

siRNA Infection Screens – Pitfalls and Promises

4th KNIME Users Group Meeting and Workshop






4.3.2011

Roger Meier - Ari Helenius lab - ETHZ

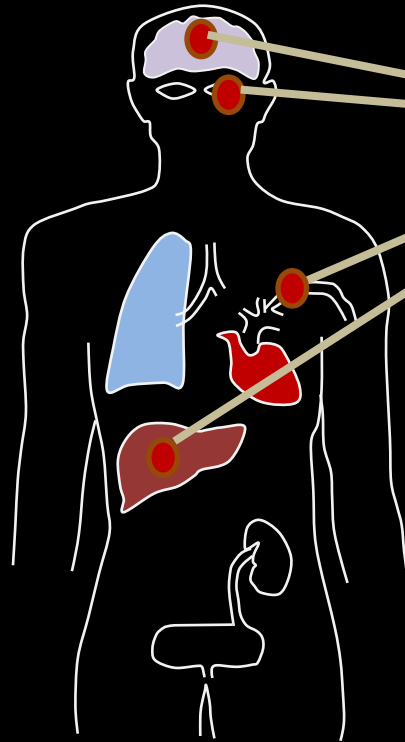
Bunyaviridae: > 350 members; 5 genera

	Genus
Plant-infecting viruses	Tospovirus
Animal-infecting viruses	Orthobunyavirus
	Phlebovirus
	Nairovirus
	Hantavirus

Bunyaviridae: Transmission

	Genus	Vector	
Plant-infecting viruses	Tospovirus	→ Thrips	
Animal-infecting viruses	Orthobunyavirus	Arthropods → Mosquitoes Members of Arboviruses	
	Phlebovirus	→ Mosquitoes Ticks	
	Nairovirus	→ Mosquitoes Culicoid flies	
	Hantavirus	→ Rodents Mouse Rats	

Human disease of *Bunyaviridae* members



Phlebovirus

Rift valley fever virus (RVFV)

- Encephalitis
- Hepatic necrosis
- Macular scarring

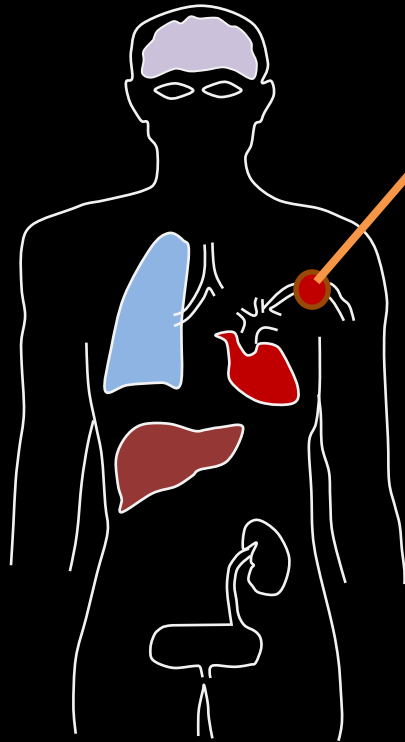


Macular scarring

Human disease of *Bunyaviridae* members

Nairovirus

- Crimean Congo virus
- Hemorrhagic fever



Human disease of *Bunyaviridae* members

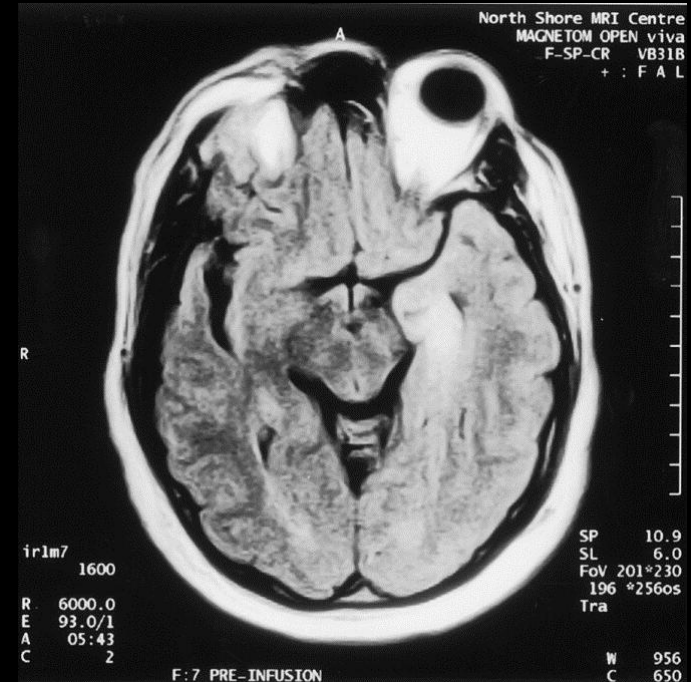
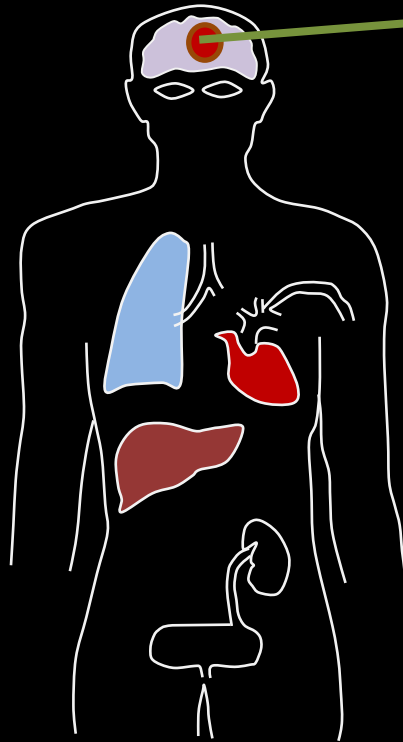
Orthobunyavirus

La Crosse virus

Bunyamwera virus

California encephalitis virus

- Encephalitis
- Neuronal necrosis



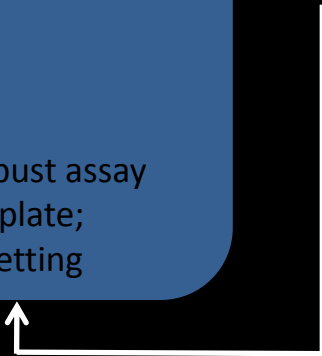
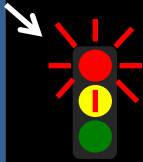
Encephalitis, left lobe (La Crosse virus)

Screen workflow

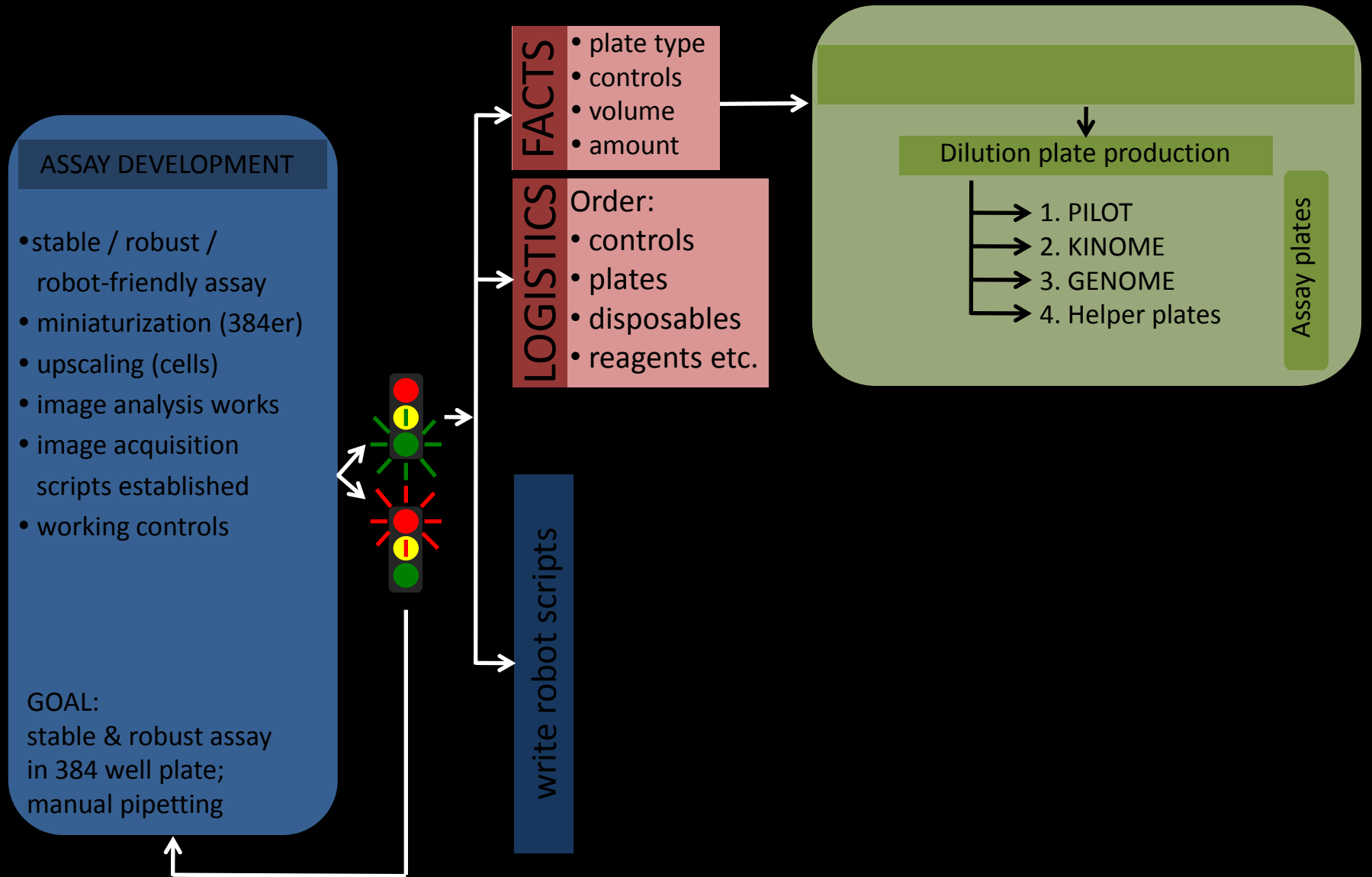
ASSAY DEVELOPMENT

- stable / robust / robot-friendly assay
- miniaturization (384er)
- upscaling (cells)
- image analysis works
- image acquisition scripts established
- working controls

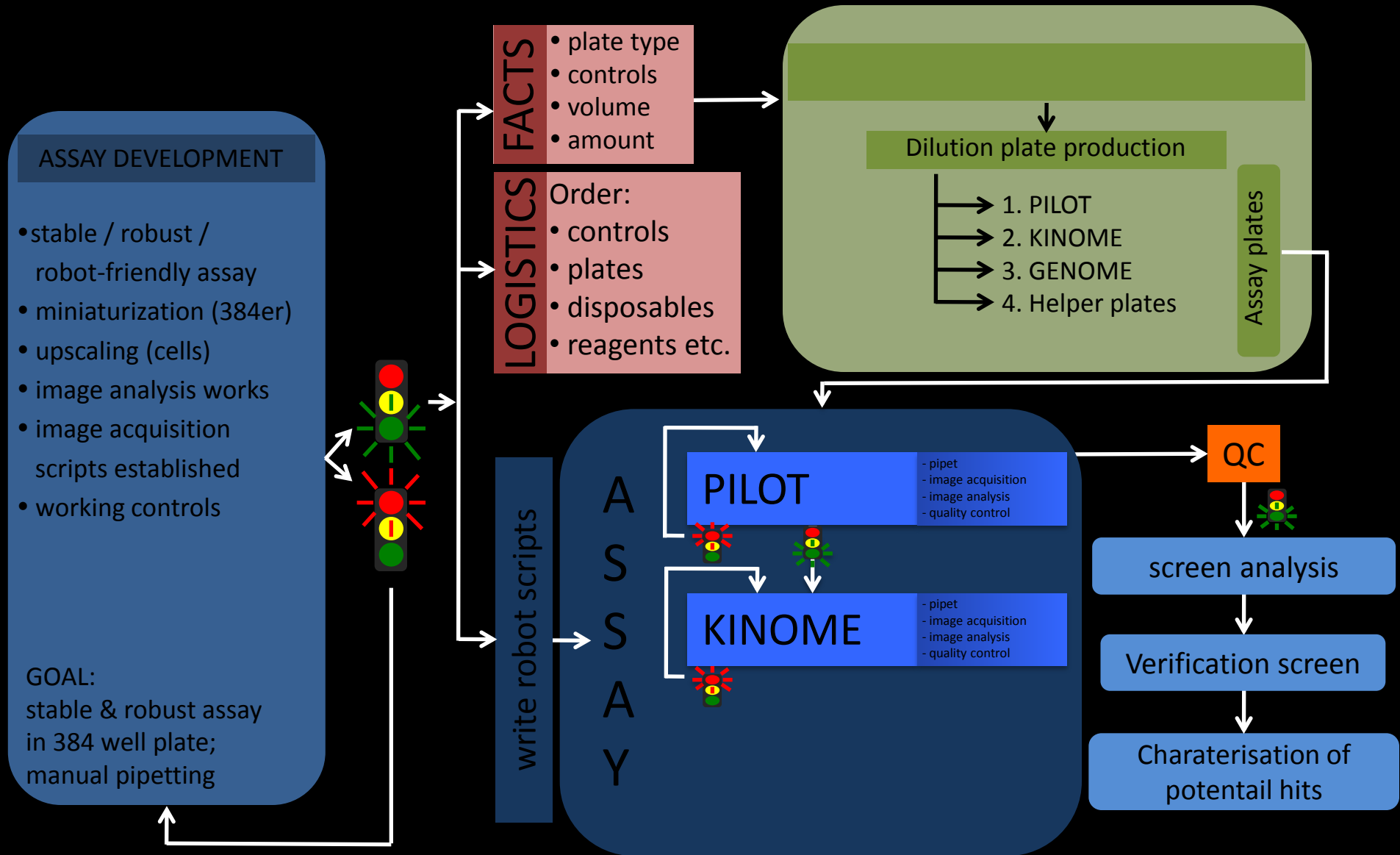
GOAL:
stable & robust assay
in 384 well plate;
manual pipetting



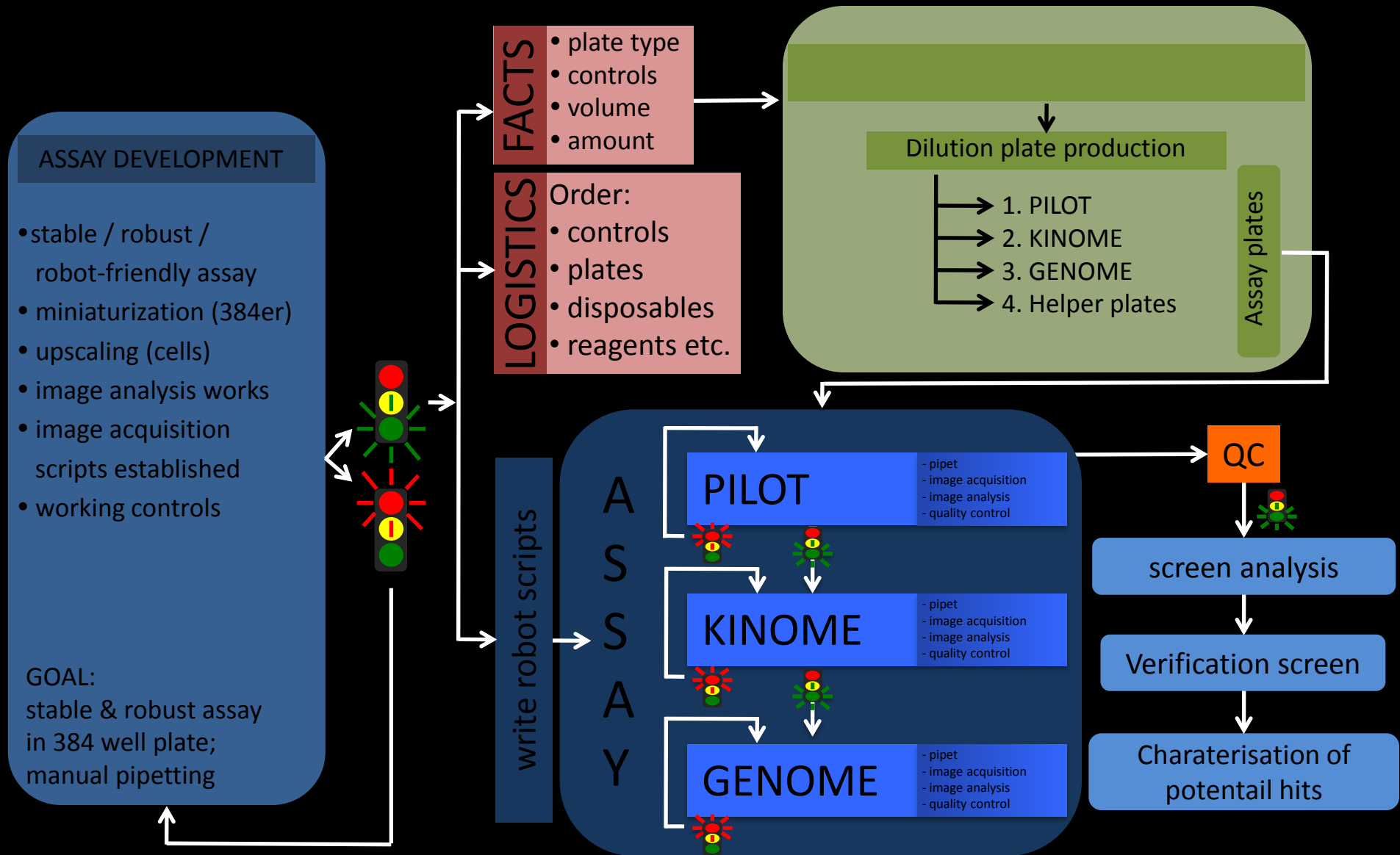
Screen workflow



Screen workflow



Screen workflow



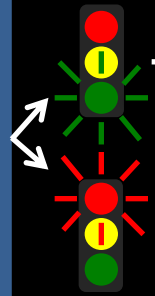
Screen workflow



ASSAY DEVELOPMENT

- stable / robust / robot-friendly assay
- miniaturization (384er)
- upscaling (cells)
- image analysis works
- image acquisition scripts established
- working controls

GOAL:
stable & robust assay in 384 well plate;
manual pipetting



FACTS

- plate type
- controls
- volume
- amount

LOGISTICS

- Order:
- controls
- plates
- disposables
- reagents etc.



Dilution plate production

- 1. PILOT
- 2. KINOME
- 3. GENOME
- 4. Helper plates

Assay plates

write robot scripts

**A
S
S
A
Y**

PILOT

- pipet
- image acquisition
- image analysis
- quality control

KINOME

- pipet
- image acquisition
- image analysis
- quality control

GENOME

- pipet
- image acquisition
- image analysis
- quality control

QC

screen analysis

Verification

Charaterisation of potentail hits



siRNA screen protocol

1.) add transfection
reagent & cells

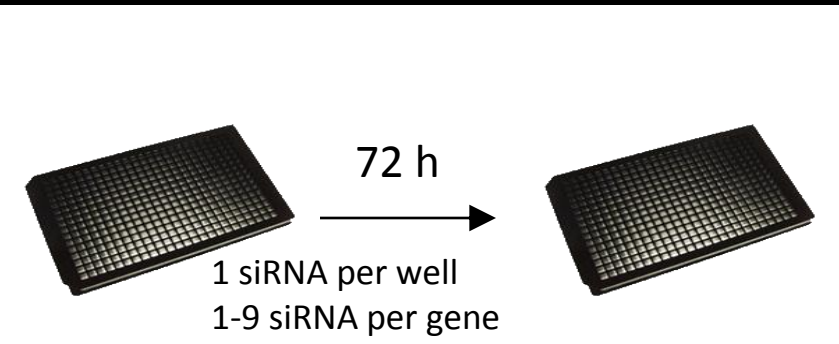


1 siRNA per well
1-9 siRNA per gene

siRNA screen protocol

1.) add transfection reagent & cells

2.) wash & virus infection



siRNA screen protocol

1.) add transfection reagent & cells

2.) wash & virus infection

3.) wash & add medium



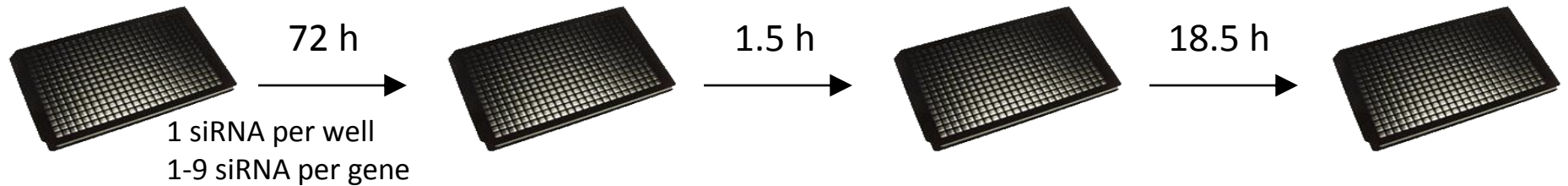
siRNA screen protocol

1.) add transfection reagent & cells

2.) wash & virus infection

3.) wash & add medium

4.) fixation & DNA & virus staining



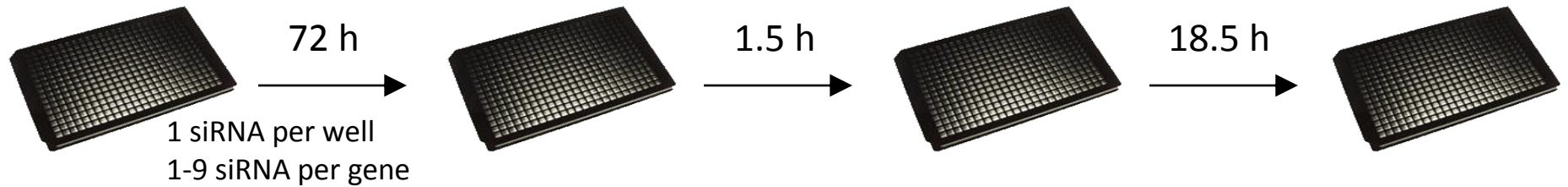
siRNA screen protocol

1.) add transfection reagent & cells

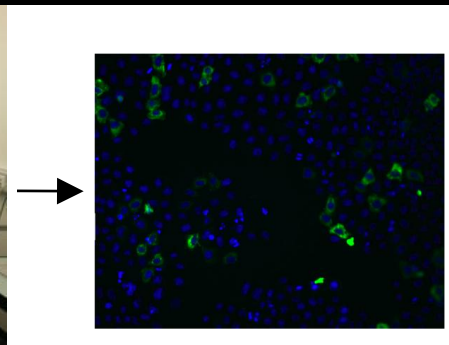
2.) wash & virus infection

3.) wash & add medium

4.) fixation & DNA & virus staining



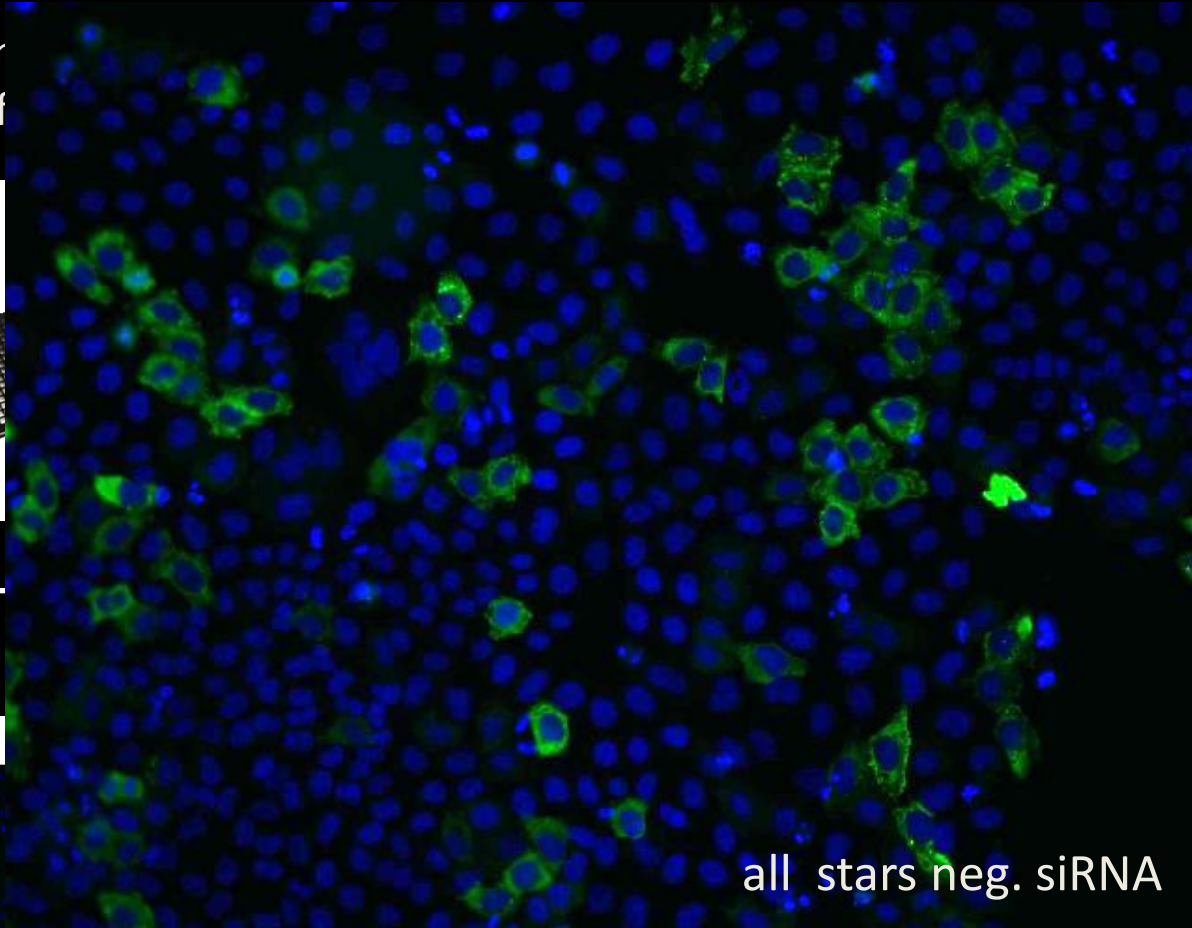
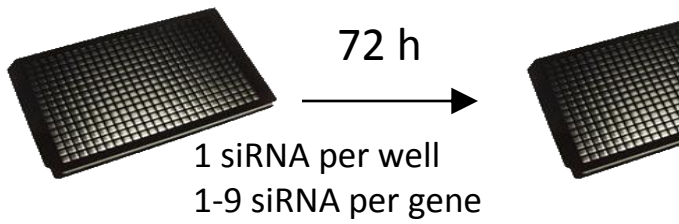
5.) microscopy



siRNA screen protocol

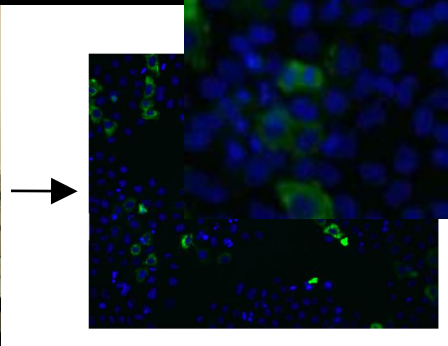
1.) add transfection reagent & cells

2.) wash virus inf



all stars neg. siRNA

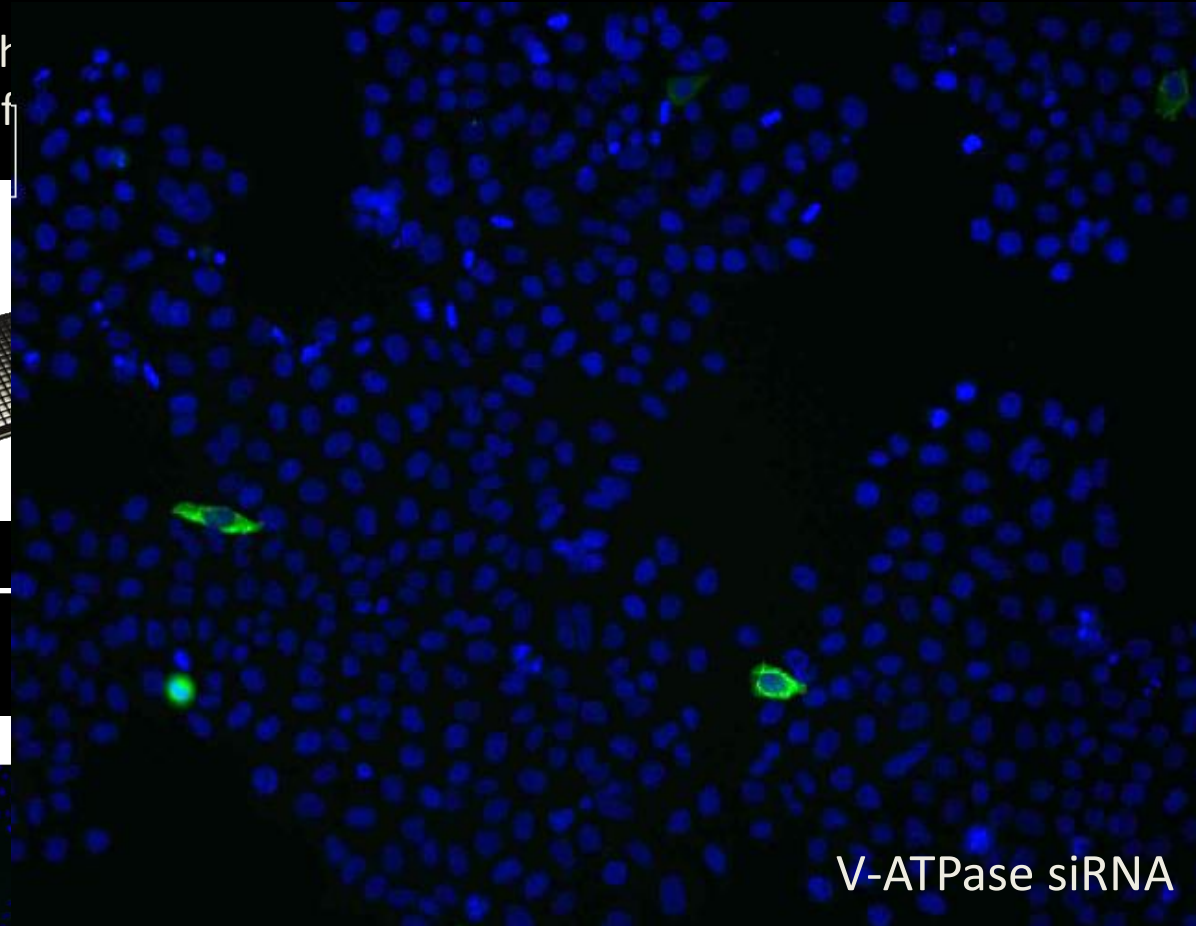
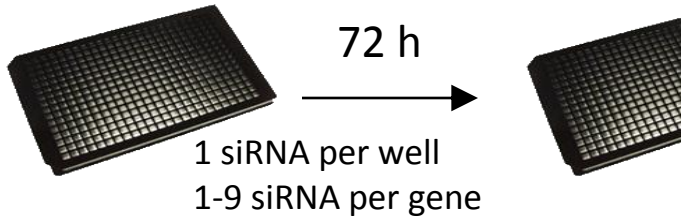
5.) microscopy



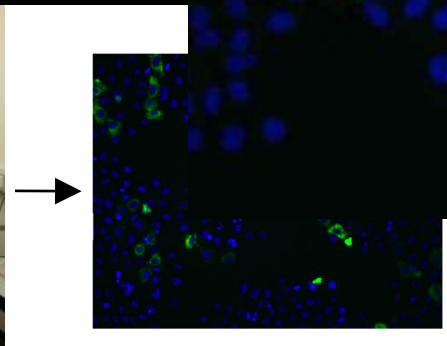
siRNA screen protocol

1.) add transfection reagent & cells

2.) wash virus inf



5.) microscopy



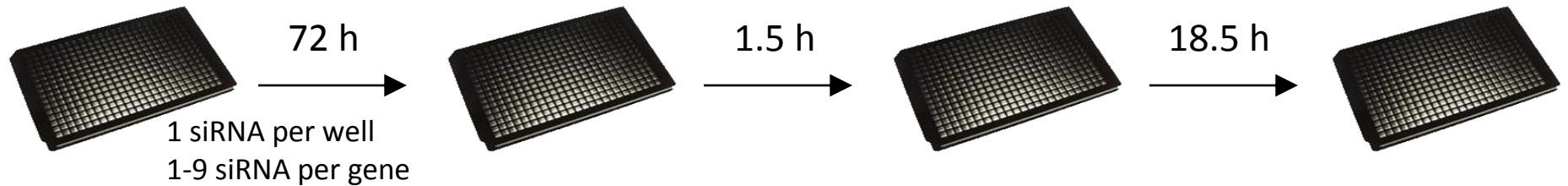
siRNA screen protocol

1.) add transfection reagent & cells

2.) wash & virus infection

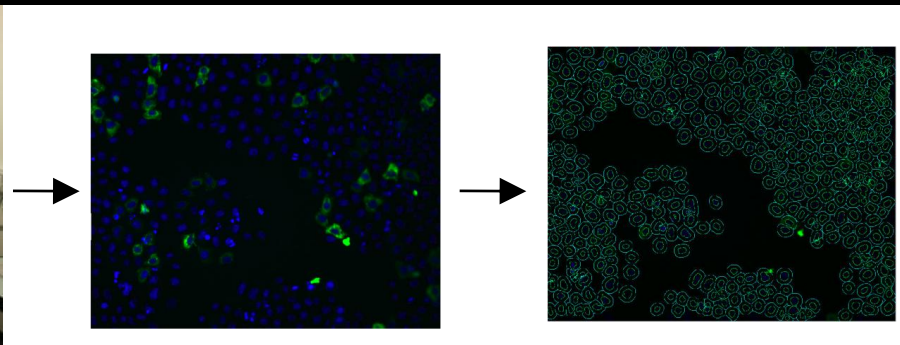
3.) wash & add medium

4.) fixation & DNA & virus staining



5.) microscopy

6.) segmentation

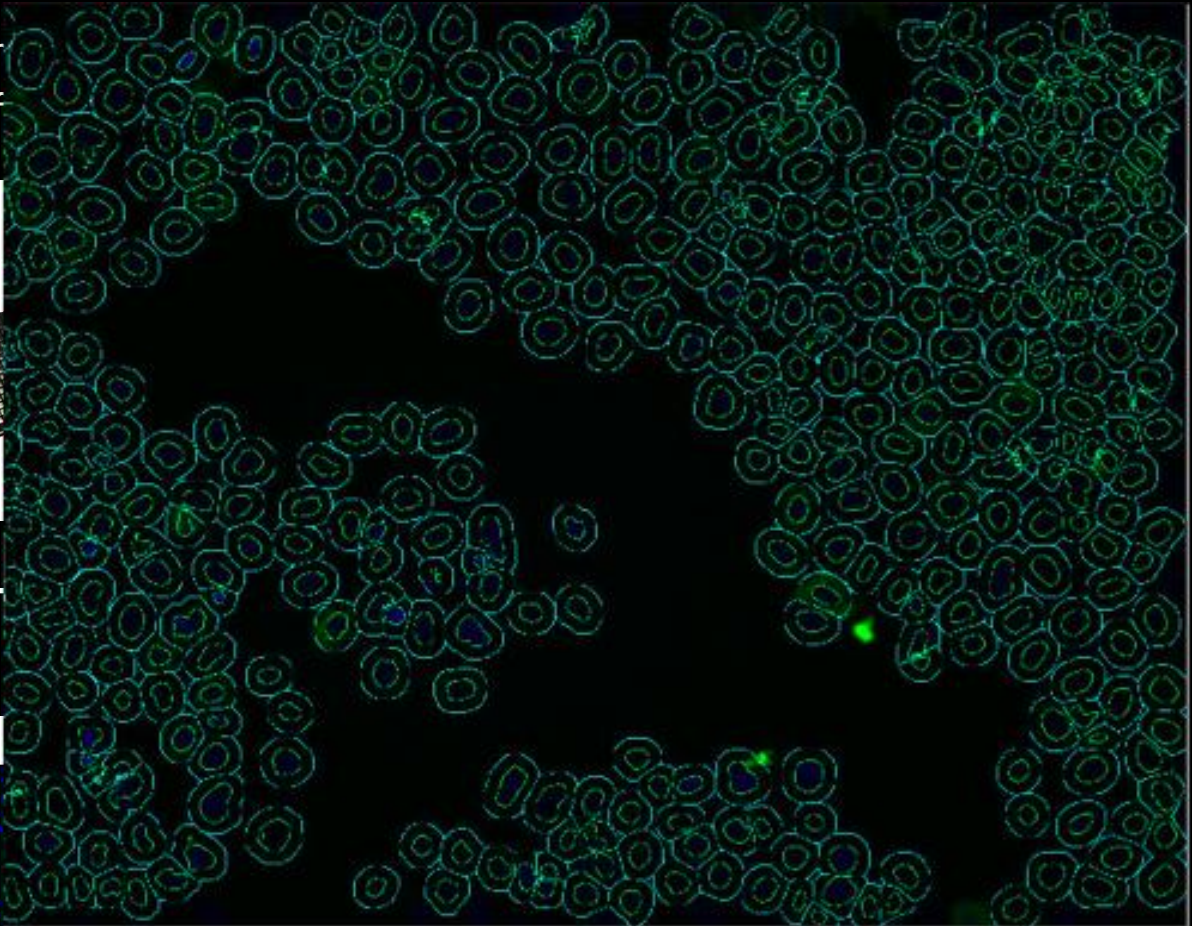
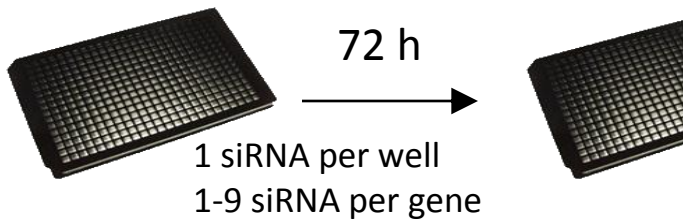


CellProfiler
<http://www.cellprofiler.org/>

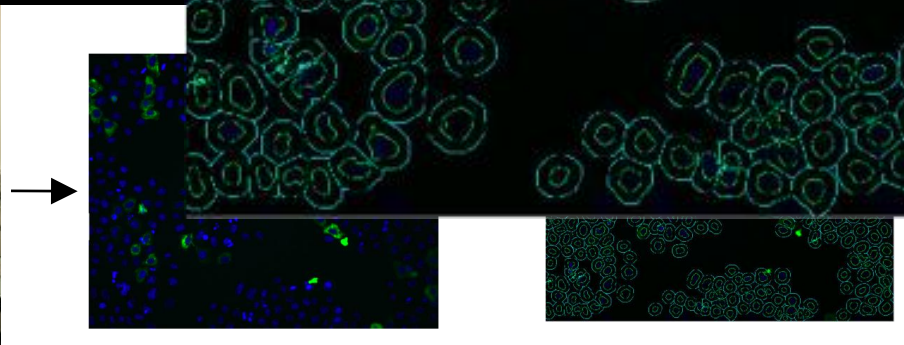
siRNA screen protocol

1.) add transfection reagent & cells

2.) wash
virus inf



5.) microscopy



CellProfiler
<http://www.cellprofiler.org/>

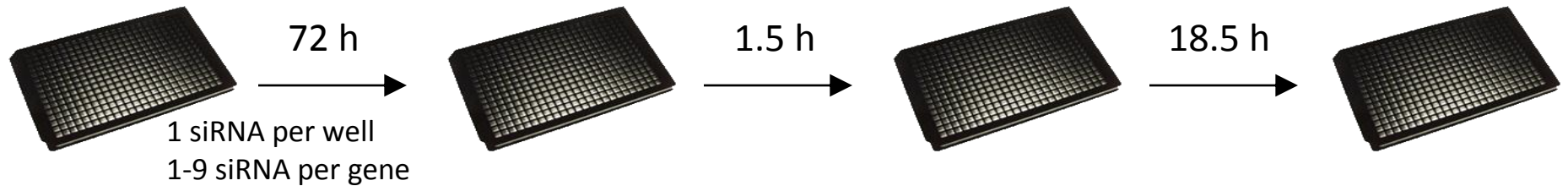
siRNA screen protocol

1.) add transfection reagent & cells

2.) wash & virus infection

3.) wash & add medium

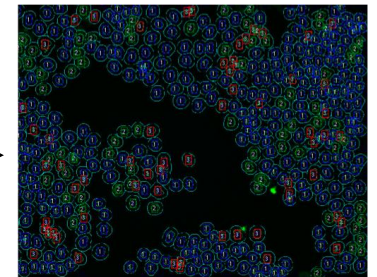
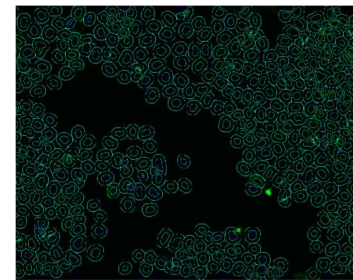
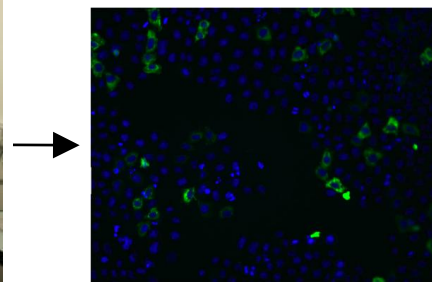
4.) fixation & DNA & virus staining



5.) microscopy

6.) segmentation

7.) classification



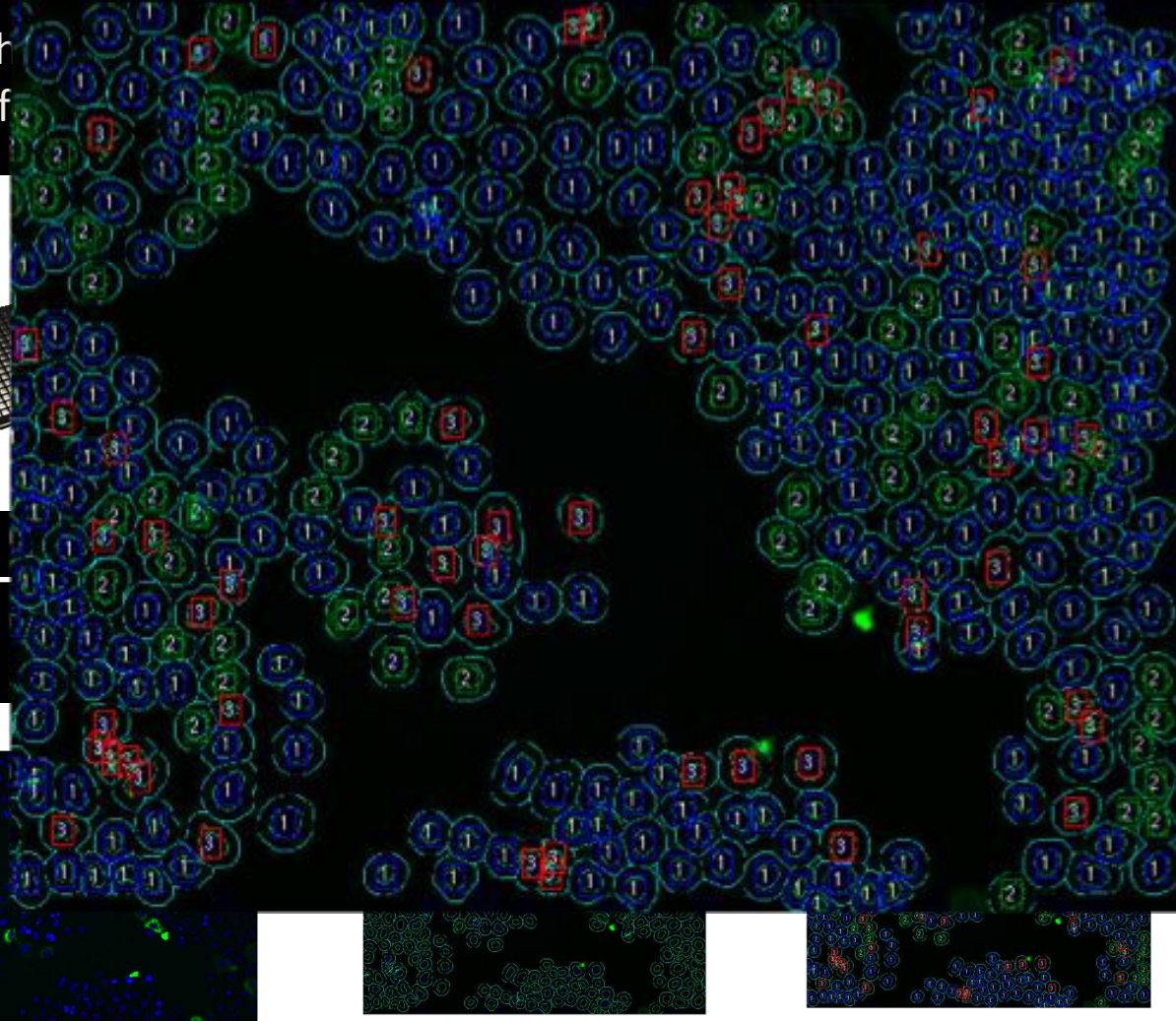
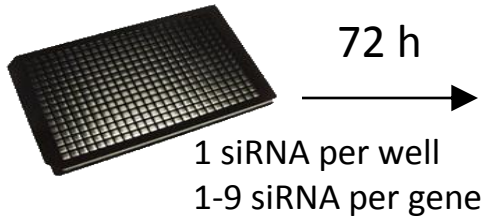
CellProfiler
<http://www.cellprofiler.org/>

Advanced Cell Classifier
<http://acc.ethz.ch/wiki.html>

siRNA screen protocol

1.) add transfection reagent & cells

2.) wash virus inf



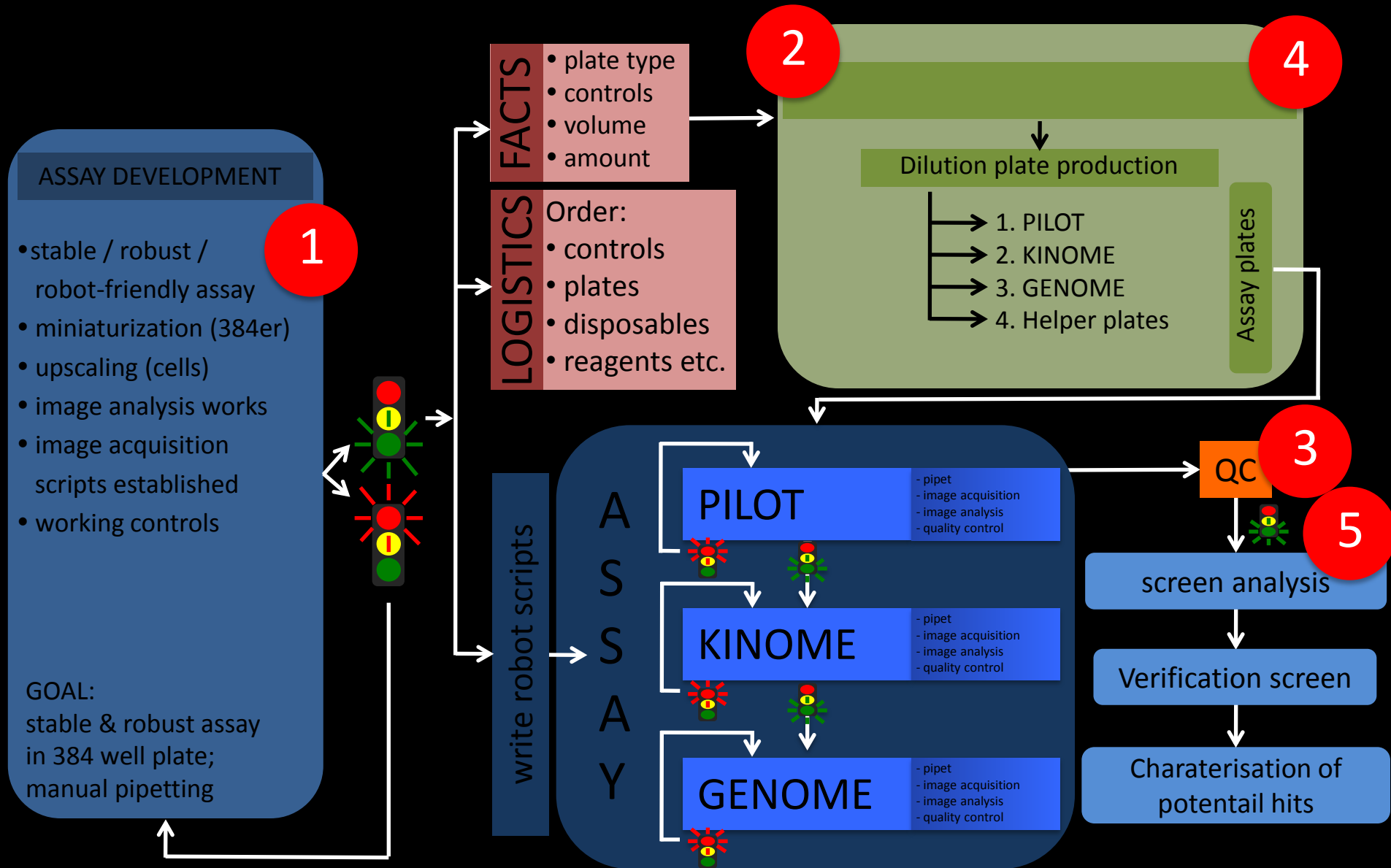
5.) microscopy



CellProfiler
<http://www.cellprofiler.org/>

Advanced Cell Classifier
<http://acc.ethz.ch/wiki.html>

Screen workflow



Challenges

- Cell type & virus strain dependent variability
- Population context dependent cell variability
- Annotation of human genome
- Reliability of siRNA libraries
- Quality control
- Layout of assay plates
- Criteria for hit definition, cell toxicity, hit validation

1

2

3

4

5

Cell type & virus strain dependent variability

1

Cell type

Same passage number throughout the whole experiment

- Produce a lot of cells of one passage number
- Freeze cells
- Thaw cells for each experiment sub-batch

Virus strain

Same virus stock throughout the whole experiment

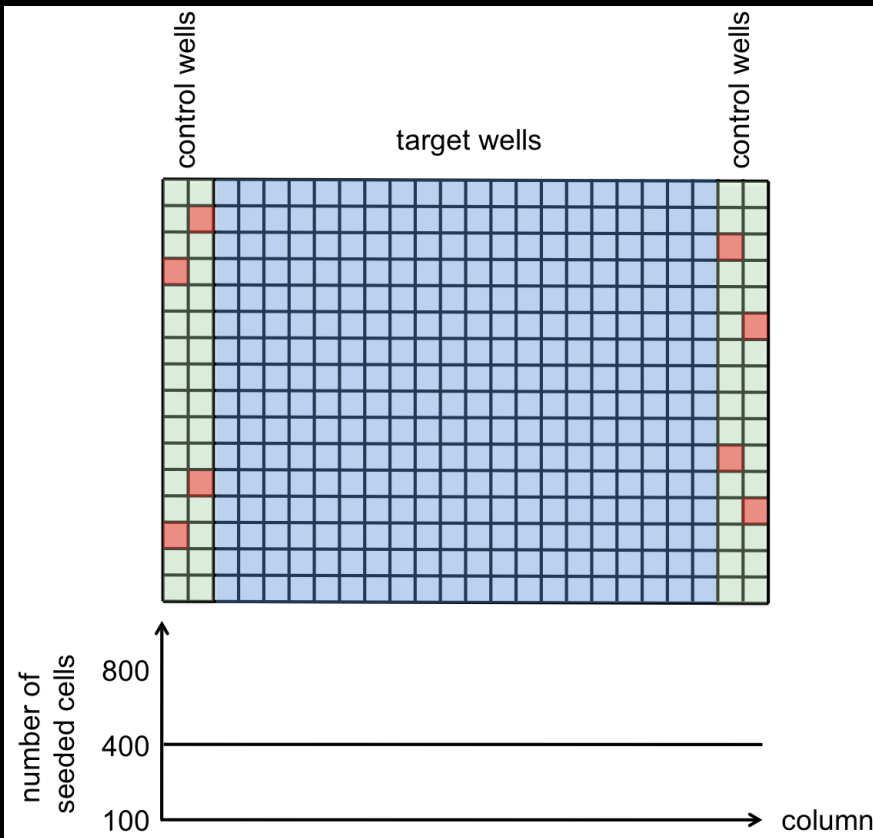
- Produce one big virus stock
- Aliquot and freeze virus
- Thaw virus for each experiment sub-batch

Population context dependent cell variability

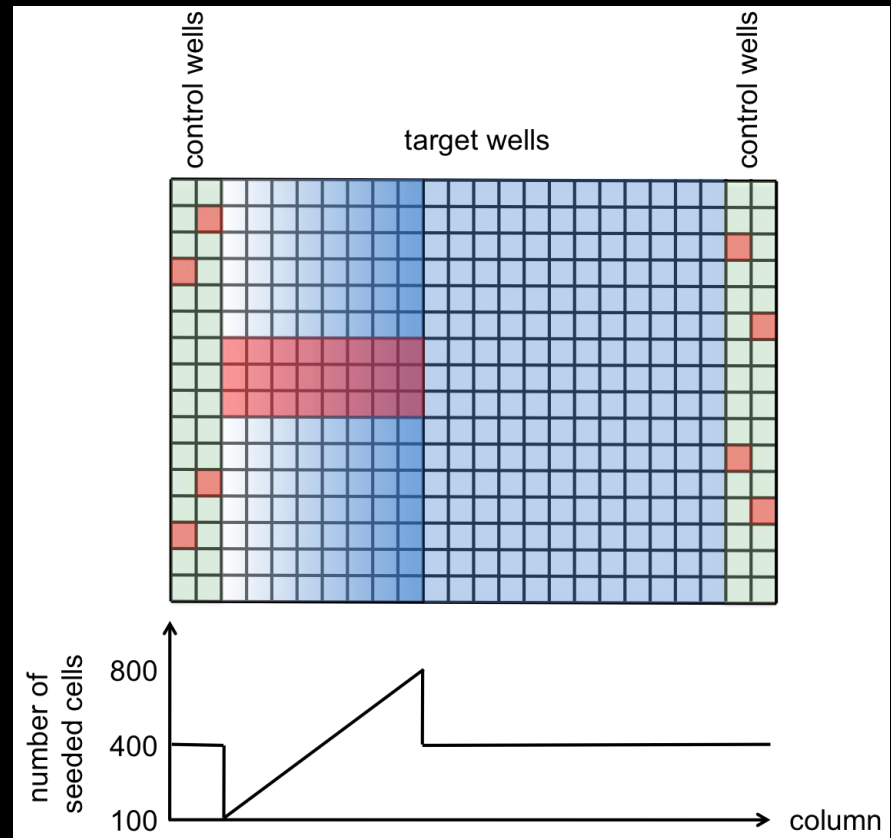
1

→ Normalization to Cell Number in Infection Assays

Library Plate Layout



Checkerboard Plate Layout

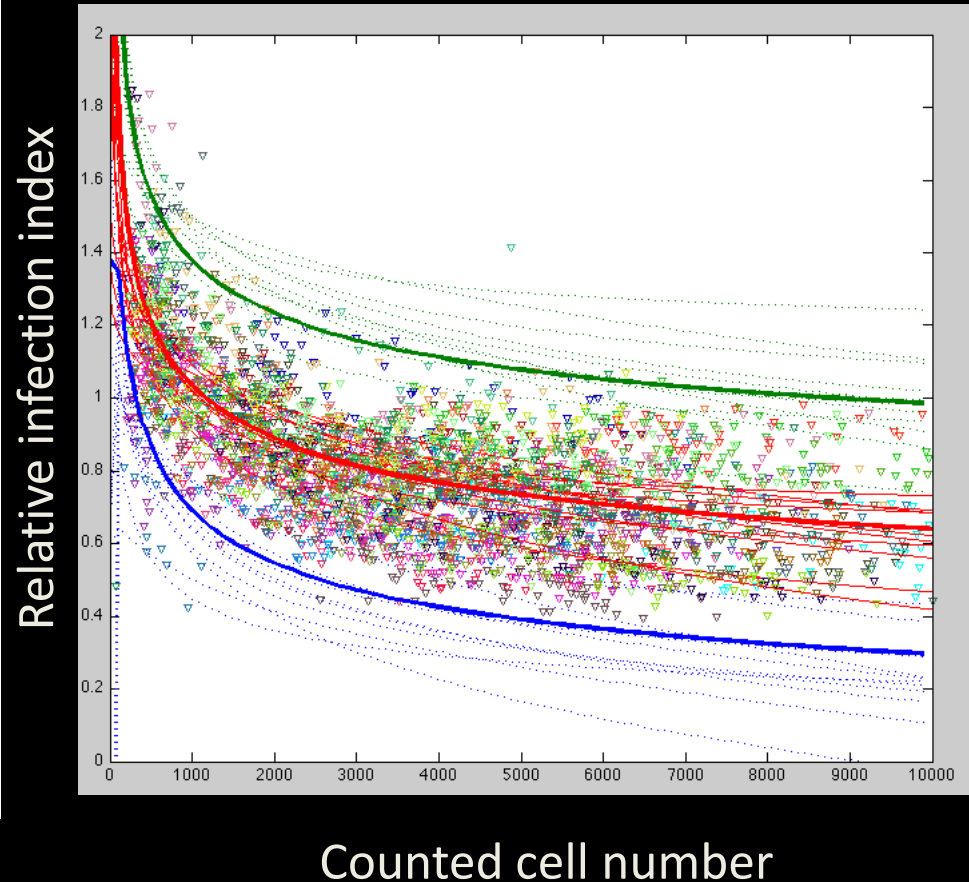
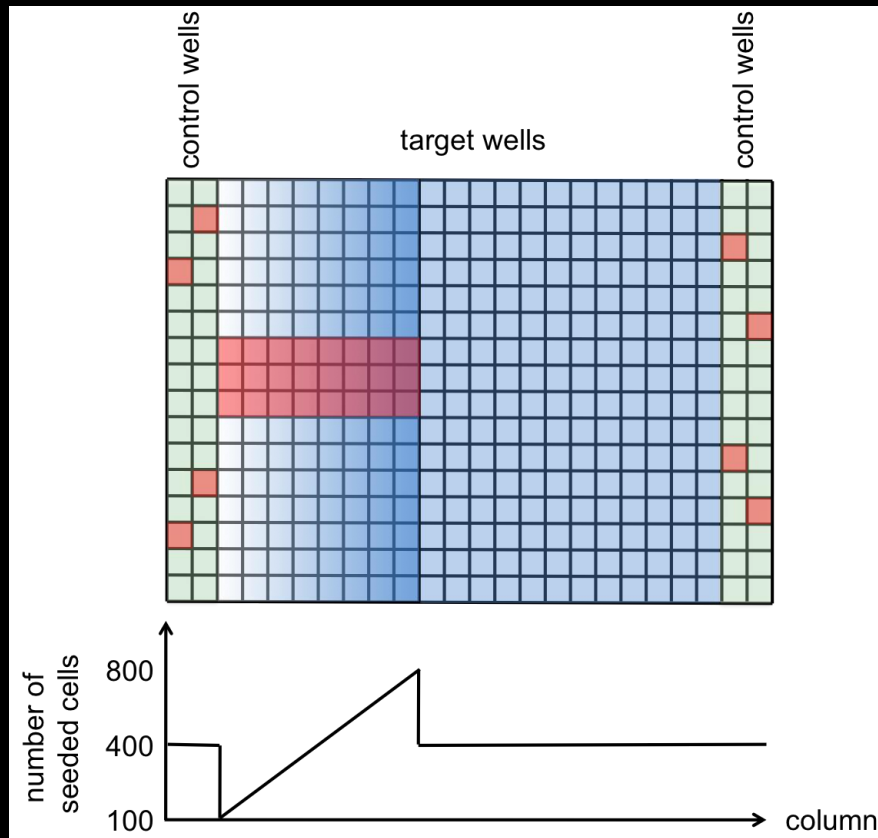


Population context dependent cell variability

1

→ Normalization to Cell Number in Infection Assays

Checkerboard Plate Layout



Annotation of human genome & Reliability of siRNA libraries

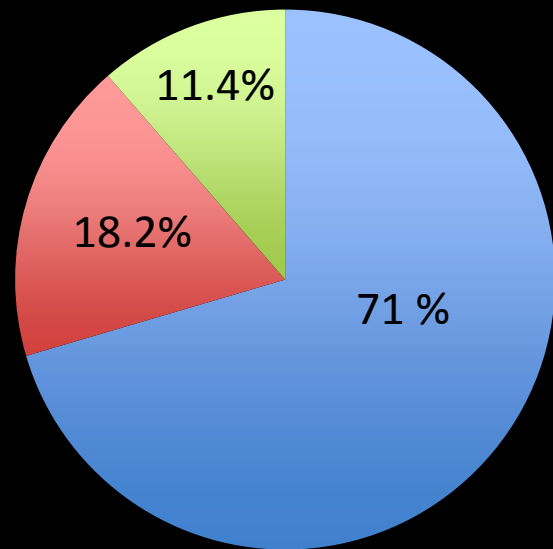
2



Qiagen genome wide siRNA library

(HsDgV3 (Human Druggable Genome siRNA Set V3); HsNmV1 (Human Refseq Xm siRNA Set V1); HsXmV1 (Human Predicted genome Set V1))

2006: 22832 genes / 90728 siRNAs



- target genes with off target(s)
- wrong predicted genes
- target genes

Annotation of human genome & Reliability of siRNA libraries

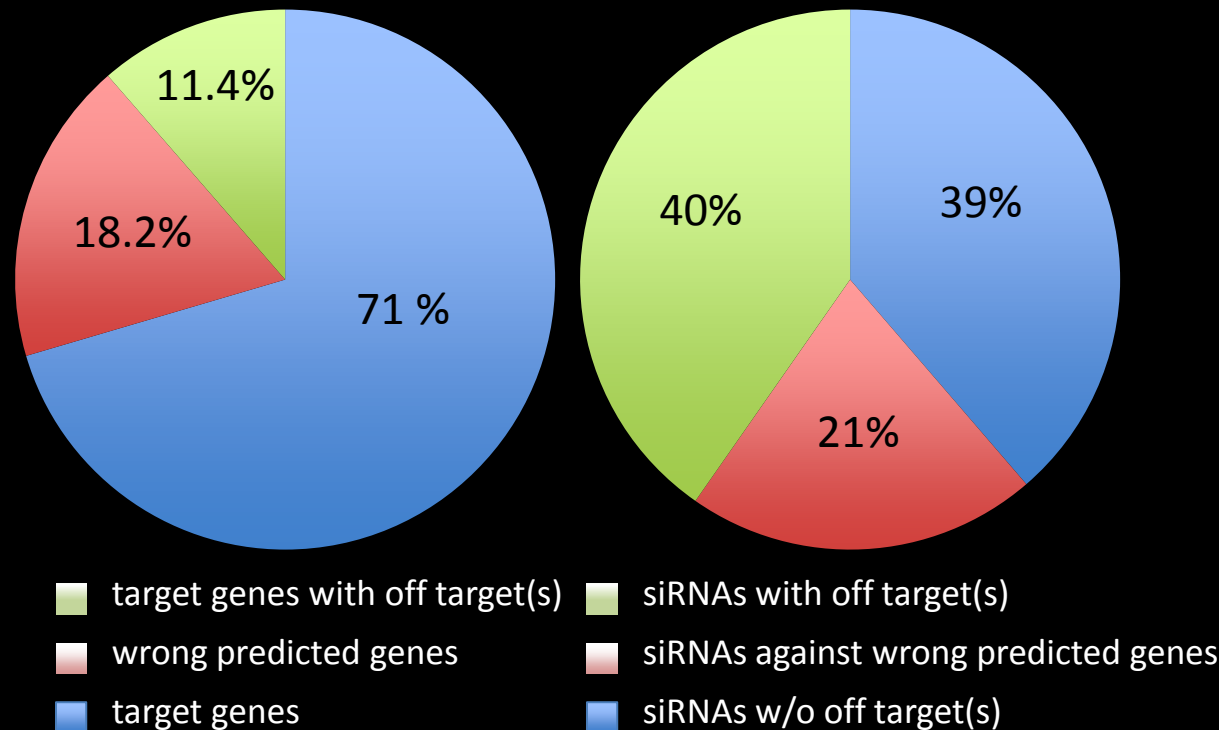
2



Qiagen genome wide siRNA library

(HsDgV3 (Human Druggable Genome siRNA Set V3); HsNmV1 (Human Refseq Xm siRNA Set V1); HsXmV1 (Human Predicted genome Set V1))

2006: 22832 genes / 90728 siRNAs



Annotation of human genome & Reliability of siRNA libraries

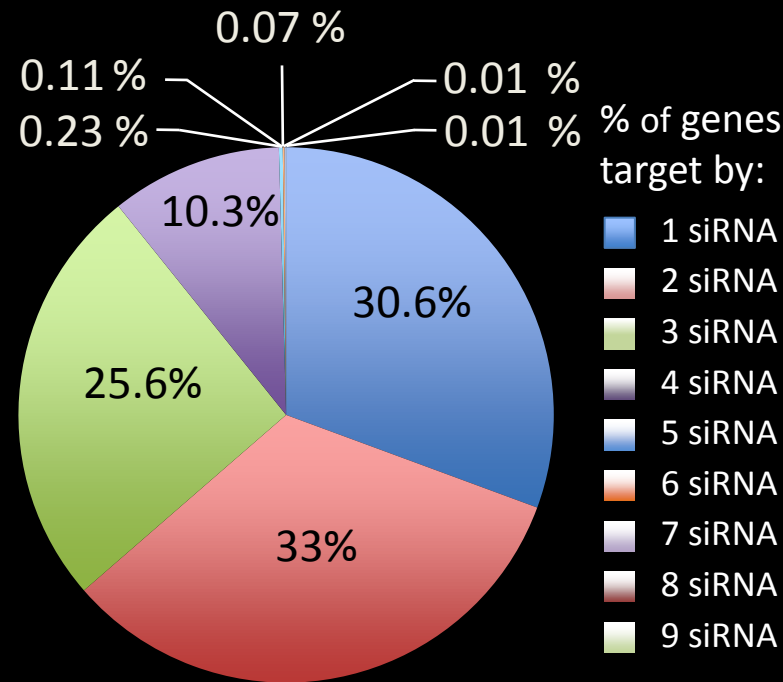
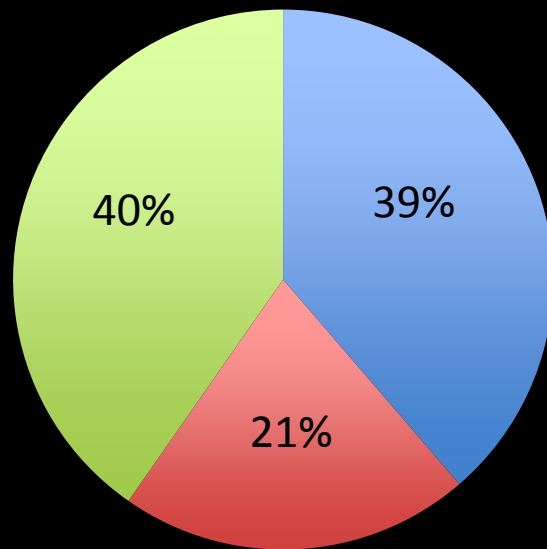
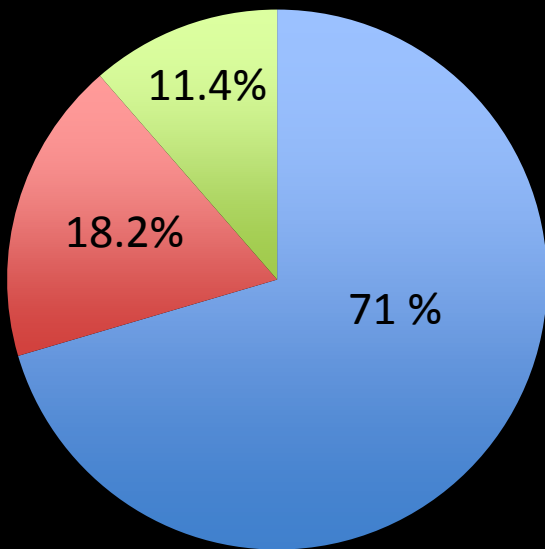


Qiagen genome wide siRNA library

(HsDgV3 (Human Druggable Genome siRNA Set V3); HsNmV1 (Human Refseq Xm siRNA Set V1); HsXmV1 (Human Predicted genome Set V1))

2006: 22832 genes / 90728 siRNAs

2010: 18'684 genes -> for clean library: 16'083 genes

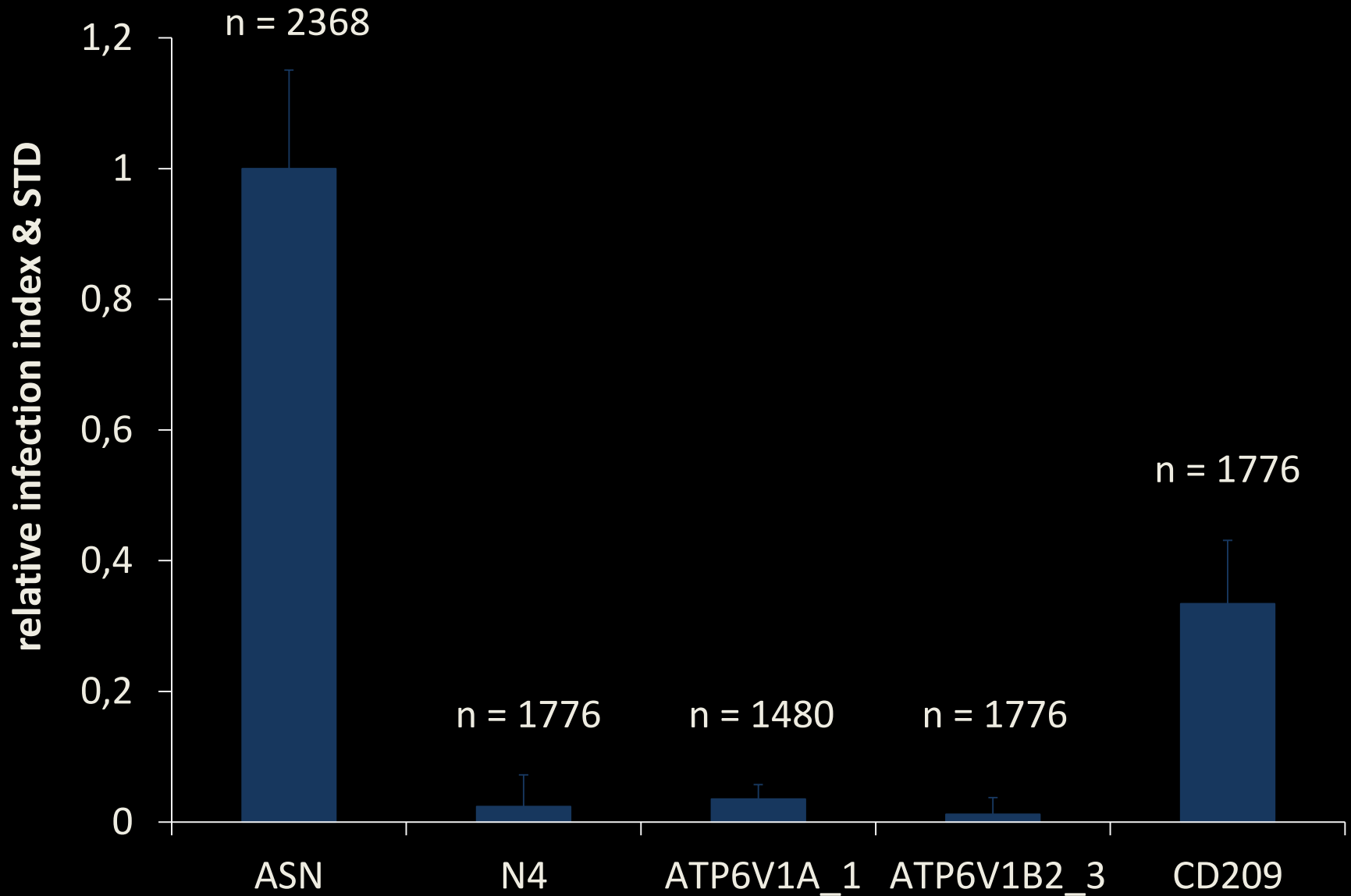


- target genes with off target(s)
- wrong predicted genes
- target genes
- siRNAs with off target(s)
- siRNAs against wrong predicted genes
- siRNAs w/o off target(s)

- % of genes target by:
- 1 siRNA
 - 2 siRNA
 - 3 siRNA
 - 4 siRNA
 - 5 siRNA
 - 6 siRNA
 - 7 siRNA
 - 8 siRNA
 - 9 siRNA

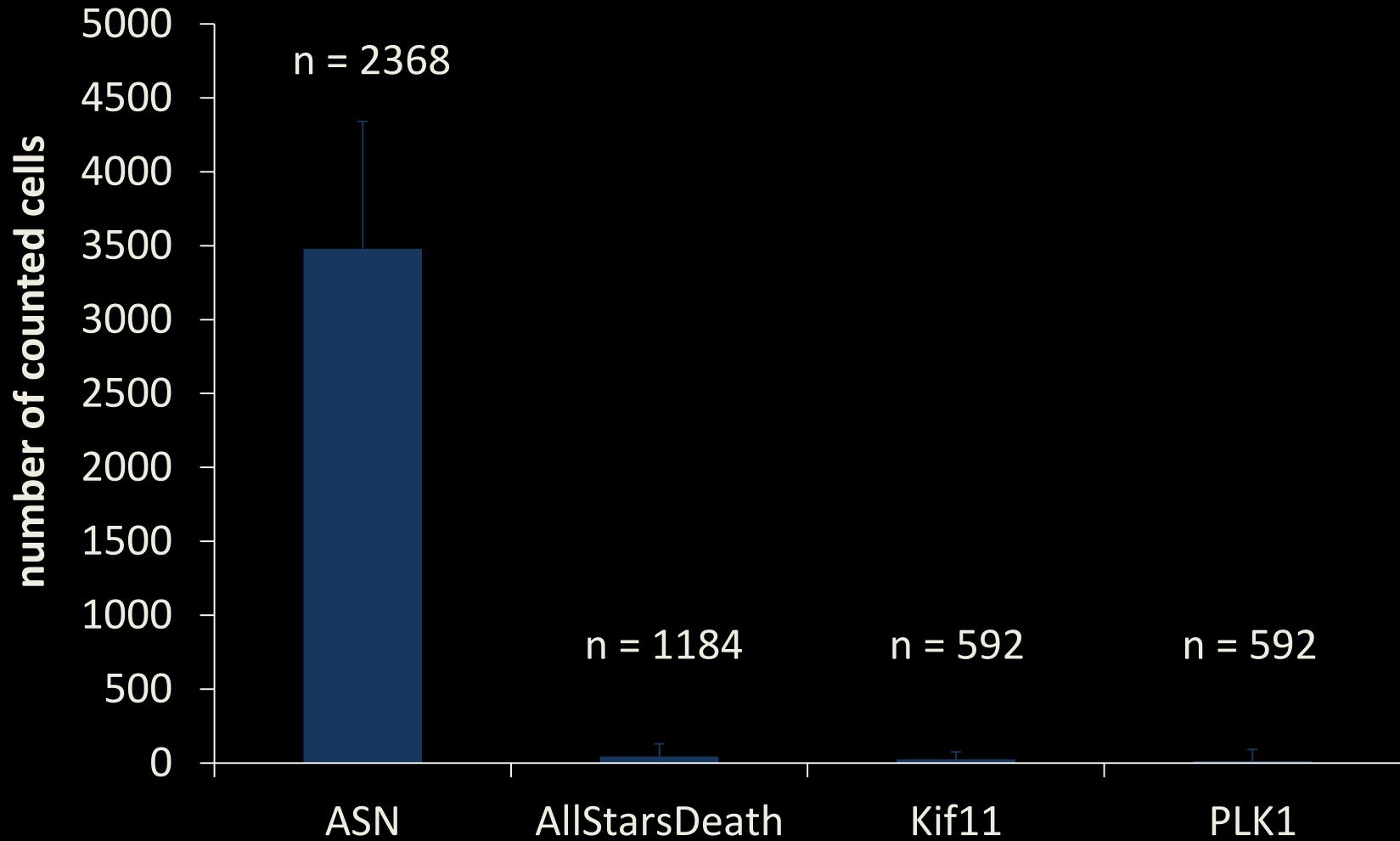
Quality control

3



Quality control

3

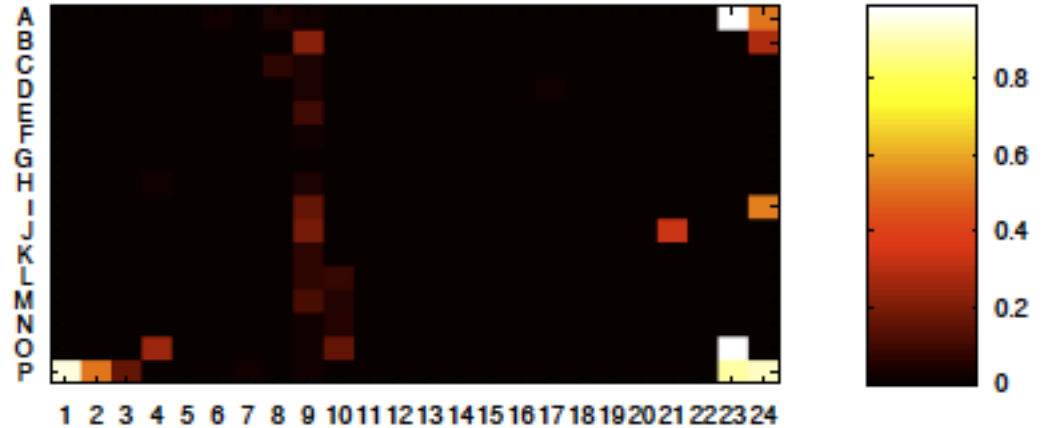


Quality control

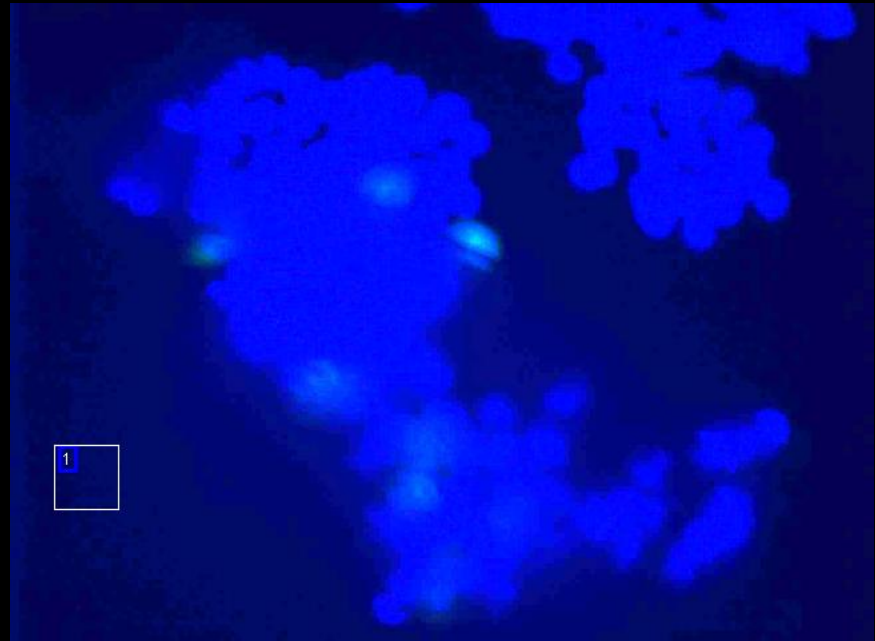
3

heat map for:
for segmentation
& out of focus
error

Possible segmentation/oof error



↓ raw data

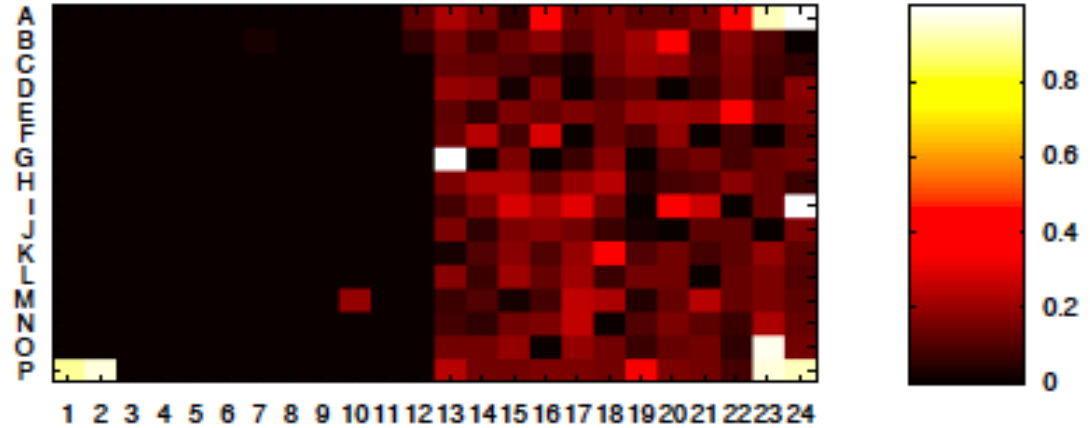


Quality control

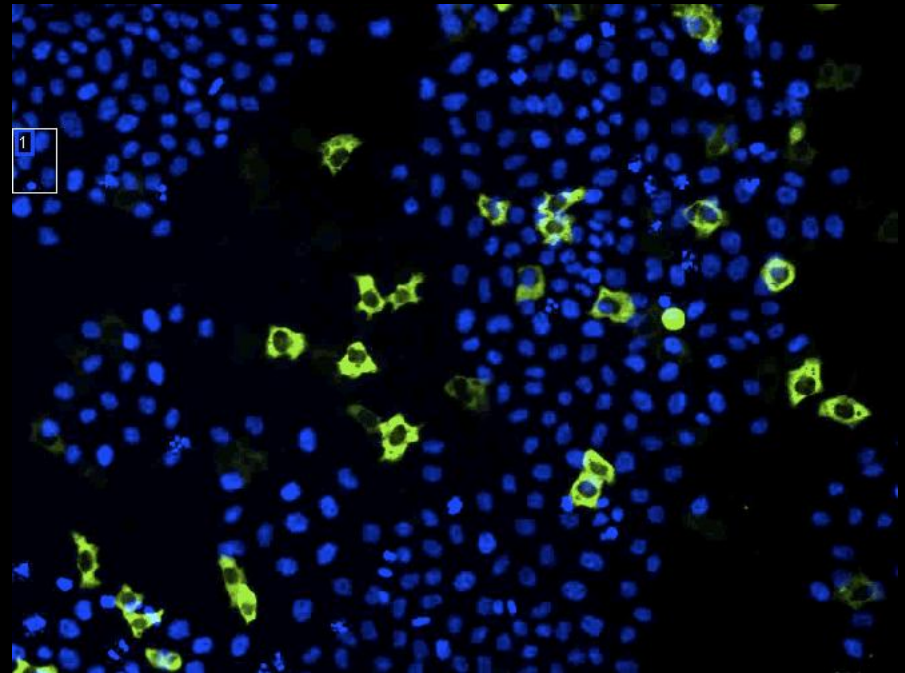
3

heat map for:
for segmentation
& out of focus
error

Possible segmentation/oof error



↓ raw data

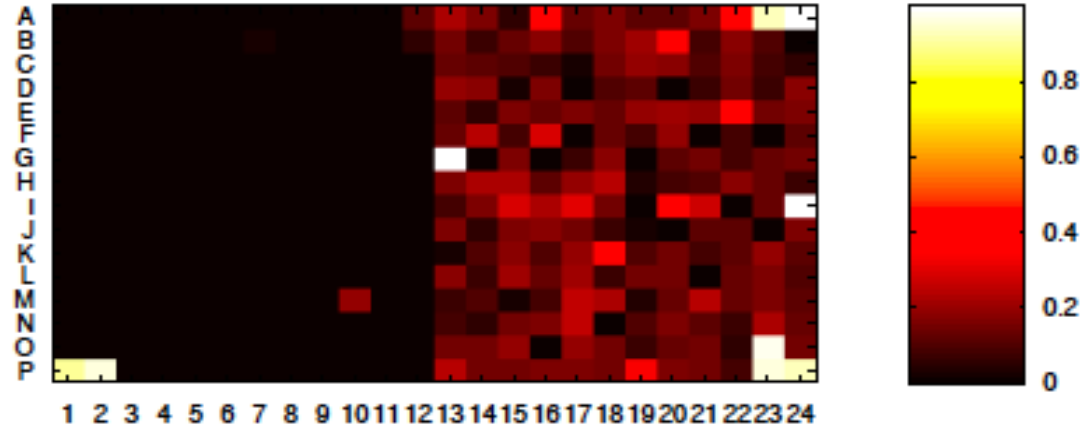


Quality control

3

heat map for:
for segmentation
& out of focus
error

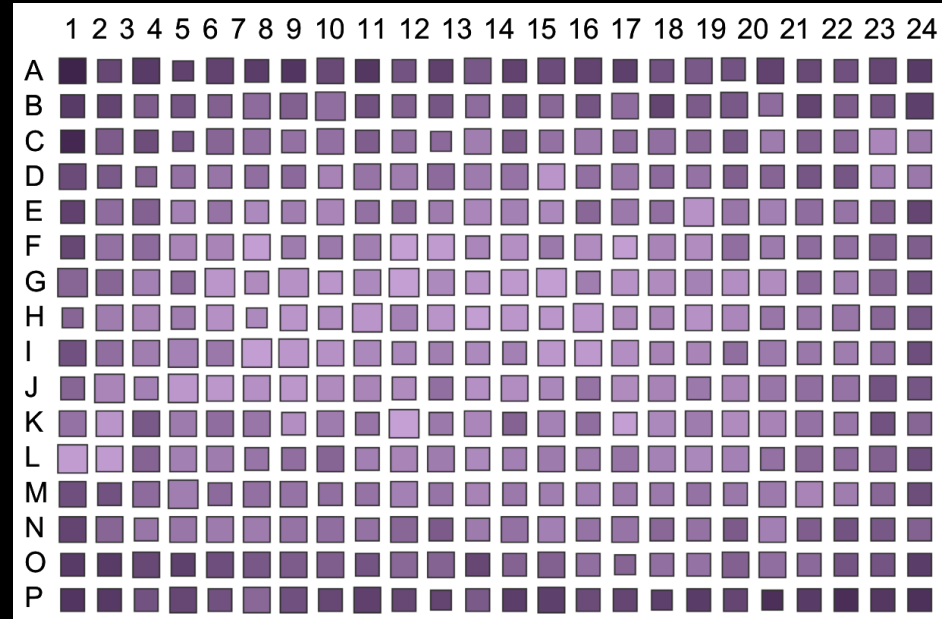
Possible segmentation/oof error



Quality control

3

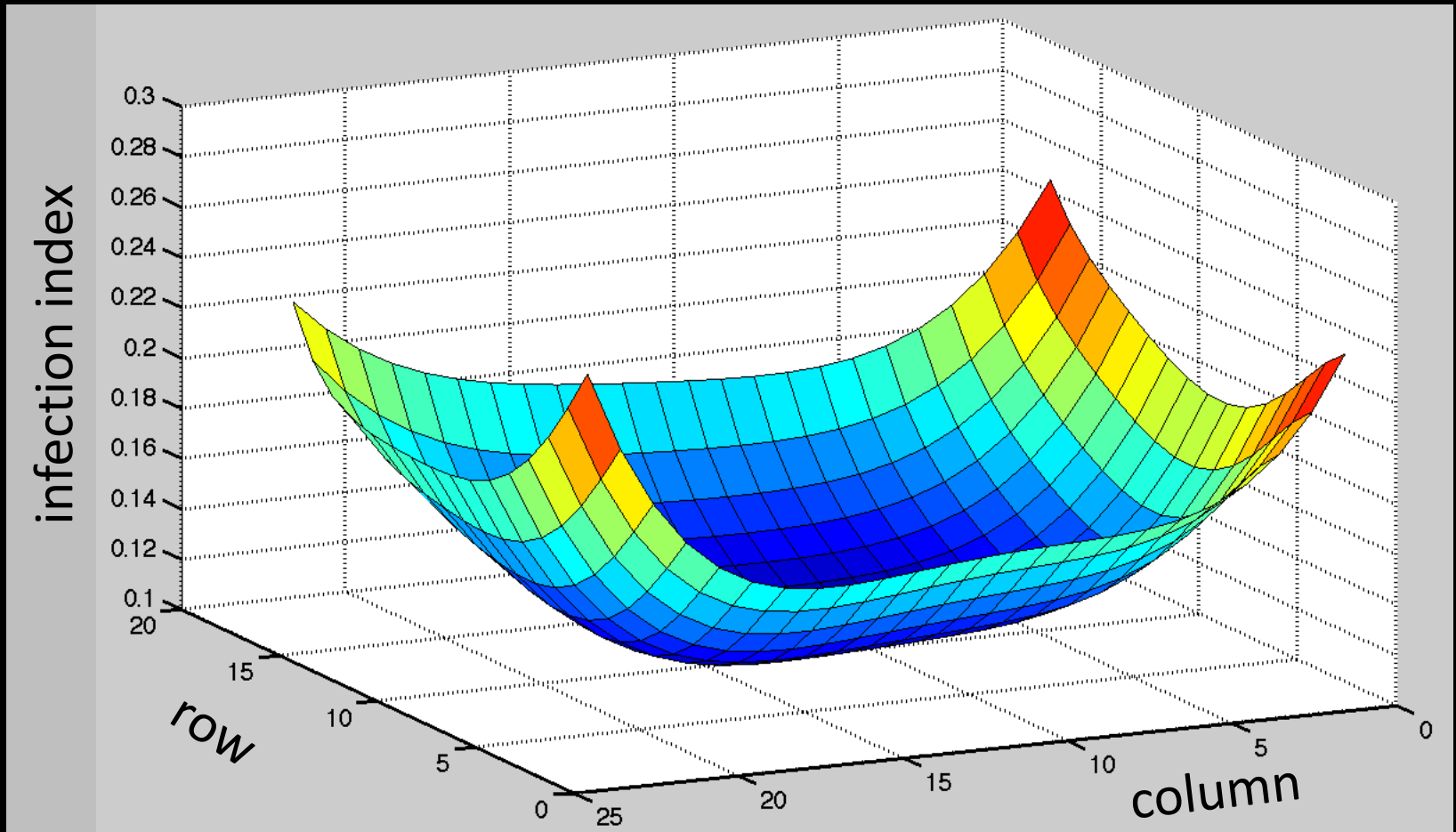
heat map for:
infection index &
number of cells



Square size \propto number of counted cells

Color intensity \propto % of infected cells

heat map for: infection index & number of cells

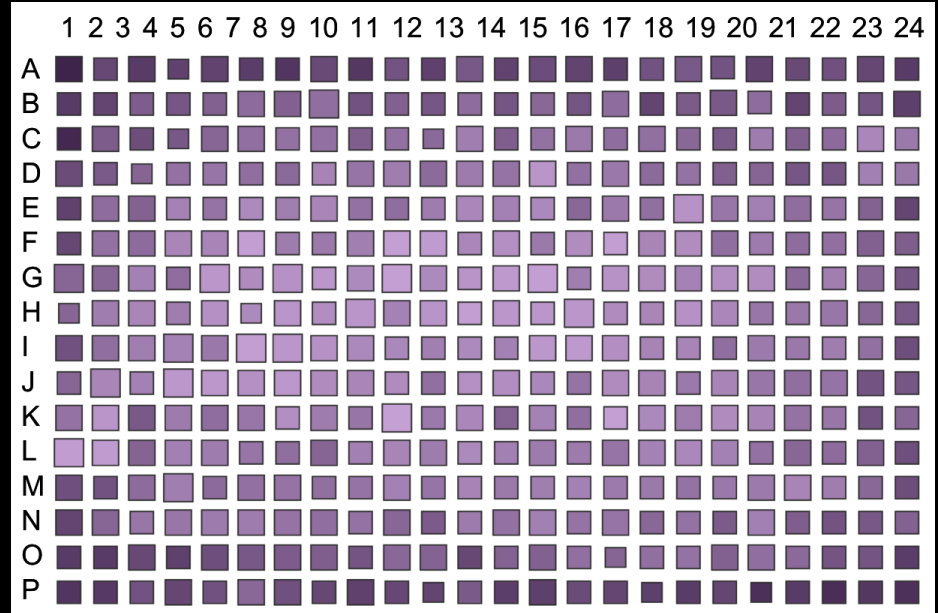


Quality control

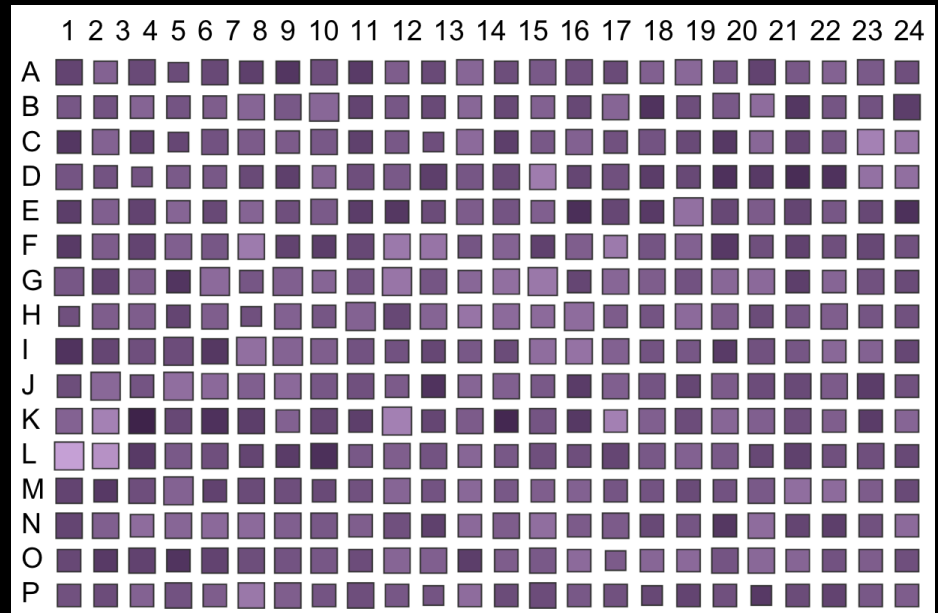
3

heat map for:
infection index &
Number of cells

Before correction



After correction



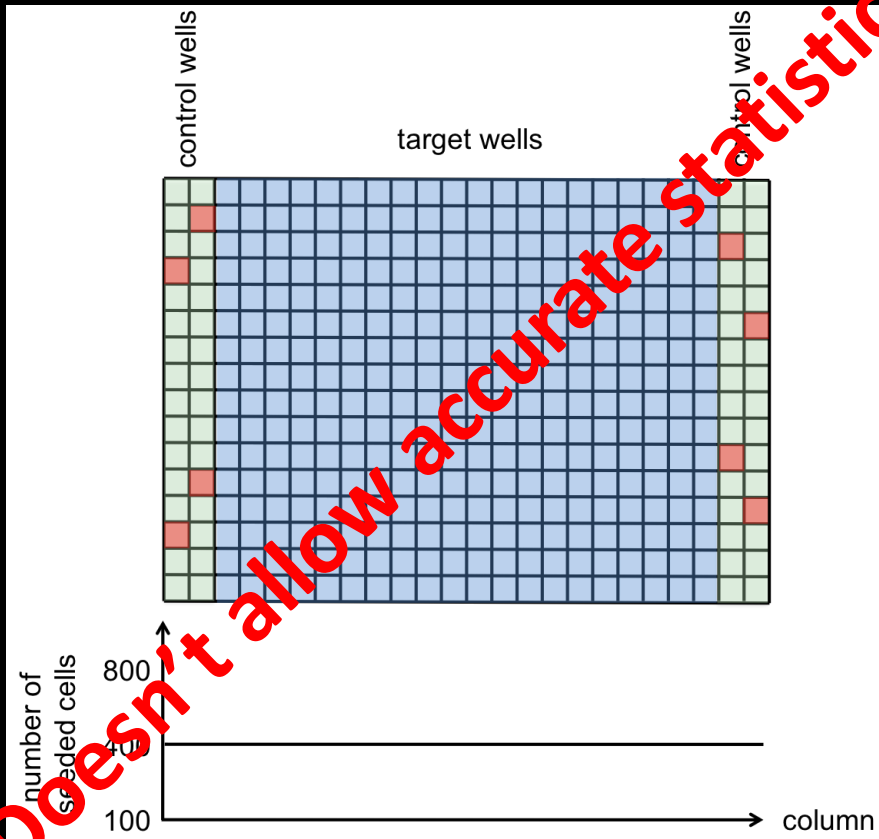
Square size \propto number of counted cells

Color intensity \propto % of infected cells

Layout of assay plates

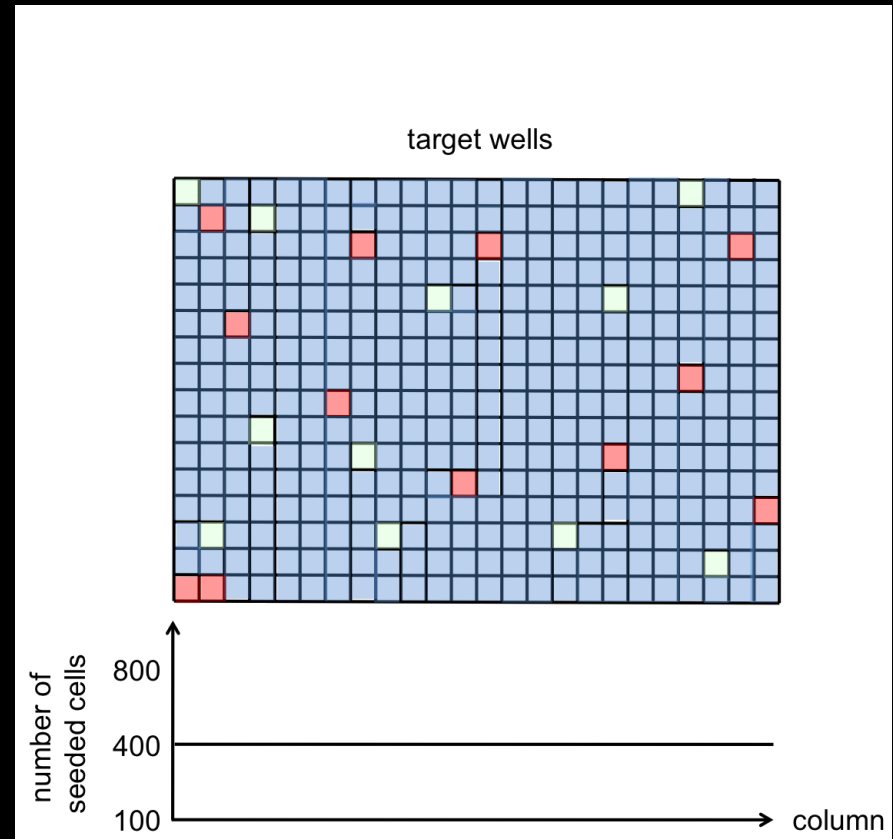
4

Library Plate Layout



Optimal Library Plate Layout

target & control siRNA fully randomized distributed (per plate)



Doesn't allow accurate statistics

- A probability-based approach for the analysis of large-scale RNAi screens
Renate König et al. 2007, Nature Methods

Method: redundant siRNA activity (RSA) analysis

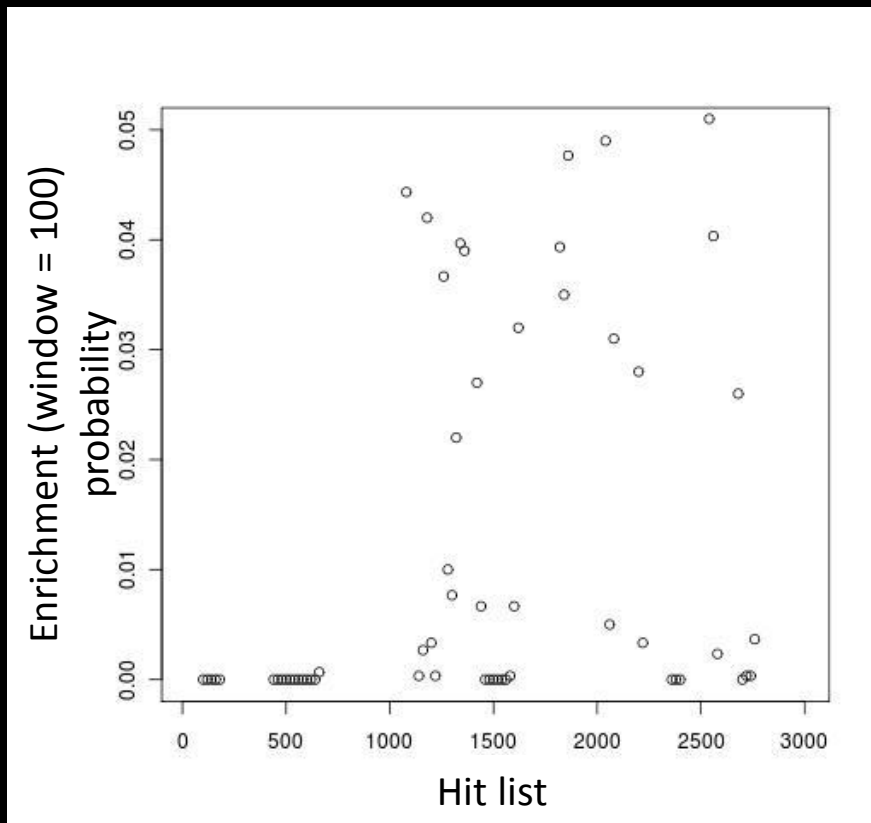
cumulative hypergeometric distribution function

$$P(N, n, m, r) = \sum_{i=r}^{\min(m, n)} \frac{\binom{n}{i} \binom{N-n}{m-i}}{\binom{N}{m}}$$

Criteria for «hit» definition

- »hit« definition: not only one definition

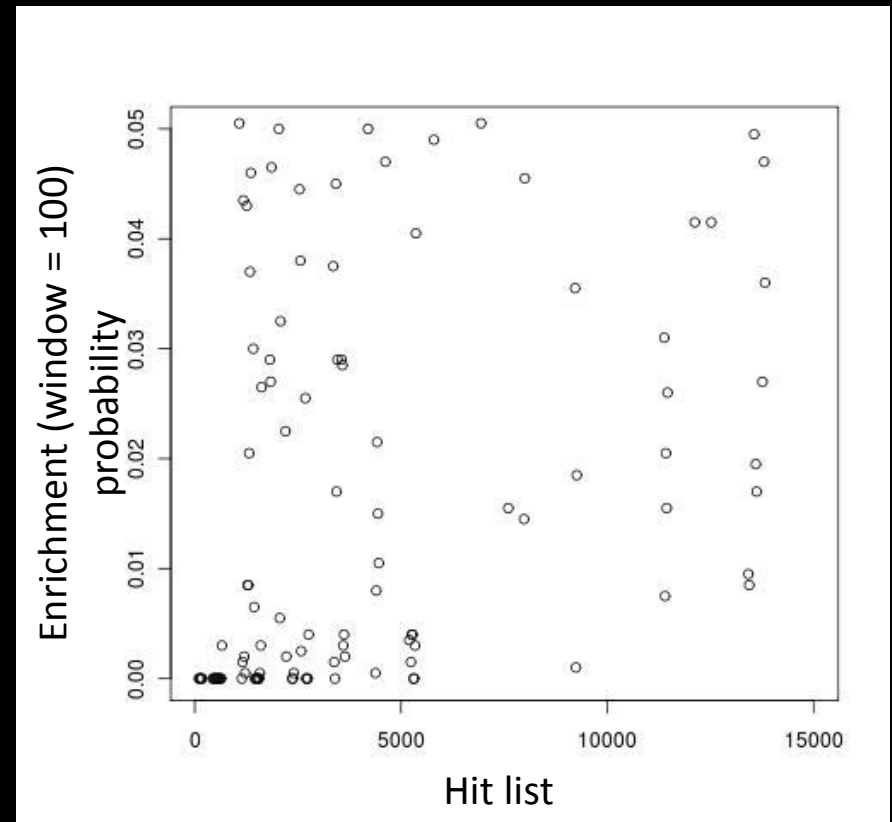
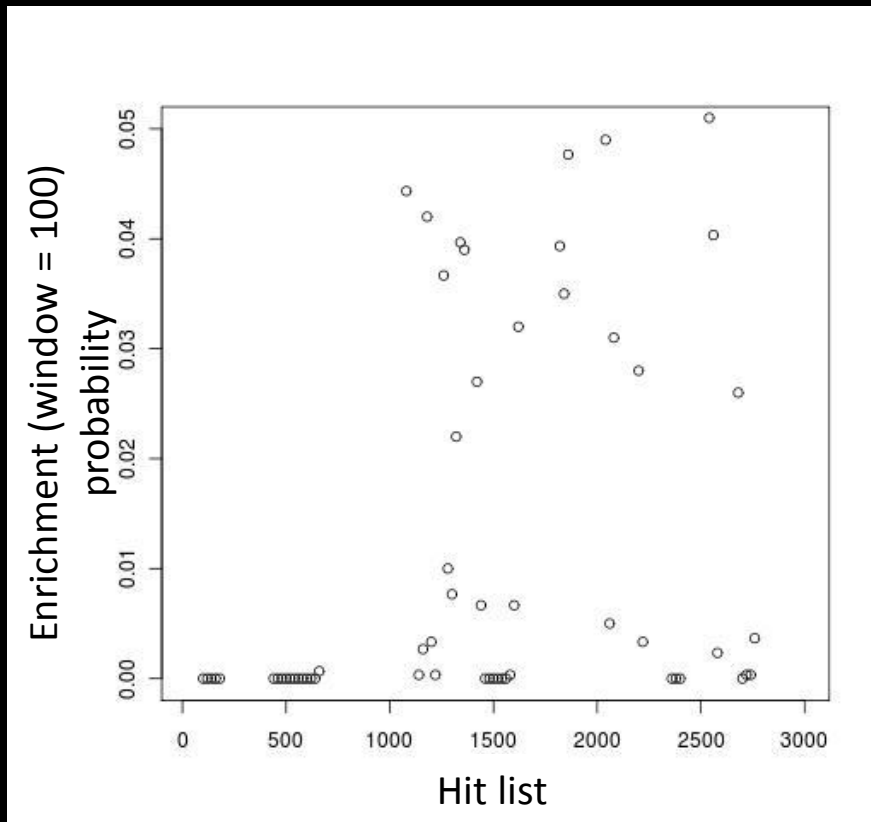
protein-protein interaction analysis: STRING <http://string-db.org/>



Criteria for «hit» definition

- »hit« definition: not only one definition

protein-protein interaction analysis: STRING <http://string-db.org/>



Criteria for cell toxicity & «hit» validation

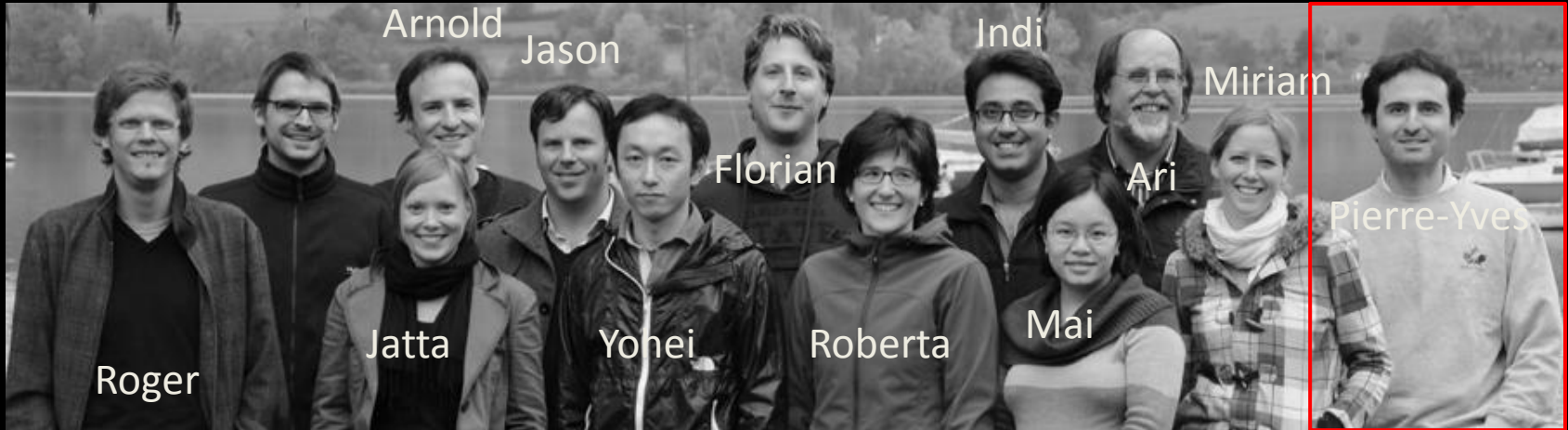
- Cytotoxicity assay: screen hits from initial high-throughput siRNA screen (e.g. MTT assay)
- «hit» validation: verification screen, protein-protein interaction analysis (DAVID, STRING), WB, rescue exp., drug exp., dominant-negative construct

Summary screen analysis

- 1.) correction to »plate effect«
- 2.) correction of infection index to cell number
- 3.) plate wise normalization
- 4.) redundant siRNA activity (RSA analysis) (König, 2007 Nature Methods)
- 5.) protein-protein interaction analysis: DAVID <http://david.abcc.ncifcrf.gov/home.jsp>
STRING <http://string-db.org/>
- 6.) »hit« definition: top 1600 proteins
- 7.) »hit« verification

Acknowledgments

Ari and his lab ...



Thomas, Samuel, Stefanie, Marco, Christine, Heithem, Sarah, Andreas, Thomas, Benjamin

RNAi Imaged-based Screening Center (RISC), ETHZ

Csúcs Gábor

Andreas Vonderheit

Michael Stebler

Karol Kozak

Peter Horvath

Institute of Molecular Life Science, UZH

Christian von Mering

Andrea Franceschini

Seminar for statistics, ETHZ

Cornelia Schwierz

Lukas Rosinus

IBC staff, ETHZ

Anton Lehmann

Maria Cerdeira-Casares

Nicola Graf

Roland Stuber

Thesis committee

Michèle Bouloy

Hubert Hilbi

Alexandra Trkola