Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system

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Drawing circuit diagrams is hard.

Golgi stain

GFP

Serial electron microscopy
“Wouldn’t it be nice if...”
Cre/lox can excise or invert

Incompatible Lox sites give distinct recombinations:

- **LoxP**: ATAACTTTCGTATA GCATACAT TATACGAAGTTAT
- **Lox2272**: ATAACTTTCGTATA GGATACTT TATACGAAGTTAT
- **LoxN**: ATAACTTTCGTATA GTATACCT TATACGAAGTTAT
1.0: Two possible excisions
1.1: Three possible excisions
2.0: One possible inversion
2.1: Putting it all together

c Brainbow-2.1

Construct

Promoter (CMV/Thy1)

Transient Cre recombination

Outcomes

Test in vitro

No Cre

+ Cre

[Diagram showing gene expression and recombination processes]
Transiently inducing Cre in vivo

- Force neurons to choose one color and stick with it -- for time lapse, etc
- Cross with CreERT2 animals: Cre fused to estrogen receptor, tamoxifen inducible
- Dose animals with tamoxifien once
Multiple constructs give a whole spectrum of colors

Copy number = 8 in one strain, 16 in another

Estimate: 89 colors (visual) 166 colors (computer)
Forcing single-copy eliminates the rainbow

- Flp/FRT excises extra copies
- Single colors only
- Also possible: loss of inter-copy recombination
Circuit mapping!

- Quantify # of cells synapsing onto a given cell
- Color throughout neuron is nearly constant; good enough for computer tracing
Contributions

- Randomly express one of up to four genes (scalable to more?)
- If many copies of construct, combinatorial expression → fill color space
- Works with transient or chronic recombinase expression
- Neural circuit tracing (and glial territory mapping)
- Other applications / variations?