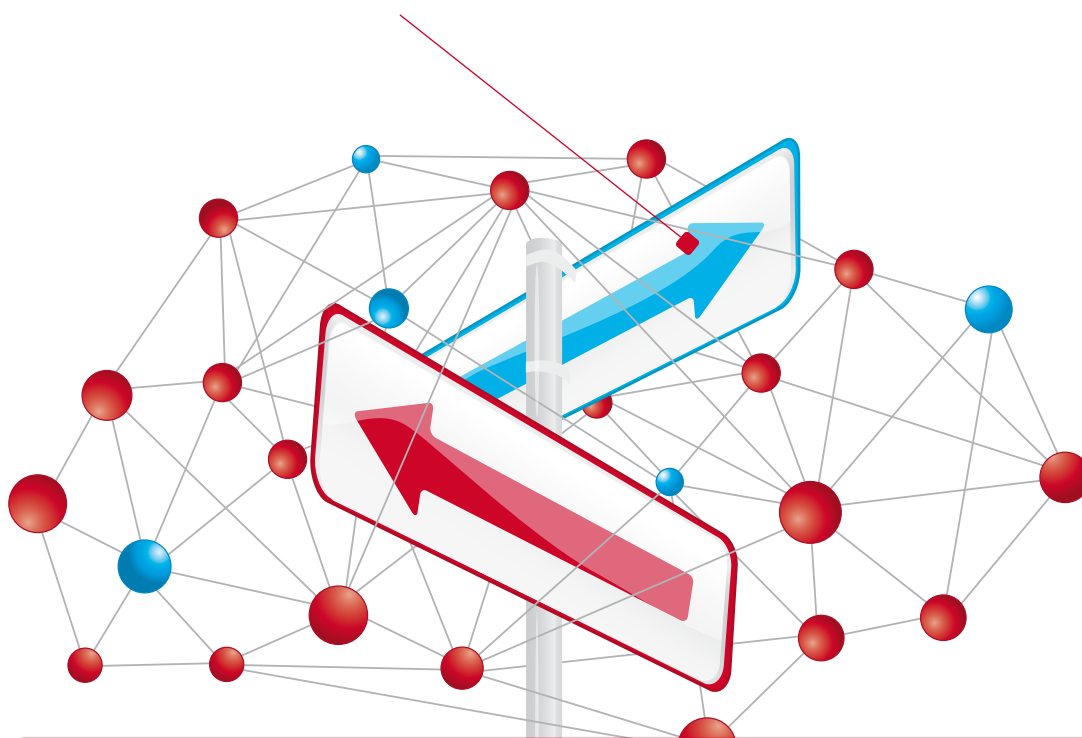


CaptiVate™ Services

Profiling of Signalling Pathways in Human Cells
Multiplexed Analysis of Protein Complexes



- Find novel interactors of your protein of interest
- Monitor protein interactions in response to drugs or extracellular stimuli
- Investigate the dynamics of cell signalling pathways
- Apply CaptiVate™ to target deconvolution and mode of action finding

CaptiVate™ Services

CaptiVate™ is a state-of-the-art mass spectrometry-based detection platform developed to identify the composition of protein complexes involving your protein of interest.

We offer a comprehensive service with less than 12 weeks from design to final data. The only comprehensive service package on the market.

❏ Choose one or several key proteins of interest

- We generate stable human cell lines expressing the protein(s) of interest
- We perform highly specific, streamlined pulldowns to isolate the complexes
- We analyse complexes and PTMs using highly sensitive LC-MS/MS

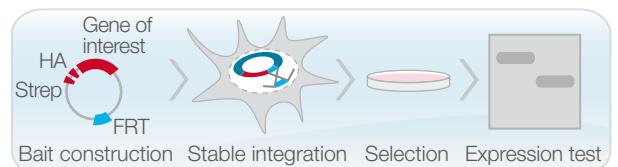
❏ You receive a filtered list of complex partners for your protein(s) of interest and a report with all experimental parameters

■ Project workflow



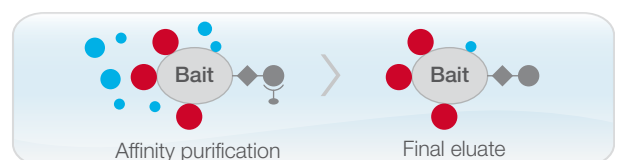
PART I – Bait construction / stable cell line generation

- Selection of one or several proteins of interest (baits) by customer
- N- or C-terminal tagging by Strep-HA purification tags
- Generation of stable cell line expressing bait(s)



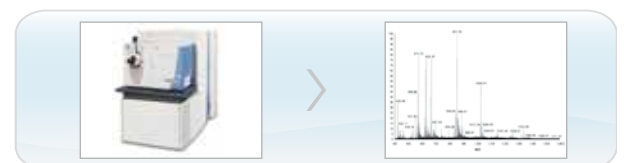
PART II – Affinity purification of protein complexes

- Each affinity purification is carried out in duplicate
- Verification of purification efficiency by Western Blotting
- Optional verification by silver gel



PART III – Mass spectrometry analysis and background filtering

- Sample preparation and analysis by direct LC-MS/MS
- Hits are analyzed using several search algorithms and statistical evaluation programs
- Background filtering against contaminant database
- You receive a complete report and a list of interactors ranked according to confidence



Technological Benefits

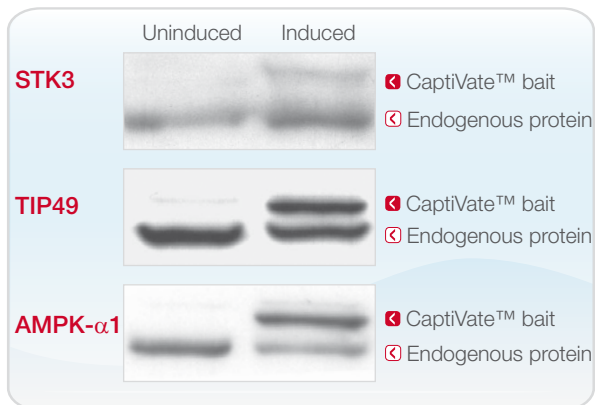
Sensitive

LTQ Orbitrap analysis ensures discovery of weak interactions and rare interactors. Compared with analysis on a conventional ion trap (LTQ), analysis of purified protein complexes on an LTQ Orbitrap identifies significantly higher numbers of interactors.



Physiological

Regulated single copy expression of bait protein ensures low level expression and physiological assembly of protein complexes.



Comparison of protein expression levels of tagged CaptiVate™ bait proteins with endogenous protein levels. HEK293 cell lines expressing tagged bait proteins were analysed by immunoblotting either prior to (uninduced) or after induction of bait protein expression (induced) using antibodies against the indicated proteins.

Specific

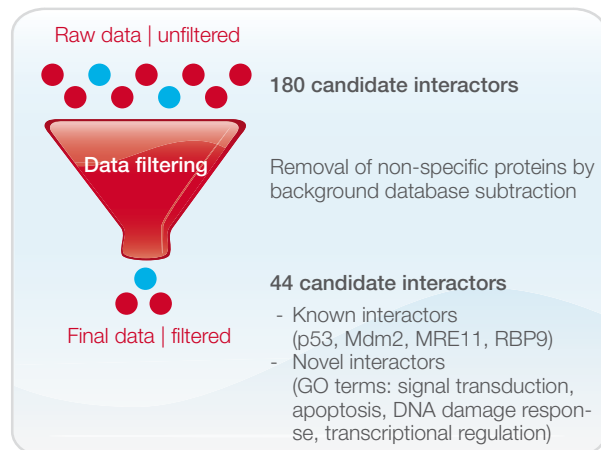
Stringent affinity purification and removal of non-specific interactors by database filtering.

Reproducible

The CaptiVate™ workflow ensures unsurpassed reproducibility (85% reproducibility between replicate purifications, see Glatter *et al.*, 2009), substantially higher than other complex purification methods (e.g. TAP tagging: 65% reproducibility, see Gavin *et al.*, 2006).

Analysis of interactors of p53 from HEK293 cells

CaptiVate™ affinity purification experiment using human p53 as bait.



Analysis using LTQ: 17 interactors after data filtering
Analysis using LTQ Orbitrap: 44 interactors after data filtering

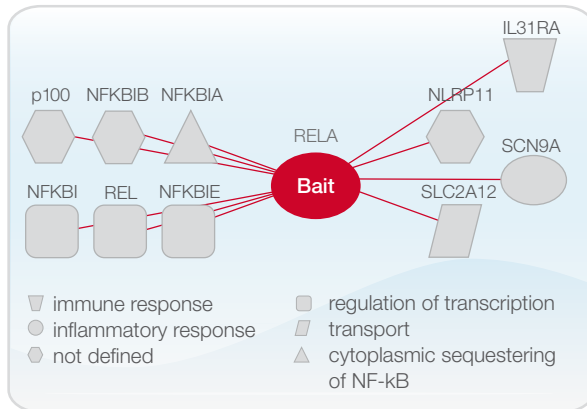


Interested?

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Applications

Identify native protein complexes



Cytoscape graph of proteins associated with the NF-kB subunit p65, purified from unstimulated HEK293 cells.

Benefits

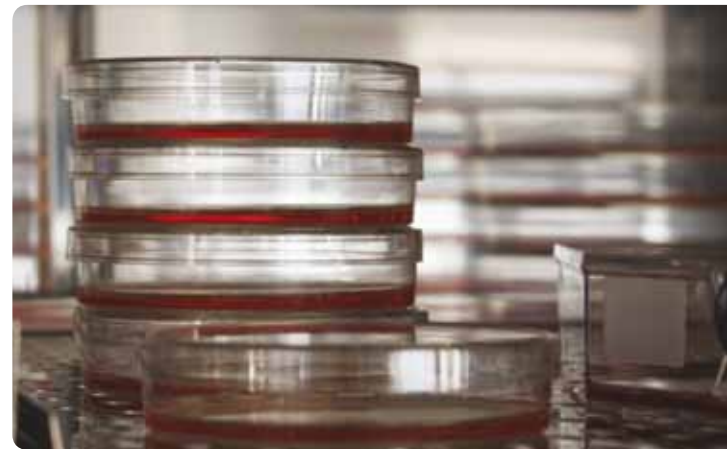
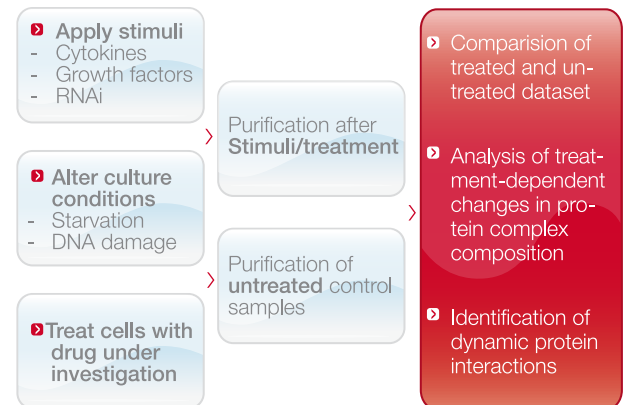
- Quick and comprehensive service — from cloning to data analysis in 12 weeks
- Unsurpassed sensitivity and specificity thanks to physiological expression levels and background filtering
- Identify treatment effects and analyze signalling dynamics in response to drug treatment or RNAi perturbation
- Multiplexing allows simultaneous analysis of multiple baits

Available cell lines

- HEK293 - Human embryonic kidney cells
- INS-1 - Beta pancreatic islet cells
- CHO - Chinese hamster ovary
- 3T3 - Primary mouse embryonic fibroblast cells cultured by the '3T3 protocol'

Please inquire for custom cell line generation!

Investigate stimuli-dependent changes in cellular pathways and protein complex composition



References

Glatter *et al.* (2009) **An integrated workflow for charting the human interaction proteome: insights into the PP2A system.** *Molecular Systems Biology* 5:237

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