

Guide to completing the HDBR metadata spreadsheet.

Column A. Dataset name – please enter your HDBR project number e.g., **200123**.

Column B. Image name - the name of the file e.g. **11613,1,Embryo,CS23,171_arl3.ome.tiff**.

Column C. Source Name - the HDBR sample number, plus the slide and section numbers if known. e.g. **Embryo 11613, slide 171, section 2**.

	A	B	C
1	Dataset Name	Image Name	Source Name
2			

Columns D-F. For HDBR human samples these columns are always the same:

D	E	F
Characteristics [Organism]	Term Source 1 REF	Term Source 1 Accession
Homo sapiens	NCBITaxon	9606

D. Homo sapiens.

E. NCBITaxon.

F. 9606.

Column G. Please enter the stage of the sample e.g. CS19 or 12PCW.

Column H. Please enter the type of experiment, e.g. Immunohistochemistry, In Situ hybridisation, Immunofluorescence, RNAScope etc.

G	H
Characteristics [Developmental Stage]	Protocol REF

Columns I to Q are for the antibody details (if used). There are columns for 3 antibodies, please add more columns if necessary. If no antibodies were used, please leave blank.

I	J	K	L	M	N	O	P	Q
Experimental Condition [Antibody 1]	Antibody Identifier 1	Comment [Antibody 1]	Experimental Condition [Antibody 2]	Antibody Identifier 2	Comment [Antibody 2]	Experimental Condition [Antibody 3]	Antibody Identifier 3	Comment [Antibody 3]

Each antibody has 3 columns as follows:

- I. The name of the antibody e.g. ISL1.
- J. The accession number of the mRNA, e.g. NM_002202.
- K. The supplier and catalogue number of the antibody e.g. R&D Systems AF1837.

Columns R S and T are for the probe details. Please add more columns if there are more than 3 probes.

R	S	T
Comment [Probe1]	Comment [Probe2]	Comment [Probe3]

For a duplex RNAScope experiment the probes would be e.g.:

- R. ACDBio 410701
- S. ACDBio 312341-C2

Columns U-Z are for the gene details. Each gene needs its Ensembl identifier and official gene symbol, which can be found at <https://www.genenames.org/>. For example:

Comment [Gene Identifier 1]	Comment [Gene Symbol 1]	Comment [Gene Identifier 2]	Comment [Gene Symbol 2]	Comment [Gene Identifier 3]	Comment [Gene Symbol 3]

T. ENSG00000138175
U. ARL3

There are columns for 3 genes. Please add extra columns for more genes.

Column AA. Image file path. Please leave this blank. It will be completed by HDBR staff at the upload stage.

Comment [Image File Path]

Columns AB to AF – Fluorescent visualisation.

AB	AC	AD	AE	AF
Channels	Channel 1 colour and Min Max values	Channel 2 colour and Min Max values	Channel 3 colour and Min Max values	Channel 4 colour and Min Max values
e.g. 1 - DAPI; 2 - ISL1; 3 - HB9; 4 - SOX2	e.g. Blue 963 - 10834	e.g. Green 492 - 8929	e.g. Red 729 - 5197	e.g. Purple 1030 - 5874

AB. For fluorescent images please list the channels here. e.g. **1 - DAPI; 2 - ISL1; 3 - HB9; 4 - SOX2**

AC to AF. For each channel, please state the colour and the min-max histogram values. Please feel free to add further columns for more channels.

Column AG – Chromogenic visualisation.

AG
Visualisation Method

For brightfield images please state the chromogen e.g. **DAB**, **Fast red** etc.

Please use column AH to describe the staining pattern in as much detail as possible. For double labelled/multichannel images, please describe each expression pattern separately.

Expression Pattern Description

More information in this section allows us to annotate the images in greater detail and will help to improve the HDBR Gene Expression Portal (<https://portal.hdbratlas.org/>).

If you need any further guidance on completing this form, contact HDBR@ncl.ac.uk.

Thank you.