EMBRYONIC PHENOTYPING AT ICS / MCI

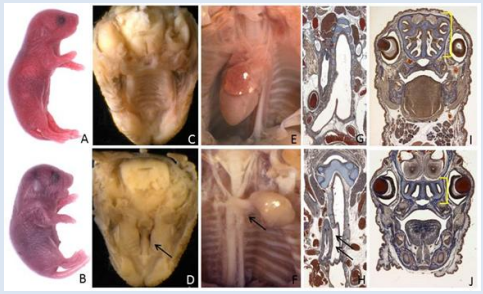
Olivia Wendling, Manuel Mark , Didier Hentsch, Jean-Luc Vonesch, Hugues Jacobs, Mohammed Selloum, Tania Sorg and Yann Hérault

Results from the EUMODIC program

	Statistics E18.5				Statistics E10.5			
Gene	HOM	TOTAL	HOM %	lethality	HOM	TOTAL	HOM %	lethality
Ube2b (ubiquitin-conjugating enzyme E2B)	2	7	**28**	neonatal				
Fabp3 (fatty acid binding protein3)	4	13	**30**	neonatal				
Fkbp10 (FK506 binding protein 10)	11	46	**23**	neonatal				
Orc4l (origin recognition complex, subunit 4)	2 (A)	18	**11**	neonatal + earlier	2 (a)	7	-	around or after E10.5
Sfrs4 (serine/arginine-rich splicing factor 4)	4	37	**11**	neonatal + earlier	2	5	-	
Jmjd5 (lysine (K)-specific demethylase 8)	4	64	**6**	neonatal + earlier	9 (b)	30	**30**	around E10.5
Fpgs (folylpolyglutamyl synthetase)	1	21	**5**	neonatal + earlier	0	10	-	
Ddx52 (DEAD (Asp-Glu-Ala-Asp) box polypeptide 52)	2	25	**8**	neonatal + earlier	0	9	-	
Ift20 (intraflagellar transport 20)	0	25	**0**	early lethality	3Ø	13	**23**	after E10.5 ?
Ino80 (INO80 homolog (S. cerevisiae))	0	42	**0**	early lethality	0	33	**0**	before E10.5 (d)
Mrpl10 (mitochondrial ribosomal protein 10)	0	47	**0**	early lethality	0	6	-	*increase stats*
Ift122 (intraflagellar transport 122)	0	20	**0**	early lethality	4 (e)	20	**20**	after E10.5
Eif2b5 (eukaryotic translation initiation factor 2B, subunit 5 epsilon)	0	19	**0**	early lethality	0	30	**0**	before E10.5 (f)
cops4 (COP9 (constitutive photomorphogenic) homolog, subunit 4)	0	25 (B)	**0**	early lethality	0	8	-	*increase stats*
Gar1 (Nola1) (GAR1 ribonucleoprotein homolog (yeast))	0	23	**0**	early lethality	0	13	-	before E10.5 (g)
Timm50 (translocase of inner mitochondrial membrane 50 homolog	0	24	**0**	early lethality	0	5	-	*increase stats*

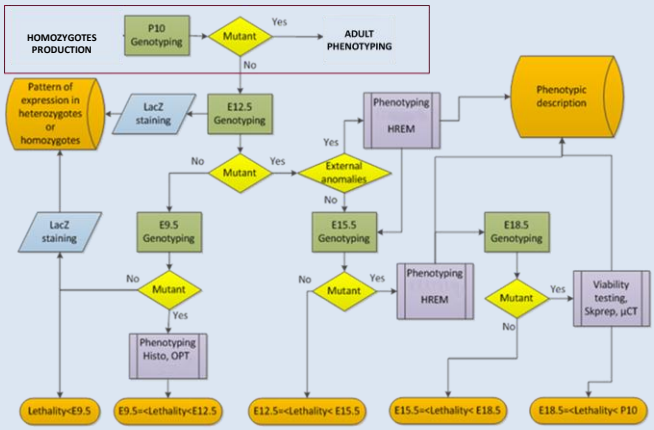
(A) no external abnormality
(B) 5 failed - genotyping issue

(a) external abnormalities on the two mutant embryos
(b) external abnormalities on 6 mutant embryos
© external abnormality on 1 mutant embryo
(d) 9 resorptions/under developped embryo with failed
(e) external abnormalities on 4 mutant embryos (publi
(f) 12 resorptions that could not be genotyped
(g) 11 resorptions that could not be genotyped



Abnormalities of *Jmjd5* knockout mice at stage E18.5. A, C, E, G, I: WT fetuses; B, D, F, H, J: mutant fetuses. A, B: external views of fetuses during the "viability test". C to F: photomicrographs of the palate and heart taken during necropsy. G to J: histological sections of the trachea and the nasal cavities. Jmjd5 knockout mice are growth-retarded. They are unable to breath and remain cyanotic. They display micrognathia, tracheal cartilage abnormalities, cleft palate, retro-oesophageal subclavian artery, and hypoplasia of ethmoid turbinates.

IMPC embryonic phenotyping



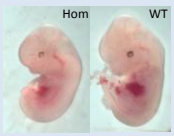
The DELPHIKOM project : Decoding Embryonic Lethal PHenotypes in Knock-Out Mouse

Operational workflow for determining the window of lethality (bottom) and for phenotyping embryonic lethal mouse lines . Histo: histological analysis; HREM: High Resolution Episcopic Microscopy; OPT: Optical Projection Tomography; µCT: micro-Computed Tomography; skprep: skeletal preparations

Current status of IMPC lines

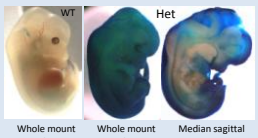
	Statistics E12,5		Statistics E15,5		Statistics E9,5	
Gene	HOM	TOTAL	HOM	TOTAL	HOM	TOTAL
Nxn1 (nucleoredoxin)- **ref line 1**	6	23	3	10		
Yipf5 (Yip1 domain family, member 5)	0	27	-	-		0
Ino 80	2	25				
Prdm10 (PR domain containing 10)	1	5				

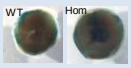
Nxn1 – Reference line

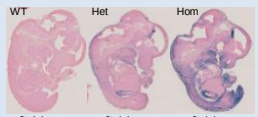


Gross morphology at stage E12.5
Micrognathia, frontonasal mass hypoplasia (not penetrant)

Gross morphology at stage E15.5
Abnormal body flexure, micrognathia

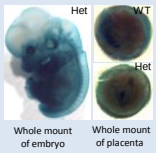




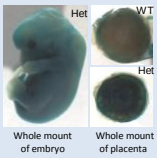


LacZ expression at stage E12,5

Yipf5 line



Ino 80 line

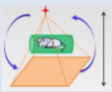


LacZ expression at stage E12.5

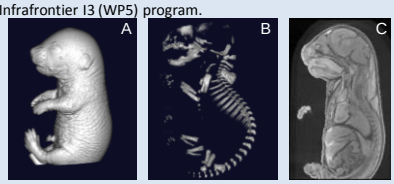
Implementing 3D imaging

X-Ray micro-computed tomography (micro-CT)

	In vivo	Ex vivo (skyscan 1172)
Specimen holder	Does not move	Moves
Scan duration	Fast: 17 sec (max 4min30)	Long
Picture reconstruction	1 min	15-90 min
Resolution	10-148 µm	1-25 µm
Doses	Low : 12-85 mGy	High
Field of view	10-76 mm	Max 50 mm



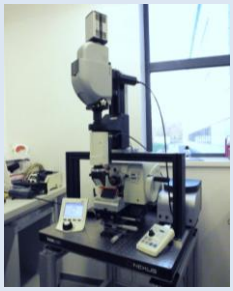
Micro-CT has become an option to image mouse embryos since protocols are available to provide contrast to soft tissues (e.g. Lugol). ICS/MCI has acquired a Quantum Fx µCT high-speed *in vivo* system for adult and embryonic phenotyping. The technology improvements are performed within the Infrafrontier I3 (WP5) program.



Scan settings
Energy: 90 kV, 160 µA
Scan time : 2 min
FOV: 20 mm
Resolution : 40 µm

Imaging of stage E18.5 fetuses. A, 3D display of the fetus ; B, 3D display of the skeleton (fetus fixed in formalin without contrast agent); C, 2D image display of a sagittal section (fetus fixed in formalin for 24h and immersed for one week in 100% Lugol)

HREM-C (High Resolution Episcopic Microscopy- Confocal)



HREM is an interesting alternative to histology. Dr Timothy Mohun, (MRC, London) who co-invented HREM, helped us in implementing the system at the ICS/MCI in cooperation with the IGBMC imaging center. Based on principles similar to HREM, our HREM-C system is aimed at replacing classical microscopy by confocal microscopy. The use of confocality will permit the achievement of optical sections delivering high contrast and low background images. It will also permit to increase the throughput, as optical sectioning will shorten physical sectioning.

ICS /MCI HREM-C device (available January 2015)

OPT (Optical Projection Tomography)

OPT is an interesting alternative to micro-CT for analyzing the anatomy of mouse embryos at stage E12 or younger. We have secured funding (Phenomin) to implement the technology at the ICS/MCI with the help of the IGBMC imaging center and Dr. Mark Henkelman, Toronto Center for Phenogenomics.

ICS /MCI OPT device (available September 2015)