New Product

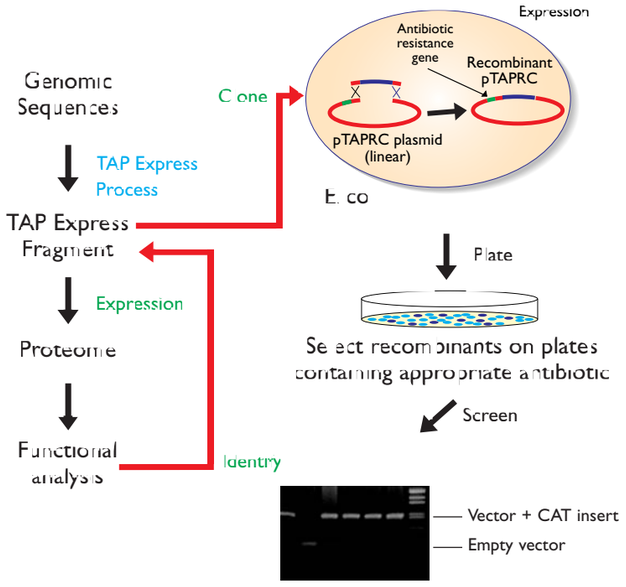
Smart PCR cloning for mammalian expression

TAP Express™ Cloning System

The revolutionary TAP Express™ technology has become the method of choice for rapid transient expression in mammalian cells (see article on page 8). However, sometimes it is useful to have your gene of interest cloned in an expression vector so that it can be readily propagated and maintained. The TAP Express™ Cloning System allows you to achieve this goal easily and rapidly by eliminating the traditional ligation and incubation steps.

Simplified protocol.
Once you obtained the TAP Express PCR Fragment, which includes an optimized 5' human cytomegalovirus (CMV) promoter, your gene of interest, and a 3' terminator, all it takes is a transformation step to clone this expression cassette into a vector (Figure 1). Simply mix the TAP Express PCR Fragment with the supplied linear TAP Express cloning/expression vector, pTAPrc , and use the mixture to transform the SMART™ Chemically Competent *E. coli*. By taking advantage of the carefully designed sequence overlaps between the TAP Express PCR Fragment and pTAPrc, the engineered endogenous recombinase activity in the SMART *E. coli* is able to join the two linear DNA fragments. You can then easily pick the correct recombinant clones by growing the cells on a plate containing kanamycin.

Figure 1. How TAP Express Cloning System Works.



The SMART competent cells are designed to contain specific recombinases and the linearized pTAPrc cloning plasmid. After transformation, the SMART E. coli cells biologically recombine TAP Express promoter and terminator sequences with identical end sequences on the linear pTAPrc cloning plasmid. TAP Express fragments are biologically inserted by the SMART E. coli recombinase into the pTAPrc plasmid at a high rate and with directional specificity.

Advantages of TAP Cloning.
TAP Express cloning offers several distinct advantages over other cloning methods. With TAP Express Cloning, your gene of interest is essentially directly cloned in the correct orientation for protein expression. There is:

- No need for restriction digest
- No need for ligase, topoisomerase, or incubation
- No need to screen for cloning orientation

In addition to the time-saving features listed above, TAP Express cloning allows you to clone the exact sequence you want because there is no restriction of multiple cloning sites or enzyme recognition sites

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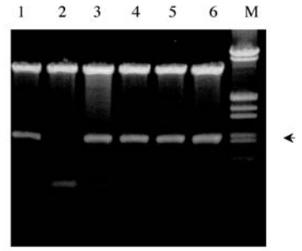
Figure 2. Efficiency of TAP Express Cloning System



PCR amplified TAP Express fragment encoding CAT reporter gene (0.5 µg) was directly mixed with the linear TAP Express cloning vector (0.2 µg) and transformed into competent *E.coli* followed by selection on LB agar plate containing 100 µg/ml Kanamycin. Miniprep DNA samples were isolated from six randomly selected colonies, restricted using *Pst* I and *Bam*H I and analyzed by agarose gel electrophoresis. Clones containing the TAP Express insert yielded a band around 800 bp (Lanes 1, 3-6 indicated by the arrow), while the background blank vector showed a 200 bp fragment (Lane 2).

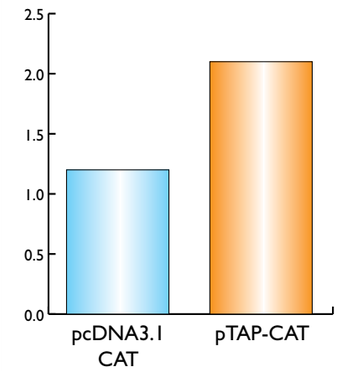
(*e.g.* topoisomerase I). This makes TAP Express cloning an ideal PCR cloning method for expressing native proteins.

Great cloning efficiency.
To demonstrate the effectiveness of TAP Express cloning, TAP Express Fragment encoding CAT reporter gene was mixed with the linear pTAPrc vector and the mixture was transformed into SMART *E. coli*. The transformed cells were plated onto an LB agar plate containing 100 µg/ml kanamycin. Plasmid DNA miniprep samples were isolated from five randomly selected colonies. The result in Figure 2 shows that five out of the six colonies are the desired recombinants. When using our recommended protocol, you can consistently get 50% to 80% positive transformants.

Solid expression results.
In addition to fast and easy cloning, expression vector generated using TAP Express cloning system offers superior expression results. This is the result of the highly optimized promoter/enhancer sequence within the TAP Express fragment. Figure 3 shows the comparison of expression levels of chloramphenicol acetyl transferase (CAT) from pTAPrc and pcDNA3.1 vectors. The result shows that the TAP cloning clearly outperforms the competing method.

Complete Kit.
The TAP Express cloning system contains everything you need to perform 10 cloning experiments, including linear pTAPrc vector, SMART chemically competent *E. coli*,

Figure 3. Expression of CAT using TAP cloning and pcDNA3.1



$5x10^5$ CHO cells were transfected in 6-well plates using 2 mg plasmid DNA plus GenePORTER transfection reagent. 48 hours post transfection, cells were lysed and assayed using CAT-ELISA kit from Roche.

SOC medium, transformation control, and a comprehensive manual. For your convenience, the SMART *E. coli* is provided in the 50 µl single-use format. The TAP Express cloning system is a great companion kit for the TAP Express Rapid Gene Expression System. Perform your PCR cloning the smart way. Call and order today.

Product	Quantity	Catalog no.	Price
TAP Express™ Cloning System (include SMART Chemically Competent *E. coli*)	10 reactions	TAPC2010	$150