

## Get High Stringency and High Specificity Ligation-Dependent Mutation Screening with Ampligase® Thermostable DNA Ligase

An element critical to the success of many ligation-dependent mutation detection methods (Figure 1) is a DNA ligase with a high degree of hybridization stringency and ligation specificity. A review of the literature reveals that EPICENTRE's Ampligase® Thermostable DNA Ligase is the most frequently used enzyme for ligation-dependent mutation screening applications such as Single Nucleotide Polymorphism (SNP) detection (see below).

Ampligase Thermostable DNA Ligase catalyzes NAD-dependent ligation of adjacent 3'-hydroxy and 5'-phosphorylated

termini in duplex DNA. Derived from a thermophilic bacterium, the enzyme is stable and active at much higher temperatures than conventional DNA ligases. The enzyme has a half-life of 48 hours at 65°C and more than 1 hour at 95°C. Ampligase DNA Ligase has been shown to be active for at least 500 thermal cycles (denature 94°C/anneal, extend 80°C) or 16 hours of cycling. This exceptional thermostability enables extremely high hybridization stringency conditions and specificity. Ampligase DNA Ligase has no detectable activity in ligating blunt-ended DNA and has no activity on RNA or RNA:DNA hybrids.

### Benefits of Ampligase® Thermostable DNA Ligase

- Half-life of 48 hours at 65°C and greater than 1 hour at 95°C.
- Active for at least 500 thermal cycles or 16 hours of cycling.
- Enables extremely high hybridization stringency.
- Extremely high ligation specificity.
- Assayed to ensure absence of detectable exo- and endonucleases and RNases.

## Selected publications citing Ampligase® Thermostable DNA Ligase for mutation detection and gene expression studies\*

For a more complete list of Ampligase Thermostable DNA Ligase citations, go to: [www.epicentre.com/ampligase\\_cite.asp](http://www.epicentre.com/ampligase_cite.asp)

### Mutation and Single Nucleotide Polymorphism (SNP) detection

Alsmadi, O.A. *et al.* (2003) *BMC Genomics* **4**(21), [www.biomedcentral.com/1471-2164/4/21](http://www.biomedcentral.com/1471-2164/4/21).

Beck, I.A. *et al.* (2002) *J. Clin. Microbiol.* **40**(4), 1413.

Pickering, J. *et al.* (2002) *Nucl. Acids Res.* **30**(12), e60.

Faruqi, F.A. *et al.* (2001) *BMC Genomics* **2**(4), [www.biomedcentral.com/1471-2164/2/4](http://www.biomedcentral.com/1471-2164/2/4).

Qi, X. *et al.* (2001) *Nucl. Acids Res.* **29**(22), e116.

Nakazawa, E. *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**, 360.

Dille, B.J. *et al.* (1993) *J. Clin. Micro.* **31**, 729.

Birkenmeyer, L. and Armstrong, A.S. (1992) *J. Clin. Micro.* **30**, 3089.

Barany, F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 189.

Wu, D.Y. and Wallace, R.B. (1989) *Genomics* **4**, 135.

### Trinucleotide Repeat Expansion Detection (RED)

Sirugo, G. *et al.* (1997) *Human Molec. Gen.* **6**(3), 403.

Sirugo, G. and Kidd, K.K. (1995) *EPICENTRE Forum* **2**(3), 1.

Schalling, M. *et al.* (1993) *Nature Genetics* **4**, 135.

### Gene Expression Studies and DNA Microarrays

Christian, A.T. *et al.* (2001) *Proc. Natl. Acad. Sci. USA* **98**(25), 14238.

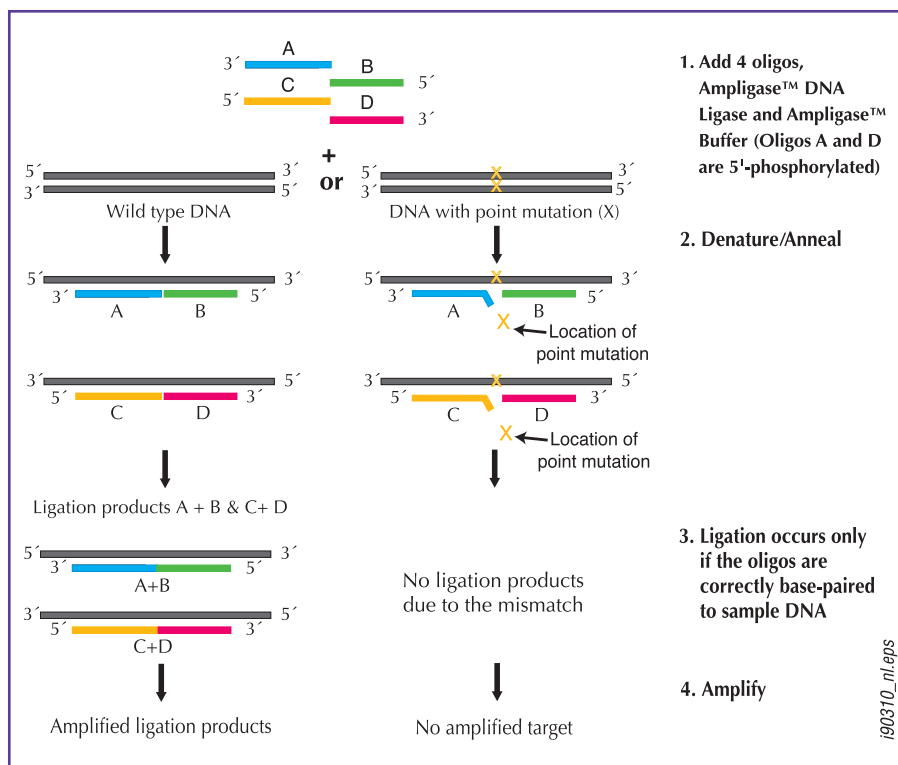
Nallur, G. *et al.* (2001) *Nuc. Acids Res.* **29**(23), e118.

### Mutagenesis

Moore, D.S. and Michale, S.F. (1995) *EPICENTRE Forum* **2**(4), 4.

Rouwental, G.J. *et al.* (1993) *BioTechniques* **15**, 68.

\* Some of the publications cited may describe methods covered by patents or patent applications in certain countries. Because purchase of Ampligase Thermostable DNA Ligase does not include a license to perform any patented procedure, users of this product may be required to obtain a license depending upon the particular application and country in which the product is used.



**Figure 1. Schematic of mutation screening using ligation amplification.** The existence of a point mutation at the site of ligation interferes with oligonucleotide ligation, resulting in no ligation product. The lack of an amplification product indicates the presence of a point mutation at the ligation site. Oligos can also be designed so ligation occurs in the presence of the mutant template.

[www.epicentre.com/ampligase.asp](http://www.epicentre.com/ampligase.asp)

**Ampligase® DNA Ligase Kit**

A8101	5 U/μl	1,000 U
A30201	5 U/μl	5,000 U

Contains 1,000 or 5,000 units of Ampligase® DNA Ligase, Ampligase® 10X Reaction Buffer, and Ligation Control DNA.

**Ampligase® Enzyme & Buffer**

A0102K	100 U/μl	2,500 U
A32250	5 U/μl	250 U
A32750	5 U/μl	750 U
A3202K	5 U/μl	2,500 U

25 μl of Ampligase® 10X Reaction Buffer is supplied with each 50 units of Ampligase® DNA Ligase.

**Ampligase® DNA Ligase**

A0110K	100 U/μl	10,000 U
A0125K	100 U/μl	25,000 U
A3210K	5 U/μl	10,000 U
A3225K	5 U/μl	25,000 U

Supplied as enzyme only; Reaction Buffer is not included.

**Ampligase® 10X Reaction Buffer**

A1905B	5 ml
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**Ampligase® 1X Storage Buffer**

A3201S	1 ml
A3205S	5 ml

## Transcribe 2'-Modified RNAs, Including RNAs Resistant to RNase A, Using Mutant T7 and SP6 R&DNA™ Polymerases

Like wild-type T7 and SP6 RNA Polymerases, EPICENTRE's mutant T7 and SP6 R&DNA™ Polymerases\* synthesize transcripts from a DNA template cloned downstream from a phage T7 or SP6 promoter. However, in addition to the canonical rNTPs, both T7 and SP6 R&DNA Polymerases can efficiently incorporate deoxyribonucleotides (dNTPs) and 2'-modified ribonucleotides, such as 2'-fluorine-dNTPs, and 2'-NH<sub>2</sub>-dNTPs, to prepare transcripts with mixed nucleotide compositions. RNA transcripts containing both 2'-fluorine-dCTP and 2'-fluorine-dUTP are particularly useful because they are not degraded by A-type RNases. Using these modified RNAs significantly reduces stability problems typically encountered when working with RNA.

Use mutant T7 and SP6 R&DNA Polymerases to:

- Synthesize transcripts containing RNase A-resistant 2'-fluorine-dCMP and 2'-fluorine-dUMP.
- Synthesize transcripts of mixed dNMP/rNMP or 2'-modified-dNMP/rNMP composition.

### Quality Control

T7 or SP6 R&DNA Polymerases are function-tested for synthesis of full length RNA transcripts using rATP, rGTP, rUTP and dCTP. Both enzymes are free of detectable DNA exo- and endonuclease, RNase, and *E. coli* RNA polymerase activities.

### Reference

Padilla, R. and Sousa, R. (1999) *Nucl. Acids Res.* 27(6), 1561.

[www.epicentre.com/t7\\_rdna.asp](http://www.epicentre.com/t7_rdna.asp)

**T7 R&DNA™ Polymerase**

D7P9201K	1,000 U	50 U/μl
D7P9205K	5,000 U	50 U/μl

**SP6 R&DNA™ Polymerase**

D6P9301K	1,000 U	50 U/μl
D6P9305K	5,000 U	50 U/μl

Each Polymerase is supplied with 5X Reaction Buffer, DTT Solution and MnSO<sub>4</sub> Solution

**2'-Fluorine-dCTP**

R2F110C	1 μmole	50 mM
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**2'-Fluorine-dUTP**

R2F110U	1 μmole	50 mM
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\* T7 and SP6 R&DNA Polymerases are covered by U.S. patent no. 5,849,546 and patents pending. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased product(s) solely for life science research.