

WP16: Gene based screens

Standardised manual or automated in situ hybridisation (ISH) protocols of sectioned or whole mount tissues were developed for in-depth molecular characterisation of novel mutant models, covering aspects of manual ISH, with both radioactive (³⁵S-UTP) and non-radioactive (digoxigenin) probes, and one SOP concerning automated ISH with non-radioactive probes. Two annexes to these SOPs have also been produced, with recommendations to work in RNase-free conditions and to optimise specificity and sensitivity of the procedures since manual section protocols are variable depending on tissue. This action has been coordinated with EUREXPRESS protocols. Four of these SOPs have been going through a cross-validation process between the ICS and GSF/IDG groups, analysing the expression patterns of three genes on adult and embryonic sections and cross-testing all steps from the preparation of samples, sectioning, probe synthesis and ISH with radioactive versus non-radioactive probes to test relative sensitivity (for the 3 genes selected, the two probes yielded similar results). The procedures provided in these SOPs have now been considered complete and standardised and can be provided to the scientific community.

Another project initiated this year is the generation of a EUMORPHIA mouse tissue bank. In conjunction with WP11, the NKI has generated a bank of both frozen and fixed tissues, consisting of tumour tissues and matched controls. These can be distributed as tissue microarrays and can be extended to other organ systems. WP11 has also compiled a 200-entry database of all commercially available Abs that work on fixed mouse tissue but could be extended to frozen samples to complement existing databases (NCI).