Biorbyt DNA-Shuffling Kit

Cat#: orb533434 (User Manual)

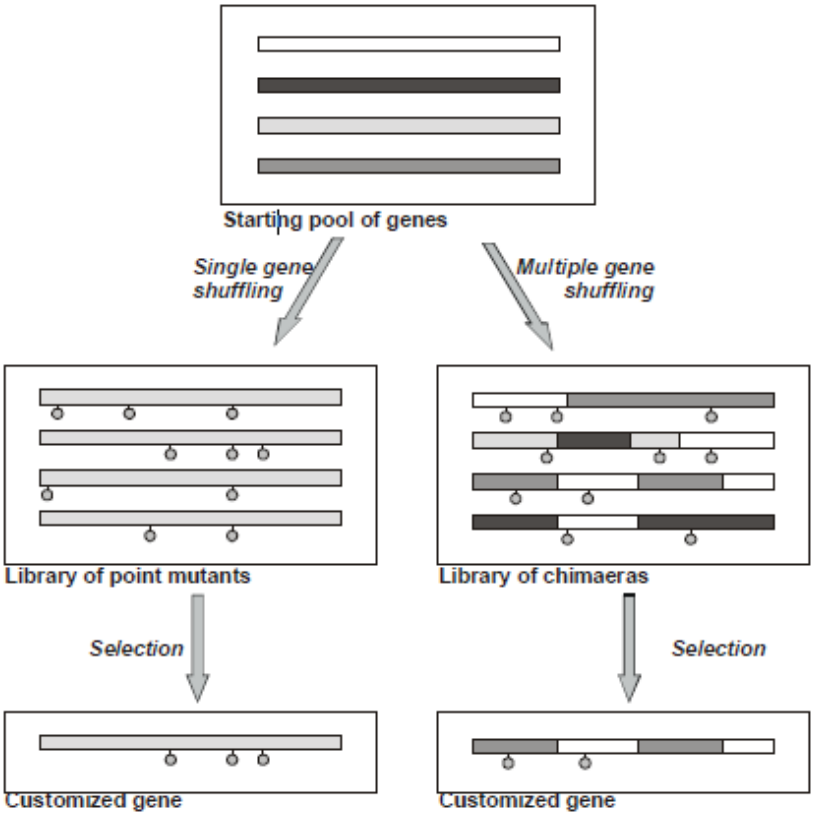

Fig.1: General types of DNA shuffling

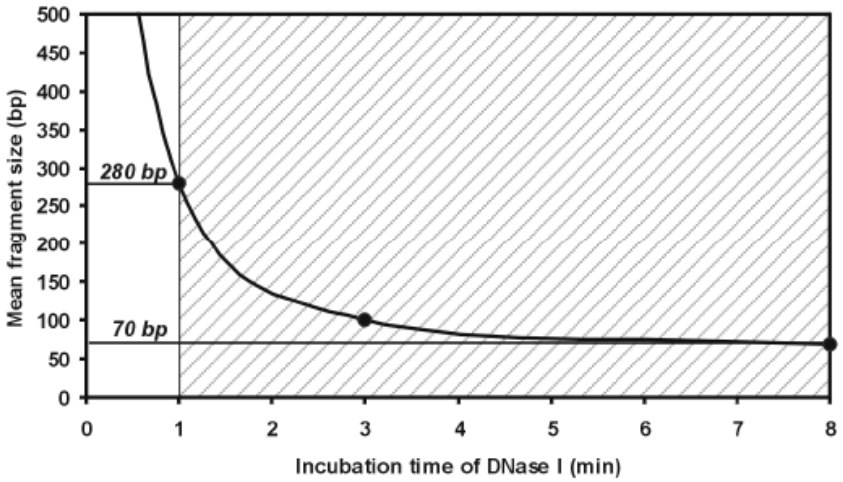

Fig. 2: DNase I digestion of a 1000 bp DNA fragment as a function of incubation time (1 µg DNA + 0.1 unit DNase I at 37°C)

Shipping: shipped on gel packs
Storage Conditions: store at -20 °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**
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**
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**
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:

DNase I (yellow cap) 0.1 units/µl, 100 µl
Digestion Buffer (blue cap) 10x concentration, 100 µl
DNase Stop Solution (yellow cap) 100 µl
Taq Polymerase (red cap) 5 units/µl, 40 µl
Shuffling Buffer (green cap) 10x concentration, 200 µl
dNTP Mix (white cap) 10 mM each dNTP (dATP, dCTP, dGTP, dTTP), 40 µl
PCR-grade Water (white cap) 3x 1 ml

Random Mutagenesis by DNA Shuffling

DNA shuffling is a powerful technique for directed evolution of proteins *in vitro*. It generates structural diversity by recombination of gene fragments originating from one or several related genes.
DNA shuffling can be divided into single gene shuffling and multiple gene shuffling (Fig. 1). In single gene shuffling only one gene is digested and subsequently reassembled resulting in point mutations at a rate of approx. 0.7%.

The major application of DNA shuffling in protein evolution is multiple gene shuffling (often referred to as molecular breeding). In this technique several homologous DNA sequences are digested and subsequently reassembled. The result is a library of chimaeric genes containing additional point mutations.

A pivotal step in DNA shuffling is the digest of the gene of interest for production of fragments of appropriate size. Therefore, all reagents in the Kit are optimised to obtain fragments of the desired size within a convenient time frame (Fig. 2). Usually best results were achieved using fragments with mean sizes of 70-280 bp. Note that a complete DNase I digest results in very short fragments that cannot be amplified by subsequent PCR.

Recommended assay preparation
1. DNase I digest of gene(s) of interest
• For a total volume of 50 µl, use 5 µl of 10x Digestion Buffer in an appropriate vial and add 0.5-2 µg of each starting DNA
• Add PCR-grade Water to a final volume of 50 µl
• Add 0.1 u (1.0 µl) DNase I per µg of starting DNA
• Depending on the desired fragment size incubate at 37°C for up to 8 min (refer to Fig. 2)
• Quench the digest by addition of 5 µl DNase Stop Solution followed by heat inactivation of the DNase I at 75°C for 10 min.
• Isolate fragments of the desired size range by agarose gel electrophoresis and purify using standard procedures.

2. First PCR (without primers)
• For a 50 µl reaction, take 5 µl of the 10x Shuffling Buffer in an appropriate vial and add the purified DNA fragments from step 1 to a final concentration of 10-20 ng/µl
• Add 1 µl of dNTP Mix
• Add 2.5 units (0.5 µl) of Taq Polymerase
• Add PCR-grade Water to a final volume of 50 µl
• Recommended thermo cycling conditions:

1. Denaturation: 94°C 90 sec
2. Annealing: 55°C 30 sec
3. Extension: 72°C 30 sec
Number of cycles: 30-45
• Purify PCR product using standard procedures

3. Second PCR (with primers)
• For a 50 µl reaction, take 5 µl of the 10x Shuffling Buffer and add 2 µl of the PCR product from step 2
• Add 1 µl of dNTP Mix
• Add primers to a concentration of 0.8 µM each
• Add 2.5 units (0.5 µl) of Taq Polymerase
• Add PCR-grade Water to a final volume of 50 µl
• Recommended thermo cycling conditions:

1. Denaturation: 94°C 30 sec
2. Annealing: 55°C 30 sec
3. Extension: 72°C 30 sec
Number of cycles: 15

Selected References:

Crameri *et al.* (1998) DNA shuffling of a family of genes from diverse species accelerates directed evolution. *Nature* **391**:288.
Patten *et al.* (1997) Applications of DNA shuffling to pharmaceuticals and vaccines. *Curr. Opin. Biotechnol.* **8**:724.
Stemmer (1994) DNA shuffling by random fragmentation and reassembly. In vitro recombination for molecular evolution. *Proc. Natl. Acad. Sci. USA* **91**:10747.
Stemmer (1994) Rapid evolution of a protein in vitro by DNA shuffling. *Nature* **370**:389.