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DATA SHEET

Version: 2
Revision date: 14/03/2023

1. Identification

Product name

PickMutant™ DNA-Shuffling Kit
Random Mutagenesis by DNA Shuffling

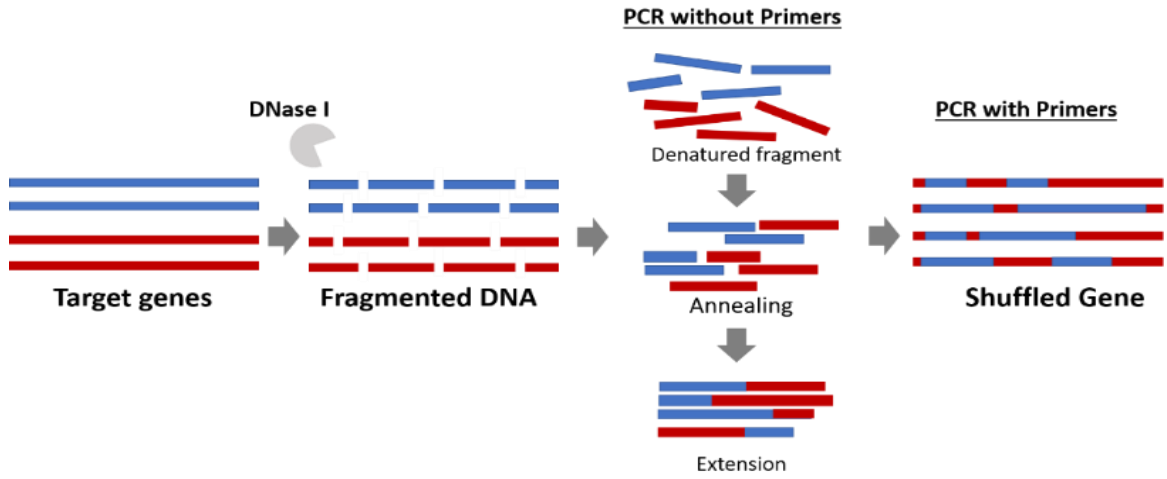
Cat. No MT030

2. Description

PickMutant™ DNA-Shuffling Kit is based on the method Developed by Stemmer.

Stemmer introduced DNA shuffling, the first homologous recombination method, in 1994. DNA shuffling involves the digestion of a gene by DNase I into random fragments, and the reassembly of those fragments into a full-length gene by primerless PCR: the fragments prime on each other based on sequence homology, and recombination occurs when fragments from one copy of a gene anneal to fragments from another copy, causing a template switch, or crossover event. While randomly recombining the DNA sequences, the technique also introduces new point mutations at a relatively high rate **(0.7%).**

DNA shuffling consists of four steps: **(i)** preparation of genes to be shuffled, **(ii)** fragmentation with DNase I, **(iii)** reassembly by thermocycling in the presence of a DNA polymerase, and **(iv)** amplification of reassembled products by a conventional PCR.



3. Composition

Item	Volume
Taq Polymerase (5 U/µl)	40 µl
10x PCR Buffer	500 µl
10 mM dNTP Mix	80 µl
DNase I solution (0.1U/ µl)	100 µl
10x Reaction Buffer	200 µl
Stop solution	100 µl
PCR-grade Water	3 x 1.5 ml

Notes: Provided material for 30 reactions.

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4. Storage specifications

Store at **-20 °C** and avoid freeze/thaw cycles.

5. Applications

- Generation of a library of mutants.
- Slightly different genes coding for the same product
- Homologous recombination of genes
- Generation of gene libraries
- Recombination of sequence blocks
- In vitro molecular evolution

6. Further information

Product Use Limitations

This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer

The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

References

Stemmer, W. P. C. (1994) DNA shuffling by random fragmentation and reassembly: In vitro recombination for molecular evolution. Proc. Natl. Acad. Sci. USA 91, 10,747–10,751.

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RECOMMENDED PCR ASSAY (50 µl assay)

A. FRAGMENTATION

1. Combine the following in a 0.2 ml PCR tube:
   - 5 µl 10X DNase I buffer
   - 0.5-2 µg parent DNA
   - Water to bring total volume to 50 µl. Equilibrate the reaction at 15˚C for 5 minutes in a thermal cycler.
2. Add 0.5-2 µl DNase I.
3. Incubate the reaction at 15˚C for 3-10 minutes. *
4. Stop the reaction by adding 1µl Stop solution and heating to +75 ºC for 10 minutes for complete inactivation of DNase I.
5. Isolate fragments of the desired size range by agarose gel electrophoresis and purify using standard procedures.

* *The incubation time with DNase I is a critical parameter for generating fragments of the desired size. Before attempting this step, prepare enough parent DNA to digest small aliquots with varying incubation times (30 s to 10 min). Then, select an optimal condition for a larger-scale digestion. Increasing DNase I concentration and/or incubation times will decrease fragment sizes and therefore more recombination sites will be introduced into the reconstructed genes. Note that a complete DNase I digest results in very short fragments that cannot be amplified by subsequent PCR.*

B. FRAGMENT REASSEMBLY (First PCR without primers)

1. Combine the following in a 0.2 ml PCR tube:
   - 5 µl 10X PCR Buffer
   - 10-20 ng/µl purified DNA
   - 1 µl of dNTP Mix
   - 2.5 units (0.5 µl) of Taq Polymerase
   - PCR-grade Water to bring total volume to 50 µl
2. Run the assembly reaction using the Recommended thermocycling conditions: 3 min at 94˚C followed by 30-45 cycles of 94˚C for 90 sec, 55˚C for 30 sec and 72˚C for 30 sec.
3. Purify PCR product using standard procedures.

C. AMPLIFICATION (Second PCR with primers).

1. Combine the following in a 0.2 ml PCR tube:
   - 5 µl 10X PCR Buffer
   - 1-2 µl of the PCR product from First PCR
   - 0.8 µM(final) forward and reverse primers
   - 1 µl of dNTP Mix
   - 2.5 units (0.5 µl) of Taq Polymerase
   - PCR-grade Water to bring total volume to 50 µl
2. Run the PCR reaction using the Recommended thermocycling conditions: 3 min at 94˚C followed by 15-30 cycles of 94˚C for 30 sec, 55˚C for 30 sec and 72˚C for 30 sec.