Living Colors®
User Manual Volume II:

Reef Coral Fluorescent Proteins

AmCyan
AsRed
DsRed
DsRed-Express
HcRed
ZsGreen
ZsYellow

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I. Introduction

Living Colors® Reef Coral Fluorescent Proteins (RCFPs) provide a valuable, non-invasive approach for investigating biological events in living cells and tissues. Ranging in color from cyan to far red, RCFPs can be used to visualize, track, and quantify many different cellular processes, including protein synthesis and turnover, protein translocation, gene expression, and cell lineage. Because they require no additional substrates or cofactors for their fluorescence, Reef Coral Fluorescent Proteins are ideal for use in live cell assays. And because of their distinctive spectra, they can be readily multiplexed—that is, combined for the simultaneous detection of two or more events in the same cell or mixed cell population.

Origins of Reef Coral Fluorescent Proteins

Reef Coral Fluorescent Proteins are derived from a group of reef corals native to the Indian and Pacific oceans (Matz *et al.,* 1999). The wild-type cDNAs were originally isolated by Matz and coworkers, while searching for GFP homologues in the colored body parts of *Anthozoan* corals (Matz, *et al*., 1999). Using a PCR-based 3'-RACE (Rapid Amplification of 3' cDNA ends) method and a set of degenerate primers corresponding to different domains in *Aequorea victoria* Green Fluorescent Protein (GFP), they amplified a number of cDNAs encoding a group of GFP-like fluorescent proteins, now known as AmCyan, ZsGreen, ZsYellow, DsRed, AsRed, and HcRed (Matz, *et al*., 1999; Lukyanov, *et al.,* 2000; and Gurskaya, *et al.,* 2001).

Optimized for bright fluorescence and fast chromophore maturation

To adapt these proteins for use as *in vivo* reporters, a series of mutations have been introduced into the corresponding full-length cDNAs to produce RCFPs with higher solubility, brighter emission, and more rapid chromophore maturation. In addition, human codon-optimized versions of each cDNA have been created for efficient translation in mammalian cells (Table I).

Specific information concerning the derivation and optimization of each RCFP can be found in the corresponding Vector Information Packet, provided with each vector and available on our web site. These packets describe the essential features of the corresponding RCFP—including the position and effect of certain key mutations—and they cite literature references when available.

I. Introduction *continued*

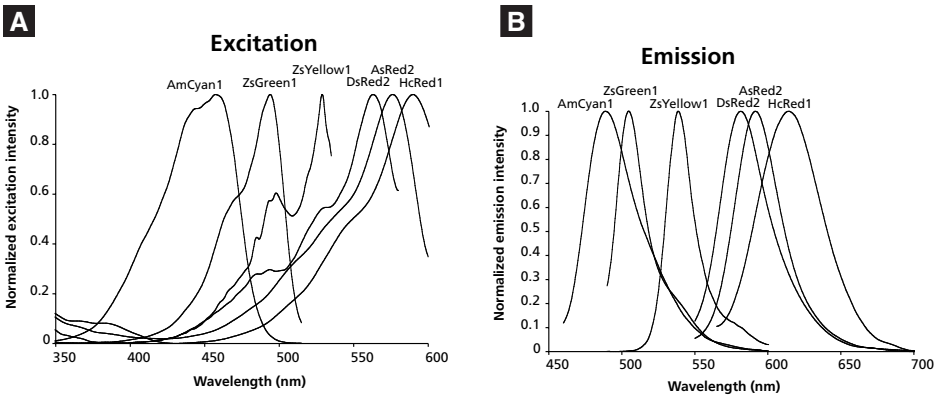

Figure 1. Excitation and emission spectra of Living Colors® Reef Coral Fluorescent Proteins. Panel A. Excitation. **Panel B.** Emission. Curves are normalized; the height of each curve is not an indication of relative signal strength per fluorophore. The spectra for DsRed-Express (not shown) are similar to those for DsRed2.

TABLE I. GENERAL INFORMATION AND NOMENCLATURE

RCFP[a]	Species of origin	Human codon-optimized	Notable properties[b]
AmCyan AmCyan1	*Anemonia majano*	No Yes	Brighter than ECFP; photostable alternative to ECFP
ZsGreen ZsGreen1	*Zoanthus* sp.	No Yes	Brighter than EGFP
ZsYellow ZsYellow1	*Zoanthus* sp.	No Yes	True yellow emission; ideal for multcolor applications
DsRed-Express	*Discosoma* sp.	Yes	Preferred DsRed for FACS[c]
DsRed2	*Discosoma* sp.	Yes	Increased solubility variant of DsRed1
AsRed2	*Anemonia sulcata*	Yes	
HcRed1	*Heterectis crispa*	Yes	Far-red fluorescence; highly soluble; dimer

[a] The amino acid sequences of AmCyan and AmCyan1 are identical, only the codon usage has been changed to enhance the translation in mammalian cells. Similarly, ZsGreen has the same amino acid sequence as ZsGreen1; and ZsYellow has the same amino acid sequence as ZsYellow1.

[b] ECFP and EGFP = Enhanced Cyan and Green Fluorescent Proteins, respectively.

[c] Because of the low level of residual green emission from DsRed-Express, cells expressing this protein are readily separated from those expressing a true green fluorescent protein such as EGFP or ZsGreen.

I. Properties of Reef Coral Fluorescent Proteins

A. Structure and Formation of the RCFP Fluorophore

The RCFP fluorophore is believed to consist of a cyclized tripeptide located on the central α-helix in the core of the protein. It forms autocatalytically, within several hours of protein syntheis by way of an intramolecular reaction involving a highly conserved set of amino acid side chains, just as in GFP (Matz *et al.,* 1999; Gross *et al.*, 2000). The formation of the fluorophore does not require any other agents except molecular oxygen.

B. Photophysical Properties of RCFP Proteins

RCFPs range in color from cyan to far-red (Table II). In general, they have broad excitation spectra and narrow emission spectra (Figure 1). Like the enhanced color variants of *Aequorea* GFP, RCFPs can be detected in cells and tissues without having to add additional cofactors or substrates, and they are extremely stable, making it easy to monitor fluorescence over extended periods of time.

RCFP monomers share structural homology to *Aequorea victoria* green fluorescent protein (Wall *et al.*, 2000; Yarbrough *et al.*, 2001). Unlike GFP, however, RCFP monomers self associate in solution to form higher order multimers such as dimers and tetramers. Most are believed to be obligate tetramers with structures similar to that of wild-type DsRed (Yarbrough *et al.*, 2001). The only exception so far noted is HcRed1, shown to be a dimer (Gurskaya *et al.,* 2001).

Because of their multimeric structures and their tendency to aggregate, RCFPs are not generally recommended for use as fusion tags. Still, many published studies show that RCFPs can be fused to a protein of interest without interfering with its normal biological function (Shulz *et al.,* 2002; Nelson *et al.,* 2002; Engqvist-Goldstein *et al.,* 2001; Tsuboi *et al.,* 2002; Nechushtan *et al.,* 2001). Some RCFPs such as DsRed2 and DsRed-Express, improved through mutagenesis, are considered to be more soluble, and therefore less prone to aggregation, than other RCFPs (Table II). Nevertheless, if you are primarily interested in constructing fluorescent fusion proteins for localization studies, we suggest you first try one of our enhanced GFP color variants: ECFP, EGFP, or EYFP. If desired, compare your results to those obtained with a corresponding RCFP fusion to find out whether the RCFP interferes with the function or distribution of the protein.

II. Properties of Reef Coral Fluorescent Proteins *continued*

TABLE II. PHOTOPHYSICAL PROPERTIES OF REEF CORAL FLUORESCENT PROTEINS

Protein	Excitation Max (nm)	Emission Max (nm)	Time to detection[b] (hr)	Brightness relative to EGFP	Quaternary structure	Utility as a reporter	Utility in fusions
Reef Coral Fluorescent Proteins							
AmCyan1[a]	458	489	8–12	+++	Tetramer	+++	+
ZsGreen1[a]	493	505	8–12	++++	Tetramer	++++	+
ZsYellow1[a]	529	539	8–12	++	Tetramer	+++	+
DsRed-Express	557	579	8–12	+++	Tetramer	+++	++
DsRed2	563	582	24	+++	Tetramer	+++	++
AsRed2[a]	576	592	8–12	++	Tetramer	+++	+
HcRed1	588	618	16	+	Dimer	+	+++
***Aequorea Victoria* GFP variants**							
ECFP	439	476	8–12	+	Monomer	+	++++
EGFP	484	510	8–12	+++	Monomer	+++	++++
EYFP	512	529	8–12	+++	Monomer	+++	++++

[a] Prone to aggregation

[b] As measured by FACS analysis using transiently transfected mammalian cell cultures

II. Properties of Reef Coral Fluorescent Proteins *continued*

C. *Discosoma* sp. Red Fluorescent Protein (DsRed) and Its Variants

Wild type *Discosoma sp.* red fluorescent protein, known commercially as DsRed, was originally isolated by Matz *et al.* (1999), who referred to the protein as drFP583. Since then, numerous variants have been developed, including DsRed2, DsRed-Express, and DsRed1-E5—the Fluorescent Timer (Figure 2).

Wild-type DsRed has been extensively studied and characterized (Baird *et al.*, 2000; Gross *et al.*, 2000; Heikal *et al.*, 2000; Jakobs *et al.*, 2000; Vrzheshch *et al.,* 2000; Wall *et al.*, 2000). Much of the work has been devoted to learning more about the DsRed fluorophore: its structure, as well as its light absorbing and emitting properties.

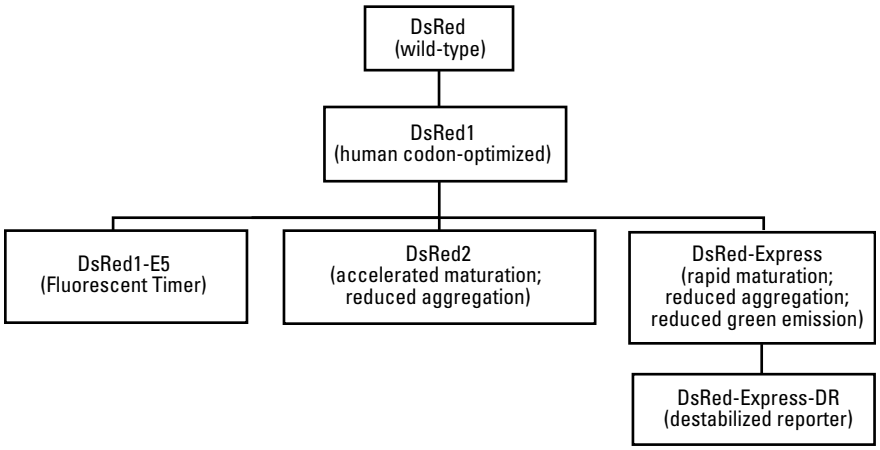

Figure 2. Genealogy of DsRed proteins.

II. Properties of Reef Coral Fluorescent Proteins *continued*

1. DsRed2

DsRed2 is a faster-maturing, more soluble variant of DsRed1 (July 2001 *Clontechniques*). It contains six amino acid substitutions (Table III), which result in the more rapid appearance of red fluorescence and reduce the protein's tendency to aggregate. Although it probably forms the same tetrameric structure as DsRed1 (Yarbrough *et al.,* 2001), DsRed2 is less prone to forming insoluble aggregates, often observed in bacterial and mammalian cell systems expressing DsRed1.

2. DsRed-Express

DsRed-Express is a variant of DsRed1 improved through a combination of site-directed and random mutagenesis (Bevis, B. J., *et al.,* 2002). It contains nine amino acid substitutions (Table III), which not only improve the protein's maturation and solubility, but also reduce the level of residual green emission, making DsRed-Express the preferred DsRed for flow cytometry. The reduction in green fluorescence allows for complete separation of red-emitting and true green-emitting populations (July 2002 *Clontechniques*). Though DsRed-Express appears to have a lower quantum yield and extinction coefficient than either DsRed2 or DsRed1 (Bevis, B. J., *et al.,* 2002), its lower intensity is offset by its accelerated maturation, which ultimately results in a more rapid build-up of red fluorescence in the cell.

TABLE III. A COMPARISON OF THE POINT MUTATIONS IN DsRed2 & DsRed-Express

DsRed2	DsRed-Express	Effect
R2A K5E K9T	R2A K5E N6D	Enhanced solubility
V105A I161T S197A	T21S H41T N42Q V44A	Accelerated maturation
	C117S T217A	Reduced green emission

II. Properties of Reef Coral Fluorescent Proteins *continued*

3. DsRed1-E5 (Fluorescent Timer)

DsRed1-E5, the Fluorescent Timer, a variant of DsRed1, contains two amino acid substitutions (V105A and S197T) which increase its fluorescence intensity and endow it with a distinct spectral property: As the protein ages, it changes color (Figure 3; Terskikh *et al.*, 2000). Shortly after its synthesis, DsRed1-E5 begins emitting green fluorescence. But as time passes, the fluorophore undergoes additional changes that shift its fluorescence to longer wavelengths—when fully matured the protein is bright red. In mammalian cells transfected with a Tet-inducible DsRed1-E5 expression vector, the green-to-red transition starts about 3 hours after the protein first becomes fluorescent (Terskikh *et al.*, 2000).

The protein's predictable color shift can be used to follow the on and off phases of gene expression (e.g., during embryogenesis and cell differentiation). And with DsRed1-E5, the green and red emissions are easily detected by fluorescence microscopy and flow cytometry. During the first few hours of DsRed1-E5 expression *in vivo*, host cells appear green. Continued expression results in a mixture of green and red fluorescence—host cells appear yellow when the emissions are overlayed. If expression stops, host cells will gradually turn red. Thus DsRed1-E5 lets you track not only up-regulation, but also down-regulation of gene expression. For more information about the properties and utility of DsRed1-E5, please see Terskikh *et al.* (2000) and April 2001 *Clontechniques*.

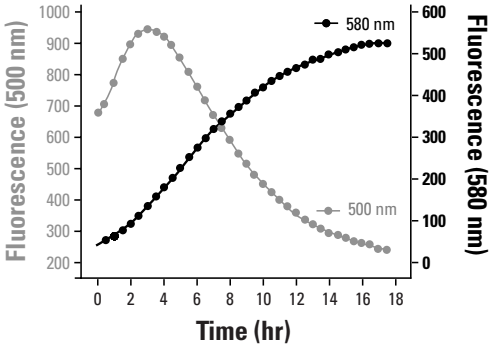

Figure 3. The Fluorescent Timer (DsRed1-E5) changes from green to red over time. Measurements were made using recombinant DsRed1-E5, freshly purified from an overnight *E. coli* culture. The protein was purified at 4°C using a TALON™ Metal Affinity Resin and ice-cold buffers. Purified protein was stored on ice until $t = 0$ hr, when it was placed at 37°C and the first measurement was recorded.

II. Properties of Reef Coral Fluorescent Proteins *continued*

4. DsRed-Express-DR (destabilized reporter)

DsRed-Express-DR is a destabilized variant of DsRed-Express. In contrast to the original protein, which is extremely stable, DsRed-Express-DR has a short half-life, making it well suited for studies that require rapid reporter turnover. DsRed-Express-DR was constructed by fusing the C-terminus of the protein to amino acid residues 422–461 of mouse ornithine decarboxylase (MODC), one of the most short-lived proteins in mammalian cells (Li, X., *et al.,* 1998). This region of MODC contains a PEST sequence that targets the protein for degradation, resulting in rapid protein turnover (Li, X., *et al.,* 1998; Rechsteiner, M., *et al.,* 1990).

DsRed-Express-DR can be used as an *in vivo* reporter of gene expression (*Clontechniques* October 2002). Because of its rapid turnover rate, its expression from a promoter of interest provides a more accurate assessment of the promoter's activity over time than does the more stable DsRed-Express. Other destabilized RCFP vectors include pZsGreen1-DR and pHcRed1-DR.

5. How pH Affects DsRed Fluorescence

DsRed's absorption and emission properties change very little with pH. According to Baird and coworkers, only extremely alkaline (≥pH12) or moderately acidic (≤pH 5) conditions depress DsRed's fluorescence (Baird *et al.,* 2000). For most *in vitro* studies of DsRed we generally use aqueous solutions (e.g., 10 mM Tris-HCl) buffered between pH 8.0–8.5.

6. How Chemical Reagents Affect DsRed Fluorescence

Unlike *Aequorea victoria* green fluorescent protein, DsRed fluorescence is sensitive to mild denaturants, such as sodium dodecyl sulfate (SDS). Fully denatured DsRed does not fluoresce.

7. DsRed Resists Photobleaching

DsRed resists photobleaching—its fluorescence remains stable during the times typically required to make measurements with a spectrofluorometer or standard fluorescence microscope (Clontech Laboratories, Inc. , unpublished observations; Baird *et al*., 2000).

II. Properties of Reef Coral Fluorescent Proteins *continued*

D. *Heteractis crispa* Far-Red Fluorescent Protein (HcRed1)

1. HcRed1

HcRed1 was generated by random and site-directed mutagenesis of a non-fluorescent tetrameric chromoprotein from the reef coral *Heteractis crispa* (Gurskaya *et al.,* 2001). Early rounds of random mutagenesis were used to produce variants with extreme far-red fluorescence and rapid maturation rates. After isolating the brightest variant, investigators used site-directed mutageneis to optimize the protein's solubility. The final humanized variant, HcRed1 (HcRed-2A in Gurskaya *et al.,* 2001), was selected not just because of its bright far-red fluorescence, but also because of its apparent dimeric nature.

Because of its far red-shifted fluorescence, HcRed1 is easily distinguished from our other fluorescent proteins, including DsRed2, and DsRed-Express (April 2002 *Clontechniques*). In one example, a FACSVantage™ flow cytometer equipped with an argon/krypton laser (which emits a 568 nm line) was used to separate a mixed population of cells expressing either DsRed2 or HcRed1 into spectrally distinct populations (April 2002 *Clontechniques*). In another experiment, three subcellular compartments were labeled and clearly visualized by cotransfecting cells with localization vectors encoding HcRed1, ECFP, and EYFP (April 2002 *Clontechniques*). Of all the RCFPs in the Living Colors family, HcRed1 is the only member known to be a dimer, and is therefore considered to be the most suitable choice for protein localization studies.

2. HcRed1-DR (destabilized reporter)

HcRed1-DR is a destabilized variant of HcRed1. Its construction is identical to that used for DsRed-Express-DR (discussed above). Like DsRed-Express-DR, it has a short half-life, making it well suited for measuring changes in gene expression.

III. Expression of Reef Coral Fluorescent Proteins

A. Suitable Host Organisms and Cells

RCFPs can be expressed in a wide variety of prokaryotic and eukaryotic cell types. They are well tolerated by mammalian cells, and have therefore proven to be useful for creating stably transfected cell lines and transgenic organisms (Handler & Harrell, 2001; Feng *et al.,* 2000). Mammalian cell lines tested so far by us include HEK 293, HeLa, 3T3, Jurkat, and HT1080. Others have reported that RCFPs can be expressed and detected in *Saccharomyces cerevisiae, E. coli, C. elegans*, *Drosophila*, *Xenopus,* Zebrafish (pers. communications), and mouse (Feng *et al.,* 2000).

To date, DsRed and its variants are the most widely used RCFPs, and many different examples of their use can now be found in the literature. For specific examples, we suggest you search our citations database, available at **www.clontech.com**, as well as other public databases for published studies relevant to your area of interest.

B. Vectors for Expressing Reef Coral Fluorescent Proteins

Clontech offers a complete line of vectors for expressing RCFPs in mammalian cells and bacteria (Table IV). For more detailed information, including sequences, cloning sites, and plasmid maps, please refer to the corresponding Vector Information Packet provided with each vector and available on our our web site at **www.clontech.com.**

1. Bacterial expression vectors

Our bacterial vectors are primarily intended to serve as sources of RCFP cDNA. MCS regions flank the cDNA insert so that it can be easily excised and shuttled into other expression systems. These vectors can also be used in bacteria for bulk production of the RCFP protein. Expression is driven by the *lac* promoter (P_{lac}) and, therefore, can be induced by isopropylthio-β-D-galactoside (IPTG). The proteins are expressed as fusions with several amino acids, including the first five amino acids of the *LacZ* protein. Each vector contains an ampicillin resistance gene and the pUC ori.

2. Mammalian expression vectors

Our fusion vectors contain the immediate-early promoter from cytomegalovirus (CMV IE) for strong constitutive expression of the gene *in vivo*. They also contain an upstream Kozak consensus sequence to further enhance the translation efficiency. These vectors can also be used as transfection markers since the empty vector will express RCFP.

III. Expression of Reef Coral Fluorescent Proteins *cont.*

TABLE IV. LIVING COLORS® EXPRESSION VECTORS

Vector Type (naming convention)	Host	Promoter	Selection Prok.	Selection Euk.	Uses/Purpose
All RCFPS					
Fusion (pRCFP-N1, or pRCFP-C1)	Mammalian	CMV IE	Kan	Neo	Express a protein of interest as a fusion to the N- or C-terminus of any RCFP
Promoterless (pRCFP-1)	Mammalian	None	Kan	Neo	Monitor transcription from different promoters and promoter/enhancer combinations
Bacterial (pRCFP)	Bacterial	lac	Amp	None	Intended primarily as a convenient source of the fluorescent protein cDNA; flanking restriction sites allow for easy excision of the full-length coding sequence.
Selected RCFPs					
Subcellular Localization (pRCFP-structure)	Mammalian	CMV IE	Kan	Neo	Ready-to-use vectors for labeling various subcellular structures: mitochondria, nucleus, endoplasmic reticulum, or peroxisomes.
Bicistronic (pIRES2-RCFP)	Mammalian	CMV IE	Kan	Neo	Contain an internal ribosome entry site (IRES), which permits translation of two ORFs from a single mRNA. Thus cells expressing the fluorescent protein also express the gene of interest and can be selected by flow cytometry

III. Expression of Reef Coral Fluorescent Proteins *cont.*

C. Expression in Mammalian Cells

Vectors may be transfected into mammalian cells by a variety of techniques, including those using calcium phosphate (Chen & Okayama, 1988), DEAE-dextran (Rosenthal, 1987), various liposome-based transfection reagents (Sambrook *et al.*, 1989), and electroporation (Ausubel *et al.*, 1994). Any calcium phosphate transfection procedure may be used, but we recommend using the CalPhos™ Mammalian Transfection Kit (Cat. No. 631312). Likewise, any liposome-mediated transfection procedure may be used. For further information on cell culture techniques, see Freshney (1993).

The efficiency of a mammalian transfection procedure is primarily dependent on the cell line. Therefore, when working with a cell line for the first time, we recommend you compare the efficiencies of several transfection protocols. This test can best be accomplished using pDsRed-Express-CMV, which has the CMV immediate early promoter for high-level expression in most mammalian cell lines. The length of time required for fluorescence to initially become detectable and the total elapsed time needed to attain the maximum fluorescent signal in a given cell population following transfection will depend, in part, on the particular cell line used. In most cases, DsRed-Express may be detected by fluorescence microscopy, FACS analysis, or fluorometry within 8–12 hours of transfection.

As an alternative to standard transfection, you may want to try one of our viral-mediated gene transfer and expression systems. We offer adenoviral, retroviral, and baculoviral based systems for expressing proteins in many different cell types. Adenovirus, for example, enables you to efficiently infect both dividing and non-dividing cell lines. You can construct your own recombinant adenovirus using a Adeno-X Expression System, or use one of our premade Adeno-X Marker Viruses, expressing either EGFP or DsRed2 (see Related Products).

D. Sequencing Primers for Confirming In-Frame Fusions

To sequence junctions upstream or downstream of the DsRed, DsRed1, DsRed2, and DsRed-Express coding regions:

- DsRed-N Sequencing Primer (Cat. No. 632386)
  For wild-type DsRed N-terminal gene fusions
- DsRed-C Sequencing Primer (Cat. No. 632389)
  For wild-type DsRed C-terminal gene fusions
- DsRed1-N Sequencing Primer (Cat. No. 632387)
  For DsRed1, DsRed2, and DsRed-Express N-terminal gene fusions
- DsRed1-C Sequencing Primer (Cat. No. 632388)
  For DsRed1, DsRed2, and DsRed-Express C-terminal gene fusions

IV. Detection of Reef Coral Fluorescent Proteins

A. Microscopy

1. Conventional Fluorescence Microscopy

a. Filter sets

Table V, below, lists filter sets suitable for the detection of RCFPs by fluorescence microscopy. Although you can achieve satisfactory results using standard filters sets, such as FITC filters to detect ZsGreen and rhodamine or propidium iodide filters to detect DsRed and its variants, optimized filter sets for the detection of Reef Coral Fluorescent Proteins have been developed by Chroma Technology Corp (**www.chroma.com**) in collaboration with Clontech Laboratories, Inc. .

TABLE V. FILTER SETS RECOMMENDED FOR THE DETECTION OF RCFPS

	Optimized Filters from Chroma Technology Corp				Standard Filters
RCFP	**Filter set name**	**Excitation**	**Dichroic**	**Emission**	**Common Name**
AmCyan1	AmCyan	D440/40x	470dcxr	D500/40m	ECFP
ZsGreen1	ZsGreen	HQ470/35	Q490lp	HQ520/40	FITC/EGFP
ZsYellow1	ZsYellow	HQ500/40	530dclp	HQ550/40m	EYFP
DsRed2[a]	DsRed	D540/40x	570dclp	D600/50m	TRITC/ Phycoerythrin
DsRed-Express	DsRed	D540/40x	570dclp	D600/50m	TRITC/ Phycoerythrin
AsRed2[a]	AsRed	D540/40x	570dclp	HQ620/60m	TRITC/ Phycoerythrin
HcRed1	HcRed	HQ575/50x	610dclp	HQ640/50m	Texas Red

[a] The DsRed2 and AsRed2 filter sets are interchangeable.

b. Light source

The emission intensity from a light source can vary with wavelength. Because the intensity of the excitation light directly affects the brightness of a reporter, it is important to compare the excitation spectra of your chosen reporters with the emission spectrum of the light source. Conventional fluorescence microscopes are equipped with either a mercury- or xenon-arc lamp. Both lamps are suitable for exciting fluorescent proteins. But be aware that, whereas the light intensity from a xenon lamp deviates very little across the blue, green, yellow, and red regions, the emission from a mercury lamp has sharp peaks in the cyan and red regions. Therefore, fluorophores excited by cyan and red light may appear brighter when excited by a mercury lamp. Check the manufacturers' specifications for details about your lamp(s).

IV. Detection of Reef Coral Fluorescent Proteins *continued*

c. Detection systems

In conventional microscopy, fluorescence is usually detected with either a photographic (35-mm) or digital camera (which frequently harbors a cooled charge-coupled device, or CCD). Recording an image with a 35-mm camera may require a longer-than-average exposure time, possibly photobleaching the sample. Digital cameras, on the other hand, are usually more sensitive than 35-mm cameras but (unless you use a color digital camera) require image analysis software to produce "pseudo-colored" data.

d. Multicolor analysis

With the development of optimized filter sets and the introduction of our red fluorescent proteins, it's now possible to separate as many as three fluorescent reporters—cyan, yellow, and red—using conventional fluorescence microscopy. Some investigators have even distinguished all four colors (cyan, green, yellow, and red) using flow cytometry (Hawley *et al.,* 2001; July 2003 *Clontechniques*). In fact, many examples of multicolor analysis can now be found in the literature, and we recommend you survey the literature for help in deciding which combination will work best for you. Here, we offer the following recommendations for two- and three-color analyses (Figure 4).

2. Laser-scanning microscopy

See Table VI for laser lines recommended for the excitation of RCFPs.

3. Multi-photon laser-scanning microscopy

Multi-photon excitation microscopy has won the approval of many researchers who seek alternatives for resolving fluorescently labeled proteins *in vivo*. Its advantages have been widely reported (Piston, D. W., 1999; Potter, S. M., 1996; Marchant, J. S., 2001). Chief among them is the ability to excite fluorophores with low-energy infrared light. IR light not only penetrates tissue more effectively than visible light but also minimizes photobleaching and causes less photodamage. In principle, all Living Colors Fluorescent Proteins are suitable for use in multi-photon applications.

IV. Detection of Reef Coral Fluorescent Proteins *continued*

First Color	Second Color	Third Color
AmCyan1	ZsYellow1	HcRed1
	DsRed2	
	DsRed-Express	
	AsRed2	
	HcRed1	ZsYellow1
ZsGreen1	DsRed2	
	DsRed-Express	
	AsRed2	
	HcRed1	
ZsYellow1	AmCyan1	HcRed1
	HcRed1	AmCyan1
DsRed2	AmCyan1	
	ZsGreen1	
DsRed-Express	AmCyan1	
	ZsGreen1	
AsRed2	AmCyan1	
	ZsGreen1	
HcRed1	AmCyan1	ZsYellow1
	ZsYellow1	AmCyan1
	ZsGreen1	

Figure 4. Recommended color combinations.

IV. Detection of Reef Coral Fluorescent Proteins *continued*

B. Flow Cytometry

Laser lines and filter sets suitable for the detection of RCFPs by flow cytometry are listed in Table VI. See also Table VII for typical instrument configurations.

TABLE VI. DETECTION OF RCFPS BY FLOW CYTOMETRY

	Excitation requirement		Emission
RCFP	**Laser line (nm)**	**Laser**	**Filter /bandpass (nm)**
AmCyan1	405	VioFlame Solid State	485/22 or 530/30
	407	Point Source Solid State	485/22 or 530/30
	413	Krypton	485/22 or 530/30
	458	Argon	485/22 or 530/30
	458	Spectrum	485/22 or 530/30
	488	Argon	530/30
ZsGreen1	488	Argon	519/20, 520/40, or 530/30
ZsYellow1	488	Argon	530/30, 550/30, or 585/42
	514	Argon	550/30, or 585/42
	531	Spectrum	585/42
DsRed2	488	Argon	555/20, 580/30, 585/42, or 610/20
	514	Argon	555/20, 580/30, 585/42, or 610/20
	531	Spectrum	555/20, 580/30, 585/42, or 610/20
	568	Spectrum	610/20 or 630/30
DsRed-Express	488	Argon	555/20, 580/30, 585/42, or 610/20
	514	Argon	555/20, 580/30, 585/42, or 610/20
	531	Spectrum	555/20, 580/30, 585/42, or 610/20
	568	Spectrum	610/20 or 630/30
AsRed2	488	Argon	580/30, 585/42, 610/20, or 630/30
	514	Argon	580/30, 585/42, 610/20, or 630/30
	531	Spectrum	580/30, 585/42, 610/20, or 630/30
	568	Spectrum	610/20 or 630/30
HcRed1*	568	Spectrum	610/20, 630/30, or 660/20
	633	HeNe	660/20
	635	Red Diode	660/20

* Note that HcRed1 cannot be efficiently excited by a 488-nm laser line.

IV. Detection of Reef Coral Fluorescent Proteins *continued*

TABLE VII. TYPICAL INSTRUMENT CONFIGURATIONS

Instrument	Laser	Laser line (nm)
FACScan™ System	Argon (L1)	488
FACSCalibur™ System	Argon (L1) Red Diode (L2)	488 635
FACStar™ Plus System (typical setup)	Argon (L1) HeNe (L2)	488 633
FACSVantage™ SE System (typical setup)	Argon (L1) Krypton (L2) HeNe (L2 or L3)	488 407 633
LSR System (typical setup)	Argon (L1) HeCd (L2) HeNe (L3)	488 325 633
LSR II System (typical setup)	Argon (L1) HeNe (L2) UV (L3) Violet (L4)	488 633 355 405
FACSAria™ Cell-Sorting System (typical setup)	Argon (L1) HeNe (L2) Violet (L3)	488 633 407
FACSArray™ Bioanalyzer System	Green Diode (L1) Red Diode (L2)	532 635

C. Detecting Fluorescent Proteins in Ethanol-Treated Cells

When expressed as soluble products, fluorescent proteins may leak from cells treated with ethanol or methanol. To prevent such loss we suggest using paraformaldehyde instead of ethanol or methanol.

D. Antibodies for the Detection of RCFPs

Clontech offers a wide variety of monoclonal and polyclonal antibodies for the detection of Living Colors Fluorescent Proteins by Western blotting, immunoprecipitation, and immunocytochemistry (Table VIII). The antibodies are especially useful for analyzing N- and C-terminal fusions following their expression *in vivo*. Because RCFPs and *Aequorea victoria* GFP variants are derived from different organisms, the antibodies raised against these two families do not cross react. Because each RCFP is a unique protein encoded by a distinct gene rather than a mutant variant of a single fluorescent protein (as in the *A. victoria* family), it has been possible to develop highly specific antibodies for individual RCFPs (Table VIII).

IV. Detection of Reef Coral Fluorescent Proteins *continued*

TABLE VIII. LIVING COLORS® ANTIBODIES

Antibody Name Cat. No.	Antibody Characteristics	Suitability for Applications		
		Western Blot	Immuno-precipitation	Immuno-cytochemistry
A.v. Monoclonal Antibody (JL-8) 632380	Affinity-purified mouse monoclonal (IgG2a)	+++	++	+++
Full-Length A.v. Polyclonal Antibody 632382	Rabbit polyclonal serum, generated using full-length GFP protein	++	+++	not recommended
A.v. Peptide Antibody (polyclonal) 632377	Rabbit polyclonal, mixture of three affinity-purified peptide Ab's to GFP	+++	++	+++
GFP Monoclonal Antibody 632375	Affinity-purified mouse monoclonal	+++	not recommended	+
DsRed Monoclonal Antibody 632393	Mouse monoclonal recognizes DsRed and its variants	+++	not determined	not determined
DsRed Polyclonal Antibody 632397	Rabbit polyclonal serum recognizes DsRed and its variants	+++	++	+++
HcRed Polyclonal Antibody 632452	Rabbit polyclonal recognizes HcRed1 as well as N- and C-terminal fusions to HcRed1	+++	not recommended	not recommended

+++ = well suited
+ = poorly suited

V. Purified Recombinant Reef Coral Fluorescent Proteins

A. General Information

Recombinant Reef Coral Fluorescent Proteins from Clontech (Table IX) are produced in *E. coli,* and then affinity purified by metal ion affinity chromatography (IMAC) using TALON™ Resin. The purified recombinant proteins contain a 6xHN, N-terminal epitope tag. The tag does not interfere with the proteins' characteristic spectral properties, but if you wish to remove it, you can do so by digestion with enterokinase (EK); there is a single EK site between the tag and the protein. **For an EK digestion protocol, please refer to the EK manufacturer's guidelines.**

Recombinant Reef Coral Fluorescent Proteins can be used as positive controls and as standards in studies involving their expression *in vivo*. Their spectral properties are identical to those of the proteins expressed in mammalian cells. You can use them as standards to calibrate fluorometers, and as positive controls to verify analysis by SDS-PAGE and Western blot, using the appropriate antibodies. Additional applications include isoelectric focusing and fluorescence activated cell sorting (FACS). Longterm expression studies of stably transfected mammalian cell cultures have shown these proteins to be well tolerated. Thus, it is reasonable to expect that the recombinant proteins, though produced in *E. coli*, are suitable for microinjection into mammalian cells and tissues. **Please note, however, that we have not established specific protocols for using recombinant red fluorescent proteins in isoelectric focusing, FACS, or microinjection.** In Part B, below, we offer some general guidelines for SDS-PAGE and Western blotting.

TABLE IX. GENERAL PROPERTIES OF RECOMBINANT RED FLUORESCENT PROTEINS

Protein	Mass (kDa)[a]	Ex. Max (nm)	Em. Max (nm)	Western blot analysis[b]
rDsRed-Express	~25.7	557	579	DsRed Monoclonal Antibody (Cat. No. 632393) or DsRed Polyclonal Antibody (Cat. No. 632397)
rDsRed2	~25.7	563	582	DsRed Monoclonal Antibody DsRed Polyclonal Antibody
rHcRed1	~25.7	588	618	HcRed Polyclonal Antibody (Cat. No. 632452)

[a] Calculated mass of monomer. Though DsRed-Express, DsRed2, and HcRed1 each have a calculated mass of ~25.7 kDa, they usually run above 29 kDa on SDS-polyacrylamide gels. DsRed2 and DsRed-Express are believed to form the same tetrameric structure as wild-type DsRed. HcRed1 is most likely a dimer (Gurskaya, N.G., *et al.,* 2001).

[b] Recommended Living Colors antibody.

V. Purified Recombinant Proteins *continued*

B. Western Blotting: General Guidelines

Use a standard procedure for polyacrylamide gel electrophoresis (PAGE) to resolve the proteins on a one-dimensional gel (Laemmli, 1970). Just prior to use, allow the recombinant protein to thaw at room temperature, and then place the tube on ice. Prepare an aliquot of the protein according to the protocol appropriate for your electrophoresis system. Gel loading recommendations depend on the size of the gel and the method of detection: Coomassie blue staining or Western blotting. See Table X, below.

TABLE X. SDS-PAGE AND WESTERN BLOT DETECTION

		Loading Recommendations (amount of protein per lane)[a]	
Apparatus	**Method of detection[b]**	**Purified recombinant protein**	**Cell/tissue lysate**
Minigel	Coomassie blue staining	~0.5–1 µg	~25–75 µg
Minigel	Western blot (antibody-based detection)	~5–50 ng	200 µg

[a] If you are adding recombinant fluorescent protein to a total cell/tissue lysate or other crude sample, the amount of total protein loaded per lane must be optimized for the particular application.

[b] Generally, Living Colors recombinant red fluorescent proteins will not fluoresce on an SDS gel or Western blot.

VI. Troubleshooting Guide

A. Potential Difficulties Encountered Using RCFPs

- Long delay between transfection and detection of fluorescence

  If you fail to detect fluorescence from constructs in your system, there are several possible explanations. These include use of an inappropriate filter set, expression of the RCFP below the limit of detection, and failure of the protein to form the fluorophore. Check your vector construct to be sure your gene was inserted in frame with the RCFP gene. Some proteins, when fused to the N- or C-terminus of an RCFP, could interfere with the formation of the chromophore or with the assembly of the fluorescent protein into its oligomeric structure. The expression of an RCFP fusion *in vivo* can be confirmed by Western blotting with the appropriate antibody.

B. Considerations for Mammalian Expression

- Use vectors expressing the human codon-optimized variant of the RCFP you wish to express.
- Verify the identity of your plasmid construct and check the concentration with a restriction digest. Confirm that all subcloning steps have been done correctly. Be aware that specific restriction sites in some vectors are inactivated by methylation.
- Check the transfection efficiency of your experimental system using a second reporter plasmid that contains the same promoter element such as pEGFP-N1 (Cat. No. 632318).
- When using RCFPs to study protein localization, be aware of possible perturbing effects. Because of their proposed multimeric structures and tendency to aggregate, RCFPs may interfere with the normal function and localization of your protein of interest. Therefore, we generally recommend you use one of our enhanced GFP color variants to monitor protein localization *in vivo*. If desired, compare your results to those obtained with a corresponding RCFP fusion.

C. Optimizing Microscope/FACS Applications

- In general, you can see the fluorescence better in a darkened room after your eyes have adjusted.
- Filter sets for detecting RCFPs are available from Chroma Technology Corp (www.chroma.com).
- DsRed fluorescence may be sensitive to some nail polishes used to seal coverslips (though black nail polish does not appear to inhibit DsRed fluorescence). Instead of nail polish for mounting coverslips, we recommend molten agarose, rubber cement, or a commercial mounting solution such as ProLong® Antifade Kit (Molecular Probes).
- Intense excitation of fluorescent proteins for extended periods may generate free radicals that are toxic to living cells. This problem can be minimized by using longer wavelengths to excite the fluorophore.

VII. References

Aronheim, A., Engelberg, D., Li, N., al-Alawi, N., Schlessinger, A. & Karin, M. (1994) Membrane targeting of the nucleotide excahnge factor Sos is sufficient for activating the Ras signaling pathway. *Cell* **78**:949–961.

Atkins, D. & Izant, J. G. (1995) Expression and analysis of the green fluorescent protein gene in the fission yeast *Schizosaccharomyces pombe. Curr. Genet.* **28**:585–588.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1994) In *Current Protocols in Molecular Biology* (John Wiley and Sons, NY), Vol. 1, Ch. 5 and 9.

Bevis, B. J., Glick, B. S. (2002) Rapidly maturing variants of the *Discosoma* red fluorescent protein (DsRed). *Nat Biotechnol* **20**:83–7.

Baird, G. S., Zacharias, D. A. & Tsien, R. Y. (2000) Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc. Natl. Acad. Sci. USA* **97**:11984–11989.

Carey, K. L., Richards, S. A., Lounsbury, K. M. & Macara, I. G. (1996) Evidence using a green fluorescent protein-glucocorticoid receptor that the RAN/TC4 GTPase mediates an essential function independent of nuclear protein import. *J. Cell Biol.* **133**(5):985–996.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. & Prasher, D. C. (1994) Green fluorescent protein as a marker for gene expression. *Science* **263:**802–805.

Chalfie, M. & Kain, S., Eds. (1998) *GFP: Green Fluorescent Protein Properties, Applications, and Protocols* (Wiley-Liss, New York).

Chen, C. & Okayama, H. (1988) Calcium phosphate-mediated gene transfer: a highly efficient transfection system for stably transforming cells with plasmid DNA. *BioTechniques* **6**:632–638.

Cheng, L., Fu, J., Tsukamoto, A. & Hawley, R. G. (1996) Use of green fluorescent protein variants to monitor gene transfer and expression in mammalian cells. *Nature Biotechnol.* **14**:606–609.

Cormack, B. P., Valdivia, R. & Falkow, S. (1996) FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* **173**:33–38.

Crameri, A., Whitehorn, E. A., Tate, E. & Stemmer, W. P. C. (1996) Improved green fluorescent protein by molecular evolution using DNA shuffling. *Nature Biotechnol.* **14**:315–319.

Ellenberg, J., Lippincott-Scwartz, J. & Presley, J. F. (1999) Dual-color imaging with GFP variants. *Trends Cell Biol.* **9**:52–56.

Ellenberg, J., Lippincott-Scwartz, J. & Presley, J. F. (1998) Two-color green fluorescent protein time-lapse imaging. *Biotechniques* **25**:838–846.

Feng, G., Mellor, R. H., Bernstein, M., Keller-Peck, C., Nguyen, Q. T., Wallace, M., Nerbonne, J. M., Lichtman, J. W. & Sanes, J. R. (2000) Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* **28**:41–51.

Fey, P., Compton, K. & Cox, E. C. (1995) Green fluorescent protein production in the cellular slime molds *Polysphondylium pallidum* and *Dictyostelium discoideum. Gene* **165**:127–130.

Flach, J., Bossie, M., Vogel, J., Corbett, A., Jinks, T., Willins, D. A. & Silver, P. A. (1994) A yeast RNA-binding protein shuttles between the nucleus and the cytoplasm. *Mol. Cell. Biol.* **14:** 8399–8407.

Freshney, R. I. (1993) *Culture of Animal Cells,* Third Edition (Wiley-Liss, NY).

Green, G., Kain, S. R. & Angres, B. (2000) Dual color detection of cyan and yellow derivatives of green fluorescent protein using conventional fluorescence microscopy and 35-mm photography. *Meth. Enzymol.* **327**:89–94.

Gross, L. A., Baird, G. S., Hoffman, R. C., Baldridge, K. K. & Tsien, R. Y. (2000) The structure of the chromophore within DsRed, a red fluorescent protein from coral. *Proc. Natl. Acad. Sci. USA* **97**:11990–11995.

VII. References *continued*

Gurskaya, N. G., Fradkov, A. F., Terskikh, A., Matz, M. V., Labas, Y. A., Martynov, V. I., Yanushevich, Y. G., Lukyanov, K. A. & Lukyanov, S. A. (2001) GFP-like chromoproteins as a source of far-red fluorescent proteins. *FEBS Lett.* **507**:16–20.

Haas, J., Park, E-C & Seed, B. (1996) Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr. Biol.* **6**:315–324.

Hancock, J. F., Cadwallader, K., Paterson, H. & Marshall, C. J. (1991) A CAAX or a CAAL motif and a second signal are sufficient for plasma membrane targeting of ras proteins. *EMBO J.* **13**:4033–4039.

Handler, A. M. & Harrell, R. A., 2nd. (2001) Polyubiquitin-regulated DsRed marker for transgenic insects. *Biotechniques* **31**:820, 824–828.

Hawley, T. S., Telford, W. G., Ramezani, A. & Hawley, R. G. (2001) Four-color flow cytometric detection of retrovirally expressed red, yellow, green, and cyan fluorescent proteins. *Biotechniques* **30**:1028–1034.

Heikal, A. A., Hess, S. T., Baird, G. S., Tsien, R. Y. & Webb, W. W. (2000) Molecular spectroscopy and dynamics of intrinsically fluorescent proteins: coral red (dsRed) and yellow (Citrine). *Proc. Natl. Acad. Sci. USA* **97**:11996–12001.

Heim, R., Prasher, D. C. & Tsien, R. Y. (1994) Wavelength mutations and posttranslational autoxidation of green fluorescent protein. *Proc. Natl. Acad. Sci. USA* **91:**12501–12504.

Heim, R. & Tsien, R. Y. (1996) Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence resonance energy transfer. *Curr. Biol.* **6**:178–182.

Jakobs, S., Subramaniam, V., Schonle, A., Jovin, T. M. & Hell, S. W. (2000) EFGP and DsRed expressing cultures of Escherichia coli imaged by confocal, two-photon and fluorescence lifetime microscopy.*FEBS Lett.* **479**:131–135.

Jiang, W. & Hunter, T. (1998) Analysis of cell-cycle profiles in transfected cells using a membrane-targeted GFP. *BioTechnique*s **24:**349–350.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680–685.

Li, X., Zhao, X., Fang, Y., Jiang, X., Duong, T., Fan, C., Huang, C-C. & Kain, S. (1998) Generation of destabilized green fluorescent protein as a transcription reporter. *J. Biol. Chem.* **273:**34970–34975.

Lukyanov, K. A., Fradkov, A. F., Gurskaya, N. G., Matz, M. V., Labas, Y. A., Savitsky, A. P., Markelov, M. L., Zaraisky, A,G,, Zhao, X,, Fang, Y., Tan, W. & Lukyanov, S. A. (2000) Natural animal coloration can Be determined by a nonfluorescent green fluorescent protein homolog. *J. Biol. Chem.* **275**:25879–25882.

Lybarger, L., Dempsey, D., Patterson, G. H., Piston, D. W., Kain, S. R. & Chervenak, R. (1998) Dual-color flow cytometric detection of fluorescent proteins using single-laser (488-nm) excitation. *Cytometry* **31**:147–152.

Marchant, J. S., Stutzmann, G. E., Leissring, M. A., LaFerla, F. M. & Parker, I. (2001) Multiphoton-evoked color change of DsRed as an optical highlighter for cellular and subcellular labeling. *Nat. Biotechnol* **19**:645–649.

Matz, M. V., Fradkov, A. F., Labas, Y. A., Savitsky, A. P., Zaraisky, A. G., Markelov, M. L. & Lukyanov S. A. (1999). Fluorescent proteins from nonbioluminescent Anthozoa species. *Nature Biotech.* **17**:969–973.

Miyawaki, A., Llopis, J., Heim, R., McCaffery, J., Adams, J., Ikura, M. & Tsien, R. (1997) Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin. *Nature* **388**:882–887.

Moriyoshi, K., Richards, L. J., Akazawa, C., O'Leary, D. D. & Nakanishi, S. (1996) Labeling neural cells using adenoviral gene transfer of membrane-targeted GFP. *Neuron* **16**:255–260.

VII. References *continued*

Ormö, M., Cubitt, A. B., Kallio, K., Gross, L. A., Tsien, R. Y. & Remington, S. J. (1996) Crystal structure of the *Aequorea victoria* green fluorescent protein. *Science* **273:**1392–1395.

Patterson, G., Day, R. N., Piston, D. (2001) Fluorescent protein spectra. *J. Cell Sci.* **114**:837–8.

Piston, D. W. (1999) Imaging living cells and tissues by two-photon excitation microscopy. *Trends Cell Biol.* **9**:66–69.

Potter, S. M., Wang, C. M., Garrity, P. A. & Fraser, S. E. (1996) Intravital imaging of green fluorescent protein using two-photon laser-scanning microscopy. *Gene* **173**:25–31.

Rechsteiner M. (1990) PEST sequences are signals for rapid intracellular proteolysis. *Semin. Cell Biol.* **1**:433–440.

Ropp, J. D., *et al.* (1995) *Aequorea* green fluorescent protein (GFP) analysis by flow cytometry. *Cytometry* **21**:309–317.

Rosenthal, N. (1987) Identification of regulatory elements of cloned genes with functional assays. *Methods Enzymol.* **152**:704–709.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Terskikh, A., Fradkov, A., Ermakova, G., Zaraisky, A., Tan, P., Kajava, A. V., Zhao, X., Lukyanov, S., Matz, M., Kim, S., Weissman, I. & Siebert, P. (2000) "Fluorescent Timer": protein that changes color with time. *Science* **290**:1585–1588.

Vrzheshch, P. V., Akovbian, N. A., Varfolomeyev, S. D. & Verkhusha, V. V. (2000) Denaturation and partial renaturation of a tightly tetramerized DsRed protein under mildly acidic conditions. *FEBS Lett.* **487**:203–208.

Wall, M. A., Socolich, M. & Ranganathan, R. (2000) The structural basis for red fluorescence in the tetrameric GFP homolog DsRed. *Nat. Struct. Biol.* **7**:1133–1138.

Yang, T. T., Sinai, P., Green, G., Kitts, P. A., Chen, Y. T., Lybarger, L., Chervenak, R., Patterson, G. H., Piston, D. W. & Kain, S. R. (1998) Improved fluorescence and dual color detection with enhanced blue and green variants of the green fluorescent protein. *J. Biol. Chem.* **273**:8212–8216.

Yarbrough, D., Wachter, R. M., Kallio, K., Matz, M. V. & Remington, S. J. (2001) Refined crystal structure of DsRed, a red fluorescent protein from coral, at 2.0-Å resolution. *Proc. Natl. Acad. Sci. USA* **98**:462–467.

Yu, J. & van den Engh, G. (1995) Flow-sort and growth of single bacterial cells transformed with cosmid and plasmid vectors that include the gene for green-fluorescent protein as a visible marker. Abstracts of papers presented at the 1995 meeting on “Genome Mapping and Sequencing”, Cold Spring Harbor. p. 293.

***Clontechniques* references** (in chronological order):

Living Colors Red Fluourescent Protein. (October 1999) *Clontechniques* **XIV**(4):2–6.

Living Colors Fluorescent Timer. (April 2001) *CLONTECHniques* **XVI**(2):14–15.

Living Colors DsRed2. (July 2001) *Clontechniques* **XVI**(3):2–3.

Living Colors HcRed. (April 2002) *Clontechniques* **XVII**(2):12–13.

Living Colors DsRed-Express (July 2002) *Clontechniques* **XVII**(3):16–17.

Destabilized DsRed-Express and HcRed Vectors (October 2002) *Clontechniques* **XVII**(4).

Reef coral fluorescent proteins (July 2003) *Clontechniques* **XVIII**(3):6–7.

VIII. Related Products

For a complete listing of all Clontech products,
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Product	Cat. No.
• RCFP Expression Vectors	many
• Enhanced GFP Expression Vectors	many
• CalPhos™ Mammalian Transfection Kit	631312
• CLONfectin™ Transfection Reagent	631301
• Adeno-X™ Expression System 1	631513
• Adeno-X™ Expression System 2 with Creator™ Technology	many
• Adeno-X DsRed2 Marker Virus	632417
• Adeno-X EGFP Marker Virus	630115
• Retro-X™ System	631508

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