Requirements for Submission of Data to the HDBR Database.

We aim to make all gene expression data generated from HDBR material accessible and as widely available as possible, by uploading it to a public database.

Images are our preferred way to receive the data, although we can accept the original microscope slides where images are not available. Low power images should be at least 500x700 px and 72dpi. We can accept all standard image formats (.jpg, .bmp, .tif etc) as well as Leica .svs and .scn, and Zeiss .czi. If you have a more unusual format please contact us at <u>HDBR@ncl.ac.uk</u>.

There are several options for transferring the data to HDBR. Small datasets can be emailed to us at the address above. If the data is already online (Dropbox etc), please provide us with a link. For large datasets please contact us to discuss your requirements. Alternatively a USB stick/hard drive can be mailed to:

HDBR Newcastle University Biomedicine West Wing International Centre for Life Times Square Newcastle upon Tyne NE1 4EP

Guidelines for Submission of Data (images and slides)

The information we require is as follows:

- 1. Name and contact details of the person submitting the data, and of the PI of the project.
- 2. The gene or protein being detected.
- 3. The method used i.e. in situ hybridisation or immunohistochemistry.
- 4. The accession number and full nucleotide sequence of the probe, whether it is DNA or RNA, sense or antisense. For immunohistochemistry we need the supplier and catalogue number of the antibody, together with the species it was raised in.
- 5. The visualisation method used e.g. probes labelled with digoxigenin and visualised with NBT/BCIP, or secondary antibodies labelled with biotin and visualised with DAB.
- 6. Information about the specimen Carnegie stage, days post conception or developmental weeks.
- 7. Original data image(s).
- 8. A description of the staining pattern, in as much detail as possible.

Data can be entered into the database in two ways, either spatially mapped into a 3D context, or as a series of unmapped 2D images.

Guidelines for Quality of Images used in 3D data mapping:

The data will be spatially mapped to a representive digial 3D embryo of simiar stage to that which was used to generate the results. This data will be entered into a confidential local database that cannot be accessed by anyone other than HDBR staff. We will contact you once this has been completed and before uploading the data to the publicly accessible database to ensure we have your consent to share your data . It is very important that the sections/images being used to map data are of sufficient quality to allow the extraction of the data to be reliable. The image should be of sufficient size, magnification, contrast and brightness to see the expression clearly. The sections should also contain minimal, or preferably no, folds or tears as these create artefacts when the expression is thresholded.

Good

Quality

The sections below are two examples of the optimal data to be used for mapping purposes. The illumination is even and bright, the sections are completely intact while the slides are clearly devoid of debris and artefact. The staining is very strong and easily discernible from the background making the mapping process easier.



Unsuitable Section Data

The sections below are all examples data unsuitable for mapping (although they could be text annotated – see below). The section on the left is of a non-radioactive in situ experiment. The slide is very dirty with many artefacts, while the section itself has many folds and is very pale with little or no contrast between staining and background, making interpretation very difficult. The centre section, also non-radioactive in situ data, although clean, is over stained and the signal cannot easily be distinguished from the background. The radioactive in situ section on the right is folded over on itself and so will not be able to be mapped effectively. The folds in the tissue appear lighter than other areas and so may be incorrectly shown in the mapping process as signal data.



Guidelines for Submission of Images not to be mapped:

Individual images or series of images can also be submitted to the database without being mapped into a 3D context. In this case the images should be of good enough quality, size, contrast and brightness to allow the expression to be distinguished easily. As there is no spatial information from the 3D model it is important to provide an annotated copy of the experimental section, or an adjacent histologically stained section, for orientation and comparison with the gene expression.

Please complete the form below for each experiment.

Contact details:

Name of person submitting data	
HDBR project number	
email address	
Address	
Name of PI of project	
PI Address	
PI email address	

Gene details:

Gene name	
Gene symbol	

Experiment details:

In situ hybridisation (please tick)	Immunohistochemistry
Probe name	
Gene accession number : NM_	Gene name:
Probe extends from nucleotide	to nucleotide Additional details
DNA or RNA	

Probe labelled with	
Visualisation method	

Antibody name	
Supplier	
Catalogue number	
Species in which AB was raised	
AB labelled with	
Visualisation method	
Embryo No _CS_slide No_gene _AS/S	background (high, moderate, clear)
EXAMPLE: N225_CS22_PAX6_AS	Clear
(add rows as required)	

Description of the expected pattern of expression and any other comments:

Please give as much detail as possible. Our usual image file naming convention is: sample ID no._stage_slide number_section no._gene. For an in situ experiment we would include sense or antisense too eg 12345_CS23_100_3_PAX6_AS

If you require more information please email us at hdbr@ncl.ac.uk.