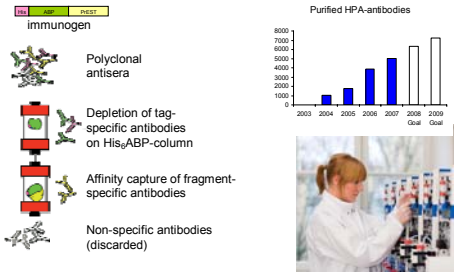
# Polyclonal Antibody Generation – Correlations With Immunogen Solubility

**Johanna Steen, Sophia Hober, Jenny Ottosson**
***Royal Institute of Technology, School of Biotechnology, Department of Proteomics, Albanova University Center, SE-106 91 Stockholm, Sweden***

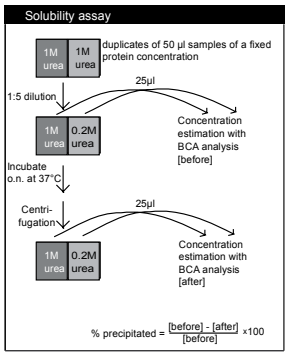
## Abstract
Within the Swedish Human Protein Atlas project polyclonal antibodies are generated in large scale against all human proteins for protein profiling in normal and cancer tissues. The immunogens are fragments of the human proteins expressed and produced recombinantly in *E. coli* and assayed routinely for solubility. In affinity-purifications of sera mono-specific antibodies are retrieved in up to mg/ml-range. With data from over five thousand affinity-purified antibodies, a positive correlation between solubility of the recombinant immunogen and acquired amount of antibody has been observed.

## Antibody generation



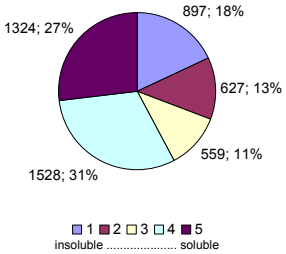

Antibodies are produced by immunisation of rabbits followed by affinity purification of the polyclonal antisera. Protein Epitope Signature Tags (PrEST, unique fragments of human proteins) produced recombinantly in fusion with a $His_6$ABP-tag (hexahistidine-Albumin Binding Protein) are used as the immunogen. Through a two-step affinity purification procedure monospecific antibodies to human proteins are acquired.

## Solubility assay

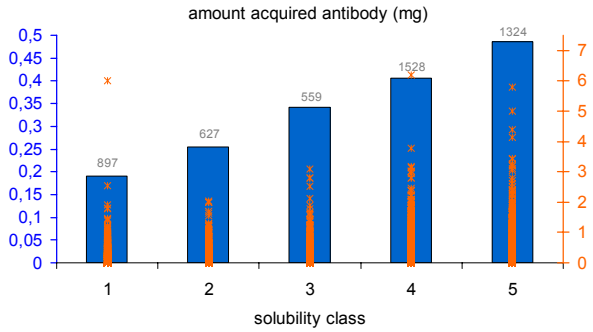



**Solubility class:** 1: 80-100%; 2: 60-80%; 3: 40-60%; 4: 20-40%; 5: 0-20% precipitated protein
**Ref:** Stenvall M et al. (2005) BBA, Proteins and Proteomics, 1752(1), 6-10

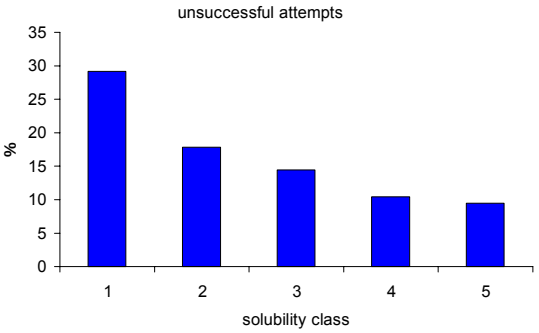
All $His_6$PrEST-proteins used as immunogens are routinely assayed for solubility in an assay developed in-house. The assay is carried out in a 96-well microtiter-format and allows for the high throughput capacity required. So far nearly 15000 samples have been assayed.




## Immunogen solubility and success of antibody generation

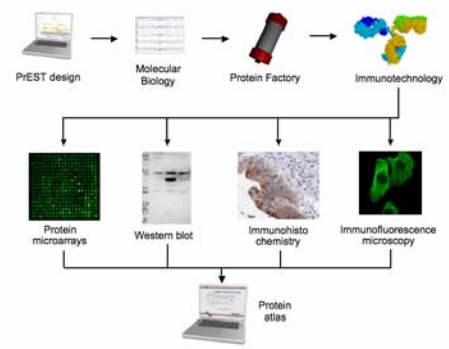



Amount of acquired affinity-purified antibody per solubility class. Average value (blue bars, left y-axis) as well as individual datapoints (orange marker, right y-axis). The number of datapoints within each solubility class is shown in grey.




Fraction of immunogens per solubility class which generated no affinity-purified antibody (amount acquired =0mg)

## Workflow of the Swedish Human Protein Atlas project




## Conclusions:
When correlating solubility data of the immunogens with the acquired amount of antibody after affinity purification purification we can see that the average amount is higher for the more soluble proteins. Furthermore, is the chance of acquiring affinity purified antibodies higher for the more soluble proteins as the fraction of sera that does not contain any antibodies after affinity purification is increased for the more insoluble immunogens. However, there is a large spread within the data sets for each solubility category. That is, the most insoluble proteins may give good amounts of affinity-purified antibody but the likelihood seems less.

## Acknowledgements:
The Swedish Human Protein Atlas programme is funded by grants from Knut and Alice Wallenberg Foundation.