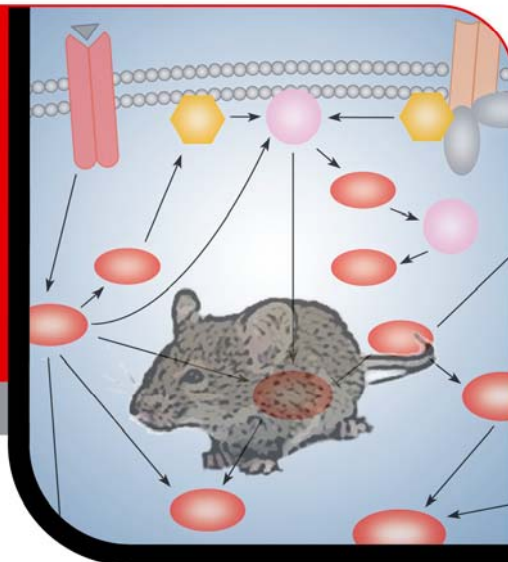


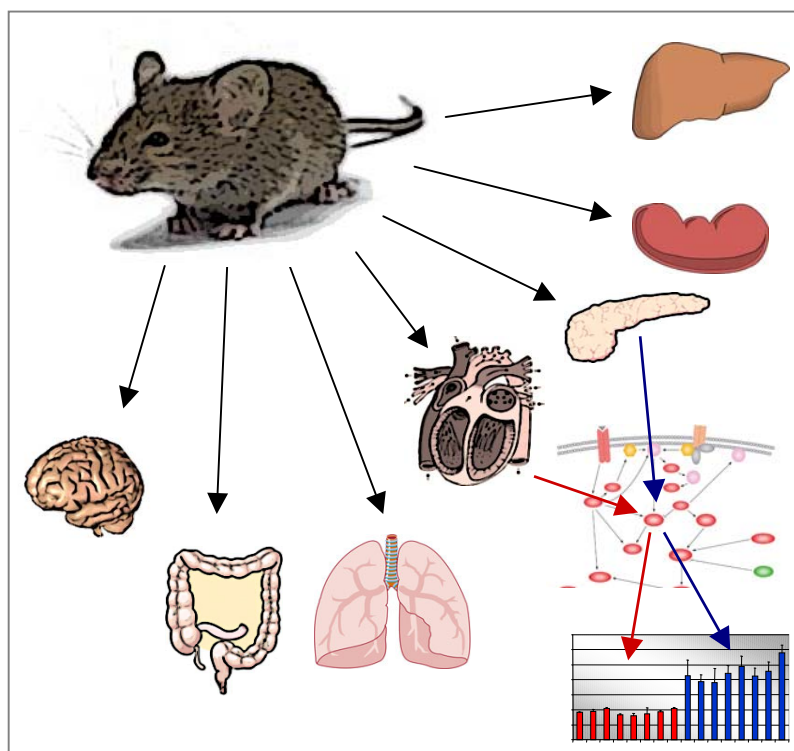
## ZeptoMARK Reverse Arrays Generation of a Pathway Activity Atlas



### Organ-Specific Pathway Activity Profiles

Mouse models are particularly useful to understand the effect of compounds in complex organisms and to gain new knowledge about molecular disease mechanisms. The treatment of an animal model system with compounds followed by analysis of relevant organs at the signaling pathway level will provide scientists with an excellent overview of the specific compound's activities in multiple cellular signaling pathways.

The creation of a pathway activity atlas for canonical signaling events would begin with the establishment of **expression levels** of proteins involved in signaling pathways in various organs or tissues.



The range of protein expression values within a group of animals belonging to the same mouse strain will provide a range of **normal values** for protein expression for the corresponding organs and tissues.

A second step would involve the measurement of the **activity levels** of signaling pathways, indicated by, for example, phospho- /non-phospho-ratios of pathway components.

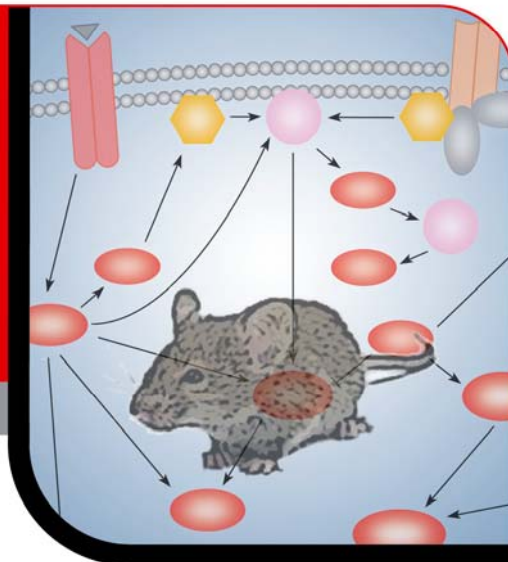
Having established "standardized" activity levels for pathways of specific mouse strains/models and organs, a true measurement of a compound's

effect is possible by comparing levels in treated mice with the standard levels.

To facilitate molecular target and biomarker discovery and validation, the standardized analysis of protein and pathway activity profiles for multiple inbred mouse strains provides a correlation between genetic variability, pathway activities and risk potential for disease.

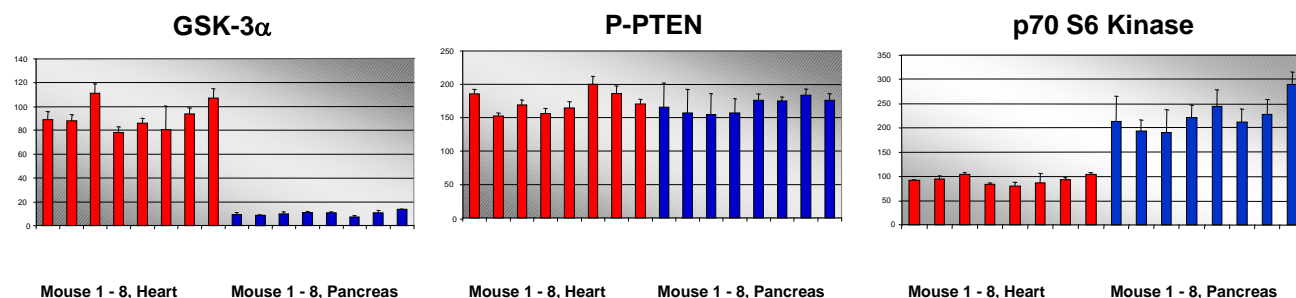
An extremely versatile, multi-target, low sample consumption and reproducible method is required for such analyses, which is provided by ZeptoMARK Reverse Arrays.

# ZeptoMARK Reverse Arrays Generation of a Pathway Activity Atlas



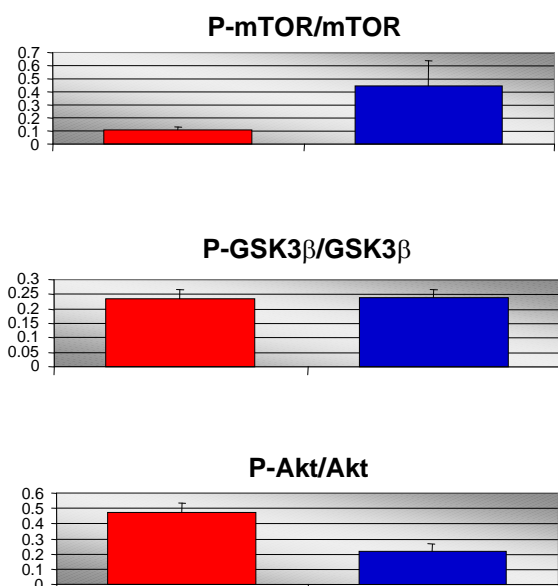
## Normal Distribution for Signaling Pathway Proteins in Mouse Pancreas and Heart Tissue

Eight different C57/B6 mice were dissected and the tissues were analyzed with ZeptoMARK Reverse Arrays. This system utilizes extremely small sample quantities for spotting - only 1 mg of tissue is necessary to measure hundreds of proteins and their modifications. Tissue from ten different mouse organs was investigated for 14 proteins and their respective 14 phosphorylated forms. The following graphs represent a small excerpt from the data which was generated, and clearly underline the very small extent in biological variation in expression levels of the proteins and their phosphorylated forms between the different mice.



The red bars show the results for the heart in the eight mice, whereas the blue bars represent the pancreas results for the same eight mice.

## Normal Distribution for Phospho- /Non-Phospho Ratio



The ratios of phospho- to non-phosphorylated proteins were calculated and as expected, the ratios between protein pairs are quite different between tissues. The small error bars from these results indicate the robustness of the approach for measuring the “standard signalome”. Treating mice with compounds and looking at the deviation from the “standard signalome” provides a clear indication about the effect of the compounds. Additionally, the comparison of standard levels of protein expression in different mouse models, or mouse species, could provide the researcher with new biomarkers.

Zeptosens is a full solution supplier of reverse array systems and services to create pathway activity atlases for study of animal disease models, molecular targets, biomarkers and organ toxicity.