

**Frequently Asked Questions (FAQ's):
TAP Express™ Rapid PCR Expression System**

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1. **What is the TAP Express Rapid PCR Expression System?**

The TAP Express Rapid Gene Expression is the first method that allows researchers to significantly increase the speed of gene functionality screening by making PCR products transcriptionally active without cloning into an expression vector. The TAP Express System eliminates the need for time-consuming cloning, transformation, and plasmid purifications, allowing researchers to go from gene amplification to cell transfection or injection into animals in as little as 1 day.

2. **How does the TAP Express System work?**

There are two PCR steps involved in the TAP Express process. The first PCR step incorporates unique TAP DNA sequences onto the 5' and 3' ends of the gene-of-interest via gene-specific TAP PCR primers. The second PCR step incorporates the high efficiency TAP Express Promoter and TAP Express Terminator onto the first-step PCR product. After the second PCR step is complete, the gene-of-interest is ready for expression.

3. **What level of gene expression can be seen with the TAP Express System both *in vitro* and *in vivo*?**

TAP Express fragments give essentially identical expression to plasmid-based constructs both *in vitro* and *in vivo*.

4. **How many genes-of-interest can I amplify using the TAP Express System?**

The TAP Express Kit provides enough reagents to do 100 PCR amplifications or to amplify 100 genes-of-interest.

5. **How much DNA will each TAP Express procedure yield?**

Each TAP Express two-step amplification procedure carried out in 50 μ l volume should yield at least 5 μ g of DNA.



6. What is the maximum length gene that can be amplified with the TAP Express System?

The maximum length fragment that can be amplified with the TAP Express System depends largely on the polymerase and conditions used. Some common genes that have been amplified with TAP Express include GFP, CAT, and beta-Galactosidase. For larger genes, a high-fidelity polymerase and optimized conditions selected for longer sequences should be used.

7. How do mutants generated during PCR affect the efficacy of the TAP Express process?

Mutant PCR products produced during the TAP Express process have essentially no effect on gene functionality screening. Even using a polymerase with no proofreading ability, the percentage of mutants generated in the entire PCR fragments pool with a functionally significant effect on protein activity will be negligible.

8. Does TAP Express completely replace traditional cloning of genes?

TAP Express replaces traditional cloning at the stage of functional screening. This is particularly advantageous when a large numbers of genes are to be analyzed. For example, using traditional cloning the time and labor necessary to analyze 30 genes would take months. However, with TAP Express system, screening this many genes is now possible within a few days.

Of course, once gene(s) with the desired function have been identified, cloning would be necessary to verify the gene sequences and to do further gene structure/function analysis studies. Towards these latter applications, Gene Therapy Systems has made available the TAP Express Cloning Kit (Cat. # TAPC2010) an enzyme-free cloning system that allows for easy and rapid cloning of any TAP-generated fragment.

9. Can I perform stable transfection in mammalian cells using the TAP Express System?

The TAP Express System is designed for high-efficiency transient transfection in mammalian cells. In order to achieve stable transfection, it is necessary to co-transfect the TAP Express Fragment carrying your gene-of-interest with a vector carrying an antibiotic resistance gene.

10. Can an epitope tag be incorporated into a TAP Express fragment so that an antibody can be used to screen for the expression product?

The epiTAP Express (HA) Rapid PCR Expression System (Cat. #'s TAP010120 & TAP010220) allows the incorporation of the commonly used hemagglutinin (HA) epitope tag into any TAP Express fragment so that the expression product can be screened or purified using commercially available antibodies.

11. What type of promoter and terminator are used in the TAP Express System?

The TAP Express Promoter is a modified CMV promoter, of approximately 800 bp, which has been optimized for high-levels of transcription and translation in a wide variety of mammalian cells and tissues. The TAP Express Terminator is a modified SV40 terminator, of approximately 200 bp, which has been optimized for efficient termination.

12. Can I use my own transfection reagent to transfect my cells with the TAP Express fragment?

The TAP Express Fragment can be successfully transfected using almost any currently available transfection reagents. Of course, we recommend our own GenePORTER™ Transfection Reagent, which is included in the kit.



13. Can I use my own promoter with the TAP Express System?

The TAP Express System is designed for use with the high-efficiency TAP Express promoter supplied in the kit, and we cannot guarantee it will work with any other promoter.

14. Are there any published references for the TAP Express System?

Yes. Liang, X., *et al.*, (2001) Transcriptionally Active PCR (TAP): High Throughput Gene Expression Using Genome Sequence Data. *J. Biol Chem.* **277**: 3593.

15. Will the TAP Express Fragment contain sequences other than my gene-of-interest that will be translated into peptides, polypeptides or proteins?

No. The only product transcribed from the TAP Express Fragment will be the gene-of-interest.

