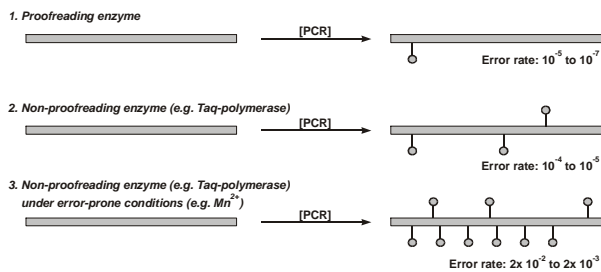




## JBS Error-Prone Kit

Random Mutagenesis by Error-Prone PCR

Cat. No.	Amount
PP-102	15 reactions



Enhanced mutational rate by error-prone PCR compared to standard PCR reactions.

**For *in vitro* use only!**

**Shipping:** shipped on gel packs

**Storage Conditions:** store at  $-20\text{ }^{\circ}\text{C}$

**Additional Storage Conditions:** avoid freeze/thaw cycles

**Shelf Life:** 12 months

### Description:

#### JBS Mutagenesis Series

Within three billion years of evolution, nature has produced a plethora of proteins simply by repeated cycles of random mutagenesis followed by *in vivo* selection for superior function of the encoded proteins. This example of natural evolution has guided researchers within the last two decades to develop strategies for *in vitro* permutation of proteins.

Among the variety of strategies applied, three major powerful techniques have emerged.

#### Random Mutagenesis by dNTP Analogs

This method is based on the incorporation of mutagenic dNTP analogs, such as 8-oxo-dGTP and dPTP, into an amplified DNA fragment by PCR. The mutagenic dNTPs are eliminated by a second PCR step in the presence of the four natural dNTPs only, resulting in a rate of mutagenesis of up to 20%.

→ **JBS dNTP-Mutagenesis Kit #PP-101**

#### Random Mutagenesis by Error-Prone PCR

Developed by Caldwell & Joyce (1992) this method introduces mutations in the gene of interest using a PCR reaction under conditions that induce an increased error-rate of the DNA-polymerase. The rate of mutagenesis achieved by error-prone PCR is in the range of 0.6-2.0%.

→ **JBS Error-Prone Kit #PP-102**

#### Random Mutagenesis by DNA Shuffling

Developed by Stemmer (1994) DNA shuffling generates libraries by random fragmentation of one gene or a pool of related genes, followed by the reassembly of the fragments in a self-priming PCR reaction. This method allows the recombination of sequences from different, related genes. The overall rate of mutagenesis is approx. 0.7%.

→ **JBS DNA-Shuffling Kit #PP-103**

Jena Bioscience now offers all components necessary for each of these techniques 'ready-to-go' in a separate kit, accompanied by a streamlined documentation that maximizes success.

### Content:

Taq Polymerase (red cap)

5 units/ $\mu\text{l}$ , 20  $\mu\text{l}$

10x Reaction Buffer (blue cap)

10x concentration, 100  $\mu\text{l}$

10x Error-prone Solution (yellow cap)

10x concentration, 100  $\mu\text{l}$

dNTP Error-prone Mix (white cap)

unbalanced dNTP ratio (dATP, dCTP, dGTP, dTTP), 40  $\mu\text{l}$



## JBS Error-Prone Kit

Random Mutagenesis by Error-Prone PCR

PCR-grade Water (white cap)  
1 ml

### Random Mutagenesis by Error-Prone PCR

The standard DNA polymerases used in conventional PCR reactions display error rates that are usually not suitable for directed mutagenesis experiments. For example, proofreading enzymes such as *Pfu* exhibit error rates in the range from  $10^{-6}$  to  $10^{-7}$  whereas non-proofreading enzymes like *Taq* Polymerase show error rates in the range from  $10^{-4}$  to  $10^{-5}$ . This rate however, can be significantly enhanced by modifying the following parameters of a PCR-reaction:

- Higher  $Mg^{2+}$ -concentration of up to 7 mM
- Partial substitution of  $Mg^{2+}$  by  $Mn^{2+}$
- Optimized dNTP concentrations at unbalanced rates

### Recommended assay preparation

- For a 50  $\mu$ l reaction, take 5  $\mu$ l of 10x Reaction Buffer in a sterile vial and refer to Tab. 1.
- Add 2  $\mu$ l dNTP Error-prone Mix.
- Add template and appropriate primers. Note that depending on the template the required concentration can be up to 10 times higher compared to standard PCR.
- Add 0.4-1  $\mu$ l *Taq* Polymerase as recommended.
- Add PCR-grade Water to a final volume of 45  $\mu$ l.
- Add 5  $\mu$ l of 10x Error-prone Solution. Note that the solution should be added last to the reaction mixture to prevent precipitation and must be protected from oxidation (oxidation of  $Mn^{2+}$  to  $Mn^{3+}$  may destroy the polymerase).

Tab. 1: Amounts of components for error-prone PCR conditions (50  $\mu$ l PCR assay)

Component	Amount	Final conc.	Cap
10x Reaction Buffer	5 $\mu$ l	1x	blue
dNTP Error-prone Mix	2 $\mu$ l	unbalanced ratio	white
Primers		20-100 pmol	
Template		3-100 fmol / 2 - 50 ng	
<i>Taq</i> Polymerase	0.4-1 $\mu$ l	2-5 units	red
PCR-grade Water	Fill up to 45 $\mu$ l		white
10x Error-prone Solution	5 $\mu$ l	1x	yellow

### Recommended thermocycling conditions

Denaturation	94°C	30 sec
Annealing <sup>1)</sup>	approx. 45-68°C	30 sec
Extension <sup>2)</sup>	72°C	1 min

Number of cycles: 30

<sup>1)</sup> The annealing temperature depends on the melting temperature of the primers.

<sup>2)</sup> The elongation time depends on the length of the fragments to be amplified. A time of 1 min per kbp is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary for each new template DNA and/or primer pair.

### Selected References:

Kim *et al.* (2001) Improvement of tagatose conversion rate by genetic evolution of thermostable galactose isomerase. *Biotechnol. Appl. Biochem.* **34**:99.

Daugherty *et al.* (2000) Quantitative analysis of the effect of the mutation frequency on the affinity maturation of single chain Fv antibodies. *PNAS* **97**:2029.

Wan *et al.* (1998) In vitro evolution of horse heart myoglobin to increase peroxidase activity. *Proc. Natl. Acad. Sci. USA* **95**:12825.

Cline *et al.* (1996) PCR fidelity of *Pfu* DNA polymerase and other thermostable DNA polymerases. *Nucleic Acids Res.* **24**:3546.

Vartanian *et al.* (1996) Hypermutagenic PCR involving all four transitions and a sizeable proportion of transversions. *Nucleic Acids Res.* **24**:2627.

Cadwell *et al.* (1992) Randomization of genes by PCR mutagenesis. *PCR Meth. Appl.* **2**:28.

Kunkel (1992) DNA replication fidelity. *J. Biol. Chem.* **267**:18251.