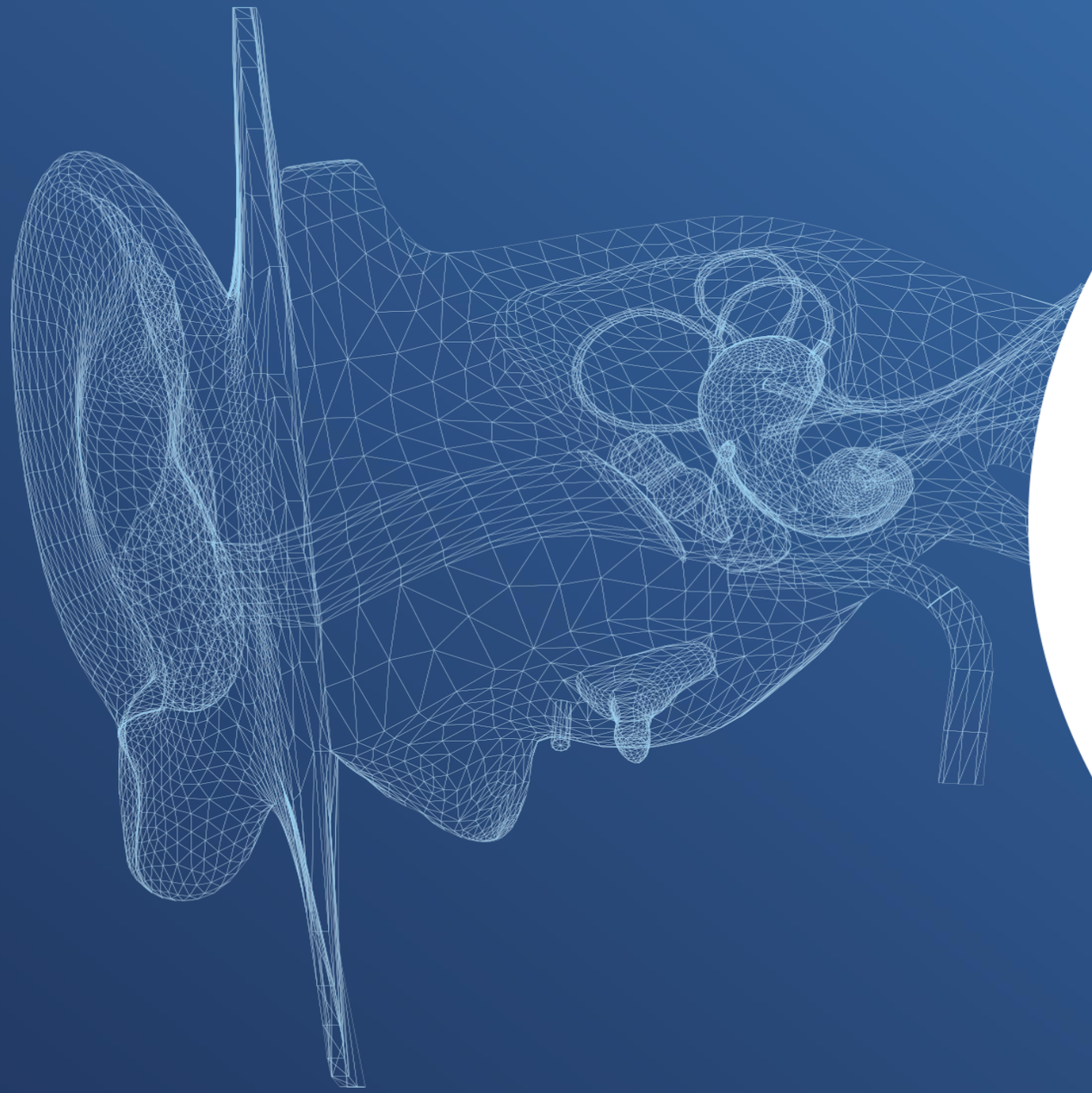

Sensorion

Characterization of Dual AAV Vector Otoferlin

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CASSS Bioassays

JULY 19-21, 2022

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Presentation outline

- Sensorion overview
- Gene Therapy Otoferlin program : Dual AAV vectors
- Key considerations in CMC for dual AAV vectors
- Evaluation of full-length human otoferlin protein expression
- Determination of the optimal ratio of dual AAV vectors

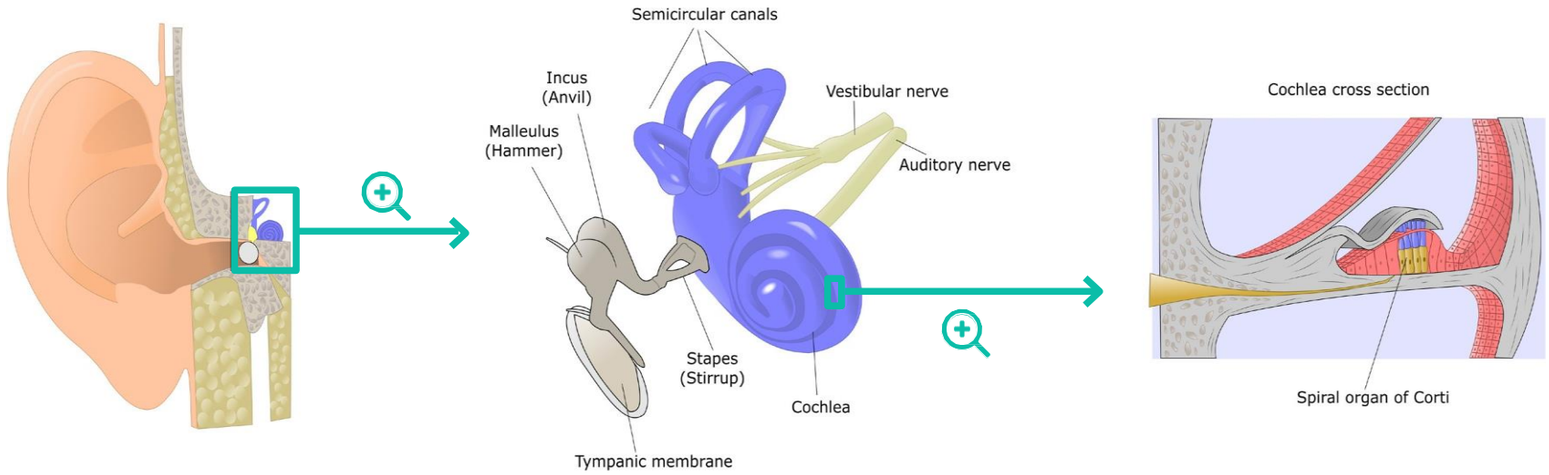
Overview - Sensorion

- Clinical stage biopharmaceutical French company
- ≈ 45 employees, offices in Montpellier and Paris
- Unique R&D technology platform to expand understanding of physiopathology and etiology of inner ear related diseases, and to select the best targets and modalities for drug candidates
- CMC Capabilities to develop at scale high quality clinical candidates
- One small molecule in Phase II
- Gene Therapy program in collaboration with Institut Pasteur, Hôpital Necker-Enfants malades and Fondation pour l'audition – Powerful consortium to advance the development of GTx program*

** Audinnove project, partially funded by ANR as part of the Investment Avenir Program (ANR 18 RHUS 0007)*

The Inner Ear is one of the most delicate organ in the human body

Inner Ear Biology



KEY FACTS

Limited number of hair cells:

- **3,500 Inner Hair Cells**
- **12,000 Outer Hair Cells**

Hair cells do not naturally regenerate

According to the WHO*:

~ 466m people affected by disabling hearing loss worldwide
~ 700m people projected to be affected by 2050

**World Health Organization, 2021 World report on Hearing*

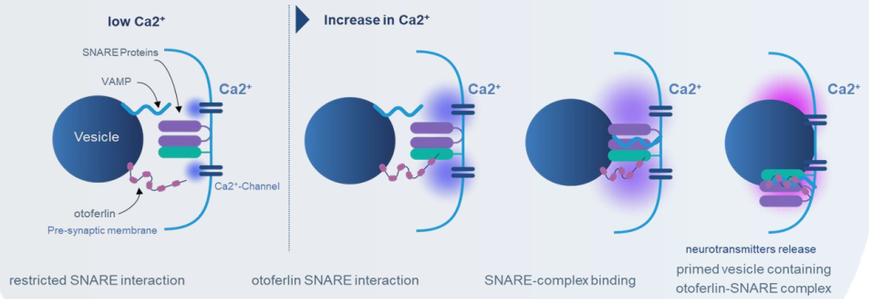
Otoferlin Gene Therapy program

Target: Reversing the severity of the disease

DFNB9:

- Profound sensorineural prelingual non-syndromic hearing loss
- up to 8% of all cases of congenital hearing loss
- Prevalence ~20,000 in the USA + EU
- Incidence ~1100 per year in USA + EU

Restore Otof function: Ca2+ sensor for vesicle fusion and vesicle pool replenishment at auditory hair cell ribbon synapses



Model illustrating calcium regulation of otoferlin/SNARE interaction in the hair cell. – Adapted from Ramakrishnan *et al.* 2014

OTOF-GT

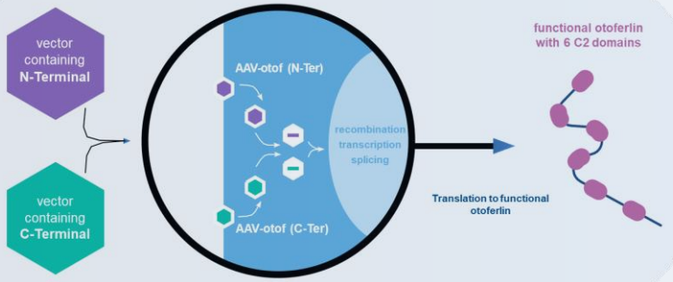
Audinnove consortium - Treating congenital hearing impairment

- understand the epidemiology
- build awareness of the emerging gene therapies

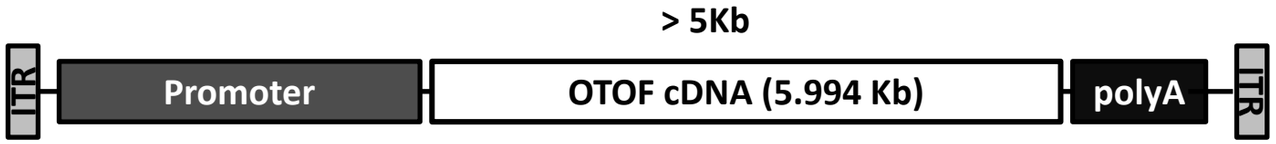
AUDINNOVE CONSORTIUM MEMBERS

Sensorion · INSTITUT PASTEUR · Necker Enfants Malades Hôpital Universitaire · Fondation pour l'Audition

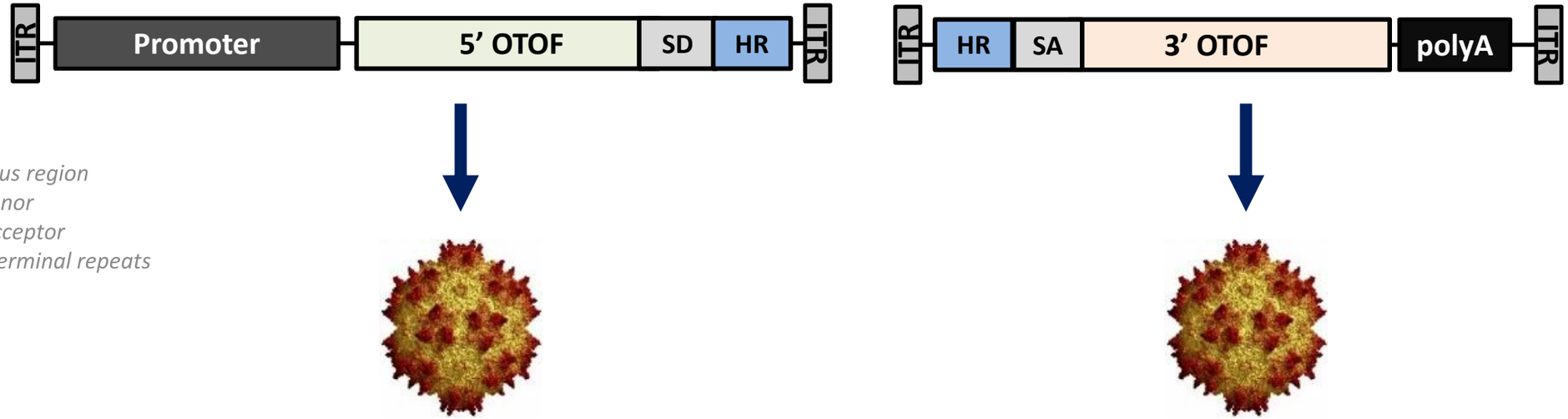
Dual AAV strategy



Dual hybrid AAV otoferlin vectors



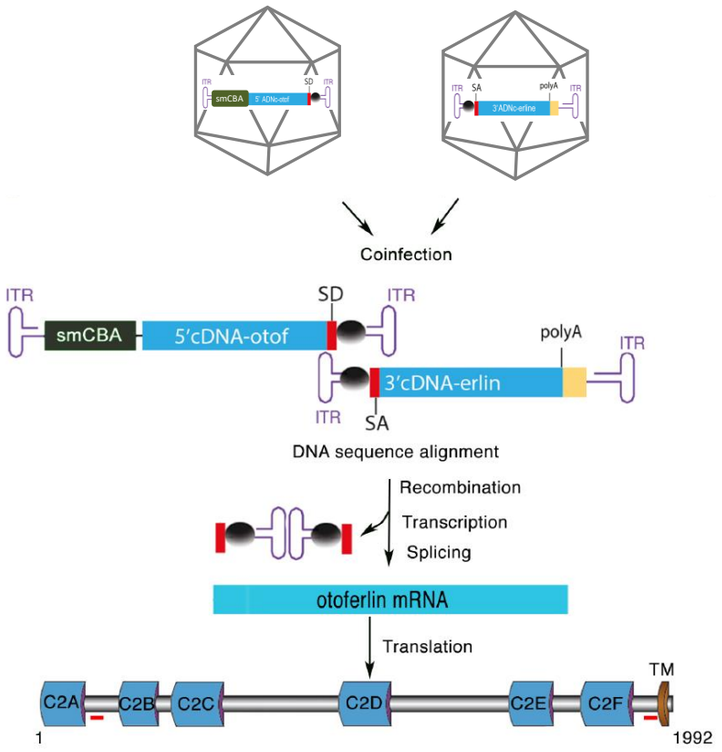
Dual Hybrid strategy:



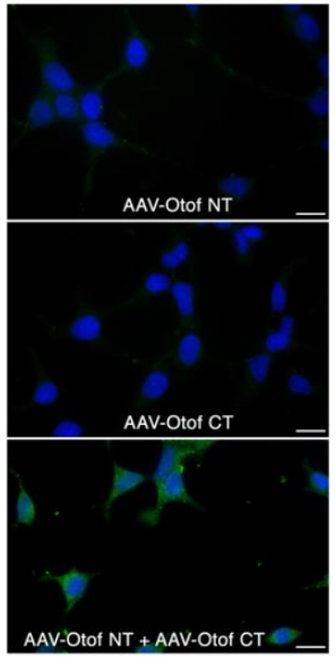
HR - homologous region
SD - splicing donor
SA – splicing acceptor
ITR - inverted terminal repeats

Proof of Concept of dual otoferlin AAV vectors

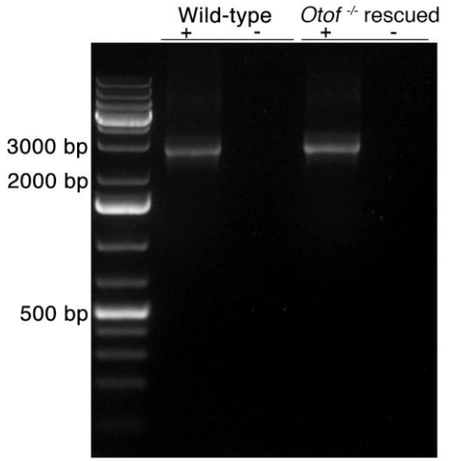
Schematic dual AAV vectors for Otoferlin delivery



Expression of Otoferlin in HEK293



RT-PCR analysis of Otoferlin in mice

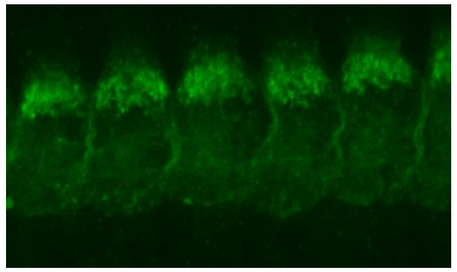


Akil et al, PNAS, 2019

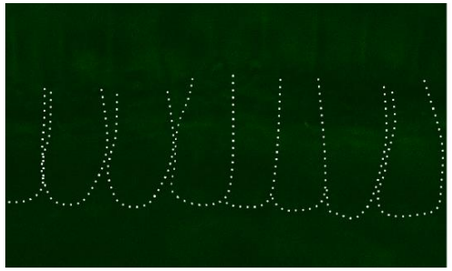
Proof of concept of dual otoferlin AAV in mouse model

Cochlear stereocilia in wild type, Otof -/- and Otof -/- injected with AAV2-OTOF vector
Expression of Otof protein in cochlear receptors

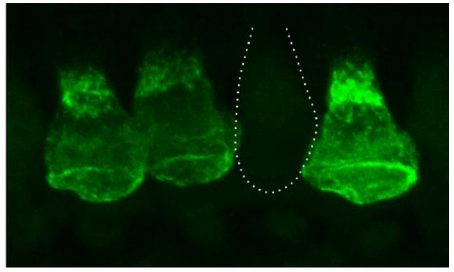
Normal mice



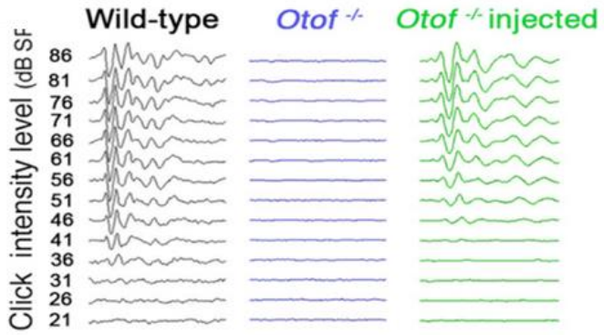
Otof-/-mice

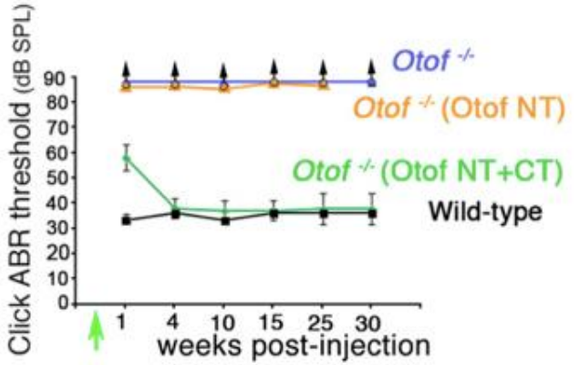


Treated Otof -/-mice



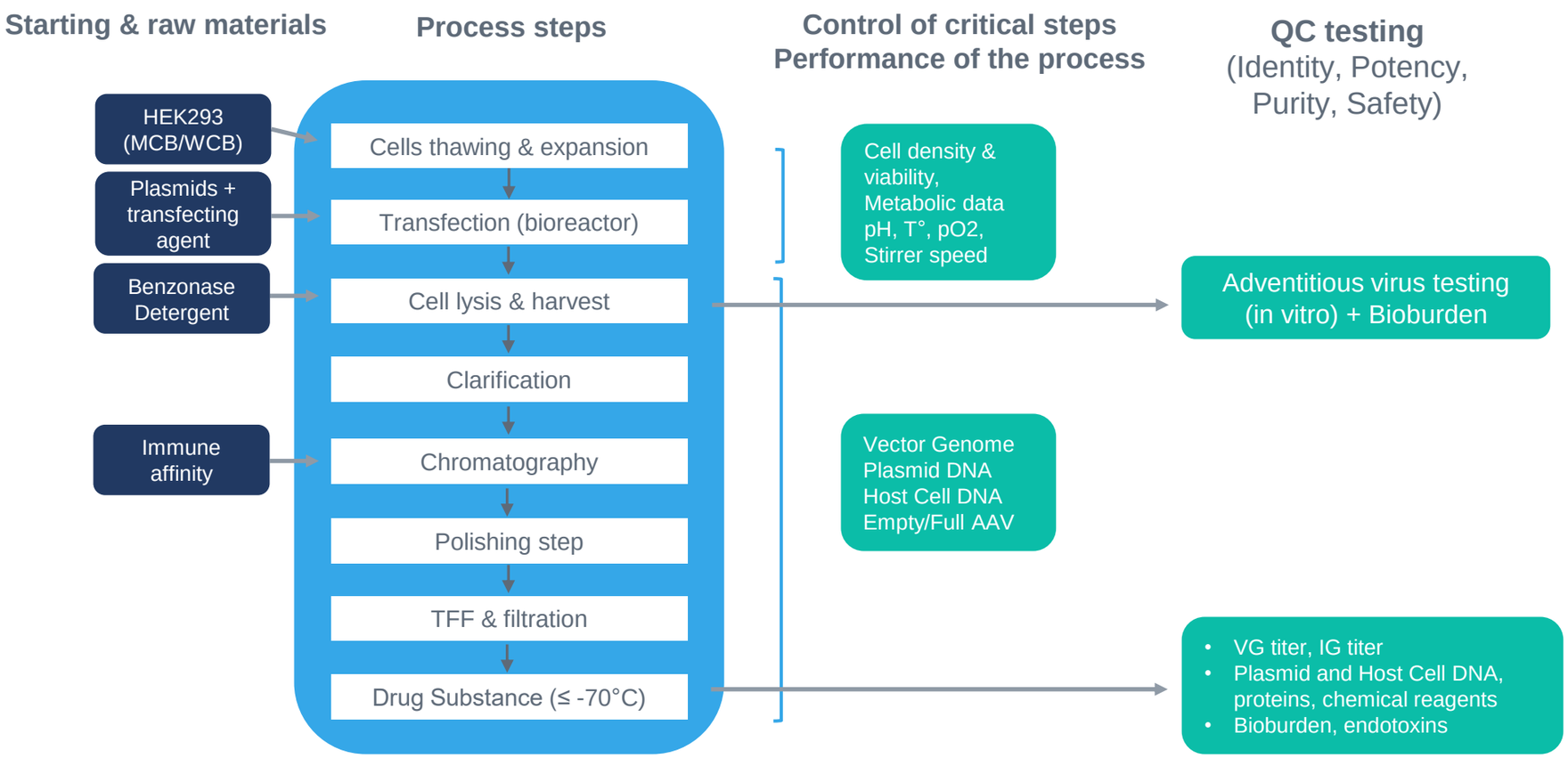
Restoration of auditory function in Otof -/- using Dual AAV2-OTOF





Akil et al, PNAS, 2019

Overview of 1st Generation Large Scale Manufacturing Platform



- Total recovery: 20-30%
- Two bulk DS for GT-OTOF

Manufacturing of dual AAV drug Product: Optimized process & controls

Pooling of DSs

DS vector 1 (stored at ≤ -70°C)

DS vector 1 thawing

DS vector 2 (stored at ≤ -70°C)

DS vector 2 thawing

Pooling of DSs

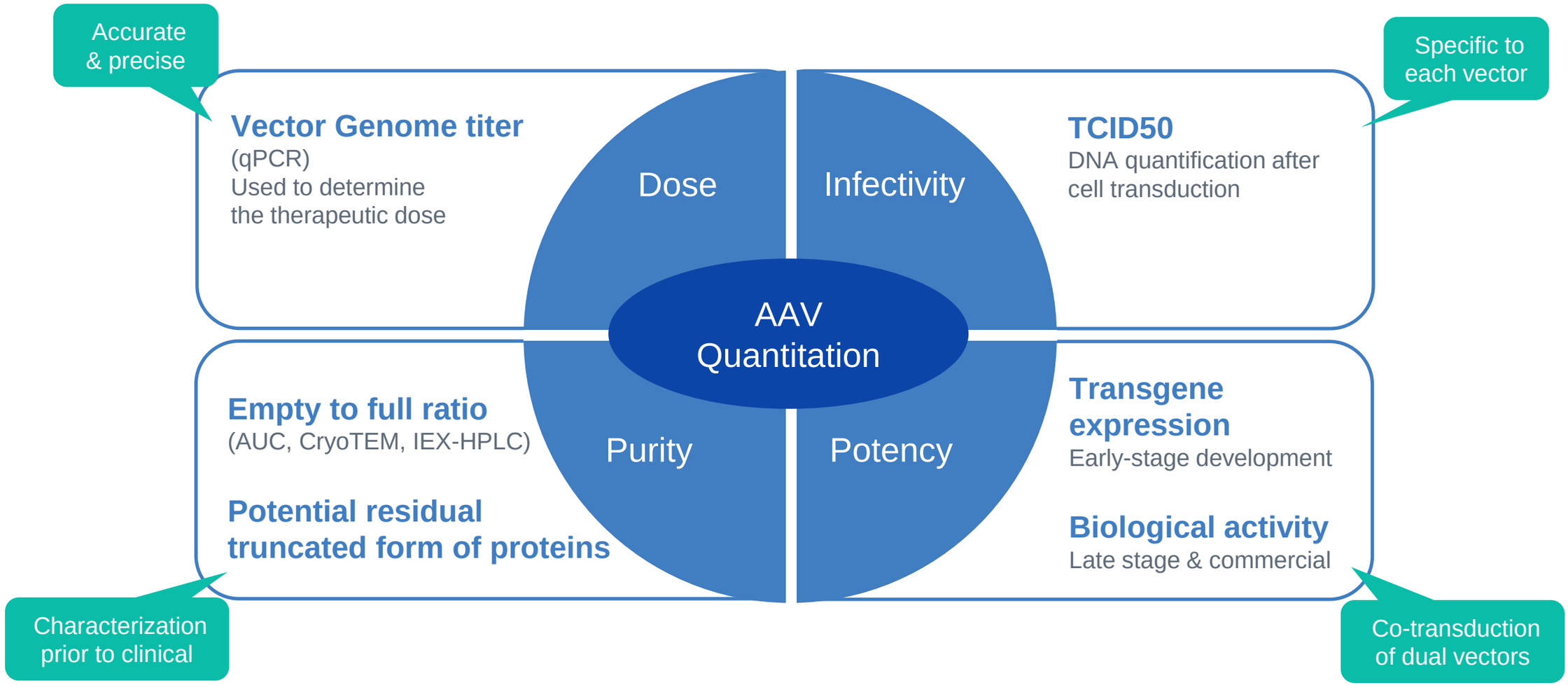
Sterile filtration

Distribution into vials

Storage at ≤ -70°C

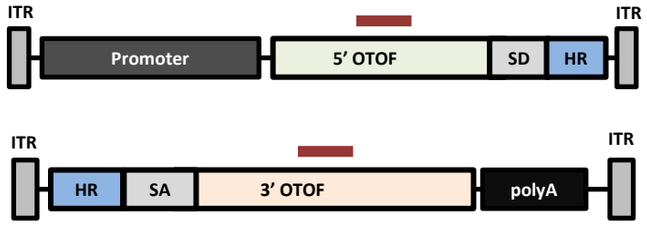
DP (mix of vectors 1 & 2 at defined ratio)

Analytics Capabilities: AAV specific testing

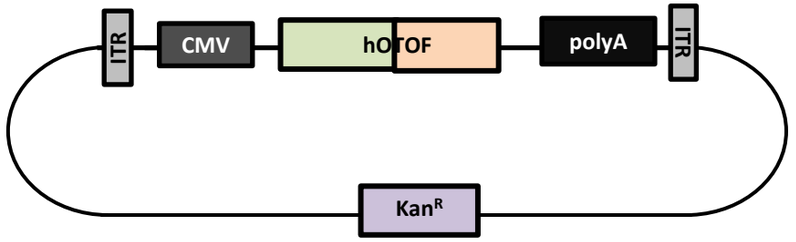


AAV quantitation assay

- **Vector Genome titer assay**
  - Release testing of DS & DP and a stability-indicating testing
  - Use for calibrating DP concentration at a ratio 1:1 of each vector
  - Need to be accurate and precise (CV ≤ 15%, FDA requirement)
  - Specific: Targeting OTOF DNA sequence for each vector
  - One DNA standard curve for both vector (qPCR)



qPCR primer-probe set specific of each vector



Plasmid with OTOF cDNA full length

VG titration assay: method qualification

ICH Q2R1 – validation of analytical procedures

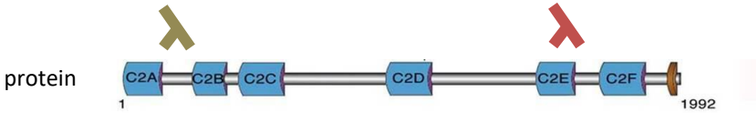
Characteristics	Parameters Evaluated	Acceptance Criteria	Results Obtained
Specificity	• In silico alignment • Negative Controls : water, PBS, HEK293 DNA, cross reaction of dual OTOF vector • Positive control: specific for the 5' or 3' part of hOTOF DNA sequence	• No amplification of other human mRNA • Ct ≥ 35 for negative controls • Ct < 35 for positive control	• Blast • Ct ≥ 35 • Ct < 35
Linearity	• Coefficient of determination of the standards • Efficiency	• R^2 ≥ 0.98 • 90 % ≤ efficiency ≤ 105%	• 5' OTOF method: R^2=0.999, eff= 100% • 3' OTOF method: R^2=0.999, eff=102%
Range	• 3 assays with standards from 1E+07 to 1E+01 copies/µL	• CV ≤ 30%	• R^2: CV< 0.15% • Standard curve: CV < 7%
Intra-assay precision	• Quantification of 1E+07, 1E+04 and 1E+01 copies in 10 replicates in one assay	• CV of quantities ≤ 30%	• 5' OTOF method: CV ≤ 14.0% • 3' OTOF method: CV ≤ 20.0%
Repeatability (Inter-assay precision)	• 3 independent assays	• CV of quantities ≤ 30%	• 5' OTOF method: CV ≤ 16.4% • 3' OTOF method: CV ≤ 11.8%
Accuracy	• 20 independent VG titrations	• Titer within +/- 3 SD and +/- 0.5 log10	• Titer within +/- 2 SD and +/- 0.3 log10

All acceptance criteria are met : transfer & GMP readiness

AAV quantitation assays

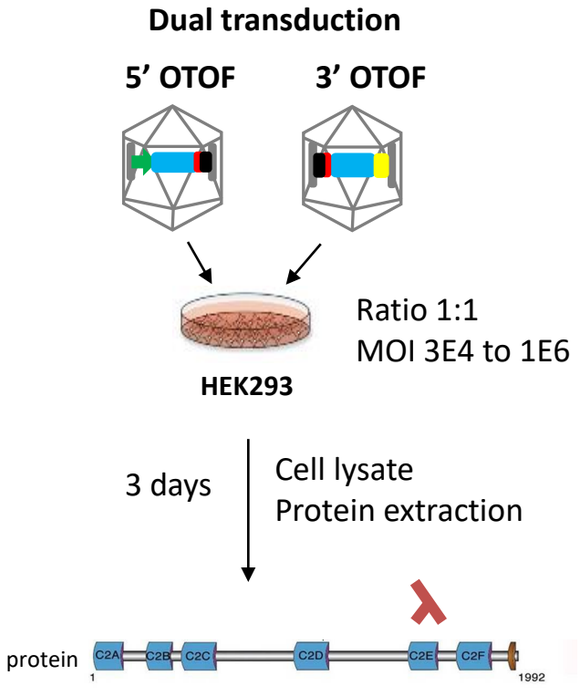
- **Gene expression assay based on the quantification of mRNA**
  - Primers/probe targeting the recombination region
  - Use to ensure the recombination processing efficiency
  - Product characterization

- **Gene expression assay based on the detection of otoferlin protein**
  - Release testing for DP (mix of vectors)
  - A stability-indicating testing
  - Qualitative or semi-quantitative assay
  - Antibodies recognized Nter and Cter region



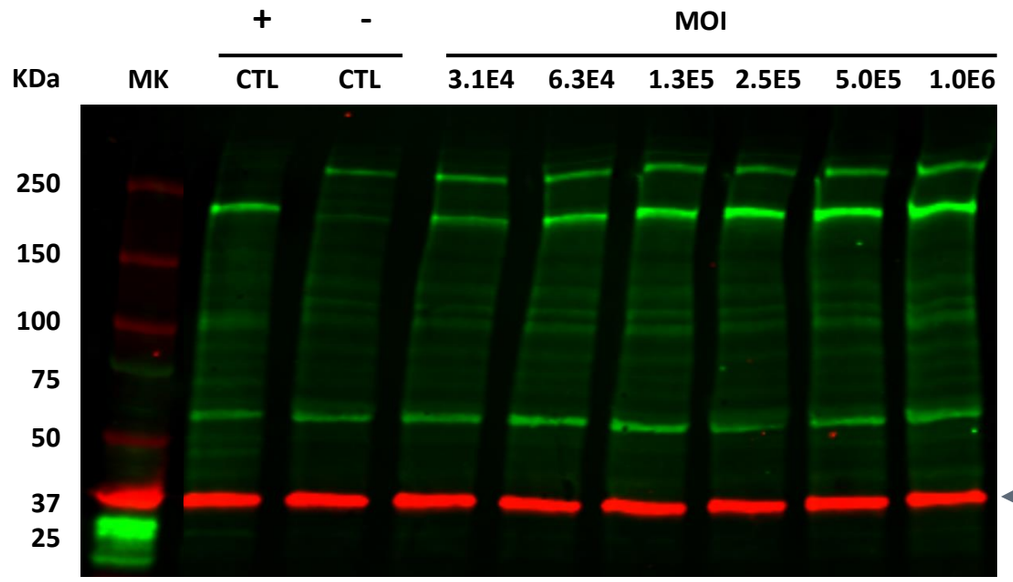
- **Use for extensive characterization to demonstrate**
  - Production of the correct full-length mRNA and protein
  - No truncated protein produced by the mix vectors (after recombination)
  - No truncated protein produced by individual vector

Full-length Otoferlin protein expression in vitro assay



Immunodetection of otoferlin protein
Anti-otoferlin Ab against Cter domain

WB analysis - Anti-Otof Ab against Cter domain

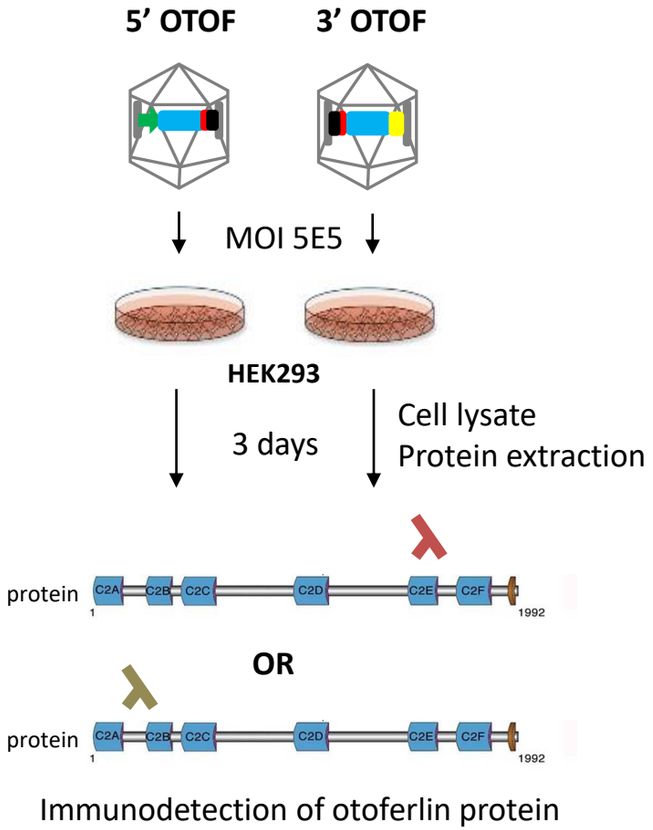


* Non-specific Ab detection. Bands also observed in non-transduced cells

Positive control is cells transfected with the plasmid encoding the hOTOF full length

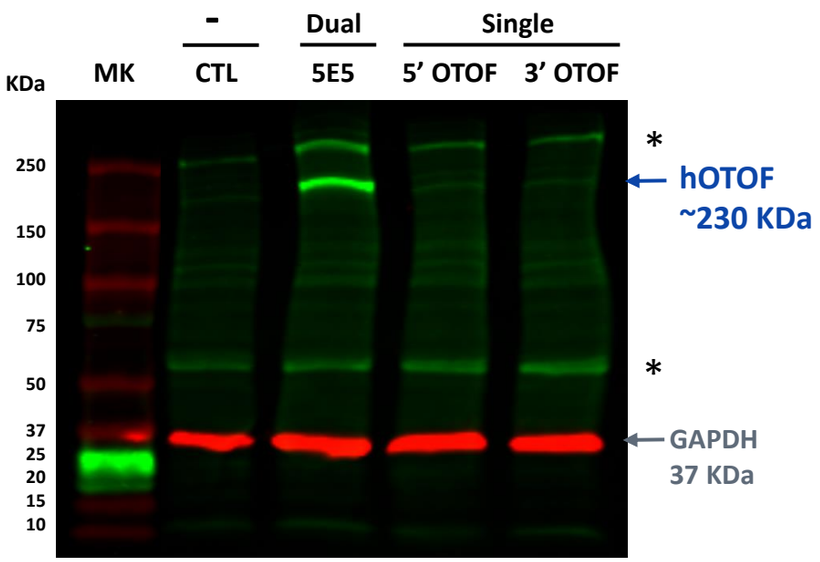
- Dose response – expression is function of the MOIs
- Dual AAV results in full length Otoferlin protein expression

In vitro expression assay with single vector



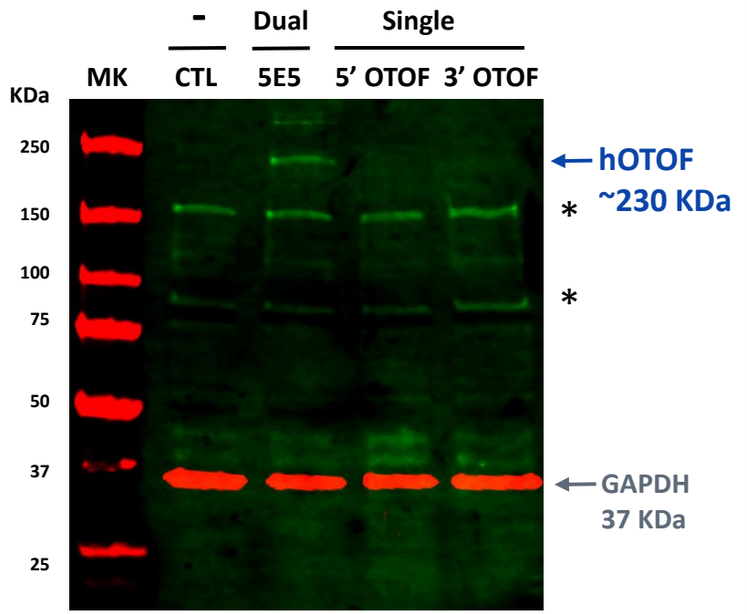
Immunodetection of otoferlin protein

Anti-Otof Ab against Cter domain



* Non-specific Ab detection

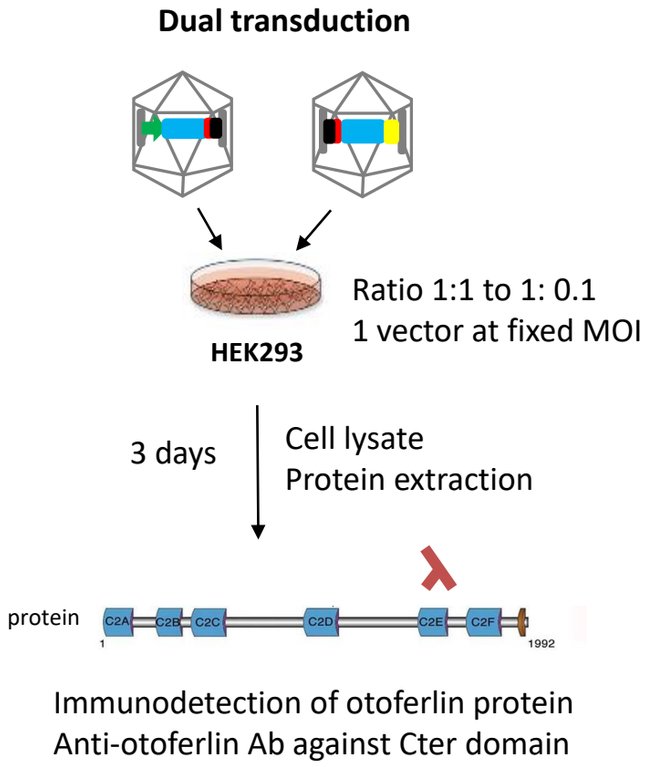
Anti-Otof Ab against Nter domain

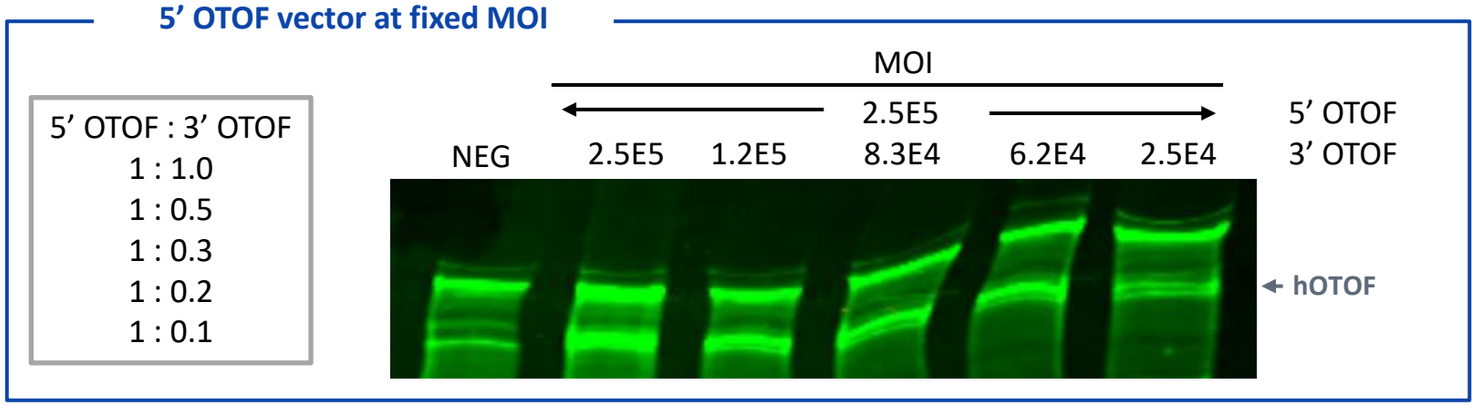


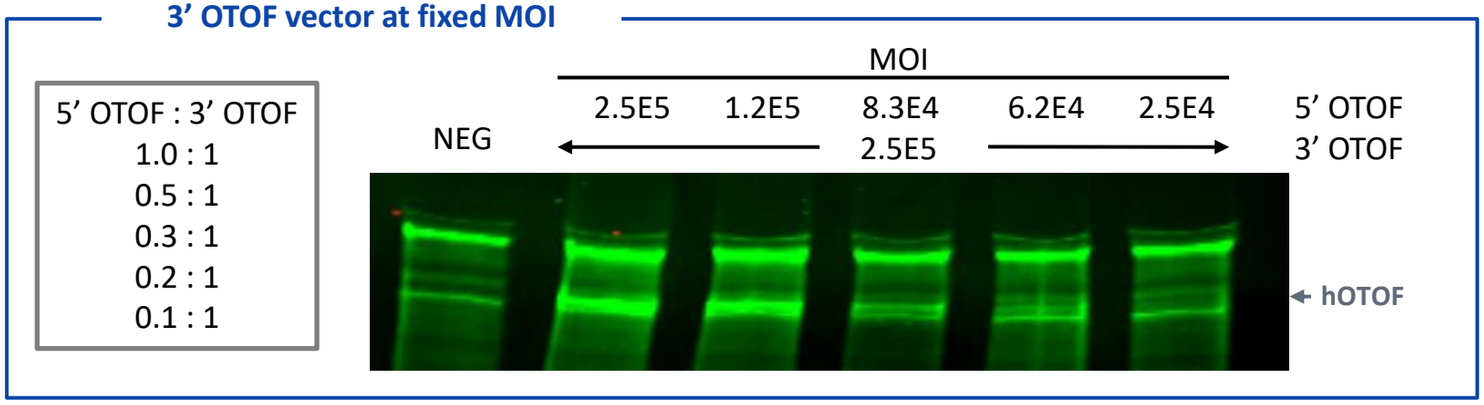
* Non-specific Ab detection

- No detectable proteins were observed by Western Blot analysis when cells are transduced with individual vector

Determination of optimal dual vector ratio



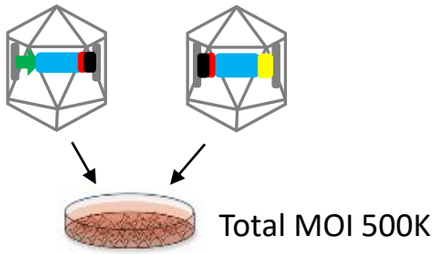




- Full length otoferlin protein expression is function of the limiting vector

Determination of optimal dual vector ratio

Dual transduction

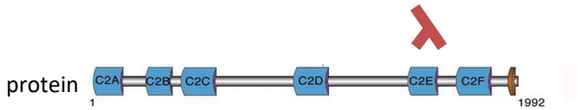


Total MOI 500K

HEK293

3 days

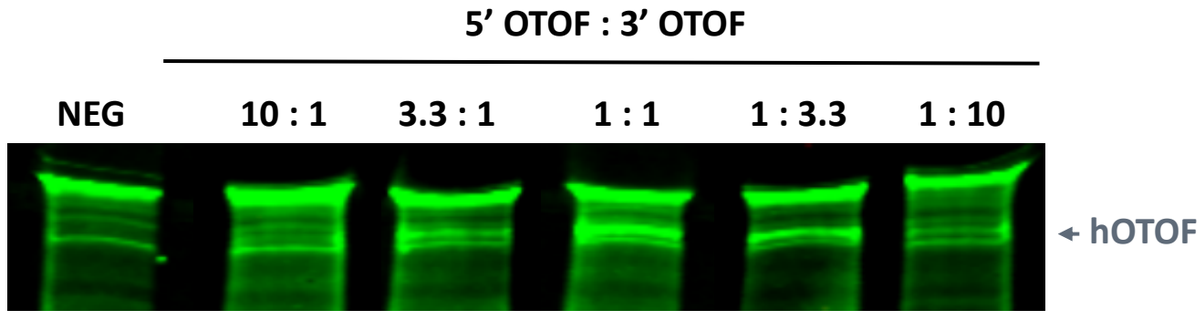
Cell lysate
Protein extraction



Immunodetection of otoferlin protein
Anti-otoferlin Ab against Cter domain

5’ OTOF : 3’ OTOF
10 : 1
3.3 : 1
1 : 1
1 : 3.3
1 : 10

WB analysis - Anti-Otof Ab against Cter domain



- A 1:1 ratio showed the highest level of full-length otoferlin protein expression

Conclusions

- A thorough control strategy (VG, IG, RT-PCR…) must be implemented very early in development

- Transduction with dual OTOF vectors results in an efficient recombination and a full-length otoferlin expression
- No undesired truncated otoferlin proteins were detected after in vitro transduction with dual vector or each individual vector
- A 1:1 ratio of dual vector shows to be optimal for efficient expression of full length otoferlin protein

- Hearing restoration demonstrated in Otof $^{-/-}$ mouse model when using dual AAV vector

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- Géraldine Honnet
- Nora Yang
- Valérie Salentey
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THANK YOU