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Image based Protein-Protein interaction analysis

Fluoppi

Fluoppi Ver.2 : Ash-Red

(Ash-MNL/MCL + Monti-Red-MNL/MCL)

Code: AM-8012M

Amalgaam

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Introduction

Fluoppi is a technology providing an easy way to visualize protein-protein interactions (PPIs) with a high signal to noise ratio. It employs an oligomeric assembly helper tag (Ash-tag) and a tetrameric fluorescent protein tag (FP-tag) to create detectable fluorescent foci when there are interactions between two proteins fused to the tags. By way of example, genetic fusion of protein X with FP-tag, and Y with Ash-tag creates a tetrameric fluorescent fusion protein X-FP and an oligomeric fusion protein Y-Ash respectively (Fig. 1). Because each fusion protein has multiple Xs or Ys, interaction between X and Y causes large lattice like complexes where the fluorescence by X-FP is concentrated and detectable as fluorescent foci (Fig. 2).

Anti-Ash-tag monoclonal antibody (mAb) (code: M223-3) recognizes the joint region between Ash-tag and flexible linker (Fig. 3a). **Fluoppi Ver.2 : Ash-Red** contains an additional amino acid at the C terminal of Ash-tag expressed by pAsh-MNL, which make it possible to be recognized by the mAb (Fig. 3b)

Fluoppi Ver.2 : Ash-Red (code: AM-8012M) includes 4 expression plasmids as listed in section 2. A tetrameric red fluorescent protein, Monti-Red, is employed as a FP-tag as describe above.

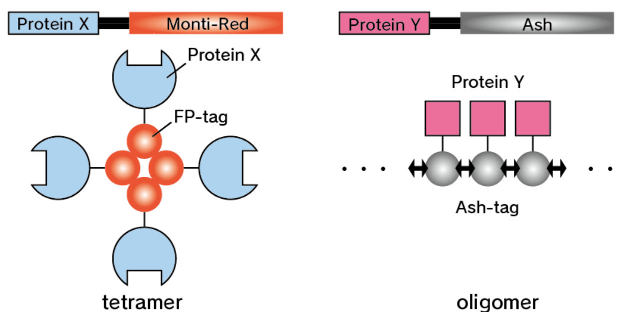


Figure 1 | Key components of Fluoppi technology

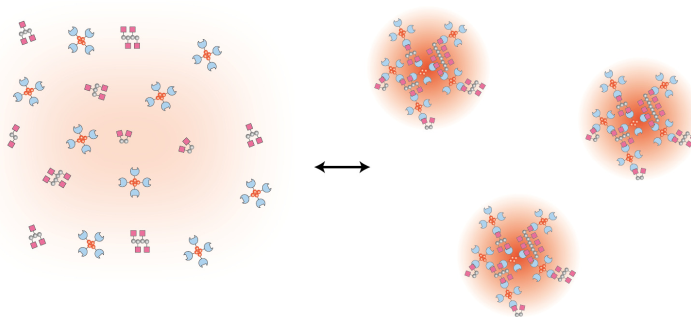


Figure 2 | Mechanism of action

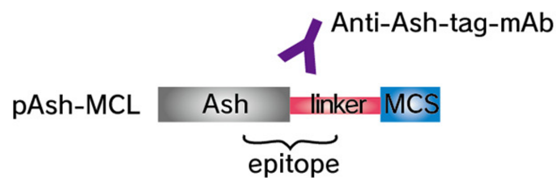


Figure 3a | Epitope of Anti-Ash-tag mAb

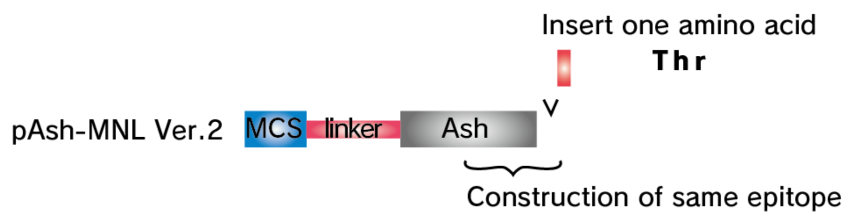


Figure 3b | Construction of same epitope

1. Product Components and Storage Condition

Plasmids	Vial color	Form
pAsh-MNL Ver.2	White	10 µg: Dry form
pAsh-MCL	White	10 µg: Dry form
pMonti-Red-MNL	Red	10 µg: Dry form
pMonti-Red-MCL	Red	10 µg: Dry form

Reconstitution in 10-50 µL of sterilized distilled water.

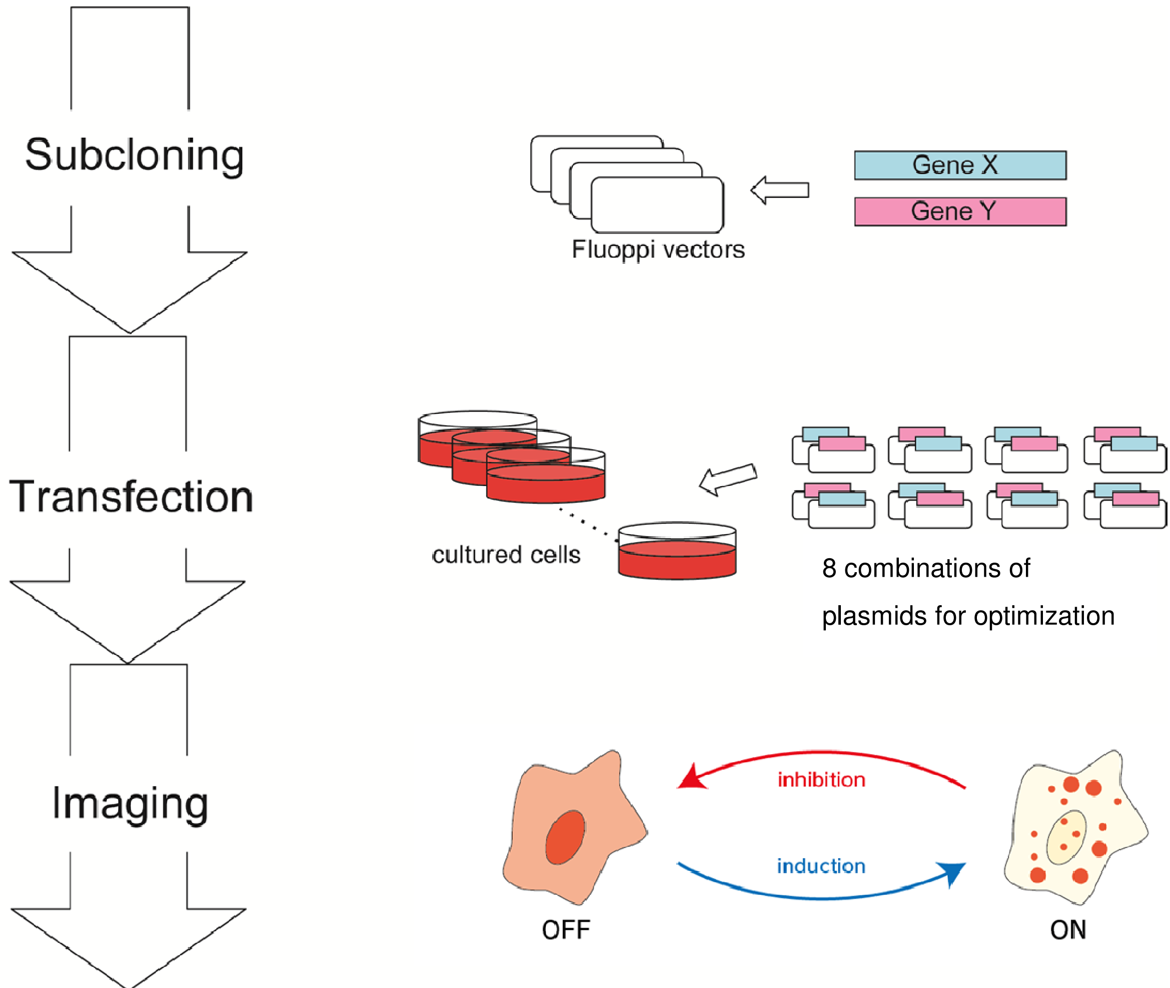
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

2. Additional Materials Required

- Restriction enzymes which can be used for constructing Fluoppi plasmids (BamH I, Kpn I, Pst I, EcoR I, Xho I, Hind III, Not I)
- Subcloning related materials (Thermocycler, DNA polymerase, DNA Ligase)
- Competent cells, LB-Kanamycin agar plates, LB-Kanamycin medium
- Cell culture related materials (Mammalian cells, Cell culture medium, Cell culture dish, Plate)
- Transfection reagent
- Buffer for imaging (HBSS, PBS, Good's Buffer)
- Fluorometric detector (Fluorescence microscopy, Plate imager)

3. Procedure

Overview of Fluoppi Procedure



Subcloning

Construct your Fluoppi plasmids as listed in Table 1. Preparing all 8 constructs listed in the table is recommended for optimization.

Table 1 | The eight possible constructs

	pMonti-Red-MNL	pMonti-Red-MCL	pAsh-MNL	pAsh-MCL
X	X-Monti-Red	Monti-Red-X	X-Ash	Ash-X
Y	Y-Monti-Red	Monti-Red-Y	Y-Ash	Ash-Y

MN-Forward primer and MC-Reverse primer can be used to verify your insert sequences.

•MN-Forward (18 mer): 5'- CGCCCCATTGACGCAAAT-3'

•MC-Reverse (19 mer): 5'- AGGTGTGGGAGGTTTTTTA-3'

The annealing sites are described in Figure 4 and Figure 5.

Note:

The insertion should not destroy the reading frame. Stop codon must be removed to insert the gene of interest into 5'-end of Fluoppi tags. An initial translation codon ATG (Methionine) must be added to the 5'-end for genes of truncated proteins fused to the N-terminal of tags.

Transfection

Co-transfect each pair of plasmid into the cells in each well respectively. This may be done by using an either commercially available transfection reagent according to the manufacturer's instructions or any in-house method appropriate to the cell type. Eight possible combinations and four negative control combinations of the plasmids are listed in Table 2.

Table 2 | Recommended co-transfection pairs

	Monti-Red		Ash	
1	X-Monti-Red	&	Y-Ash	
2	X-Monti-Red	&	Ash-Y	
3	Monti-Red-X	&	Y-Ash	
4	Monti-Red-X	&	Ash-Y	
5	Y-Monti-Red	&	X-Ash	
6	Y-Monti-Red	&	Ash-X	
7	Monti-Red-Y	&	X-Ash	
8	Monti-Red-Y	&	Ash-X	
9	X-Monti-Red	&	Ash	Negative Control for 1 and 2
10	Monti-Red-X	&	Ash	Negative Control for 3 and 4
11	Y-Monti-Red	&	Ash	Negative Control for 5 and 6
12	Monti-Red-Y	&	Ash	Negative Control for 7 and 8

pAsh-MNL encodes Ash-tag only and therefore can be used as a negative control plasmid.

Note:

Depending on the nature of your protein of interest, tetrameric fusion fluorescent proteins may cause foci like structure by itself (Karasawa et al. 2003). To avoid misinterpretation of your Fluoppi result, it is important to observe the images of negative controls listed in Table 2.

Imaging

Twenty-four hours after transfection, the cells can be imaged by a fluorescence microscopy. It is recommended to displace the cell cultured medium to HBSS with 20 mM HEPES. The filters set for mCherry/Texas-Red can be used for Monti-Red.

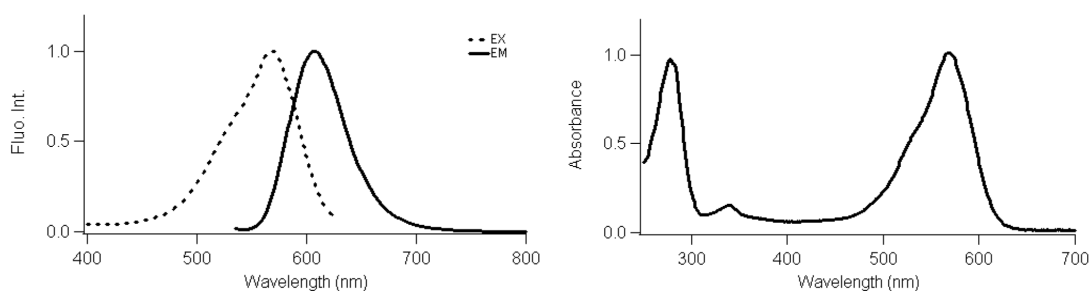
NOTE

Fluoppi Does Not Guarantee

Detection of all Protein-Protein Interactions.

4. Properties of Fluorescent protein “Monti-Red”

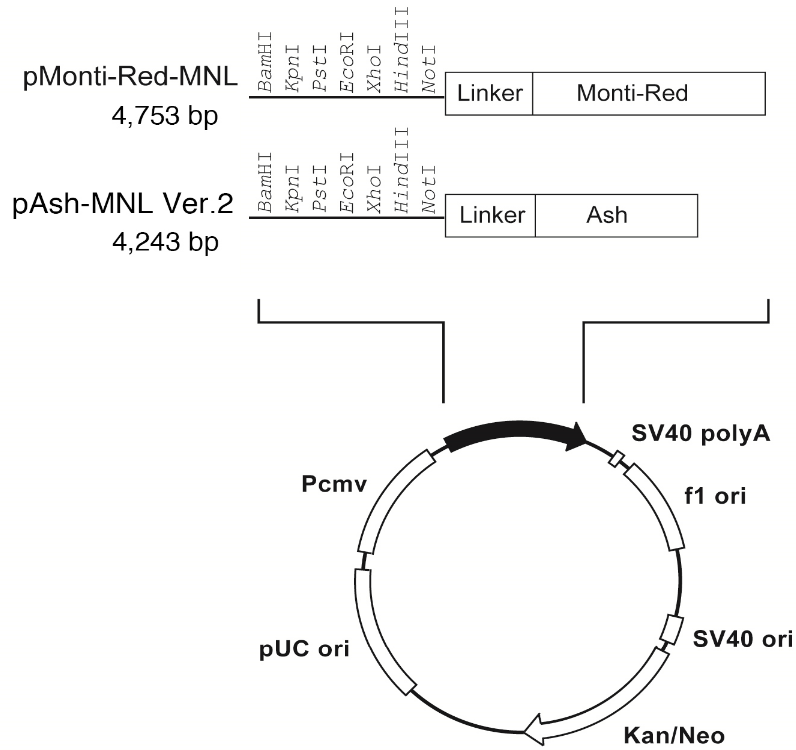
Monti-Red, a mutant fluorescent protein derived from Keima-Red which was originally cloned from the stony coral (Montipora sp.), forms tetramer and absorbs light maximally at 571 nm and emits red light at 607 nm. Fluorescent signal of Monti-Red can be detected by using filter sets for Texas Red or similar fluorescent dyes.



Fluorescent protein	Excitation/Emission maximum (nm)	Extinction coefficient ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	pKa
Monti-Red	571/607	83,000 (571 nm)	0.3	5.5

Extinction coefficient was measured by the alkali denaturation method.

5. Plasmid Maps



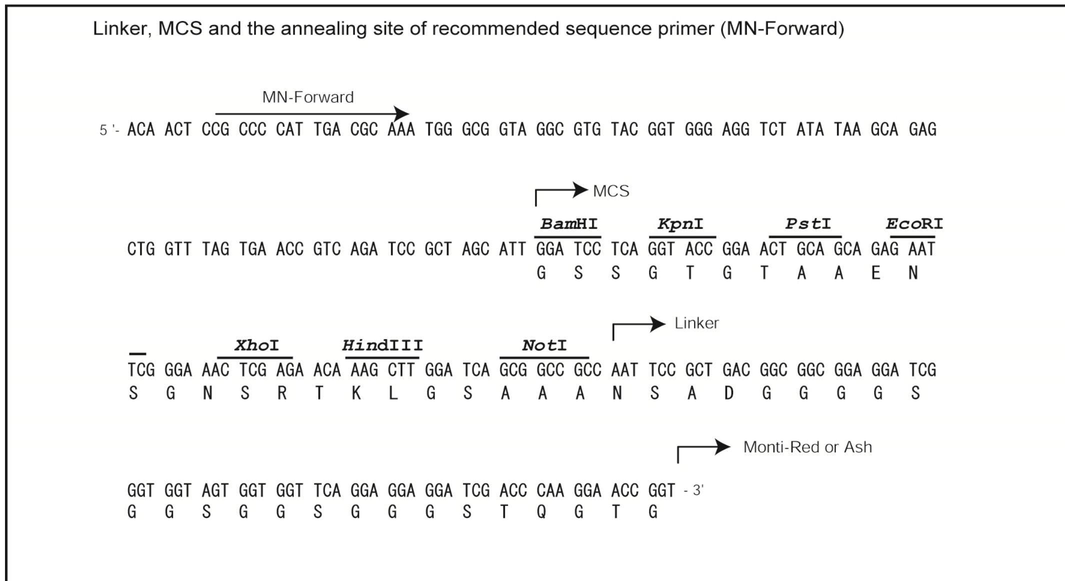
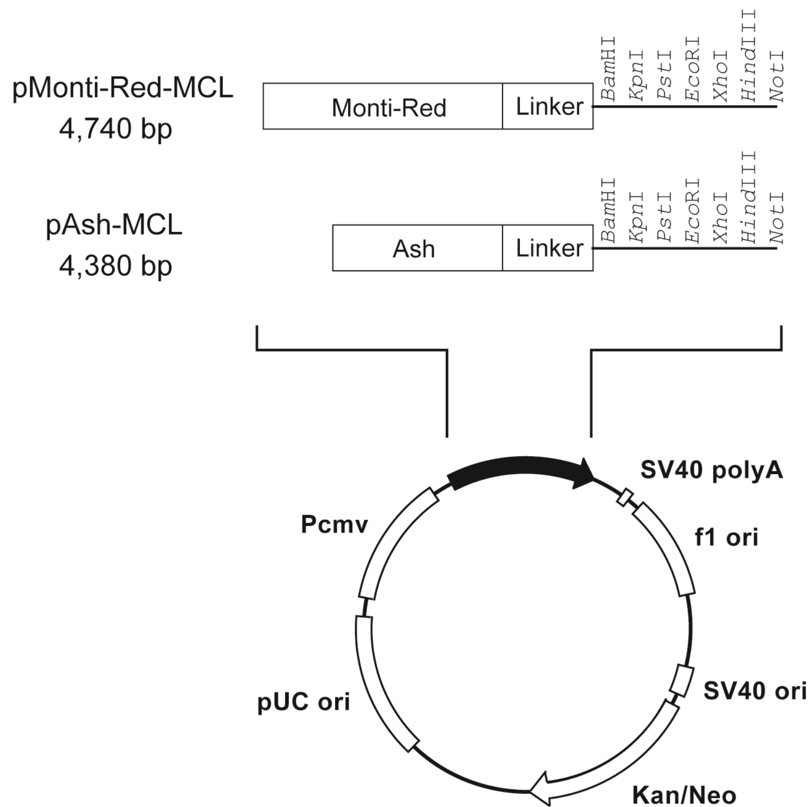


Figure 4 | Plasmid Map of pMonti-Red-MNL and pAsh-MNL Ver.2



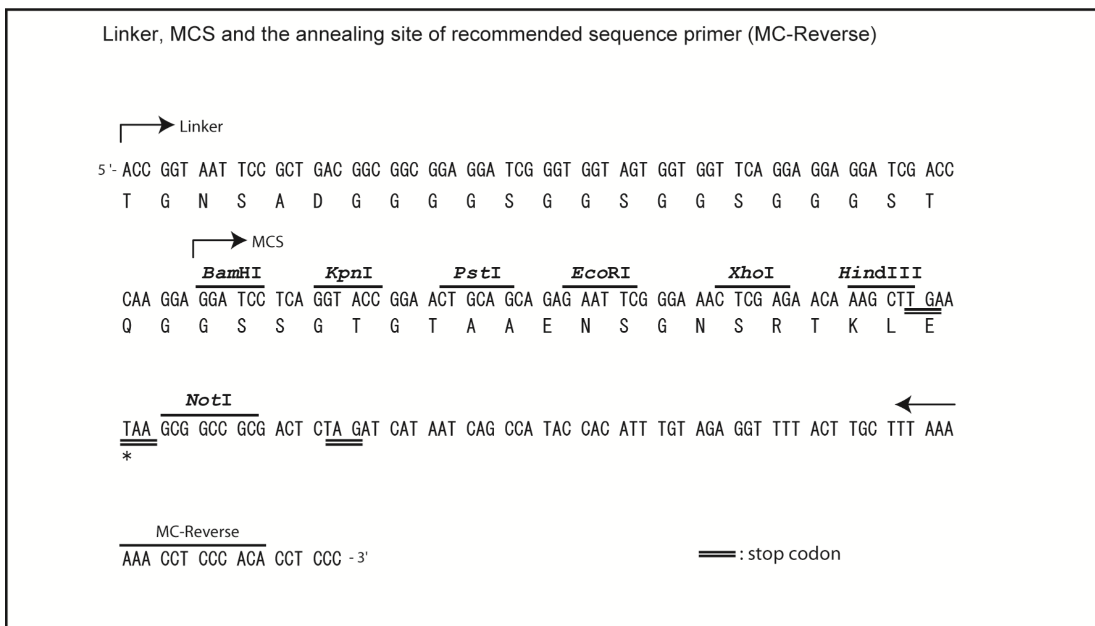


Figure 5 | Plasmid Map of pMonti-Red-MCL and pAsh-MCL

Sequence information of all 4 plasmids can be downloaded from our website.

http://ruo.mbl.co.jp/product/flprotein/fluoppi.html

Fluoppi plasmids contain the following elements.

- Cytomegalovirus (CMV) promoter for high level expression in a wide range of mammalian cells
- Kanamycin / Neomycin resistance gene
- Multiple cloning site (MCS): restriction enzyme site (BamH I, Kpn I, Pst I, EcoR I, Xho I, Hind III and Not I)
- Flexible linker to relieve steric hindrance between the protein of interest and Fluoppi tags

6. Reference

1) Koyano F *et al*. Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature (2014) [PMID: 24784582]

7. License

This technology was developed by Amalgaam, an MBL group company and has been filed for international patent applications. MBL grants non-profit research organizations an international, royalty-free, non-exclusive, limited license for non-commercial, research purpose only. This license excludes the right to sell or transfer the product, its components, or modifications to any third party. Any other uses by non-profit research organizations, or any uses by for-profit organizations regardless of commercial or non-commercial purpose, require a license.

8. Related products

AM-8011M	Fluoppi Ver.2 : Ash-hAG (Ash-MNL/MCL + hAG-MNL/MCL)
AM-8012M	Fluoppi Ver.2 : Ash-Red (Ash-MNL/MCL + Monti-Red-MNL/MCL)
AM-8201M	Fluoppi : Ash-hAG [p53-MDM2]
AM-8202M	Fluoppi : Ash-hAG [mTOR-FKBP12]
AM-VS0801M	humanized Azami-Green for Fluoppi (phAG-MNL/MCL)
AM-VS0802M	Monti-Red for Fluoppi (pMonti-Red-MNL/MCL)
M223-3	Anti-Ash-tag mAb
SI-8010*	Fluoppi : Ash-hAG-Lenti pCMV (Ash-Neo-MNL/MCL + hAG-Puro-MNL/MCL)
SI-8011*	Fluoppi : Ash-hAG-Lenti pCMV (Ash-Puro-MNL/MCL + hAG-Neo-MNL/MCL)
SI-8020*	Fluoppi : Ash-hAG-Lenti pEF1a (Ash-Neo-MNL/MCL + hAG-Puro-MNL/MCL)
SI-8021*	Fluoppi : Ash-hAG-Lenti pEF1a (Ash-Puro-MNL/MCL + hAG-Neo-MNL/MCL)

* Anti-Ash-tag mAb (M223-3) reacts with proteins expressed by pAsh-MCL and pAsh-MNL Ver.2 (components of code no. AM-8011M and AM-0812M) but not with Ash-MNLs including lentiviral vectors (components of code no. SI-8010, SI-8011, SI-8020, SI-8021).