HDBR In-House Gene Expression Service (IHGES)

As a service to Registered Users, the HDBR will perform *in-situ* hybridisation or immunohistochemistry experiments on human embryonic/foetal material for a charge per gene/protein studied. Only one gene may be studied per registered project, all new projects are subject to a project registration fee.

Summary of the IHGES

1. Projects qualify for the IHGES, if they are registered with the HDBR and involve the analysis of gene expression patterns by *in situ* hybridisation or immunocytochemistry on human embryonic or foetal sections of HDBR origin.

2. HDBR core-funded staff will receive users' cDNA plasmids, cloned into an appropriate expression vector. Probes will be prepared and *in situ* hybridisation studies performed on the human developmental stages and tissues appropriate for the users' project.

3. The *in situ* hybridisation study will be performed in two parts: a pilot project to determine probe suitability (each probe tested will be charged separately) and the specificity and abundance of the detectable signal. If a specific signal is detectable, then a full study will be carried out. The HDBR will provide users with a limited number of electronic images showing the results of the pilot study, supplied in PDF format. The complete results of the full study will be provided via the web or on CD as appropriate for publication and/or other types of data presentation. In instances where no detectable signal can be detected, a project will be closed after the initial pilot study, following discussions with the user.

4. HDBR staff will assist users in the interpretation of their gene expression patterns in relation to embryonic/foetal anatomy, and to relevant literature studies provided by the user.

5. The HDR will consider immunocytochemistry projects on a strictly case by case basis. The charging scheme for each immunohistochemisrtry project is negotiable based upon the amount of work-up needed for each antibody used.

Users must agree to:

1. Provide DNA templates per gene in a form ready for transcription to prepare RNA probes. This can be either:

   cDNA fragments cloned into plasmid vectors (e.g. Bluescript) so that they are ready for linearization

   OR

   RNA polymerase tagged primers which can be used to generate DNA templates by PCR.

   The HDBR staff will use these DNA templates to generate digoxygenin labelled RNA probes using a T3, T7 or SP6 RNA polymerase

2. Aid in the preparation of a linearised template for RNA probe production by providing:

   (i) Restriction map of the DNA constructs, indicating cutting sites for commonly available restriction enzymes.
   (ii) Information on the orientation of the DNA inserts
   (iii) Computer generated restriction maps, using all the commonly available restriction enzymes of the sequence of the cDNA inserts.
   (iv) Any other information, such as conserved regions, coding regions, 5'UTR or 3'UTR, etc.
   (v) Full sequence of any primers sent.

3. Agree to HDBR staff sequencing the insert(s) for quality control purposes

4. Consult HDBR staff early in preparation of their plasmid vector(s) to ensure that compatibility issues do not delay the start of the project. In the event that HDBR encounters problems with a particular plasmid construct, it is the responsibility of the users to provide a different construct from the same gene.

5. Abide by the conditions of use of the HDBR (see Registration Form) as these also apply to the IHGES.

6. Agree to publication authorship (usually middle) to the member of HDBR lab personnel who carries out the gene expression studies. This applies only to papers directly reporting results of the study. Authorship for senior HDBR members is negotiable, depending on the level of their input into the project. Generally, we do not expect that senior HDBR members will be authors on most studies.

7. Pay for the costs of an initial pilot study prior to the HDBR commencing the IHGES project.

8. Agree to the publication of all results generated by this project to be included in a publicly accessible database following any publication arising from the IHGES or after 12 months from receiving the final project report whichever is the sooner.

The HDBR agrees to:

1. Keep all information provided by users strictly confidential throughout the course of the study.

2. Not to distribute the users DNA clones to any third party without the users explicit permission.

3. Carry out the *in situ* hybridisation and/or immunocytochemistry experiments to the best of its ability within a reasonable time-frame (to be agreed with users at the time of registration). HDBR cannot be held responsible for delays resulting from poor quality vector plasmids or incorrect information provided by users.

Stages in the progress of a typical* in situ *hybridisation project

1. The users provide the HDBR with at least 20µg purified, lyophilised plasmid DNA per probe *OR* at least 10µl of 100µM RNA polymerase binding sequence tagged primer. Usually DNA templates to generate 3 RNA probes will be tested initially.

   The purified plasmid DNA should reach the HDBR within 1 week of growing the culture. It is expected that plasmid DNA will be prepared using a commercial kit (e.g. Qiagen) or suitable technique to ensure that there is no contamination from protein or degradation of DNA. If a column based method is used the plasmid DNA should be eluted using water rather than buffer

   Further details of the design of the RNA polymerase binding sequence tagged primers should be sought from the HDBR prior to the user synthesising oligonucleotides.

   Receipt of the plasmid DNA or primers by the HDBR marks the start of the project.

2. Normally antisense (experimental) and sense (control) transcript probes will be prepared and compared in initial studies. A plasmid vector should be chosen to facilitate preparation of these probes.

3. A small number of slides will be used in a pilot experiment and, provided satisfactory results are obtained, the HDBR proceeds to perform *in situ* hybridisation on the requested range of human embryonic/foetal sections. Stages and tissues for analysis are as agreed at the time of project registration.
4. A limited number of representative results from hybridised sections are captured electronically and provided to users in the form of PDF images.

5. Follow-up studies are then planned using a verified probe from the pilot study. Following the pilot study, if further probes are required these will be tested at the pilot study rate of £400 per probe.

6. A main study will be performed looking at expression through varying stages of development as appropriate and with consultation between users and HDBR staff on interpretation of expression patterns.

7. Final images are prepared electronically for publication or other types of presentation and will be supplied either by CD or via the web. A final report of the project is sent to the user, making the completion of the project.

7. 12 months after the final report has been sent, the data is uploaded to a public database.

Charging scheme

Users are assumed to have already secured peer-reviewed grant funding to support their gene expression project. The HDBR will charge users for the cost of materials and for a proportion of the HDBR labour costs involved in the IHGES gene study. The rest of

the HDBR labour costs for the IHGES gene study and for the collection of material are core-funded.

The prices listed below apply to each study involving analysis of a SINGLE GENE.

All new project registrations – Project registration fee £300.

UK research organisations – Pilot study £400 per probe tested. Main study - £1250.

Non-UK research organisations will be charged at the same rate and should contact the Resource Manager at either hdbr@ncl.ac.uk or hdbr@ich.ucl.ac.uk. Please contact the Resource Manager if there are any problems with being invoiced in £ sterling.

Commercial organisations please contact the Resource Manager at either hdbr@ncl.ac.uk or hdbr@ich.ucl.ac.uk to inquire about feasibility and charging schemes.

A full study involves probe preparation, testing and in situ hybridisation OR immunocytochemistry to a maximum of 100 human embryonic/foetal slides. This equates to a 'standard' gene study that could involve the analysis of gene expression in approximately 3 different developmental stages or organs. This cost is likely to cover the entire scope of the research for most gene studies. Should further work be necessary, then this would be charged as a new gene study. If both in situ hybridisation and immunocytochemistry are requested then this will be charged as two separate gene studies.

MRC/Wellcome Trust funded Human Developmental Biology Resource

Application for In House Gene Expression Service (IHGES)

User Name :

Institution :

Please fill in the following for each gene separately:

1. The title of your HDBR registered project?

2. The name of the gene you intend to study?

3 The stages (range) of human embryonic development you would like to study.

4 The organs or anatomical regions that you are interested in.

5 Current knowledge about the expression of the gene. Please provide published and unpublished data.

6 If other IHGES gene studies have been requested for this registered project, please state the order of priority to be given to each gene study.

Application for IHGES continued.

Please provide the following information for each gene separately:

Gene name:	
Gene symbol	
Gene accession number:	
Probe coverage of accession number (nt-nt)	
Host species of antibody (IHC only)	
Probe Name(s) a b c	
Vector Name a b c	
Size of vector (kb) a b c	
Size of insert(s) (bp or kb) a b c	
Position of insert(s) a b c	
Total size (kb) of constructs a b c	
Enzyme(s) for linearization of plasmid a antisense : sense : b antisense : sense : c antisense : sense :	
Any special conditions required for plasmid digestion? a b c	
Full primer sequences a forward: reverse: b forward: reverse: c forward: reverse:	

RNA polymerase for RNA probe production a antisense : sense : b antisense : sense : c antisense : sense :	

Other information that must accompany your application:
(Please tick boxes to indicate items are enclosed)

☐ Restriction map of the clone(s)
- □ Restriction map as predicted by computer and/or
- □ Restriction map as produced by digestion experiments.

☐ Orientation of insert(s) within the vector.

☐ Others
- □ Positions of conserved motifs within the insert, if any.
- □ Positions of translated regions, if any.

Where did you hear about us
Please tick

MRC/Wellcome information	
HDBR Website	
HDBR Flyer	
Paper citation	
Conference	
Recommendation	
If other please specify	

Finance Details

This information is required for invoicing. Two separate purchase order numbers are required: An invoice will be sent when the gene study application is accepted and the pilot study will commence after payment is received. If a full study is requested an invoice for the additional fee will be sent and the full study will begin after payment is received.

Person or Company Name	
Full Address of Finance Dept.	
Finance Dept. Tel. no. **Fax. no.**	
Contact person (Finance Dept)	
Purchase Order no. Piolt Study	
Purchase Order no. Main Study	
To which gene does this purchase order refer:	
VAT registration no. (EU Countries only)	

The prices for a SINGLE GENE STUDY (research organisations or not-for-profit organisations):

Pilot study: **£400**

Full study: additional **£1250**

Non-UK research organisations will be charged at the same rate and should contact the Resource Manager at either hdbr@ncl.ac.uk or hdbr@ich.ucl.ac.uk.

If there are any problems with being invoiced in £ sterling, or if you are a commercial organisation, please contact the Resource Manager at either hdbr@ncl.ac.uk or hdbr@ich.ucl.ac.uk