Applications for Addgene's Fluorescent Plasmids



Addgene has assembled a collection of over 4,500 fluorescent plasmids in a rainbow colors and applications including UV, photoactivatable, near-infrared, far red, and everything in between for single and multicolor imaging.





Optogenetics (for monitoring and measuring cellular activity with light) - The field of optogenetics integrates optics and genetic engineering approaches and technology to detect, measure, and control molecular signals, cells, and groups of cells in order to understand their activity and the effects of alterations to this activity. Addgene's collection represents two functions in optogenetics: **actuators** (genetically-encoded tools for light-activated control of neurons; e.g. microbial opsins) and **sensors** (genetically-encoded reporters of molecular signals; e.g. calcium indicators). (Browse the plasmids and learn more: http://www.addgene.org/optogenetics/)



FRET- The Förster resonance energy transfer (FRET) process transfers energy from an excited donor fluorophore to an acceptor protein and is measured using fluorescence microscopy. Since the transfer of energy does not occur by emission of a photon, the acceptor molecule is not required to be fluorescent. FRET is used to study protein-protein interactions (where each protein is separately fused to a donor or acceptor molecule) or conformational changes within a protein (where the donor and acceptor are both fused to the same protein). (Browse the plasmids and learn more: http://www.addgene.org/fluorescent-proteins/FRET/)



Subcellular Localization - Determining where your protein of interest is subcellularly localized can help with understanding its function. By examining the overlap between your tagged protein and a marker fusion protein using fluorescence microscopy, you can assess whether your protein is targeted to a known cellular structure.

(Browse the plasmids and learn more: http://www.addgene.org/fluorescent-proteins/localization/)



In vivo **Imaging** (for single or multicolor imaging) - Because signals emitting below 650nm are highly absorbed by hemoglobin causing undesirable background signals, stable imaging of individual plasmids or protein-protein interactions in whole mammals and live organs requires genetically encoded fluorescent probes specifically engineered to emit near or above 650nm. (Browse the plasmids and learn more: *http://www.addgene.org/fluorescent-proteins/in-vivo/*)



Empty Backbones (for creating fusion proteins) - By fusing your protein of interest with a FP tagged plasmid, you can study your protein's localization and/or function using fluorescent microscopy. (Browse the plasmids and learn more: http://www.addgene.org/fluorescent-proteins/plasmid-backbones/)

For questions about plasmids, shipping, or ordering, please contact help@addgene.org

Addgene plasmids are \$65 USD and are available worldwide to academic scientists.

See Addgene's Entire Fluorescent Plasmid Collection www.addgene.org/fluorescent-proteins

Addgene's Fluorescent Protein Collection

Fluorescent proteins are genetically encoded probes that are used extensively by life scientists for many applications. The original green fluorescent protein (GFP) was cloned in 1992 (Prasher et al., Gene, 1992), and since then scientists have engineered numerous GFP-variants and non-GFP proteins that result in a diverse set of colors.



Consider Excitation and Emission

- Each fluorophore has a specific excitation and emission range with at least one peak.
- Check to make sure your imaging equipment is compatible with your FPs.
- Make sure your FPs' excitation/emission spectra are sufficiently apart when multi-color imaging.

Some Additional Considerations:

- As FPs are exposed to excitation light, the fluorescent molecules lose the ability to emit light (known as photostability, photobleaching).
- FPs all have different levels of brightness. Brightness is calculated as the product of the extinction coefficient and quantum yield, divided by 1,000.

The excitation/emission, intensity, or maturation time of your FP may be altered by the pH, oxygen levels, or temperature of the environment that FP is expressed in. Browse popular fluorescent plasmids below or view the entire fluorescence collection at *www.addgene.org/fluorescent-proteins*

FP	Excitation	Emission	Sample Empty Backbone Plasmids
EBFP2	383nm	448nm	pBad-EBFP2 (#14891) (Bacterial expression) pEBFP2-Nuc (#14893) (Mammalian expression)
Cerulean	433nm	475nm	Cerulean (#15214) (Mammalian expression)
ECFP	433nm	475nm	pE5c (#17465) (Mammalian, Bacterial, Yeast, Insect expression)
Clover	505nm	515nm	pcDNA3-Clover (#40259) (Mammalian expression)
EGFP	488nm	507nm	GFP-ERK1 (#14747) (Mammalian expression) pK7WGF2::hCas9 (#46965) (Plant expression)
Venus	515nm	528nm	pCAGGS-ChR2-Venus (#15753) (Mammalian expression) ampkar (#35097) (Bacterial expression)
EYFP	513nm	527nm	pCAG-YFP (#11180) (Mammalian expression)
mOrange	548nm	562nm	pCAG2LMKOSimO (#20866) (Mammalian expression)
mCherry	587nm	610nm	pDS221 (#34981) (Yeast expression) pCS2+8NmCherry (#34936) (Mammalian, Sea urchin, Zebrafish, Xenopus expression)
mNeptune	600nm	650nm	pcDNA3-mNeptune2-N (#41645) (Mammalian expression)
iRFP670	643nm	670nm	piRFP670-N1 (#45457) (Mammalian expression)



Read more and get additional information about using FPs, visit www.addgene.org/fluorescent-proteins

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