Advanced characterization of human hepatocytes xenotransplanted mice as predictive pharmacological tool system for human liver-targeted gene therapy

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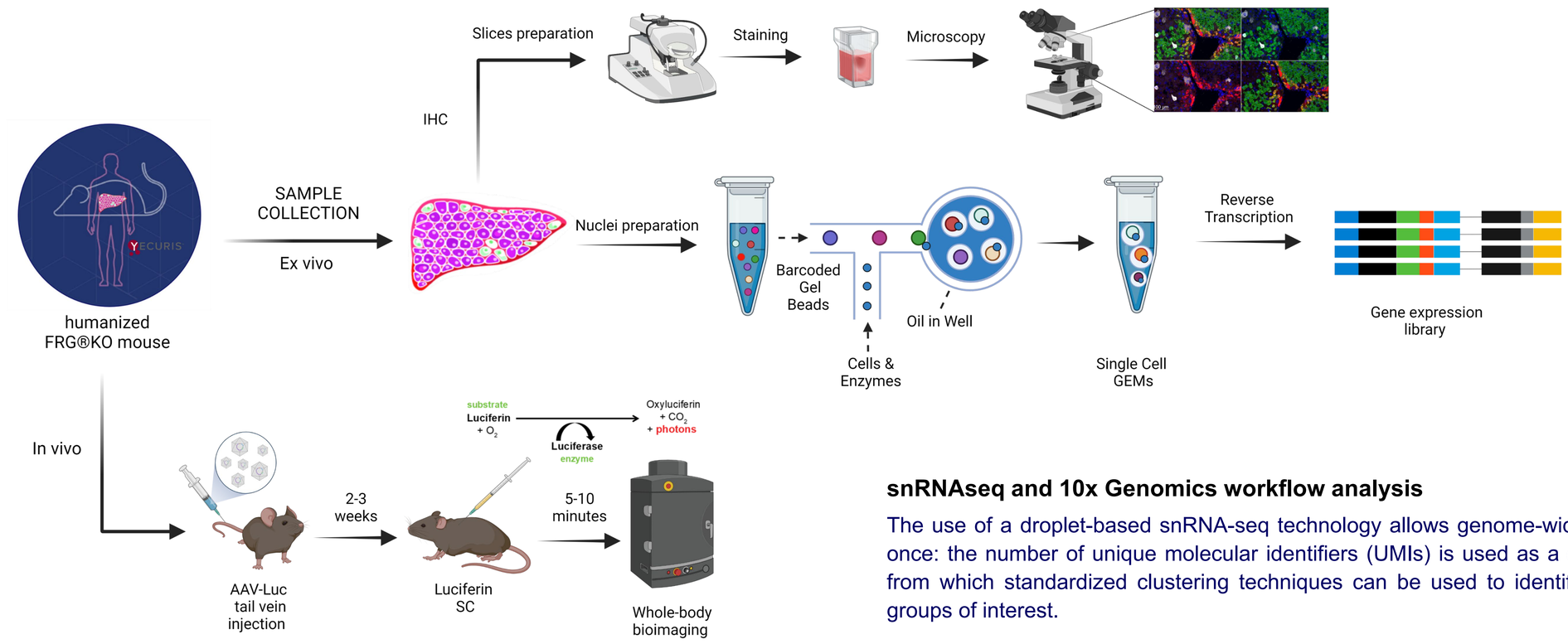
In vivo AAV transduction of human hepatocytes xenografted FRG®KO mice

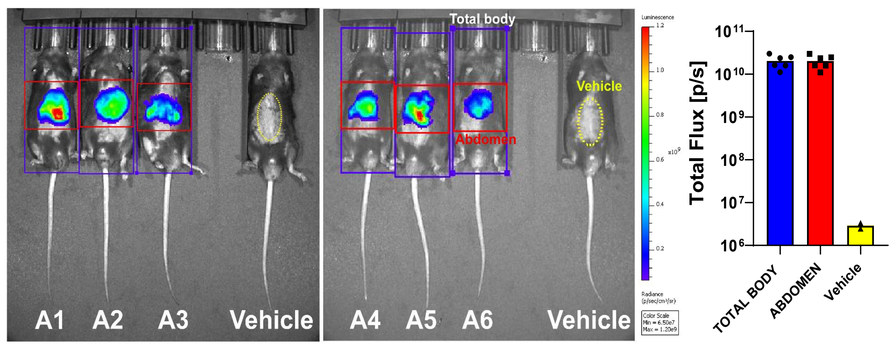
Our standardized workflow allows the *in vivo* screening of AAV-derived viral vectors in xenotransplanted mice, with a focus on improved human liver tropism and species-specific transduction of the payload.
Our strategy complements *in vivo* bio-imaging expression measures with *ex vivo* histological and molecular analysis to study tissue biodistribution at a resolution refined to the individual hepatocyte.



snRNAseq and 10x Genomics workflow analysis

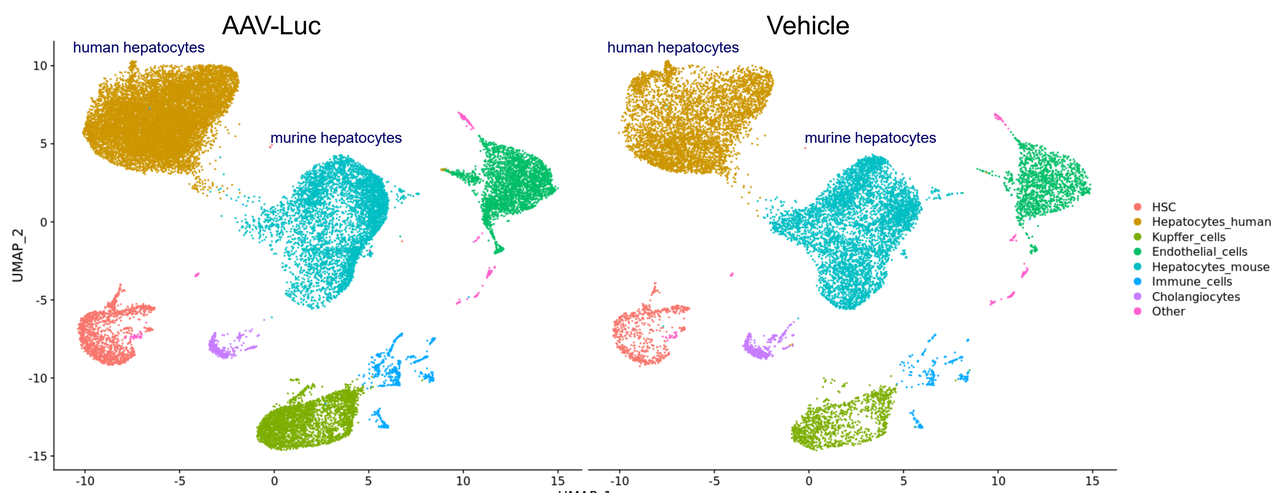
The use of a droplet-based snRNA-seq technology allows genome-wide expression profiling of thousands of cells at once: the number of unique molecular identifiers (UMIs) is used as a direct representation of gene expression level, from which standardized clustering techniques can be used to identify cell-type population, and experimental sub-groups of interest.

In vivo biodistribution of transgene expression



In vivo whole-body bio-luminesce imaging measures the photons emitted as a result of an enzymatic reaction transforming the substrate Luciferin into oxy-Luciferin. The un-biased quantitative readout [p/s] is calculated for each standardized ROI in order to allow inter- and intra-individual (longitudinal) comparisons

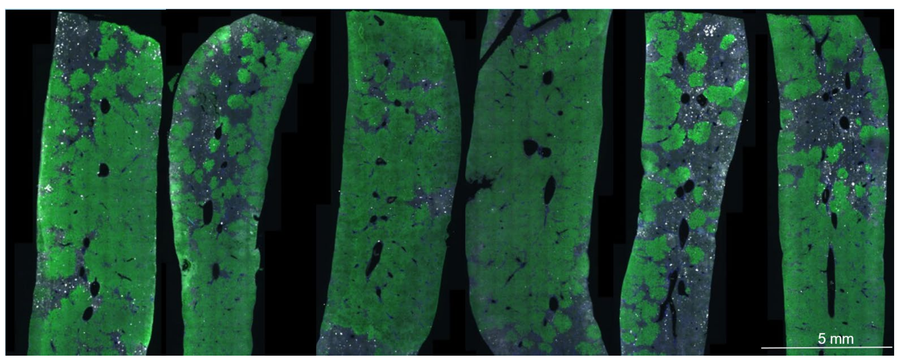
AAV-Luc treatment does not affect cells distribution in the annotated clusters

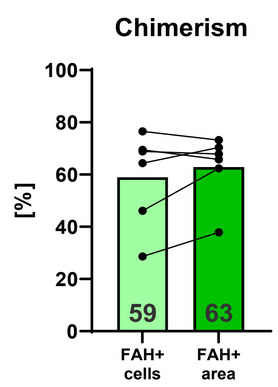


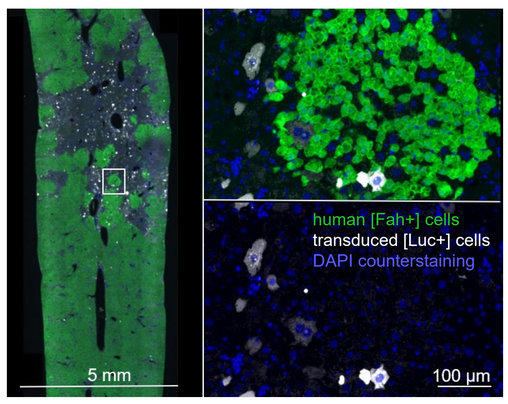
Uniform manifold approximation and projection (UMAP) plot representation of > 43,000 cells (nuclei) yielding 8 major cell types. UMAP plots are split between treatments (AAV and Vehicle) to illustrate clusters identified in both groups. Differences in cell abundance and transcriptional heterogeneity between subpopulations within groups can be further investigated in detail.

IF detection of transgene expression in human vs. murine hepatocytes

The combination of immunofluorescent labelling of the payload and detection of cell-identifying markers further allows for the analysis of spatial biodistribution within the organ, such as cell-type targeting and zonation effects

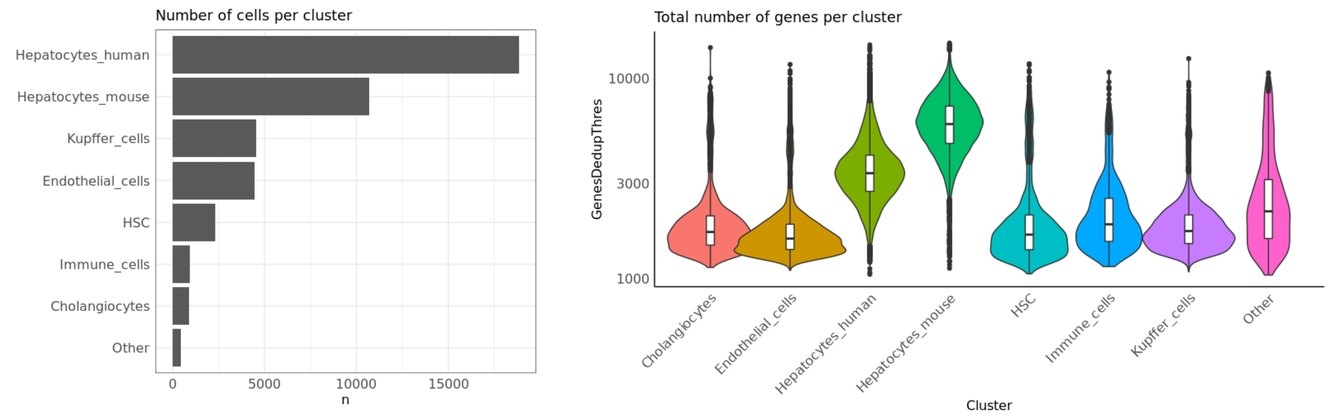






Whole sections were analyzed using Visiopharm image analysis software. The total number of cells [DAPI+], human hepatocytes [Fah+] and transduced cells [Luc+] were determined using the staining intensity in the corresponding channel. The overall degree of chimerism can also be estimated via the ratio of Fah+ area and whole section area with reasonable accuracy.

Quantification of cells and transcriptome abundance in each annotated cluster

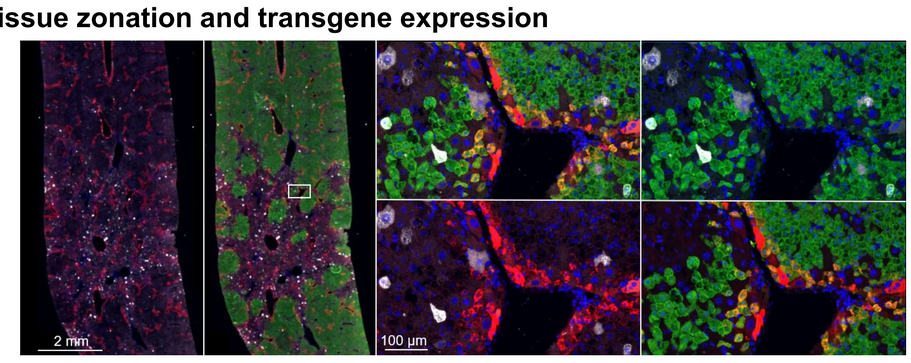


Number of cells per cluster (left panel) was calculated including cells expressing < 25% mitochondrial counts and at least 1000 genes were retained after a quality control step. Number of genes per cluster (right panel) is further visualized for each one of the cell-type clusters identified, where the violin plot widths are proportional to the density of the distribution.

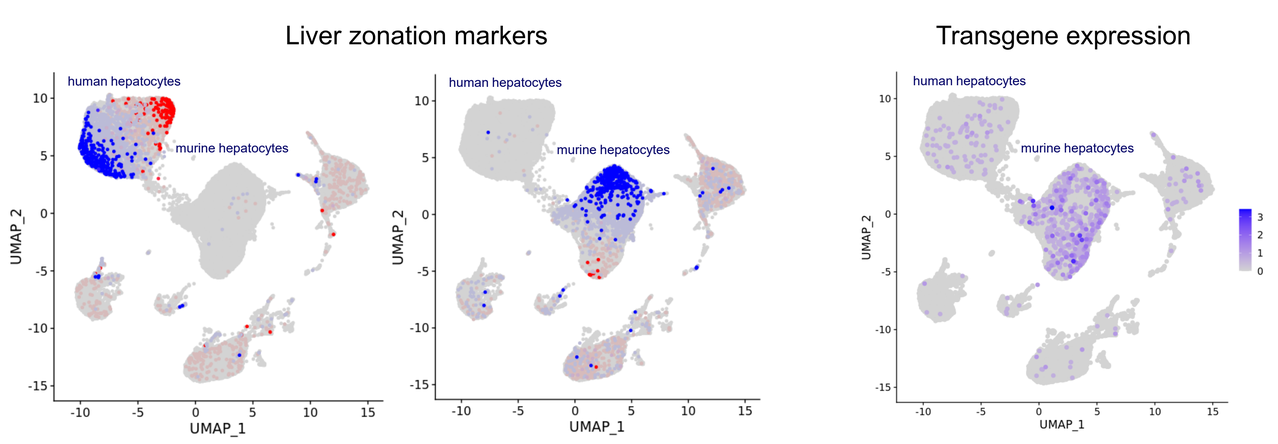
Spatial- and cell type-specific transgene expression is further annotated

Advanced characterization of single cells within the identified clusters enables the molecular identification of experimental populations of interest relative to e.g. treatment potency and biodistribution, with the potential to highlight additional pharmacodynamic or toxicological outcomes

Tissue zonation and transgene expression



Tissue zonation and topographical distribution of payload transduction were assessed by differential immuno-labelling of cell-identifying markers. In the example, **peri-central [GS+]** hepatocytes are highlighted.



Expression of genes of interest mapped onto the UMAP embedding. Markers of liver zonation were used to highlight **peri-central [GluI+]** vs. **peri-portal [Sds+]** hepatocytes in the human and murine populations, respectively. Additionally, transgene expression in those population is further highlighted. The color scale represents a range of 0 (grey, no expression) to 10 (red or blue, highest expression).

- Primary human hepatocytes xenotransplanted mice represent an increasingly interesting system to investigate the translational potential of liver-targeted gene therapy solutions
- Our standardized workflow allows the quantification of cell-type- and species-specific tropism of AAV-derived gene therapy products, with a resolution refined to the individual human hepatocyte
- The use of complimentary analytics vastly improves the translational value of this model system in the development of novel gene therapies
- Potential to expand the outlined analytical workflow to include ISH (RNAscope) as well as spatial RNA sequencing approaches

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