Stratech

NZYtech
genes & enzymes

MOLECULAR BIOLOGY

DNA cloning and mutagenesis kits

EASY CLONING & EXPRESSION

STANDARD CLONING

DNA MUTAGENESIS

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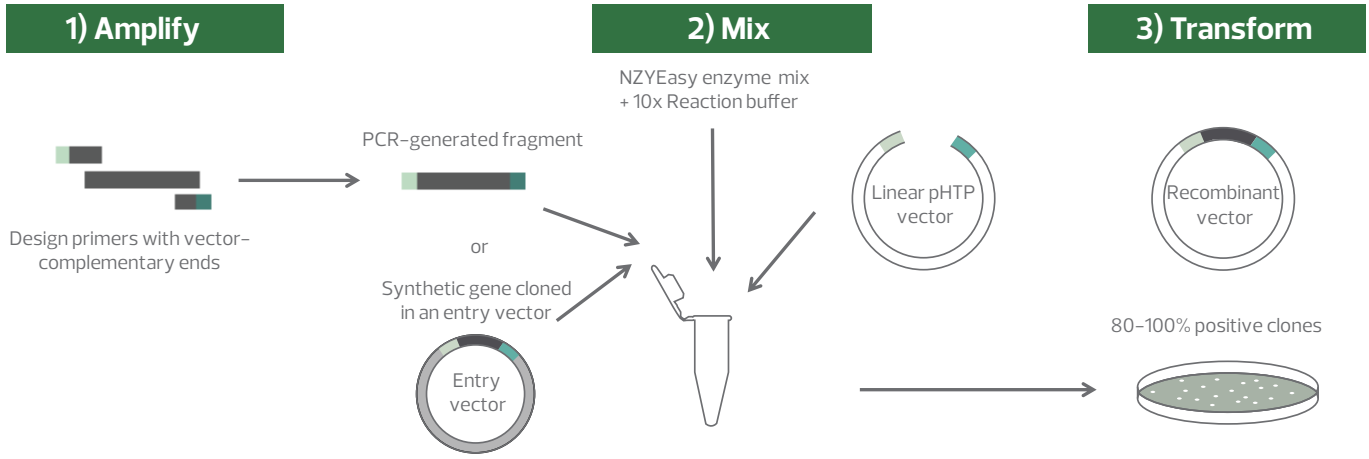
DNA CLONING KITS

EASY CLONING & EXPRESSION

The NZYEasy Cloning & Expression System was designed to allow directional cloning of PCR-generated fragments or synthetic genes previously cloned in pUC-vectors into a linearized pHTP vector in a single reaction mediated by NZYEasy enzyme mix. The system allows achieving high cloning efficiencies and does not require the use of DNA ligases. In addition, no further treatment (e.g. restriction digestion, phosphorylation or blunt-end polishing) of the inserts is required.



NZYTech provides ready-to-use pHTP vectors in separate kits. The portfolio of pHTP prokaryotic expression vectors, which includes a different range of fusion tags, offers the possibility to quickly assay levels of expression and solubility of the desired protein in multiple expression vectors simultaneously. Choose the pHTP vectors that most suits your experiments on the table below:

Vector	Features	Comments	Kit Cat. No.
pHTP0	*lac* promoter and *lacZ_a* reporter	High-copy number cloning vector for the NZYEasy Cloning & Expression System; allows blue/white screening during cloning (if adding IPTG and X-GAL)	MB281
pHTP1	N- and/or C-terminal 6xHis	Standard vector for high-level protein expression in *E. coli*	Kit I, MB282
pHTP2	N- and/or C-terminal 6xHis; N-terminal LLDsbC	Leader Less DsbC promotes cytoplasmic isomerization of disulfide bonds	Kit II, MB319
pHTP3	N- and/or C-terminal 6xHis; N-terminal mutDsbC	Inactive DsbC promotes cytoplasmic solubilization without isomerization of disulfide bonds	Kit III, MB320
pHTP4	N- and/or C-terminal 6xHis; N-terminal DsbC	DsbC promotes periplasmic isomerization of disulfide bonds	Kit IV, MB321
pHTP7	N- and/or C-terminal 6xHis; N-terminal DsbA	DsbA promotes periplasmic formation of disulfide bonds	Kit VII, MB322
pHTP8	N- and/or C-terminal 6xHis; N-terminal Trx	Trx enhances solubility of tagged proteins	Kit VIII, MB323
pHTP9	N- and/or C-terminal 6xHis; N-terminal GFP	GFP is a reporter molecule that allows monitoring protein localization	Kit IX, MB324
pHTP10	N- and/or C-terminal 6xHis; N-terminal NusA	NusA enhances solubility of tagged proteins	Kit X, MB325
pHTP11	N- and/or C-terminal 6xHis; N-terminal GST	GST enables glutathione-based affinity purification of tagged proteins while enhancing protein solubility	Kit XI, MB326
pHTP13	N- and/or C-terminal 6xHis; N-terminal GB1	GB1 enhances solubility of tagged proteins	Kit XIII, MB327
pHTP14	N- and/or C-terminal 6xHis; N-terminal KSI	KSI enhances solubility of tagged proteins	Kit XIV, MB328
pHTP16	N- and/or C-terminal 6xHis; N-terminal CpA	CpA enhances solubility of tagged proteins	Kit XVI, M3290
pHTP17	N- and/or C-terminal 6xHis; N-terminal CpB	CpB enhances solubility of tagged proteins	Kit XVII, MB330

PCR-generated fragments can be cloned into the pHTP0 cloning vector (included in the NZYEasy Cloning kit) or, alternatively, into one of the various kanamycin-resistant pHTP expression vectors (included in the different NZYEasy Cloning & Expression kits) without the need to go through the tedious and laborious intermediate stages.

DNA Cloning

The NZYEasy Cloning kit was designed for time-saving and cost-effective DNA cloning. It includes the pHTP0 vector (pUC-derivative) that allows blue/white screening for positive bacterial colonies (if adding IPTG and X-GAL).

NZYEasy Cloning kit

MB28101	8 reactions
MB28103	96 reactions

DNA Cloning & Expression

NZYEasy Cloning & Expression kits include different expression vectors that use the T7/*lac* promoter for regulated high-level protein expression in *E. coli* strains containing the λDE3 lysogen, such as BL21(DE3).

NZYEasy Cloning & Expression kit I

MB28201	8 reactions
MB28203	96 reactions

NZYEasy Cloning & Expression kit II

MB31901	8 reactions
MB31903	96 reactions

NZYEasy Cloning & Expression kit III

MB32001	8 reactions
MB32003	96 reactions

NZYEasy Cloning & Expression kit IV

MB32101	8 reactions
MB32103	96 reactions

NZYEasy Cloning & Expression kit VII

MB32201	8 reactions
MB32203	96 reactions

NZYEasy Cloning & Expression kit VIII

MB32301	8 reactions
MB32303	96 reactions

NZYEasy Cloning & Expression kit IX

MB32401	8 reactions
MB32403	96 reactions

NZYEasy Cloning & Expression kit X

MB32501	8 reactions
MB32503	96 reactions

NZYEasy Cloning & Expression kit XI

MB32601	8 reactions
MB32603	96 reactions

NZYEasy Cloning & Expression kit XIII

MB32701	8 reactions
MB32703	96 reactions

NZYEasy Cloning & Expression kit XIV

MB32801	8 reactions
MB32803	96 reactions

NZYEasy Cloning & Expression kit XVI

MB32901	8 reactions
MB32903	96 reactions

NZYEasy Cloning & Expression kit XVII

MB33001	8 reactions
MB33003	96 reactions

STANDARD CLONING

NZYTech's DNA cloning kits are optimized to provide high-efficiency cloning based on easy protocols with no requirement for time-consuming restriction digests. To clone PCR-amplified fragments take into account the type of DNA polymerase that was used to generate the DNA fragment and choose the appropriate cloning kit: NZY-A PCR cloning kits are designed for cloning of DNA fragments amplified using non-proofreading polymerases, while NZY-blunt PCR cloning kit is designed to clone blunt-end PCR products amplified by proofreading enzymes. For a faster DNA cloning, the speedy version of NZY-A PCR cloning kit (NZY-A Speedy PCR cloning kit) should be chosen.

Blunt-end

NZY-blunt PCR cloning kit was designed to allow the direct cloning of PCR products with blunt-ends which result from amplifications using proofreading DNA polymerases such as NZYProof DNA polymerase (MB146).

NZY-blunt PCR cloning kit	
MB12101	24 ligations + competent cells
MB12102	24 ligations

A-overhang

NZY-A PCR cloning kits were designed to clone PCR products produced by non-proofreading DNA polymerases such as NZYTaq II DNA polymerase (MB354). They take advantage of the terminal transferase activity of these polymerases which adds a single 3'-A overhang to each end of the PCR product. For a faster DNA cloning, NZYTech provides the NZY-A Speedy PCR cloning kit which allows direct cloning of PCR products with 3'-A overhangs in only 5 minutes at room temperature. Blunt-ended PCR fragments generated by amplification with proofreading polymerases can also be cloned using NZY-A PCR cloning kits after conducting an A-tailing procedure.

NZY-A PCR cloning kit	
MB05301	24 ligations + competent cells
MB05302	24 ligations

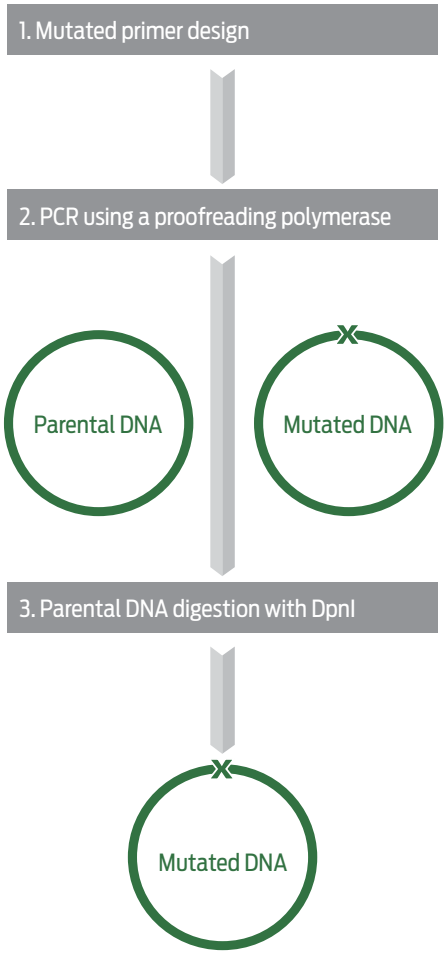
NZY-A Speedy PCR cloning kit	
MB13701	24 ligations + competent cells
MB13702	24 ligations

High efficiency cloning: >95% positive clones
Blue/white colony screening
5 minutes cloning with NZY-A Speedy PCR cloning kit

DNA MUTAGENESIS

Site-Directed Mutagenesis can help you in a range of applications allowing to edit the desired DNA sequence by incorporation of single or multiple point mutations in any type of plasmid DNA. NZYTech's Mutagenesis kits provide simple and highly efficient methods to generate point mutations and delete or insert single (or multiple) nucleotides in plasmid DNA using PCR. NZYSupreme Mutagenesis kit was recently developed to reduce labor time and increase the efficiency of DNA editing.

Mutagenesis kits contain a proofreading DNA polymerase for PCR amplification of dsDNA plasmid to be mutated. NZYSupreme Mutagenesis kit includes Supreme NZYProof DNA polymerase, an engineered highly accurate, fast and sensitive variant of NZYProof DNA polymerase (the DNA polymerase present in NZYMutagenesis kit), formulated in a 2x concentrated master mix solution. Both Supreme NZYProof and NZYProof DNA polymerases ensure high fidelity for the exponential PCR amplification, thus reducing the unwanted secondary mutations and enabling amplification of large plasmids up to 15 kb. In addition, mutagenesis system requires the provision of two synthetic oligonucleotide primers containing the desired mutation. Mutated primers can be designed following standard guidelines (overlapping primers) or using an improved methodology (non-overlapping primers) recommended on NZYSupreme Mutagenesis kit. The mutagenesis protocol includes only three main steps:



1. Mutated primer design

Primers should have between 25 and 45 bases in length, with a melting temperature (T_m) of ≥ 78 ºC; a GC content of 40% and should terminate in one or more C or G bases;

2. PCR amplification

Extension of the oligonucleotide primers with a proofreading DNA polymerase generates a mutated plasmid containing staggered nicks;

3. Digestion with DpnI

Digestion of PCR product with DpnI endonuclease for elimination of the parental methylated and hemimethylated DNA template and selection of the mutation-containing synthetic DNA (not methylated).

NZYTech provides convenient versions of the kit, which include highly efficient competent cells for mutaded plasmid recovering.

	NZYMutagenesis kit	NZYSupreme Mutagenesis kit
Primer design:	Standard (overlapping primers)	Improved (non-overlapping primers)
DNA polymerase:	NZYProof DNA polymerase	Supreme NZYProof 2x Colourless Master Mix
PCR time:	2h 30	<1h 30 min
Digestion time:	1 hour (at 37 ºC)	5-15 min (at 37 ºC)

NZYMutagenesis kit	
MB01201	10 mutations
MB01202	10 mutations + competent cells

NZYSupreme Mutagenesis kit	
MB44701	10 mutations
MB44702	10 mutations + competent cells