The French Contribution to the IMPC

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The French National Infrastructure for Mouse PHENOMENOGENOMICS

M. Selloum, M.-C. Birling, G. Pavlovic, A. Ayadi, T. Sorg, Y. Herault
C. Olivier, S. Lerondel, P. Lopes, C. Fremond,
F. Fiore, B. Malissen,
WP1: Management

WP2: Mutagenesis

WP3: Zootechny

WP4: Phenotyping

WP5: Discover of mammalian genes function / Contribution to IMPC
<table>
<thead>
<tr>
<th>Step</th>
<th>Hours</th>
<th>Days</th>
<th>Resources</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ES Cells to Mouse</td>
<td>180</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>2. Mouse to Cohort</td>
<td>180</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>3. Cohort to Phenotype</td>
<td>250</td>
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<td>250</td>
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<tr>
<td>4. Archive and Distribute</td>
<td>180</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>5. Tech and Tools Dev</td>
<td>1,2,3</td>
<td>4</td>
<td>1,3</td>
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</tbody>
</table>
Cre recombinase

KO first allele (reporter-tagged insertion allele)

tm1a

tm1b (post-Cre)
QC and procedures from ES to cohorts

- Southern blot analysis for recombination confirmation as well as confirmation of the presence of the 3’ loxP site,
- Karyotyping of 2 validated clones
- Micro-injection in 50 BALB/cN blastocysts
- Establishment of chimeras
- Breeding of the 4 best chimeras (chimerism >45%)
- GLT assessment (tm1a alleles for cryo)
- Generation of tm1b KO allele (post-Cre)
- IVF quick expansion → Het (4 males and 8 females)
- Het x Het cross → Hom (4 males and 4 females)
- Hom X Het crosses: production of cohort, fertility and viability tests (n=4) Males and females
- 1 Cohort of control per week
Genetic Tools Dev.: Speeding up the Cre-Step with Fluo marker

$\text{Rosa26}^{\text{Cre-GFP/0}}$

$\text{Rosa26}^{\text{Flp-YPF/0}}$

B6N

M-C. Birling, G. Pavlovic
Genetic Tools Dev.: and Maternal Cre effect

Rosa26<sup>WT</sup>  

Rosa26<sup>Cre-GFP(FRT-Neo-FRT)</sup>  

Rosa26<sup>Cre-GFP(FRT)</sup>  

Rosa26<sup>FLP-YFP(LoxP-Neo-LoxP)</sup>  

M-C. Birling, G. Pavlovic
Weight Curve – 5wk to 16wk

In life
- Open Field
- Modified SHIRPA/dysmorph
- Rotarod
- Acoustic Startle/PPI
- Grip Strength
- Awake ECG/Echo
- Calorimetry
- Intraperitoneal Glucose Tolerance Test
- Challenge Whole Body Plethysmography
- Body Composition (lean/fat) QNMR
- Auditory Brain Stem Response
- Slit Lamp
- Ophthalmoscope

Terminal
- Multiplex analysis
  - Hematology
  - Clinical Blood Chemistry
  - Insulin Blood Level
  - FACspleen/BM
  - Organ and Heart Weight
  - Gross Pathology (7+7)
  - Block Banking
  - Histopathology (2+2)
  - X-ray (5+5)
  - Whole mount LacZ staining

7 M + 7 F Mutant Adult Mice
TECH DEVELOPMENT

Cardiovascular

Behavior and cognition

Nutrition, metabolism

Neuromuscular system

Embryology and Reproduction

Immunology and Inflammation

Respiration, allergy

Immune responses to pathogens

Imaging

Skeleton, Tooth and Craniofacial morphology

Cytometry by time-of-flight mass spectrometry

Electrophysiology

Follow gestation by micro-ultrasonography at E5 and E8 (count the deciduas)

No or few (less than 40%) resorptions

Collect fetuses at E18.5

Externally malformed fetuses
Fix 3 litters in Bouin’s solution
No mutant detected
Collect embryos at E10.5
Autopsy (Klotz) or Serial sections (Bouin)

Externally normal fetuses
Fix 3 litters in Klotz’s solution
Mutants detected
Genotyping
Collect embryos at E10.5

High level of resorptions

Collect embryos at E10.5

β-galactosidase expression by optical tomography
Fix 2 litters in paraformaldehyde
Mutants detected
Genotyping
Fix 2 litters in Bouin’s fluid

End of analysis
(Death must occur around implantation or gastrulation)

No mutant detected

Collect embryos at E10.5
Serial sections
Landmark and methodologies for craniofacial analysis

### A. LANDMARKS

<table>
<thead>
<tr>
<th>Right</th>
<th>Median</th>
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<th>Description</th>
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<tr>
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<td>Nasale</td>
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<td>Nasion</td>
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<td>3</td>
<td></td>
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<td>Bregma</td>
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<td>4</td>
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<td>Parietal-occipital junction</td>
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<td>5</td>
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<td>Midline of the interparietal-occipital junction</td>
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<td>6</td>
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<td>Dorsal midpoint of the foramen magnum</td>
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<tr>
<td>8</td>
<td>18</td>
<td>9</td>
<td>Dorsal-most point of the incisor alveolus</td>
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<td>19</td>
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<td>Parietal-premaxillary-maxillary junction</td>
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<tr>
<td>11</td>
<td>21</td>
<td>12</td>
<td>Anterior-most point of the zygomatic arch</td>
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<td>13</td>
<td>22</td>
<td>14</td>
<td>Posterior-most point of the zygomatic-maxillary suture</td>
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<td>23</td>
<td>16</td>
<td>Anterior-most point of the zygomatic-maxillary suture</td>
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<td>17</td>
<td>24</td>
<td>18</td>
<td>Posterior-most point of the zygomatic-maxillary suture</td>
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<td>20</td>
<td>26</td>
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<td>Tip of the post-temporal fossa</td>
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<td>27</td>
<td>23</td>
<td>Anterior-most point of the anterior palatine foramen</td>
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<td>25</td>
<td>Posterior-most point of the anterior palatine foramen</td>
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<td>31</td>
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<td>Anterior-most point of the premaxillary-maxillary and anterior palatine foramen junction</td>
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<td>30</td>
<td>32</td>
<td>31</td>
<td>Maxillary-most point of the first upper molar foramen</td>
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<td>32</td>
<td>33</td>
<td>33</td>
<td>Point of greatest curvature of the posterior margin of malar process</td>
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<td>34</td>
<td>Point of greatest curvature of the squamosal retroversus process</td>
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<td>37</td>
<td>35</td>
<td>Antero-medial projection of ectotympanic in basi-sphenoid</td>
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<tr>
<td>38</td>
<td>39</td>
<td>36</td>
<td>Ventral midpoint of the foramen magnum</td>
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### B. LANDMARKS

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L. Viriot, P. Lopes, E. Velot, S. Lerondel, Y. Herault
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Cardiovascular

Behavior and cognition

Nutrition, metabolism

Neuromuscular system

Embryology and Reproduction

Immunology and Inflammation

Respiration, allergy

Immune responses to pathogens

Imaging

Skeleton, Tooth and Craniofacial morphology

Cytometry by time-of-flight mass spectrometry

Electrophysiology

Lipopolysaccharide (LPS) challenge

Pulmonary and inflammatory Function (6h)

E. Dalloneau, J. Becker, Y. Herault
Lipopolysaccharide (LPS) challenge

Pulmonary airway resistance

WT NaCl
Prmt2+/− NaCl
WT LPS
Prmt2+/− LPS

[IL-6]

PG/ml

NaCl LPS

[TNF]

PG/ml

NaCl LPS

E. Dalloneau, J. Becker, Y. Herault (J. Immunol in press)
Profiling of the mouse innate and adaptive immune system under normal and confined (BSL3) conditions via the quantitative analysis of over 200 parameters.
Standard operating procedure for quantitative analysis of the lymphoid and myeloid cells present in small blood samples.

Standard operating procedure for quantitative analysis of dendritic cells.
REACHING THE COMMUNITY  > 1000 Lab Users

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http://transgenose.cnrs-orleans.fr/taam/presentation.php
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