A genome-wide murine siRNA screen to investigate the molecular machinery governing PrPc biosynthesis and PrPsc propagation

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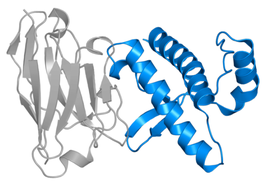
Overview

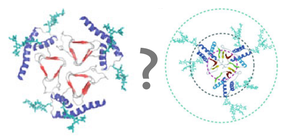
Transmissible spongiform encephalopathies are a group of devastating transmissible neurodegenerative diseases characterized by spongiform changes in the neuronal tissue, astrogliosis and microglial activation, leading to death within a short time after disease onset[x].

The **causative agents** of these diseases are **prions**, which mainly consist of **PrPsc — a misfolded conformational variant of the cellular prion protein PrPc[x,y].**

PrPc



PrPsc[z]
models



Currently, we establish a **genome-wide high-throughput siRNA screen** in murine CAD5 cells to elucidate the **regulatory networks** involved in

- Biosynthesis of PrPc
- Propagation of PrPsc

RNA interference

siRNA PrPc/PrPsc screen

RNA interference Hight Throughput Screening Platform

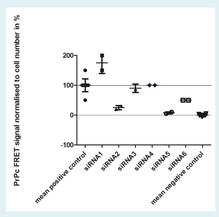
RNA interference: targeted gene knock-down

- Cells are transfected with defined concentrations of siRNAs.
- The siRNAs bind to their target mRNAs, inducing their cleavage
- Upon mRNA cleavage, translation of the gene product is inhibited.

➡The target protein is knocked-down.
➡We assess the influence of the protein knock-down on PrPc biosynthesis and PrPsc infectivity:

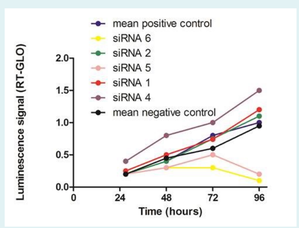
Biosynthesis of PrPc and propagation of PrPsc

- We use a FRET system to measure the PrPc/PrPsc levels upon knock down of a specific protein.



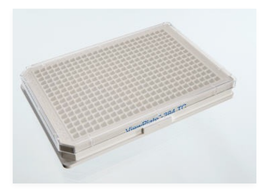
Kinetics of cell viability

- Monitor cell growth throughout the experiment.
- Assess anomalies, cytotoxic events, contaminations and relative cell numbers, used for normalization of FRET signals.



High-throughput siRNA screen

➡**Parallelizing RNA interference for genome-wide collections of siRNAs allows for high-throughput screens.**



384-well plate

The individual assays are run in parallel in 384-well plates.
We currently target a daily throughput of 480 mRNAs, including 4 siRNAs per mRNA with 2 duplicates.

Technology

- Acoustic dispensing in nL range precision (ECHO555)
- Peristaltic pump dispensing system (Biotek Multiflo FX)

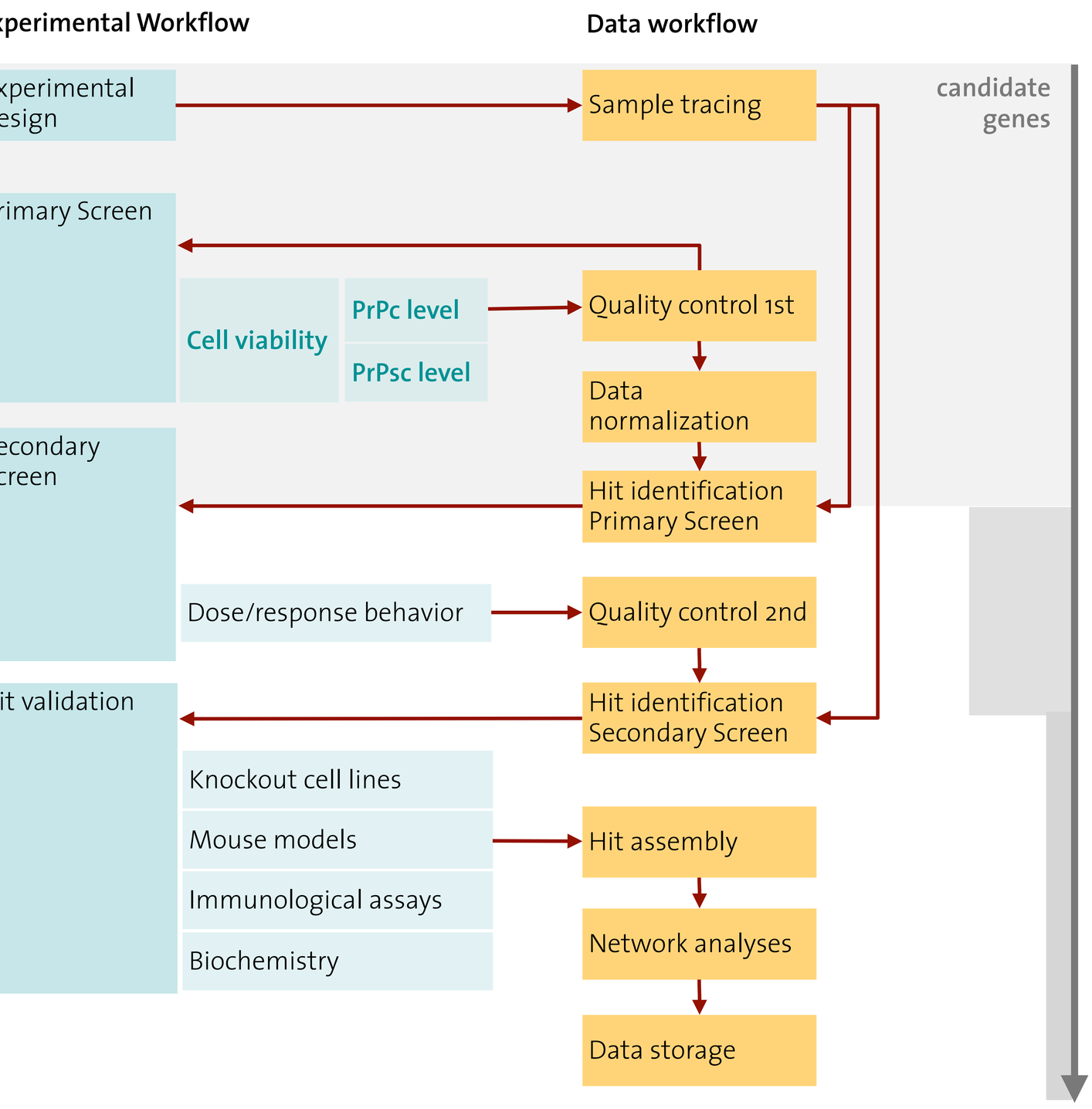
Peristaltic pump

Experimental workflow

- **Pilot Screen**: Test a library subset to ensure consistent and replicable results.
- **Primary Screen**: Rough genome screen, with the purpose to filter the bulk of negative samples.
- **Secondary Screen**: Stable, quantitative screen of remaining candidates with a dose/response approach.

Data workflow

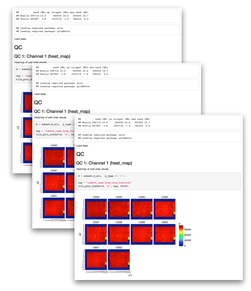
The data workflow and the experimental workflow are closely interweaved, to allow for fast feedback to the experimenter.
Data handling, including sample tracing, data storage, quality control, analyses are automated programmatically.



Data analysis

We are following the *reproducible research* paradigm:
"Data, Code and Data analysis are published together"

Example Quality control analysis:
- Experimental data and numerical and graphical quality controls are pulled together in RMarkdown documents.



Outlook

- Establish **pilot screen** with consistent and replicable results, notoriously passing quality control measures
- Conduct the workflow for our siRNA Prp system
- Increase throughput with 1536-well plates
- Fully automate the experimental workflow on robotic Labcyte Platform
- Transfer other prionoid experimental systems to the developed RNA interference high-throughput screening platform:
  - PrP, α-synuclein
  - siRNA, miRNA, CRISPR/CAS9

References

[x] Aguzzi, A.,. Calella, A. M. (2009). Prions: protein aggregation and infectious diseases. Physiological Reviews.

[y] Aguzzi, A., & Falsig, J. (2012). Prion propagation, toxicity and degradation. *Nature Neuroscience*.

[z] Surewicz, W. K., & Apostol, M. I. (2011). Prion protein and its conformational conversion: a structural perspective. *Topics in Current Chemistry*.