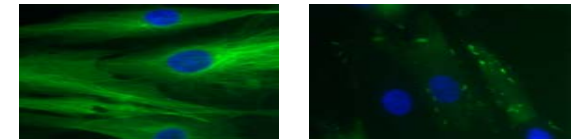


Living Molecular Probes for the next generation

George T. Hanson, Rob Batchelor, Dan Beacham, Magnus Persmark, and Michael O'Grady
 Molecular Probes - Invitrogen • 29851 Willow Creek Road • Eugene, Oregon 97402 • USA



Primary human cells treated with vinblastin, an anti-mitotic drug used to treat cancer

Introduction

For over three decades Molecular Probes™ has been the leader in fluorescence technologies, mainly focused on small molecule, organic dyes. Alexa Fluor® and BODIPY® dyes along with ion indicator dyes such as fluo-4 have been the industry standard. With researchers moving more into live cell studies and in physiologically relevant cell types, such as stem cells and primary cells, there is a demand for reagents that deliver content in a non-invasive way. We have combined the content of auto-fluorescent proteins with the “BacMam” delivery technology to create Organelle and Cellular Lights™ reagents.

Figure 1 – BacMam Technology

The “BacMam” gene delivery technology is simply the use of the insect cell virus, baculovirus to deliver genes to mammalian cells^{1,2}.

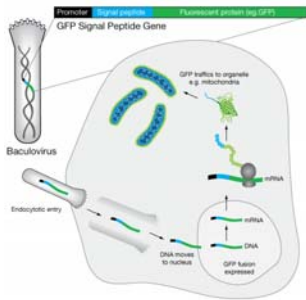


Figure 1. Schematic diagram of BacMam delivery of Organelle Lights™ mito-GFP.

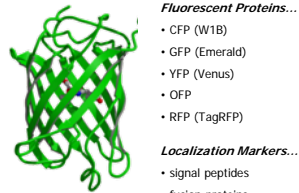
BacMam Applications Include:

- Transduction of primary and stem cells
- Protein production in mammalian cells
- HTS for GPCRs, ion channels, nuclear receptors and transporters
- Frozen, “Assay ready cells”

BacMam Virus Characteristics:

- Non-cytotoxic, transient expression
- Easy-to-use
- Non-replicating in mammalian cells
- Biosafety level 1
- Accepts inserts of 38 kb

Figure 2 – Passive Content



Fluorescent Proteins...

- CFP (W1B)
- GFP (Emerald)
- YFP (Venus)
- OFP
- RFP (TagRFP)

Localization Markers...

- signal peptides
- fusion proteins

Figure 2. Fluorescent proteins are a key component of our new “Living probes” – Organelle and Cellular Lights™ reagents. In general we use proven *Aequorea victoria* fluorescent proteins with signal sequences to direct expression to particular subcellular spaces.

Fluorescent Protein Attributes:

- spontaneous fluorescence
- multiple colors
- highly specific localization
- no background labeling
- live cell expression
- stable and “fixable” signal
- non-cytotoxic
- “gold standard” for live cell labeling

Figure 3 – Active Content

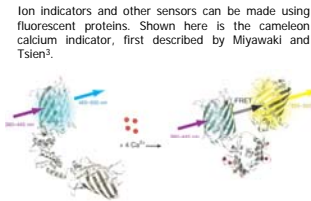
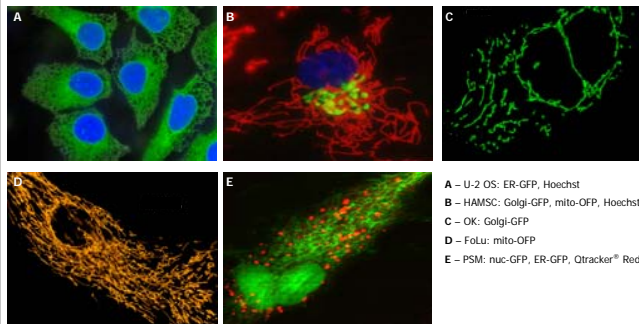


Figure 3. The cameleon calcium indicator is a Förster RET based sensor comprised of CFP, YFP and the calcium binding protein calmodulin. In the absence of calcium, a predominantly cyan fluorescence is observed. Upon calcium binding, calmodulin undergoes a large conformation change that brings CFP and YFP into an appropriate orientation and distance for more productive Förster RET. This conformational change results in a color change from cyan to yellow, hence the name cameleon.

Other Fluorescent Protein Sensors:

- chloride⁴ (halide/nitrate)
- pH⁵ (GFP H148D)
- cAMP⁶ (ICUE)
- redox⁷ (roGFP)
- kinase⁸ (AKAR)

Figure 4 – Organelle Lights™ reagents



A – U-2 OS: ER-GFP, Hoechst
 B – HAMSC: Golgi-GFP, mito-OFP, Hoechst
 C – OK: Golgi-GFP
 D – FoLu: mito-OFP
 E – PSM: nuc-GFP, ER-GFP, OTracker® Red

Figure 4. Live cell imaging of U-2 OS, primary human aortic smooth muscle cells (HAMSC), Opossum kidney cortex proximal tubule epithelial (OK), Normal Gray Fox lung fibroblast (FoLu), and Primary Skeletal Muscle (PSM) cells in full culture media with 10% serum.

Figure 5 – Premo™ biosensors

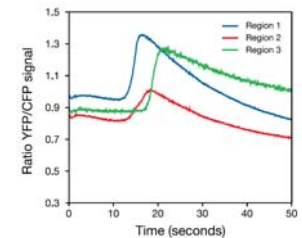
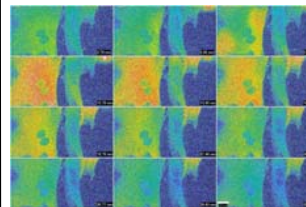
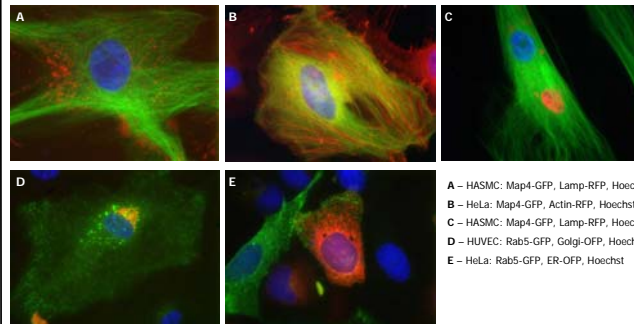


Figure 5. Calcium mobilization monitored by Premo™ cameleon in response to GPCR signaling via ATP stimulus in porcine left atrial appendage progenitor (adult stem) cells.

Figure 7 – Cellular Lights™ reagents



A – HAMSC: Map4-GFP, Lamp-RFP, Hoechst
 B – HeLa: Map4-GFP, Actin-RFP, Hoechst
 C – HAMSC: Map4-GFP, Lamp-RFP, Hoechst
 D – HUVEC: Rab5-GFP, Golgi-OFP, Hoechst
 E – HeLa: Rab5-GFP, ER-OFP, Hoechst

Figure 7. Live cell imaging of primary human aortic smooth muscle cells (HAMSC), human umbilical vein epithelial cells (HUVEC), or HeLa cells in full culture media with 10% serum.

Figure 6 – BacMam Targets

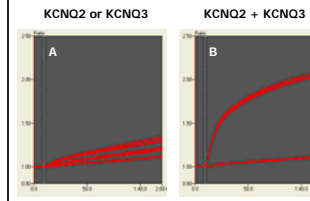


Figure 6. Ion channel targets delivered with BacMam technology. The activity of the KCNQ2/3 potassium channels was studied using the FluxOR™ potassium channel assay. A) KCNQ2 or KCNQ3 by themselves show only small amounts of ion flux, B) however when combined the two channels together show strong ion channel activity.

Gene Families Delivered With BacMam²:

- G protein-coupled receptors
- G proteins
- Ion channels (Na⁺, K⁺, & Ca²⁺)
- Nuclear receptors
- Transporters
- (shRNA)

Figure 8 – FluxOR™ K⁺ Assay

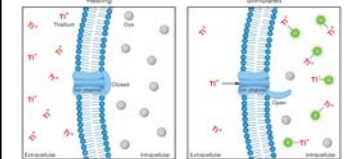


Figure 8. FluxOR™ is a universal potassium channel assay. Potassium channels will allow thallium to be used as a surrogate ion. Cells loaded with a thallium sensitive dye fluoresce green when channels are open.

Results and Conclusions

- BacMam delivery is a baculovirus-based technology used to deliver genetically encoded fluorescent markers or other genes of interest to mammalian cells.
- BacMam is non-toxic to both humans and insects.
- BacMam is also non-cytotoxic to mammalian cells in culture, including stem cells and normal human primary cells.
- Organelle and Cellular Lights™ reagents are ready-to-use BacMam virus solution that are simply applied to cells to “light-up” organelles or other subcellular structures.
- Organelle Lights™ and Cellular Lights™ reagents are great compliments to organic dyes for cellular labeling.
- Organelle and Cellular Lights™ reagents are used with live cells >6 hours after addition of reagent.
- Signal is stable for typically up to 2 weeks and is “fixable” if desired.

References

1. Boyce, F.M. & Bucher, N. (1996) *PNAS* **93**:2348-2352.
2. Kost, T.A. *et al.* (2005) *Nat. Biotech.* **23**:567-575.
3. Miyawaki, A. *et al.* (1997) *Nature* **388**:882-887.
4. Wächter, R.M. & Remington, S.J. (1999) *Curr Biol* **9**:R628-R629.
5. Eislinger, M.A. *et al.* (1999) *Biochem* **38**:5296-5301.
6. DiPilato, L.M. (2004) *PNAS* **101**:16513-16518.
7. Hanson, G.T. *et al.* (2004) *J Biol Chem* **279**:13044-13053.
8. Zhang, J. *et al.* (2001) *PNAS* **98**:14997-15002.