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Image based Protein-Protein interaction analysis

Fluoppi

Fluoppi Ver.2 : Ash-hAG

(Ash-MNL/MCL + hAG-MNL/MCL)

Code: AM-8011M

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1. 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-hAG** 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-hAG (code: AM-8011M) includes 4 expression plasmids as listed in section 2. A tetrameric green fluorescent protein, *CoralHue*® Azami-Green (hAG), is employed as a FP-tag as describe above.

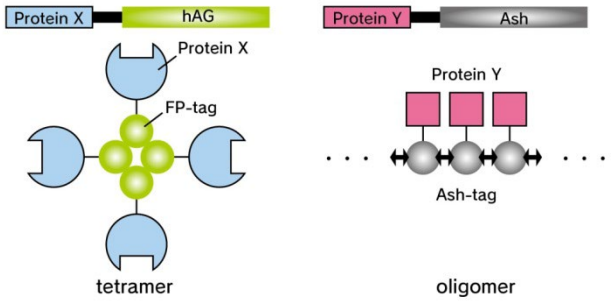


Figure 1 |Key components of Fluoppi technology

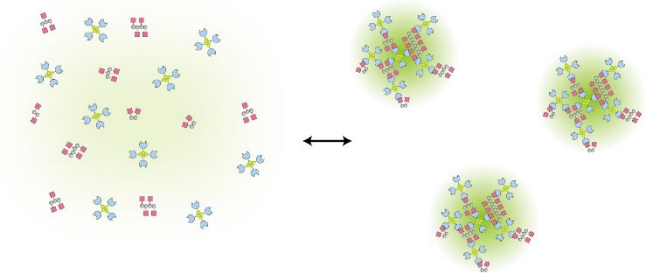


Figure 2 | Mechanism of action

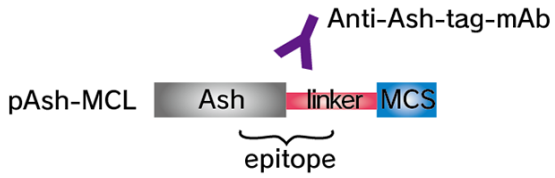


Figure 3a | Epitope of Anti-Ash-tag mAb

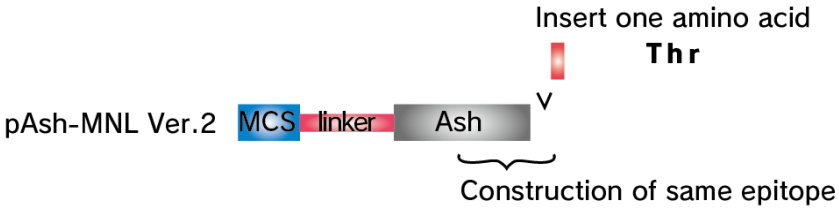


Figure 3b | Construction of same epitope

2. 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
phAG-MNL	Green	10 μg: Dry form
phAG-MCL	Green	10 μg: Dry form

Reconstitution in 10-50 μL of sterilized distilled water.

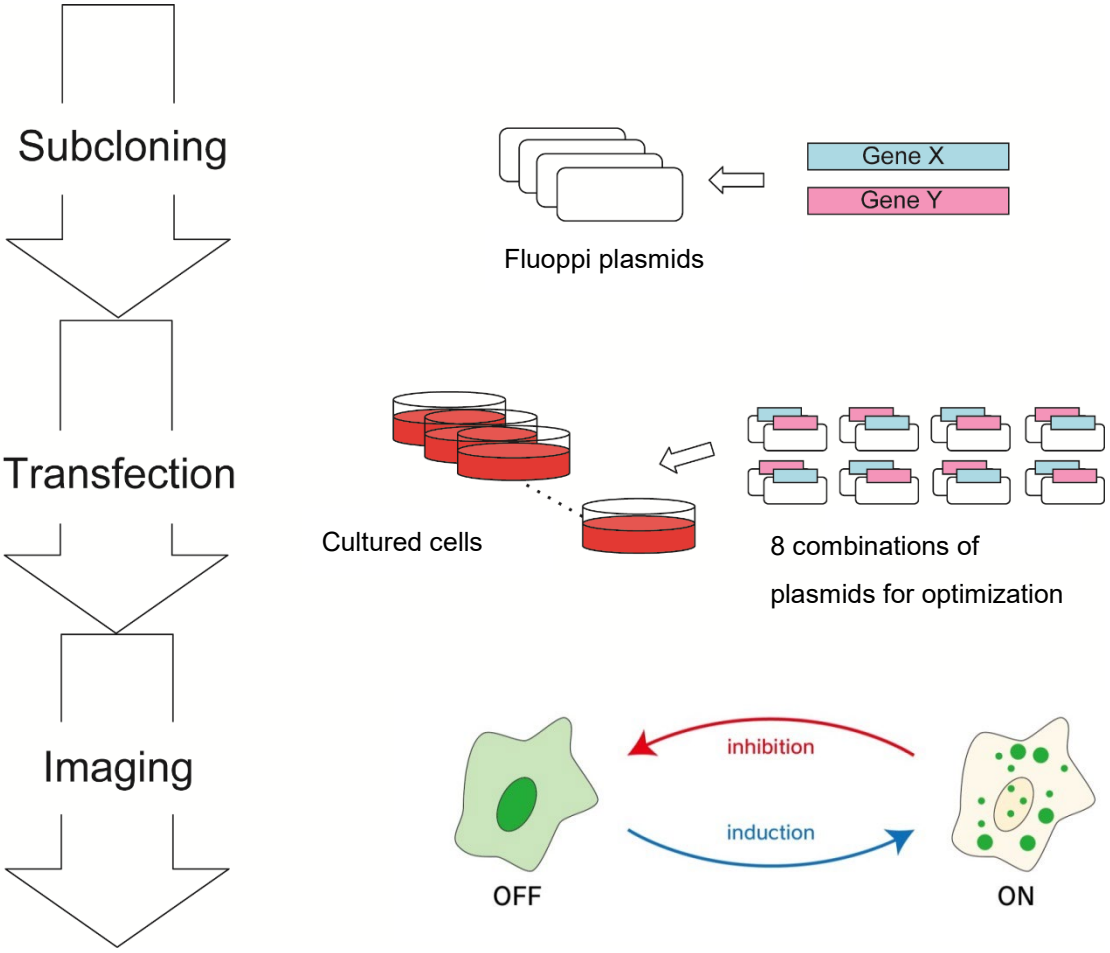
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

3. 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)

4. 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

	phAG-MNL	phAG-MCL	pAsh-MNL	pAsh-MCL
X	X-hAG	hAG-X	X-Ash	Ash-X
Y	Y-hAG	hAG-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 Figures 4 and 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 plasmids 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

	hAG		Ash	
1	X-hAG	&	Y-Ash	
2	X-hAG	&	Ash-Y	
3	hAG-X	&	Y-Ash	
4	hAG-X	&	Ash-Y	
5	Y-hAG	&	X-Ash	
6	Y-hAG	&	Ash-X	
7	hAG-Y	&	X-Ash	
8	hAG-Y	&	Ash-X	
9	X-hAG	&	Ash	Negative Control for 1 and 2
10	hAG-X	&	Ash	Negative Control for 3 and 4
11	Y-hAG	&	Ash	Negative Control for 5 and 6
12	hAG-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 GFP/FITC can be used for hAG.

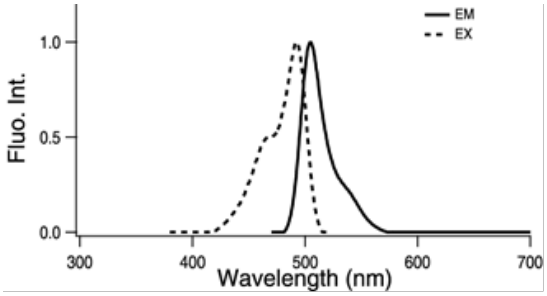
NOTE

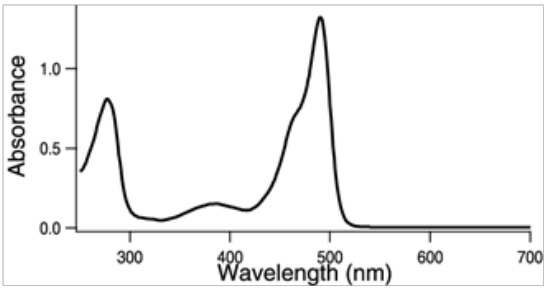
Fluoppi Does Not Guarantee

Detection of all Protein-Protein Interactions.

5. Properties of Fluorescent protein “hAG”

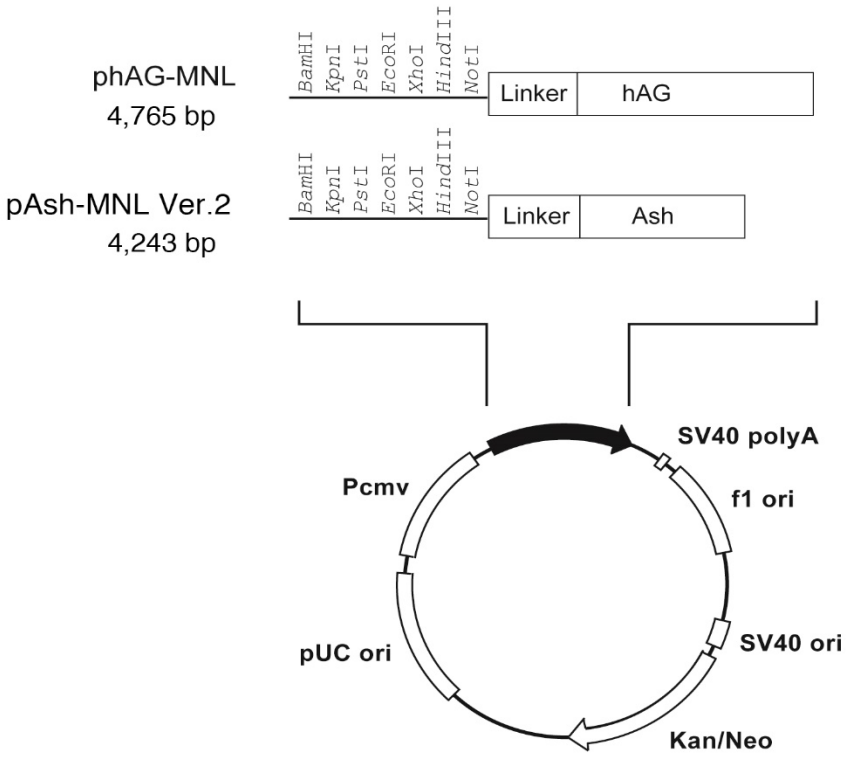
CoralHue® humanized Azami-Green (hAG), cloned from the stony coral (azami-sango in Japanese), absorbs light maximally at 492 nm and emits green light at 505 nm. hAG forms tetramer and is featured by its fast maturation and high stability nature. The gene codon is optimized for mammalian cells.





Fluorescent protein	Excitation/Emission maximum (nm)	Extinction coefficient ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	pKa
hAG	492/505	72,300 (492 nm)	0.67	<5.0

6. Plasmid Maps



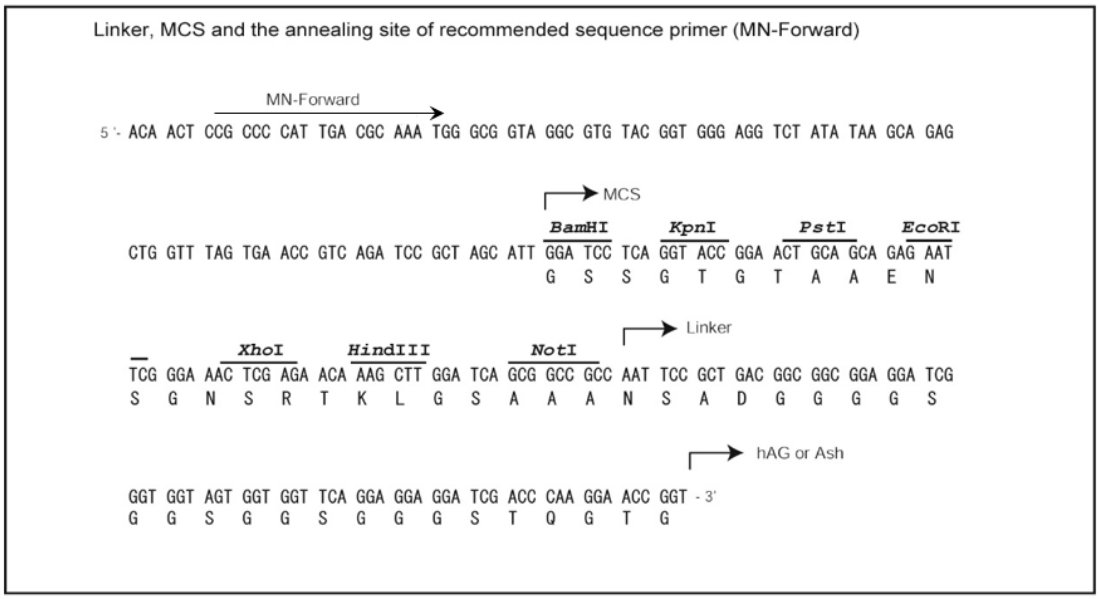
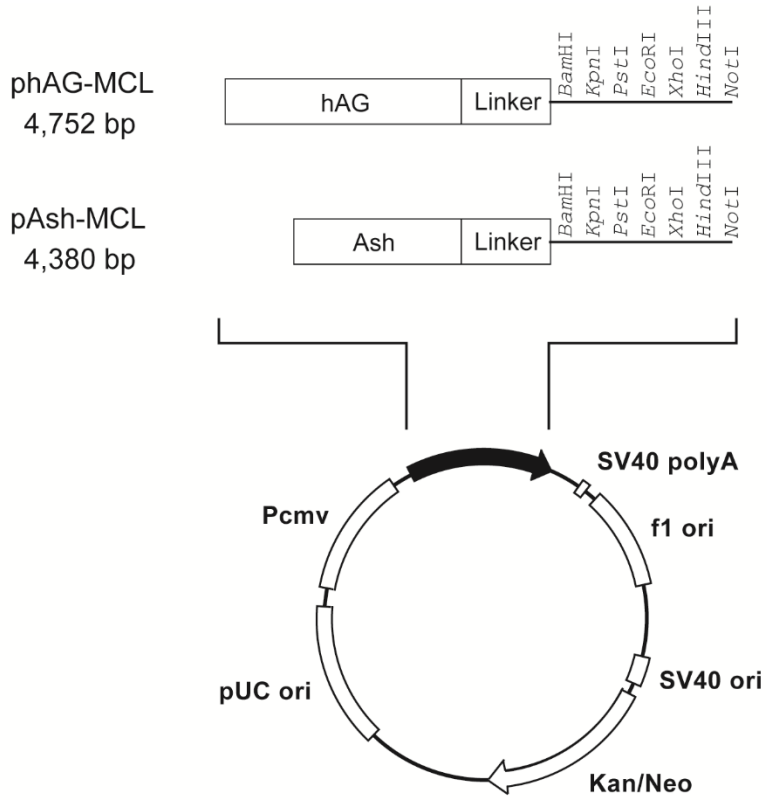


Figure 4 | Plasmid Map of phAG-MNL and pAsh-MNL Ver.2



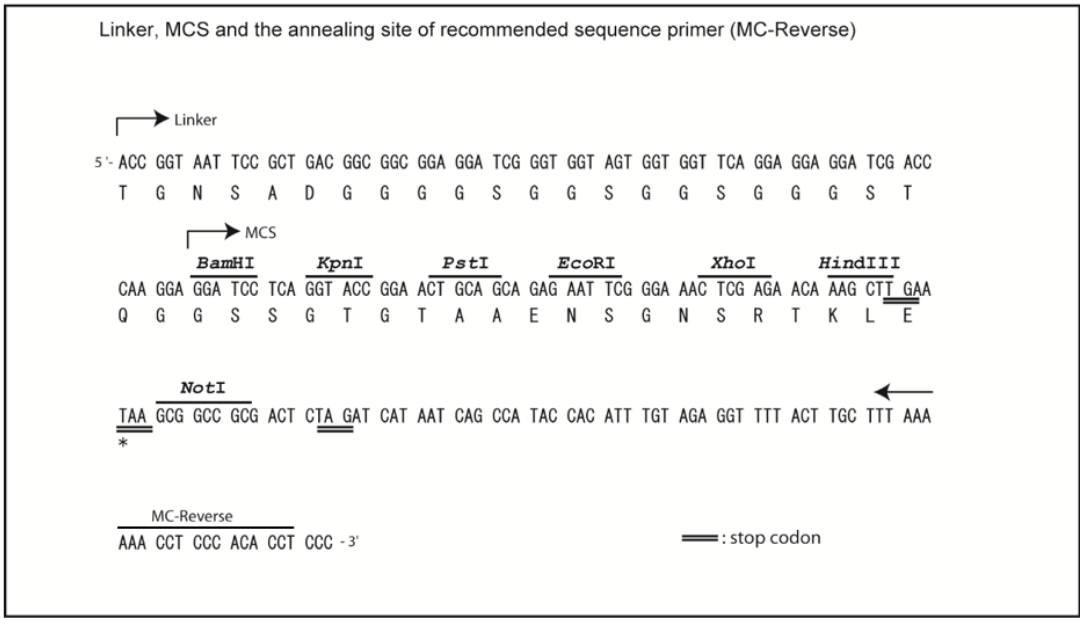


Figure 5 | Plasmid Map of phAG-MCL and pAsh-MCL

Sequence information of all 4 plasmids can be downloaded from MBL's website.
https://ruo.mbl.co.jp/product/flprotein/dna-sequence.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

7. References

1) Koyano F *et al*. Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature (2014) [PMID: 24784582]
2) Karasawa S *et al*. A green-emitting fluorescent protein from Galaxeidae coral and its monomeric version for use in fluorescent labeling. J Biol Chem. 278, 34167-71. (2003) [PMID: 12819206]

8. License

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Printed Apr. 2023/Version 2

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