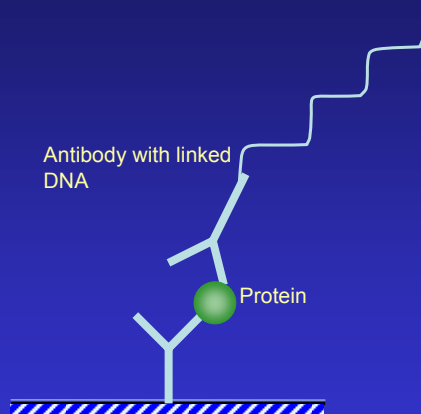


Use of Immuno-qPCR for quantifying proteins in large-scale TAP-tag collections

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Immuno-qPCR

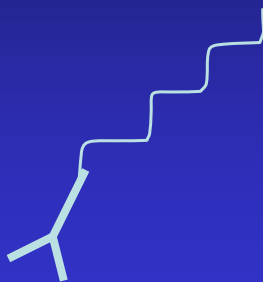


Benefits of immuno-qPCR

- Sensitive quantification.
- Long quantification range.
- Possibility to analyze many samples at the same time.

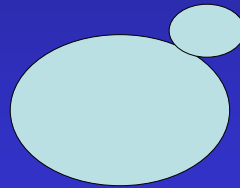
Drawback

- May be difficult and expensive to get the antibody/DNA-conjugate



Aim

- Study protein expression in *Saccharomyces cerevisiae* in large scale.
- Model organism with all 6000 genes sequenced.
- Mutant collections
 - Gene deletions
 - GFP-tag
 - TAP-tag

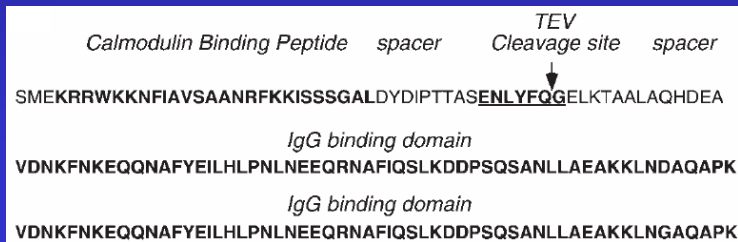


Genome wide TAP-tag collections

- Library with essentially all 6000 genes in *Saccharomyces cerevisiae* tagged.

TAP-tag

- TAP – Tandem Affinity Purification
- 186 amino acids long
- Calmodulin Binding Peptide and two copies of Protein A
- Developed for purification of proteins



Ref. Rigaut, G et al. 1999

TAP-tag

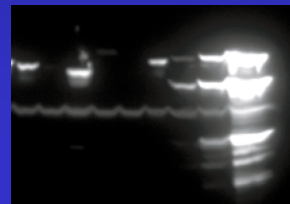
- Inserted at the C-terminal of the protein



Ref. Ghaemmaghami, S et al. 2003

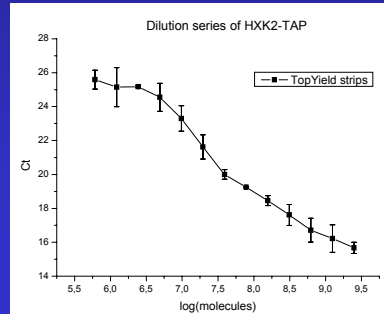
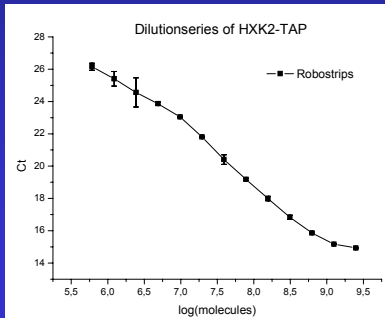
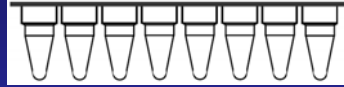
- Used for Western blot analysis

- Problems with background subtraction and quantification.

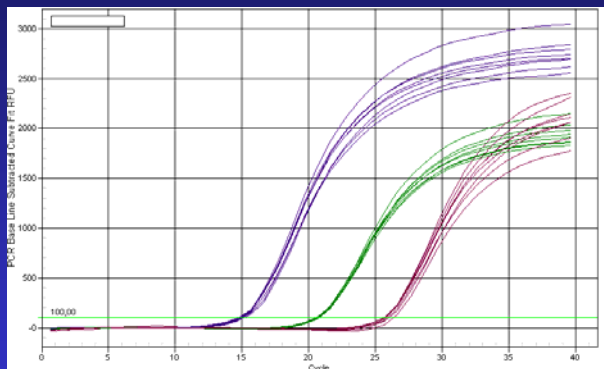


Tubes

- Robostrips (Roboscreen)
– Polycarbonate



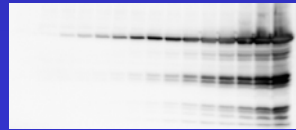
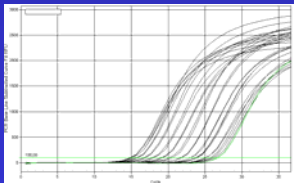
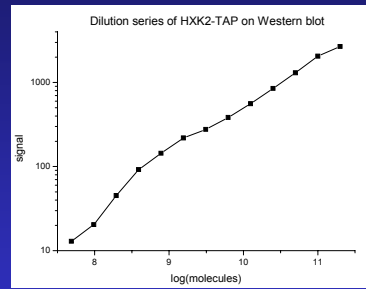
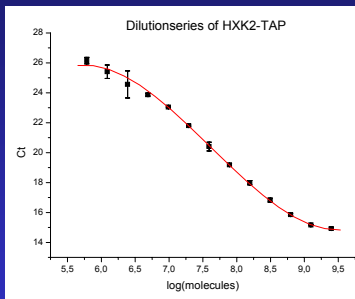
Reproducibility



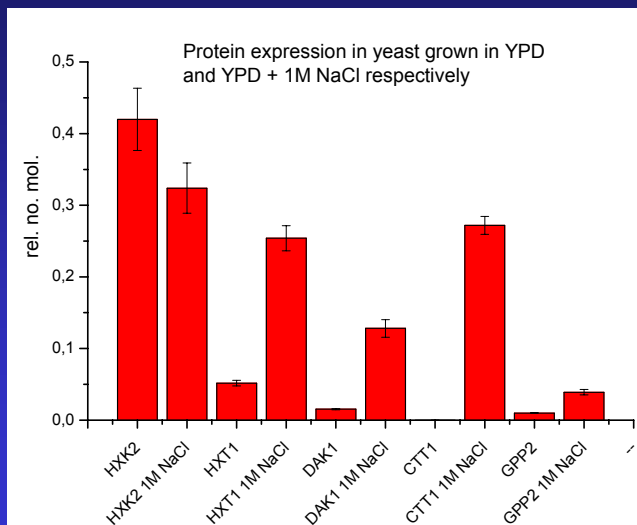
8 replicates

CV of 9%, 4% and 13% respectively

Immuno-qPCR vs Western blot



Protein expression



Fold change with salt stress

Fold change in protein expression during growth in YPD + 1M NaCl compared to growth in YPD.

Protein	Immuno-qPCR	Western
HXK2	0.8	1
HXT1	5	- a)
DAK1	8	5
CTT1	2000	- b)
GPP2	4	3

a) Smear on gel

b) Not detectable without salt stress

Future

- Automization
- Large scale analysis
- Not restricted to yeast
 - Other organisms ranging from E. Coli to humans

Acknowledgement

- Joakim Norbeck
- Mikael Kubista



tatabiocenter