8 Summary

Aim of this work was to develop a resource of *Arabidopsis thaliana* proteins as well as creating protein-microarrays for use of functional studies. Protein-microarrays were chosen as a base for these functional analyses. Besides macroarrays this method belongs to the array technologies. In both cases proteins are arrayed onto surfaced in systematic arrays. These technologies, as further enhancement of DNA array technologies, are enabling miniaturised and parallelised functional studies of proteins.
To receive the resource of *Arabidopsis thaliana*-proteins for this work, we generated two cDNA libraries, starting from mRNA of pistil and inflorescence.
The pistil cDNA-library consists of 660,800 clones in a GATEWAY compatible cloning vector and generates the base for further genome wide studies in different expression and analysis systems.
The inflorescence cDNA-library (ATM1) consists of 40,000 clones in a protein expression vector. This library was examined for protein expressing clones, using high-density protein-macroarrays. Based on this library we developed a protein expression sub-library. This sub-library consists of 5,000 protein expression clones. These clones were sequenced from the 5'-end. After extensive bioinformatics analysis we created from this expression subset a non redundant so-called unicloneset consisting of 1,502 clones. With this non-redundant set analyses are possible with the same information, as analyses with the redundant protein expression sub-library. This unicloneset (1,502 clones) was extended by 192 full length cDNA-clones for further analysis. From all 1,694 clones of the "extended unicloneset" proteins were expressed and purified in high-throughput.
The first functional study of the 1,700 *Arabidopsis*-proteins of the extended uniclone set, was the phosphorylisation by two *Arabidopsis* MAP-kinases using protein-microarrays. After establishing the first plant protein-microarray (Kersten, Feilner *et al*., 2003), we developed a proteomic method based on protein-microarrays (Kramer, Feilner *et al*., 2004). Based on this method, we established a kinase assay for *Arabidopsis* proteins. Therefore all 1,700 purified *Arabidopsis*-proteins were spotted onto nitrocellulose coated microarrays surfaces. These were used to establish a quantification system using protein kinase A (PKA), which enables a selection of potential phosphorylisation targets. Microarrays which were incubated with either

Arabidopsis MAP-Kinase 3 (MPK3) or 6 (MPK6) were analysed using this quantification system. In this study starting from ca. 1,700 Arabidopsis-proteins ca. 50 targets of MPK3 and ca. 40 targets of MPK6 were identified.

These results are a base for *in vivo* research of new identified targets and the established kinase assay can be used in particular for research of the extended unicloneset with other kinases as in general for studding further proteins, independent of origin.