

## ProteoLink™ IN VITRO EXPRESSION CLONING SYSTEM: LINKING GENES TO PROTEINS

Promega's new ProteoLink™ In Vitro Expression Cloning System (Human Adult Brain, Cat.# L6500, L6501, L6502) allows researchers to functionally screen a high-quality human brain cDNA library for genes encoding proteins of interest.

To understand how genomes direct cellular function, investigators need to identify relationships between specific DNA sequences and the encoded protein function. Such identification requires both biochemical and genetic approaches. Traditional biochemical methods involve purifying proteins based on a particular characteristic or function. The genes that encode the proteins are isolated through multiple steps that include peptide sequencing and complementary DNA (cDNA) isolation based on predicted sequences. Other methods are also used to analyze proteins; however, each presents limitations for providing a direct correlation between a specific gene and a specific protein.

Promega's ProteoLink™ In Vitro Expression Cloning System<sup>(a)</sup> (IVEC) is a systematic and broadly applicable tool that can be used to directly correlate genes with proteins. The simple, in vitro method eliminates theoretical or indirect approaches for establishing gene:protein relationships. The system is a plasmid-based human adult brain cDNA library that is expressed using Promega's Gold TNT® Express 96 Transcription/Translation System<sup>(b-d)</sup> (Figure 1).

Small plasmid pools are expressed in vitro, circumventing the traditional and laborious process of protein purification, peptide sequencing and cDNA isolation to identify the genes that encode specific proteins. Each protein pool is screened for a desired biochemical activity. Positive pools are progressively subdivided until a single cDNA encoding the active protein is isolated (1,2). Because the cDNA is cloned into a vector containing an appropriate promoter, the identified cDNAs can be expressed in mammalian cells to confirm the in vitro screening results in a physiological system (Figure 2).

### IVEC Screening Assays

There are three major categories for which investigators have successfully used IVEC screens: substrate, activity and interaction screens. These exemplify the flexibility of IVEC screening, and several recent articles have suggested that protein function can be retained in the absence of sequence conservation. Typically, the initial screens are performed on protein pools expressed from 50–100

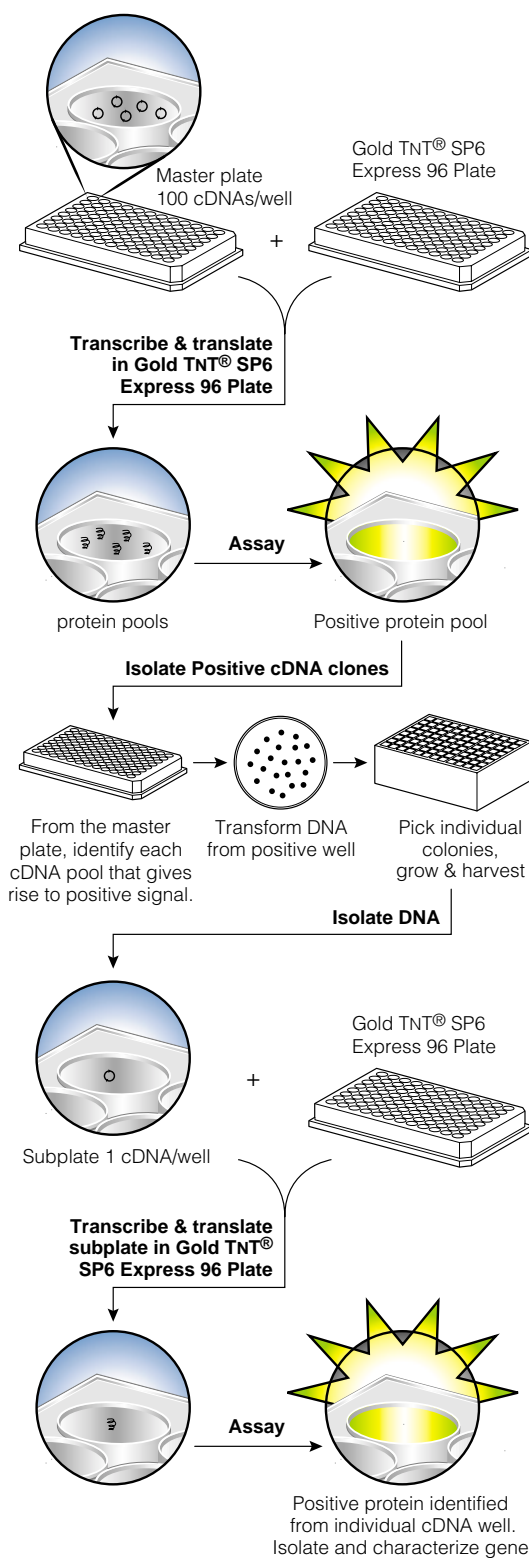


Figure 1. Schematic illustrating the typical experimental design for primary screening and subsequent isolation of a cDNA encoding a protein of interest.

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cDNAs, and single cDNAs are identified by subdivision. Subsequent experiments are performed to confirm the *in vitro* and *in vivo* activity. The libraries are constructed in bifunctional vectors that will efficiently express cDNAs *in vitro* from a phage promoter and *in vivo* by the use of a highly efficient eukaryotic promoter.

**Substrate Screens**

**Protease Substrates:** There are a number of examples of protease substrate screens using the IVEC approach (3–7). Pools of <sup>35</sup>S-labeled proteins are incubated with extracts containing differential activities (i.e., mitotic vs. interphase extracts), and increases in mobility are observed on denaturing polyacrylamide gels when a protease substrate is identified. Inhibitors of the particular protease can further confirm the specificity of the activity. A modification of this strategy was used to screen for physiological substrates of the N-end rule pathway in a mouse cDNA library (8).

**Kinase Substrates:** There are also several examples of differential extract screening to identify kinase substrates (3,9). The approach is similar to the protease substrate

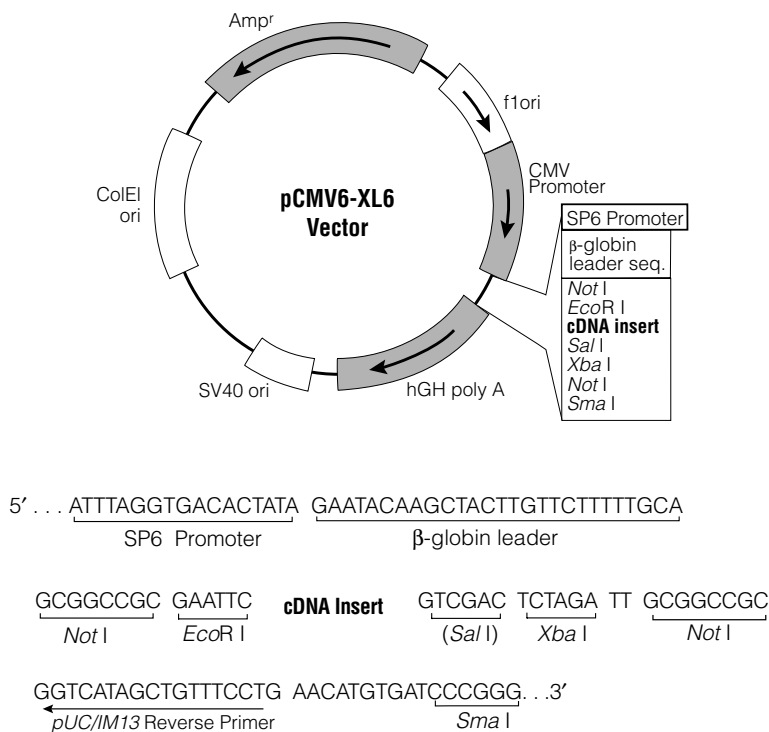
screen except that kinase substrates have a decreased mobility on denaturing polyacrylamide gels. Alternatively, purified or recombinant kinases can be used in the screen, and this approach has successfully identified a variety of casein kinase I and ERK2 substrates (10).

**Activity Screens**

Enzymatic activity screens can be performed using IVEC, the limitation being that the activity of interest should not be present in the reticulocyte lysate. Haushalter *et al.* (11) were able to find a new uracil DNA-glycosylase family member by performing an IVEC screen. Enzyme activity screens can be used to identify proteins that have activities under unusual conditions (e.g., high or low pH, temperature or ionic strength). Additionally, this approach can be used to perform protein evolution with screens for mutant proteins that have interesting properties, such as insensitivity to inhibitors.

**Interaction Screens**

IVEC approaches can be used generally for interaction screens. The advantage of this approach is that the “bait” can be a molecular complex, antibody, organelle, or even



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**Figure 2. pCMV6-XL6 Vector circle map and multiple cloning site.** The cDNA inserts have been cloned directly into the *EcoR I* and *Sal I* sites. As a result, the *Sal I* site has been destroyed.

a virus. In vitro methods also offer the potential for accurate post translational modifications. An excellent example of the importance of this is seen in the recent paper by Kanai *et al.* (12), where the observed protein:protein interaction of TAZ with the 14-3-3 proteins requires phosphorylation of a single serine residue, a post translational activity that is often present in the rabbit reticulocyte lysate system. Binding to DNA and RNA can be screened by this approach (13) as well as binding to other biologically relevant targets such as phospholipids (14). Marignani *et al.* (15) have recently discovered that LKB1, a serine-threonine kinase associated with Peutz-Jeghers Syndrome, interacts with and regulates Brg 1.

### Summary

Essentially, any assay that has successfully been used with the reticulocyte lysate system should be considered for use in an IVEC format for identifying gene:protein relationships. As more sensitive methods become available to researchers, the applications for assays based on IVEC will be expanded.

### References

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### Protocol

#### **ProteoLink™ In Vitro Expression Cloning System (Human Adult Brain) Technical Manual #TM057**

[www.promega.com/tbs/tm057/tm057.html](http://www.promega.com/tbs/tm057/tm057.html)

### Additional information:

[www.promega.com/proteolink](http://www.promega.com/proteolink)

### Ordering Information

Product	Size	Cat. #
ProteoLink™ In Vitro Expression Cloning System (Human Adult Brain) <sup>1</sup>	10 plates Gold TnT® SP6 Express 96 System 10 plates cDNA Library	L6500
ProteoLink™ In Vitro Expression Cloning System, Set 1 (Human Adult Brain) <sup>1</sup>	5 plates Gold TnT® SP6 Express 96 System 5 plates cDNA Library, Plates A–E	L6501
ProteoLink™ In Vitro Expression Cloning System, Set 2 (Human Adult Brain) <sup>1</sup>	5 plates Gold TnT® SP6 Express 96 System 5 plates cDNA Library, Plates F–J	L6502

<sup>1</sup>Human adult brain cDNA Library created by OriGene Technologies, Inc.

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(b)U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and European Pat. No. 0 553 234 have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.

(c)U.S. Pat. Nos. 5,324,637, 5,492,817 and 5,665,563, European Pat. No. 0 566 714 B1, Australian Pat. No. 660329 and Japanese Pat. No. 2904583 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.

(d)U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803 and Japanese Pat. No. 3009458 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

(e)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

(f)U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

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