# Evotec capabilities in AAV and LV application

**FOR FURTHER INFORMATION:**

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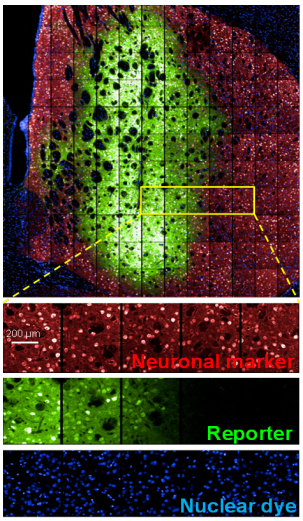
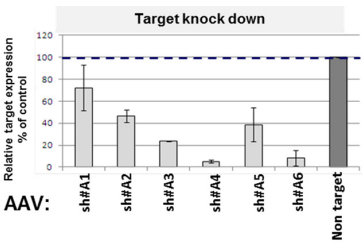
## 8 YEARS OF EXPERIENCE IN AAV PRODUCTION, QC AND *IN VITRO* / *IN VIVO* APPLICATION

- More than 8 years of experience in AAV production, QC and *in vitro* / *in vivo* application. To date, more than 30 targets have been evaluated by either knock-down or overexpression.
- Established process for production and identification of a suitable knock-down construct (10 constructs/target are examined; > 1000 small scale productions to date).
- Followed by large-scale production of selected constructs (>300 large-scale productions to date) enabling both *in vitro* and *in vivo* studies.
- Long-term experience with *in vivo* application techniques and stereotactic surgeries in mice and rats.
- More than 2 years' experience in lentivirus application. Supported by a dedicated core team (9 members, including 3 PhD scientists).



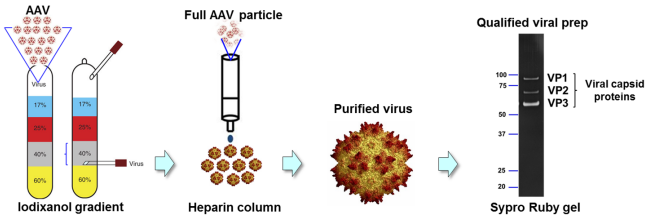

## FULLY ESTABLISHED WORKFLOW TO IDENTIFY KNOCKDOWN CONSTRUCTS: SHRNA OR CRISPR

- Custom design of shRNA or gRNA constructs, followed by cloning of AAV expression vectors.
- Small scale AAV production (up to 10 constructs/target) and AAV transduction into target cell of choice.
- Identification of the most efficient constructs by target expression or INDEL analysis.
- Option to perform *in vitro* target validation (TV) by qPCR, RNAseq, next-generation sequencing (editing frequency), MSD, TR-FRET, Singulex, and/or High-content imaging.



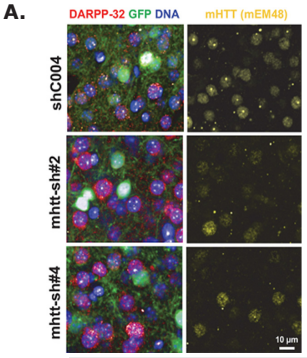
## HIGH-QUALITY VIRUS PRODUCTION AND PURIFICATION: ABLE TO DELIVER AAVS SUITABLE FOR *IN VIVO* STUDIES

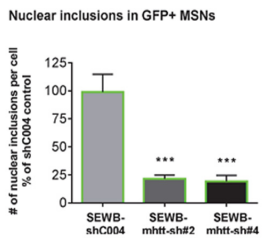
- High purity of AAV are needed for *in vivo* applications. This is achieved by gradient centrifugation and purification based on the AAV serotype. AAVs are enabled for long-term storage at -80°C.
- Standard QC checks before *in vivo* use: titer determination, purity and *in vitro* TV.




## CASE STUDY HUNTINGTON DISEASE – REGULATION OF TARGET EXPRESSION REDUCES DISEASE PHENOTYPE

- Modulation of HTT levels using shRNAs delivered by AAVs leads to a significant decrease in the number of nuclear mHTT inclusions in comparison to the control. Data are published: Carty N, et al. PLOS ONE, 2015.
- AAV2 1+2 particles encoding GFP and shRNAs directed against HTT were injected in the right ventricle of neonate zQ175 heterozygous mice (analysis at 4m of age):
  (A.) DARPP-32 and mHTT IHC staining in the GFP positive striatal region transduced with AAV2 encoding shRNA
  (B.) Quantitative analysis of mHTT nuclear inclusions in GFP positive cells of the striatum demonstrates significant knock-down in target cells




## CASE STUDY 2 HUNTINGTON DISEASE – TARGET VALIDATION AT THE NEURONAL SYNAPSE

- WT mice with a neonatal injection of AAV into the lateral ventricle
- Brains sectioned and stained after 1 month. Images collected using 60x mag on Opera (4 channels, 11 planes, 0.5 µm steps).
- Target mutant appears to act as a dominant negative