Clontech Products in Stem Cell Research

Our innovative products are helping researchers achieve progress in stem cell studies

Clontech's diverse and comprehensive product line has enabled scientists to make significant advances in a wide variety of research areas involving gene discovery, regulation, and function for over two decades. One achievement that we are particularly proud of is our contribution to stem cell research, by supplying a wide variety of products for numerous studies involving stem cells. Some of these products have been in use for many years, while others are the more recent result of our constant efforts to develop innovative technologies. Although there are many instances in which Clontech products have played a role in increasing our understanding of stem cells, we are providing the following examples from recent scientific publications, which are described in greater depth in the body of this article, to illustrate the diversity of the products used in these studies:

- **DsRed,** one of our popular **Living Colors®** fluorescent protein tags (Figure 1), was fused to specific mesenchymal stem cell proteins in order to monitor their subcellular localization during apoptosis (1).
- Our pioneering **Matchmaker™ Yeast Two-Hybrid System** (Figure 2) was used to screen primitive hematopoietic stem cell cDNA libraries for proteins involved in regulating chromatin structure (2).
- Clontech's **SMART™ PCR cDNA Synthesis Kit** (Figure 3) was used to develop a PCR-based method for identifying membrane marker proteins in order to distinguish leukemic from nonleukemic hematopoietic stem cells (3).
- Our **Adeno-X™ Tet-Off® Expression System 2,** a doxycycline-regulated adenoviral expression system, was used to transduce endothelial nitric oxide synthase (eNOS) into human mesenchymal stem cell lines and achieve therapeutic overexpression (4).

Monitoring Protein Localization in Stem Cells using Clontech's Living Colors Fluorescent Proteins

Raz, V. *et al.* (2006) Changes in lamina structure are followed by spatial reorganization of heterochromatic regions in caspase-8-activated human mesenchymal stem cells. *J. Cell Sci.* **119**(Pt 20):4247–4256.

Apoptosis is a crucial event in regulating cellular homeostasis. However, our understanding of the events surrounding stem cell homeostasis is very limited. In this study, the authors dissected early events during stem cell apoptosis using an inducible, caspase-8-dependent model system in human mesenchymal stem cells. The authors analyzed the effect of caspase-8 activation by monitoring changes in the subcellular localization of proteins involved in the organization of the nuclear lamina and the telomeres. They focused specifically on lamin A, a structural protein of the nuclear lamina, and TRF1, a telomere binding protein. In this paper, the authors expressed these proteins of interest as fusion proteins tagged with the red fluorescent protein **DsRed,** a member of the **Living Colors** product family (Figure 1). Fluorescent imaging of human mesenchymal stem cells expressing these fusion proteins unveiled a drastic reorganization of the lamina structure upon caspase-8-dependent apoptosis, followed by the degradation of lamins A and B, and chromatin degradation. Monitoring TRF1-**DsRed** expressed in human mesenchymal stem cells revealed that induction of apoptosis via the caspase-8 pathway also induced a change in telomere organization. The telomeres in apoptotic cells visualized by fluorescence microscopy had begun moving towards each other. Similar results were obtained with another labeled protein, TRF2-citrine, indicating that this change in telomere spatial organization is not due to DsRed, which can aggregate. *Editor's Note: Although aggregation can occur with the earlier version of DsRed used in this experiment, we have addressed this*

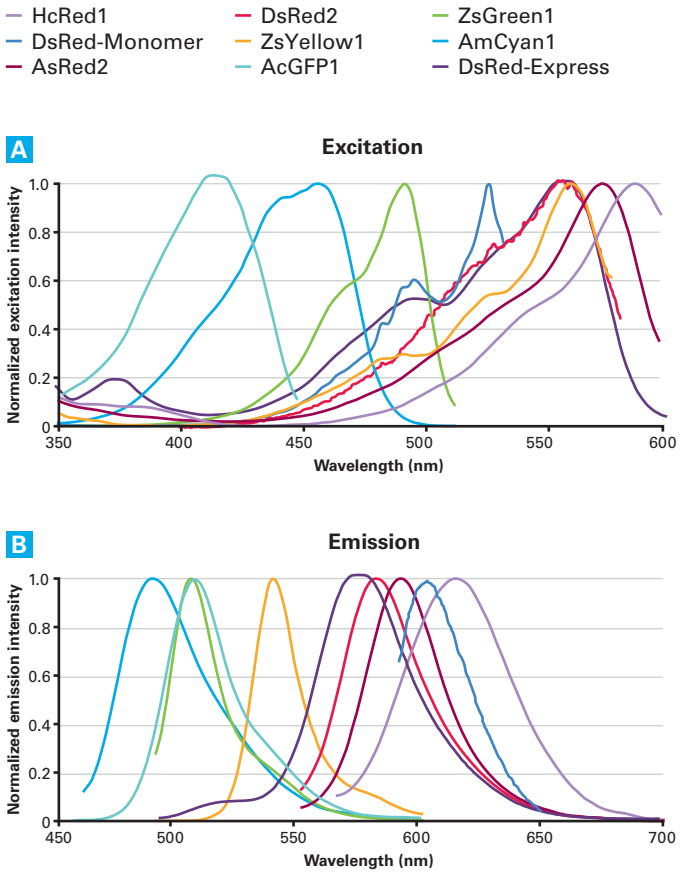

Figure 1. Excitation and emission spectra of Living Colors Fluorescent Proteins. Clontech offers the broadest spectrum of colors available in fluorescent protein tags. **Panel A.** Excitation spectra. **Panel B.** Emission spectra.

Clontech Products in Stem Cell Research...continued

problem in later versions, such as DsRed2, DsRed-Monomer, and DsRed-Express. The use of **DsRed** in this study provided a more detailed insight into the early rearrangement of the nuclear structure in apoptotic human mesenchymal stem cells.

Identification of Stem Cell Regulatory Proteins using our Matchmaker Yeast Two-Hybrid System

Chagraoui, J. *et al.* (2006) E4F1: a novel candidate factor for mediating BMI1 function in primitive hematopoietic cells. *Genes Dev.* **20**(15):2110–2120.

The authors studied the protein BMI1, a polycomb group (PcG) protein. This family of proteins, first discovered in fruit flies, can remodel chromatin in such a way that transcription factors cannot bind to promoter sequences in DNA. Mutations in the PcG genes have been associated with increased severity and invasiveness of cancer processes. PcG genes are essential for the proliferation of hematopoietic stem cells (HSC) and derivatives such as T and B cells. Since the role of BMI1, which is detected mainly in primitive human bone marrow cells, is most well-characterized with respect to HSC proliferation and self-renewal, identification of factors that mediate BMI1 function in HSC is important. In this study, the authors used Clontech's **Matchmaker Yeast Two-Hybrid System** (Figure 2) with BMI1 as the bait to screen a fetal cDNA library, enriched for primitive hematopoietic cells. The transcription factor E4F1 was identified as an interaction partner. The yeast two-hybrid assay was further used to define the interaction surfaces between BMI1 and E4F1. From their studies, the authors concluded that E4F1 is a key modulator of BMI1 activity in primitive hematopoietic stem cells.

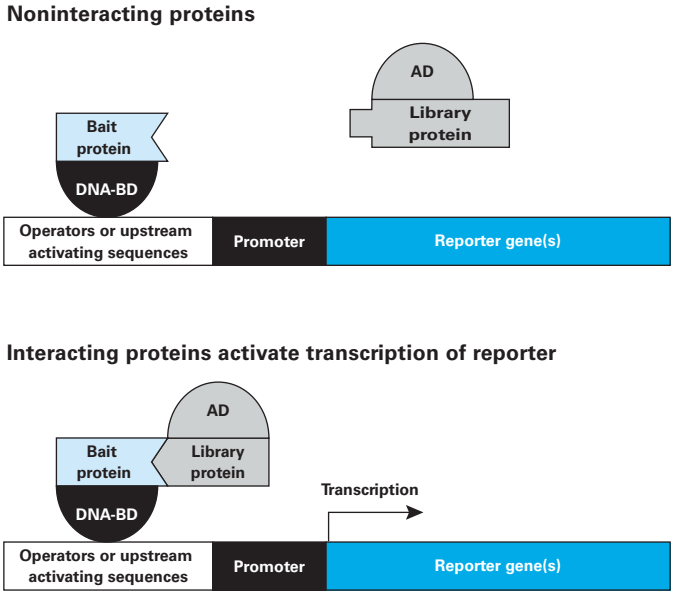

Figure 2. Principle of the Matchmaker Yeast Two-Hybrid System.

Identification of Stem Cell Membrane Proteins using our SMART PCR cDNA Synthesis Kit

Hosen, N. *et al.* (2007) CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. *Proc. Natl. Acad. Sci. USA* **104**(26):11008–11013.

Acute myeloid leukemia cannot be treated with standard chemotherapeutic methods because they do not eliminate leukemic stem cells (LSC), the self-renewing component of the disease. In order to find a cure that specifically targets those LSCs, it is first necessary to identify markers on the cell surface of LSCs that distinguish them from nonleukemic hematopoietic stem cells. Using a signal sequence trap PCR method combined with Clontech's **SMART PCR cDNA Synthesis Kit** (Figure 3), the authors were able to identify CD96 as a membrane marker protein for LSCs. The results indicate that LSCs can be distinguished from normal hematopoietic stem cells by the presence of CD96. This finding may open the door for a more specific therapy by targeting CD96 positive LSCs without harming normal hematopoietic stem cells.

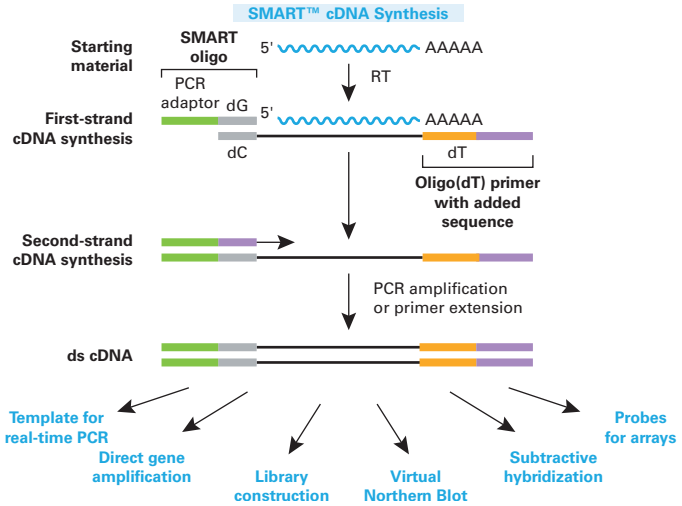

Figure 3. Overview of the SMART cDNA synthesis method.

Clontech Products in Stem Cell Research...continued

Gene Transduction & Therapeutic Overexpression in Stem Cells using our Adeno-X Tet-Off Adenoviral Expression System 2

Kanki-Horimoto, S. *et al.* (2006) Synthetic vascular prosthesis impregnated with mesenchymal stem cells overexpressing endothelial nitric oxide synthase. *Circulation* **114**(1 Suppl):I327–I330.

Coronary artery disease is a serious illness that is aggravated by endothelial dysfunction, which can cause irreversible heart muscle damage. Many patients must undergo multiple surgeries, which may result in a shortage of graft materials. Small caliber vascular prostheses have an extremely high failure rate due to clot formation and occlusion. It has been found that grafts releasing nitric oxide may alleviate these problems. Mesenchymal stem cells (MSCs) can differentiate into vascular endothelium and produce arteriogenic cytokines in a paracrine fashion. MSCs are also easy to transduce with viral vectors. In this study, the authors created expanded polytetrafluoroethylene grafts that were seeded with MSCs that had been transduced with an adenovirus expressing endothelial nitric oxide synthase (eNOS). The eNOS gene was amplified from a rat cDNA library and cloned into the pDNR-CMV plasmid obtained from Clontech's doxycycline-regulated adenoviral vector system, the **Adeno-X Tet-Off Expression System 2.** An adenovirus expressing eNOS was generated using eNOS-pDNR-CMV and pLP-Adeno-X-TRE, followed by site-specific Cre-*loxP* recombination (Figure 4). For control purposes, the authors also cloned and expressed the *lacZ* gene into an adenovirus using the same methodology. Adenovirus was used at a multiplicity of infection of 2,000 to transduce MSCs, which then yielded high levels of eNOS activity. The authors concluded that they successfully generated small caliber prostheses that expressed bioactive eNOS. This finding may lead to vasculoprotective hybrid vascular prostheses.

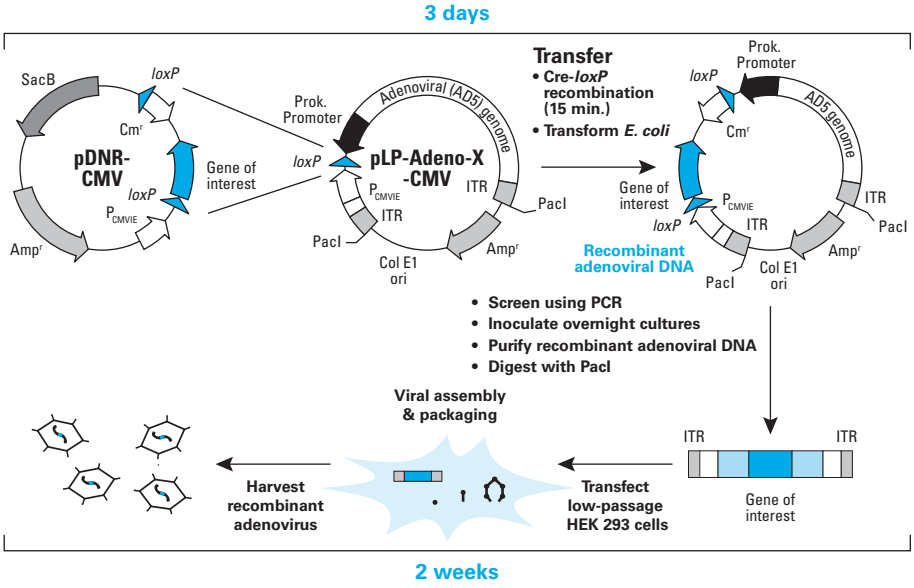

Figure 4. Overview of the Adeno-X Expression System 2. The Adeno-X Expression System 2 reduces the overall timeline for constructing recombinant adenovirus vectors. Using Cre-*loxP* recombination, the standard system generates a recombinant adenoviral construct in which gene expression is regulated by the CMV major immediate-early promoter/enhancer. Other Adeno-X expression systems feature a promoterless adenoviral Acceptor Vector (for use in shRNA or tissue-specific applications) or an adenoviral Acceptor Vector that includes a Tet-response element (TRE) for doxycycline-inducible expression applications.

Product	Size	Cat. No.
DsRed Fluorescent Protein Vectors	20 µg	many
Matchmaker Two-Hybrid System 3	each	630303
SMART PCR cDNA Synthesis Kit	7 rxns	634902
Adeno-X Tet-Off Expression System 2	5 rxns	631058

Notice to Purchaser

Please see the CMV Sequence, DsRed-Express, DsRed-Monomer, Living Colors® Fluorescent Protein Products, Matchmaker™ Two-Hybrid System, SMART™ Amplification Products, and Tet-Based Expression Products licensing statements on page 42.

References

1. Raz, V. *et al.* (2006) *J. Cell Sci.* **119**(Pt 20): 4247–4256.
2. Chagraoui, J. *et al.* (2006) *Genes Dev.* **20**(15):2110–2120.
3. Hosen, N. *et al.* (2007) *Proc. Natl. Acad. Sci. USA* **104**(26):11008–11013.
4. Kanki-Horimoto, S. *et al.* (2006) *Circulation* **114**(1 Suppl):I327–I330.

Ref. 2 (Genes & Development, Volume 20, J. Chagraoui, S. L. Niessen, J. Lessard, S. Girad, P. Coulombe, M. Sauvageau, S. Meloche & G. Sauvageau, "E4F1: a novel candidate factor for mediating BMI1 function in primitive hematopoietic cells," pages 2110–2120, ©2006) was cited and summarized with permission from Cold Spring Harbor Laboratory Press.