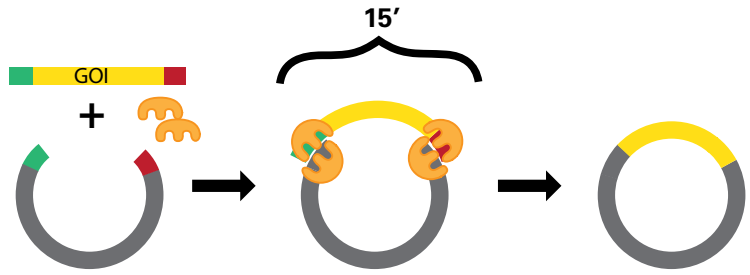
In-Fusion® HD Cloning Plus

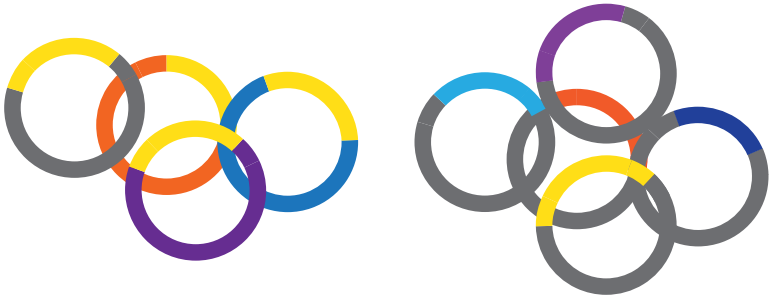
that's GOOD science!®

Fast, accurate cloning for simple to complex projects



Seamless • Directional
ANY insert • ANY vector • ANY locus

Large & small inserts or vectors
Point mutations • Multiple fragments
Build your own vector



One GOI - different vectors
One vector - different inserts
Libraries

TaKaRa

Clontech TaKaRa cellartis

In-Fusion® Cloning:

Ideal for...

- Single-insert cloning
- Multiple-insert cloning
- Large-insert cloning
- Adding small tags
- Direct cloning into large vectors (up to 46-kb cosmids)
- Site-directed mutagenesis (single or multiple deletions, insertions, or substitutions)
- HTP Cloning

The right clone every time.

Good science relies on a solid foundation of inspiration matched with technical expertise. Every decision has an impact, starting with experimental design. This is why we are dedicated to delivering best-in-class products backed by expert scientific support. We understand that streamlined methods and high-performance reagents enable you to push your research to its fullest potential.

In-Fusion Cloning kits are unique in their ability to allow easy, accurate, seamless cloning without the use of ligase. From the simple to the complex, you can recover your final construct on the second day! In just one quick reaction, you can clone ANY insert into ANY vector at ANY locus, without the hassle of subcloning, unwanted extra bases, or inconvenient restriction sites.

The reaction is specific and directional, ensuring an exceptionally high rate of cloning accuracy in all applications. Our standards for accuracy involve more than simple transformation efficiency. Why waste time screening a large number of colonies? With background levels approaching zero, you can pick just a few colonies and be confident that you have the right clone.

As the first step in so many experiments, successful cloning is key to recovering meaningful data. The cloning tools you use can be the difference between wasted time and fast, consistent results.

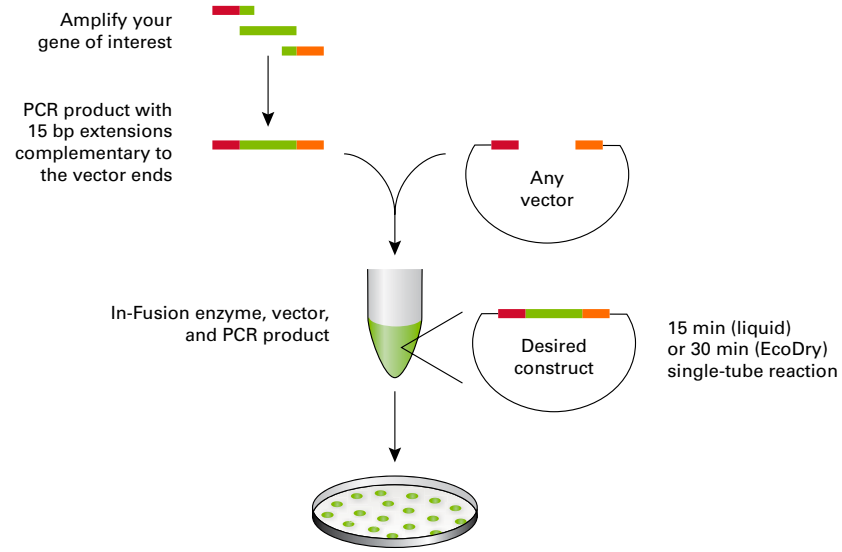


Figure 1. In-Fusion Cloning protocol.
The key to In-Fusion Cloning is 15 bp of homology between your insert(s) and linearized vector backbone. Add these 15 bp to the 5′ end of your forward and reverse primers, and PCR amplify your desired insert(s). Linearize your vector, by restriction digest or inverse PCR. Combine in a single tube containing the In-Fusion enzyme mix, perform the In-Fusion cloning reaction, and transform into competent cells.

that's GOOD science!®

One Cloning System for multiple applications

Clone multiple fragments with ease!

Using In-Fusion, cloning multiple fragments simultaneously is as easy as cloning a single fragment and it can be done in a single reaction! Just combine the PCR fragments with appropriate complementary ends and a linear vector, then incubate with the In-Fusion enzyme. This saves weeks and months that would otherwise be spent screening clones and sub-cloning. The ability to easily, rapidly and precisely clone many fragments at once will help to speed up the generation of complex target constructs in your lab. And don't just take our word for it; Zhu et al. successfully constructed a three domain immunoglobulin fusion protein by means of a four-piece In-Fusion reaction (See Fig. 2 below adapted from Zhu *et al.* (2007) BioTechniques 43(3): 354-359.).

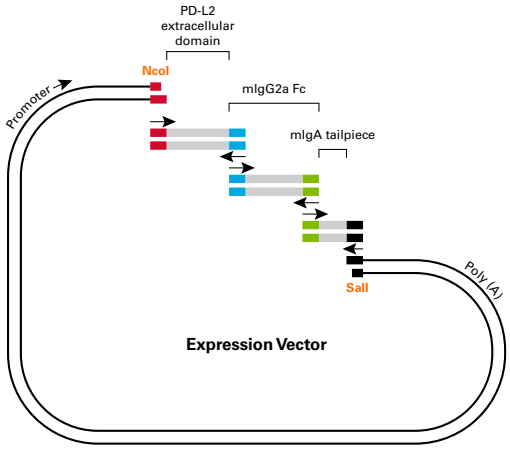


Figure 2 : Seamless construction of a three-domain immunoglobulin fusoin protein with a four piece In-Fusion reaction.
Segments were generated by PCR using primers that contained a 15 bp overlap with the adjacent segment, and 20-30 bp of segment specific sequence. Colored regions indicate overlap regions with 15 bp of identify. Arrows indicate PCR primers. The segments were joined to a NcoI-SalI-digested expression vector in a ligase-free In-Fusion reaction.

Site-directed mutagenesis

In-Fusion cloning can be used for both cloning and site-directed mutagenesis including insertions, deletions and substitutions, eliminating the need for a separate kit for mutagenesis. Save money, time, and space in your laboratory by using this one universal system for genetic manipulation, including the engineering of both single and multiple mutations quickly and efficiently in a single reaction. Mutagenesis can be performed easily by combining the strength of the In-Fusion enzyme with inverse PCR using mutagenic primers (See Fig. 3 below).

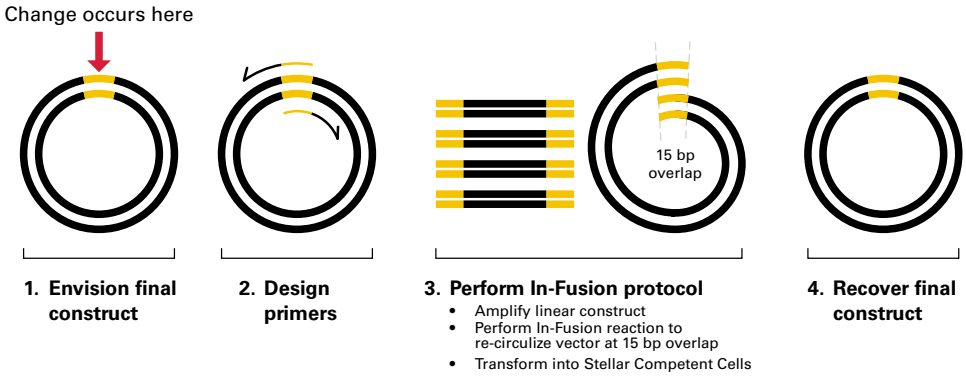


Figure 3 : Procedure for performing mutagenesis with In-Fusion systems.
The area where mutagenesis occurs is shown in yellow.

HTP Cloning for protein expression and production studies

Obtaining recombinant proteins in a soluble form suitable for crystallization remains a bottleneck for high-throughput (HTP) structural biology. The Oxford Protein Production Facility (OPPF) has created the pOPIN vector suite for use with Clontech's In-Fusion cloning system. This custom vector suite in combination with the In-Fusion enzyme delivers a versatile, precise, and seamless one-step cloning process (i.e., no unwanted residues in the translated protein), that can be readily adapted to high-throughput structural biology studies. Incorporating the In-Fusion system into the OPPF HTP pipeline enabled parallel vector construction, expression screening, and purification, accelerating the protein production process (Berrow *et al.*, 2007, Nucleic Acids Res. 35(6):e45).

In-Fusion HD Cloning Plus Systems

Complete, optimized systems

Each system fully equips you for every step, bringing the price per reaction in line with the true total cost of your experiment. The components have been optimized to work together efficiently, and provide you with a high out-of-the-box success rate.

Our complete range of In-Fusion HD Cloning Plus kits allows you to choose the version that best fits your experimental needs.

The flexibility and speed of the In-Fusion mechanism means it can be easily adapted for nearly every cloning application.

Complete systems include:

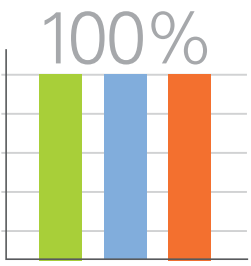
- **CloneAmp™ HiFi PCR Premix**
- **PCR product treatment** (either Cloning Enhancer or a NucleoSpin Gel and PCR Cleanup kit)
- **In-Fusion HD Enzyme Premix**
- **Stellar™ Competent Cells**

Be Green!

The In-Fusion HD EcoDry System can be stored at room temperature.

Table I. Selection Guide for In-Fusion HD Cloning Systems

	In-Fusion HD Cloning Plus System	In-Fusion HD Cloning Plus CE System	In-Fusion HD EcoDry™ Cloning Plus System
System format	liquid	liquid	lyophilized
Ability to customize reaction volume and/or plasticware	✓	✓	
Easily scalable for high-throughput applications		✓	
Minimize handling with pre-aliquoted, lyophilized components			✓
Store reagents at room temperature			✓
Fast, enzymatic removal of background DNA and PCR residue (for PCR that produces a single, clean band)		✓	
Suitable for PCR and gel extraction when PCR produces >1 product	✓		✓



Exceptional cloning accuracy with In-Fusion HD Cloning.

Consider the following scenario:

The Experiment	The Results
One vector	26/26 colonies (100% cloning accuracy)
Three inserts	Sequence-verified
One 15-minute reaction	Low background (single colony on no-insert plate)

In-Fusion technology reliably demonstrates high cloning accuracy in all applications. This is especially useful with more challenging projects such as multiple-insert cloning, where high background and false positives present a bigger problem.

PCR Polymerases

A crucial element required for successful cloning is an error-free insert. A PCR polymerase capable of amplifying your templates for both cloning and colony screening is key to seeing accurate results. Clontech offers a wide range of DNA polymerases to suit every cloning project.

- **CloneAmp HiFi PCR Premix**
- **SapphireAmp® Fast PCR Master Mix**
- **PrimeSTAR® GXL DNA Polymerase**

Table III. Selection Guide for DNA Polymerases for Cloning

CloneAmp HiFi PCR Premix	SapphireAmp Fast PCR Master Mix	PrimeSTAR GXL DNA Polymerase
Optimized for use with In-Fusion Cloning	Analyze without purification Restriction digest Gel electrophoresis	Robust, high-fidelity PCR in all conditions
Extremely low error rate (see Fig.4 below)	Colony PCR in ~1 hour	Excels with long templates
Quick priming & extension (5sec/kb)	Master mix includes dye (see Fig.5 below)	GC- or AT-rich targets
Templates up to 10 kb		Templates with repeats
GC-rich templates		

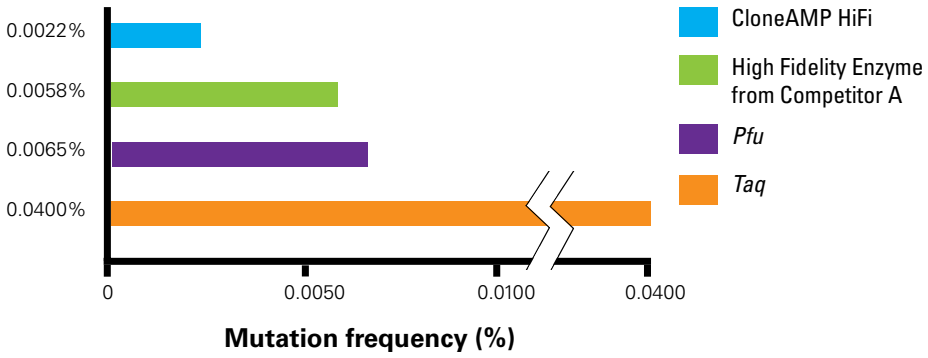


Figure 4 : Mutation frequency of CloneAmp Hifi compared to other high-fidelity PCR enzymes
Mutation frequency of CloneAmp HiFi Polymerase compared to other high-fidelity PCR enzymes. Eight arbitrarily selected GC-rich regions were amplified with CloneAmp HiFi Polymerase or other DNA polymerases using a *Thermus thermophilus* HB8 genomic DNA template, and cloned into suitable plasmids. Multiple clones were selected for each amplification product and subjected to sequence analysis. DNA fragments amplified using CloneAmp HiFi Polymerase yielded only 12 mismatched bases per 542,580 total bases—lower than an alternative high-fidelity enzyme from Company A, and 10-fold lower than Taq DNA polymerase.

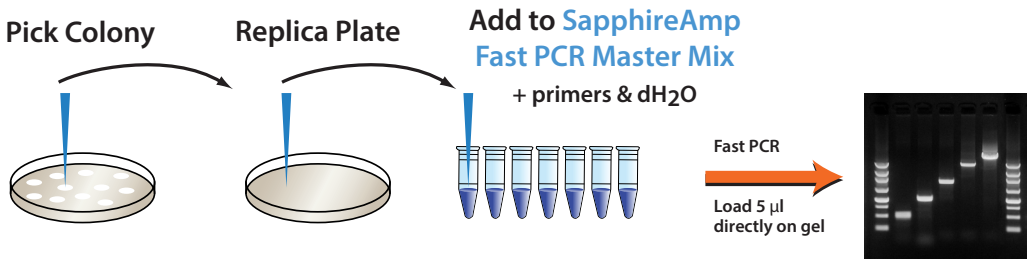


Figure 5. Colony PCR protocol with SapphireAmp Fast PCR Master Mix.
Colony PCR can be set up with two basic steps: 1. Pick a colony ; 2. Inoculate a replica plate.

PRODUCTS

Cat. #	Product	Size
In-Fusion Cloning		
638909 638910 638911 638920	In-Fusion HD Cloning Plus (Liquid system, includes: In-Fusion HD Enzyme Premix, CloneAmp HiFi PCR Premix, Stellar Competent Cells, and NucleoSpin Gel and PCR Clean-Up kit)	10 Rxns 50 Rxns 100 Rxns 96 Rxns
638916 638917 638918 638919	In-Fusion HD Cloning Plus CE (Liquid system, includes: In-Fusion HD Enzyme Premix, CloneAmp HiFi PCR Premix, Stellar Competent Cells, and Cloning Enhancer)	10 Rxns 50 Rxns 100 Rxns 96 Rxns
638912 638913 638914 638915	In-Fusion HD EcoDry Cloning Plus (Lyophilized system, includes: In-Fusion HD Enzyme Premix, CloneAmp HiFi PCR Premix, Stellar Competent Cells, and NucleoSpin Gel and PCR Clean-Up kit)	8 Rxns 24 Rxns 48 Rxns 96 Rxns
PCR Polymerases		
639298	CloneAmp HiFi PCR Premix	200 Rxns
RR350A RR350B	SapphireAmp Fast PCR Master Mix	160 Rxns 800 Rxns
R050A R050B	PrimeSTAR GXL DNA Polymerase	250 Units 1,000 Units

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