Comparison of *in ovo* CRISPR/Cas9 and Embryonic Stem cell based strategies to produce germ-line modifications of the mouse genome.


Academic Year 2016 - 2017
PROGRAMME OF THE
MOUSE GENETICS COURSE

2016 - 2017

January 9 – February 3, 2017
(Project presentations: February 14, 2017)

The Course on Mouse Genetics will be at
The Institut Pasteur
Pavillon Louis Martin (Building 09)
28, rue du Docteur Roux
75724 PARIS Cedex 15

Theoretical training: Room n° 3 (Building 06)
Practical training: Ground floor - Pavillon Louis Martin (Building 09)
DESCRIPTION OF THE COURSE

The mouse has become the most powerful experimental system we have for mammalian research. The mouse had genetic and physiological systems and a metabolism that truly resembles the human condition. It is also a technically adept system, permitting facile, powerful experimentation. Finally, the mouse model is practically feasible, cheap and easily transported from one laboratory to the other. However working with mice requires specific knowledge. The Mouse Genetics course is an intensive laboratory and lecture course of five weeks. It is composed of lectures given by internationally renowned scientists. The Mouse Genetics course gives specific knowledge and explores the cutting edge of research in embryology, Mendelian and complex traits genetics, genomics and gene manipulation.

The maximum number of participants is 18, which includes a selected number of Master 2 students from the Universities of Paris 6, Paris 7 and Paris 11 and foreign postgraduate students in an interactive classroom setting. The course is intended to be a platform of excellence in which students can meet and closely interact with worldwide top-level scientists to discuss, exchange ideas and establish valuable contacts in the perspective of establishing a network of mouse geneticists at an early stage in their careers. Students will be able to understand the importance of basic research and of a broad interdisciplinary approach to contribute to human and animal health improvement. We also expect to provide orientations and mentoring to help students in their future career.

The 2016-2017 course includes a number of practical sessions, focused respectively on mouse anatomy and pathology, embryology, genetic mapping of Mendelian and complex traits, behavioral studies, manipulation of the mouse genome, and phenotypic characterization.

More specifically, the attendees will:

- Map a recessive mutation,
- Study the genetics of the predisposition to systemic lupus erythematosus, a complex trait,
- Analyze deep sequencing data,
- Search candidate genes in critical intervals,
- Manipulate embryonic stem (ES) cells,
- Produce induced pluripotent stem (iPS) cells from fibroblasts,
- Examine the behavior of inbred mice,
- Characterize phenotypes associated with limb anomalies,
- Use reporter genes to track the expression of targeted genes in cultured cells and embryos,
- Perform in vitro fertilization experiment.

These hands-on experiences in the training laboratory will be completed by a series of lectures dealing with emerging topics and resources in mouse genetics, such as the importance of miRNA-directed gene regulation, novel methods to analyze cell lineages in embryos and adult mice, the use of mouse genetic reference populations to integrate phenotypic data, large-scale ENU mutagenesis programs, sophisticated methods to generate conditional mutations, large-scale standardized and comprehensive phenotypic programs, the analysis of predisposition to metabolic or infection diseases, and mouse genome-wide association and systems genetics to identify main players of metabolism regulation.

At the end of each session students are expected to present and discuss their experiments. The final exam of the course consists in the design and presentation of an imaginary follow-up project based on the critical analysis of recent scientific articles. Students submit a written document (4 – 5 pages) and then discuss their project following a slide presentation. The final evaluation is based on an oral presentation, a report on the practical work sessions and the final exam.
Programme
- WEEK 1 -

THEORETICAL TRAINING: Formal Genetics; Mouse anatomy; The mouse genetic populations; Physiology of reproduction; Embryonic and adult stem cell; Mapping populations; The mechanisms of meiosis.

PRACTICAL TRAINING: Anatomy of rodents, necropsy of mutant mice; Pathology of rodents; Mapping a recessive mutation in the mouse; The analysis of behaviour in mice.

Monday January 9 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Instructor</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 - 10:30</td>
<td>Welcome and introduction to the course.</td>
<td>Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
<td></td>
</tr>
<tr>
<td>10:30 - 12:00</td>
<td>Theoretical training: Elements in formal genetics (part one).</td>
<td>Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
<td></td>
</tr>
<tr>
<td>13:30 - 14:00</td>
<td>Health and safety: rules.</td>
<td>Murielle ALMOUSSA (Institut Pasteur – Paris)</td>
<td></td>
</tr>
<tr>
<td>14:00 - 15:30</td>
<td>Theoretical training: The mouse and its reproduction.</td>
<td>Laurent TIRET (Ecole Nationale Vétérinaire d’Alfort Maisons-Alfort- France)</td>
<td></td>
</tr>
<tr>
<td>15:45 - 17:30</td>
<td>Theoretical training: The mouse genetic populations.</td>
<td>Xavier MONTAGUTELLI (Institut Pasteur - Paris)</td>
<td></td>
</tr>
</tbody>
</table>

Tuesday January 10 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Instructor</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-10:30</td>
<td>Theoretical training: Elements in formal genetics (part two).</td>
<td>Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
<td></td>
</tr>
<tr>
<td>10:45-12:30</td>
<td>Theoretical training: Mouse pathology.</td>
<td>Gregory JOUVION (Institut Pasteur - Paris)</td>
<td></td>
</tr>
<tr>
<td>13:15-17:00</td>
<td>Theoretical training: Anatomy of rodents. Necropsy of mutant mice.</td>
<td>Céline ROBERT (Ecole Nationale Vétérinaire d’Alfort Maisons-Alfort- France)</td>
<td></td>
</tr>
<tr>
<td>17:00-17:45</td>
<td>Presentation of the attendees (15’ talk, 5’ questions)</td>
<td>Sophia EVANS: &lt;Title : Not provided&gt;</td>
<td>Graciela RABADAN MORAES: Myeloproliferative diseases</td>
</tr>
</tbody>
</table>

Wednesday January 11 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Instructor</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-11:00</td>
<td>Theoretical training: Genetic analysis in the mouse</td>
<td>Xavier MONTAGUTELLI (Institut Pasteur - Paris)</td>
<td></td>
</tr>
<tr>
<td>11:15-12:30</td>
<td>Practical training 3: Mapping a recessive mutation in the mouse.</td>
<td>Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
<td></td>
</tr>
<tr>
<td>13:30-15:30</td>
<td>Theoretical training: The early development of the mouse embryo.</td>
<td>Jérôme ARTUS (Université Paris Sud —Inserm U935 Villejuif - France)</td>
<td></td>
</tr>
<tr>
<td>15:45-17:15</td>
<td>Theoretical training: Small animal optical in vivo imaging.</td>
<td>Régis TOURNEBIZE (Institut Pasteur - Paris)</td>
<td></td>
</tr>
</tbody>
</table>
### Mouse Genetics course 2016-2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>17:30-19:00</td>
<td>Presentation of the attendees (15' talk, 5' questions)</td>
</tr>
<tr>
<td></td>
<td><strong>Alix GOUPIL</strong>: Extra-centrosomes behavior in polyploid cells</td>
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<tr>
<td></td>
<td><strong>Vitalina VOLOCHTCHOUK</strong>: Analyse de la mutation hot-spot p53&lt;sup&gt;Y217C&lt;/sup&gt; chez la souris</td>
</tr>
<tr>
<td></td>
<td><strong>Nasim RAMEZANI</strong>: &lt;Title : not provided &gt;</td>
</tr>
<tr>
<td></td>
<td><strong>Julie CODZAMANIAN</strong>: Characterization of an epithelial prostatic population that is resistant to castration.</td>
</tr>
</tbody>
</table>

#### Thursday January 12 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-10:00</td>
<td><strong>Practical training 3</strong>: Mapping a recessive mutation in the mouse.</td>
</tr>
<tr>
<td></td>
<td>- Discussion on the mapping strategy of the patchwork mutation.</td>
</tr>
<tr>
<td>10:00-12:00</td>
<td><strong>Practical training 3</strong>: Mapping a recessive mutation in the mouse (step 1)</td>
</tr>
<tr>
<td></td>
<td>Microsatellites PCR amplification from DNA of backcross mice.</td>
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<tr>
<td></td>
<td>Agarose gel electrophoresis: preparation of the gels.</td>
</tr>
<tr>
<td>13:00-15:00</td>
<td><strong>Practical training 2</strong>: Pathology of rodents.</td>
</tr>
<tr>
<td></td>
<td>- Examination of histological virtual slides.</td>
</tr>
<tr>
<td>15:15-17:45</td>
<td><strong>Practical training 3</strong>: Mapping a recessive mutation in the mouse (step 1).</td>
</tr>
<tr>
<td></td>
<td>- Migration of the PCR products.</td>
</tr>
<tr>
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<td>- Analysis of mapping data using Gene-Link.</td>
</tr>
</tbody>
</table>

#### Friday January 13 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:45-11:15</td>
<td><strong>Theoretical training</strong>: The analysis of behaviour in mice.</td>
</tr>
<tr>
<td>11:15-11:45</td>
<td><strong>Practical training 4</strong>: Presentation of the alternate sessions of the afternoon.</td>
</tr>
<tr>
<td></td>
<td>Format of the data to be recorded.</td>
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</table>

**Afternoon**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:00-15:00</td>
<td><strong>Practical training 3</strong>: Mapping a recessive mutation in the mouse (step 2).</td>
</tr>
<tr>
<td>or</td>
<td></td>
</tr>
<tr>
<td>15:15-17:15</td>
<td>Microsatellites PCR amplification.</td>
</tr>
<tr>
<td></td>
<td>Agarose gel electrophoresis: preparation of the gels.</td>
</tr>
<tr>
<td>13:00-15:00</td>
<td><strong>Practical training 4</strong>: Behaviour analysis in inbred mice.</td>
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<tr>
<td>or</td>
<td></td>
</tr>
<tr>
<td>15:15-17:15</td>
<td>General monitoring and locomotion test (1&lt;sup&gt;st&lt;/sup&gt; session).</td>
</tr>
</tbody>
</table>
THEORETICAL TRAINING: The early development of the mouse embryo; The pluripotent stem cells; The embryonic and adult stem cells; Epigenetics and genomic imprinting; Cell lineage analysis; Techniques for rodent genome editing; Mouse mutagenesis; Optical in vivo imaging; Mouse behaviour analysis.

PRACTICAL TRAINING: Maintenance and differentiation of mouse ES cells; Reprogramming mouse fibroblast into iPS; Manipulation of Cre/loxP system and fluorescent proteins; Mapping of a recessive mutation using polymorphic markers; Behaviour analysis in the mouse.

Monday January 16 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Speaker/Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-10:30</td>
<td>Theoretical training: Transgenic models to study the regulation of gene expression.</td>
<td>Sophie VAULONT (Institut Cochin - INSERM U1016 CNRS UMR 8104 - Paris)</td>
</tr>
<tr>
<td>10:45-12:15</td>
<td>Theoretical training: Techniques for rat genome editing: lentiviral vectors and gene-specific nucleases.</td>
<td>Séverine REMY (Université de Nantes - France)</td>
</tr>
<tr>
<td>14:00-15:30</td>
<td>Visit to the museum and in the Louis Pasteur apartment and crypt.</td>
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</tr>
<tr>
<td>15:45-17:45</td>
<td>Practical training 3: Mapping a recessive mutation in the mouse (step 2).</td>
<td>Xavier MONTAGUTELLI Jean-Jacques PANTHIER Murielle ALMOUSSA (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>17:45-18:30</td>
<td>Presentation of the attendees (15' talk, 5' questions).</td>
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</tbody>
</table>

Tuesday January 17 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Speaker/Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-11:00</td>
<td>Theoretical training: Pluripotent stem cells, from the teratocarcinomas to the perspectives in regenerative medicine in man.</td>
<td>Michel COHEN-TANNOUDJI (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>11:15-12:45</td>
<td>Presentation of the attendees (15' talk, 5' questions).</td>
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<td></td>
<td>Sofia ARCHONTIDI: TCF12 roles in oligodendrogial development and oligodendrogliomas</td>
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<tr>
<td></td>
<td>Laura Nalleli GARRIDO CASTILLO: Inflammation, calcium signaling and prostate cancer</td>
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<tr>
<td></td>
<td>Sofia GAID: Deregulation of signaling pathway in medulloblastoma.</td>
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<tr>
<td></td>
<td>Ebrahim HASSAN: Influenza virus interaction with the human innate immune system.</td>
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</tr>
<tr>
<td>Afternoon</td>
<td>Practical training switching between genetic mapping and behaviour testing. (pairs 1 to 4 / pairs 5 to 9).</td>
<td></td>
</tr>
<tr>
<td>14:00-16:00</td>
<td>Practical training 3: Mapping a recessive mutation in the mouse (step 3).</td>
<td>Xavier MONTAGUTELLI Jean-Jacques PANTHIER Murielle ALMOUSSA (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>16:15-18:15</td>
<td>or Microsatellites PCR amplification.</td>
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<tr>
<td></td>
<td>- Agarose gel electrophoresis : preparation of the gels.</td>
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</tr>
</tbody>
</table>
### Mouse Genetics course 2016-2017

14:00-16:00 **Practical training 4**: Behaviour analysis in inbred mice.

or

16:15-18:15 General monitoring and locomotion test (2\textsuperscript{nd} session).

At 15:00 Superovulation: I.P. administration of Pregnant Mare Serum Gonadotrophin (PMSG) to female mice (for embryos at day 12 of development [E12] on February 1\textsuperscript{st}).

18:15-19:00 Presentation of the attendees (15’ talk, 5’ questions).

**Mylène BRUN**: The regulation of Notch2 in the Spleen Marginal Zone Lymphoma (SMZL).

**Romane DURAND**: Importance de la voie p53 dans les syndromes de dysfonctionnement télomérique.

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### Wednesday January 18 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Instructor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-10:00</td>
<td><strong>Practical training 3</strong>: Mapping a recessive mutation in the mouse (step 3). - Migration of PCR products.</td>
<td>Xavier MONTAGUTELLI, Jean-Jacques PANTHIER, Murielle ALMOUSSA (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>10:00-12:00</td>
<td><strong>Practical training 4</strong>: Behaviour analysis in inbred mice. - Statistical analysis and discussion.</td>
<td>Sylvie GRANON-CRESSANT (Université Paris Sud XI - CNRS UMR 8195 Orsay - France)</td>
</tr>
<tr>
<td>12:00-12:45</td>
<td><strong>Practical training 3</strong>: Mapping a recessive mutation in the mouse (step 3). - Photos of the agarose gels.</td>
<td>Xavier MONTAGUTELLI, Jean-Jacques PANTHIER, Murielle ALMOUSSA (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>13:30-14:00</td>
<td><strong>Practical training</strong>: Presentation of cell culture practices.</td>
<td>Muriel ALMOUSSA (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>14:00-19:00</td>
<td><strong>Practical training 5</strong>: Maintenance and differentiation of mouse ES cells. - Observation and splitting of reprogrammable mouse embryonic fibroblasts.</td>
<td>Michel COHEN-TANNOUDJI, Sandrine VANDORMAEL-POURNIN, Murielle ALMOUSSA (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td></td>
<td><strong>Practical training 6</strong>: Reprogramming mouse fibroblasts into iPS - Observation and splitting of reprogrammable mouse embryonic fibroblasts.</td>
<td>Jérôme ARTUS (Université Paris Sud - Inserm U935 Villejuif - France), Boris BARDOT (UMR 7147 CNRS/Institut Curie - Paris)</td>
</tr>
<tr>
<td></td>
<td><strong>Practical training 7</strong>: Manipulation of the Cre/loxP system and fluorescent proteins. - Transfection of a Cre expression plasmid into Flox Fluo HEK cells</td>
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<tr>
<td></td>
<td>At 15:00 Superovulation: I.P. administration of Pregnant Mare Serum Gonadotrophin (PMSG) to female mice (for embryos at day 11 of development [E11] on February 1\textsuperscript{st}).</td>
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### Thursday January 19 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Instructor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-10:30</td>
<td><strong>Practical training 3</strong>: Mapping a recessive mutation in the mouse (step 3). - Data collection and final analysis.</td>
<td>Xavier MONTAGUTELLI, Jean-Jacques PANTHIER, Murielle ALMOUSSA (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>10:45-12:45</td>
<td><strong>Theoretical training</strong>: “Genomic imprinting”</td>
<td>Déborah BOURC'HIS (INSERM U935, CNRS UMR 3212, Institut Curie - Paris)</td>
</tr>
<tr>
<td></td>
<td>At 13:00 Superovulation : IP administration of Human Chorionic Gonadotrophin (hCG) to female mice (E12) and and mating with Kit\textsuperscript{W-acl2\textsuperscript{+}}, Myf5\textsuperscript{lacZ\textsuperscript{+}}, Dct-lacZ\textsuperscript{+}, et Pax3\textsuperscript{a2cl2\textsuperscript{+}} males.</td>
<td></td>
</tr>
</tbody>
</table>
### Mouse Genetics course 2016-2017

13:30-15:30 **Theoretical training:** Conditional mutagenesis in the Mouse.  
Frédéric JAISSER  
(Centre de Recherche des Cordeliers, INSERM U.872 - Paris)

At 15:00  
Superovulation: I. P. administration of Pregnant Mare Serum Gonadotrophin (PMSG) to female mice (for embryos at day 10 of development [E10] on February 1st).

15:45-17:15 **Theoretical training:** Rodent models for studying addiction  
Morgane BESSON  
(Institut Pasteur - Paris)

17:30-19:00  
Presentation of the attendees (15' talk, 5' questions).

Sebastian GREGORICCHIO: Epigenetics in Acute Myeloid Leukemia (AML): role of Spi-1/PU.1 in the PcG complex modulation  
Laura MONIOT-PERRON: Hox gene clusters: 3D-organisation in fruit fly  
Mathilde DURA: Répression des transposons dans la gamétogenèse mâle chez la souris  
Tiphanie MÉRE: Investigation of key genes in typical and atypical human sex-determination

### Friday January 20 2017

9:00-10:30 **Theoretical training:** Mutagenesis and high throughput phenotyping of the mouse - in search of new genes and new gene functions.  
Sara WELLS  
(MRC Harwell - Oxford United Kingdom)

10:45-12:15 **Theoretical training:** The mouse mutagenesis programs using ENU and homologous recombination.  
Jean-Jacques PANTHIER  
(Institut Pasteur - Paris)

13:30-19:00 **Practical training 5:** Maintenance and differentiation of mouse ES cells  
- Differentiation of ES cells into embryoid bodies.  
- Clonal growth of ES cells in various culture conditions.  
Michel COHEN-TANNOUDJI  
Sandrine VANDORMAEL-POURNIN  
Murielle ALMOUSSA  
Jérôme ARTUS  
(Boris BARDOT  
(UMR 7147 CNRS/Institut Curie - Paris)

**Practical training 6:** Reprogramming mouse fibroblasts into iPSCs  
- Changing medium of reprogrammable mouse embryonic fibroblasts.  
Practical training 7: Manipulation of the Cre/loxP system and fluorescent proteins.  
- Flox Fluo HEK cell subculturing.

At 13:00  
Superovulation: IP administration of Human Chorionic Gonadotrophin (hCG) to female mice (E11) and mating with KitW<sup>Wt</sup>a<sup>alb</sup>W<sup>as</sup>, Myf5<sup>Wt</sup>a<sup>alb</sup>W<sup>as</sup>, Dct-lacZ<sup>+</sup>, and Pax3<sup>3md2</sup> males.

At 15:00  
Superovulation: I.P. administration of Pregnant Mare Serum Gonadotrophin (PMSG) to female mice (for embryos at day 9 of development [E9] on February 1st).

### Saturday January 21 2017

At 13:00  
Superovulation: IP administration of Human Chorionic Gonadotrophin (hCG) to female mice (E10) and mating with KitW<sup>Wt</sup>a<sup>alb</sup>W<sup>as</sup>, Myf5<sup>Wt</sup>a<sup>alb</sup>W<sup>as</sup>, Dct-lacZ<sup>+</sup>, Msx1<sup>lacZ</sup>, Msx2<sup>lacZ</sup> males.

### Sunday January 22 2017

At 13:00  
Superovulation: IP administration of Human Chorionic Gonadotrophin (hCG) to female mice (E9) and mating with KitW<sup>Wt</sup>a<sup>alb</sup>W<sup>as</sup>, Myf5<sup>Wt</sup>a<sup>alb</sup>W<sup>as</sup>, Dct-lacZ<sup>+</sup>, and Pax3<sup>3md2</sup> males.
Mouse Genetics course 2016-2017

Programme
- WEEK 3 -

THEORETICAL TRAINING: Conditional mutagenesis in the Mouse; Formal Genetics; Ethics and animal welfare; Multicolored transgenic mice; Optogenetics; Deep sequencing analysis; Phenotypic analysis in mice; Structural variant analysis in the mouse genome.

PRACTICAL TRAINING: Phenotypic analysis of skeleton mouse mutants; The use of reporter genes in mouse embryos; RNA interference and read-through; Observation of E9.5-E13.5 embryos; Manipulation of early embryos and in vitro fertilization; Maintenance and differentiation of mouse ES cells; Manipulation of Cre/loxP system and fluorescent proteins.

Monday January 23 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity Description</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-10:30</td>
<td>Theoretical training: Reflections on the Cre/loxP system.</td>
<td>Michel COHEN-TANNOUDJI (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>10:45-12:15</td>
<td>Theoretical training: Biogenesis of mammalian small RNAs.</td>
<td>Constance CIAUDO (Swiss Federal Institute of Technology Zurich - Switzerland)</td>
</tr>
<tr>
<td>13:30-15:00</td>
<td>Theoretical training: Characterizing regulatory sequences in the mouse genome : power and limits of genetics.</td>
<td>Benoît ROBERT (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>15:00-18:00</td>
<td>Practical training 8: Phenotypic analysis of skeleton mouse mutants Effects of mutations at Sonic hedgehog (Shh) and Extra-toes/Gli3 loci. - Dissection of foetuses at E14.5, fixation in 4% PFA. - Yolk sac sampling and freezing to genotype the foetuses.</td>
<td>Yvan LALLEMAND Benoît ROBERT Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>18:00</td>
<td>Practical training 11: Superovulation : IP administration of Pregnant Mare Serum Gonadotrophin (PMSG) to female mice (for the in vitro fertilization experiment on January 26th). Practical training: Change medium and observe ES/iPS/EB.</td>
<td></td>
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</table>

Tuesday January 24 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity Description</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-9:30</td>
<td>Practical training 8: Effects of mutations at Sonic hedgehog (Shh) and Extra-toes/Gli3 loci. - Post fix washing of the embryos in 1× PBS.</td>
<td>Yvan LALLEMAND Benoît ROBERT (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>9:30-11:00</td>
<td>Discussion: The scientific project for the examination.</td>
<td>Michel COHEN-TANNOUDJI, Jean-Jacques PANTHIER, Xavier MONTAGUETTELI (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>11:15-12:45</td>
<td>Theoretical training: The embryonic and adult stem cells.</td>
<td>Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>14:00-19:30</td>
<td>Practical training 5: Maintenance and differentiation of mouse ES cells - Plating embryod body for further differentiation. - Observation of various embryo-derived stem cell lines.</td>
<td>Michel COHEN-TANNOUDJI Sandrine VANDORMAEL-POURNIN Murielle ALMOUSSA Jérôme ARTUS Boris BARDOT (Institut Pasteur – Paris) Université Paris Sud - Inserm U935 - Villejuif - France UMR 7147 CNRS/Institut Curie - Paris</td>
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<td>Practical training 7: Manipulation of the Cre/loxP system and fluorescent proteins. - Fixation of Flox Fluo HEK cells and microscopic observation.</td>
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<td>Practical training 10: Observation of fluorescent proteins in Transgenic ovaries. - Dissection and observation at the stereomicroscope.</td>
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<td>Practical training 8: Effects of mutations at Sonic hedgehog (Shh) and Extra-toes/Gli3 loci. - Fixation of the embryos in 70% ethanol.</td>
<td>Yvan LALLEMAND Benoît ROBERT Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
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### Wednesday January 25 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Instructor(s)</th>
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</thead>
<tbody>
<tr>
<td>9:00-10:30</td>
<td><strong>Theoretical training</strong>: The mouse as a model for cancer studies: the oncogenes.</td>
<td>Boris BARDOT (UMR 7147 CNRS/Institut Curie - Paris)</td>
</tr>
<tr>
<td>10:45-12:15</td>
<td><strong>Theoretical training</strong>: The mouse as a model for cancer studies: the suppressor genes.</td>
<td>Franck TOLEDO (UMR 7147 CNRS/UPMC-Institut Curie - Paris)</td>
</tr>
<tr>
<td>13:15-18:30</td>
<td><strong>Practical training 8</strong>: Effects of mutations at Sonic hedgehog (Shh) and Extra-toes/Gli3 loci. - Alcian blue staining. - DNA extraction from the frozen foetuses. - PCR reaction using specific primers. - Migration of the PCR products and assessment of the genotypes.</td>
<td>Yvan LALLEMAND Benoît ROBERT (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>At 17:00</td>
<td>Superovulation: IP administration of Human Chorionic Gonadotrophin (hCG) to female mice and mating with males (in vitro fertilization experiment).</td>
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### Thursday January 26 2017

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<thead>
<tr>
<th>Time</th>
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<th>Instructor(s)</th>
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<tbody>
<tr>
<td>9:00-12:00</td>
<td><strong>Practical training 11</strong>: In vitro fertilization. - Collection of sperm and artificial capacitation. - Collection of oocytes. - in vitro fertilization.</td>
<td>Franck BOURGADE Angélique VINCENT Jean JAUBERT (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>12:00-12:30</td>
<td><strong>Practical training 8</strong>: Effects of mutations at Sonic hedgehog (Shh) and Extra-toes/Gli3 loci. Washing of the embryo in 70% Ethanol, 1% HCl.</td>
<td>Yvan LALLEMAND Benoît ROBERT Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>13:30-15:00</td>
<td><strong>Theoretical training</strong>: DeepSeq data analysis</td>
<td>Christophe ANTONIEWSKI (Drosophila Genetics and Epigenetics Université Pierre et Marie Curie - Paris)</td>
</tr>
<tr>
<td>15:00-17:30</td>
<td><strong>Practical training 11</strong>: In vitro fertilization. - Washing and culture of the fertilized mouse eggs</td>
<td>Franck BOURGADE Angélique VINCENT Jean JAUBERT (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>17:30-18:00</td>
<td><strong>Practical training 8</strong>: Effects of mutations at Sonic hedgehog (Shh) and Extra-toes/Gli3 loci. Transfer embryos in 0.1% NaOH.</td>
<td>Yvan LALLEMAND Benoît ROBERT (Institut Pasteur - Paris)</td>
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### Friday January 27 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Instructor(s)</th>
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</thead>
<tbody>
<tr>
<td>9:00-11:00</td>
<td><strong>Theoretical training</strong>: Structural variant analysis in the mouse genome.</td>
<td>Binnaz YALCIN (University of Lausanne - Lausanne - Suisse)</td>
</tr>
<tr>
<td>11:00-12:30</td>
<td><strong>Theoretical training</strong>: The mouse genetic reference populations.</td>
<td>Xavier MONTAGUTELLI (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>13:30-15:30</td>
<td><strong>Theoretical training</strong>: Introduction to Quantitative Genetics.</td>
<td>Xavier MONTAGUTELLI (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>15:45-16:00</td>
<td><strong>Practical training 8</strong>: Effects of mutations at Sonic hedgehog (Shh) and Extra-toes/Gli3 loci. Transfer embryos in 0.1% NaOH.</td>
<td>Yvan LALLEMAND Benoît ROBERT Jean-Jacques PANTHIER (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td>16:00-17:30</td>
<td><strong>Practical training 11</strong>: In vitro fertilization. Mouse eggs after fertilization: observation at the 2-cell stage, and further in vitro culture to the blastocyst stage.</td>
<td>Franck BOURGADE Angélique VINCENT Jean JAUBERT (Institut Pasteur - Paris)</td>
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<tr>
<td>Time</td>
<td>Activity</td>
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| 17:30-18:00 | **Practical training 8**: Effects of mutations at *Sonic hedgehog*  
*Shh* and *Extra-toes/Gli3* loci.  
Transfer embryos in 0.1% NaOH. |
| 18:00    | **Practical training**: Change medium and observe ES/iPS/EB.             |
THEORETICAL TRAINING: Genetic analysis of complex traits; Mammalian small RNAs; The mouse as a model for cancer studies; Modifiers of phenotype; Systems genetics of metabolism; Host genetic control of infectious diseases.

PRACTICAL TRAINING: Examples of genetic analyses of complex traits; RNA interference and read-through; Web data mining; Maintenance and differentiation of mouse ES cells; Reprogramming mouse fibroblast into iPS; Intestinal organoid culture.

Monday January 30 2017

9:00-11:00 Theoretical training: Transgenic and optical approaches to image cells in situ: application to brain structures and development.
Jean LIVET
(Institut de la vision - Paris)

11:00-12:30 Practical training 11: In vitro fertilization.
Observation the blastocysts obtained by in vitro fertilization.
Non-surgical embryo transfer.
Franck BOURGADE
Angélique VINCENT
Jean JAUBERT
(Institut Pasteur - Paris)

13:30-19:00 Practical training 12: Intestinal organoid culture.
- Intestinal crypt isolation and dissociation;
- Plating in matrigel with growth factors
Michel COHEN-TANNOUDJI
Aurélien RAVEUX
Aline STEDMAN
Béatrice ROMAGNOLO
Silvia FRE
Guillaume JACQUEMIN
(Institut Pasteur - Paris)

Tuesday January 31 2017

9:00-12:30 Practical training 13: Genetic predisposition to systemic lupus erythematosus: analysis of crosses between inbred strains of mice.
Xavier MONTAGUTELLI
(Institut Pasteur - Paris)

13:45-18:00 Practical training 5: Maintenance and differentiation of mouse ES cells.
- Observation of differentiated embryoid bodies
- Alkaline phosphatase staining of ES cell clones.
Michel COHEN-TANNOUDJI
Sandrine VANDORMAEL-POURNIN
Murielle ALMOUSSA
(Institut Pasteur - Paris)
Jérôme ARTUS
(Université Paris Sud - Inserm U935 Villejuif - France)
Boris BARDOT
(UMR 7147 CNRS/Institut Curie - Paris)

Wednesday February 1 2017

9:00-12:30 Practical training 9: The use of lacZ and GFP reporter genes in embryology.
- Isolation of the embryos and foetuses at E9, E10, E11 and E12 stages
- Fixation and X-Gal staining.
Jean-Jacques PANTHIER
(Institut Pasteur - Paris)

13:30-18:00 Practical training 14: Data mining in websites.
- Critical search for information in genetic databases.
- Identification of candidate genes for the patchwork mutation using the genetic mapping data.
- The use of synten homologies between mouse and man.
Jean JAUBERT
(Institut Pasteur - Paris)
## Mouse Genetics course 2016-2017

### Thursday February 2 2017

<table>
<thead>
<tr>
<th>Time</th>
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<th>Instructor</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>9:00-10:45</td>
<td><strong>Theoretical training:</strong> Cell lineage analysis methods in the mouse.</td>
<td>Sigolène MEILHAC</td>
<td>Institut Pasteur - Paris</td>
</tr>
<tr>
<td>11:00-13:00</td>
<td><strong>Practical training 9:</strong> The use of lacZ and GFP reporter genes in embryology</td>
<td>Jean-Jacques PANTHIER</td>
<td>Institut Pasteur - Paris</td>
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<td></td>
<td>Observation and description of the X-Gal staining in wild-type and mutant embryos.</td>
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<tr>
<td>14:00-16:30</td>
<td><strong>Practical training:</strong> DeepSeq data analysis.</td>
<td>Christophe ANTONIEWSKI</td>
<td>Drosophila Genetics and Epigenetics</td>
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<td>Université Pierre et Marie Curie - Paris</td>
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<tr>
<td>16:45-18h30</td>
<td><strong>Practical training 8:</strong> Effects of mutations at Sonic hedgehog (Shh) and Extra-toes/Gli3 loci. Data analysis, final discussion.</td>
<td>Yvan LALLEMAND Benoît ROBERT</td>
<td>Institut Pasteur - Paris</td>
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<tr>
<td>18:00</td>
<td><strong>Practical training 4:</strong> Change medium and observe minigut organoid.</td>
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### Friday February 3 2017

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<tr>
<th>Time</th>
<th>Session</th>
<th>Instructor</th>
<th>Location</th>
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<tbody>
<tr>
<td>9:00-10:30</td>
<td>Hercule Poirot in mouse genetics</td>
<td>Jean-Jacques PANTHIER Michel COHEN-TANNOUDJI Xavier MONTAGUTELLI</td>
<td>Institut Pasteur - Paris</td>
</tr>
<tr>
<td>10:45-12:15</td>
<td><strong>Theoretical training:</strong> Systems Genetics of Metabolism.</td>
<td>Ewan WILLIAMS</td>
<td>ETH Zürich - Switzerland</td>
</tr>
<tr>
<td>13:30-14:30</td>
<td><strong>Practical training:</strong> Observe minigut organoid.</td>
<td>Michel COHEN-TANNOUDJI</td>
<td>Institut Pasteur - Paris</td>
</tr>
<tr>
<td>14:30-16:00</td>
<td><strong>Theoretical training:</strong> Ethics and animal welfare.</td>
<td>Xavier MONTAGUTELLI</td>
<td>Institut Pasteur - Paris</td>
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<tr>
<td>16:00</td>
<td>Farewell snack: cakes, petit fours and drinks.</td>
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## Programme
- **WEEK 5** -

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<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Activity</th>
<th>Instructor/Coordinator</th>
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<tbody>
<tr>
<td><strong>Thursday February 9 2017</strong></td>
<td>9:30-12:00</td>
<td>Practical training 15: Visit of the Mouse Genetics Engineering Center. Pairs 1, 2, 3, 4 and 5.</td>
<td>Francina LANGA-VIVES (Institut Pasteur - Paris)</td>
</tr>
<tr>
<td><strong>Friday February 10 2017</strong></td>
<td>9:30-12:00</td>
<td>Practical training 15: Visit of the Mouse Genetics Engineering Center. Pairs 6, 7, 8 and 9.</td>
<td>Francina LANGA-VIVES (Institut Pasteur - Paris)</td>
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<tr>
<td><strong>Tuesday February 14 2017</strong></td>
<td>9:00-18:00</td>
<td>Oral examination: The presentation should last 13 minutes, followed by 7 minutes for questions.</td>
<td>JURY:</td>
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<td>Jérôme ARTUS, Université Paris-Sud</td>
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<td>Benoît ROBERT, Institut Pasteur</td>
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<td>Sylvie GRANON-CRESSANT, Université Paris-Sud</td>
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<td>Franck TOLEDO, Université Paris 6</td>
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<td>Alexis LALOUETTE, Université Paris 7</td>
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<td>Laurent TIRET, Ecole Nationale Vétérinaire d’Alfort</td>
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<td>Denis HOUZELSTEIN, CNRS</td>
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<td>Michel COHEN-TANNOUDJI, Institut Pasteur</td>
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<td>Xavier MONTAGUTELLI, Institut Pasteur</td>
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<td>Jean-Jacques PANTHIER, Institut Pasteur et Ecole Nationale Vétérinaire d’Alfort</td>
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ORGANIZATION OF EXAMINATIONS

- **First oral exam**
  Presentation of the past and current scientific project
  The presentation should last 15 mn, followed by approximately 10 to 15 minutes for questions
  Mark on a 1-20 scale, coefficient 1

- **Continuous student assessment during the practical training**
  Based on the results and conclusions of the experiments as presented in the workbook.
  Mark on a 1-20 scale, coefficient 2

- **Final written and oral examination**
  Presentation of an imaginary 3-year research project
  
  o **Written project**
  Fictional project in a 4/5 page document that should be submitted to the members of the jury no later than Friday 8th at 3:00 pm.
  This document should include:
  - Project title, and 5 key-words
  - Introduction and Objectives (max 1 page)
  - Working hypothesis (max 1 page).
  - Description of the project (max 2 pages). The planned experiments should be presented in a logical order. It is not necessary to give a detailed description of the methods and techniques.
  - A short justification of the chosen methods and techniques will be appreciated.
  - Expected results and impact (1/2 page)
  - Appendices:
    - Most significant state of art references
    - Figures
  Concerning the number of references, take into account that a research project is not a review, thus it is not meant to be exhaustive. The project incorporates knowledge acquired in the Mouse Genetics Course. The project is only limited by your imagination, therefore all the technical and scientific means are available.

  o **Organization of the oral presentation**
  The presentation is open to the public.
  Slides (Powerpoint or other supported format).
  The presentation should last 13 minutes, followed by 7 minutes for questions.
  The Final oral and written examination will be marked on a 1-20 scale, coefficient 7.