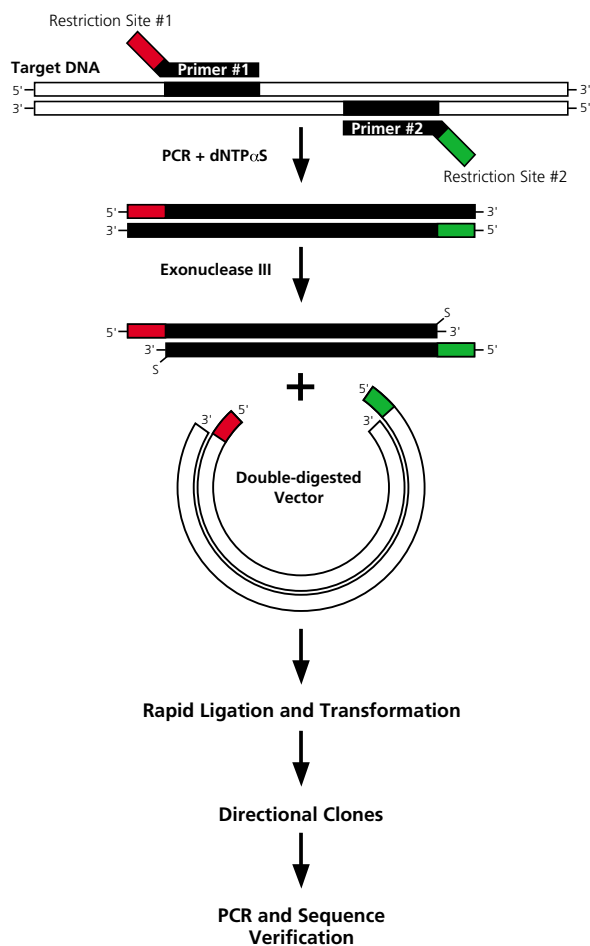


# CLONING AND EXPRESSION



## Overview of Director™ Strategy.

Amplify targets using gene-specific primers containing 5 base overhangs corresponding to the cohesive ends of the pre-digested vector in the presence of specially formulated ExoClone™ dNTP mix. Perform short digestion with Exonuclease III to sequentially degrade 3'-ends of PCR products to expose cohesive ends. Select a Director-Ready™ vector or prepare your own. Perform rapid ligation and transformation of construct. Verify Clones.

## Director™ Universal Cloning System

Sigma's Director Universal Cloning system is an ExoClone™ technology-based solution that provides a rapid, universal, and directional method to facilitate the streamlined cloning and expression of numerous genes. Previously, streamlined cloning and expression of numerous genes had been limited by methods that require the use of base recognition enzymes. Because the Director Universal Cloning System does not rely on such enzymes, typical problems of internal cutting sites and rearrangements seen with traditional methods and other systems are overcome. The complete system includes a universal PCR kit and either of two Director-Ready™ vector kits.

PCR in the Director system uses a specially formulated dNTP mix containing dATPαS, dGTPαS and Exonuclease III. Primers are gene-specific with universal 5 base overhangs that provide directionality and pairing with Director-Ready vectors. After PCR, cohesive 5'-termini of the amplicon are produced by Exonuclease III digestion instead of being generated by traditional base recognition enzymes. Incorporation of dA/GTPαS into the amplicon provides a convenient method for protecting the amplicon from overdigestion by Exonuclease III.<sup>1</sup> The nucleotide mix in the kit is formulated so that the amplicon terminates at a statistically determined array of 3'-dA/GαS sites.<sup>2</sup> The PCR products are ready for directional ligation into any expression vector previously digested with 5' overhang producing enzymes (i.e. Director-Ready vectors).

Director-Ready vectors are designed to optimize efficient directional cloning of PCR products. The typical cloning efficiency using these vectors is greater than 90% (transformants with a positive insert in the desired orientation).

## Features & Benefits

- **Universal** – Streamlined cloning of numerous genes without concerns of internal cutting sites or rearrangements
- **Directional** – Use of ExoClone technology provides new method of generating cohesive ends
- **Versatile** – Prepare your own vector or use our Director-Ready vectors
- **High efficiencies** – Typical cloning efficiency using the Director-Ready vectors is greater than 90%
- **Rapid** – Simple procedure increases throughput while decreasing hands-on time

## References

1. Andersson, S., et al., *J. Biol Chem.*, **264**, 8222-8229 (1989).
2. Thomsen, D.R., et al., *Proc. Natl. Acad. Sci. USA*, **81**, 659-663 (1984).

Product Code	Description	Size
<a href="#">RDC-1</a>	Director™ Universal PCR System	1 kit
<a href="#">RDC-LIG1</a>	Director-Ready™ pFLAG-MAC™ Set	1 set
<a href="#">RDC-L3</a>	Director-Ready™ pFLAG-CMV™-2 Set	1 set

Note: [RDC-1](#) is sufficient for 25 reactions.