



BacMam gene delivery of targeted fluorescent proteins, Organelle Lights™ for HCS

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Abstract

Examining cellular events in four dimensions is often necessary for scientists interested in cellular pathways and development. Organelles are used as landmarks for cell morphology, development, and subcellular localization. Our new line of Organelle Lights™ reagents expands fluorescent imaging possibilities, providing more information in each experiment by allowing subcellular localization in living or fixed cell preparations.

Molecular Probes® Organelle Lights™ reagents contain a baculovirus that upon entry into mammalian cells^(1,2) directs the expression of autofluorescent proteins targeted to specific subcellular compartments and organelles via signal peptide or protein fusions. The Organelle Lights™ reagents were developed to provide a convenient, highly efficient and robust tool for delivering organelle-targeted fluorescent proteins to a variety of mammalian cells.

Here we describe how Organelle Lights™ reagents work in mammalian cells, can be used as HCS tools, and can multiplex with other Organelle Lights™ reagents, traditional organic dyes, and Qdot® nanocrystals. The combination of the information learned from the Organelle Lights™ with the Premo™ Biosensors will result in targeted intracellular imaging and quantitation tools for high throughput and high content screening efforts.

Figure 1 – Organelle Lights™ Workflow

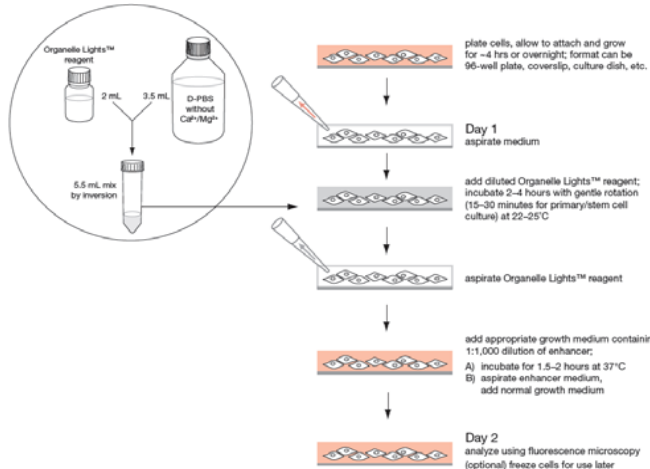
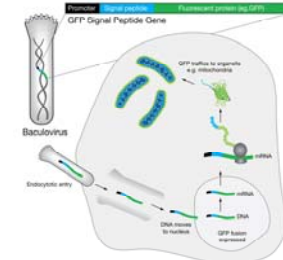


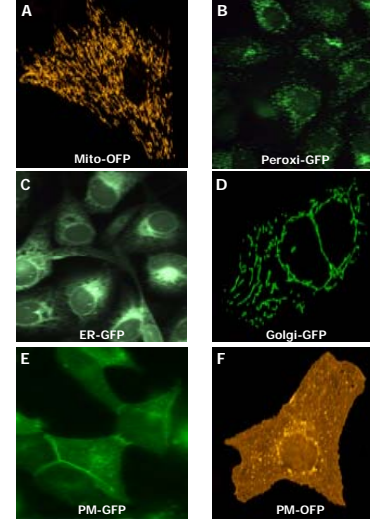
Figure 2 – Content & Delivery



The combination of targeted fluorescent proteins with BacMam delivery technology yields easy-to-use, genetically encoded reagents for cell-based assays.

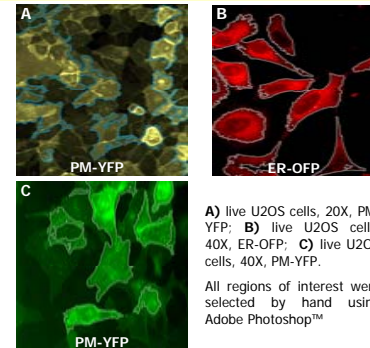
- Easy-to-use
- Biosafety Level 1
- Multiple day expression
- Stable to fixation
- Multiplex with dyes and Qdot® nanocrystals
- HCS compatible

Figure 3 – Organelle Lights™ in cells



A) fixed Indian Muntjac deer epidermis fibroblast cell, 100X, Mito-OPF; **B)** fixed U2OS (human osteosarcoma cell line) cells, 63X, Peroxi-GFP; **C)** fixed U2OS cells, 63X, ER-GFP; **D)** fixed OK cell, 100X, Golgi-GFP; **E)** live U2OS cells, 63X, PM-GFP; **F)** live U2OS cell, 100X, PM-OPF

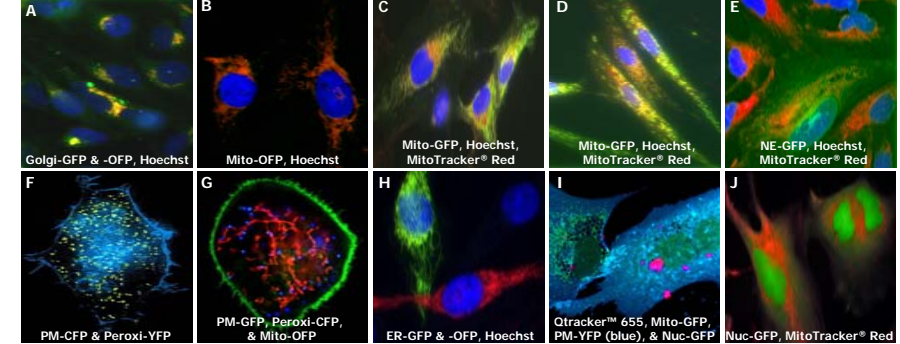
Figure 4 – Organelle Lights™ as HCS tools



A) live U2OS cells, 20X, PM-YFP; **B)** live U2OS cells, 40X, ER-OPF; **C)** live U2OS cells, 40X, PM-YFP.

All regions of interest were selected by hand using Adobe Photoshop™

Figure 5 – Multiplex Organelle Lights™ with organic dyes and Qdot® nanocrystals



A) Live HeLa cells, 40X, Golgi-GFP, Golgi-OPF, and Hoechst 33342; **B)** Live HeLa cells, 63X, Mito-OPF and Hoechst; **C)** live HeLa cells, 40X, Mito-GFP, MitoTracker® Red, and Hoechst; **D)** live Muntjac cells, 40X, Mito-GFP, MitoTracker® Red, and Hoechst; **E)** live HeLa cells, 40X, NE-GFP, MitoTracker® Red, and Hoechst; **F)** live HEK293GT cell, 63X, PM-CFP and Peroxi-YFP; **G)** live CHO cell, 63X, PM-GFP, Peroxi-CFP, and Mito-OPF; **H)** live U2OS cells, 63X, ER-GFP, ER-OPF, and Hoechst; **I)** live U2OS cells, 63X, Qtracker™ 655, Mito-GFP, PM-YFP, and Nuc-GFP; **J)** live HeLa cells, 63X, Nuc-GFP and MitoTracker® Red

Table 1 – Organelle Lights™ reagents

Target	Targeting sequence	CFP (440/480)	GFP (488/510)	YFP (514/528)	OPF (548/565)
Endoplasmic Reticulum (ER)	ER signal sequence of calreticulin & KDEL (ER retention signal) (3)				
Golgi	Golgi-resident enzyme N-acetyl-galactosaminyltransferase-2 (4)				
Plasma Membrane	Myristoylation/palmitoylation sequence from Lck tyrosine kinase (5)				
Mitochondria	Leader sequence of E1 alpha pyruvate dehydrogenase (6)				
Nuclear envelope (NE)	Nesprin 1alpha C-terminal transmembrane domain (aa 923-982) (7)				
Nucleus	SV nuclear localization sequence (C-terminus) (8)				
Peroxisome	Peroxisomal C-terminal targeting sequence (9)				
Cytoplasm	Nuclear export sequence (C-terminus) (10)				
Actin, tubulin, MAP4	Entire coding sequence		In progress		In progress

Conclusions

- Organelle Lights™ products are engineered baculoviruses that upon entry into living mammalian cells direct expression of targeted fluorescent proteins in cells
- Organelle Lights™ reagents can be used for High Content Screening
- Organelle Lights™ products can be multiplexed with other Organelle Lights™ reagents, organic dyes, and Qdot® nanocrystals

References

1. Kost, TA (2005) Nature Biotech 23:567
2. Kost, TA & Condeelis, JP (2002) Trends in Biotech 20:173
3. Fliegel, L (1989) JBC 264(36) 21522-8
4. Storrer, B (1998) JCB 143(6) 1505-21
5. Kavouridis, PS (1997) EMBO 16(16) 4983-98
6. Hanson, GT (2004) JBC 279(13) 13044-53
7. Zhang, Q (2001) JCS 114: 4485-98
8. Dingwall, C (1991) TIBS 16(12) 478-81
9. Gould, SJ (1989) JCB 108: 1657-64
10. Chevalier, SA (2005) BMC-R 2(70) 1-11