Lecture c+10

Genetic engineering for neuroscience
Making the brain fabulous

Lichtman Lab
Proteins to record neural activity

GCaMP

FRET in Cameleon Calcium Ion Indicators

Figure 1
Proteins to control neural activity

Fiberoptic Control of Locomotion in ChR2 Mouse
Protein synthesis – central dogma
Expressing artificial proteins

- Microinjection
- Viral Vectors
- Lipofection
- Electroporation

Nucleus

Cell Membrane
Creating animals with artificial genes
Central Dogma of Biology
Promoter + Transcription factors determine when/where genes are expressed
Homologous recombination

- Homologous chromosomes
- Sister chromatids

Exchange by recombination

Double-strand break

Resection

3' 5' 3' 5'

Stand invasion, D-loop formation, DNA synthesis

DSBR

SDSA

Second end capture, DNA synthesis, Ligation

Branch migration, Resolution

Non-crossover (uncommon)

or

Crossover (common)

Strand displacement annealing

DNA synthesis, Ligation

Non-crossover
Introducing a transgene

Electroporation

Homologous recombination

Gene of interest

Selection with resistance marker
Further inbreeding leads to homozygous (double KO)
Foster mother

Chimera mouse  Normal mouse

Chimera mouse  Normal mouse

Heterozygous for gene knock out  Normal mouse  Normal mouse

Breeding to produce a mouse homozygous for the gene knockout
Tissue specific expression

• Find genes that are expressed in specific brain areas.

• Design DNA constructs that will be integrated under the same promoter.
The internet:

mouse.brain-map.org
Thy1 vs RGS9
IER5 vs. TCF7L2
Transgenic animals

Generating transgenic animals can take many months or longer...
CRE – recombinase
Cre-LoxP system

LoxP sequence bound by Cre

[Diagram showing translocation, deletion, and inversion processes involving Cre-loxP interactions]

Jackson Laboratory
Cre-dependent expression

Promoter IoxP Stop IoxP Gene OI

+ Cre

Promoter IoxP Gene OI

Promoter IoxP Gene OI IoxP

+ Cre

Stop IoxP

IoxP IoxP gene OI
Reversible Cre-mediated recombination

Permanent Cre-mediated lox site excision
Cre-expressing cell + floxed viral construct

Cre-expressing

Non-Cre expressing

Infect

Cre expressing
Active gene

Non-Cre expressing
Inactive gene
Eg. Thy-1 Cre

jaxmice.jax.org/strain/006143.html
The Brainbow
RGB colorspace

Three fluorophores can encode many colors
Brainbow 1.0 & 1.1

a) XFP combinations

<table>
<thead>
<tr>
<th>Outcome for each copy</th>
<th>Resulting colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>C C C</td>
<td>Blue</td>
</tr>
<tr>
<td>C C Y</td>
<td>Light blue</td>
</tr>
<tr>
<td>C Y Y</td>
<td>Blue-green</td>
</tr>
<tr>
<td>Y Y Y</td>
<td>Green</td>
</tr>
<tr>
<td>Y Y H</td>
<td>Light green</td>
</tr>
<tr>
<td>Y Y R</td>
<td>Orange</td>
</tr>
<tr>
<td>Y R R</td>
<td>Red</td>
</tr>
<tr>
<td>R R R</td>
<td>Magenta</td>
</tr>
<tr>
<td>R R C</td>
<td>Purple</td>
</tr>
<tr>
<td>R C C</td>
<td>Grey</td>
</tr>
</tbody>
</table>

b) Oculomotor nerve

c) Dentate gyrus
• Today:
  – Genetic engineering to target protein expression by cell type (and/or brain region)

• Next time:
  – 3d imaging in the brain (mulit-photon microscopy)
  – Neural circuit dynamics (synaptic plasticity)