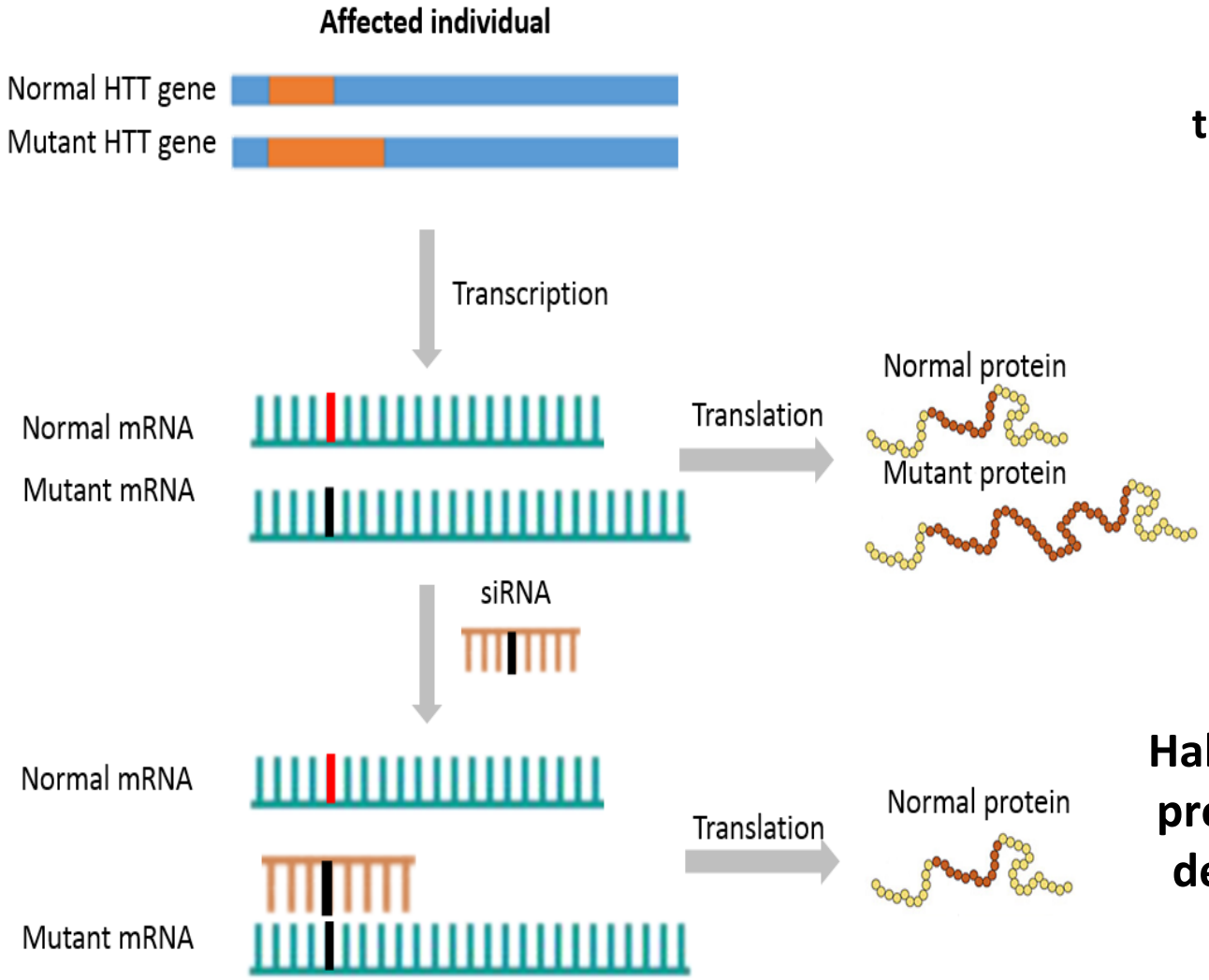
Identification of SNPs that are associated with the mutated HTT allele and that can be used for allele-specific targeting

- 24 SNPs ➔ represent 90% of patient
- 4 are preserved in high percentages



Strategy

Use of **siRNA** (small interfering RNA) to target specific SNPs on the mutated HTT allele



Regulation of the post-transcriptional HTT expression

- siRNAs degrade the mRNA using molecular machinery of the cells
- siRNAs are sequence-specific
- Construction of specific siRNA is possible

Half wild-type amount of HTT protein is enough for normal development and neuronal function

Our project

❖Construct the vector

- CAV-2 adenovector

❖In vitro experiments using iPS cells

- Better lifespan in culture than primary cells.
- High capacity of proliferation due to the similarity with Embrionic Stem cells (ES)

❖In vivo experiments using mice

- Mice FVB BACHD
- Mice FVB YAC18

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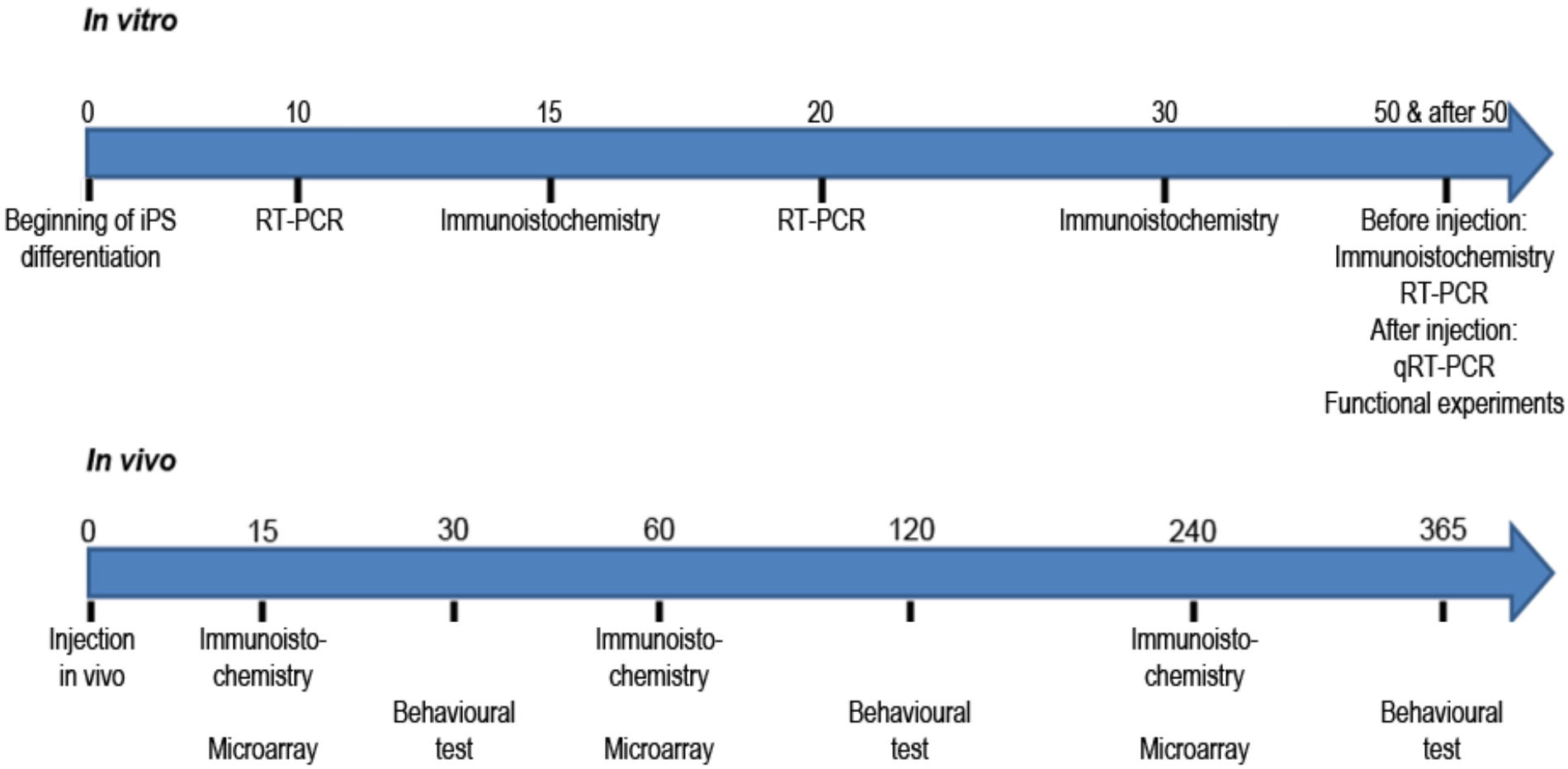
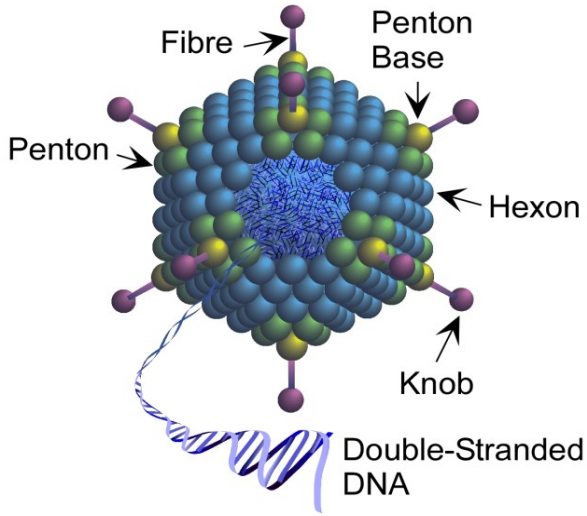
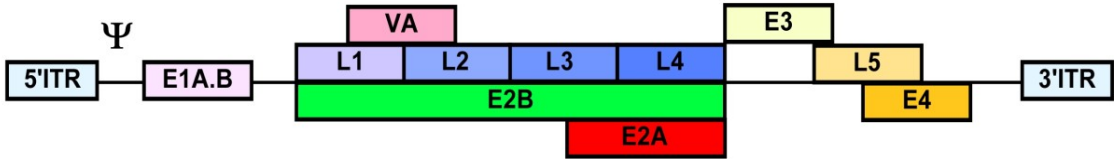
Trials time-line



CAV-2 gutless derived vector

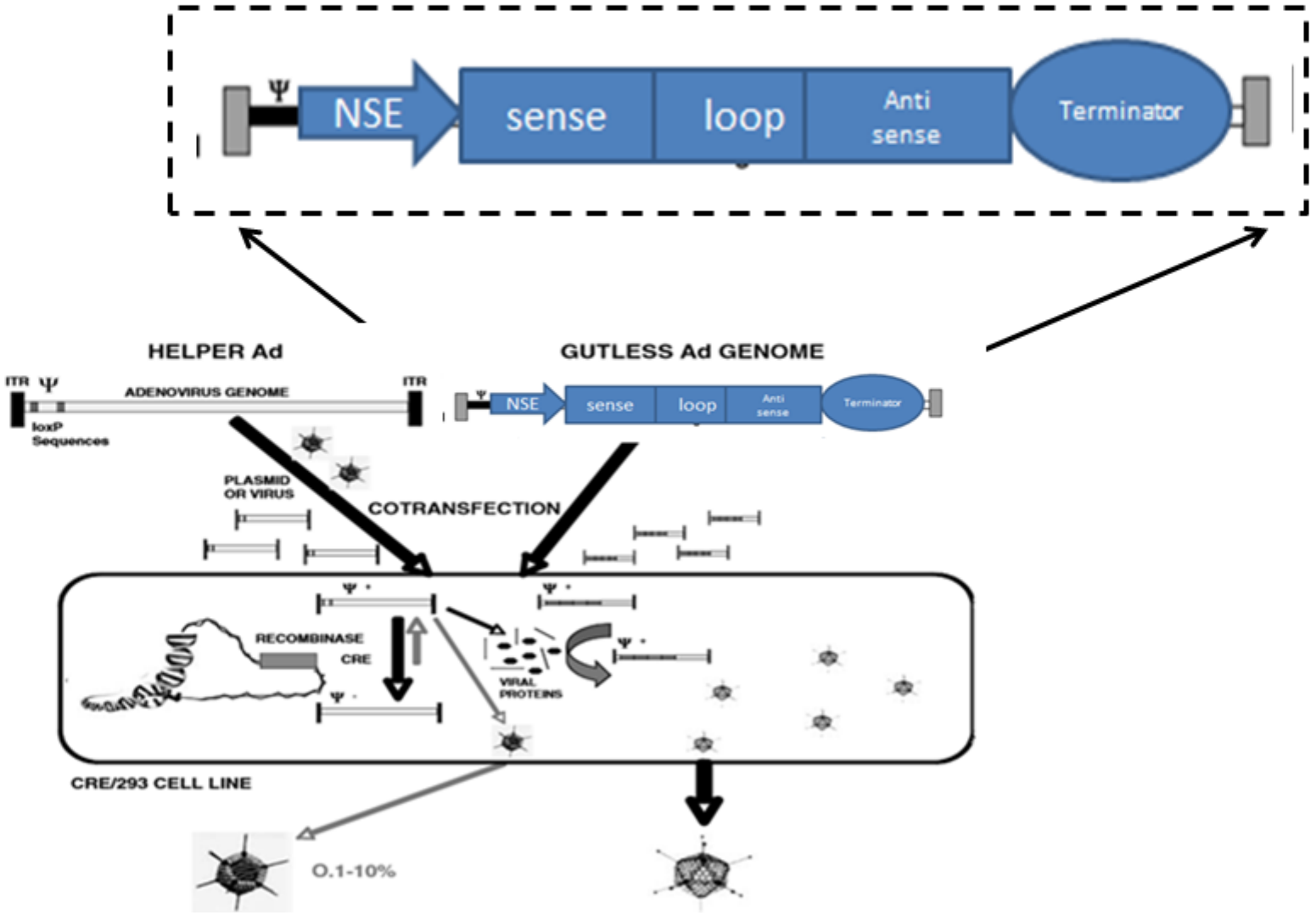
- Preferential transduction of neurons in the brains
- CAV-2 axonal transport can also be >100-fold more efficient than HAdV type 5 (HAdV5) vectors and lentivirus vectors
- CAV-2 transduced neurons can also express a transgene for at least 1 year in vivo
- 30-kb cloning capacity in helper-dependent (HD) CAV-2 vectors





Vector

Structure and insertion of the sequence in Cav-2 vector called by us CAVington



Promoter

For the specific expression in the neuronal cells we use the NSE promoter

- High expression in the neuronal cell
- Well-characterized promoter

Target

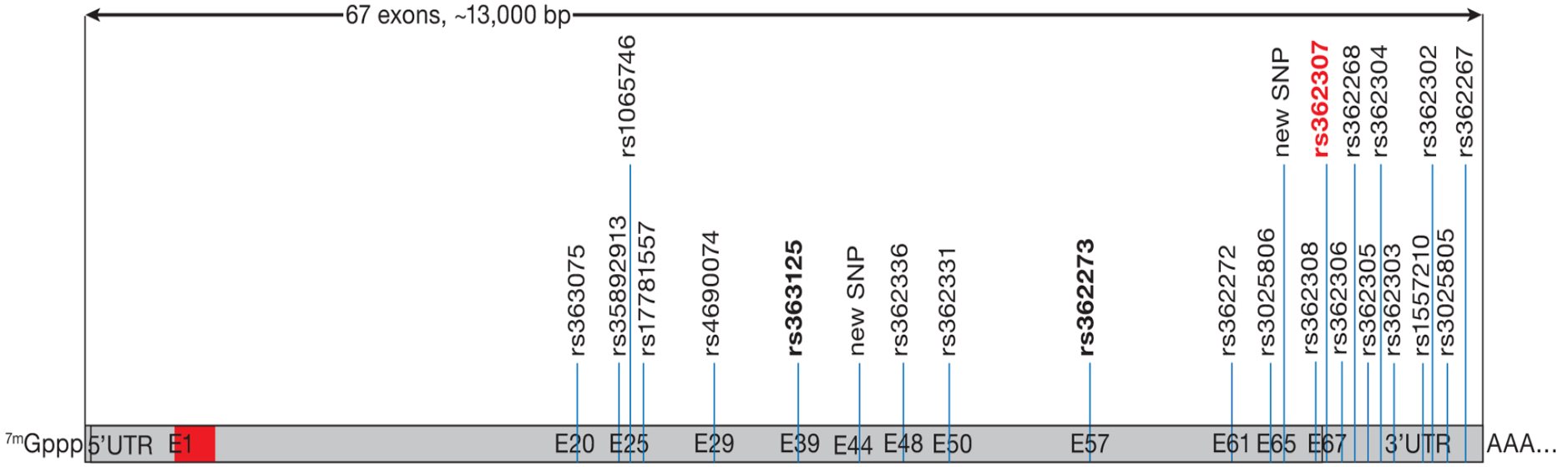
We use siRNA to target SNPs

[siRNA], nM

3′-CACGAAGAUCGCAACUUCAUG-5′
5′-UCUUCUAGCGUUGAAGUACUG-3′

Rs362307

We target the most conserved SNP in the HD-population

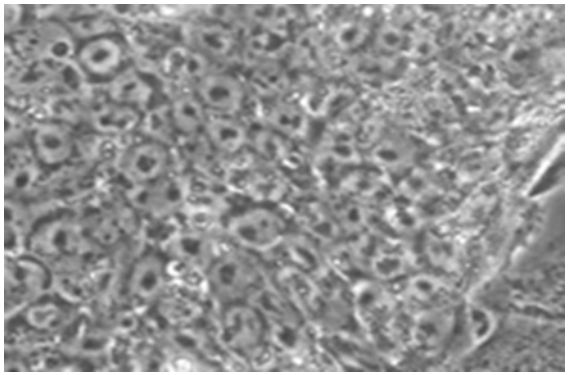


$(CAG)_n$, normal: n = 6~36; mutant: n > 36

In vitro studies

Use of induced pluripotent stem cells (iPS)

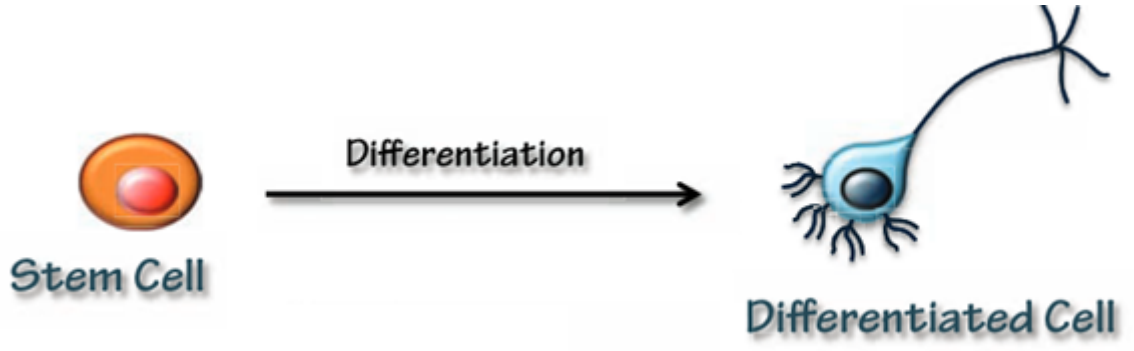
- High capacity of proliferation due to the similarity with Embrionic Stem cells (ES)
- Better lifespan in culture than primary cells



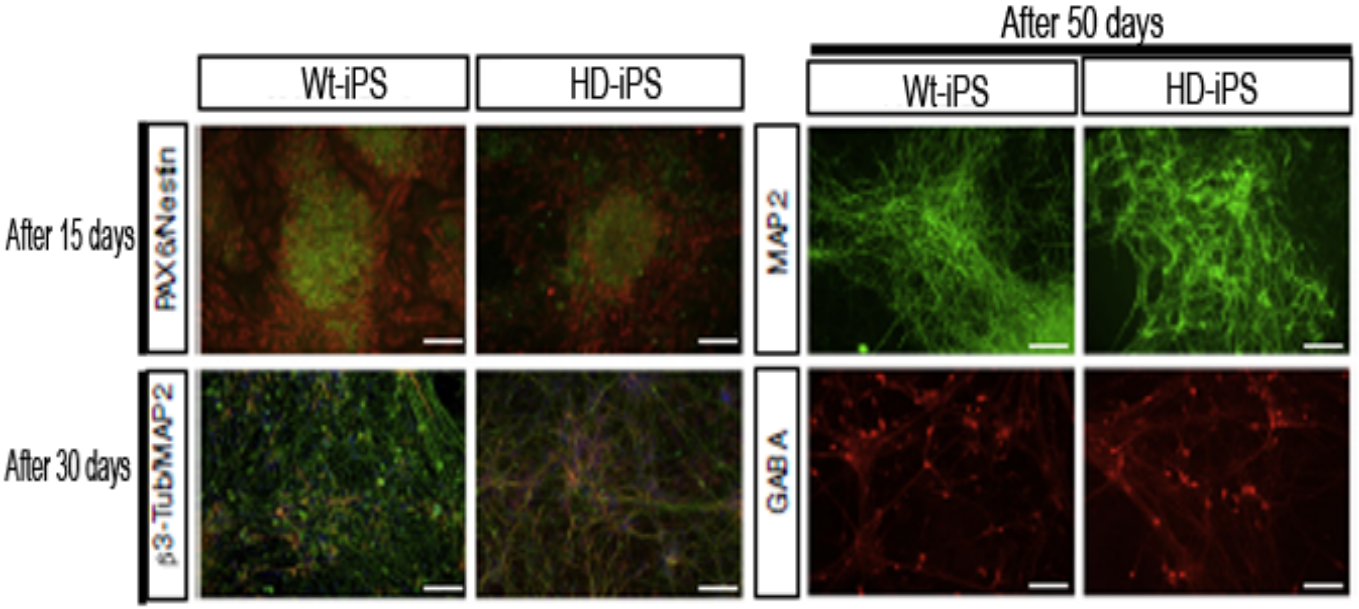
HD-iPS cells are gently donated from Coriell institute

Neuronal differentiation of HD-iPS cells

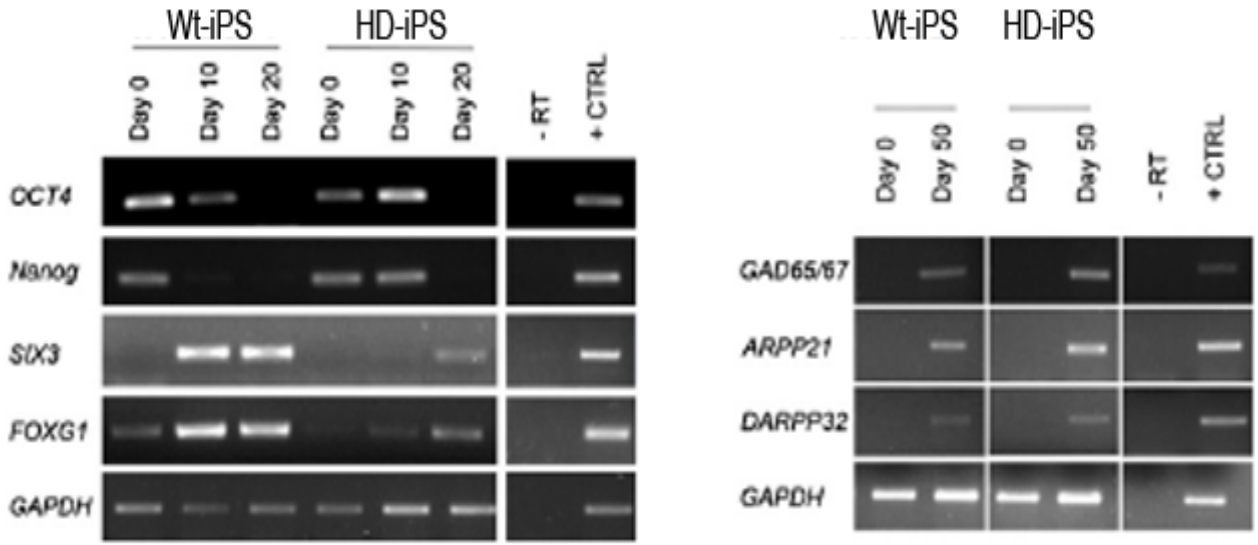
We need to obtain neuronal cells from HD-iPS.
It is possible to apply a recently published monolayer protocol for neuronal differentiation (Chambers et al., 2009).



Neuronal differentiation of HD-iPS cells



Immunoistochemistry is possible to detect differentiation markers



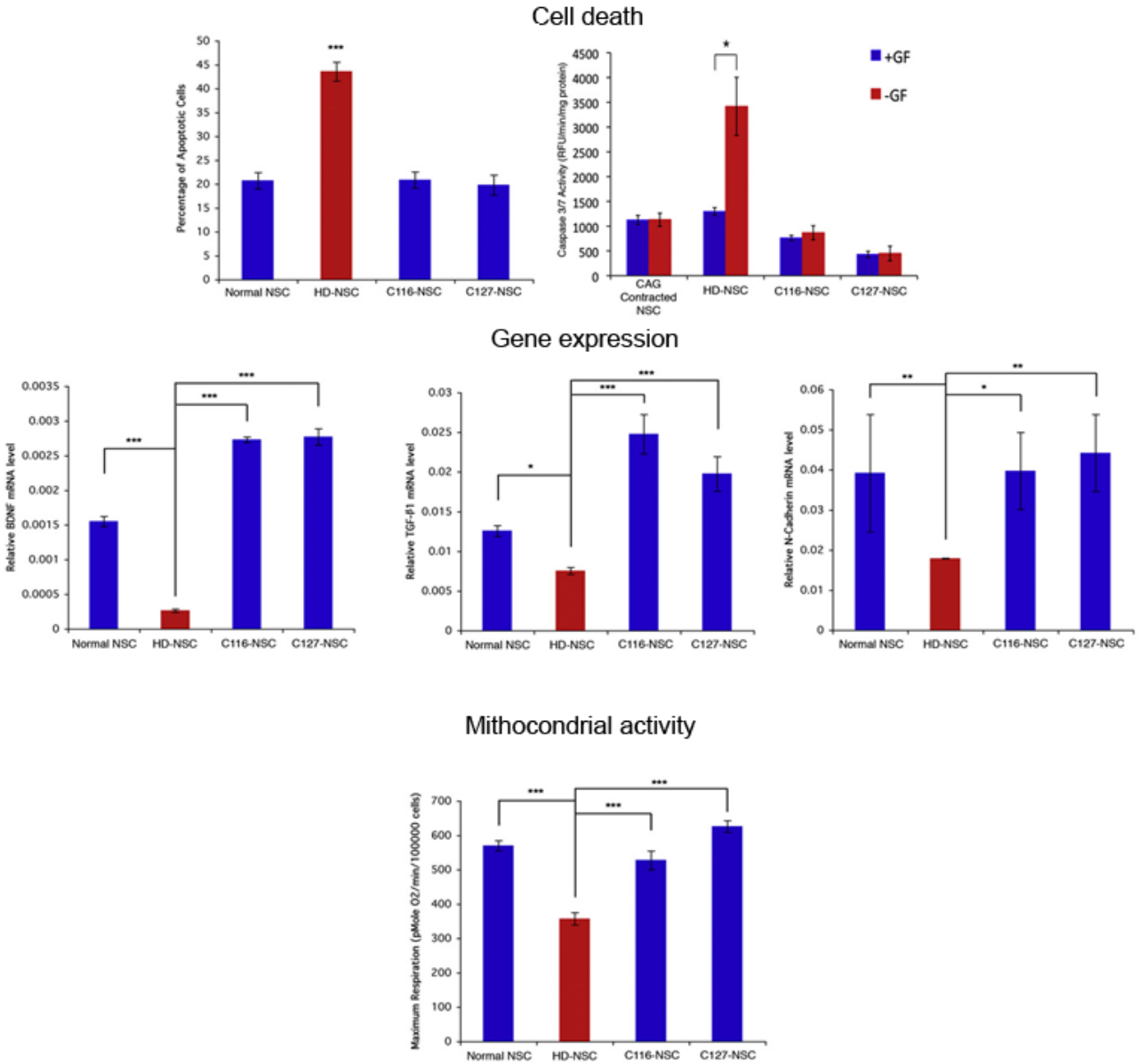
RT-PCR during neuronal induction

Transduction of HD-iPS derived neuronal cells and inspection on RNAi construct efficiency

- Transduction of HD-iPS derived neuronal cells with our adenovector containing our RNAi costruct
- Check the reduced expression of the mutated allele due to the effect of the interference:

  qRT-PCR → give quantitative data on specific RNA due to the use of specific primers. Less expensive than RNAseq

Functional studies on induced neuronal cells



We expect that the specific allele silencing with RNAi bring to reverse disease phenotypes in a similar way

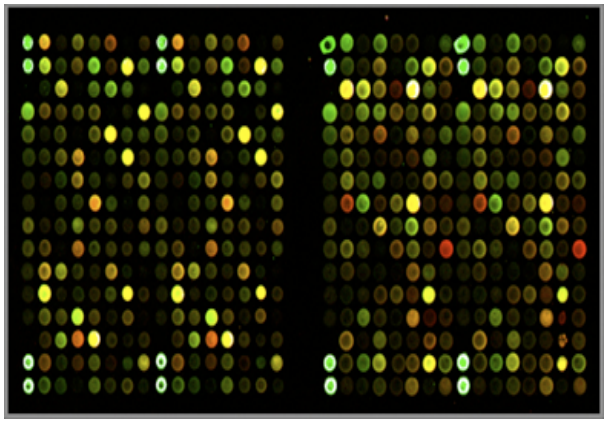
Test in-vivo

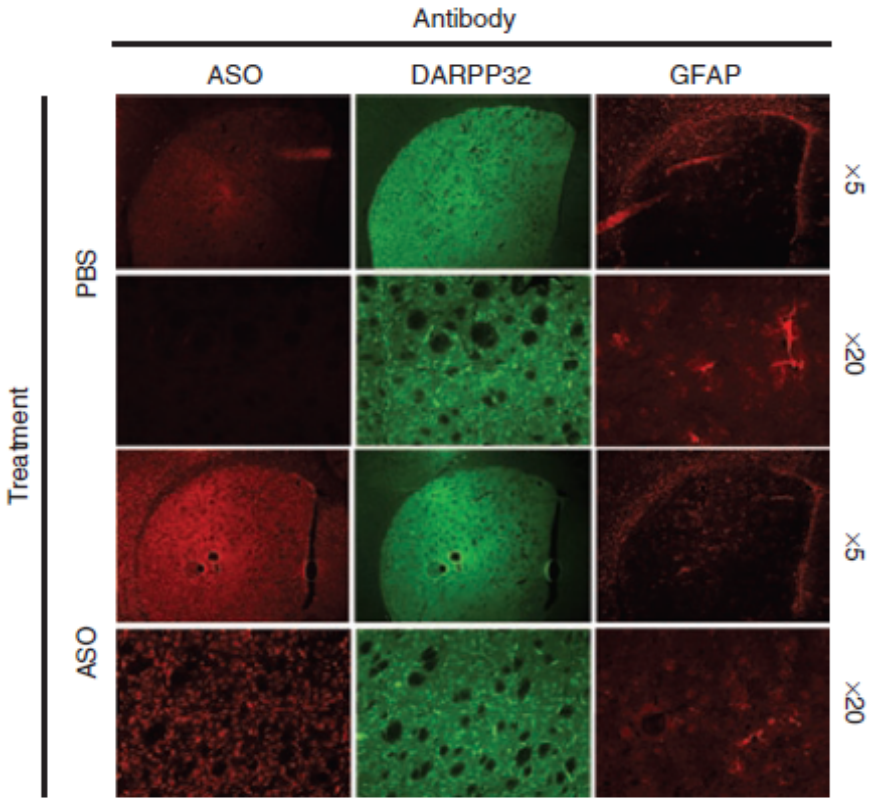
Intra-striatal injection of thirty-six mice at the age of 12 weeks with :

- CAVington
  - four YAC18
  - four BACHD
  - four WT
- Empty adenovector
  - four YAC18
  - four BACHD
  - four WT
- Control
  - four YAC18
  - four BACHD
  - four WT

Efficiency of adenovector

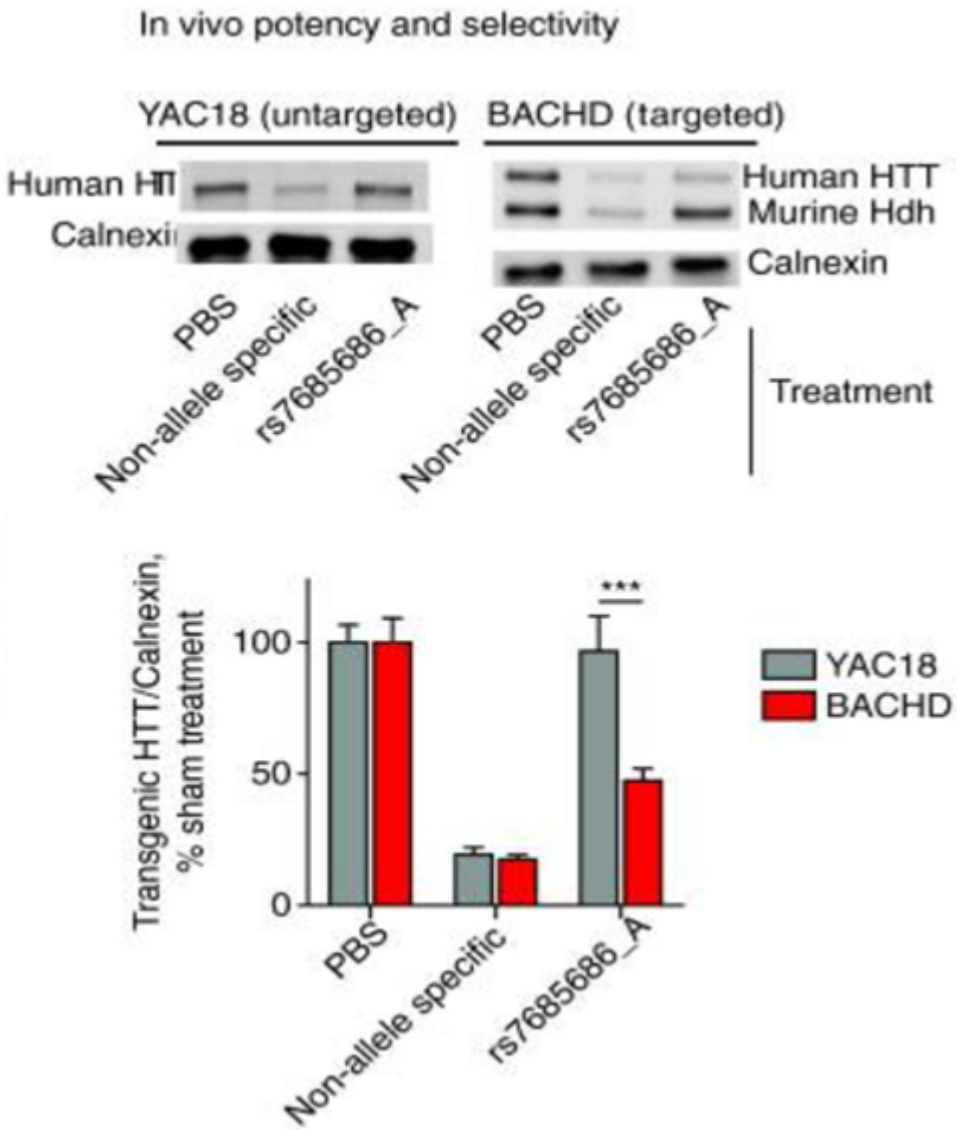
- Analyze with microarray to evaluate the expression of adenovector and his influence on neuronal gene expression





- ASO are potent and selective after acute delivery to the murine central nervous system (CNS).
- Our siRNA expression have to be limited only in neuronal cells

Efficiency of adenovector



Western-Blot

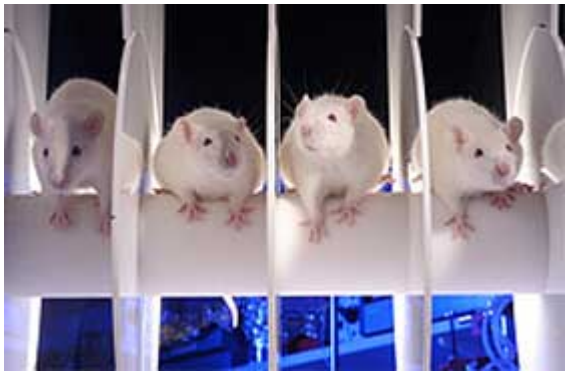
14 days post-injections

MutantHTT levels were examined. Both YAC18 and BACHD transgenes are reduced after injection with non-allele-specific ASO , while the ASO targeting rs7685686_A is only potent in the BACHD striatum.

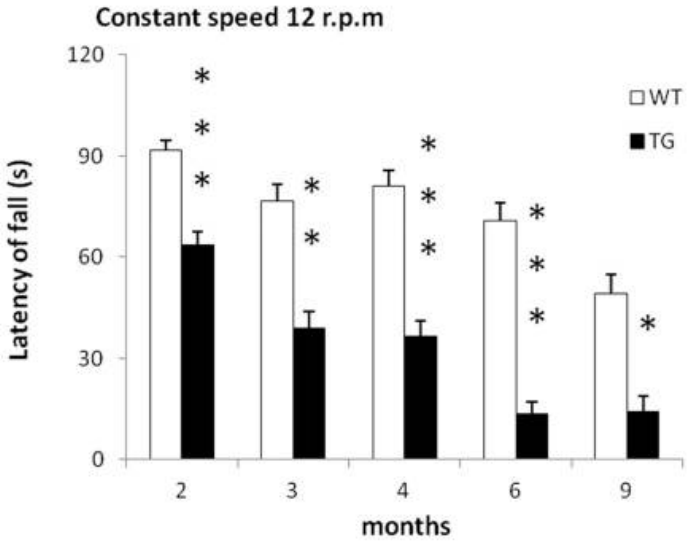
The use of siRNA allow to benefit from interference molecular machinery so the efficency of the strategy could be higher.

Behavioural experiments

- Open field tests → Behavioural tests based on mouse deambulation
- Light-Dark tests → Anxiety test
- Rotarod → locomotor activity, example: latency to fall off the rod



Rotarod



We expect that the adenovector will restore the WT activity

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Price of the project

	Company	Price single	Amounts	Total
CAV-2 vector production kit	Microbix Biosystems Inc.	$1,430.00	1	$1,430.00
HEK293 Cre4 Cells	Microbix Biosystems Inc.	$660.00	1	$660.00
qPCR Kits	life technologies	$382.00	1	$382.00
IPS	Coriell Institute for Medical Research	donate	2	$0.00
Mice FVB:BACHD	The Jackson Laboratory	$310.70	12	$3,728.40
MiceFVB:YAC 18	The Jackson Laboratory	$301.60	12	$3,619.20
Wt Mice	The Jackson Laboratory	$232.8	12	$2,793.60
GeneChip Array Set	Affymetrix	$159	14	$2,226.00

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