

CellPainter[™] Organelle Marker Plasmids

Application Guide

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Package Contents and Related Products

The following components are included:

- One (1) vial containing the organelle marker clone as 10 ug lyophilized plasmid DNA.
- 1 copy of Certificate of Analysis
- 1 copy of Application Guide

Store at -20°C for long-term storage.

The cDNA clone is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, clones are guaranteed to be stable for 12 months.

Related, Optional Reagents

For amplification of the plasmid DNA:

Competent *E. coli* cells

LB agar plates with ampicillin, 100 µg/ml

LB broth

(10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl. Adjust pH to 7.0 with 1 N NaOH)

DNA purification reagents

For cell culture, transfection and cell preparation:

Six-well tissue culture plates or chamber slides

Cell culture media with FBS or serum-free media

Transfection reagent

Cell fixing reagent and slide mounting reagents

Related OriGene Products

TrueORF tagged cDNA clones

<http://www.origene.com/orf>

TrueClone™ full length cDNA clones

<http://www.origene.com/cdna/>

HuSH™ shRNA Plasmids

<http://www.origene.com/rnai/>

VERIFY™ Tagged Antigens

<http://www.origene.com/lysate/>

Validated Antibodies

<http://www.origene.com/antibody/>

Functional Proteins

<http://www.origene.com/protein/>

TurboFectin 8.0 Transfection Reagent

<http://www.origene.com/cdna/turbofectin.msp>

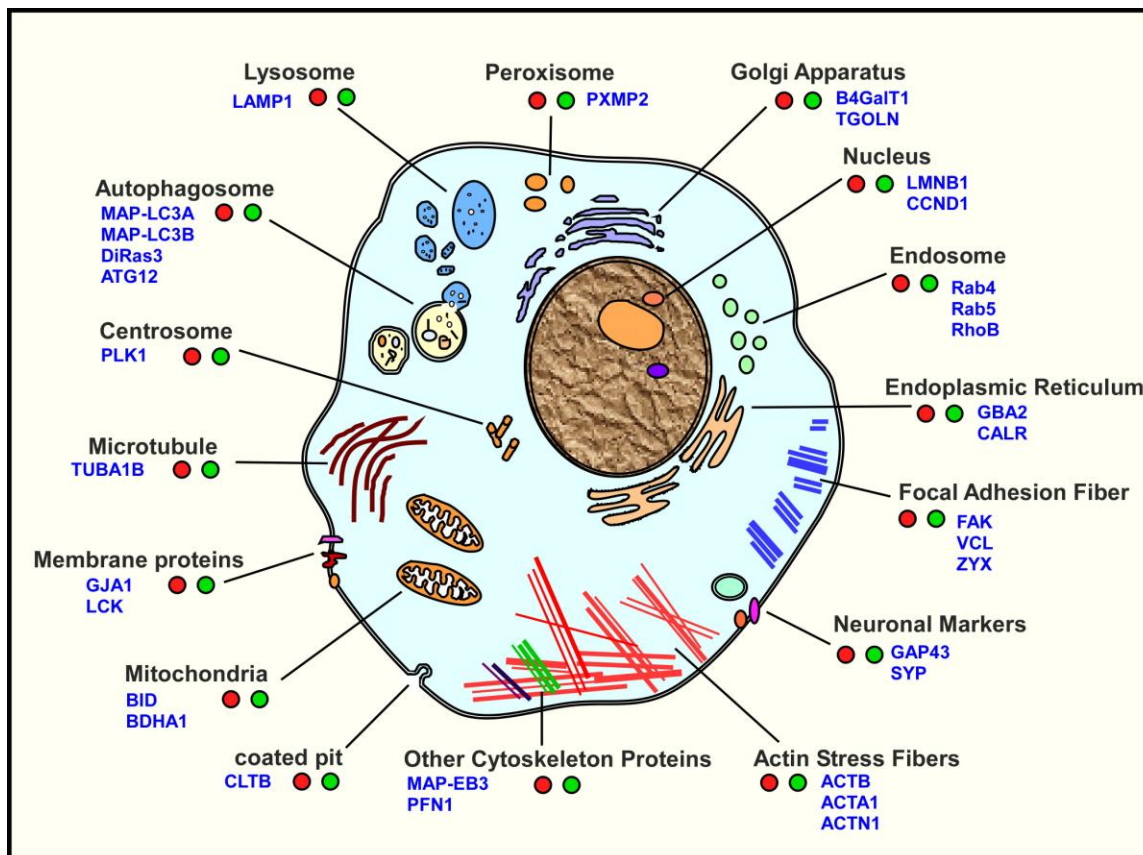
Organelle Maker Descriptions:

CellPainter™ Organelle Markers are GFP- or RFP-tagged [TrueORF](#) cDNA clones that encode organelle-specific or structure-specific proteins. The proteins are fused in-frame with different fluorescent proteins and allow clear visualization of the organelles or structures. ([View](#) the full set of validation data in our photo gallery)

Organelle markers represent a small subset of the TrueORF collection, our genome wide offering of tagged cDNA clones. Over 37,000 TrueORF clones are offered with tagging options. Learn more at www.origene.com/ORF

Applications:

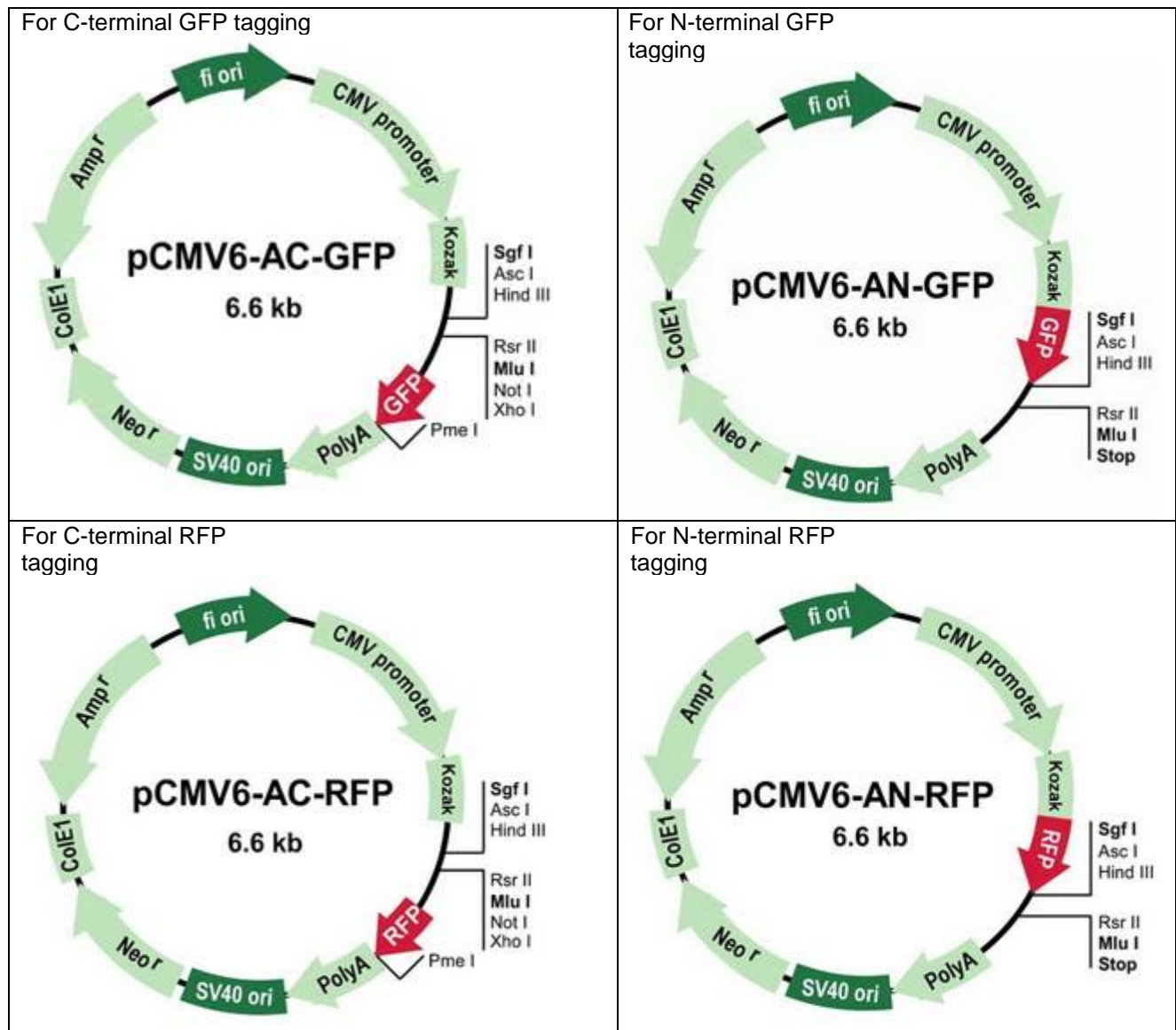
1. Cell organelle marking without antibodies or chemicals
2. Monitoring of protein trafficking
3. Study organelle morphology and dynamics
4. Protein co-localization with organelles
5. Fraction tracking during enrichment or purification



All CellPainter™ Organelle marker plasmids are constructed using OriGene's TrueORF destination vectors. The fluorescent protein tags (turboGFP or turboRFP) are fused at the N- or C-terminus of the marker protein.

Currently, four types of vectors have been used to create the organelle marker plasmids.

All of the vectors can be selected with ampicillin (100ug/ml) in *E.coli* and with the neomycin analog, G418, in mammalian cells.



All organelle marker clone vectors are described in detail on OriGene's website at http://www.origene.com/cdna/cellPainter_Vector.aspx

Experimental Procedures

Transfection Optimization

Although the transfection protocols below have been proven to result in highly efficient transfection, it is encouraged to carefully optimize the reaction conditions for each individual cell type. The following variables should be considered:

Cell density (% confluence at transfection): The recommended confluence for most cell types at transfection is 50-70%. Determine the optimal cell density for each cell type for maximal efficiency and maintain the density in all experiments for reproducibility.

Media conditions: TurboFectin 8.0 performs better in complete growth medium compared to serum-free medium. No media change is necessary before or after the transfection.

DNA purity and concentration: OriGene recommends highly purified, sterile DNA prepared on ion-exchange columns. It is important to remove the contamination of endotoxin from the DNA prep for maximal transfection efficiency. The optimal DNA concentration for transfection is 1-3 ug per well for a 6-well plate. Refer to Table 1 for more details.

TurboFectin 8.0 to DNA ratio: The standard ratio is 3 uL of TurboFectin to 1 ug of DNA. It is recommended to titrate the ratio by adjusting the TurboFectin from 2-8 uL per 1 ug DNA. Refer to Table 1 for more details.

Table 1. Recommended starting transfection conditions for TurboFectin 8.0

Culture plate	10cm Dish	6-well	12-well	24-well	48-well	96-well
surface area (cm ²)	59	9.6	3.8	1.9	1.0	0.35
volume of complex (uL)	600	100	50	20	10	5
TurboFectin 8.0 (uL)	18	3	1.5	0.6	0.3	0.15
DNA volume (uL; 1ug/uL stock)	6	1	0.5	0.2	0.1	0.05

Protocol for transient transfection (adherent cells)

A sample protocol is listed here for experiments performed in 6-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate.

Cell Plating

On the day before transfection, plate cells at a density of 1-3 x 10⁵ cells in complete growth medium per well of a 6-well plate to obtain 50-70% confluence on the following day.

Incubate overnight.

Complex formation (perform this step immediately before transfection)

In a sterile plastic tube, add 100 uL of serum free medium.

- Add the appropriate amount of TurboFectin 8.0 (2-6 uL per 1 ug DNA) into tube. Mix completely by gentle pipetting.
- Incubate at room temperature for 5 minutes.
- Add plasmid DNA (1-3 ug per well) to the TurboFectin-containing media prepared above. Mix by gentle pipetting.
- Incubate at room temperature for 15-30 min.

2. Transfection in complete culture medium
 - a) If necessary, remove spent medium from the cells prepared in Step 1 and replace with 2 mL of fresh complete medium per well.
 - b) Add the mixture prepared in Step 2 dropwise to the cells. Gently rock the dish to distribute the complex evenly.
 - c) Incubate for 24-48 hours.

Note: With TurboFectin, no medium change is necessary. If you wish to remove the complex, remove the medium 4-24 hours post-transfection and replace with fresh complete medium.

Protocol for Confocal Microscopy:

1. At 30-36 hrs post-transfection, wash cells 1 x with cold PBS and then fix cells with 4% formaldehyde in PBS for 10-20 min at room temperature. Do not incubate the cells longer than 48 hrs post-transfection. Wash cells 3 x with cold PBS.
2. Carefully transfer the cover slip from well with cells face down to a glass slide with one drop of any commercial mounting medium with or without DAPI (for nuclear staining). We recommend VECTASHIELD® or VECTASHIELD® with DAPI mounting medium (Vector Laboratories. <http://www.vectorlabs.com>) which provides strong initial fluorescence and retards photo bleaching. Seal cover slips with nail polish or a plastic sealant for long-term storage. Mounted slides should be stored at 4 °C. The fluorescence will remain for several days.
3. View slides by confocal fluorescence microscopy within 24 - 48 hours post-fixation.

Note: Clear and distinguishable cell organelle markers sometimes depend on many factors such as cell type, transfection time, DNA transfection rate, stimulating reagents or co-expressing proteins, etc. Users lacks experience may have negative, weak, or messy images. Nuclear proteins usually provide strong images for most of cells and then could be used as a positive control.

If immunofluorescence staining required as a co-stain, follow the standard immunofluorescence staining protocol.

Protocol for Stable Transfection:

Perform a transfection as described above (protocol for transient transfection). At 24 hrs post-transfection, passage the cells (at 1:10 or higher dilution) into fresh growth medium containing the selective agent, G418. We recommend that a kill curve be performed to determine the optimal G418 concentration for your cell type. A mock transfection should be performed in parallel as a control. Grow and passage the cells as necessary, maintaining selection pressure by keeping the selective agent in the growth medium. After 1-2 weeks, a large number of the cells will be killed; the cells that remain growing in the selective medium have retained the expression plasmid, which stably integrates into the genome of the targeted cells. Monitor the mock control to ensure the cells are dying.

Frequently Asked Questions

For questions not addressed here, please contact OriGene's Technical Support professionals. You may dial 888-267-4436 from any US location, or 301-340-3188 outside the US. E-mail inquiries to techsupport@origene.com are also invited.

1. What are the fluorescent proteins used for the organelle markers?

The fluorescent proteins are turboGFP and turboRFP from Evrogen. Both proteins are super bright with brightness % compared to EGFP of 112 and 187, respectively. They are both fast maturing, allowing detection of fluorescence as early as 8-10 hrs post transfection. In spite of their dimeric structures, tGFP and tRFP have been successfully used as fusion protein for subcellular structure labeling. All OriGene's organelle markers are validated for correct labeling.

2. What is the excitation/emission wavelength of tGFP?

The excitation/ emission max = 482/ 502 nm. tGFP can be detected using common fluorescence filter sets for EGFP, FITC, and other green dyes. We recommend Omega Optical filter sets QMAX_Green, XF100_2, XF100_3, XF115_2, and XF116_2.

3. What is the excitation/emission wavelength of RFP?

The excitation/emission max=553/574 nm. tRFP can be detected using common fluorescence filter sets for Texas Red. We recommend Omega Optical filter sets QMAX_Yellow, XF108_2, XF101_2, and XF111_2. tRFP can also be detected using TRITC filter set.

4. Can I use a regular fluorescent microscope to obtain organelle images?

Conventional fluorescent microscope can be used for viewing some of the organelle or subcellular structures. However the images are usually lack the desired clarity. Confocal microscope is strongly recommended for obtaining sharp organelle images. All of OriGene's validation data was obtained using a confocal microscope. <http://www.origene.com/assets/documents/trueorf/FP-Images.pdf>

5. What antibody can I use to detect the tGFP fusion protein on a Western blot?

We recommend the catalog# AB513 (rabbit polyclonal against denatured turboGFP) sold from Evrogen. <http://www.evrogen.com/products/antibodies/AB-TurboGFP.shtml>

6. What antibody can I use to detect the RFP fusion protein on a Western blot?

We recommend the catalog# AB231 (rabbit polyclonal against turboRFP) from Evrogen. <http://www.evrogen.com/products/antibodies/AB-tRFP.shtml>

7. Can I make stable mammalian cell lines with the organelle markers?

Yes. All organelle marker plasmids carry a neomycin selection gene.

8. What positive control should I use with these markers?

Optional nuclear protein marker, such as Cyclin D1, is available at an additional cost. If you have an antibody specific for an organelle, you can also use that as a reference point.

9. Where can I find the sequence and cloning sites for my Organelle marker?

All organelle markers are cloned into the SgfI and MluI sites. The vector sequence are available from website at http://origene.medigent.com/cdna/cellPainter_Vector.aspx

10. Which vector should I use as a negative control for my GFP-tagged or RFP-tagged marker?

The corresponding empty vector can serve as negative control. Detailed info can be found at http://www.origene.com/cdna/cellPainter_Vector.aspx

11. Can I shuttle my ORF using your PrecisionShuttle system to change my fluorescent tag?

Yes. All organelle markers can be easily shuttled among any of the Destination vectors using the PrecisionShuttle system. For further information, please see our website at <http://www.origene.com/cdna/trueorf/destinationvector.aspx>

12. How are the organelle marker tested?

Each organelle marker plasmid is tested by transfection into HEK293 cells followed by imaging via confocal microscopy. Appropriate organelles or subcellular structures were labeled and the data can be viewed online at <http://www.origene.com/assets/documents/trueorf/FP-Images.pdf>

13. I am writing a paper for publication and need to describe this product. How should I cite?

We recommend that you refer to the product by its specific catalog number and refer to us as OriGene Technologies (Rockville, MD). Furthermore, we'd love to hear from you when your paper is published. Inform us and we will send a gift.