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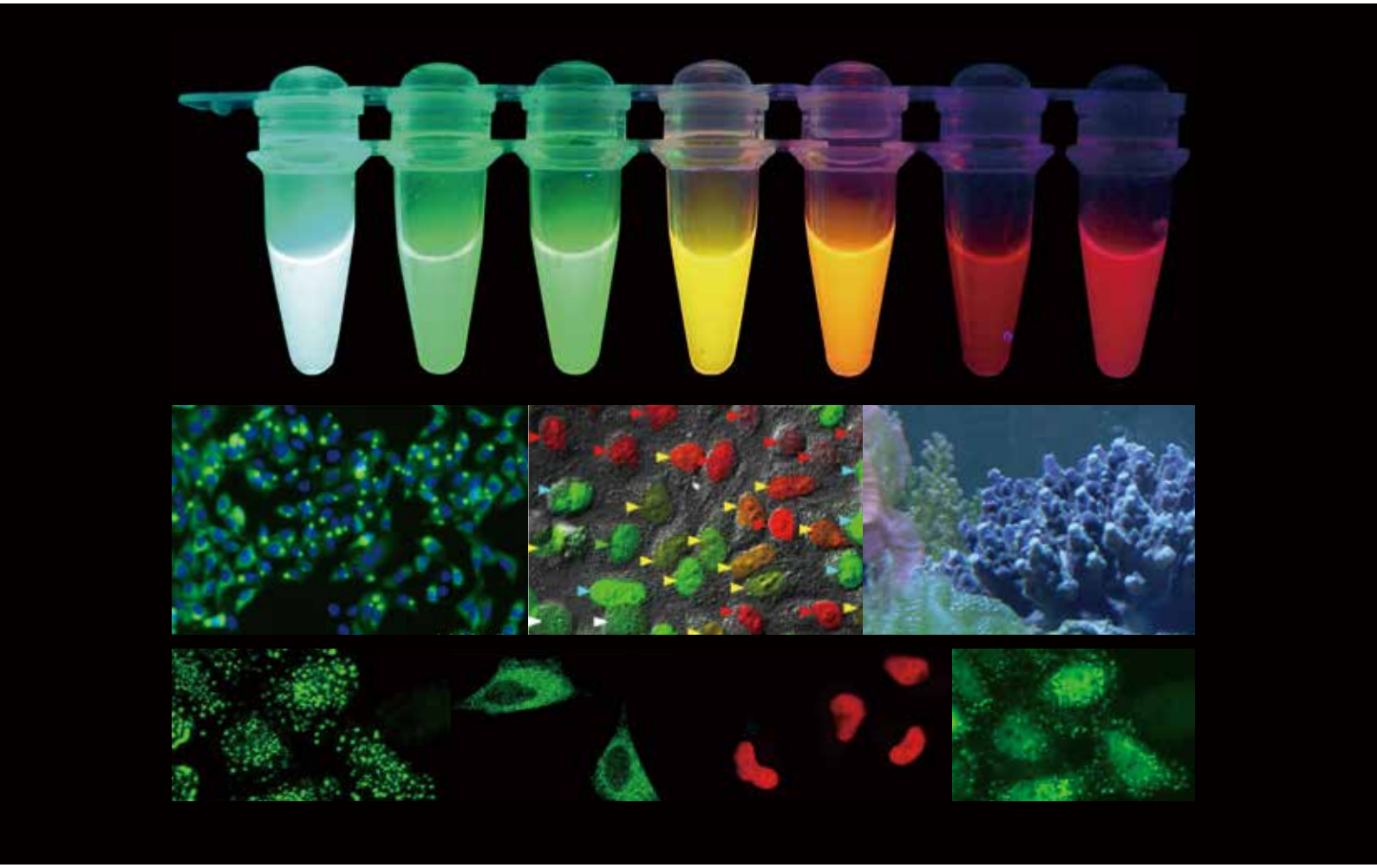
Amalgaam
Fluorescent proteins



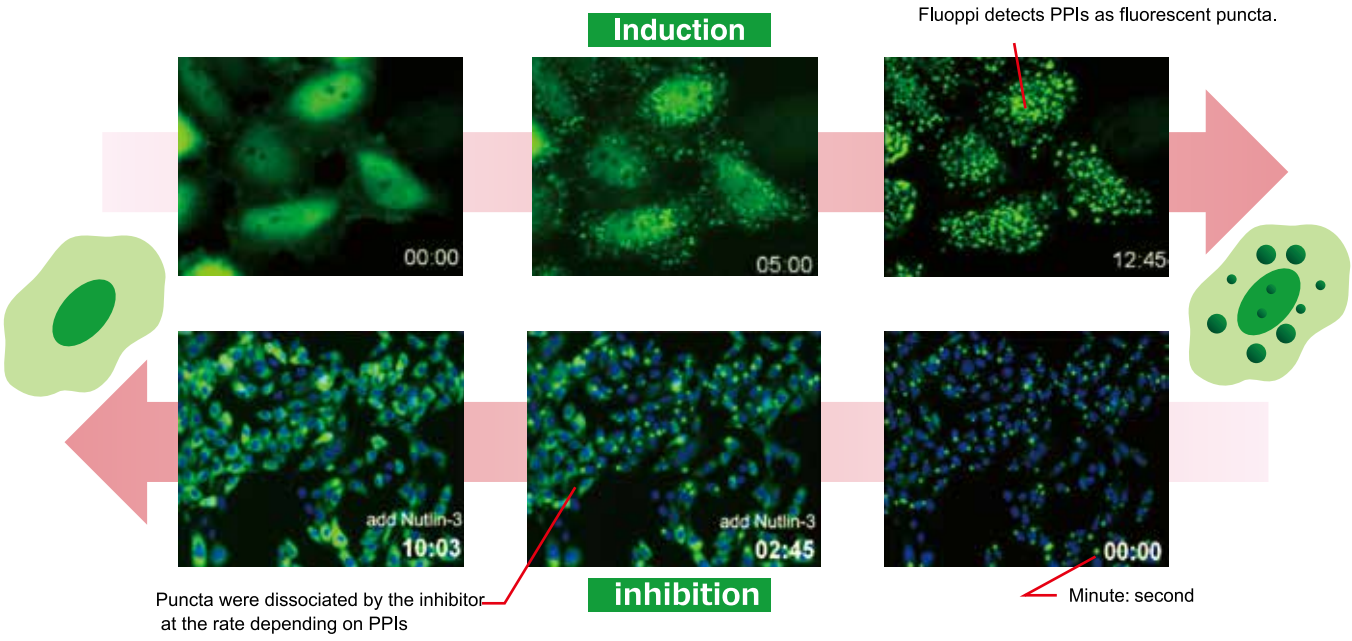
- Protein-Protein Interactions Detection System
- Advanced Fluorescent Indicator
- Basic Fluorescent Proteins
- Antibodies

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

Fluoppi

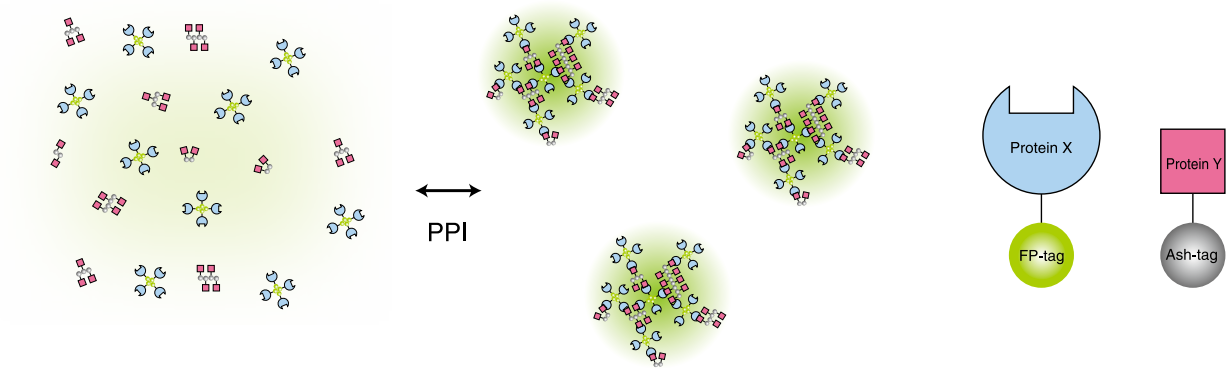
Novel technology for detecting PPIs

- **Visualize PPI as fluorescent "Puncta" in living cells**
- **"Reversible" puncta-formation**
- **Easy to construct an experimental system**
- **Ideal tool for drug screening**



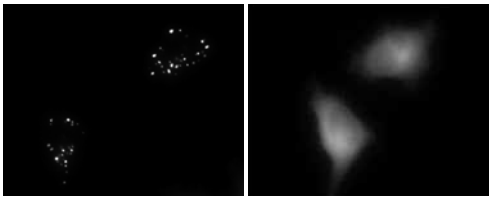
Mechanism of action

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 puncta 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. Because each fusion protein has multiple Xs or Ys, interaction between protein X and Y causes large lattice like complexes where the fluorescence by X-FP is concentrated and detectable as fluorescent puncta.

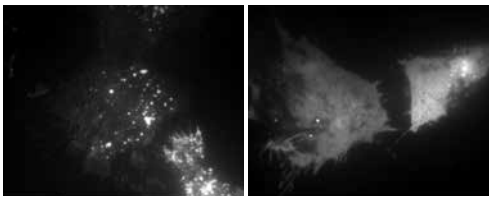


Localization

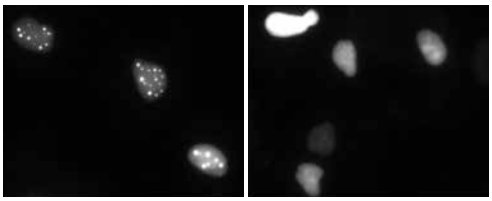
Because location of puncta is not restricted to specific site inside the cell, Fluoppi can detect PPIs at several subcellular localizations such as cytosol, nucleus, and juxtamembrane.
The left pictures represent puncta at several subcellular localizations, and the right pictures are negative controls which express hAG tagged protein and Ash-tag without fusing any proteins.
The images of juxtamembrane are taken by Total Internal Reflection Fluorescence Microscopy (TIRFM).



PPI in the cytosol　　Negative control



PPI at juxtamembrane　　Negative control

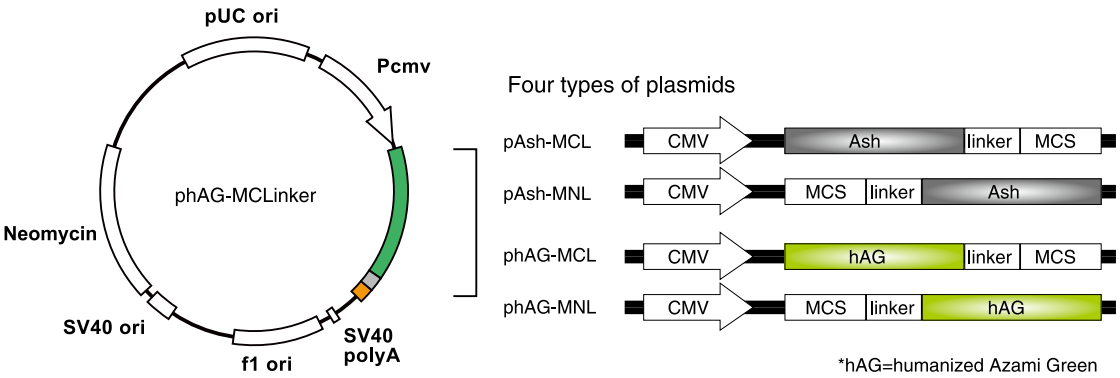


PPI in the nucleus　　Negative control

Workflow

Fluoppi tags are able to work in both *N* and *C* terminal fusion. We have several plasmid vectors which include CMV promoter, Fluoppi tag, flexible peptide linker, Multiple Cloning Site (MCS) and Neomycin resistant gene.
At first, proteins X & Y of your interest are fused to FP-tag and Ash-tag respectively. We recommend to prepare all the eight possible constructs to identify the best workable combination.

Because fluorescent signal of Fluoppi is very high, conventional fluorescence microscopy can be used to image the cell.
If the proteins interact with each other upon expression, fluorescent puncta will be detected. Formation of puncta is reversible so that they can be dissociated and the fluorescent signal will spread over the cell by PPI inhibitors, and vice versa by PPI inducers.



"Flexible"peptide linker (22 aa):
N term.- NSADG GGGSG GSGGS GGGST QG – *C* term.

Fluorescent proteins	Code No.	Products	Volume
Monti-Red (Red)	AM-8012M	Fluoppi Ver.2 : Ash-Red (Ash-MNL/MCL + Monti-Red-MNL/MCL)	10 μg each
	AM-VS0802M	Monti-Red for Fluoppi (pMonti-Red-MNL/MCL)	10 μg each
hAG (Green)	AM-8011M	Fluoppi Ver.2 : Ash-hAG (Ash-MNL/MCL + hAG-MNL/MCL)	10 μg each
	AM-8201M	Fluoppi : Ash-hAG [p53-MDM2]	10 μg each
	AM-8202M	Fluoppi : Ash-hAG [mTOR-FKBP12]	10 μg each
	AM-VS0801M	humanized Azami-Green for Fluoppi (phAG-MNL/MCL)	10 μg each

- This kit consists of four types of plasmids (pAsh-MCL, pAsh-MNL, phAG-MCL, phAG-MNL)
- The use of these products requires a license from MBL Co., Ltd. MBL grants non-profit research organizations the right to use the product for non-commercial research purpose. For commercial entities a commercial license is required. For more information, please contact support@mbl.co.jp
- Fluoppi does not guarantee detection of all Protein-Protein Interactions.
- The fluorescent proteins used in product, hAzami-Green and Monti-Red, differ from each other in fluorescence and other properties.