Validation of Commercial tool Antibodies

The Antibody Society Webcast series – Antibody Validation #7

It's a Knockout

Simon L. Goodman
Science and Technology Program Manager
The Antibody Society

Antibody Validation: a 9-part series

1.	Andreas Pluckthun	:	The different antibody formats
2.	Glenn Begley	:	Antibodies and the reproducibility crisis in biological science
	Cecilia Williams	:	The Erß story – is your antibody like this?
3.	Jan Voskuil	:	Beware the supplier OEM
	Andy Chalmers	:	Finding antibodies in the Antibody Databases
4.	Anita Bandrowski	:	Which antibody are you looking for? The RRID
	Jan Voskuil	:	Points to note on the supplier datasheets
5.	Giovanna Roncador:	:	Correct positive and negative controls in validation
6.	Aldrin Gomes	:	Standard technology: "even" Western blots are non-trivial
	Jim Trimmer	:	IHC issues in brain sciences
7.	**Travis Hardcastle**	**:**	**Cell KO technology**
	Alejandra Solache	**:**	**Validating Antibodies with KO technology**
8.	Mike Taussig	:	Validating antibodies using array technologies
	Fridjhof Lund-Johansen	:	Mass spectroscopy for mass validation
9.	Andrew Bradbury	:	Why publish sequences?
	Andreas Pluckthun	:	What are the coming alternatives ?

ANTIBODY SOCIETY

It‘s a Knockout

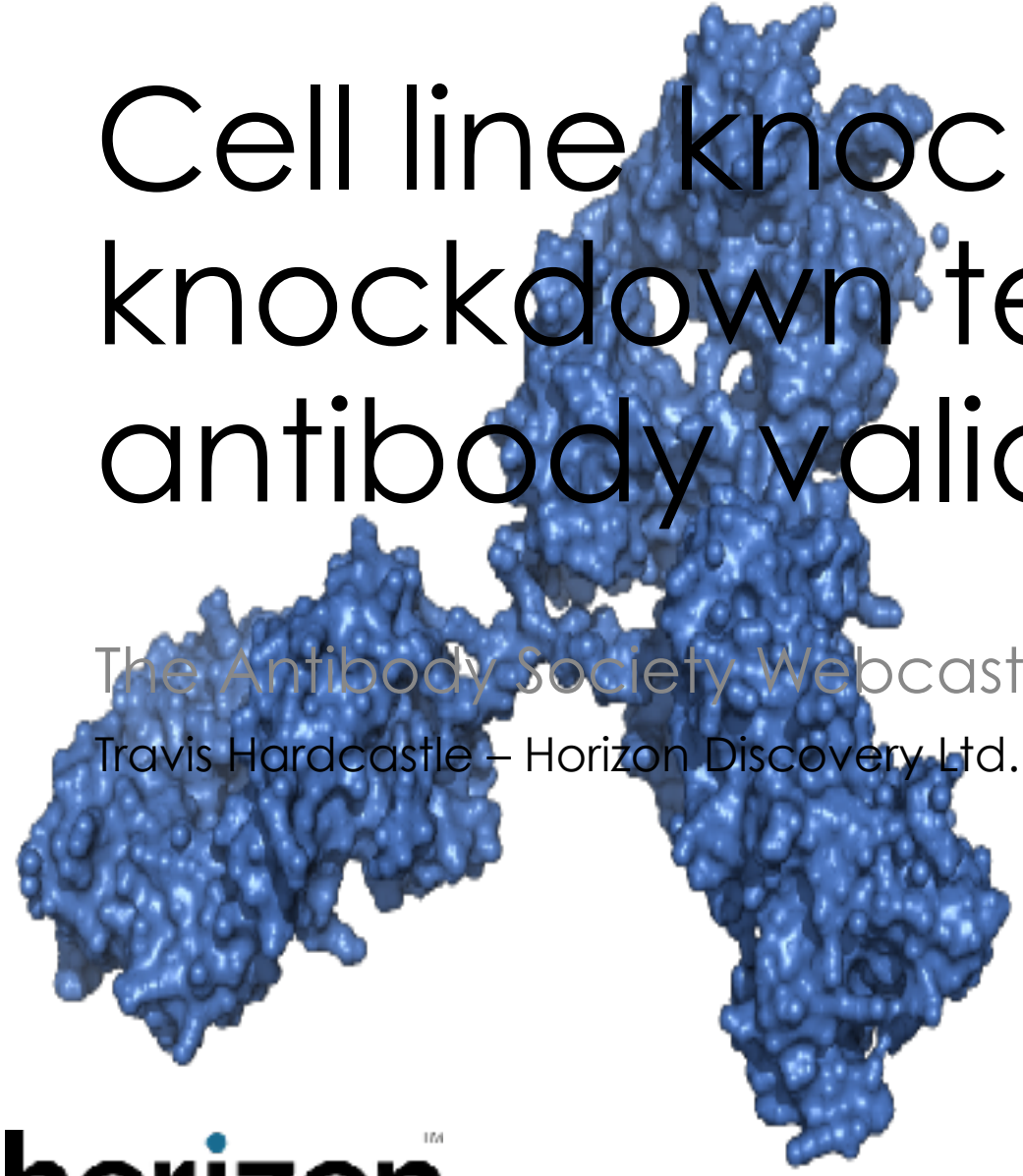
The Antibody Society Webcast series – Antibody Validation #7

Travis Hardcastle — Horizon Discovery, Cambridge, U.K.

Alejandra Solache — Abcam, UK.

ANTIBODY SOCIETY

Cell line knock-out & knockdown technologies in antibody validation



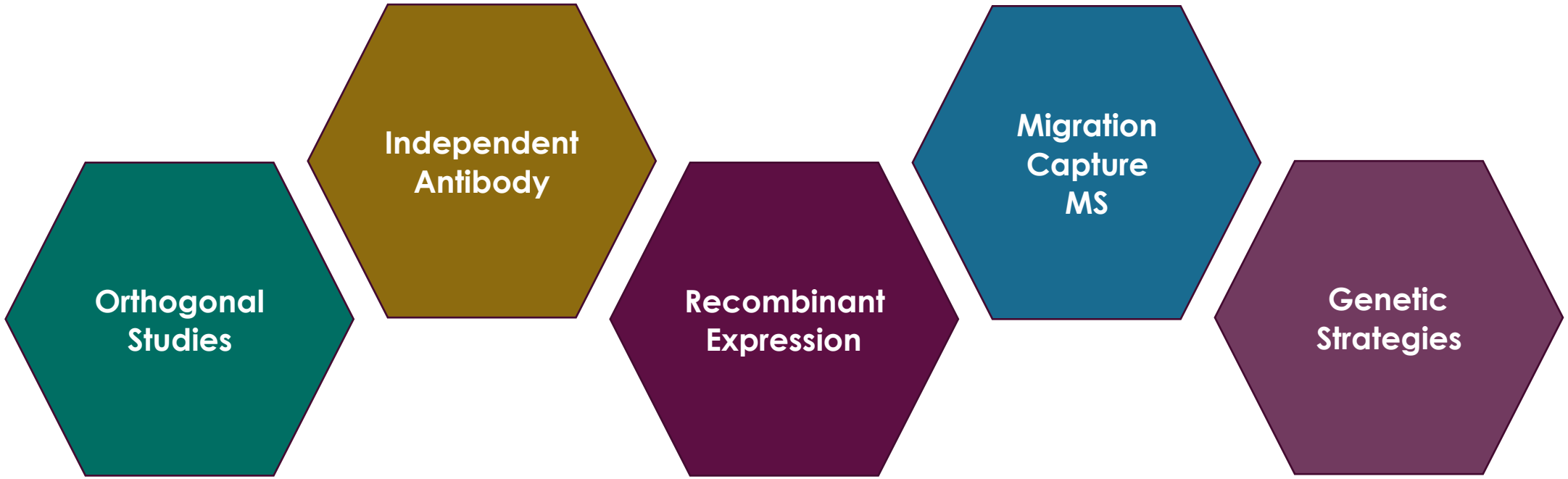
The Antibody Society Webcast series - Antibody Validation #7

Travis Hardcastle – Horizon Discovery Ltd.

horizon™
INSPIRED CELL SOLUTIONS

ANTIBODY SOCIETY

The Five Methods of Validation

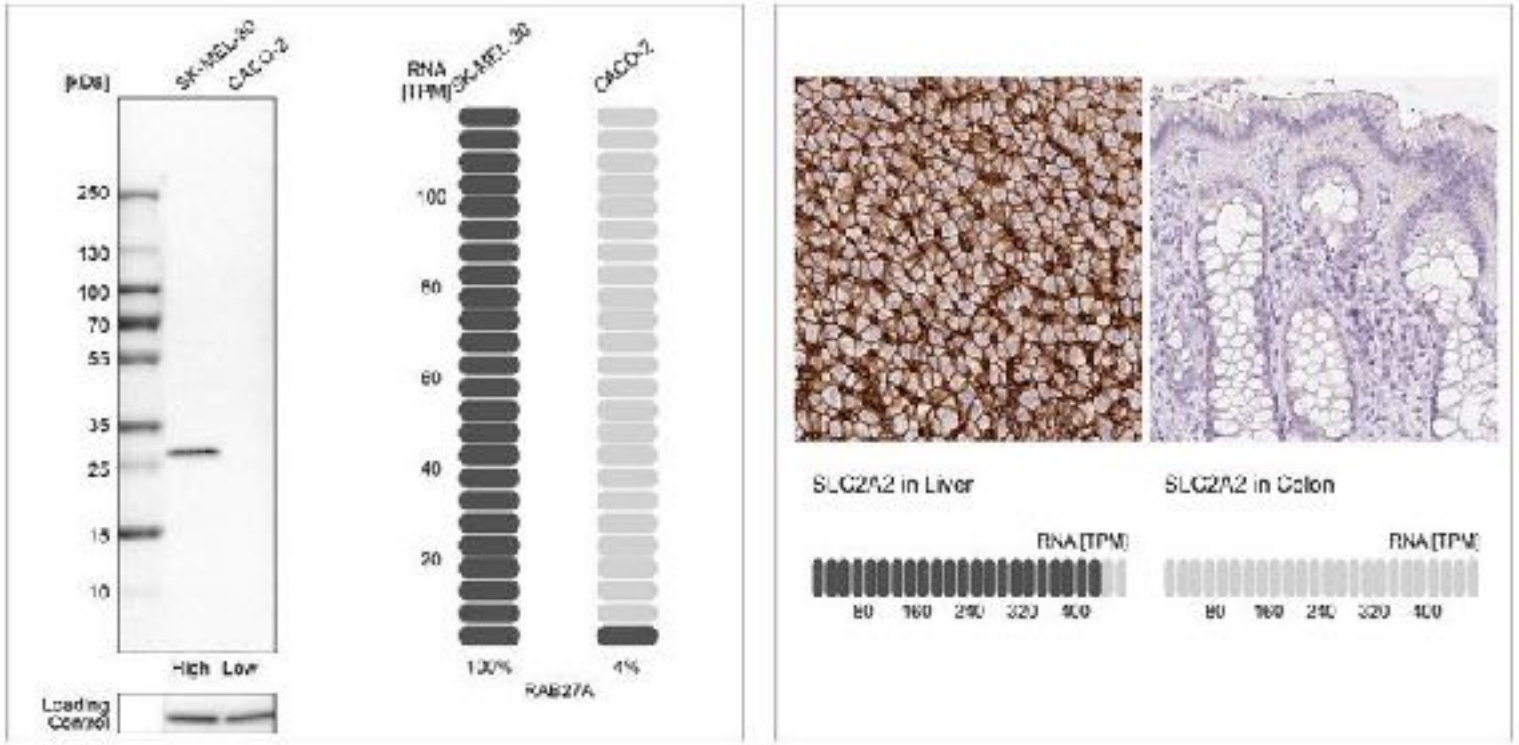


Adopted from: Uhlen, M. et al. (2016). A proposal for validation of antibodies. *Nature Methods* **13**, 823-827

Validation by orthogonal studies

Orthogonal Studies

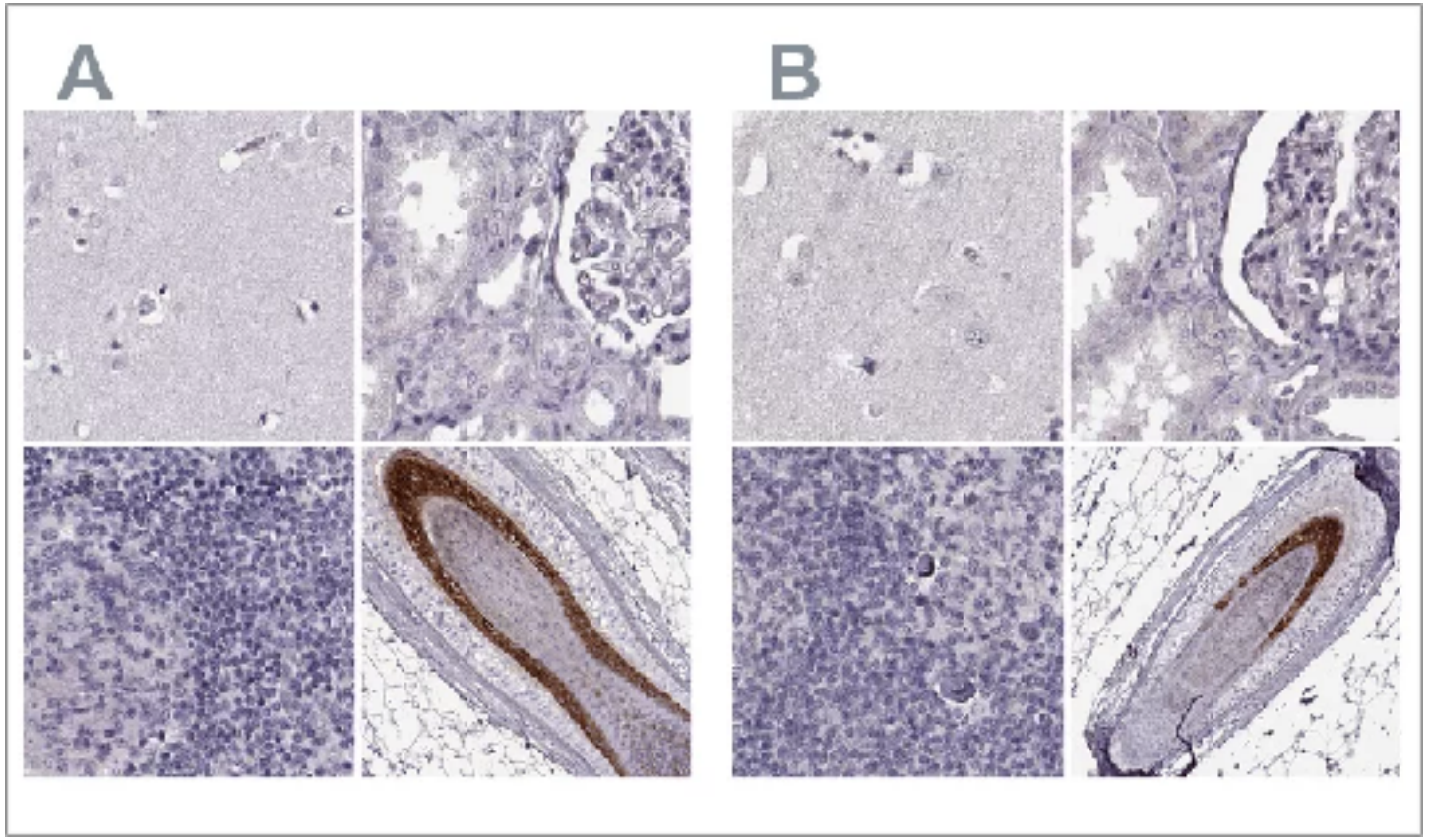
- The antibody is validated by comparing the results with a non-antibody based method across multiple samples



Validation by independent antibodies

- Antibody specificity is demonstrated by comparing two antibodies targeting different regions of the same protein

Independent Antibody

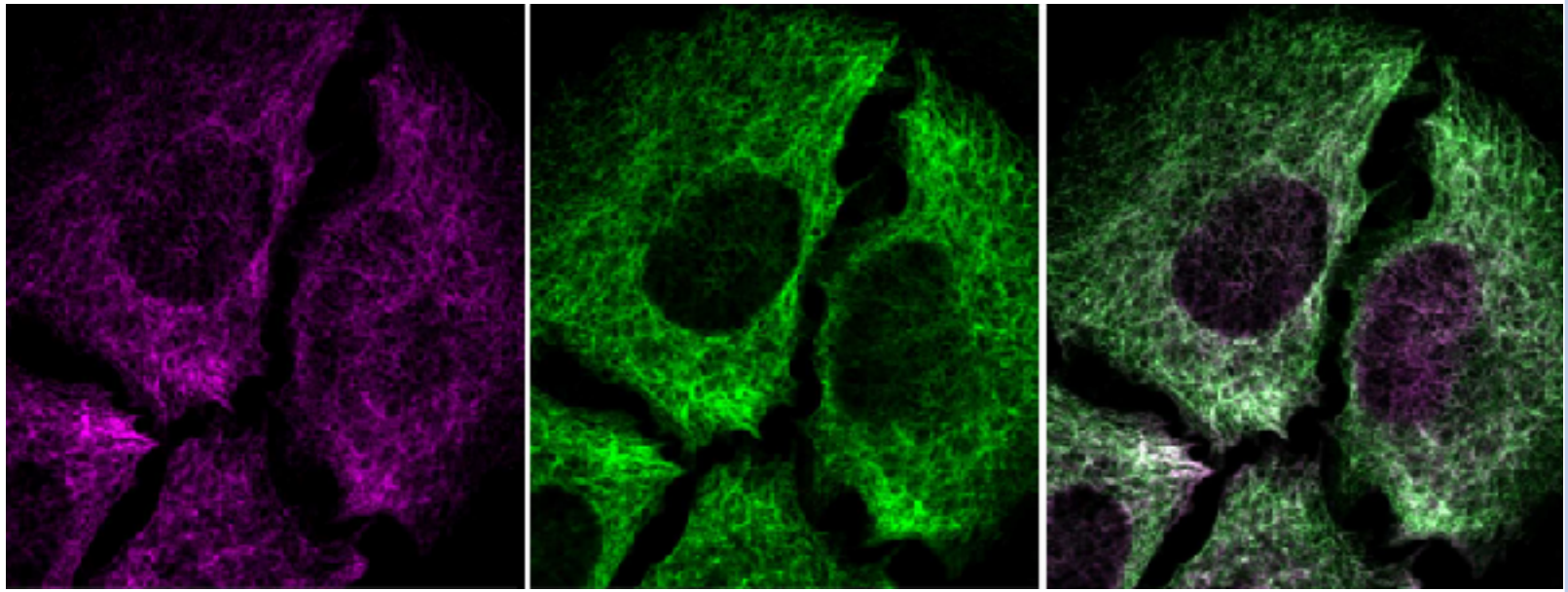


Two anti-TCHHL1 antibodies staining cerebral cortex, kidney, lymph node, & skin

Validation by recombinant expression

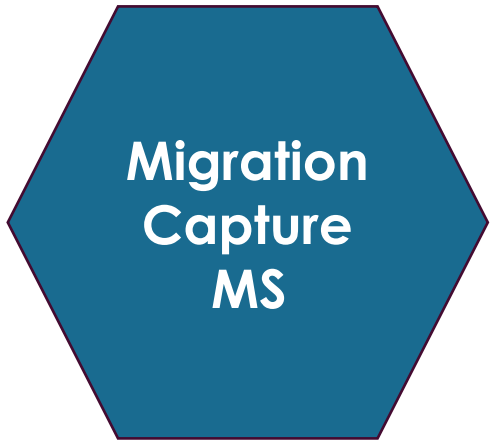
- The antibody binding is verified using and over-expressed or tagged version of the target protein

Recombinant Expression



Anti-NES antibody (green), GFP tagged nestin protein (in purple)

Validation by migration capture MS



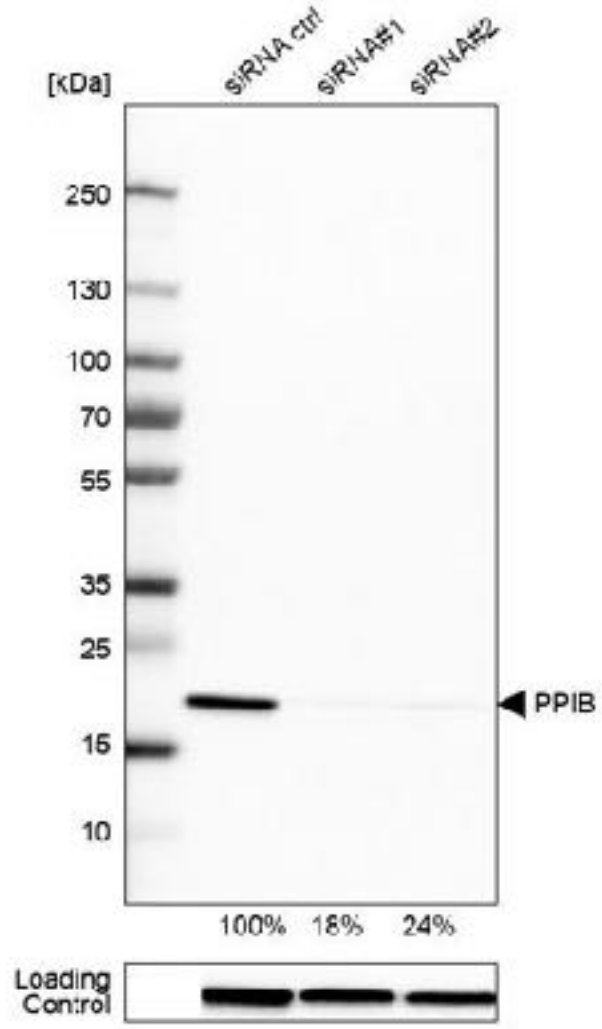
The staining pattern and the protein size detected by the antibody is compared with results obtained by a capture Mass Spectrometry (MS) method.

Presence of target protein verified by Mass Spectrometry

Limitation: When target can not be detected in Mass Spectrometry

Validation by genetic strategies

Genetic Strategies



- The antibody specificity is confirmed by downregulating the target protein on a genetic level using siRNA or CRISPR-Cas9

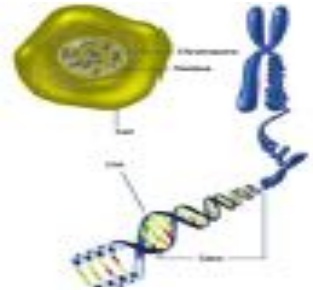
Genetic strategy: Tools to decrease gene expression

RNAi

(siRNA, shRNA, microRNA)

Gene Editing

(CRISPR-Cas9)

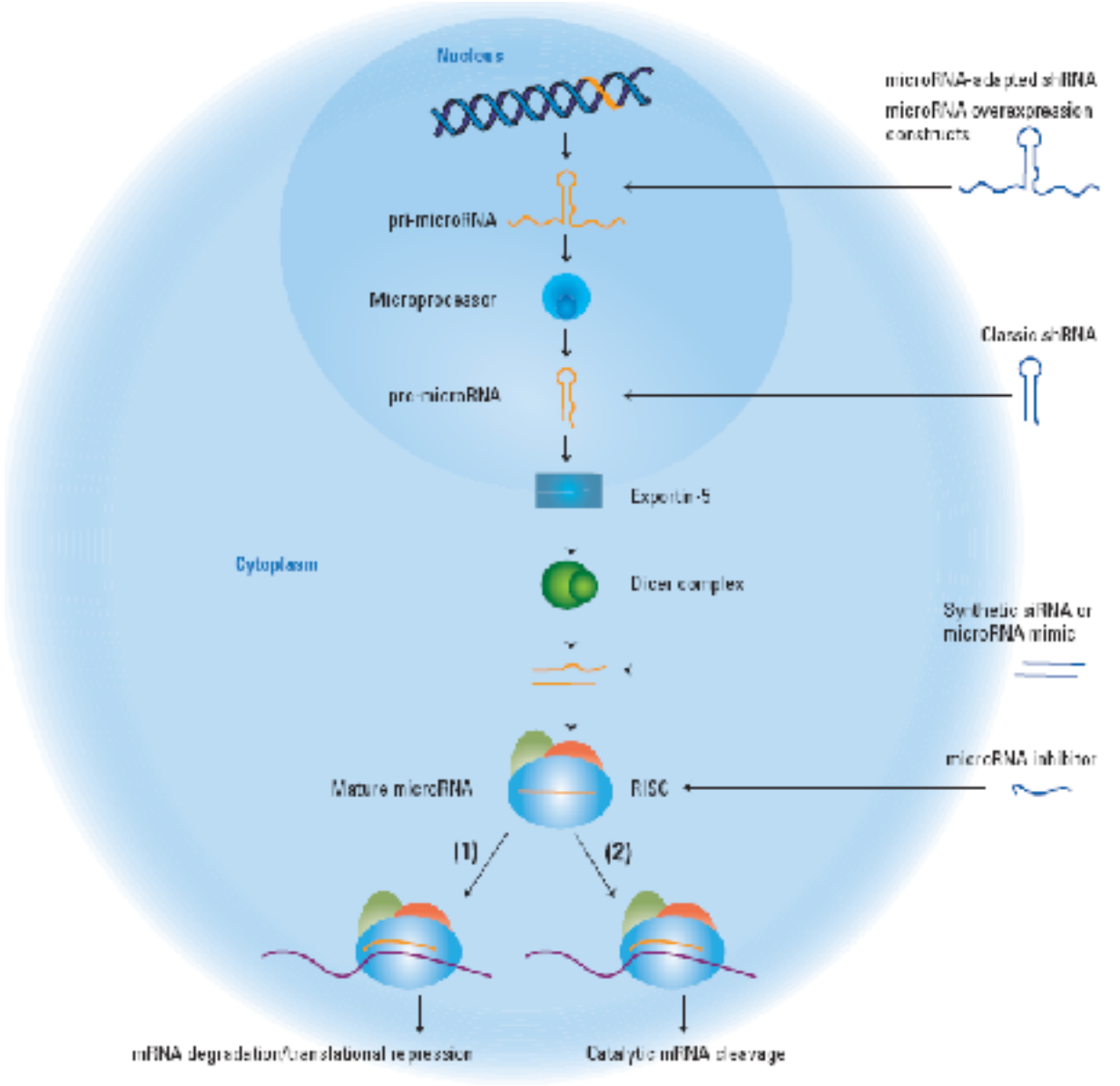


Gene

off

siRNA advantages

- Fast, as knockdowns can often be performed in 72-96h, antibodies can be tested in a relatively quick fashion.
- Easy to test multiple cell backgrounds unlikely to have the same protein composition leading to unspecificities.
- Test sensitivity due to changes in expression
- Compare the expression between treated cells, mock transfected and NTC transfected cells

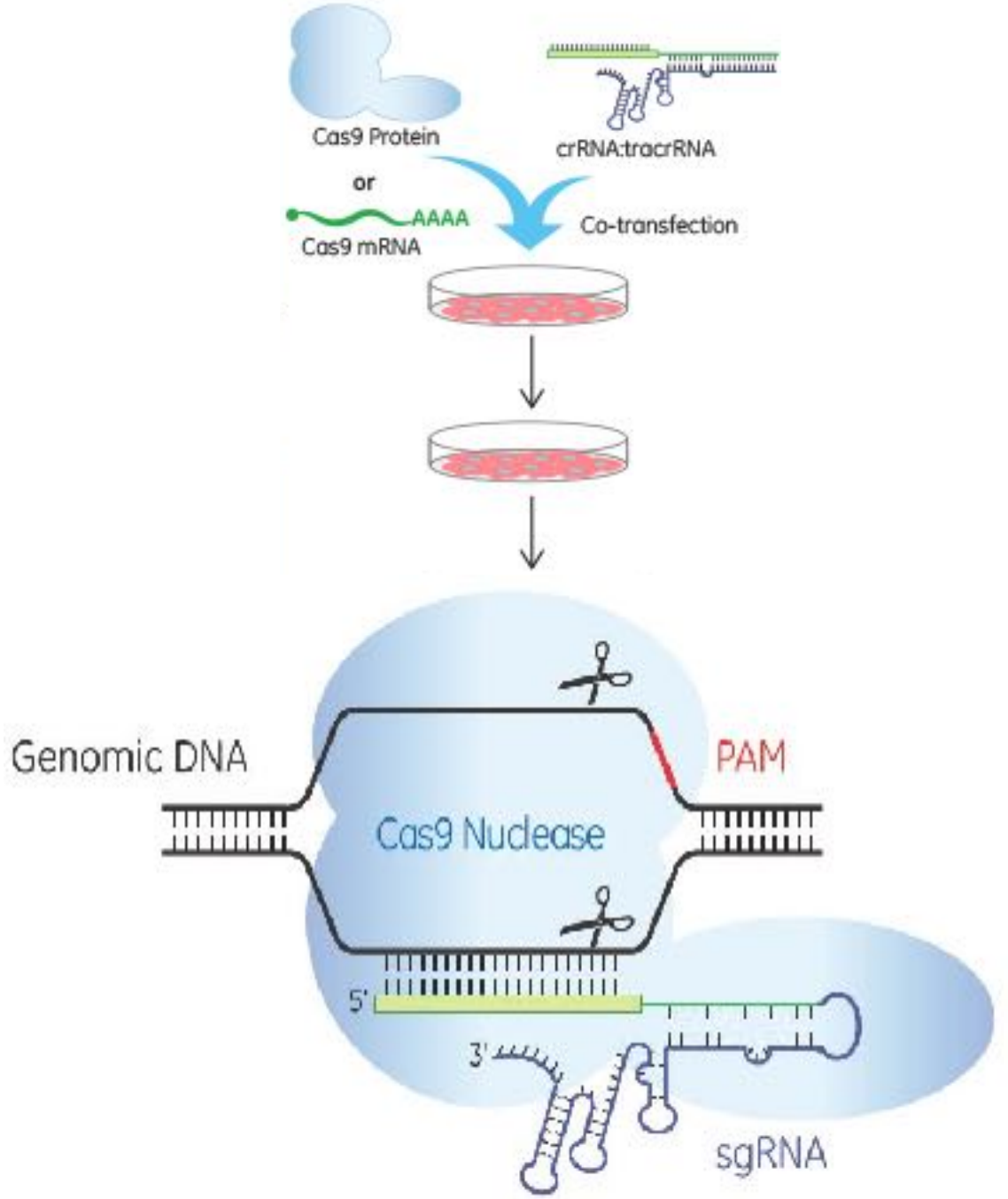


What is a CRISPR-Cas system?

- Mechanism of adaptive immunity in bacteria and archaea

- Evolved to adapt and defend against foreign genetic material (i.e., phage, horizontal gene transfer, etc.)

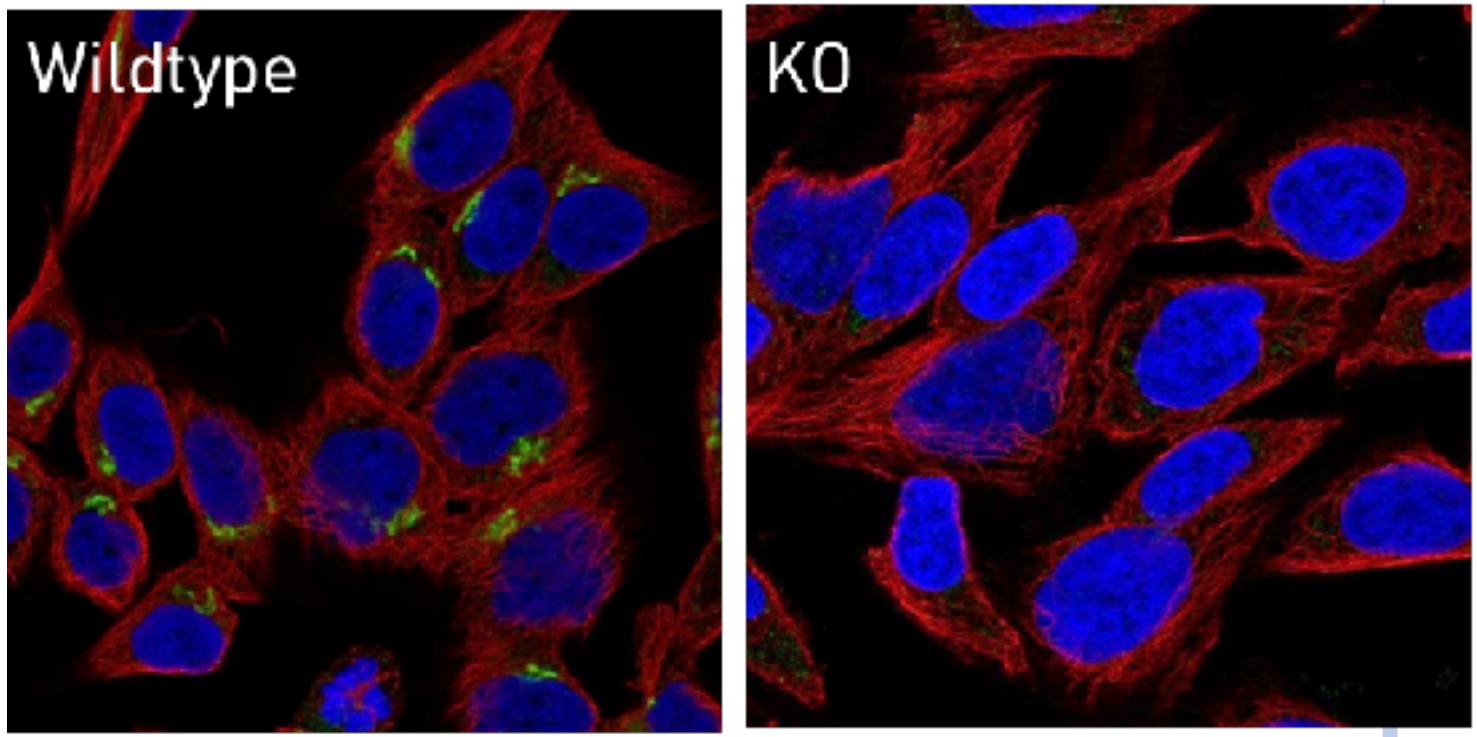
- Requires:
  - *Cas9 Nuclease – creates double-strand break*
  - *Guide RNA – recruits Cas9 and directs target cleavage*



ANTIBODY SOCIETY

Knock out cell line advantages

- Validated at the genetic level. Functional protein expression can be ablated by introduction of frameshift mutations into the coding sequence, or the epitope can be excised completely
- Many KO cell lines have already being used to validate antibodies by commercial suppliers



The parallel use of wildtype and KO cell lines provides a valuable tool to control for research reagents quality

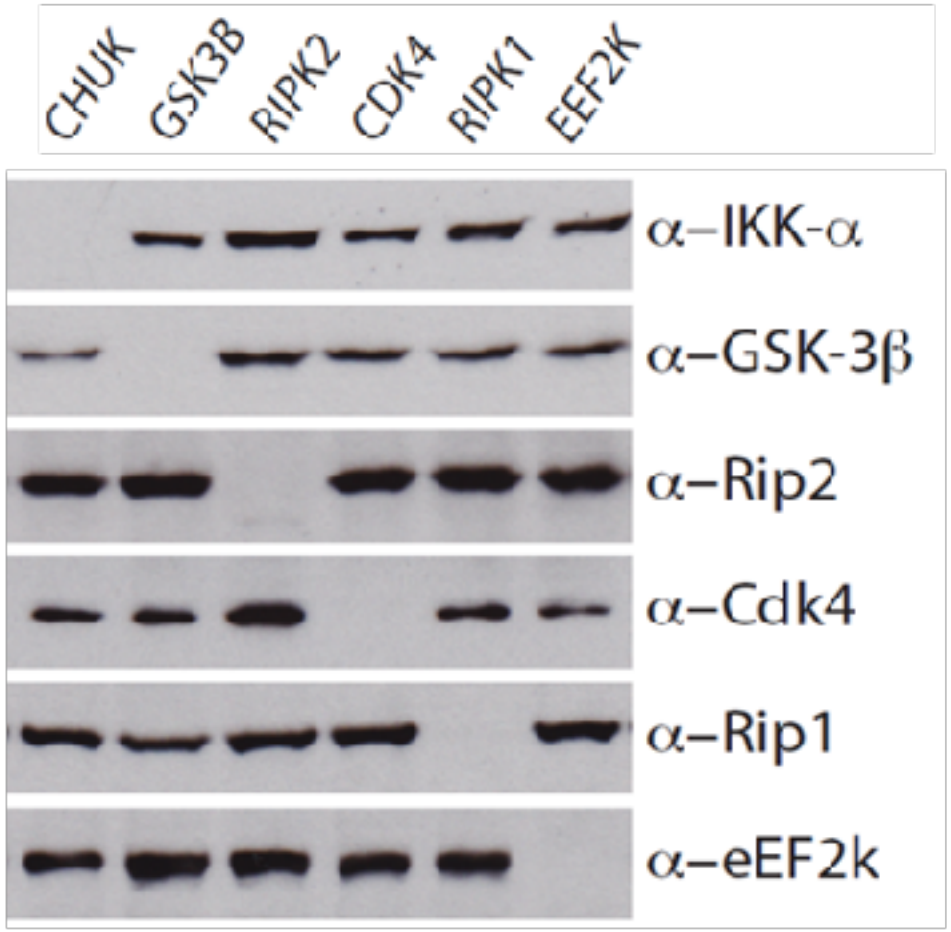
Images courtesy of Dr Emma Lundberg, Cell Profiling facility. KTH Royal Institute of Technology

Green	SLC30A6
Blue	Nucleus
Red	Microtubules

ANTIBODY SOCIETY

Ready-to-go™ knockout cell lines

- CRISPR knock out cell lines without risking your resources
- Generate multiple clones to control for off-target effects
- Pair KO cells with matched parental cells
- 9,000 gene targets available
- 3.000 gene targets ready to ship
    - 7,500+ off-the-shelf clones
- Custom projects capability



A selection of gene edited KO cell lines. Western blot data confirms absence of protein, and so validates the antibody used.

ANTIBODY SOCIETY

Thank you

horizon™
INSPIRED CELL SOLUTIONS

ANTIBODY SOCIETY

Cell line knock-out & knockdown technologies in antibody validation

The Antibody Society Webcast series - Antibody Validation #7

Travis Hardcastle – Horizon Discovery Ltd.

horizon™
INSPIRED CELL SOLUTIONS

ANTIBODY SOCIETY

Cell line knock-out & knockdown technologies in antibody validation

The Antibody Society Webcast series - Antibody Validation #7

Alejandra Solache
Vice-President of New Product Development
Abcam, UK.

abcam

ANTIBODY SOCIETY