For Research Use Only.
Not for use in diagnostic procedures.

Amalgaam
MBL

Fucci (*Fluorescent Ubiquitination-based Cell Cycle Indicator*) series

pFucci-S/G₂/M Green (N+C) (Cloning vector)

Code No.	Quantity
AM-V9034M	20 µg

VECTOR DESCRIPTION:

AM-V9034M pFucci-S/G₂/M Green (N+C) is a Cloning vector encoding ***CoralHue***® humanized monomeric Azami-Green 1 (hmAG1) fused to a part of human Geminin (hGeminin). pFucci-S/G₂/M Green (N+C) can trace the silhouette of individual cells in S/G₂/M phases with fluorescence.

"Fucci" stands for *Fluorescent Ubiquitination-based Cell Cycle Indicator.*

Geminin is an inhibitor of the DNA replication licensing factor. It accumulates during the S, G₂, and M phases, but is degraded during G₁ phase by ubiquitin-mediated proteolysis. A part of hGeminin (1-60) is also degradable in a cell cycle dependent manner.

CoralHue® hmAG1 sequence is codon-optimized for higher expression in mammalian cells. ***CoralHue***® monomeric AG 1 (mAG1) has been generated from tetrameric ***CoralHue***® Azami-Green (AG).

SOURCE: The ***CoralHue***® AG gene was cloned from a stony coral (*Galaxea fascicularis*).

FORMULATION: Dry form. Reconstitute with distilled water or TE before use.

PURITY: A260/A280 > 1.5

STORAGE: Stored at -20°C

SEQUENCE LANDMARKS:
Fucci-S/G₂/M Green (N+C) gene (including stop codon): bases 2264-3154
Ampicillin resistance gene: bases 200-1059
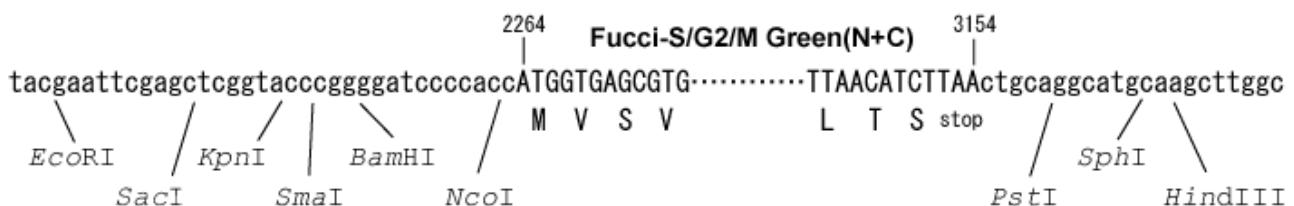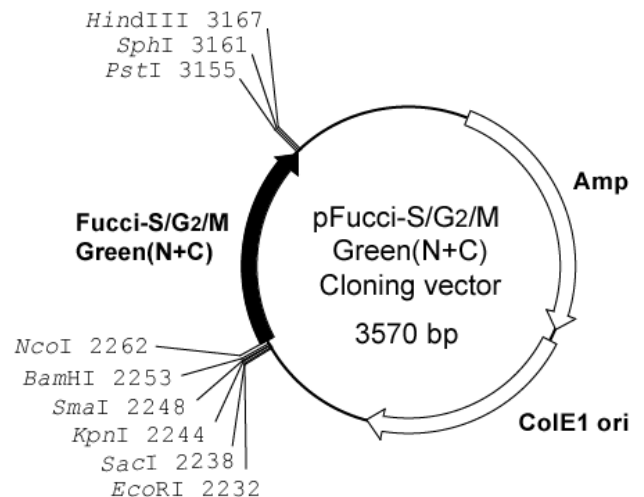*Col*E1 origin: bases 1062-2002

REFERENCES:
1) Sakaue-Sawano, A., *et al., Cell.* **132**, 487-498 (2008)
2) Nakayama, K. I., *et al., Nat. Rev. Cancer.* **6**, 369-381 (2006)
3) Blow, J. J., and Dutta, A., *Nat. Rev. Mol. Cell Biol*. **6**, 476-486 (2005)
4) Nishitani, H., *et al., J. Biol. Chem.* **279**, 30807-30816 (2004)
5) Karasawa, S., *et al., J. Biol. Chem.* **278**, 34167-71 (2003)
6) Nishitani, H., *et al*., *Nature.* **404**, 625-628 (2000)

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

NOTICES:

1) pFucci-S/G₂/M Green (N+C) (Cloning vector) is not expression vector. When pFucci-S/G₂/M Green (N+C) is expressed in any cells, the cDNA must be transferred to appropriate expression vectors by your own.
2) Val (encoded by GTG) is inserted as the second amino acid of ***CoralHue***® hmAG1 to form the Kozak sequence.
3) It is recommended that Fucci be stably expressed.

RELATED PRODUCTS:

AM-V9030M   pFucci-S/G$_2$/M Green (N+C)-Hyg (Expression vector)
AM-V9014M   pFucci-S/G$_2$/M Green (Cloning vector)
AM-V9016M   pFucci-S/G$_2$/M Green (Expression vector)
AM-V9010M   pFucci-S/G$_2$/M Green-Hyg (Expression vector)
AM-V9001M   pFucci-G$_1$ Orange (Cloning vector)
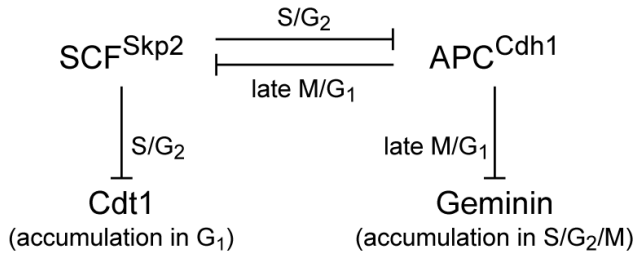AM-V9003M   pFucci-G$_1$ Orange (Expression vector)
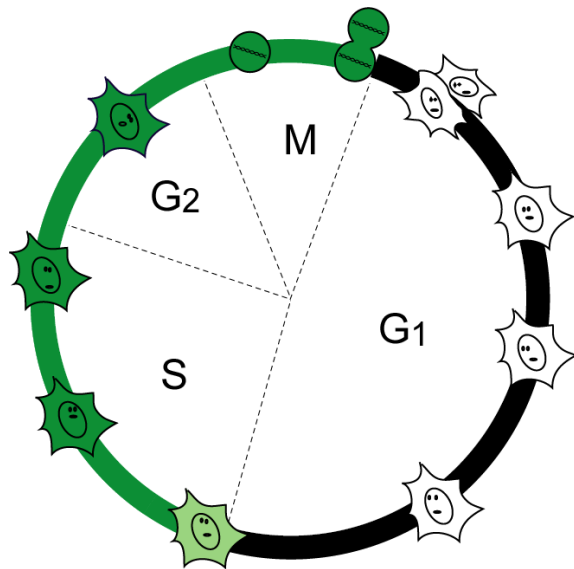

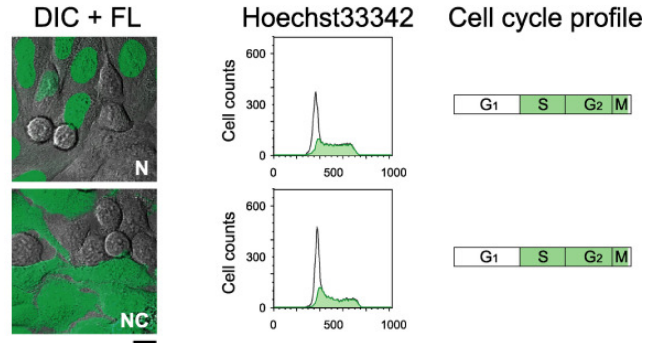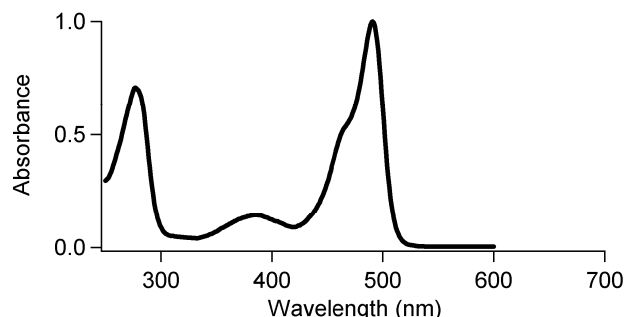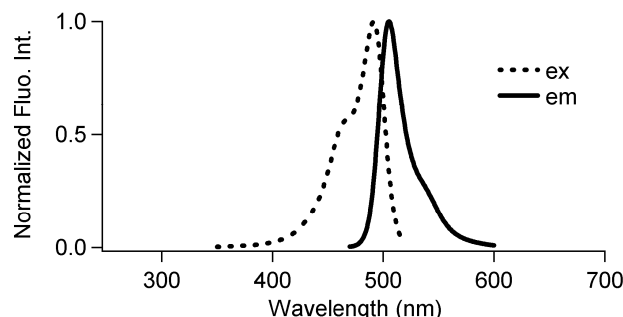Fig 1. Cell cycle regulation by SCF^{Skp2} and APC^{Cdh1}

Fig 3. HeLa cells stably expressing S/G$_2$/M Markers.
(DIC + FL) Typical differential interference contrast (DIC) and fluorescence (FL) images of HeLa cells stably expressing constructs. Distribution patterns are indicated as follows: N, nucleus; C, cytosol; NC, nucleus and cytosol. Scale bar, 10 μm.
(Hoechst33342) HeLa cells stably expressing S/G2/M markers were stained with Hoechst33342 and analyzed using fluorescence-activated cell sorting.
(Cell cycle profile) Cell cycle phases highlighted by fluorescence are colored.

Fig 2. Schematic of the cell cycle specific fluorescence of Fucci-S/G$_2$/M Green (N+C).

CoralHue® **mAG1:** 226 amino acids

	Excit./Emiss.Maxima (nm)	Extinction Coefficient(M^{-1}cm^{-1})	Fluorescence Quantum Yield	pH sensitivity
mAG1	492/505	55,500 (492 nm)	0.74	pKa=5.8

Fucci-S/G$_2$/M Green (N+C)
1) DNA sequence

ATGGTGAGCGTGATCAAGCCCGAGATGAAGATCAAGCTGTGC
ATGAGGGGCACCGTGAACGGCCACAACTTCGTGATCGAGGGC
GAGGGCAAGGGCAACCCCTACGAGGGCACCCAGATCCTGGAC
CTGAACGTGACCGAGGGCGCCCCCCTGCCCTTCGCCTACGAC
ATCCTGACCACCGTGTTCCAGTACGGCAACAGGGCCTTCACC
AAGTACCCCGCCGACATCCAGGACTACTTCAAGCAGACCTTC
CCCGAGGGCTACCACTGGGAGAGGAGCATGACCTACGAGGAC
CAGGGCATCTGCACCGCCACCAGCAACATCAGCATGAGGGGC
GACTGCTTCTTCTACGACATCAGGTTCGACGGCACCAACTTC
CCCCCCAACGGCCCCGTGATGCAGAAGAAGACCCTGAAGTGG
GAGCCCAGCACCGAGAAGATGTACGTGGAGGACGGCGTGCTG
AAGGGCGACGTGAACATGAGGCTGCTGCTGGAGGGCGGCGGC
CACTACAGGTGCGACTTCAAGACCACCTACAAGGCCAAGAAG
GAGGTGAGGCTGCCCGACGCCCACAAGATCGACCACAGGATC
GAGATCCTGAAGCACGACAAGGACTACAACAAGGTGAAGCTG
TACGAGAACGCCGTGGCCAGGTACTCCATGCTGCCCAGCCAG
GCCAAGGGGATATCCATCACACTGGCGGCCGCTCGAGATGAAT
CCCAGTATGAAGCAGAAACAAGAAGAAATCAAAGAGAATATA
AAGAATAGTTCTGTCCCAAGAAGAACTCTGAAGATGATTCAG
CCTTCTGCATCTGGATCTCTTGTTGGAAGAGAAAATGAGCTG
TCCGCAGGCTTGTCCAAAAGGAAACATCGGAATGACCACTTA
ACATCT

2) Amino acid sequence

MVSVIKPEMKIKLCMRGTVNGHNFVIEGEGKGNPYEGTQILDLN
VTEGAPLPFAYDILTTVFQYGNRAFTKYPADIQDYFKQTFPEGY
HWERSMTYEDQGICTATSNISMRGDCFFYDIRFDGTNFPPNGPV
MQKKTLKWEPSTEKMYVEDGVLKGDVNMRLLLEGGGHYRCDFKT
TYKAKKEVRLPDAHKIDHRIEILKHDKDYNKVKLYENAVARYSM
LPSQAKGYPSHWRPLEMNPSMKQKQEEIKENIKNSSVPRRTLKM
IQPSASGSLVGRENELSAGLSKRKHRNDHLTS