In-Fusion Cloning

In-Fusion® Cloning: Accuracy, Not Background

Unmatched performance for precise, seamless cloning.

- Fast, single-tube cloning protocol
- High cloning accuracy for single and multiple fragments
- Low background—get the right clone every time

that's GOOD science!™

Fast, Easy Cloning without Ligation

In-Fusion Cloning kits allow ligation-independent, directional cloning of PCR products into **any** vector, at **any** site of linearization (Figure 1). The cloning reaction takes as little as 15 minutes, and enables you to build even complicated constructs in just one step (1, 2).

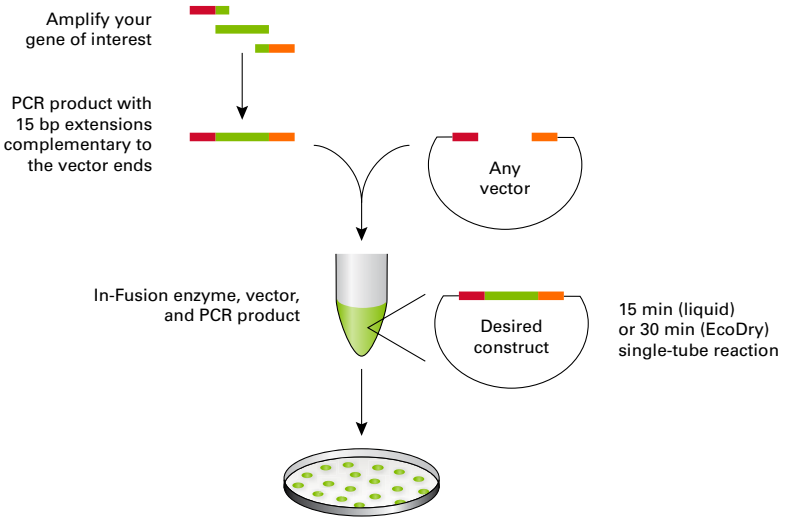

Figure 1. In-Fusion cloning protocol.

How Does In-Fusion Technology Compare with Another Cloning System?

At first glance, In-Fusion Cloning technology has a lot in common with a method developed by Gibson and colleagues (3). Both systems:

- Provide options that eliminate restriction digest steps
- Have relatively fast, simple protocols with just a few steps and reagents
- Are seamless—that is, they don't add extra bases between joined fragments
- Allow for multiple DNA fragments to be cloned in a single reaction

However, there are also some critical differences in workflow and performance. In-Fusion technology has a faster protocol, provides lower background, and reliably demonstrates higher cloning accuracy—especially where more complex cloning projects are concerned. Under more challenging conditions and shorter reaction times, Gibson's system demonstrates a problematic issue with high levels of background, something it has in common with more traditional ligation and TA cloning methods.

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Putting In-Fusion Technology to the Test

In-Fusion HD Cloning technology was put up against Gibson's method in side-by-side experiments. In all instances, the cloning vector used was pUC19, linearized with BamHI. Each cloning system was tested under the recommended conditions of its own cloning protocol, and additional testing was done with Gibson's enzyme mix for multiple-insert cloning. Gibson's method states the incubation time should be increased from 15 minutes to 60 minutes for 4-fragment (3-insert) assemblies. In an effort to make a more direct comparison with In-Fusion Cloning, this multiple-insert experiment with Gibson's enzyme mix was also run at the shorter In-Fusion reaction time. All cloning reactions were then transformed into Stellar™ Competent Cells, and 1/10th of each reaction was plated. Colony counts and clone sequence verification were used to evaluate the results of each reaction. Sequencing data is the most precise measurement of cloning accuracy, and thus gives a much more informative analysis than simply comparing the total number of colonies. Results are shown below (Tables I and II).

Table I. Results for multiple-insert cloning. Three fragments (in addition to the linearized vector backbone of 2.7 kb) were used as inserts in each reaction: MBP (1.1 kb), PROF12 (0.7 kb), AcGFP1 (0.7 kb). Total finished plasmid size was 5.2 kb.

Multiple-Insert Cloning

	In-Fusion HD Cloning	Gibson's Method	
Conditions	Incubate at 50°C for 15 min	Incubate at 50°C for 15 min	Incubate at 50°C for 60 min
Vector + Inserts	89 colonies	111 colonies	392 colonies
Negative Control (no insert)	1 colony	39 colonies	78 colonies
Cloning Accuracy	100% (26/26 correct colonies)	19% (5/26 correct colonies)	73% (19/26 correct colonies)

Table II. Results for single-insert cloning. One fragment (MBP; 1.1 kb) was used as an insert in each reaction with a linearized vector backbone (2.7 kb). Total finished plasmid size was 3.8 kb.

Single-Insert Cloning

	In-Fusion HD Cloning	Gibson's Method
Conditions	Incubate at 50°C for 15 min	Incubate at 50°C for 15 min
Vector + Insert	635 colonies	401 colonies
Negative Control (no insert)	1 colony	39 colonies
Cloning Accuracy	100% (26/26 correct colonies)	96% (25/26 correct colonies)

Accuracy Counts—Get the Right Clone, Every Time

For single-insert reactions, In-Fusion technology showed the expected high level of cloning accuracy. Gibson's technology showed a comparable level of accuracy when using In-Fusion Cloning conditions. However, the number of colonies seen in the negative control for Gibson's method were far higher than with In-Fusion Cloning, and point to a much more precise, reliable cloning mechanism when using the In-Fusion enzyme.

The background observed when using In-Fusion technology was consistently lower than that observed when using Gibson's method, regardless of the number of inserts or reaction conditions. This difference was especially striking with multiple-insert cloning, where the total number of colonies are generally reduced, and false positives present a bigger problem. In-Fusion Cloning's high accuracy shines under the demands of multiple-fragment cloning—the negative control reaction gave an exceptionally low colony count, and the cloning accuracy reached 100%. By comparison, Gibson's cloning method was found lacking whether it was performed using In-Fusion Cloning's conditions, or Gibson's recommended conditions, which take four times as long!

In-Fusion Cloning Delivers where the Competition Falls Short

While both cloning methods provided good accuracy with single-fragment cloning, In-Fusion technology was the clear winner in terms of background, speed, and overall accuracy—especially when more complicated cloning projects were considered. In providing such a high level of cloning accuracy, In-Fusion technology reveals that the real measure of success is not in sheer numbers of colonies, but instead in the number of correct, error-free colonies. Researchers should be able to expect the right clone every time, and In-Fusion Cloning makes that possible.

We have also observed that In-Fusion technology has an additional advantage over Gibson's method with regard to maximum vector size (46 kb cosmid vs. 20.5 kb plasmid). This additional flexibility means one In-Fusion reaction can take the place of a packaging system, quickly and accurately assembling a large vector that would otherwise be too unwieldy for cloning.

In-Fusion HD Cloning Plus kits provide everything needed for a cloning experiment, including high-fidelity PCR polymerase, cloning master mix, competent cells, and reagents to treat PCR products prior to cloning (either Cloning Enhancer or a NucleoSpin Gel and PCR Clean-Up kit). Where the competing technology provides the bare minimum, In-Fusion Cloning kits fully equip the researcher for all steps, ensuring a high out-of-the-box success rate, and aligning the price per reaction with the true total cost of the experiment.

References

1. Chen, C. G., *et al.* (2014) *Nucleic Acids Research* **42**(4):e26
2. Lestini, R., *et al.* (2013) *Nucleic Acids Research* **41**(22):10358–10370
3. Gibson, D. G.,*et al.* (2009) *Nature Methods* **6**(5):343–345.

PRODUCTS

Cat. #	Product	Size
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
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

Prices are subject to change without notice. Please check www.clontech.com for current pricing.