Mutations in the DNA encoding for isocitrate dehydrogenases (IDH) mutations are established molecular markers in 70-80% of stage II or III in glioblastoma. Among IDH1 mutations, R132H is the most common mutation (90%) in glioma. IDH1-R132H is expressed in other cancers so the visualization of the dynamics of the cancerous cells within the brain, and their lineage tracking using an animal model could help as an early predictor for grades 2 and 3 glioma. There have been various promising experiments on mice regarding vaccinations, or immunizations targeting IDH1-R132H.

Biological question: Develop a cancer brainbow animal model expresssing IDH1^{R132H} in the brain subventricular zone (SVS) to visualize expansion and spread of the oncogene IDH1^{R132H}. Investigate cell migration in term of speed and diffusion across tissues.

- We will use the study by Bardella et al. to generate a mouse model of glioma by expressing IDH1-R132H in the subventricular zone (SVZ) in the brains of adult mice. The IDH1$^{fi(R132H)/+}$ knockin mice[1] showed hydrocephalsys and expanded lateral ventricles with an accumulation of D isomer of 2-hydroxyglutarate (D2HG) and a reduction of alpha-ketoglutarate (α-KG).
- Once the germline transmission of the mutant allele established, we will engineer and validate a cancer brainbow mouse model. We will follow the methodology detailed in [1]. The cancer rainbow (crainbow transgene has 4 positions that either express an inert fluorescent protein (position 0), or 3 spectrally resolvable fluorescent proteins paired with an oncogenic mutation of choice (positions 1-3) which in our case, is the mutated IDH1^{R132H} gene. Each R132H allele is co-expressed with TagBFP (cyan), mTFP1 (yellow), or mKO (magenta). The construct is further optimized to provide a rich fluorescent protein palette (XFP) by the expression of a membrane-targeted and chemically inducible near-infrared fluorogen-activating peptide (Fap-Mars1) as a control.
- We will then follow a multi-step process to analyze the collective mobility of the investigated brain cancer cells:
  1. Brain mouse organoids will be then made according to established protocols.
  2. We will then use an RNA Protein detection assay such as the RNAScope Kits from ACD to identify mRNA targets in FFPE tissues sections from Crainbow mice brain.
  3. We will then follow a single-cell RNASeq protocol which will involve in turn a few steps:
     o Cell isolation (we can use a single-cell isolator for single cell capture)
     o Build of gene matrices (or expression matrix in scRnaSeq methodology)
     o Feature selection → Dimensionality reduction → Cell-cell distance matrix
     o Unsupervised clustering for identification of cell populations
  4. Excitation laser will be performed for image acquisition, preprocessed to remove noise, followed by various filtering techniques, and then analyzed.

Having identified clusters of subpopulations, we will then be able to identify cell lineage by their unique color and perform a displacement analysis using the following equations
Assuming that the cells follow a Brownian motion, the cells

$$P(r,t) = \frac{1}{\sqrt{2}}$$

The mean squared displacement which describes the displacement of the cells as the average distance of the subpopulations of cells (identified by the same color) in a given time interval, τ:

$$\text{MSD} = \frac{1}{N} \sum_{n=1}^{N} |r^n(\tau) - r^n(0)|$$ where N: total number of trajectories for all measured subpopulations

$r^n(0)$: starting position of the nth cell trajectory
$r^n(\tau)$: position of the nth cell after τ

Instantaneous speed, V, at the ith step of a cell as the displacement

$$\eta = |\mathbf{v}(t_i)| = \frac{r(t_i) - r(t_{i-1})}{t_i - t_{i-1}}$$
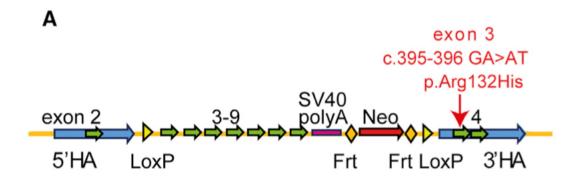
The migration speed of a cell, V, is the mean over the entire length of the trajectory from i = 1 to the total number of steps L: $V = \frac{1}{L} \sum_{i=1}^{L} \eta_i$
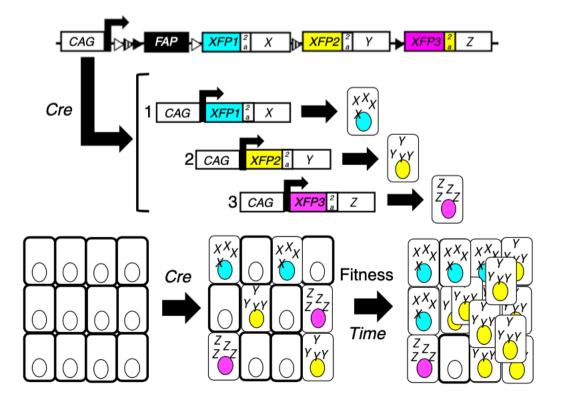
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[1] The knock-in region comprised a 5' loxP site, a wildtype min-gene (exons 3-9 and 3'UTR), an SSV40 polyA signal, a Neo^R cassette flanked by Frt sites, and a 3' loxP site.The LoxFtNwCD vector was injected into C57BL/6 ESS cells. The Neo^R cassette was removed using Cre-mediated recombination and the R132H allele was knocked-in *in vivo*.

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Expression matrix	Feature selection	Dimensionality reduction	Cell-cell distances	K-nearest neighbours
(n) cells	(n) cells	(n) cells	(n) cells	cell

Main methods:

	HVG	PCA	K-means	Louvain
	M3Drop	tSNE	Hierarchical	Infomap
	Spike-ins	Diffusion maps	DBSCAN	densityCut
				SNN-Cliq

Clustering