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# ViraPower™ Adenoviral Expression System

Quickly and easily get high-level transient gene expression in any mammalian cell type. With the ViraPower™ Adenoviral Expression System[19,28,118] you can:

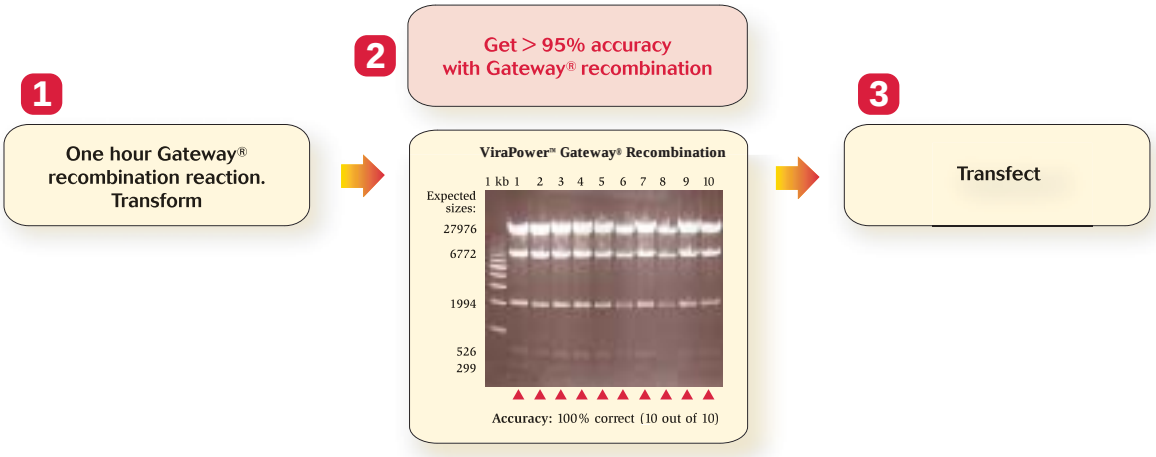
- Save days of time and avoid tedious hands-on cloning procedures
- Rapidly clone your gene with > 95% efficiency
- Transiently express your gene in dividing and non-dividing cells from the CMV or your own promoter

The ViraPower™ Adenoviral Expression System frees you from the monotony of lengthy cloning steps, delivering your transient expression results faster.

## Gateway® advantage for incomparable adenoviral cloning

Gateway® Technology provides the ideal route to the ViraPower™ Adenoviral Expression System. Using Gateway® Technology to clone your gene of interest means there's no need for shuttle vectors, inefficient homologous recombination steps, and time-consuming DNA manipulations. Plus Gateway® Technology uses a simple one-hour recombination reaction with >95% accuracy (Figure 1). Use the ViraPower™ Adenoviral System for the ultimate in fast, powerful adenoviral cloning.

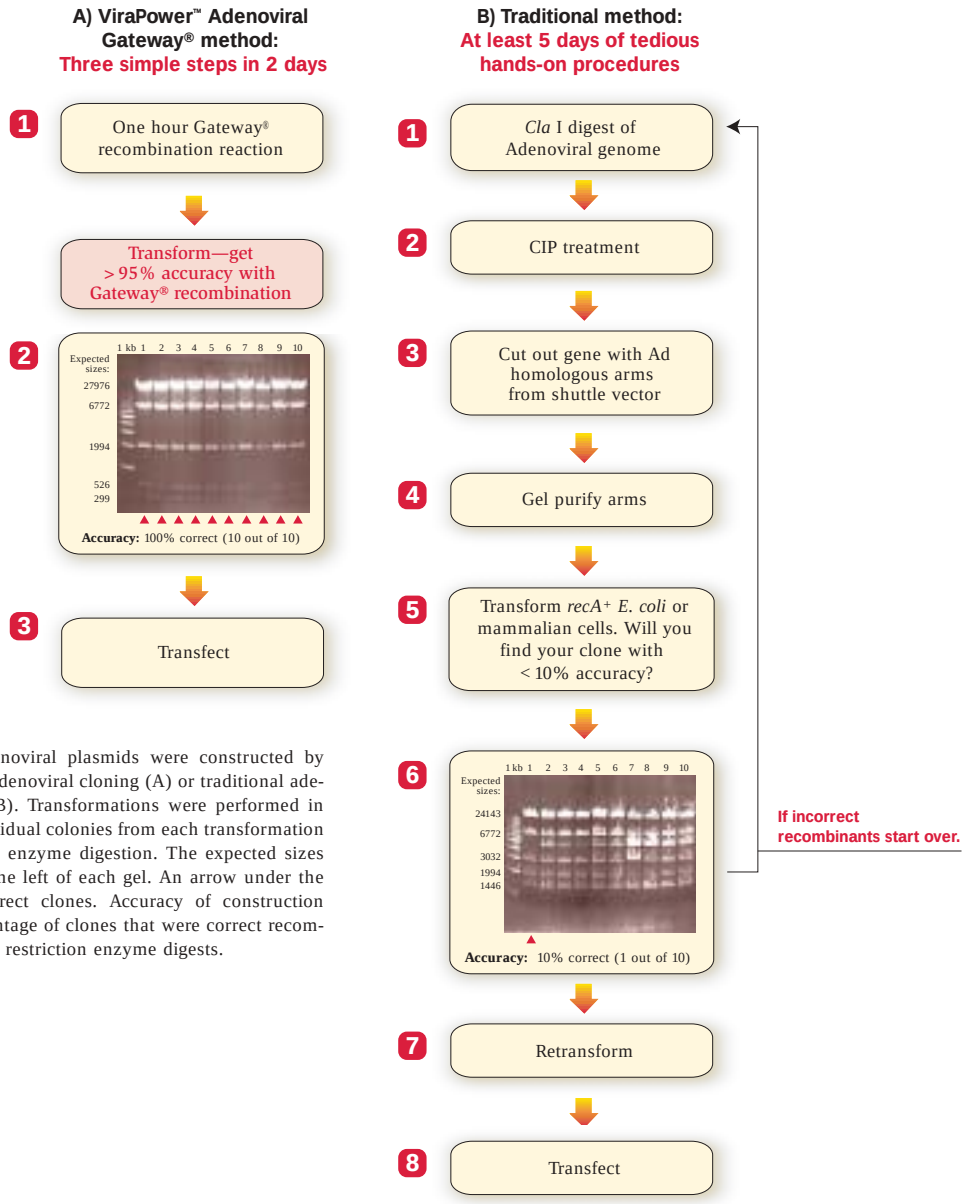
**Figure 1 - Three simple steps to fast and efficient adenoviral cloning**



# Unmatched adenoviral cloning efficiency

The Gateway® Technology in the ViraPower™ Adenoviral System circumvents the need for traditional homologous recombination in *recA+* bacteria or mammalian cells, or cumbersome *in vitro* ligation protocols. This provides you with a faster and easier way to harvest viral DNA for your adenoviral expression experiments. Any gene flanked by *att*L sites can be recombined into a ViraPower™ Adenoviral Gateway® Expression Vector. Using the ViraPower™ Adenoviral System with Gateway® Technology you'll get > 95% cloning efficiency with a simple one-hour recombination reaction (Figure 2A). In contrast, when using traditional cloning methods, the large adenoviral genome is highly unstable in *recA+* bacteria, resulting in low DNA yields and typically < 10% correct recombinant clones (Figure 2B). Since the efficiency of Gateway® recombination yields almost exclusively the correct construct, you can even use this system for high-throughput experiments. No more tedious protocols. No more waiting for days just to find you need to start over again. Assure your cloning success with the ViraPower™ Adenoviral System.

**Figure 2 - Gateway® advantage for incomparable adenoviral cloning speed**

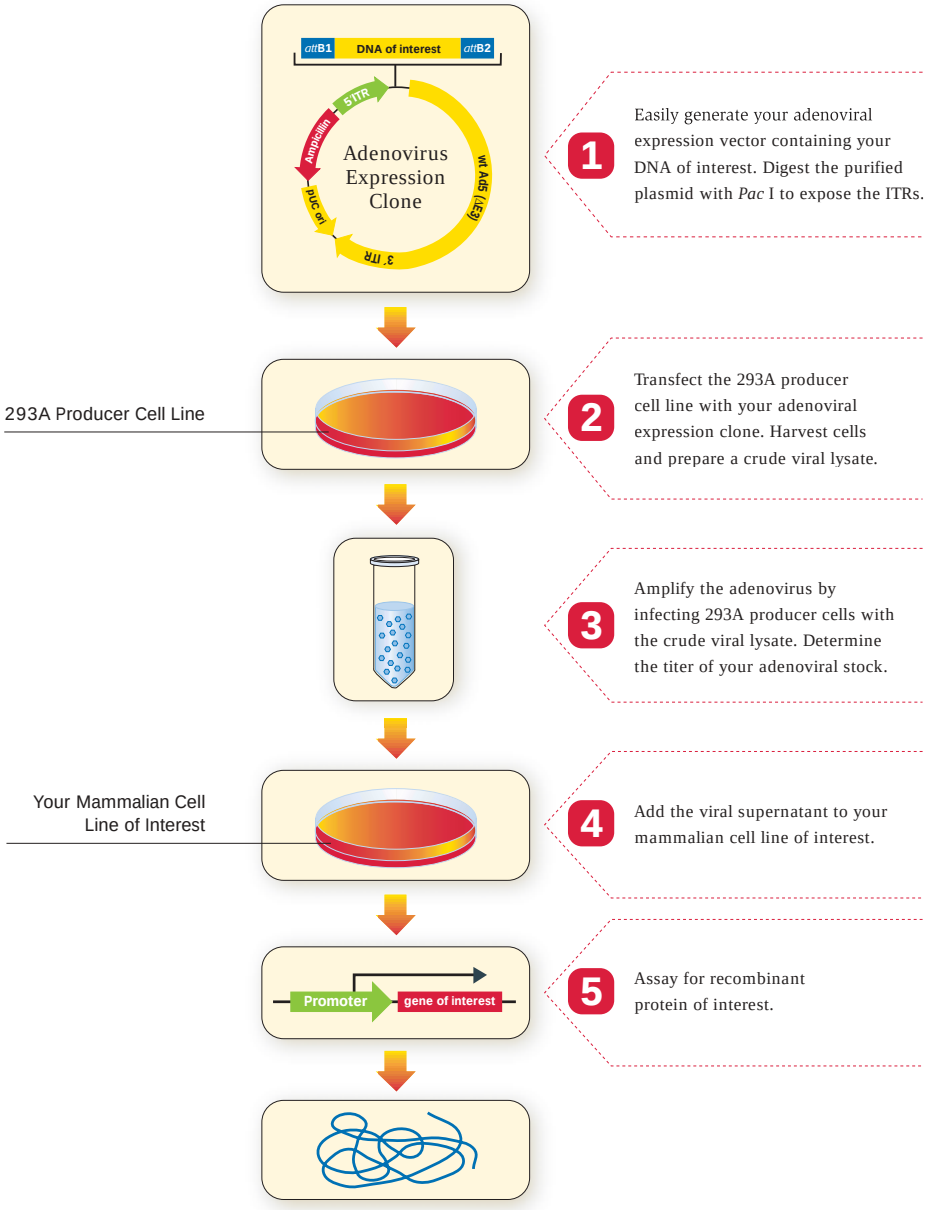



Recombinant adenoviral plasmids were constructed by either Gateway® adenoviral cloning (A) or traditional adenoviral cloning (B). Transformations were performed in parallel. Ten individual colonies from each transformation were screened by enzyme digestion. The expected sizes are indicated to the left of each gel. An arrow under the gel indicates correct clones. Accuracy of construction reflects the percentage of clones that were correct recombinants, based on restriction enzyme digests.

# High-level transient expression

High-level transient gene expression with the ViraPower™ Adenoviral Expression System is easily achieved (Figure 3). First, recombine your gene of interest from Gateway® entry clone into a pAd-DEST™ Adenoviral Gateway® Vector (see the following section on Adenoviral Gateway® Vectors for map and vector descriptions). Then, transfect the resulting expression construct into the 293A Cell Line to produce an adenoviral stock, typically with titers ranging from 1 x $10^7$ to 1 x $10^8$ pfu/ml. This stock can be amplified and concentrated to titers as high as 1 x $10^{12}$ pfu/ml. Use this viral stock to transduce your target mammalian cell line of choice. Adenovirus enters target cells by binding to the Coxsackie/Adenovirus Receptor (CAR) (1). After binding to the CAR, the adenovirus is internalized via integrin-mediated endocytosis (2), followed by active transport to the nucleus. Once in the nucleus, high levels of the gene of interest are transiently expressed. With the ViraPower™ Adenoviral System you can be assured of successful transient gene expression in any mammalian cell type.

**Figure 3 - How ViraPower™ Adenoviral Expression Systems work**



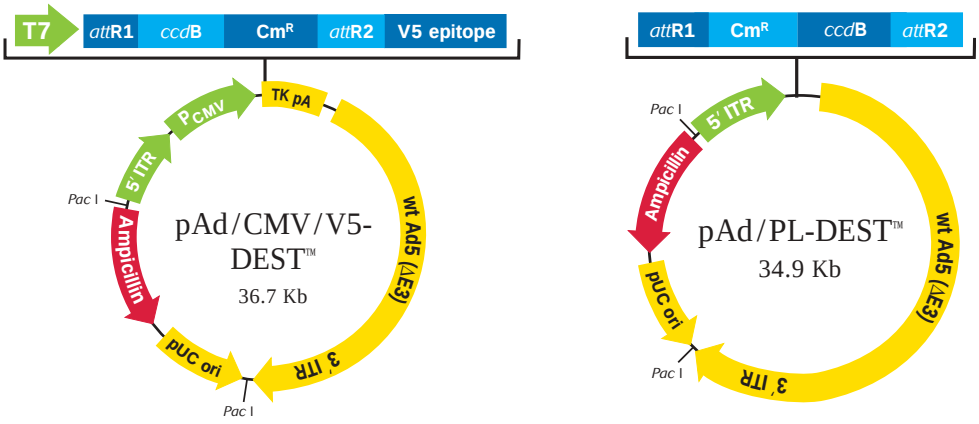
# ViraPower™ Adenoviral Gateway® Vectors for easy and efficient cloning

In the ViraPower™ Adenoviral Expression System two pAd-DEST™ Vectors (Figure 4) are available for fast, easy, and accurate Gateway® cloning into an adenoviral expression construct. Both pAd/CMV/V5-DEST™ and pAd/PL-DEST™ are E1- and E3-deleted, resulting in the production of replication-incompetent virus. High-level expression can be achieved in most mammalian cells using pAd/CMV/V5-DEST™, where your gene of interest is driven by the human cytomegalovirus (CMV) promoter. Alternatively, pAd/PL-DEST™ allows you to clone in your promoter and sequence of choice. Both pAd/CMV/V5-DEST™ and pAd/PL-DEST™ contain elements required for packaging of the expression construct into virions (*e.g.*, 5´ and 3´ ITRs, encapsidation signal, and adenoviral late genes). Once you have constructed your adenoviral vector, the ViraPower™ Adenoviral Expression System offers you a number of advantages:

- Efficient delivery of your gene of interest to actively dividing and non-dividing mammalian cells in culture or *in vivo*
- High-titer adenoviral stock production with the 293A Cell Line that can be concentrated to as high as 1 x $10^{12}$ pfu/ml
- Simplified, efficient cloning allows high-throughput applications
- Enhanced biosafety due to the use of a replication-incompetent virus as a gene delivery vehicle

Choose the ViraPower™ Adenoviral vector that works best for you, and get fast, accurate Gateway® cloning combined with an efficient expression system for any mammalian cell type.

**Figure 4 - pAd/CMV/V5-DEST™ and pAd/PL-DEST™ Vectors**




* See Gateway® online seminar at www.invitrogen.com for information on selection and preparation of an entry clone required for recombination into ViraPower™ Adenoviral Gateway® Expression Vectors.

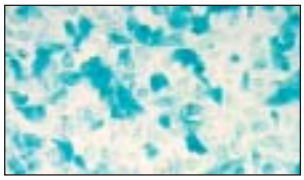
# Powerful expression

Adenoviral systems are popular platforms for reliable gene delivery and high-level transient expression in any mammalian cell type (Table 1). The ViraPower™ Adenoviral Expression System overcomes the tedious and time-consuming manipulations, screening, and multiple transformations required with traditional adenoviral vector construction methods. The ViraPower™ Adenoviral System uses Gateway® Technology for fast, easy, and accurate cloning into a pAd-DEST™ vector. Expression levels are equivalent to those seen with traditional adenoviral vectors. Use of this system allows the creation of replication-incompetent (E1- and E3- deleted) adenoviral particles that deliver and express high levels of your gene of interest in essentially any mammalian cell type (Figure 5). The pAd-DEST™ vectors are available with the 293A Cell Line. The 293A producer cell line contains the E1 gene sequences required for producing high-titers of recombinant virus, and is optimized to facilitate viral production and titering.

**Table 1 - Choose the best viral system for your experiments**

| Viral System | Transient expression | | Stable expression | | | |
|---|---|---|---|---|---|---|
| | Dividing Cells | Non Dividing Cells | Dividing Cells | Neuronal Cells | Drug or Growth Arrested Cells | Contact Inhibited Cells |
| Adenovirus | • | • | | | | |
| Retrovirus | • | | • | | | |
| Lentivirus | • | • | • | • | • | • |

**Figure 5 - High levels of β-galactosidase expression in HT1080 Cells**



Adenoviral plasmid DNA containing the *lac*Z open reading frame was generated in the Gateway® pAd/CMV/V5-DEST™ vector. Virus was produced in 293A cells and the crude viral supernatants were used to transduce HT1080 cells. Forty-eight hours post-transduction, cells were stained for β-galactosidase activity.

# High-titer producer 293A Cell Line for use with the ViraPower™ Adenoviral Expression System

The 293A Cell Line facilitates the initial production, amplification, and titering of replication-incompetent adenovirus. The 293A cells contain a stably integrated copy of the E1 gene that supplies the E1 proteins (E1a and E1b) required to generate recombinant adenovirus *in trans*. This cell line has also been selected for a flattened morphology to make the titering procedure easier.

## Advance your transient expression research

With the ViraPower™ Adenovival Expression System you'll simplify your cloning and get results in any mammalian cell type faster. Call and order the ViraPower™ Adenovival System today.

## Ordering information

| Product | Quantity | Cat. no. |
|---|---|---|
| ViraPower™ Adenoviral Gateway® Expression Kit* | 1 kit | K4930-00 |
| ViraPower™ Adenoviral Promoterless Gateway® Expression Kit* | 1 kit | K4940-00 |
| pAd/CMV/V5-DEST™ Gateway® Vector | 6 µg | V493-20 |
| pAd/PL-DEST™ Gateway® Vector | 6 µg | V494-20 |
| 293A Cell Line | 3 x $10^6$ cells | R705-07 |

*Kits contain Gateway® DEST Vector and 293A Cell Line.

[19,28,118]Products mentioned above are subject to the Limited Use Label Licenses indicated by the superscript numbers. Please refer to the Invitrogen web site or catalog for the Limited Use Label Licenses corresponding to the numbers indicated.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

**References:**
1. Bergelson, J.M., *et al.* (1997) *Science* **275:** 1320-1323.
2. Russell, W.C. (2000) *J. Gen. Virol.* **81:** 2573-2604.

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