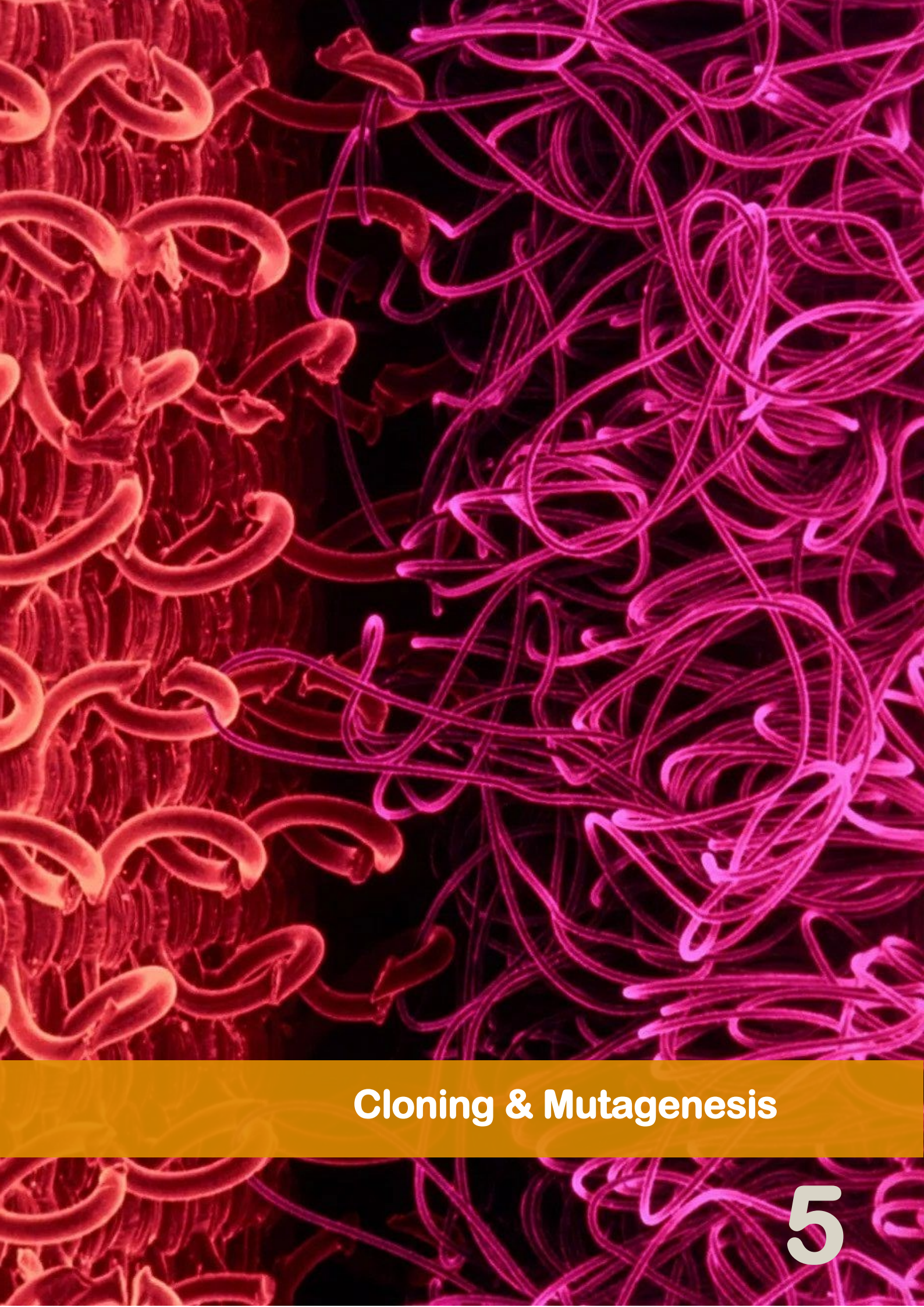


Cloning & Mutagenesis

5

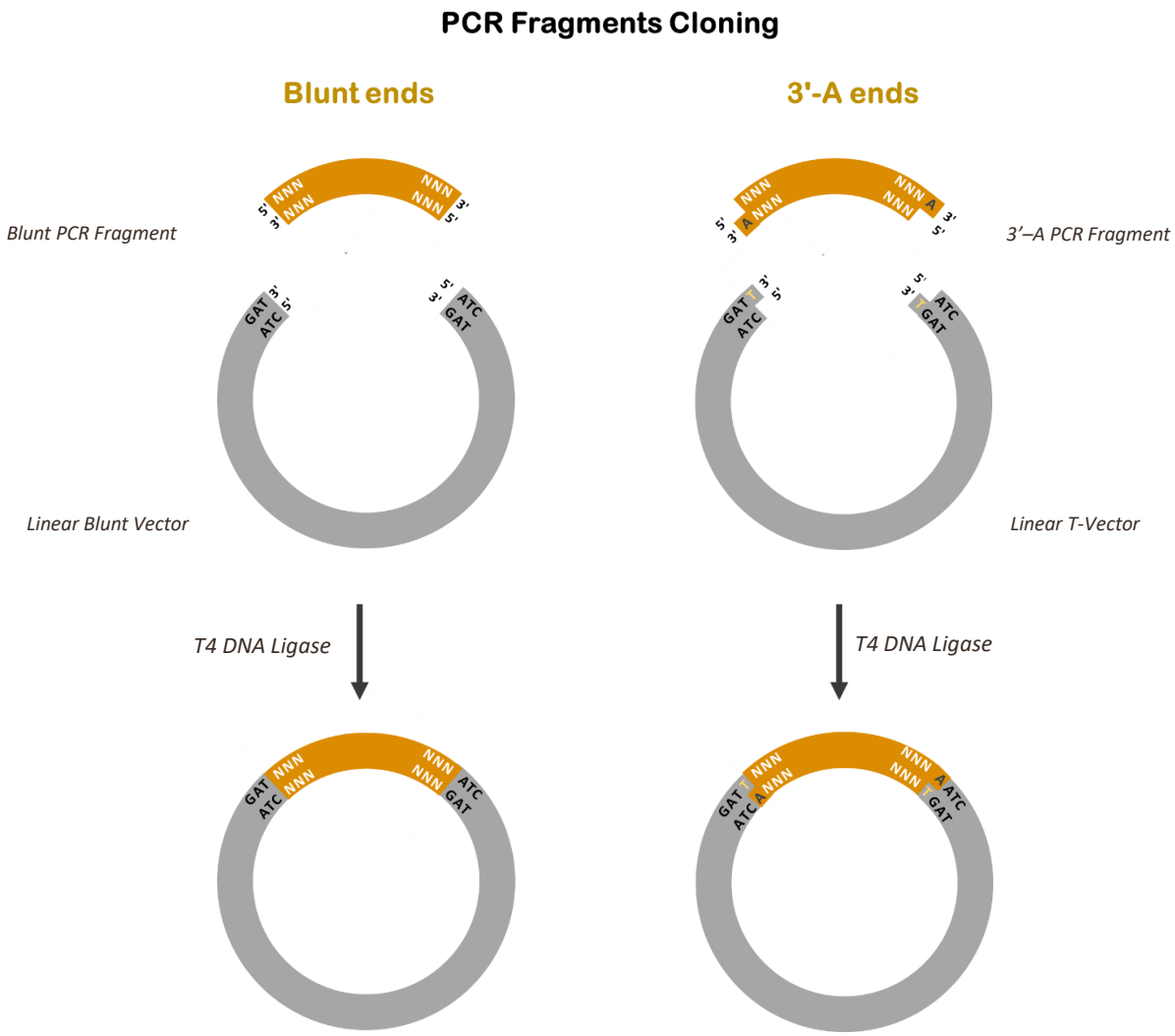
CLONING

T4 DNA LIGASE

T4 DNA Ligase is a recombinant ATP-dependent DNA ligase from bacteriophage T4 produced in *Escherichia coli*. It has been widely used in various applications ranging from molecular cloning, library construction and high-throughput DNA sequencing. This enzyme efficiently catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini juxtaposed in duplex DNA or RNA.

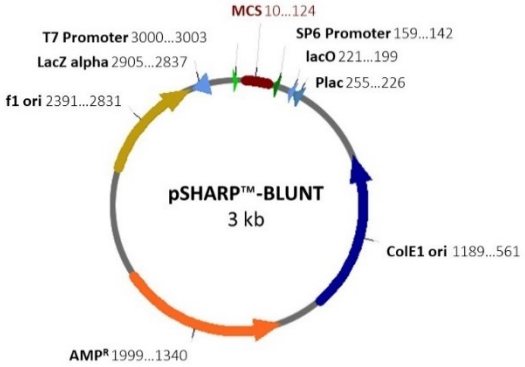
REFERENCES	DESCRIPTION	FORMAT
TBZ0326	T4 DNA LIGASE	200 U
TBZ0327	T4 DNA LIGASE	1000 U

PCR FRAGMENTS CLONING KITS



pSHARP™-BLUNT

The kit includes a blunt linear vector, obtained through innovative technology that ensures an efficient cloning process of blunt PCR fragments with minimal background. Cloning does not require phosphorylated primers or restriction enzymes. The vector includes identical restriction sites on both sides of the cloning site to facilitate subsequent subcloning processes.



Features

- Linear vector ready to use.
- Minimal background.
- Selection blue/ white.
- High number of white recombinant clones.
- *Eco*RI restriction site on both sides of MCS.
- T7 and SP6 promoters included.

Applications

- Cloning PCR fragments produced with high fidelity enzymes or blend polymerases.
- Subcloning of cloned PCR fragment.
- *In vitro* transcription.
- Sequencing.

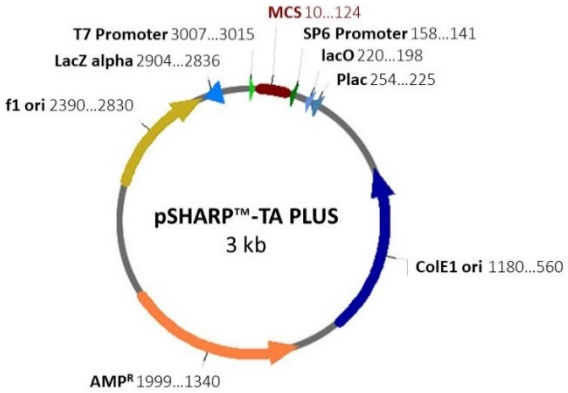
REFERENCES	DESCRIPTION	FORMAT
TBK0800	pSHARP™ -BLUNT VECTOR CLONING KIT	10 rxn
TBK0801	pSHARP™ -BLUNT VECTOR CLONING KIT	20 rxn

Complementary Products

- ✓ **TIARIS™ High-Fidelity DNA Polymerase Kit** (TBK1033)
- ✓ **Pfu DNA Polymerase** (TBK0086, TBK0087)
- ✓ **T7 RNA Polymerase** (TBZ0216)
- ✓ **HIGH-Q™ Spin-Column Gel Extraction & Cleanup Purification Kit** (TBK0191, TBK0192)

pSHARP™-TA PLUS

It is an ideal kit to clone 3'-A PCR fragments. The kit includes a linearized vector with thymine added at the 3' end. Complementarity with adenine, added template-free to the PCR fragment, promotes successful cloning.



Features

- Linear vector ready to use.
- Low background.
- Selection blue/ white.
- High number of white recombinant clones.
- T7 and SP6 promoters included.

Applications

- Cloning PCR fragments produced with non-proofreading DNA polymerases.
- Subcloning of cloned PCR fragment.
- *In vitro* transcription.
- Sequencing.

REFERENCES	DESCRIPTION	FORMAT
TBK0806	pSHARP™ -TA PLUS VECTOR CLONING KIT	10 rxn
TBK0807	pSHARP™ -TA PLUS VECTOR CLONING KIT	20 rxn

Complementary Products

- ✓ **STOUT™ Recombinant Taq DNA polymerase Master Mix** (TBK0029, TBK0030)
- ✓ **STOUT™ Red PCR Master Mix** (TBK0026, TBK0027)
- ✓ **HIGH-Q™ Spin-Column Gel Extraction & Cleanup Purification Kit** (TBK0191, TBK0192)

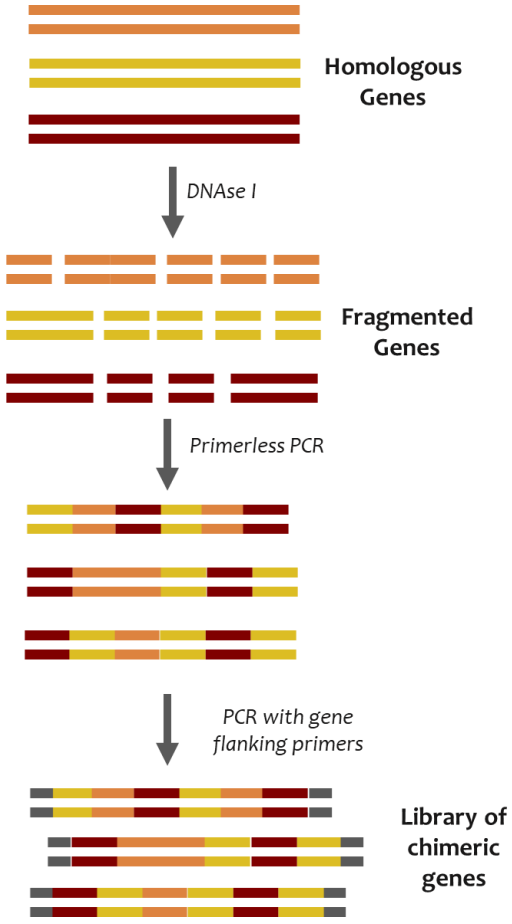
MUTAGENESIS

DNA SHUFFLING

TrapMutant™ DNA Shuffling Kits

The shuffling technique is a powerful tool for conducting molecular evolution of genes, ensuring broad genetic diversity. It is based on the random fragmentation of a target gene or a collection of homologous genes, which are then reassembled in two PCR steps: the first without oligonucleotides and the second flanked by primers of interest.

The reassembly of a single treated gene allows for the generation of point mutations at a rate of approximately 0.7%, while the inclusion of different homologous genes enables the creation of chimeric gene libraries with point mutations.



Features

- **0.7% point mutation rate** for one gene shuffled.
- **Homologous recombination and point mutation** at a rate of 0.7% for several genes shuffled.

Applications

- Molecular evolution of genes to obtain new protein variants.
- *In vitro* recombination of homologous or different genes.
- Library generation.

REFERENCES	DESCRIPTION	FORMAT
TBK0090	TRAPMUTANT™ DNA SHUFFLING KIT	30 rxn
TBK0091	TRAPMUTANT™ PLUS DNA SHUFFLING KIT	30 rxn

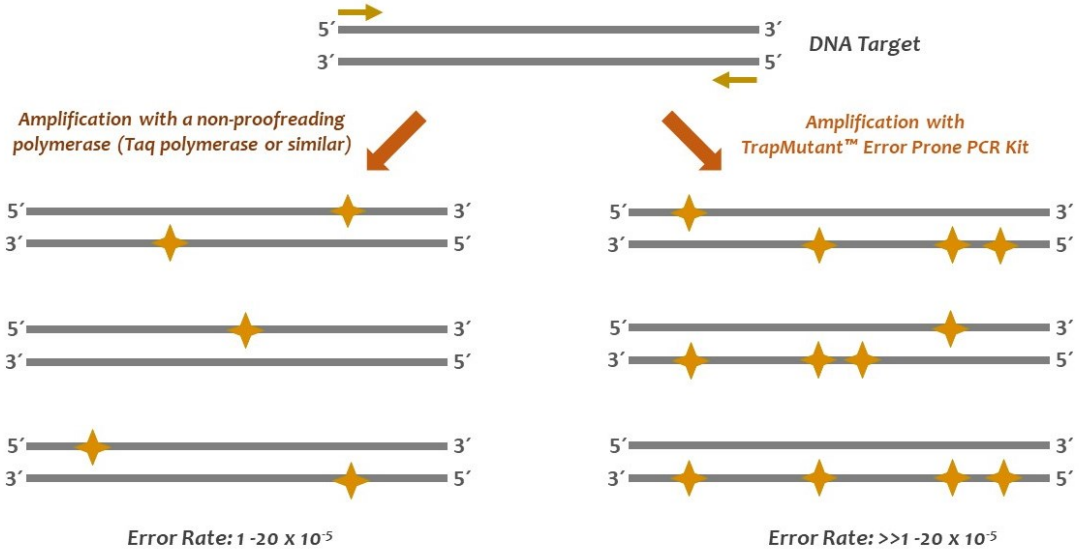
Complementary Products

✓ **HIGH-Q™ Spin-Column DNA Cleanup Purification Kit** (TBK0196, TBK0197)

ERROR PRONE

TrapMutant™ Error Prone PCR Kit

This kit utilizes the fidelity of Taq DNA polymerase, which has an estimated error rate of 1-20 x 10^{-5} bases. This inherent error rate can be modulated by adding Mn^{2+}, adjusting the Mg^{2+} concentration, and using unbalanced dNTP concentrations. Through these deliberate modifications, error-prone PCR enables controlled mutagenesis, with mutation rates typically ranging from 0.6% to 2.0%.



Features

- Efficient, **mutation rate of 0.6-2.0%**.
- **Polymerase included** has an error rate at 10^{-5} order.

Applications

- Protein Engineering to generate variants with desirable properties.
- Molecular evolution of genes to functional genomics studies or to obtain new protein variants.
- Library generation.
- Antibody Development.

REFERENCES	DESCRIPTION	FORMAT
TBK0094	TRAPMUTANT™ ERROR-PRONE PCR KIT	30 rxn

RELATED REAGENTS

X-GAL

X-GAL is a chromogenic permeable substrate to detect β-galactosidase activity. The enzyme hydrolyzes X-GAL releasing galactose and 5-Bromo-4-Chloro-3-hydroxyindole. Indole compound produced is dimerized and oxidized generating an insoluble dark blue product

IPTG

It is a synthetic compound widely used in protein expression driven by lac promoter. This compound is a non-metabolizing analogous of lactose that triggers transcription of the lac promoter.

Ampicillin, sodium salt

Ampicillin belongs to the group of beta lactam antibiotics. Its beta lactam ring structure includes an amino side chain attached to 6-amino-penicillanic acid. Ampicillin is one of the most important β-lactam antibiotics used today. Its action interrupts bacterial cell-wall peptidoglycan synthesis.

Kanamycin Sulphate

It belongs to the group of aminoglycoside antibiotics which binds to the 30S ribosomal subunit causing translocation inhibition and misreading of tRNA. Their specific interactions with bacterial ribosomal RNAs inhibits bacterial protein synthesis.

SOC Medium

SOC Medium is bacterial growth medium mostly used to culture transformed cells of *Escherichia coli.* It is a rich medium that contains a combination of salts, magnesium and glucose that stabilizes competent cells and maximizes transformation efficiency .

REFERENCES	DESCRIPTION	FORMAT
TBR0110	X-GAL	1 g
TBR0111	X-GAL SOLUTION, READY TO USE	10 mL
TBR0112	AMPICILLIN SODIUM SALT	5 g
TBR0113	AMPICILLIN SODIUM SALT	10 g
TBR0114	IPTG	5 g
TBR0115	IPTG SOLUTION 1M	5 x 1.5 mL
TBR0116	IPTG SOLUTION 0.1M	10 x 1.5 mL
TBR0118	KANAMYCIN SULPHATE	5 g
TBB0417	SOC MEDIUM	100 mL

"The answer to our questions are everywhere,
we just need to change the lens with which we see the world."

Janine Benyus

Today, in some countries, traveling by train at over 200 km/h is no longer a groundbreaking experience. However, in the early 1960s, trains rarely exceeded 110 km/h. In October 1964, for the Tokyo Olympics, Japan inaugurated the era of high-speed rail with the Shinkansen line. The first Shinkansen, known as the Series 0, boasted a maximum speed—unbelievable for the time—of 210 km/h.

When trains began reaching speeds close to 270 km/h, the operating company faced a major problem: as trains exited tunnels, they produced a loud noise that could be heard up to 400 meters away. The solution to this problem came from Eiji Nakatsu, manager of the Test Operations Department at Japan Railway West. After hearing one of his engineers remark that the train seemed to "shrink" when entering a tunnel, Nakatsu hypothesized that the sonic boom was caused by differences in air resistance inside and outside the tunnel.

The solution to this challenge lay in the question: is there a living organism that deals with sudden changes in air resistance? Nakatsu, a passionate birdwatcher and member of the Wild Bird Society of Japan, recalled the kingfisher's dives, which barely create a splash. The kingfisher's ability to transition from a low-resistance medium to water, which is 800 times denser, mirrored the issue faced by the bullet trains.

Nakatsu and his engineering team conducted a detailed study of the kingfisher's beak shape. The modified train, redesigned by Nakatsu, featured an elongated nose, extending from the original train's 6 meters to 15 meters in the Series 500 version, which debuted in 1997. Additionally, the new train had a rounded body, allowing air to flow more smoothly between the train and the tunnel walls. This design achieved speeds of 300 km/h without exceeding the noise limit of 70 decibels and reduced energy consumption by 15% compared to previous models.

Today, Shinkansen technology and infrastructure have advanced significantly, reaching regular operating speeds of up to 320 km/h with the Series N700S, launched in July 2020. Even faster models are expected by 2027. Following Japan's lead, high-speed rail systems were developed worldwide, including Italy's Diretissima (1977), France's TGV (1981), Spain's AVE (1992), South Korea's KTX (2004), and China's CRH (2007). And technology continues to progress at bullet-train speed!

##NatureInspiredBulletTrain