Advancing analysis beyond the scope of traditional Western Blot at the University of Lausanne

Séverine Lorrain, PhD

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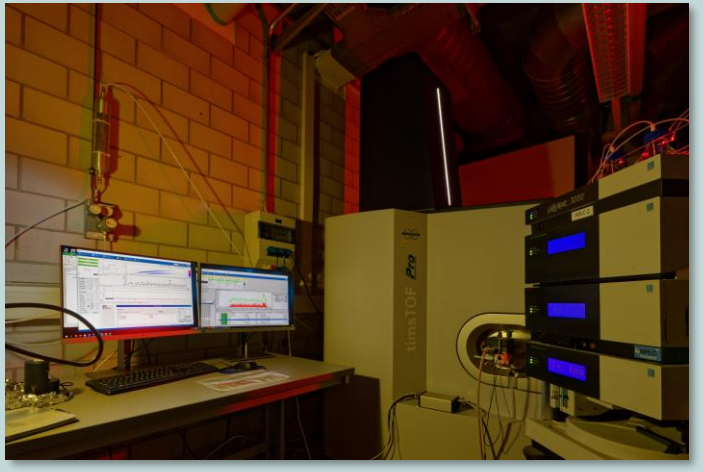
Protein Analysis Facility

The Protein Analysis Facility

A core facility supporting the local community in the analysis of single proteins and complex proteomes

MASS SPECTROMETRY

- Protein identification
- Protein quantification (label-free, isobaric/metabolic labelling)
- Analysis of post-translational modifications

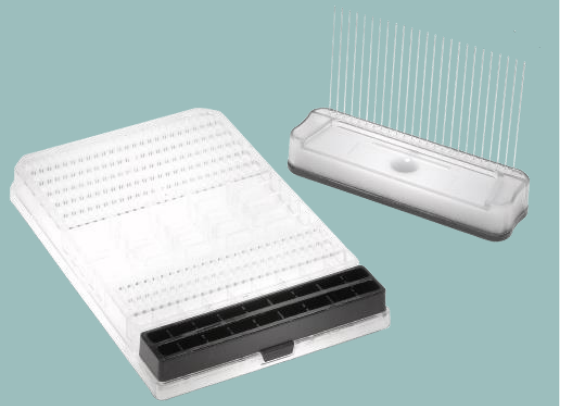


ANTIBODY-BASED PLATFORMS

Targeted quantification of proteins using antibodies

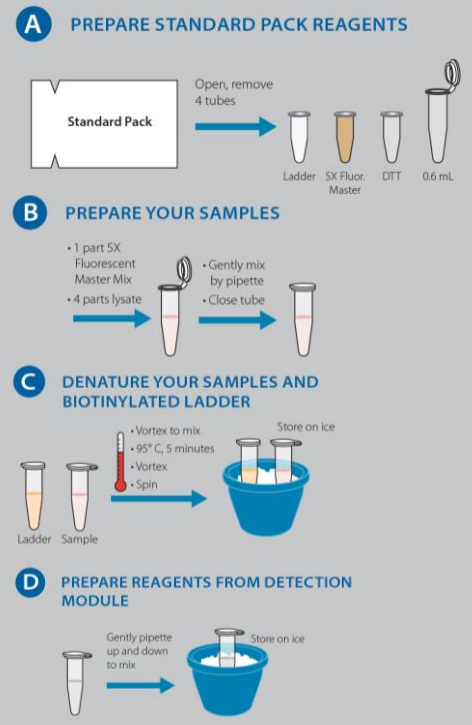
Simple Western

An automated western blot in capillaries
Courtesy of Helen Hall, 2023 Bio-Techne®



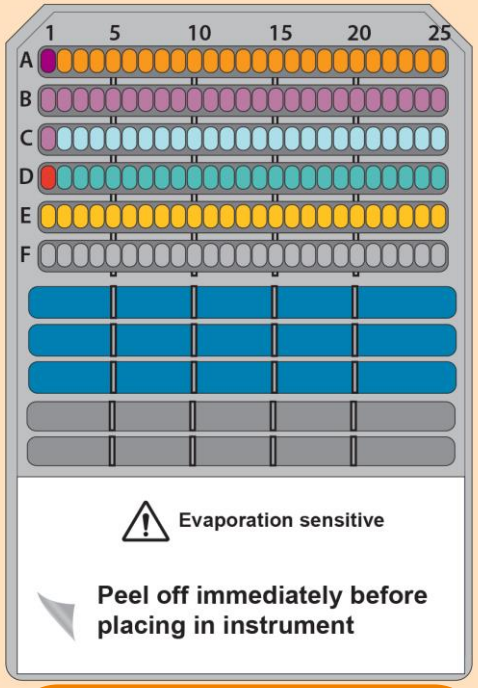
30-45 min

1. Prepare your reagents



Things you already do
- Pipette
- Mix
- Heat

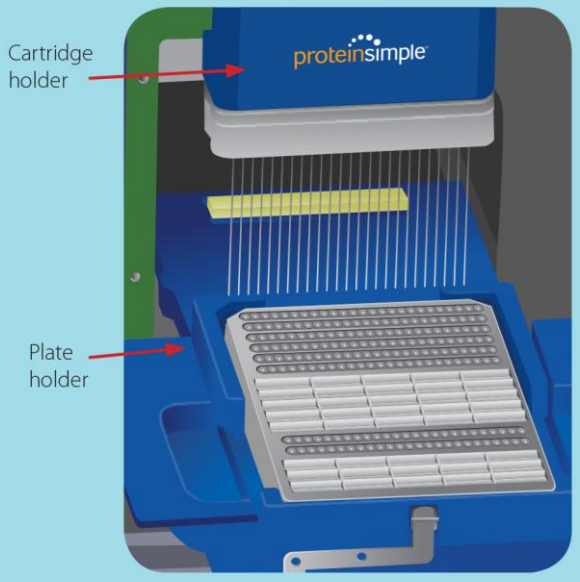
2. Pipette your plate



Things you already use
- Ladder
- Samples
- Ab diluent
- Primary Ab
- Secondary Ab
- Substrate

3. Start Abby / Jess

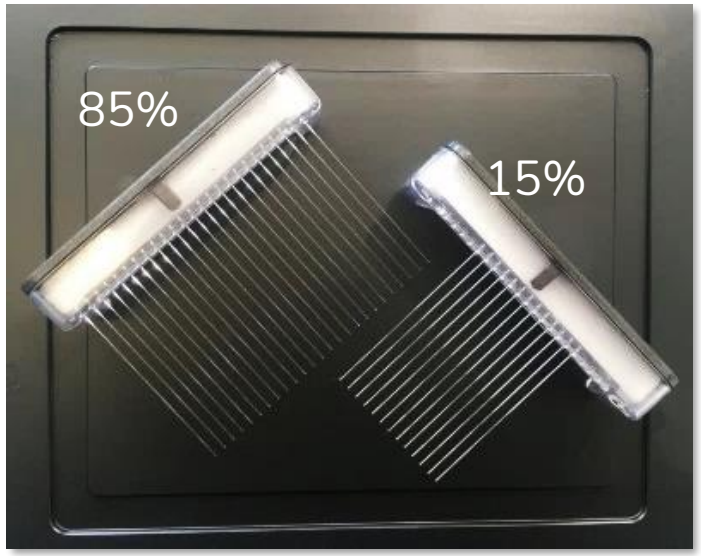
3h

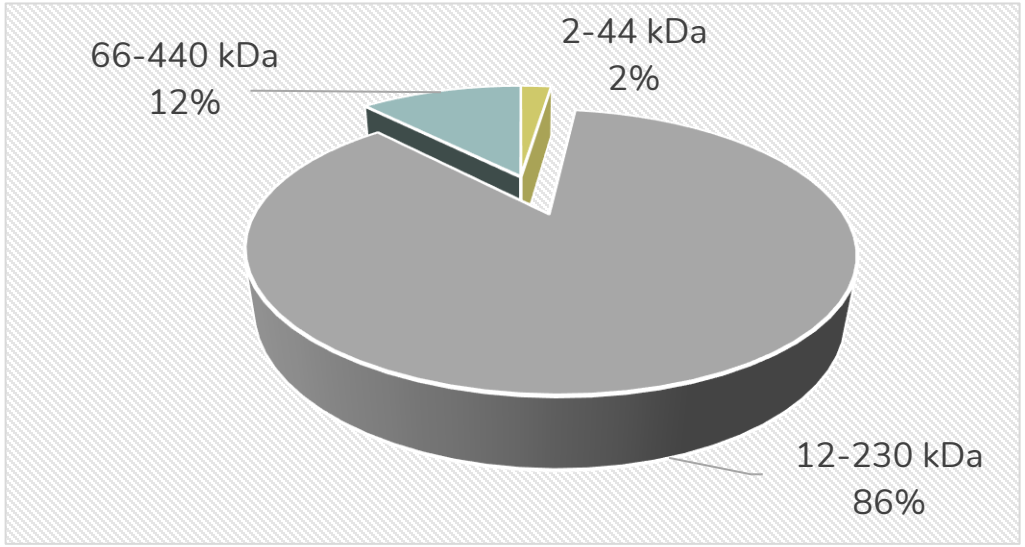


Magic happens in the capillary
- Separate
- Immobilize
- Probe
- Detect

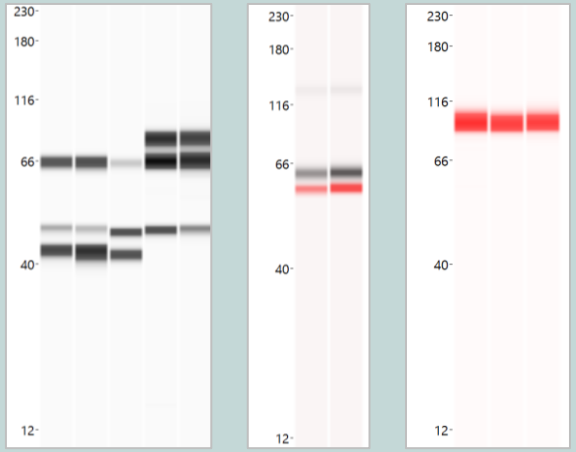
Jess in numbers

2020-2023

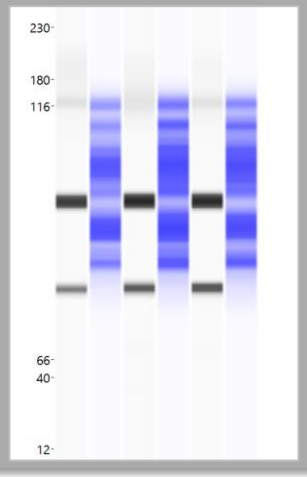




Chemiluminescence (1-4 antibodies)
Fluorescence (mostly Near Infra Red)



RePlex 20 %
Total protein Normalisation 15%



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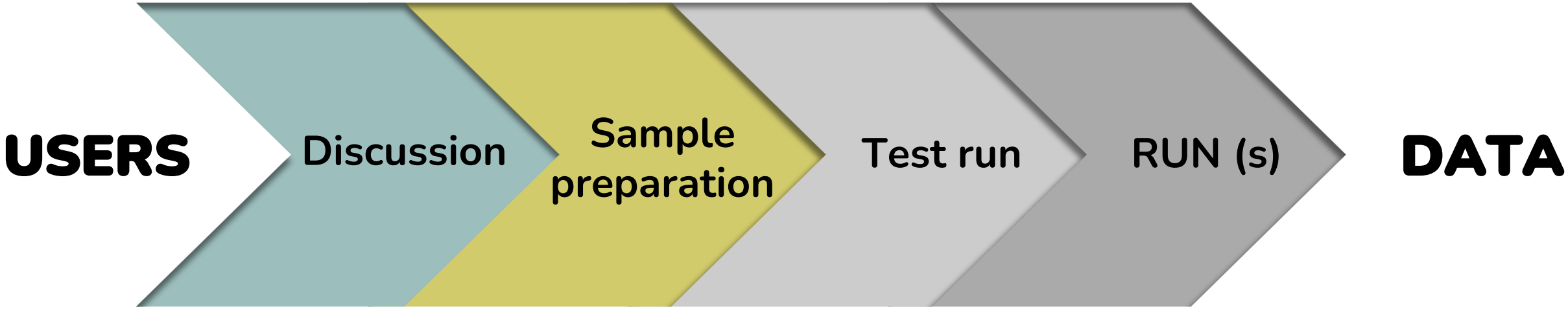
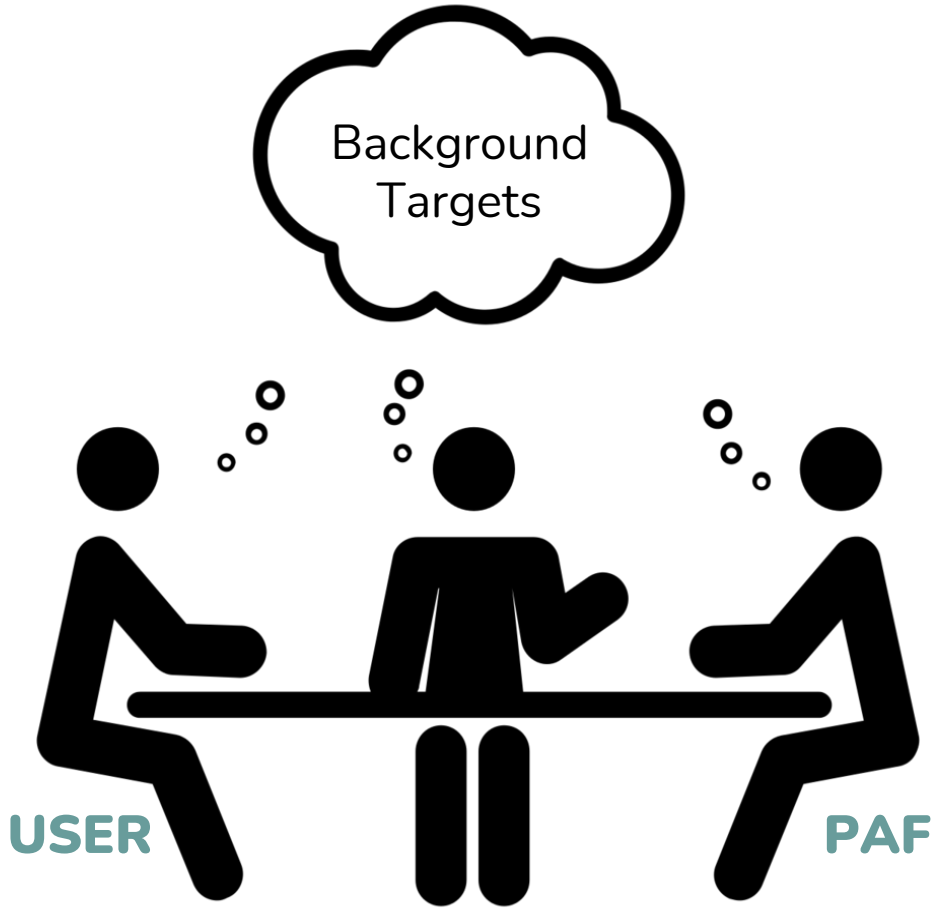
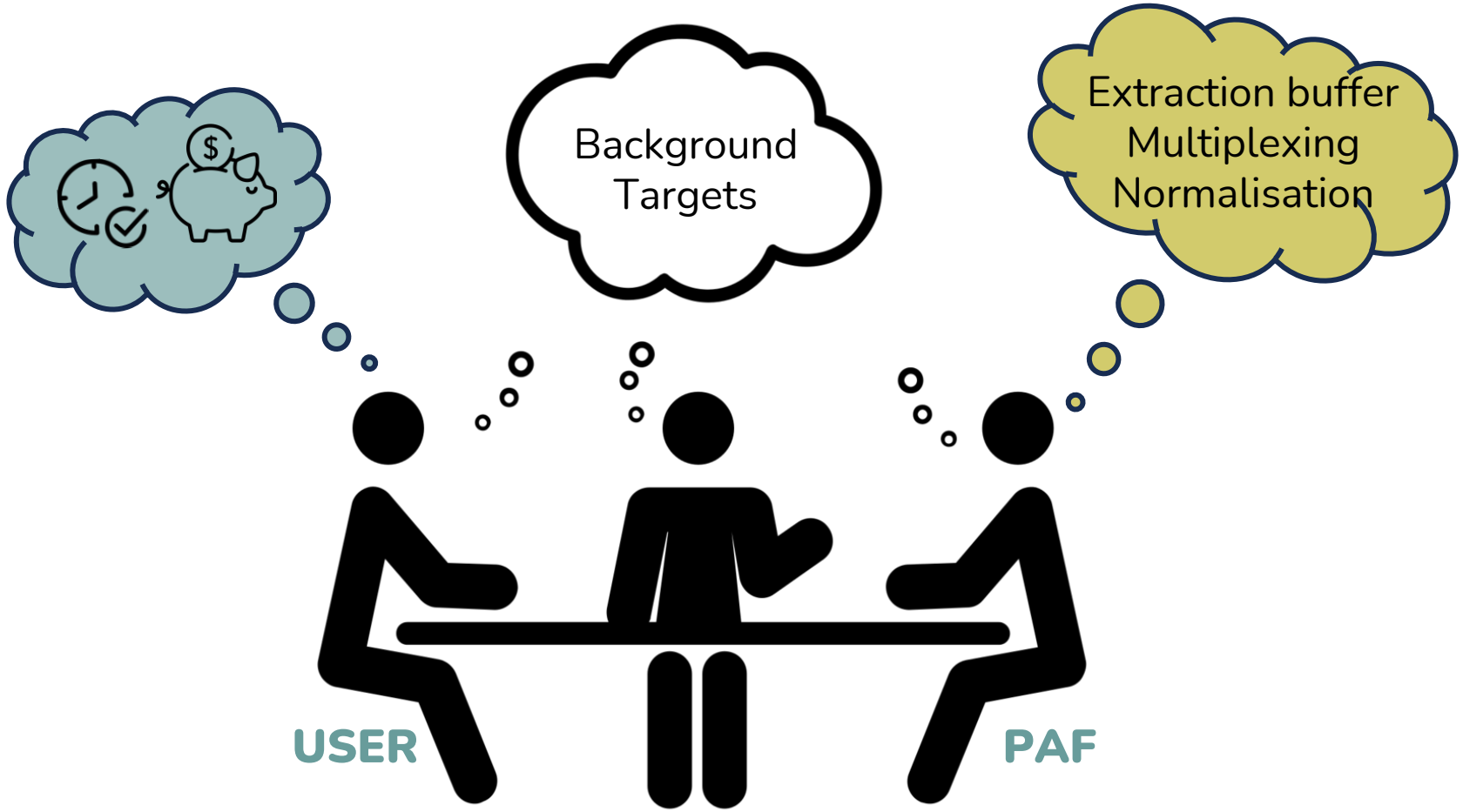
How do we proceed

To avoid common pitfalls



- No experience in western blot
- Need quantitative data
- Many samples
- Low protein amount





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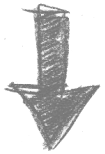
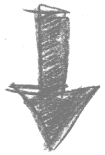
Sample prep.

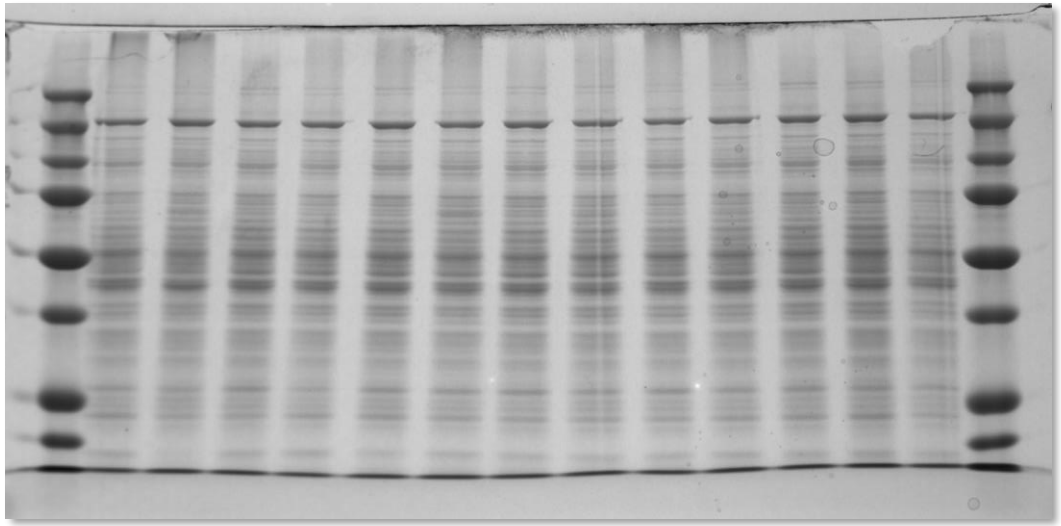
Protein extracts



Extraction



- Quantification (tryptophan fluorescence)
- Normalisation of loading amount
- Quality control (Coomasie staining)



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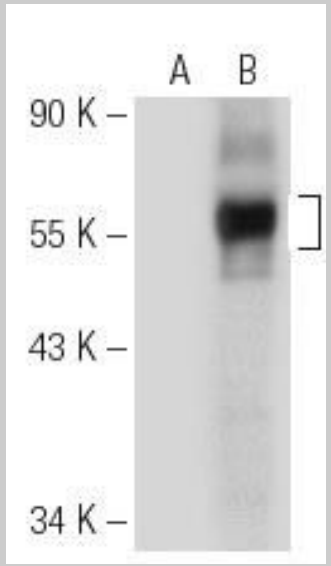
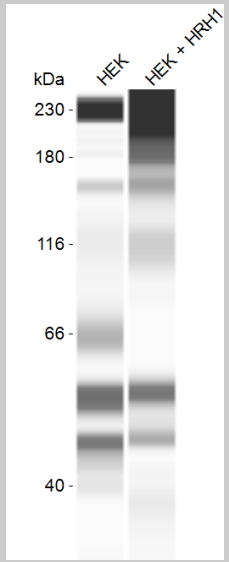
1. Antibody validation

Manufacturer



Jess



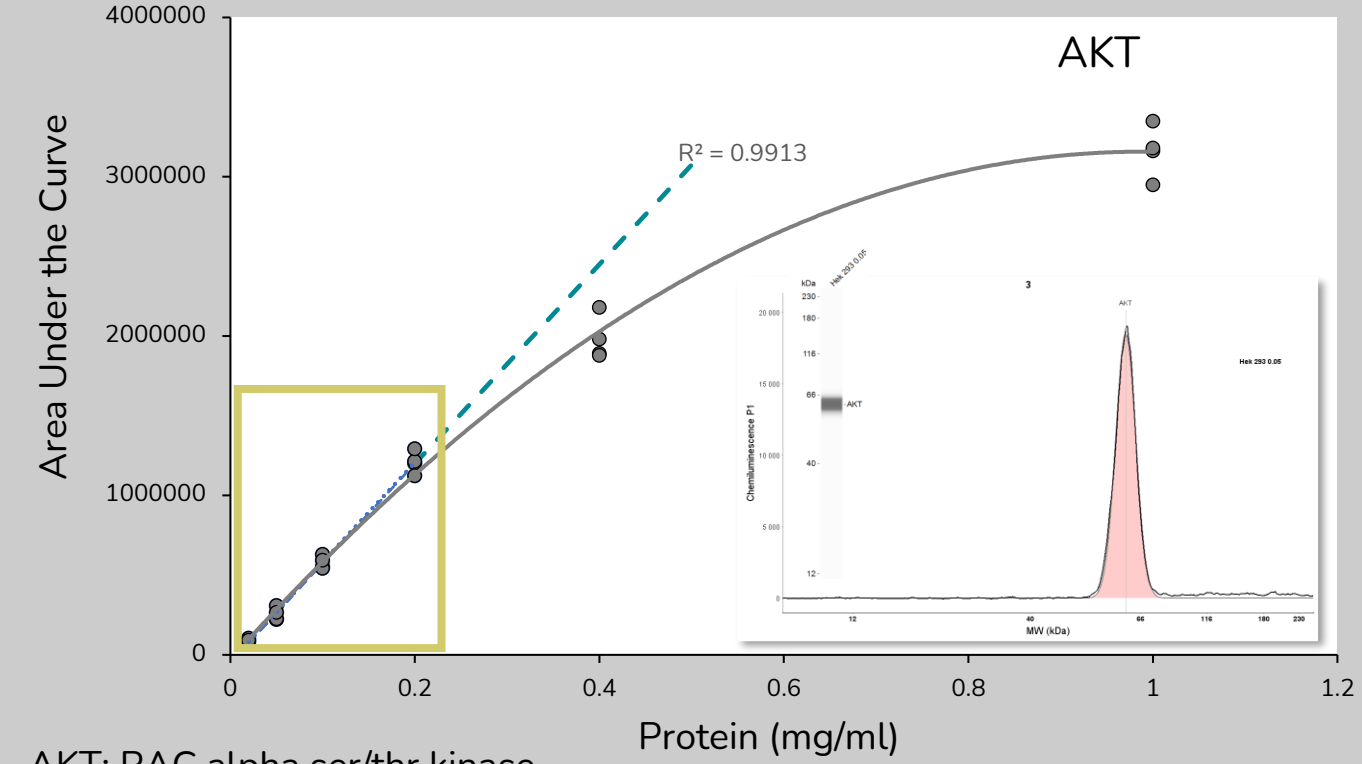
Histamine H1 Receptor

Test run

- Quick
- Flexible
- Reproductive
- Quantitative

1. Antibody validation

2. Antibody linear range



AKT: RAC alpha ser/thr kinase
HEK293 cells

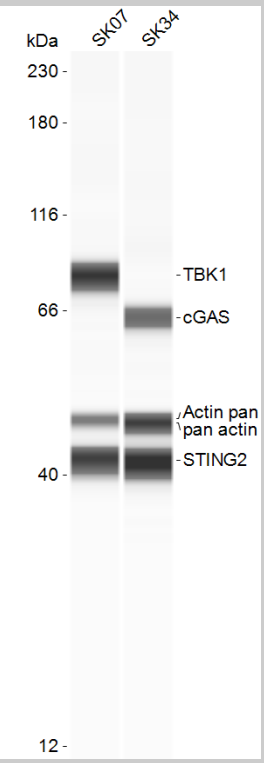
Test run

- Quick
- Flexible
- Reproductive
- Quantitative

1. Antibody validation

2. Antibody linear range

3. Mutiplexing 3-4 antibodies

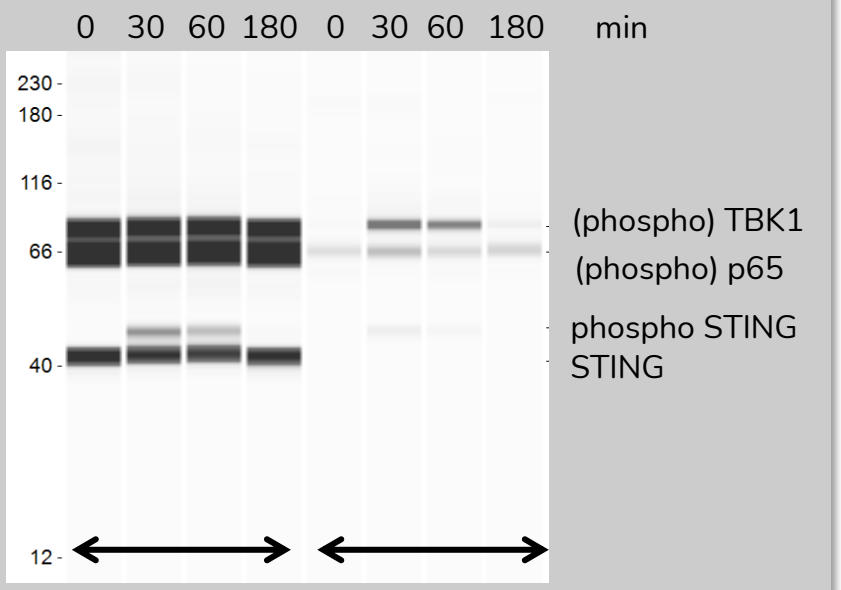


Human cells

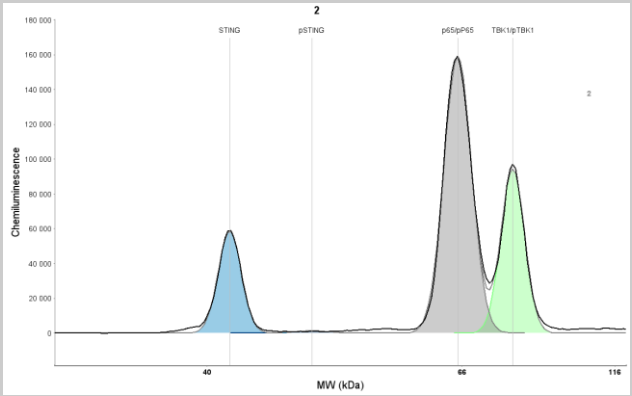
Run(s)

Katakam *et al.*, 2021

Run

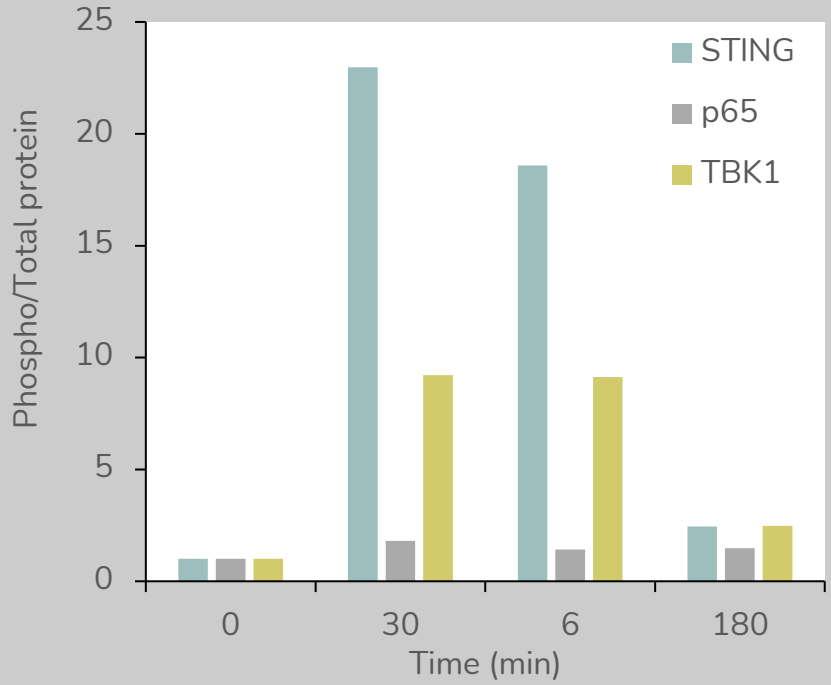


Area Under the Curve



STING: Stimulator of Interferon Gene
p65: transcription factor
TBK1: serine/threonine protein kinase
Human cells

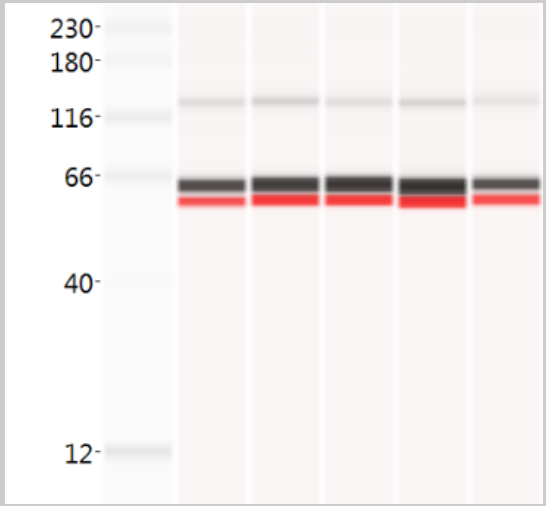
Quantification



Dealing with proteins of different intensity

Combining detection mode

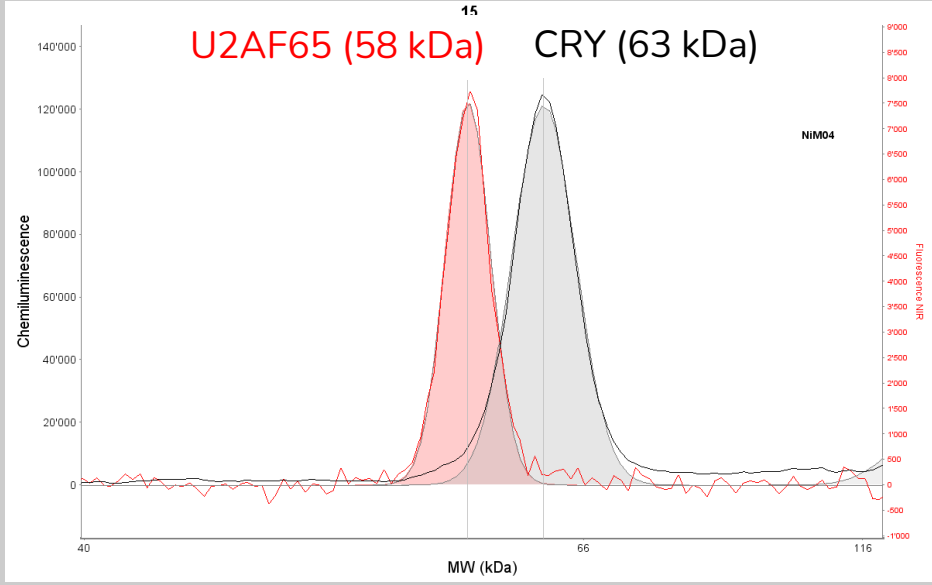
Chemiluminescence for high sensitivity
Near Infra Red for abundant proteins



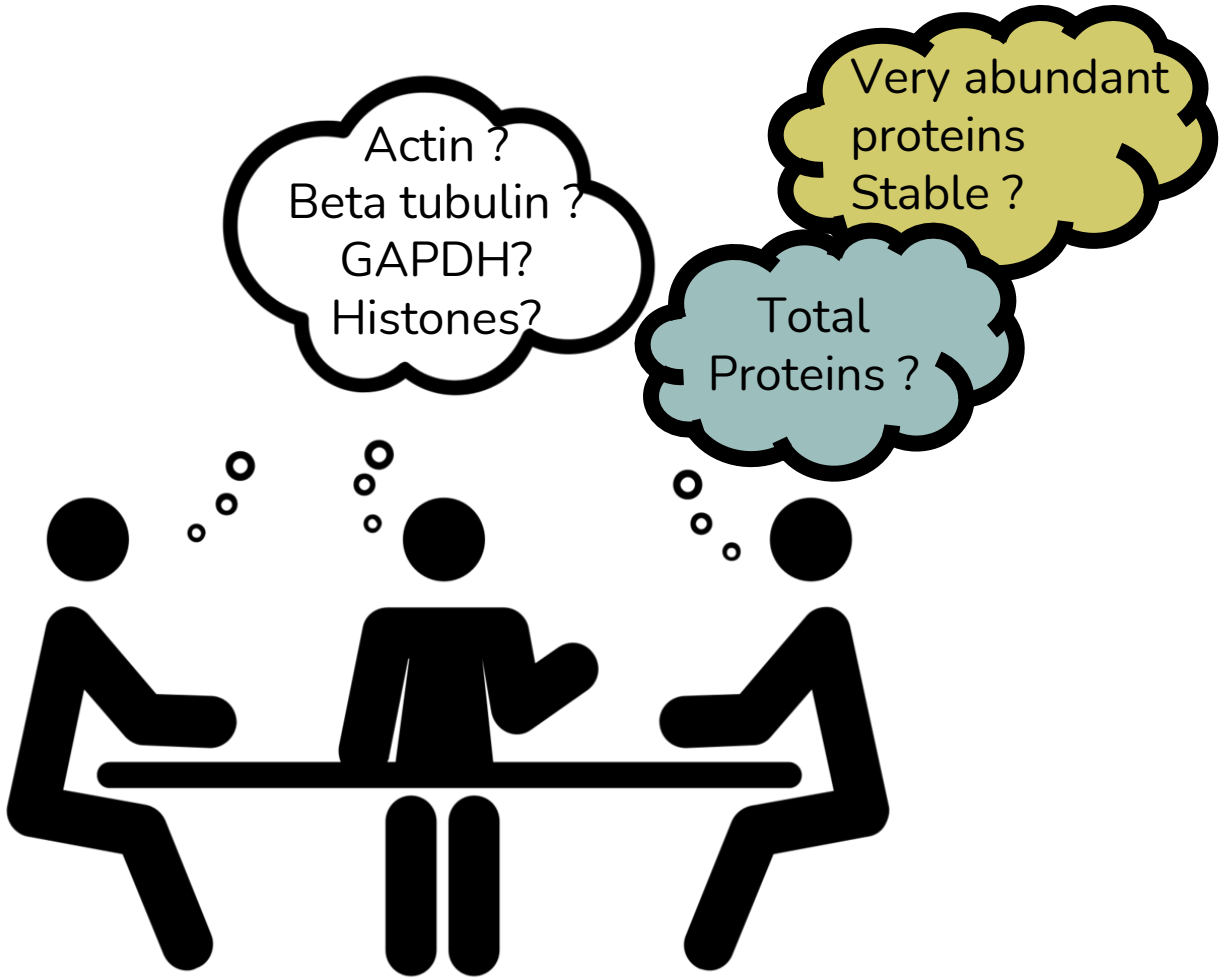
CRY (63 kDa)
U2AF65 (58 kDa)

CRY: cryptochrome
U2AF65: splicing factor, nuclear marker
Mouse cells

Area Under the Curve



Loading control



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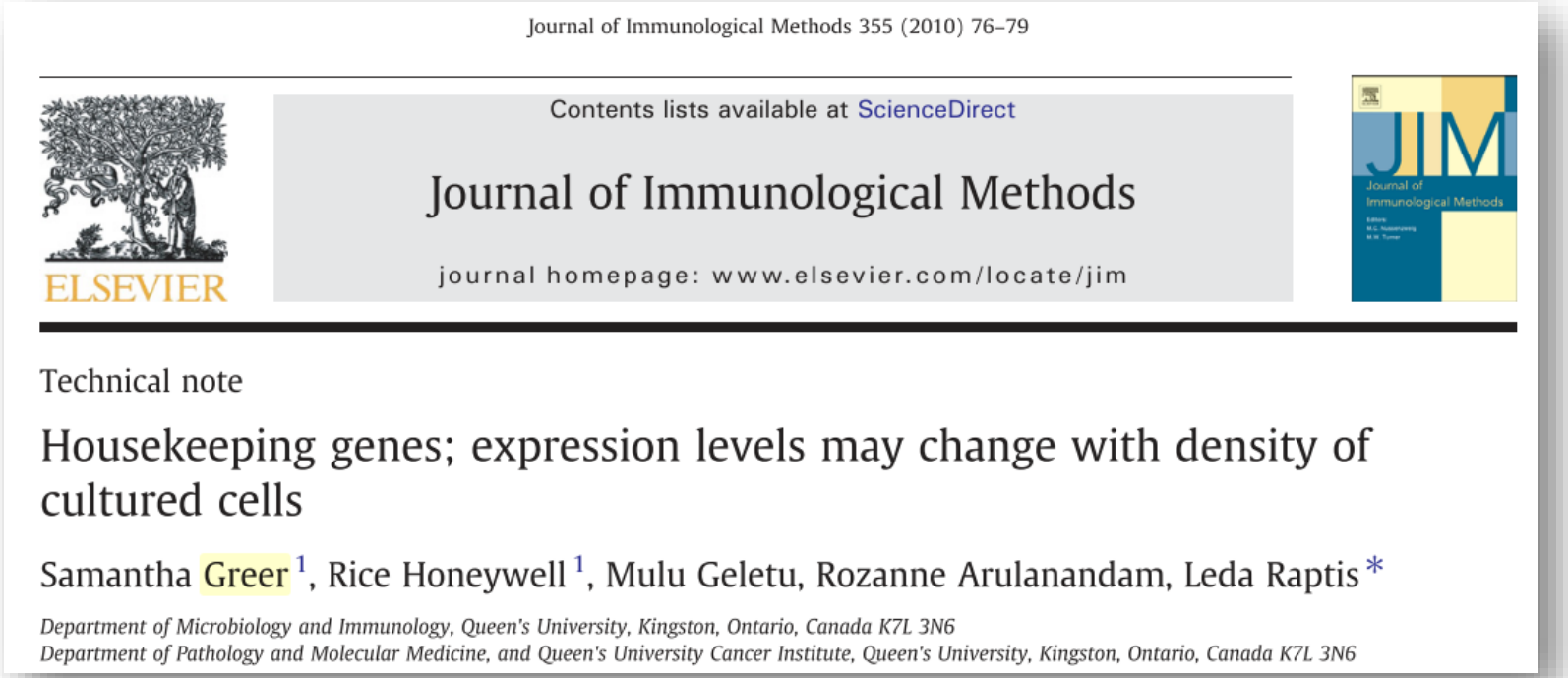
Data normalisation

Total Protein *versus* house-keeping proteins



Journal of Immunological Methods 355 (2010) 76–79

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Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Technical note

Housekeeping genes; expression levels may change with density of cultured cells

Samantha Greer [1], Rice Honeywell [1], Mulu Geletu, Rozanne Arulanandam, Leda Raptis *

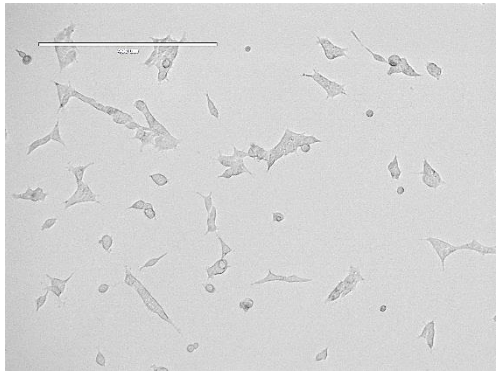
Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6
Department of Pathology and Molecular Medicine, and Queen's University Cancer Institute, Queen's University, Kingston, Ontario, Canada K7L 3N6

«Cell confluence **significantly affects the levels** of **α-tubulin** and **Glyceraldehyde-3-Phosphate Dehydrogenase** (...)

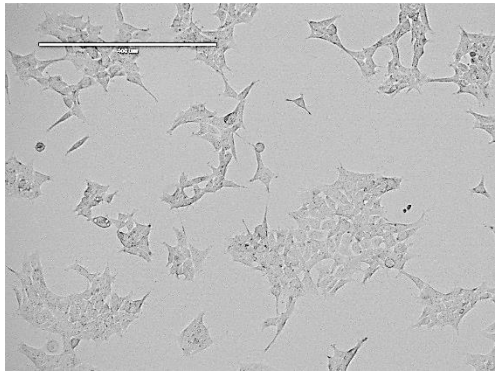
Levels of **heat-shock protein-90** (α and β) and **β-actin** remained **unchanged** at a wide range of cell densities»

Total *versus* house-keeping proteins

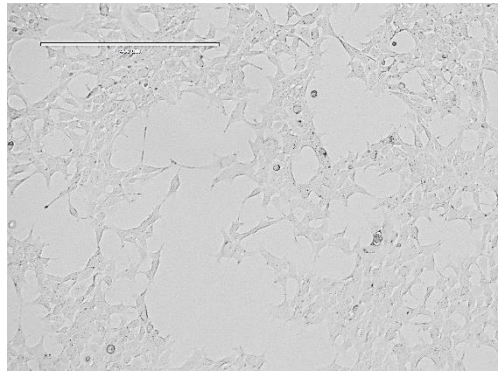
CONF1



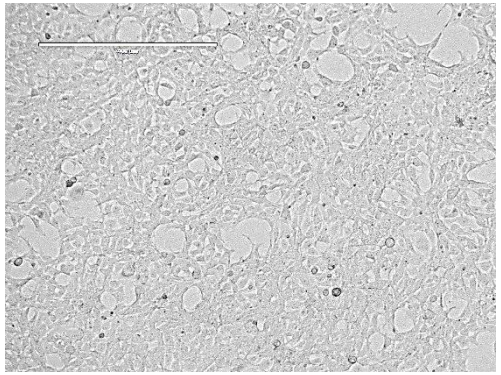
CONF3



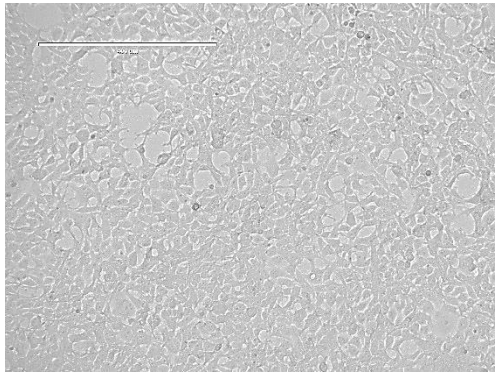
CONF5



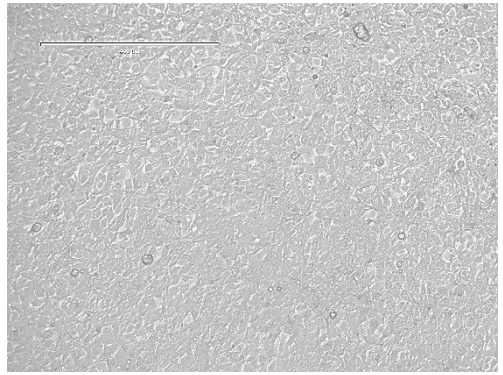
CONF6



CONF7



CONF8



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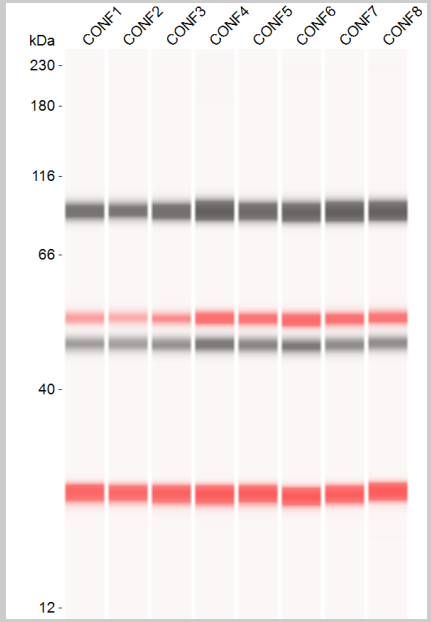
Total *versus* house-keeping proteins

Multiplex and strip and reprobe with RePlex

Probe 1 : antibodies

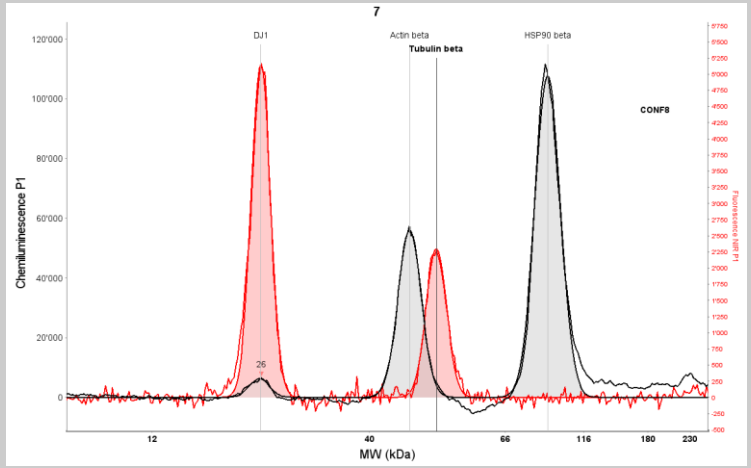


HSP90 β

Tubulin β

Actin β

DJ-1



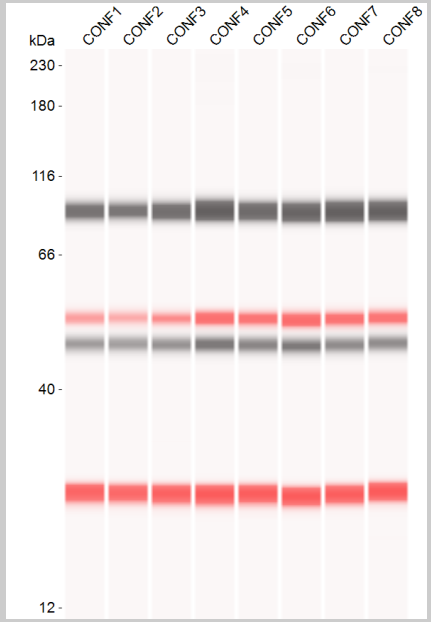
Procedure

- ✓ Antibodies specificity
- ✓ Linear range for each antibody
- ✓ Choice of detection mode (chemi *versus* Near Infra Red)
- ✓ Multiplexing

Total *versus* house-keeping proteins

Multiplex and strip and reprobe with RePlex

Probe 1 : antibodies



HSP90 β

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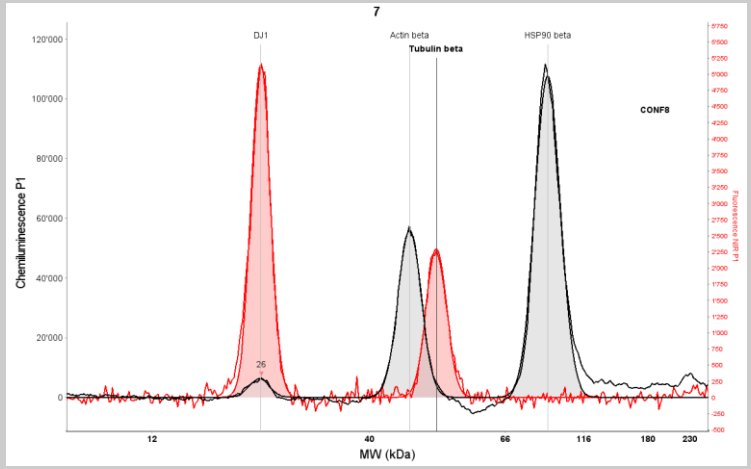
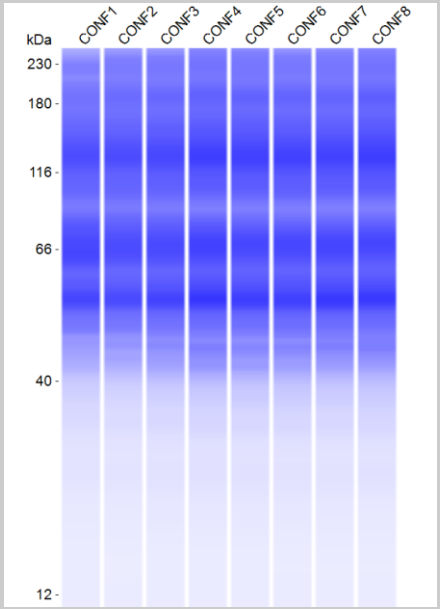
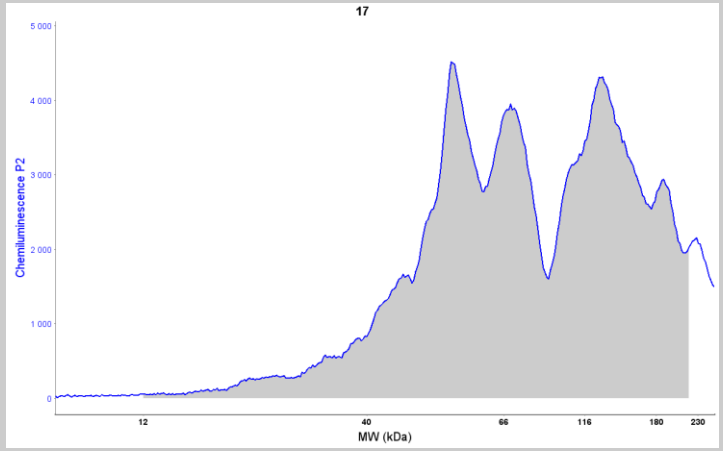


Probe 2 : total protein detection

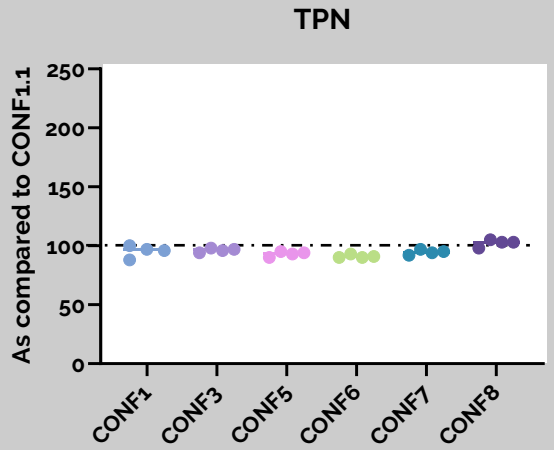


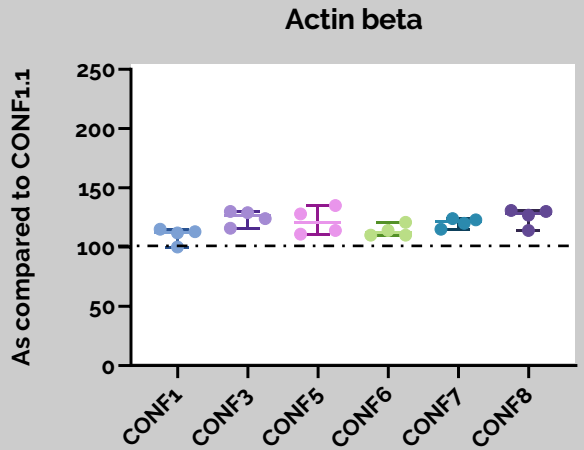


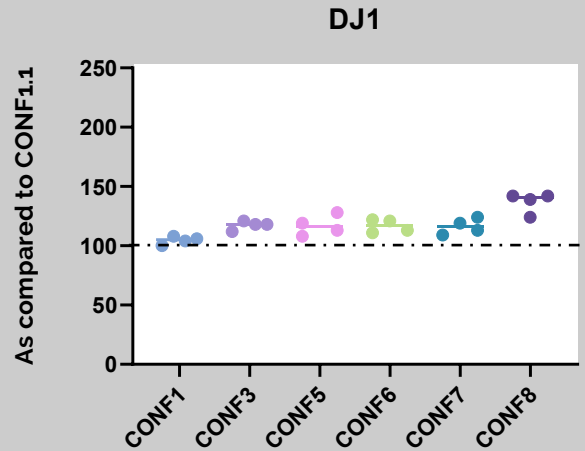
ReThink ReUse RePlex™

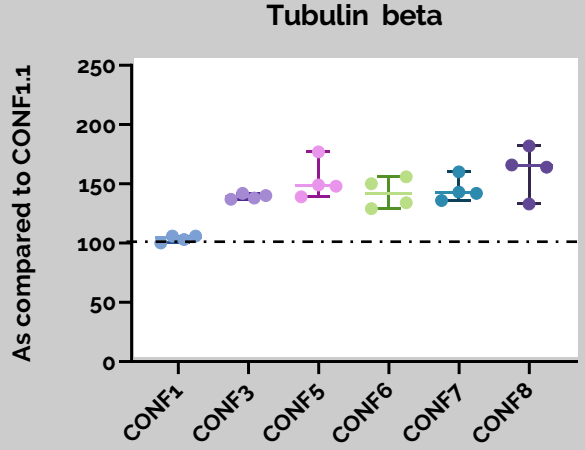
Total *versus* house-keeping proteins

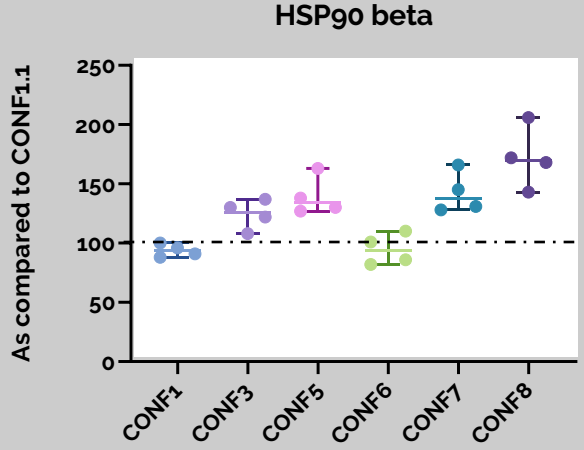
«House-keeping» protein signal depends on cell density











- 6 samples
- 4 technical replicates
- 0.6 µg / lane

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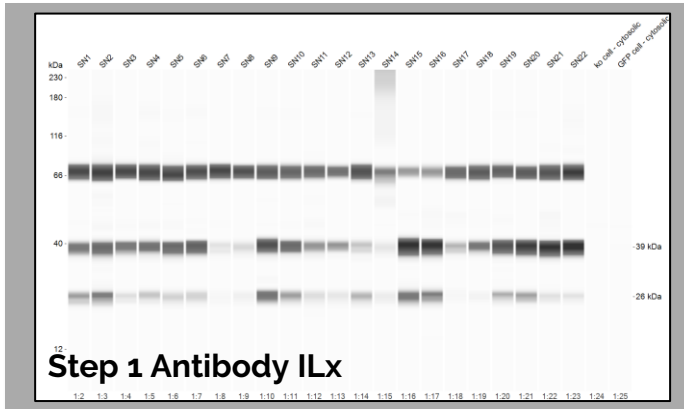
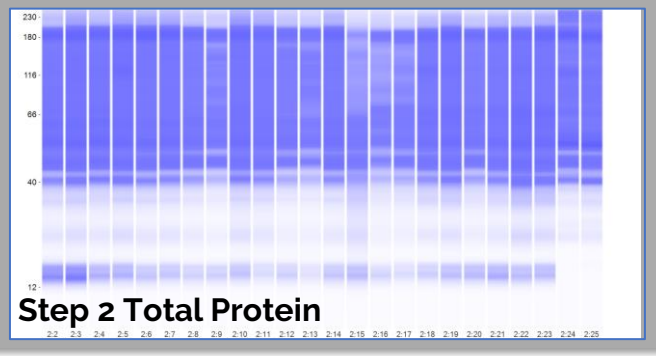
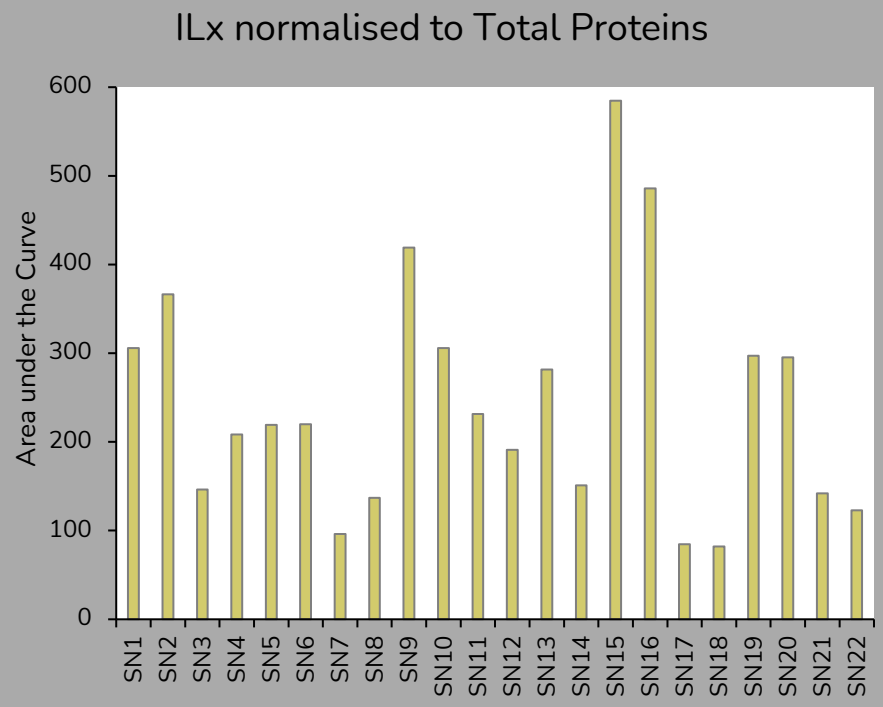
A representative run

- 24 samples
- 3 µl sample
- 0.6 µg / lane (often less)

- 30-45 min plate preparation
- 3h / 6 h (Replex) fully-automated run
- 3 runs / 24 hours
- Reproducible







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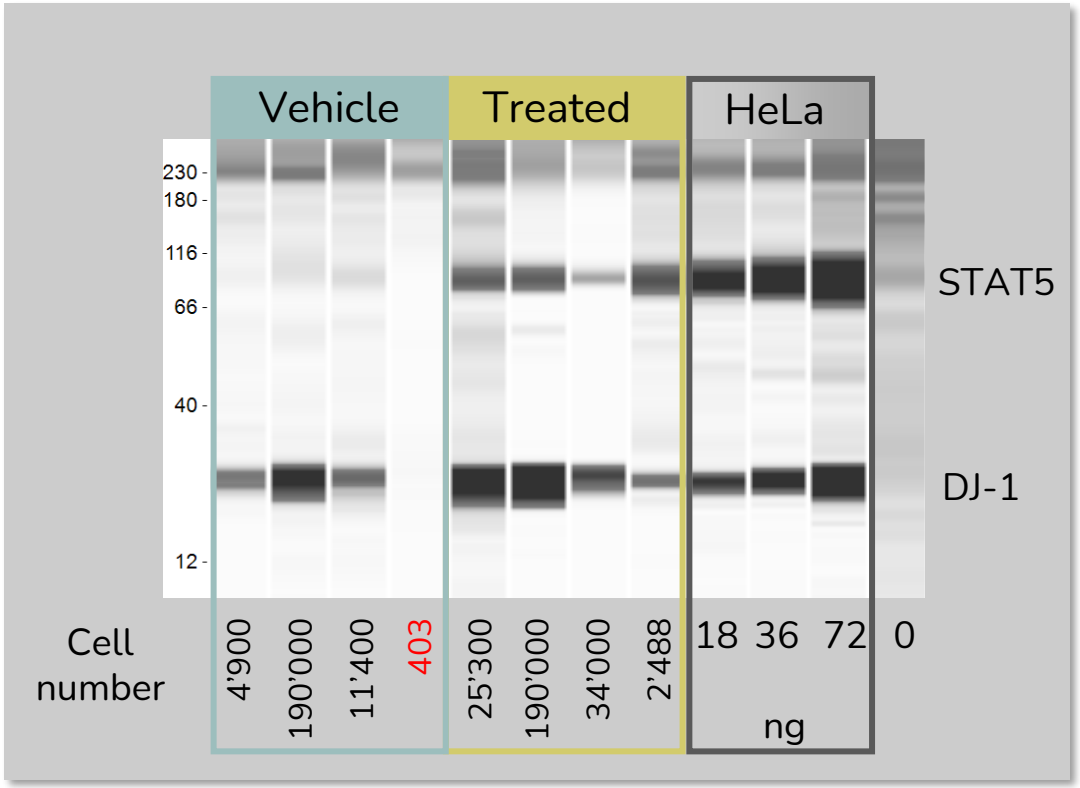
Challenging Jess with low cell numbers

FAC sorted cells

- Few cells: 400 – 200’000
- <15 µl protein sample
- Low protein /unknown concentration

One shot experiment !

- 3 µl protein
- Multiplex



Strongly dependant on target proteins **AND** antibody quality

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Thank you for your attention!

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