VOLUME 3 • ISSUE 1

# GeneDex

Editor: Michele Nealen

## Tag your protein using a **PrecisionShuttle** vector!

Overexpression of a target protein can be monitored and measured when that protein is expressed as a fusion construct with a fluorescent or other epitope tag. The TrueORF product line is a new generation of cDNA clones based on OriGene's TrueClone Collection. Unlike TrueClones, a TrueORF clone enables expression of the encoded transcript as a tagged protein, which facilitates multiple downstream applications that utilize an anti-tag antibody, such as protein detection, protein purification, subcellular localization, etc.

All TrueORF inserts are housed in a pCMV6-Entry vector (Figure 1) and therefore can be easily shuttled by a simple 'cut-and-paste' mechanism (Figure 2) into any of the OriGene PrecisionShuttle Destination Vectors.

All TrueORF clones are purified from a single colony and are shipped as a 10 ug quantity of purified plasmid DNA. The entry vector is not only useful for subcloning an ORF into a destination vector; it can be used for immediate application in stable or transient transfection experiments, and will express a transcript tagged with Myc and Flag at the C-terminus of the ORF. Alternatively, the cDNA can be transferred into a destination vector of choice, available with a variety of types and

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**ALSO IN THIS ISSUE:**



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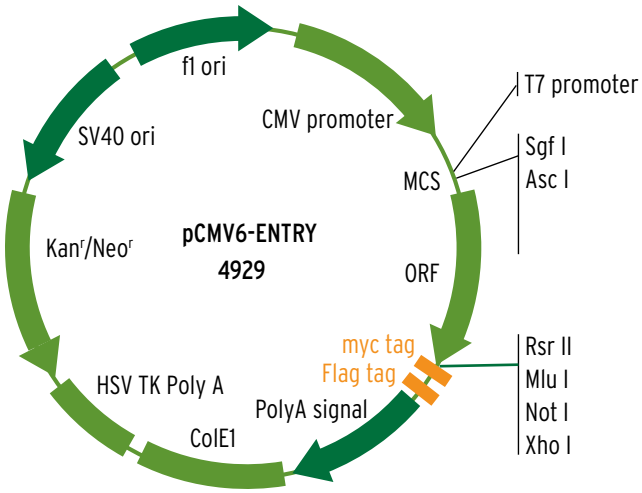


**Figure 1** The TrueORF entry vector contains many of the same features of our TrueClone vector (the CMV promoter for mammalian expression and the T7 promoter for *in vitro* transcription/translation reactions in cell-free systems), but has many additional, highly useful features. pCMV6-Entry contains a Kozak consensus sequence to facilitate protein expression in mammalian cells, C-terminal Myc and Flag tags, and a neomycin resistance cassette for selection of stable transfectants. And as the entry vector for the OriGene PrecisionShuttle system, this vector contains an identical MCS as nineteen unique destination vectors, which have different tag options (see Table 1).

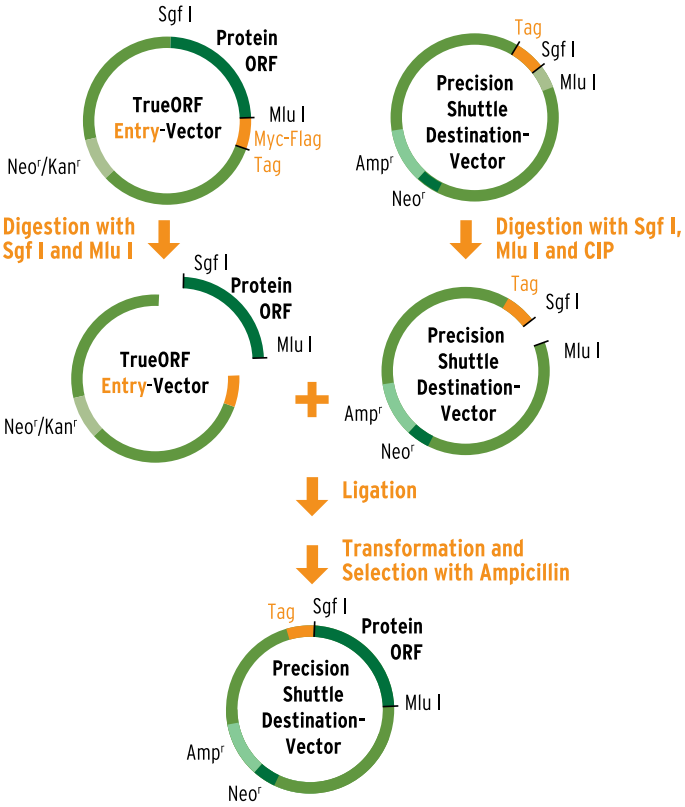


**Figure 2** Schematic of the PrecisionShuttle subcloning procedure. The entry and destination vectors are digested with Sgf I and Mlu I, which rarely cut in mammalian coding sequences. After a ligation reaction, the resulting clones are grown on ampicillin-containing medium to select for successful subcloning of the ORF into the destination vector.

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locations of fusion tags. All TrueORF destination vectors contain the same multiple cloning sites as the entry vector but a different antibiotic screening marker so that customers can easily transfer the ORF of interest into the destination vector of choice. All of the plasmids in the PrecisionShuttle vector system allow high-level target gene expression in mammalian cells or via *in vitro* translation in a cell-free system. The plasmids contain the promoter and enhancers of the human cytomegalovirus (CMV) immediate–early gene to drive mammalian gene expression, and the T7 promoter for *in vitro* transcription/translation. An optimal Kozak consensus sequence is included in the plasmid to enhance expression in mammalian cells.

## AVAILABLE ENTRY & DESTINATION VECTORS

| Calalog Number | Precision-Shuttle Vectors | Drug Selection | Cell Selection | Expression | Description |
|---|---|---|---|---|---|
| PS100001 | pCMV6-Entry | Kanamycin | Neomycin | Mammalian | C-terminal Myc and Flag® tag |
| PS100002 | pCMV6-AC-His | Ampicillin | Neomycin | Mammalian | C-terminal His tag |
| PS100003 | pCMV6-AC-Myc | Ampicillin | Neomycin | Mammalian | C-terminal Myc tag |
| PS100004 | pCMV6-AC-HA | Ampicillin | Neomycin | Mammalian | C-terminal HA tag |
| PS100005 | pCMV6-AC-Flag | Ampicillin | Neomycin | Mammalian | C-terminal Flag® tag |
| PS100006 | pCMV6-AC-Myc-His | Ampicillin | Neomycin | Mammalian | C-terminal Myc and His tag |
| PS100007 | pCMV6-AC-Myc-Flag | Ampicillin | Neomycin | Mammalian | C-terminal Myc and Flag® tag |
| PS100008 | pCMV6-AC-HA-His | Ampicillin | Neomycin | Mammalian | C-terminal HA and His tag |
| PS100009 | pCMV6-AC-Flag-His | Ampicillin | Neomycin | Mammalian | C-terminal Flag® and His tag |
| PS100010 | pCMV6-AC-GFP | Ampicillin | Neomycin | Mammalian | C-terminal T-GFP tag |
| PS100011 | pCMV6-AN-His | Ampicillin | Neomycin | Mammalian | N-terminal His tag |
| PS100012 | pCMV6-AN-Myc | Ampicillin | Neomycin | Mammalian | N-terminal Myc tag |
| PS100013 | pCMV6-AN-HA | Ampicillin | Neomycin | Mammalian | N-terminal HA tag |
| PS100014 | pCMV6-AN-Flag | Ampicillin | Neomycin | Mammalian | N-terminal Flag® tag |
| PS100015 | pCMV6-AN-His-Myc | Ampicillin | Neomycin | Mammalian | N-terminal His and Myc tag |
| PS100016 | pCMV6-AN-Myc-Flag | Ampicillin | Neomycin | Mammalian | N-terminal Myc and Flag® tag |
| PS100017 | pCMV6-AN-His-HA | Ampicillin | Neomycin | Mammalian | N-terminal His and HA tag |
| PS100018 | pCMV6-AN-His-Flag | Ampicillin | Neomycin | Mammalian | N-terminal His and Flag® tag |
| PS100019 | pCMV6-AN-GFP | Ampicillin | Neomycin | Mammalian | N-terminal T-GFP tag |
| PS100020 | pCMV6-A | Ampicillin | Neomycin | Mammalian | Not tagged |

**Table 1** Precision Shuttle entry and destination vectors are available with N-terminal or C-terminal His, Myc, HA, Flag, or T-GFP tags. All vectors can be amplified in bacterial cells using either kanamycin or ampicillin. Both entry and destination vectors can be used for either transient transfection experiments or to make stable cell lines, using the neomycin resistance gene.

The PrecisionShuttle vector system employs a basic "cut and paste" molecular cloning method (Figure 2). It is faster, cheaper, more reliable and more flexible than a recombination strategy, and no intellectual license is required for either academic or commercial users. The transfer of the ORF from the entry clone to any destination vector is a rapid process. Digestion, ligation and transformation takes as little as 3 hours (see Protocol later in this issue); different antibiotic selection markers facilitate screening of ligation products.

Two rare-cutting restriction enzymes are utilized in a simple yet powerful directional cloning method for transferring an ORF between vectors. Because of the correspondence between the multiple cloning sites (MCS) of the TrueORF entry and destination vectors, users have a precise, rapid, and high-fidelity method for transferring an ORF between a variety of vectors. Most subcloning from the entry to a destination vector involves Sgf I (present in 0.37% of human ORFs) and Mlu I (4%). In the very unusual case when Sgf I or Mlu I sites are inside an ORF, the TrueORF vector MCS provides other rare restriction sites, such as Asc I, Rsr II, and Not I so that any ORF can be shuttled from the entry vector to a destination vector by using some combination of these five rare restriction enzymes. Unlike site-specific recombination vector systems, the TrueORF Clone System does not append multiple amino acids to the amino or carboxy terminus of the protein of interest. The subcloning strategy maintains insert orientation and reading frame, eliminating the need to resequence the insert after each transfer. Because the entry and destination vectors have different antibiotic resistance genes, selection after subcloning is a very simple process.

While the PrecisionShuttle vector system can be used for any cDNA, we have developed this system to take advantage of the largest collection of human full-length cDNA clones, OriGene's TrueClone Collection. Nearly every human cDNA clone is offered in the entry vector as a TrueORF clone, and a customer can easily transfer this ORF into any destination vector. A special effort has been incorporated into the synthesis of these TrueORF clones to minimize the generation of mutations. By using a large quantity of high-quality cDNA template, a minimum of PCR cycles and a polymerase with the highest fidelity (one mutation in 40,000,000 bp), the number of mutations is very limited. No mutations were identified during the initial cloning of over 200 ORF cDNAs (ranging from 500-6000 bp) into the entry vector after full-length sequencing of each of these clones. The MCS of the PrecisionShuttle vectors was engineered to be compatible with most other commercially

**All TrueORF destination vectors contain the same multiple cloning sites as the entry vector but a different antibiotic screening marker so that customers can easily transfer the ORF of interest into the destination vector of choice.**

available vector systems including Gateway vectors (Invitrogen), pET vectors (Novagen) and Flexi vectors (Promega). In this sense, the TrueORF vector system is truly universal.

The PrecisionShuttle vector system was not only developed for individual researchers to work on one or two genes at a time, but also for high-throughput applications that require thousands of ORFs in a standard vector. Unlike recombination-based systems, this digestion-based system requires only nanograms of vector for successful ORF transfer. A specific destination DNA can be predigested and mixed with the digested TrueORF clones, then ligated and transformed into competent *E. coli* cells. The whole process can be readily adapted to a 384-well format. With the availability of over 32,000 unique full-length human cDNA clones, OriGene is in an enviable position to develop and support such high-throughput applications.

The PrecisionShuttle entry and destination vectors contain the neomycin phosphotransferase gene under the dual control of the β-lactamase promoter and the SV40 promoter. Expression of the neomycin phosphotransferase gene in mammalian cells allows stable cell selection with a neomycin analog such as G418, whereas in bacteria the gene confers resistance to kanamycin selection.

The development of the PrecisionShuttle vector system has gone through a rigorous quality control process. Both the entry vector and the destination vectors have been validated for transient and stable mammalian cell transfections using a T-GFP marker (data not shown). The N-terminal and C-terminal fusion tags have been validated by Western blot analysis (shown in Figure 3).

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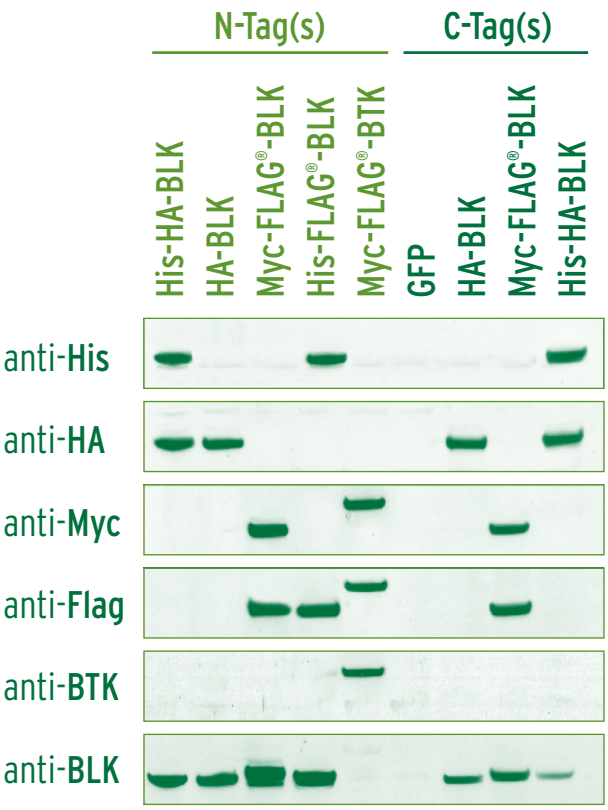


Figure 3. Western blot analysis of proteins expressed from N-terminally and C-terminally tagged PrecisionShuttle vectors. Each lane of the blot contains the whole cell lysate from an overexpression experiment using a PrecisionShuttle vector. BLK represents human B lymphoid tyrosine kinase (NM_001715); BTK represents human Bruton agammaglobulinemia tyrosine kinase (NM_000061). These two cDNAs were cloned into the destination vectors identified at the top of the blot. T-GFP represents one empty destination vector, pCMV6-AC-GFP, used for cloning C-terminal T-GFP fusion proteins. Specific antibodies against BLK and BTK detected the same size proteins as antibodies against the N-terminal and C-terminal tags.

## PRECISIONSHUTTLE VECTOR DETAILS

**TrueORF Entry Vector (pCMV6-Entry)** OriGene's TrueORF clones contain the fully sequenced ORFs of the genes of interest in pCMV6-Entry. This construct can be directly used for transient or stable transfection of cultured mammalian cells or for *in vitro* protein expression. It also can act as a donor of the protein-coding region using the Sgf I and Mlu I sites. The different antibiotic resistance genes carried on the TrueORF vectors (kanamycin resistance in the entry vector and ampicillin resistance in the destination vectors) facilitate the selection of successfully transferred protein-coding regions from entry to destination vectors. The entry vector also contains C-terminal tags of Myc and Flag, and the neomycin resistance gene.

**N-terminally tagged PrecisionShuttle destination vectors (pCMV6-AN)** These vectors are designed for expressing N-terminally tagged proteins in mammalian cells or using *in vitro* protein expression systems. The plasmids allow protein expression via the human cytomegalovirus (CMV) intermediate-early enhancer/promoter in mammalian cells and via the T7 RNA polymerase promoter in cell-free systems. The vectors contain the ampicillin resistance gene for selection in *E. coli* and the neomycin resistance gene enables stable cell selection in mammalian cells. Available tags include Myc, HIS, HA, Flag, and T-GFP.

**C-terminally tagged PrecisionShuttle destination vectors (pCMV6-AC)** These vectors are designed for expressing C-terminally tagged proteins in mammalian cells or using *in vitro* protein expression systems. The plasmids allow protein expression via the human cytomegalovirus (CMV) intermediate-early enhancer/promoter in mammalian cells and via the T7 RNA polymerase promoter in cell-free systems. The vectors contain the ampicillin resistance gene for selection in *E. coli* and the neomycin resistance gene enables stable cell selection in mammalian cells. Available tags include Myc, HIS, HA, Flag, and T-GFP.

**Untagged PrecisionShuttle vector (pCMV6-A)** This vector is designed for expression of the native (untagged) protein in mammalian cells or using *in vitro* protein expression systems. The vector contains the same promoters and selection markers (ampicillin and neomycin) as the N- and C-terminally tagged destination vectors.

**Nucleotide Sequences of PrecisionShuttle vectors** All sequences are available electronically on the OriGene website at http://www.origene.com/cdna/trueorf/destinationvector.mspx.

Stop spending your valuable time cloning, tagging, and subcloning. Order a TrueORF clone today! Search the OriGene website to find your TrueORF clone at http://www.origene.com/cdna/trueorf/trueorf.mspx, or give us a call at 888-267-4436 (301-340-3188 outside the US) and we'll help you find the clone that you need.

# TrueORF and PrecisionShuttle **Protocols**

### TRANSFER OF ORF FROM TRUEORF ENTRY VECTOR TO DESTINATION VECTORS

To transfer the protein-coding region from the TrueORF Entry Vector (donor) to a PrecisionShuttle destination vector (recipient), choose a destination vector with the desired tag options (Table 1). There are three types of PrecisionShuttle destination vectors, each of which is designed to express a native (untagged) protein, an N-terminally tagged protein, or a C-terminally tagged protein. Vectors which contain double tags include a short spacer (5-6 amino acid residues) between the two tags. The transfer protocol between TrueORF vectors is shown schematically on page 2, and is detailed below.*

*1. Digest the TrueORF entry clone:*

| Component | Volume |
|---|---|
| 10X restriction buffer** | 2 µL |
| Sgf I (10 U/µL) | 0.6 µL |
| Mlu I (10 U/µL) | 0.6 µL |
| nuclease-free water | 13.8 µL |
| TrueORF entry vector (100-200ng) | 3 µL |
| Total volume | 20 µL |

Incubate at 37ºC for 1 hour.

* For the 4% of the clones that have internal Sgf I or Mlu I sites, please use the appropriate combination of restriction sites as recommended by OriGene

** NEB buffer 3 has been shown to work well for a double digestion with Sgf I and Mlu I.

*2. Digest the TrueORF destination vector:*

| Component | Volume |
|---|---|
| 10X restriction buffer** | 2 µL |
| Sgf I (10 U/µL) | 0.6 µL |
| Mlu I (10 U/µL) | 0.6 µL |
| nuclease-free water | 14.8 µL |
| TrueORF destination vector (200ng) | 2 µL |
| Total volume | 20 µL |

Incubate at 37ºC for 1 hour. Add 0.4 µL calf intestine phosphatase (CIP) to the digestion, and continue to incubate at 37ºC for an additional 30 minutes.

** NEB buffer 3 has been shown to work well for a double digestion with Sgf I and Mlu I.

*3. Purify both digestions using commercial PCR purification columns and elute each in 20 µL 10 mM Tris.*

4. *Set up a ligation reaction*:

| Component | Volume |
|---|---|
| 10x T4 DNA ligation buffer | 1 µL |
| T4 DNA Ligase (4U/µL) | 0.75µL |
| nuclease-free water | 3.25 µL |
| digested DNA from Step 1 | 2 µL |
| digested DNA from Step 2 | 3 µL |
| Total volume | 10 µL |

Incubate the ligation reaction at room temperature for 1 hour.

*5. Transform the ligation reaction into high-efficiency, competent E. coli cells (1×10$^8$ CFU/µg DNA) following the appropriate transformation protocol. Plate the transformants on LB-agar plates supplemented with 100 µg/ml ampicillin.*

*6. Pick at least four colonies for subsequent DNA purification and screening. Amplify and purify the selected clone(s) by growing overnight in liquid LB-amp media, then isolating the DNA using standard plasmid purification procedures.*

*7. Confirm the insert by restriction digestion and/or vector primer sequencing using the provided primers (VP1.5 for 5′ end sequencing or XL39 for 3′ end sequencing.)*

### PRIMER DESIGN AND PCR AMPLIFICATION OF ORF

If you would like to insert a clone that is NOT a TrueORF into one of the PrecisionShuttle destination vectors, the open reading frame (ORF) of the clone must be PCR amplified in order to append cloning sites to the 5′ and 3′ ends of the sequence. Add the target sequences of the chosen restriction enzymes to the 5′ ends of the forward and reverse PCR primers; examples are shown below using Sgf I and Mlu I, which are absent in ~96% of all human ORFs.

*Forward primer with Sgf I:*
5′ GAGGC | GA | TCGCCN | NNNNNNNNNNNNNNNNNNNN 3′
*(Ns represent the sequence of the ORF beginning with the start codon, ATG)*

*Reverse primer with Mlu I:*
5′ GCGAC | GC | GTNNN | NNNNNNNNNNNNNNNNNNNN 3′
*(Ns represent the reverse complement of the ORF sequence starting with the stop codon for N-terminally tagged or untagged destination vectors. This ensures that the expressed fusion protein will end at the native C-terminal end of the ORF.*

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*For C-terminally tagged vectors, the reverse complement of the ORF sequence should start with the second-to-last codon, as the stop codon must be removed to generate a fusion protein. )*

If your ORF is one of the 4% that contain the recognition sites for Sgf I or Mlu I, another rare cutter such as Asc I, Rsr II or Not I can be used in the cloning strategy. In these cases, the sequences of these alternate restriction sites should be appended to the PCR primers, as shown below. The same primer design strategy described above should be used for design of the primers using Asc I, Rsr II, or Not I. The Ns in the forward primer represent the sequence of the ORF beginning with the start codon, ATG. The Ns in the reverse primers represent the reverse complement of the ORF sequence starting with the stop codon for N-terminally tagged or untagged destination vectors, or starting with the second-to-last codon for C-terminally tagged vectors.

*Forward primer with Asc I:*
5′ GCCGG | CG | CGCCN | NNNNNNNNNNNNNNNNNNNNN 3′

*Reverse primer with Rsr II:*
5′ GCGTC | GG | ACCGCT | NNNNNNNNNNNNNNNNNNNNNNN 3′

*Reverse primer with Not I:*
5′ GCGAC | GC | GGCCG | CTCACGCGT-
NNNNNNNNNNNNNNNNNNNNNNN 3′

We recommend using a full-length cDNA clone as the template for ORF cloning. The success rate is rather low when a cDNA pool is used as the template for a PCR cloning reaction. When the GC content of an ORF is above 75%, a special PCR buffer with DMSO or other additive should be used to increase the success rate. The recommended PCR polymerase and buffer are available from New England Biolabs (Phusion™ High-Fidelity PCR Kit, F-553S).

**PCR reaction setup:**

| Component | Volume |
|---|---|
| 5X PCR buffer | 4 µL |
| dNTPs (2.5 mM each) | 1.6 µL |
| Phusion polymerase (2U/µl) | 0.2 µL |
| Nuclease free water | 11 µL |
| Forward primer (10 µM) | 0.6 µL |
| Reverse primer (10 µM) | 0.6 µL |
| cDNA template (50-100ng plasmid) | 2 µL |
| Total volume | 20 µL |

All of the components should be kept on ice. When setting up multiple reactions, a master mix can be prepared without cDNA template or primers. After aliquotting the master mix, the cDNA template and primers can be added individually to each tube.

**PCR cycling conditions:**
The optimum Tm for annealing should be 55-60ºC. The extension time depends upon the length of the ORF. The following program is generally used for ORFs that are 500 bp-6000 bp.

| | |
|---|---|
| 95ºC, 1 min | |
| 2 cycles of | 95ºC, 10 sec<br>62ºC, 20 sec<br>72ºC, 4 min |
| 2 cycles of | 95ºC, 10 sec<br>60ºC, 20 sec<br>72ºC, 4 min |
| 2 cycles of | 95ºC, 10 sec<br>58ºC, 20 sec<br>72ºC, 4 min |
| 15 cycles of | 95ºC, 10 sec<br>56ºC, 20 sec<br>72ºC, 4 min |
| 72ºC, 10 min<br>4ºC hold | |

## CLONING OF ORF INTO THE ENTRY VECTOR

*1. Confirm that the size of the amplification product is correct by agarose gel electrophoresis, and purify the remainder of the reaction using a purification column or similar method. Elute the DNA from the purification column using 26 µL of 10 mM Tris buffer. Set up a digestion reaction as described below, substituting other restriction enzymes as appropriate.*

| Component | Volume |
|---|---|
| 10X restriction buffer | 3 µL |
| Sgf I (10U/µL) | 0.6 µL |
| Mlu I (10U/µL) | 0.6 µL |
| Purified PCR product | 26 µL |
| Total volume | ~30 µL |

Mix well, and incubate at 37ºC for 1 hour.

*2. Purify the digestion reaction using a purification column and elute using 18 µL of 10 mM Tris buffer.*

*3. Digest pCMV6-Entry with the restriction enzymes corresponding to the sequences added to the ORF. pCMV6-Entry is available from OriGene as 5 µg lyophilized DNA (catalog # PS100001). Resuspend the lyophilized DNA in 50 µL 10 mM*

*Tris buffer, and incubate for at least 30 min before use. Set up a digestion reaction as described below, substituting other restriction enzymes as appropriate.*

| Component | Volume |
|---|---|
| 10X restriction buffer | 3 µL |
| Sgf I (10U/µL) | 0.8 µL |
| Mlu I (10U/µL) | 0.8 µL |
| Nuclease free water | 15.4 µL |
| Entry vector DNA | 10 µL |
| Total volume | 30 µL |

Incubate at 37ºC for 1.5 hours, then add 0.5 µL calf alkaline phosphatase (CIP), and continue the incubation at 37ºC for another 30 min. A two hour digestion is recommended to ensure that the vector is completely digested. Dephosphorylation of the digested vector is essential to eliminate self-ligation.

*4. Purify the desired vector fragment by running the digestion reaction on an agarose gel, isolating the appropriate band, and purifying that band using a gel purification column. Elute the digested plasmid vector using 40 µL of 10 mM Tris buffer.*

*5. Set up a ligation reaction with the purified vector and insert fragments:*

| Component | Volume |
|---|---|
| 10X ligase buffer | 1 µL |
| Nuclease free water | 3.5 µL |
| T4 DNA ligase | 0.5 µL |
| Vector fragment | 2 µL |
| PCR product | 3 µL |
| Total volume | 10 µL |

Incubate the ligation reaction at room temperature for 30-60 minutes.

*6. Transform 1 µL of the ligation mixture using 20 µL high efficiency competent E. coli cells (ideally 1x10$^8$ CFU/ug). Following transformation, resuspend cells in 200 uL LB.*

*7. Plate the entire transformation reaction on a standard LB-agar plate containing 25 µg/mL kanamycin. Incubate at 37ºC overnight.*

*8. Pick at least 4-8 independent colonies from each ligation. Amplify and purify the selected clone(s) by growing overnight in liquid LB-kan media, then isolating the DNA using standard plasmid purification procedures. Confirm the insert by restriction digestion and/or vector primer sequencing.*

# How can OriGene products **benefit your research?**

The scientists at OriGene would like to collaborate with you on an ongoing research project, by providing advice and reagents that would be useful to your work. If you are interested in this kind of partnership, send an email to cDNA@origene.com with the subject line "collaboration". One of our highly trained scientists will contact you to discuss possible joint research ventures.


Already used an OriGene product in a published research article? If you have authored a paper that cites an OriGene product, send us a link to that paper, and we'll send you an OriGene "Nice Genes" T-shirt.

It's our way of saying "thanks" for relying on OriGene for your research needs.

## Trivia Question

**Who was primarily responsible for compiling the database of human genes and genetic disorders known as Mendelian Inheritance in Man?**

*Send your answer to cDNA@origene.com. The first ten correct responses received will win free OriGene merchandise, including an OriGene tote. Please include your full mailing address with your response.*

**The answer to our last trivia question:**
The human chromosome that contains the most (currently identified) genes of known sequence is chromosome 1. According to Build 36.2 on the NCBI website, chromosome 1 contains 2782 genes, nearly 900 more than any other human chromosome. This includes 1016 distinct loci.

# Frequently Asked Questions...

## about TrueORF clones and the PrecisionShuttle System

### WHY SHOULD I USE ORIGENE'S TRUEORF CLONES?

**Answer:** All TrueORF Clones are derived from OriGene's unique TrueClone Collection, and were isolated from high quality human cDNA libraries made from a variety of tissues. TrueORF Clones provide enough (10 µg) purified DNA to allow customers to directly apply these expression-ready, tagged ORF clones to experiments designed for protein expression, purification, protein-protein interaction and stable clone generation. TrueORF clones also serve as the entry vector for OriGene's PrecisionShuttle system and allow easy construction of variably tagged ORFs. All TrueORF vectors share the same multiple cloning sites (MCS), which are compatible with many other commonly used vector systems, such as Promega's Flexi system, Invitrogen's Gateway system and Novagen's pET system. Customers can easily shuttle the cDNA of a TrueORF clone between multiple TrueORF destination vectors to generate clones with different epitope tags, or transfer the ORF to other expression systems designed for specific experimental purposes.

### WHAT IS THE DIFFERENCE BETWEEN ORIGENE'S ENTRY VECTOR AND THE DESTINATION VECTORS?

**Answer:** The major differences are the antibiotic selection marker and the epitope tags or markers. The entry vector carries kanamycin resistance, while all destination vectors contain the ampicillin resistance gene. This allows simple screening for successfully subcloned products. All of the vectors have a unique combination of N- or C-terminal epitope tags or a fluorescent marker, as described in Table I on page 2.

**All TrueORF Clones are derived from OriGene's TrueClone Collection, and were isolated from high quality human cDNA libraries made from a variety of tissues.**

### WHAT RESTRICTION ENZYMES SHOULD I USE IF SGF I OR MLU I SITES ARE PRESENT IN MY ORF?

**Answer:** The recommended subcloning strategy for every TrueORF cDNA is listed in the product information on our website. While 96% of all human ORFs can use the Sgf I - Mlu I combination, some ORFs do contain internal sites for these enzymes. Most of those ORFs can be transferred using another rare cutter (Rsr II), whose restriction site is upstream of Mlu I, or Not I, whose site is immediately downstream of Mlu I. Using one of the four different subcloning combinations, any ORF can be transferred from one vector to another.

### HAS ORIGENE FULLY SEQUENCED ALL TRUEORF CLONES?

**Answer:** Not always. When transferring the cDNA into the TrueORF Entry Vector, OriGene always uses fully sequenced plasmids as templates and Phusion High-Fidelity DNA Polymerase (New England Biolabs), which has a mutation rate less than $4 \times 10^{-7}$. This ensures the highest fidelity of every TrueORF clone. After cloning into the entry vector, each of OriGene's TrueORF clones was sequenced at both the 5' and 3' ends, and the resulting sequence was matched to the corresponding reference sequence. For many ORFs 1 Kb or less in length, the 5' and 3' sequencing reads have covered the full ORF. For longer cDNAs, the ORF was not fully covered by sequencing reads.

### DO TRUEORF CLONES EXACTLY MATCH THE REFERENCE GENE SEQUENCE?

**Answer:** All TrueORF clones are guaranteed to match the ORF of the corresponding gene. However, some clones may contain nucleotide changes compared to the published reference sequences. This is due to SNPs (single nucleotide polymorphisms) reflecting the unique differences from genes expressed in different tissues and different individuals. Published references may represent a different SNP than the OriGene transcript. Should a specific SNP be required, this can be contracted from OriGene at an additional cost.

**The PrecisionShuttle destination vectors each has a unique combination of N- or C-terminal epitope tags or a fluorescent marker.**

### CAN I TRANSFER LARGE ORFS USING THIS SYSTEM?

**Answer:** Yes. It has been reported that ORFs larger than 4 Kb are unstable in recombination-based systems; conversely, our restriction digest-based vector system has no real size limitation. An ORF up to 18 Kb can be readily transferred from one vector to another.

### WHAT DOES YOUR DISCLAIMER MEAN?

**Answer:** OriGene's disclaimer for the TrueORF clones reads as follows: "Our molecular clone sequence data has been matched to the accession number below as a point of reference. Note that the complete sequence of our molecular clones may differ from the sequence published for this corresponding accession number, e.g., by representing an alternative RNA splicing form or single nucleotide polymorphism (SNP).

The NCBI RefSeq human mRNA sequences are continuously being revised, as some may have been derived from aberrantly spliced transcripts or generated by incorrect prediction of intron-exon junctions in silico. These sequences are therefore used only as a "reference" and not as a "standard". OriGene's clones are isolated from full-length cDNA libraries and may differ from the reference sequence for this reason.

### WHAT IS THE TRUECLONE GUARANTEE?

**Answer:** OriGene warrants that the product will meet specifications listed. At OriGene's discretion, free replacement of any non-conforming product will be made if OriGene is notified within 30 days of product receipt. If you experience any difficulty with any OriGene product, please contact our Technical Support Staff at 888-267-4436, or 301-340-3188 outside the US.

## **Coming Soon...**
## GFC-Transfection Arrays of cell cycle genes, proteases, or epigenetic regulatory cDNAs!

OriGene already offers many useful arrays of gene families such as protein kinases, transcription factors, and G-protein coupled receptors (GPCRs). In the coming weeks, we'll also be releasing arrays of cell cycle-related genes, proteases, and genes involved in epigenetic regulation.

Check our website (http://www.origene.com/cdna/gfc-array/) to see when these arrays become available, or send an email to techsupport@origene.com and we'll let you know when you can purchase these arrays for high-throughput functional assays. If you would like to see a different gene family or subset of the genome represented on a GFC-Transfection Array, please let us know by sending an email to techsupport@origene.com. We're always looking for ways to offer more products that our customers would find useful, and we'd love to hear from you!

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# **Genes** in the News

## TWO GENOME-WIDE ASSOCIATION STUDIES OF AGGRESSIVE PROSTATE CANCER IMPLICATE PUTATIVE PROSTATE TUMOR SUPPRESSOR GENE DAB2IP

David Duggan, Siqun L. Zheng, Michele Knowlton, Debbie Benitez, Latchezar Dimitrov, Fredrik Wiklund, Christiane Robbins, Sarah D. Isaacs, Yu Cheng, Ge Li, Jielin Sun, Bao-Li Chang, Leslie Marovich, Kathleen E. Wiley, Katarina Bälter, Pär Stattin, Hans-Olov Adami, Marta Gielzak, Guifang Yan, Jurga Sauvageot, Wennuan Liu, Jin Woo Kim, Eugene R. Bleecker, Deborah A. Meyers, Bruce J. Trock, Alan W. Partin, Patrick C. Walsh, William B. Isaacs, Henrik Grönberg, Jianfeng Xu, John D. Carpten

**J Natl Cancer Inst 2007; 99: 1836 – 44**

### A single nucleotide polymorphism in DAB2IP may be associated with aggressive prostate cancer

Genome-wide association studies can be used to identify genetic variations that are linked to increased risk of a disease. Detecting these variations is the first step in determining whether they are associated not only with increased risk, but also early development or aggressive progression of the disease, or even responsiveness to a given treatment. Two recent publications in Nature Genetics used this type of analysis to identify a chromosomal location associated with increased risk of prostate cancer (Gudmundsson et. al. 2007, Yeager et al. 2007).

Researchers from the Translational Genomics Research Institute, Wake Forest University School of Medicine, the Karolinska Institute in Sweden and Johns Hopkins Medical Institutions analyzed the single nucleotide polymorphisms (SNPs) from a large group of Swedish subjects with aggressive prostate cancer and from a large control population without prostate cancer. Over sixty thousand SNPs were analyzed by genotyping using chip technology from Affymetrix and Illumina. Those SNPs identified as being significantly associated with advanced prostatic disease in the initial analysis were subjected to confirmation in two further studies using American subjects. In the first of two follow-up studies, a group of subjects were identified from patients undergoing radical prostatectomy at the Johns Hopkins Hospital as a treatment for prostate cancer. Control subjects were chosen from the same population group being screened for prostate cancer. Seven SNPs were identified that met further statistical qualifications (conformation to Hardy-Weinberg equilibrium, allele frequency analysis, and correlation of direction of association in both studies). These seven SNPs were genotyped in a second follow-up study of patients with aggressive prostate cancer and unaffected control subjects. One SNP, rs1571801 at 9q33, was statistically significantly associated with aggressive disease in populations of European ancestry (P = 0.004) and in populations of African ancestry (P = 0.02). This SNP was also associated with a significantly increased risk of overall prostate cancer in the European-American population, but the increased risk of overall disease was not significant in the African-American population.

SNP rs1571801 maps within the gene DAB2IP, which contains two transcripts recognized in the Entrez Gene database, represented by the nucleotide sequences NM_032552 and NM_138709. DAB2IP is a Ras GTPase-activating protein (GAP) that acts as a tumor suppressor gene and is inactivated by methylation in prostate and breast cancers (Yano et al. 2005). As would be expected of a tumor suppressor gene, it is expressed in normal prostate epithelial cells but expression is decreased in prostate cancer cells. Recent studies have linked the decreased expression of DAB2IP with increased expression of EZH2 (Chen et al. 2005), a transcriptional repressor that has been identified as a significant marker of aggressive prostate cancer (Varambally et al. 2002). This information, coupled with the functional association of DAB2IP with the MAP kinase cascade, strengthens the hypothesis that SNP rs1571801 represents a potentially important indicator of aggressive prostate cancer. However, the authors caution that there remains a risk of false-positive association due to population stratification, and encourage confirmation studies to determine the validity of this association.

If confirmed, this SNP could eventually be used as a screening tool (via a small blood sample) to help doctors and patients determine the best course of treatment following the diagnosis of prostate cancer. Currently, there are markers to determine risk of prostate cancer, but none that indicate the aggressiveness of the disease. Such a marker could be used to determine whether or not aggressive treatment strategies would be advisable, and prevent potentially unnecessary radiation treatments and surgeries in patients with low risk.

**References**

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Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 2002; 419: 624 – 9.

Yano M, Toyooka S, Tsukuda K, Dote H, Ouchida M, Hanabata T, Aoe M, Date H, Gazdar AF, Shimizu N. Aberrant promoter methylation of human DAB2 interactive protein (hDAB2IP) gene in lung cancers. Int J Cancer. 2005 Jan 1;113(1):59-66.

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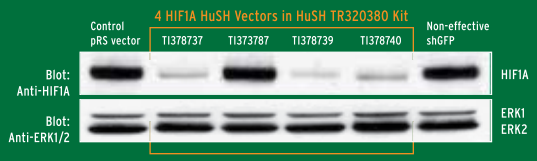
- The successful use of OriGene shRNA vectors have been recently cited
- OriGene shRNAs can be rapidly validated in HEK293T cells together with OriGene TrueClone cDNAs

### Downregulation of HIF1A Expression by HuSH Contructs




HEK239T cells were contransfected with pCMV-HIF1A cDNA together with four shRNA contructs against HIF1A. Western blot data demonstrates that three out of the four constructs significantly downregulate the cotransfected HIF1A expression. More details can be found at www.origene.com/rna

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# **High-throughput functional screening** has never been so simple to do!

The post-genomic area brought many novel and exciting genome-wide assay platforms for high-throughput screening. However, functional screening, a key element in identifying genes involved with biological processes, is still a labor-intensive, expensive process applied to just a handful of selected genes at a time.

OriGene offers a unique tool for discovery: GFC-Transfection Arrays for high-throughput functional screening by protein overexpression. These arrays are ready to use; all you have to do is add your reporter (if applicable), a transfection agent and cells, and you'll have your answer in just 48 hours. The rapidly growing arsenal of assays and readouts specifically designed for high-throughput increases the versatility of usage and makes these arrays an indispensable tool for discovery of biological processes.

### ARRAY PRODUCTION AND QUALITY

OriGene's TrueClone Collection of authentic full-length human cDNA clones in mammalian expression vectors presents an exclusive advantage in the construction of GFC-Transfection Arrays. We use only high-quality midiprep plasmid DNA that is prenormalized to the same DNA quantity (10 ng) by spectrophotometric ($OD_{260}$) analysis. This procedure eliminates most clone-to-clone and batch-to batch variability and allows us to consistently deliver a uniform, high-quality product. The arrays are printed directly from the bar-coded tubes by a robotic instrument in a validated procedure developed at OriGene. The above reagents and procedures ensure a consistent, uniform printing of the arrays. The cDNA in the arrays is lyophilized, and the arrays are stored sealed at –20°C for maximal stability.

Our current GFC-Transfection Arrays and upcoming arrays are listed below. OriGene is constantly expanding our coverage based upon customer's input, so please let us know if there is a specific array you'd like to see.

### FORMATS AVAILABLE FOR GFC-TRANSFECTION ARRAY PANELS

All GFC-Transfection Arrays are printed on clear bottom plates allowing visual inspection and readout of the transfected cells. White plates are ideal for luminescence applications while black plates are particularly useful for fluorescence and other applications. Each array is provided with two identical optimization plates for establishing the most appropriate assay conditions.

The 384-well format of most GFC-Transfection Arrays includes 3 identical sets, each containing one (1) plate of up to 352 individual cDNAs arrayed in columns 1-22. Each well contains 60 ng of plasmid DNA. The last two columns are left empty for assay controls and to allow the researcher to add additional cDNAs that are important for his or her particular use.

The 96-well format of these arrays includes 3 identical sets, each containing four (4) plates with 100 ng each of up to 88 individual cDNAs (columns 1-11, total of 352). The last column in each plate is left empty for assay controls and to allow the researcher to add additional cDNAs that are important for his or her particular use.

**GFC-Transfection Arrays are a unique tool for high-throughput functional screening by protein overexpression.**

## Currently Available GFC-Transfection Arrays

### PROTEIN KINASE GFC-TRANSFECTION ARRAY I

The Protein Kinase GFC-Transfection Array I contains 352 transfection-ready plasmids encoding various protein kinases. The array includes MAP kinase isoforms, tyrosine kinases, serine/threonine kinases, Protein Kinase C isoforms, casein kinases, cell cycle and cyclin-dependent kinases and more.

Data generated using this array in two luciferase reporter assays are shown in Figures 1 and 2.

*Continued on Page 14*

**EFFECT OF GENES IN THE PROTEIN KINASE GFC-TRANSFECTION ARRAY ON ACTIVITY OF p16-Luc REPORTER**

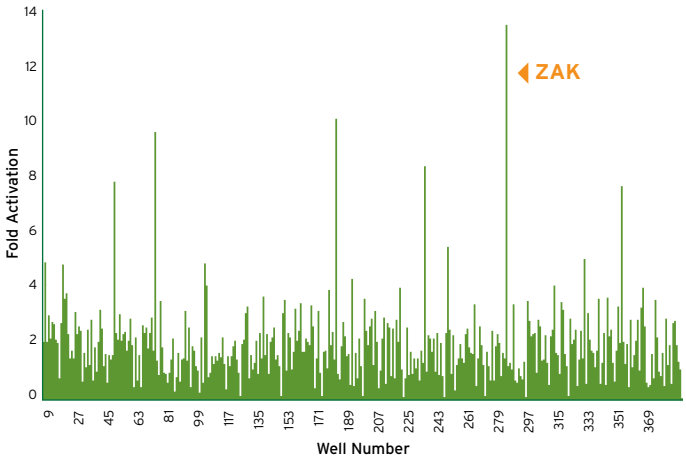



Figure 1. Reporter gene assay using a p16 reporter plasmid. The Protein Kinase GFC-Transfection Array was tested for activation of a reporter plasmid containing the cyclin-dependent kinase inhibitor 2A (CDKN2A or p16) promoter driving expression of luciferase. The array was reverse transfected with HEK293T cells using TurboFectin 8.0, and luciferase activity was scored 48 hours later using BriteLite luciferase substrate (PerkinElmer). Luciferase activity for each of the 352 cDNAs in the array is plotted against the sample number. The cDNA initiating the highest activation of the reporter is indicated. Additional genes (known and novel interactors) that activate the p16 promoter or inhibit its intrinsic activity were also detected.

**EFFECT OF GENES IN THE PROTEIN KINASE GFC-TRANSFECTION ARRAY ON ACTIVITY OF p21-Luc REPORTER**

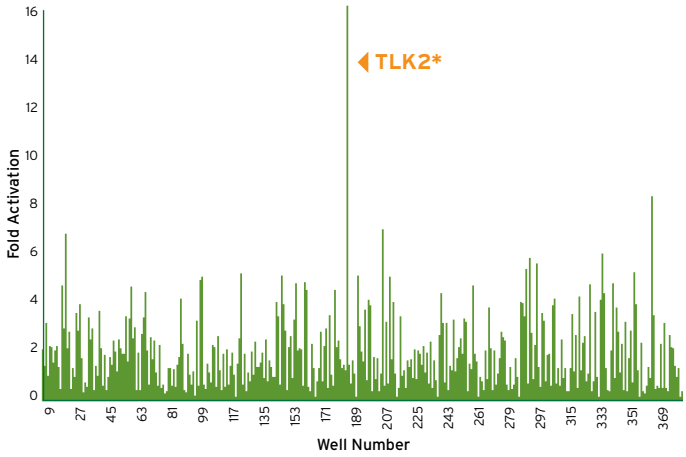



Figure 2. Reporter gene assay using a p21 reporter plasmid. The Protein Kinase GFC-Transfection Array was tested for activation of a reporter plasmid containing the cyclin-dependent kinase inhibitor 1A (CDKN1A or p21) promoter driving expression of luciferase. p21 is a known inhibitor of the activity of CDK2 or CDK4 kinase complexes suggested to be involved in tumor suppression. The array was reverse transfected with HEK293T cells using TurboFectin 8.0, and luciferase activity was scored 48 hours later using BriteLite luciferase substrate (PerkinElmer). Luciferase activity for each of the 352 cDNAs in the array is plotted against the sample number. The highest activator is marked. Additional genes (known and novel) that activate the CDKN1A promoter or inhibit its intrinsic activity were also detected.

**APOPTOSIS OF HeLa CELLS IN THE APOPTOSIS GFC-TRANSFECTION ARRAY INDUCED BY PACLITAXEL**

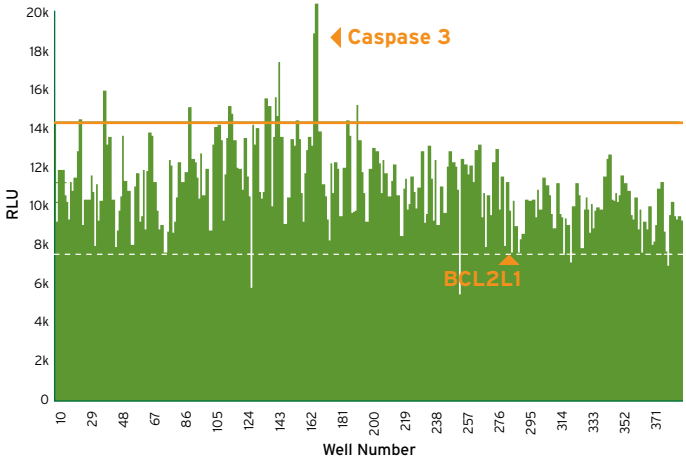



Figure 3. Apoptosis assay using paclitaxel. The effect of paclitaxel on apoptosis related genes was determined using the Apoptosis GFC-Transfection Array. The array was reverse transfected with HeLa cells using TurboFectin 8.0, and paclitaxel was added to a final concentration of 0.1 µM 32 hours later. The cells were incubated for additional 12 hours (total of 48 hours), then assayed using the Caspase 3/7 Glo assay system (Promega). Luciferase activity (RLU) for each of the 352 cDNAs in the array is plotted against the sample number. The cutoff for induction or protection from paclitaxel-induced apoptosis was calculated from the average RLU of 10 control wells +/- 3 times the standard deviation.

**CELL SURVIVAL ASSAY USING THE APOPTOSIS GFC-TRANSFECTION ARRAY**

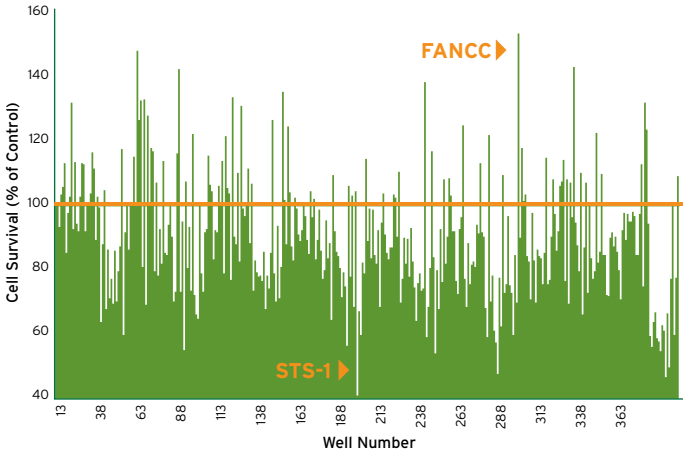



Figure 4. Cell survival assay. The effect of apoptosis related genes on the viability of HEK293T cells was determined using the Apoptosis GFC-Transfection Array. The array was reverse transfected with HEK293T cells using TurboFectin 8.0. Cell viability was tested 48 hours later with the ATPlite 1step assay (PerkinElmer). Several genes were shown to promote cell proliferation / viability by up to 50% or promote cell death by more than 50%. Other genes had variable effect on cell survival.

*Continued from Page 12*

#### GPCRS GFC-TRANSFECTION ARRAY I

The GPCRs GFC-Transfection Array I contains 286 transfection-ready plasmids encoding various G-protein coupled receptors. The set includes mostly non-olfactory GPCRs from all three major classes and utilizing various second messenger signaling methods.

#### APOPTOSIS GENES GFC-TRANSFECTION ARRAY I

The Apoptosis GFC-Transfection Array I contains 352 transfection-ready plasmids encoding various genes associated with apoptosis. The array includes: caspases, kinases/phosphatases, cell death/death domain containing proteins, BCL2 associated genes, TNF and interleukin related proteins, and more.

Data generated using this array in two apoptosis and/or viability assays are shown in Figures 3 and 4.

#### TRANSMEMBRANE GENES GFC-TRANSFECTION ARRAY I

The Transmembrane Genes GFC-Transfection Array I contains 352 transfection-ready plasmids encoding various transmembrane protein cDNAs. Many of the cDNAs in this panel are associated with enzymatic activities. The array includes UDP associated enzymes, phosphotidyl inositol related cDNAs, cytochrome related cDNAs, lectins, zinc and ring finger proteins, proteins associated with lipid or carbohydrates and more. Additional panels of transmembrane protein cDNAs are also available.

#### SECRETED PROTEINS GFC-TRANSFECTION ARRAY I

The Secreted Proteins GFC-Transfection Array I contains the first set of 352 transfection-ready cDNAs each encoding a secreted protein. The array includes cDNAs for growth factors, cytokines, chemokines, various enzymes, various ligands, matrix proteins, proteases, complement related proteins, secreted receptors, kinases and more.

#### SECRETED PROTEINS GFC-TRANSFECTION ARRAY II

The Secreted Proteins GFC-Transfection Array II contains a second, unique set of 352 transfection-ready cDNAs each encoding a secreted protein. The array includes cDNAs for growth factors, cytokines, chemokines, various enzymes, various ligands, matrix proteins, proteases, complement related proteins, secreted receptors, kinases and more.

#### TRANSCRIPTION FACTORS GFC-TRANSFECTION ARRAY

The Transcription Factors GFC-Transfection Array contains a set of 704 transfection-ready plasmids encoding many common transcription factors. The array includes homeobox proteins, nuclear receptors, forkhead proteins, zinc finger proteins, polymerase associated proteins, IRFs, DNA binding proteins, general transcription factors and more.

## Upcoming GFC-Transfection Arrays

#### CELL-CYCLE GFC-TRANSFECTION ARRAY

The Cell-Cycle GFC-Transfection Array contains 352 transfection-ready plasmids encoding genes associated with various cell cycle processes. These gene include: cell division cycle (CDC) genes, transcription factors, signal transducers and adaptors of signal transduction, cyclin-dependent kinases (CDK) and CDK inhibitors, molecular switches, genes responsive for particular cell-cycle phases, calcium binding proteins and more.

#### DNA-REPAIR GFC-TRANSFECTION ARRAY

The DNA-Repair GFC-Transfection Array contains 266 transfection-ready plasmids encoding genes participating in repair mechanisms for damaged DNA. The array includes: X-ray repair genes, UV radiation repair genes, ubiquitin associated genes, replication proteins, DNA cross-link repair genes, kinases and kinase inhibitors, and many more.

#### EPIGENETIC I GFC-TRANSFECTION ARRAY

The Epigenetic I GFC-Transfection Array contains a collection of 350 transfection-ready plasmids encoding various chromatin and chromatin modulating genes. The array includes: histones, histone modifiers, regulators of chromatin, forkhead box transcripts, transcription factors, signal transduction and signal transduction response elements, kinases and more.

#### EPIGENETIC II GFC-TRANSFECTION ARRAY

The Epigenetic II GFC-Transfection Array contains 350 transfection-ready plasmids encoding various chromatin and histone modulating genes, and telomere associated genes. The array includes transcription factors, signal transduction proteins, kinases, methyltransferases, nuclear receptors, homeobox genes, and more.

### ONCOGENES GFC-TRANSFECTION ARRAY

The Oncogenes GFC-Transfection Array includes a unique set of transfection-ready plasmids encoding almost 300 known and suspected oncogenes. The array includes human genes homologous to viral oncogenes, growth factors, RAS related proteins, myc related proteins, BCL related proteins and many more.

### TUMOR SUPPRESSOR GFC-TRANSFECTION ARRAY

The Tumor Suppressor GFC-Transfection Array includes a collection of 352 transfection-ready plasmids encoding various known and suspected tumor suppressors. The set includes p53 family and related genes, cyclin-dependent kinase inhibitors, transcription factors and signal transduction genes, nuclear receptors, kinases, phosphatases and more.

### PROTEASES GFC-TRANSFECTION ARRAY

The Protease GFC-Transfection Array includes a diverse collection of 352 transfection-ready plasmids encoding various proteases. The array includes caspases, cathepsins, serine proteases, amino- and carboxy- peptidases, matrix metallopeptidases, proteasomes, and many more.

### CHAPERONES GFC-TRANSFECTION ARRAY

The Chaperones GFC-Transfection Array contains 116 transfection-ready plasmids encoding various chaperone genes. The array includes chaperonin, DnaJ related molecules, heat shock genes and more.

### UBIQUITIN GFC-TRANSFECTION ARRAY

The Ubiquitin GFC-Transfection Array is a set of more than 250 transfection-ready plasmids encoding various gene associated with ubiquitin related processes. The array includes ubiquitin and ubiquitin-like containing molecules, ubiquitin-conjugating enzymes, ubiquitin specific peptidase, ubiquitin-activating enzymes, tripartite motif-containing proteins, ring finger proteins and more.

OriGene is developing additional arrays that include genes involved with angiogenesis, proteins containing known domains and motifs and more. OriGene is also in the process of assembling a set of transmembrane proteins arrays that will include channels, transporters, cell surface proteins, various receptors and more. These arrays will be available later this year. We value your opinion and suggestions. Please let us know which type of arrays will serve your particular interest and research.

*Continued on Page 16*

**"OriGene has done a wonderful job in creating an outstanding collection of high quality cDNAs in a single expression vector. We believe OriGene's GFC-Transfection Array will be an extremely useful resource to help us accelerate our understanding of the function and role of genes in biology in a high throughput and systematic manner."**

Brian Seed, Ph.D. MGH Molecular Biology Department and Professor of Genetics, Harvard University Medical School

**About Dr. Seed**

*Dr. Seed is a Professor of Genetics at Harvard Medical School and Director of the Center for Computational and Integrative Biology at Massachusetts General Hospital. His lab was an early developer of the methodology of expression cloning. An outgrowth of work in that area is directed at the use of automated platforms to achieve a comprehensive enumeration of genes that have specific biological functions in vivo.*

*Dr. Seed's lab is one of the pioneers that used OriGene's GFC-Transfection Array format to conduct high-throughput functional analysis of genes.*

*Continued from Page 15*

## What can I do with a GFC-Transfection Array?

The way we conduct functional screening, a key element in identifying genes involved with biological processes and assembling various pathways, is rapidly changing. OriGene GFC-Transfection Arrays for high-throughput functional screening by protein overexpression is becoming a major tool for discovery by both pharmaceutical companies and academics. All you actually need is an assay readout suitable for a 96 or 384 well format and you can use any OriGene GFC-Transfection Array. Many companies offer numerous kits and technologies for high-throughput screening assays, and the list is rapidly growing. Chances are that you will find or can easily adapt an appropriate readout for your specific needs.

Reporter gene assays can identify transcription factors, kinases, GPCRs and other genes that activate or inhibit your gene of interest. These simple to perform assays allow you to discover or assign novel genes to specific pathways such as the interferon pathway (Fig. 5). In many organisms, tools to explore promoter activation and identifying important response elements are lacking. The use of GFC-Transfection arrays can provide an answer even if the promoter is of non-mammalian origin (Fig. 6).

GFC-transfection arrays can be used to measure the effect of kinases, GPCRs, apoptosis related genes, etc. on cell viability, appearance, gene expression and various signal transduction processes (Figures 1-4). The specificity of various compounds can be validated and measured with the appropriate GFC-Transfection Arrays, thus facilitating the process of drug discovery.

Resistance to anti-cancer drugs is a major difficulty in the treatment of cancer. GFC-Transfection Arrays can identify key genes that reverse or induce resistance to anti-cancer drugs such as paclitaxel and provide key evidence for the mode of action of these agents. These arrays can be used to identify unknown receptors for viruses, bacteria, cell-cell interactions, ligands and reactive compounds. The arrays can also identify targets of antibodies and validate the specificity of these antibodies.

Cell engineering is another potential use for these arrays. GFC-Transfection Arrays provide a platform for discovering genes that can modify your target cell for a specific function or phenotype. Screening for genes that will increase protein pro-

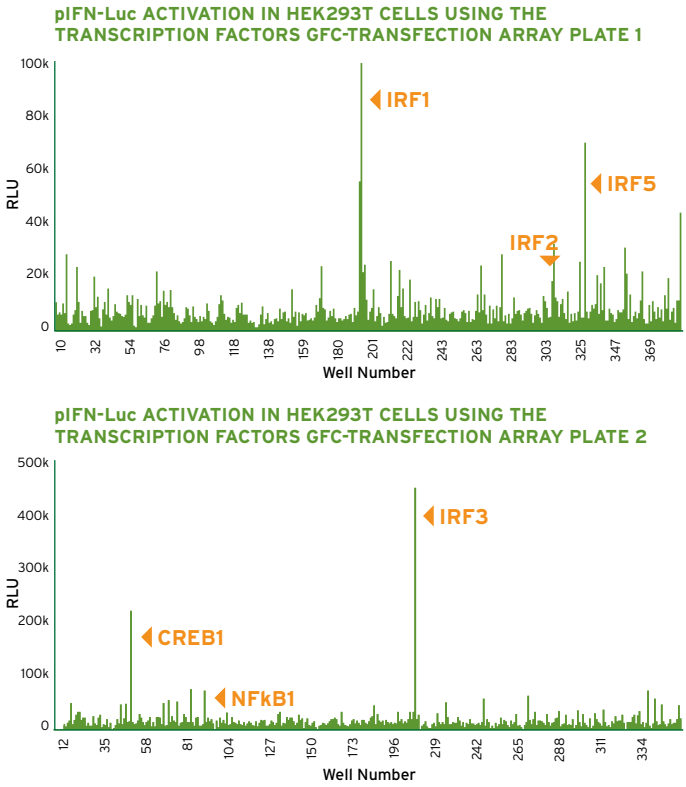



Figure 5. Reporter gene assay with pIFN reporter plasmid. The Transcription Factors GFC-Transfection Array was tested for activation of a reporter plasmid containing the interferon-β1 promoter driving expression of luciferase (pIFN-Luc). The array was reverse transfected with HEK293T cells using TurboFectin 8.0, and luciferase activity was scored 48 hours later using BriteLite luciferase substrate (Perkin Elmer). Luciferase activity for each of the cDNAs in the array is plotted against the sample number (plate 1, top; plate 2, bottom). Several of the cDNAs known to activate the reporter are indicated. Additional genes (known and novel interactors) that activate the IFN-β1 promoter or inhibit its intrinsic activity were also detected.

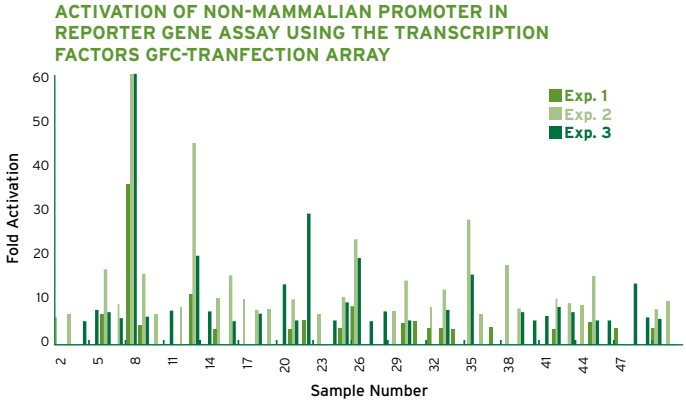



Figure 6. Reporter gene assay using a non-mammalian promoter. The Transcription Factors GFC-Transfection Array was used in three independent experiments looking for activation of a non-mammalian promoter for which no activators are known. Very few transcription factors are known in these organisms, and none are known to activate this promoter. Using the GFC-Transfection Array, we identified several potential activators of the promoter, including one cDNA that increased reporter activity by 70-100 fold.

## GFC-Transfection Arrays containing proteases, oncogenes, tumor suppressors, cell cycle regulators, DNA repair genes, and epigenetic modulators will be released soon.

duction, provide for correct or better protein folding or boost secretion of recombinant proteins are some of the applications that can be implemented with GFC-Transfection Arrays.

These useful arrays offer countless possibilities for genome-wide functional discovery. We (and many of our customers) routinely find novel genes and discoveries with GFC-Transfection Array screening. We are absolutely confident that you will have the same success – order an array today!

### GFC-TRANSFECTION ARRAYS CAN BE USED TO STUDY A WIDE RANGE OF BIOLOGICAL PROCESSES INCLUDING:

- Gene function
- Transcriptional regulation
- Cell and tissue-specific gene expression
- Cell proliferation and differentiation
- Developmental gene regulation
- Cytotoxicity
- Apoptosis
- Host-pathogen interactions
- Sensitivity to drugs and small molecules
- Mode of action of drugs and genes

### GFC-TRANSFECTION ARRAYS ARE ADAPTABLE FOR HIGH-THROUGHPUT READOUT ASSAYS INCLUDING:

- Reporter gene assays
- Receptor activation assays (e.g., G-protein coupled receptor assays)
- Nuclear translocations of protein markers
- Enzymatic assays
- Immunoassays
- Protein interaction assays
- Cell proliferation assays
- Apoptosis assays
- Cell toxicity assays
- Morphological assays

### THE UTILITY OF GFC-TRANSFECTION ARRAY METHODOLOGY HAS BEEN DEMONSTRATED BY NUMEROUS RESEARCHERS LEADING TO THESE RECENTLY PUBLISHED STUDIES:

**A genomic screen for activators of the antioxidant response element.** Yanxia Liu, Jonathan T Kern, John R Walker, Jeffrey A Johnson, Peter G Schultz, and Hendrik Luesch *Proc Natl Acad Sci U S A. 2007 Mar 20;104(12):5205-10.*

**A functional genomic approach to the mode of action of apratoxin A.** Hendrik Luesch, Smith K Chanda, R Marina Raya, Paul D DeJesus, Anthony P Orth, John R Walker, Juan Carlos Izpisua Belmonte and Peter G Schultz. *Nature Chemical Biology 2006 2(3) 158-167.*

**Transducer of regulated CREB-binding proteins (TORCs) induce PGC-1{alpha} transcription and mitochondrial biogenesis in muscle cells** Zhidan Wu, Xueming Huang, Yajun Feng, Christoph Handschin, Yan Feng, P. Scott Gullicksen, Olivia Bare, Mark Labow, Bruce Spiegelman, and Susan C. Stevenson *PNAS 2006 Sep 26;103(39):14379-84.*

**Obesity, hyperphagia and increased metabolic efficiency in Pc1 mutant mice** David J. Lloyd, Sandy Bohan, and Nicholas Gekakis *Hum. Mol. Genet., Jun 2006; 15: 1884-1893.*

**High throughput functional genomics: Identification of novel genes with tumor suppressor phenotypes** Kerstin Koenig-Hoffmann, Angelika L. Bonin-Debs, Irene Boche, Beate Gawin, Andrea Gnirke, Christoph Hergersberg, Frank Madeo, Michael Kazinski, Matthias Klein, Christian Korherr, Dieter Link, Sascha Röhrig, Rolf Schäfer and Ulrich Brinkmann. *Int. J of Cancer 2005 113(30) 434-439.*

**Activity-dependent NMDA receptor degradation mediated by retrotranslocation and ubiquitination** Kato A, Rouach N, Nicoll RA, Bredt DS. *Proc Natl Acad Sci U S A. 2005 Apr 12;102(15):5600-5.*

**Identification of the Wnt signaling activator leucine-rich repeat in Flightless interaction protein 2 by a genome-wide functional analysis** Liu J, Bang AG, Kintner C, Orth AP, Chanda SK, Ding S, Schultz PG. *Proc Natl Acad Sci U S A. 2005 Feb 8;102(6):1927-32.*

For more information on GFC-Transfection Arrays, visit OriGene's website at http://www.origene.com/cdna/gfc-array/ or contact our Technical Support Professionals at techsupport@origene.com or 888-267-4436 (301-340-3188 outside the US).

# TrueClones come in different flavors—**mouse clones now available!**

OriGene's customers often ask if we can provide high quality TrueClone cDNAs from mouse tissue or other sources. Now we can—over 5000 mouse clones can be shipped to you in a vector suitable for transient or stable expression in mammalian transfection experiments.

The full-length mouse TrueClones are delivered in the pCMV-Kan/Neo vector (Figure 1). This vector is suitable for transient transfection or stable cell generation, and can also be used to express the mouse cDNA in cell-free systems using the T7 promoter. Each mouse TrueClone is fully sequenced and is assured to represent the specified reference sequence through strict BLAST requirements. These full-length mouse cDNAs are purified by anion-exchange column chromatography to provide transfection-ready plasmid DNA.

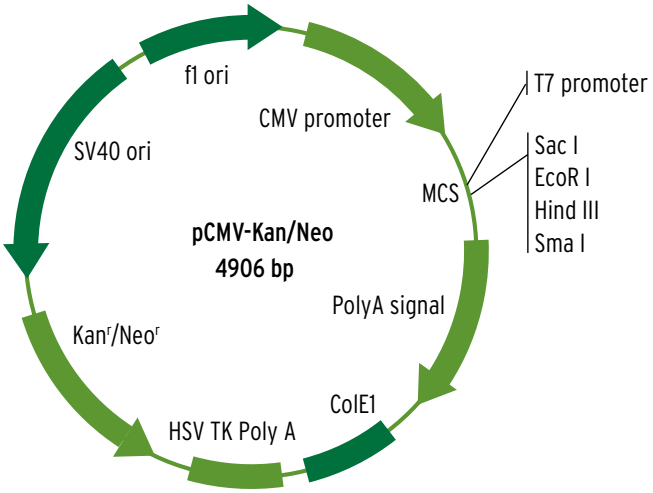
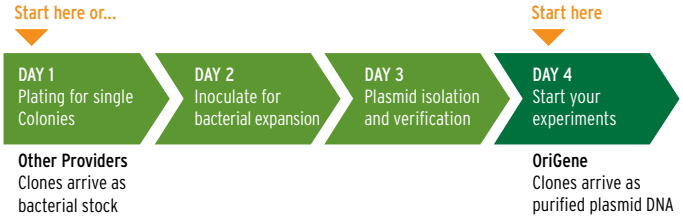



**Figure 1** The vector for all mouse TrueClones, pCMV-Kan/Neo, is 4906 bp in size. The cDNA library inserts are directionally ligated into the EcoR I and Not I sites of pCMV6-Kan/Neo vector, and can be recut from the plasmid using the flanking EcoR I / Hind III sites. The CMV promoter is upstream of the cDNA insert, which is followed by the mGH (mouse growth hormone) polyA signal. The ColE1 ori is the bacterial origin of replication, the SV40 ori allows for replication in mammalian cells and the f1 ori is the filamentous phage origin of replication, which allows for the recovery of single-stranded plasmids. Selection of the plasmid in *E. coli* by kanamycin and in mammalian cells by neomycin is conferred by the neomycin-kanamycin phosphotransferase gene. The T7 RNA polymerase can be used for generating transcripts of the cDNA by *in vitro* transcription.

TrueClones are available as 10 ug of purified plasmid DNA derived from a single colony, which are end-sequence verified before shipment. This format provides unmatched convenience and industry-leading accuracy and saves at least 3 days of routine lab work and related costs.

Most clones from other providers are provided as bacterial stocks that need to be converted to purified plasmid for identity verification and for downstream applications such as transfection, subcloning, protein expression in cell-free systems, etc. Those suppliers then ask you to verify that you received the correct stock.




OriGene realizes that such quality control should not be your job. We put in extra effort to deliver pure plasmid DNA so that you can start your project on the first day the clone arrives.

### WHAT ARE THE ADVANTAGES OF USING AN ORIGENE TRUECLONE?

TrueClones are a cost-effective and time-saving alternative to de novo cloning. Although gene cloning can be a straightforward process, it still requires substantial resources and time to clone even the most common gene into an expression vector. And many cDNAs (such as rare, large, or GC-rich transcripts) can be quite difficult to clone, and would require significant effort which even then may not be successful. A few clones are even toxic to standard cloning bacteria, posing even more obstacles to successful de novo cloning. The estimated time and effort required to isolate a clone are usually grossly underestimated.

### WHY SPEND YOUR TIME, RESOURCES AND ENERGY ON GENE CLONING WHEN A PRE-CLONED GENE IS AVAILABLE AND READY TO BE SHIPPED TO YOUR LABORATORY?

TrueClones are expression-ready and transfection-ready. All TrueClones have the strong CMV promoter upstream of the cDNA for robust mammalian expression. The plasmid can be transfected immediately for protein overexpression studies.

**Each TrueClone is derived from a cDNA library, not via PCR amplification. This means your clone contains a naturally occurring cDNA transcript, free from PCR-introduced artifacts.**

### AUTHENTICITY

Each TrueClone is derived from a cDNA library, not via PCR amplification. This means your clone contains a naturally occurring cDNA transcript, free from PCR-introduced artifacts. With a native transcript, you don't need to worry that your downstream application will be affected by unintended mutations.

### CONSISTENT VECTOR

While other vendors provide clones in a conglomeration of vectors with various antibiotic selection markers and variable expression-readiness, all mouse TrueClones are in a single vector, the pCMV6-Kan/Neo vector. Every TrueClone is ready for immediate protein overexpression in mammalian cells or in cell-free systems utilizing the T7 promoter.

The expression-readiness and the uniform vector system of the TrueClone product line render it ideal for high-throughput screening for functional genes.

### ACCURACY

Verified, pure plasmid DNA stocks will be delivered instead of bacterial stocks. No need for colony screening, plasmid preparation and restriction digestion/sequencing, which are all necessary when ordering a clone from most suppliers.

### FEATURES OF THE TRUECLONE MOUSE VECTOR

- The CMV promoter and a Kozak consensus sequence drive protein expression in mammalian cells.
- Ten (10) ug of purified plasmid is provided for immediate transfection and expression experiments.
- An antibiotic selection cassette ($Kan^r$/ $Neo^r$) confers resistance to kanamycin in *E. coli* and neomycin analogs in mammalian cells.
- The T7 promoter upstream of the ORF allows protein expression in cell-free systems.
- Each clone contains a fully verified insert sequence.

If your research requires a mouse cDNA clone, then check OriGene's website (http://www.origene.com/cdna/mouseclones.mspx) or call our Customer Service professionals to inquire how quickly we can put a mouse TrueClone in your hands. You'll be glad you saved the time you might have spent cloning it yourself, or purifying it from another company's bacterial stock. After all, you've got more important work to do!

# Citations

OriGene is proud of its customers and their track records of scholarly article publication. Here are some citations of recently published, peer-reviewed manuscripts from OriGene customers. Take a look, and see how an OriGene product may help your experiments blossom into a published research article!

### TRUECLONE

*Am J Physiol Cell Physiol.* 2007 Apr;292(4):C1305-12. **[KLF4]**

*Am J Physiol Cell Physiol.* 2007 Dec;293(6):C1895-905. **[CD8A]**

*Cancer Res.* 2007 Nov 15;67(22):10859-68 **[PRKCD]**

*Cancer Res.* 2007 Nov 15;67(22):11001-11 **[ZBTB10]**

*Cancer Res.* 2007 Dec 1;67(23):11377-85. **[PDGFD]**

*Hypertension.* 2007 Oct;50(4):636-42. **[HMOX1]**

*J Biol Chem.* 2007 Nov 23;282(47):34555-67. **[ARPP-19]**

*J Biol Chem.* 2007 Nov 23;282(47):34365-71. **[BACH1]**

*J Biol Chem.* 2007 Oct 12;282(41):30107-19. **[PRKAB1, PRKAG1, PRKAA2]**

*J Biol Chem.* 2008 Jan 18;283(3):1679-91. **HuSH-29 TESK1**

*J Biol Chem.* 2008 Jan 4;283(1):648-59. **[COMP]**

*J Biol Chem.* 2008 Jan 11;283(2):1052-63. **[NPC1]**

*J Biol Chem.* 2008 Jan 11;283(2):1064-75. **[NPC2]**

*J Biol Chem.* 2008 Jan 11;283(2):708-15. **[SOCS3]**

*J Immunol.* 2007 Oct 1;179(7):4939-44. **[IGSF22]**

*J Virol.* 2007 Dec;81(24):13783-93. **[APOE]**

*J Virol.* 2007 Dec 5 [Epub ahead of print] **[MCM2, MCM6]**

*Mol Biol Cell.* 2007 Nov 28 [Epub ahead of print] **[SERPINA1, SERPINI1 and INS]**

*Mol Cell Biol.* 2007 Dec;27(24):8683-97. **[NET1]**

*Mol Pharmacol.* 2007 Nov 7 [Epub ahead of print] **[CACNB1]**

*Nucleic Acids Res.* 2007;35(19):6598-610. **[DICER1 and XPO5]**

### POLY A+ RNA

*Mol Biol Cell.* 2007 Jul;18(7):2698-706.

### HUSH-29

*Cancer Res.* 2007 Nov 15;67(22):10859-68 PRKCD

*Genome Res.* 2007 Nov;17(11):1550-61. E2F6

# Research behind single gene targeting experiments wins a **Nobel Prize**

The Nobel Prize in Physiology or Medicine for 2007 was awarded to Drs. Mario R. Capecchi, Martin J. Evans and Oliver Smithies for their work pioneering the field of engineering specific gene modifications in mice via embryonic stem cells. The resulting technique of gene targeting is widely used in basic research and more recently, in applied therapeutics. Generating a "knockout mouse" via gene targeting has led to the understanding of the roles of numerous genes in embryonic development, adult physiology, aging and disease. Nearly half of all genes in the mammalian genome have been knocked out via gene targeting, vastly increasing our understanding of the role of those genes in human physiology.

More than five hundred different mouse models of human disorders, including cardiovascular disease, neurodegenerative diseases, diabetes and cancer, have been generated by an extension of the initial gene modification technique. Mario Capecchi and Oliver Smithies initiated the work showing that homologous recombination could be used to specifically modify genes in mammalian cells. Capecchi demonstrated that defective genes could be repaired by homologous recombination with exogenously introduced DNA. Smithies showed that endogenous genes could be targeted irrespective of their activity, suggested that all genes may be modified by homologous recombination.

The cell types initially studied by Capecchi and Smithies could not give rise to germ cells, so the DNA modifications could not be inherited. In order to produce a gene-targeted animal, another type of cell would be required. Martin Evans theorized that pluripotent mouse embryonal carcinoma (EC) cells could be used to introduce genetic material into the mouse germ line, allowing inheritance of the targeted loci. However, EC cells carried abnormal chromosomes and could not contribute to germ cell formation, so Evans established chromosomally normal cell cultures from early mouse embryos. These cells are now referred to as embryonic stem (ES) cells, and are routinely used to generate gene-targeted mice. "Knockout mouse" lines are used to study models of human disease or other single gene phenomena. The suppression of a gene's expression using knockout technology has advanced even further with the use of inducible promoters that allow control of the gene expression temporally, spatially, and/or developmentally.

Gene targeting has helped us understand the roles of many hundreds of genes in mammalian fetal development. Capecchi's research has uncovered the roles of genes involved in mammalian organ development and in the establishment of the body plan. His work has shed light on the causes of several human inborn malformations. Evans applied gene targeting to develop mouse models for human diseases. He developed several models for the inherited human disease cystic fibrosis and has used these models to study disease mechanisms and to test the effects of gene therapy. Smithies also used gene targeting to develop mouse models for inherited diseases such as cystic fibrosis and the blood disease thalassemia. He has also developed numerous mouse models for common human diseases such as hypertension and atherosclerosis.

OriGene salutes Drs. Capecchi, Evans and Smithies for their remarkable accomplishments, and their receipt of the 2007 Nobel Prize in Physiology or Medicine.

*Adapted from the press release posted on The Official Web Site of the Nobel Foundation at http://nobelprize.org/nobel_prizes/medicine/laureates/2007/press.html.*

**Nearly half of all genes in the mammalian genome have been knocked out via gene targeting, vastly increasing our understanding of the role of those genes in human physiology.**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# **Seymour Benzer**, Geneticist, is Dead at 86

By CARL ZIMMER

Seymour Benzer, a geneticist who made scientific history by discovering that genes were structured like words and who went on to do pioneering work on the ties between genes and behavior, memory and longevity, died on Nov. 30 in Pasadena, Calif. He was 86. The cause was a stroke, according to a statement from the California Institute of Technology, where he was a professor emeritus.

Dr. Benzer was born in New York City to immigrants from Poland. On summer vacations in the country as a boy, he had his first experiences in studying biology, catching frogs and dissecting them. For his 13th birthday he received a microscope, "and that opened up the whole world," Dr. Benzer recalled in a 1990 interview for an oral history project at Caltech. In 1938, he enrolled at Brooklyn College. Unwilling to bother with introductory biology classes, he ended up majoring in physics. He earned a Ph.D. at Purdue University, where he worked on a secret military project to develop crystals for carrying current in radar systems. He discovered how to make the crystals withstand high voltages, and after World War II engineers at Bell Labs built on the Purdue research to invent the world's first transistors. By then Dr. Benzer had returned to biology, becoming fascinated by the mysterious nature of genes.

In the early 1950s, scientists were still debating whether genes were indivisible units or whether they were built up from smaller parts. To answer that question, Dr. Benzer experimented with viruses that infected the gut microbe Escherichia coli. He selected viruses that could not infect a particular strain of E. coli called K because they had defective versions of a gene. Dr. Benzer knew that when two viruses infect the same host, their genetic material is sometimes blended in their offspring. By repeatedly infecting bacteria, he discovered that the blending allowed some of the resulting viruses to infect the K strain. Dr. Benzer was able to show that the viruses had swapped pieces of the same gene. In some cases, the swaps produced a working version of the gene. Dr. Benzer established from the experiments that genes are like words, built up from smaller units of DNA. The mutant viruses had misspelled genes, which could be combined into the correct sequence, like combining the first three letters of BENXRQ and the last three letters of PSDZER to produce BENZER.

"He performed one of the most conceptually beautiful experiments of all time," said Ralph Greenspan, a senior fellow at the Neurosciences Institute in San Diego. But in the mid-1960s, Dr. Benzer suddenly abandoned his viruses. "When things get to that stage, you wonder why you should be doing something somebody else is already doing," he explained in the oral history. "It's just redundant." Now Dr. Benzer wanted to understand how genes might give rise to behaviors. His inspiration came from watching his daughters grow up and noticing their widely different personalities. "If you have one daughter, you don't notice anything, but if you have a second one, you begin to wonder, 'Are we doing things differently, or is it genetic?'" he said.

After Dr. Benzer moved to Caltech in 1967, he began to study the behavior of Drosophila flies. He began to study the simplest behavior he could think of: the attraction of flies to light. Dr. Benzer and his colleagues exposed flies to toxins to trigger mutations. They then sorted through the offspring of the mutant flies to find those that behaved strangely. They found flies that did not respond to the light, others that tried to fly away from it, and many other peculiar variations.

By breeding these mutant flies, Dr. Benzer was able to pinpoint the location of some of the genes responsible for the differences in behavior. Dr. Benzer's work marked the birth of a new field called neurogenetics. Scientists are now investigating the links between genes and behaviors in other animals, including humans.

With his colleagues, Dr. Benzer went on to discover genes linked to memory in flies and to their internal body clocks. He won many awards for his research, including the National Medal of Science. But he never won the Nobel Prize, which many of his colleagues believed he deserved.

Dr. Benzer is survived by his wife, Carol Miller; two daughters, Barbara Freidin and Martha Goldberg; a son, Alexander Benzer; two stepsons, Renny and Douglas Feldman; and four grandchildren. Dr. Benzer continued his research long after the age when most scientists retire. In his late 70s, he began finding genes that control longevity in flies. "It's always very refreshing to be able to just make a clean break, start over again with something you're completely ignorant about," Dr. Benzer said. "That's very exhilarating; nothing's expected of you because you're a novice; and, with luck, you come up with something that other people were saying was impossible because they know too much."

*From The New York Times on the Web*

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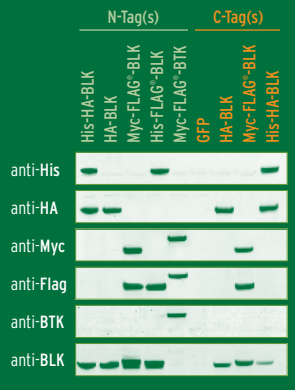


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