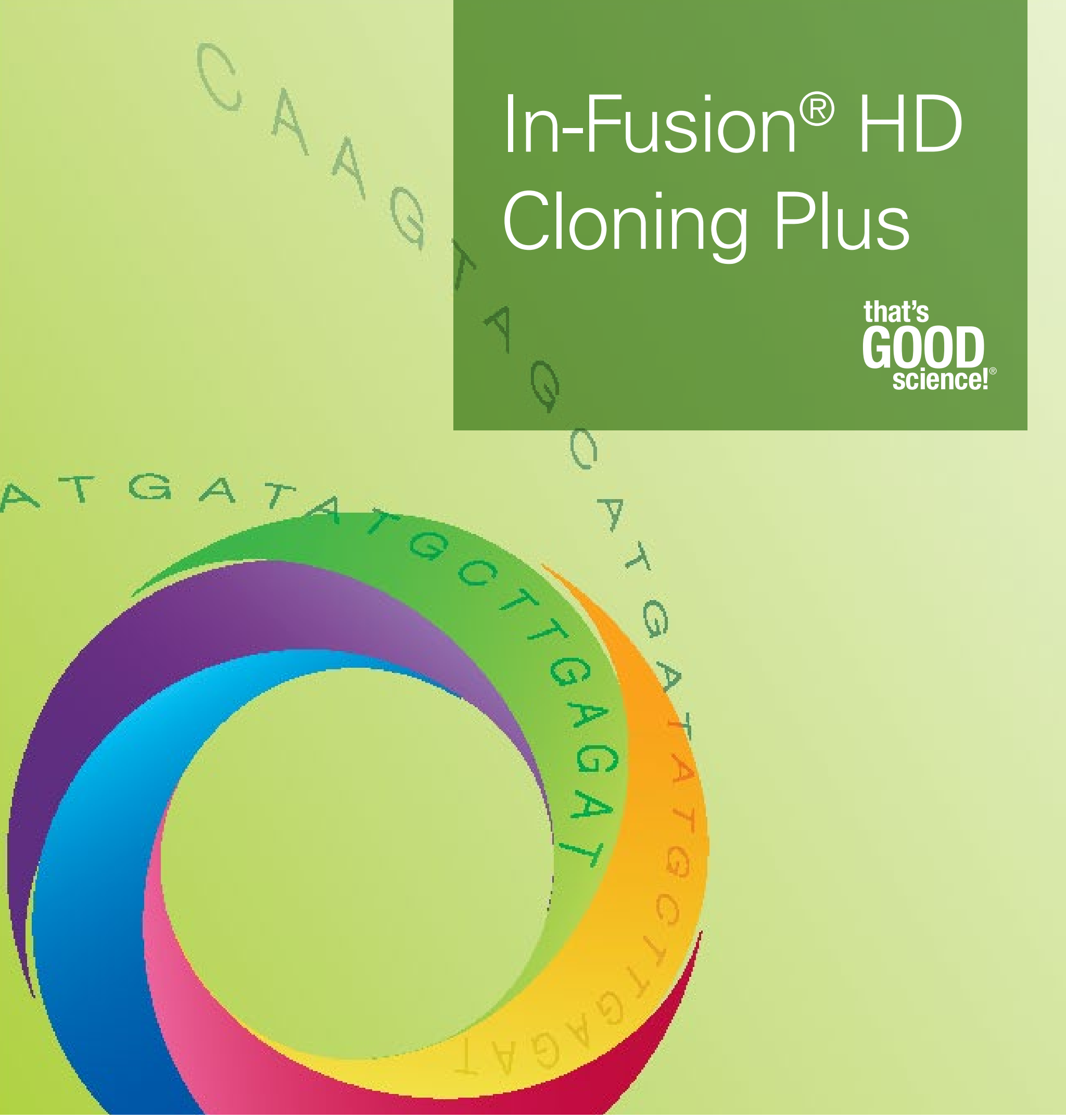
# In-Fusion® HD Cloning Plus

that's GOOD science!®

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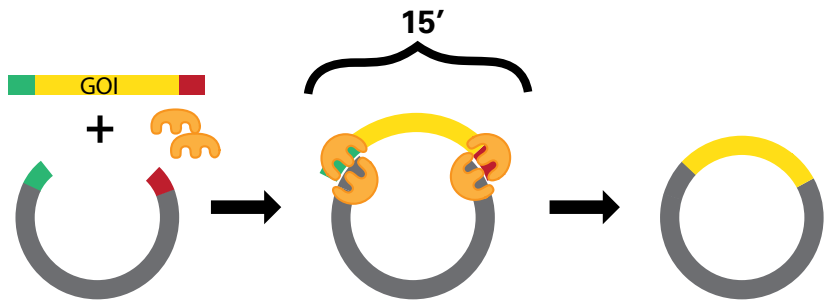
# The right clone every time

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.

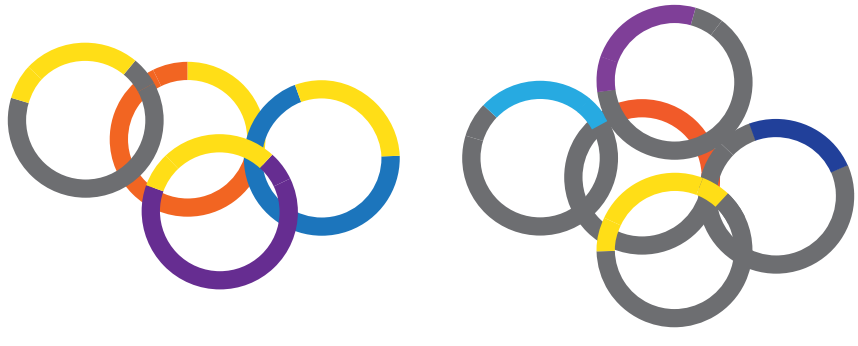



Seamless • Directional

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

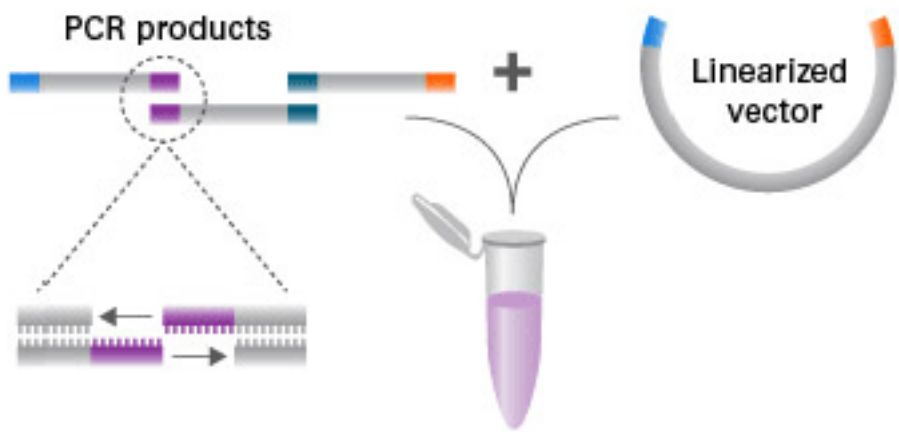
# In-Fusion cloning protocol

## 1 Amplify

each fragment/insert as a PCR product with 15-bp extensions (or 20-bp extensions for multiple fragment cloning), complementary to the linearized vector ends.

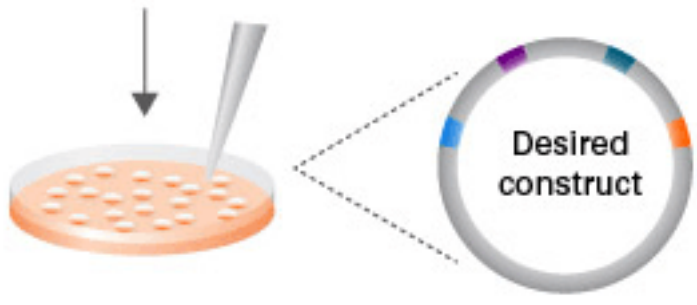

## 2 Combine

in a single tube, the In-Fusion enzyme mix, the linearized vector, and PCR insert(s), and incubate for 15 min (liquid enzyme) or 30 min (EcoDry enzyme) at 50°C.




## 3 Transform

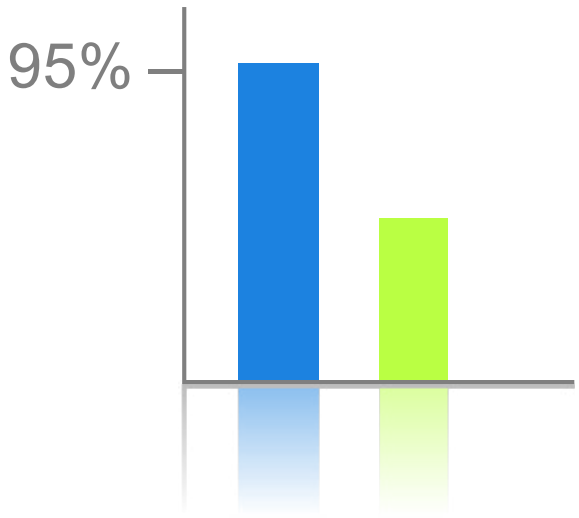
competent cells with the In-Fusion cloning reaction. Plate on selective media and screen for positive clones.



# Primary advantages

## Cloning success

- 10+ years of progressive research
- Clones ANY insert directly into ANY vector at ANY locus
- Seamless and directional construction



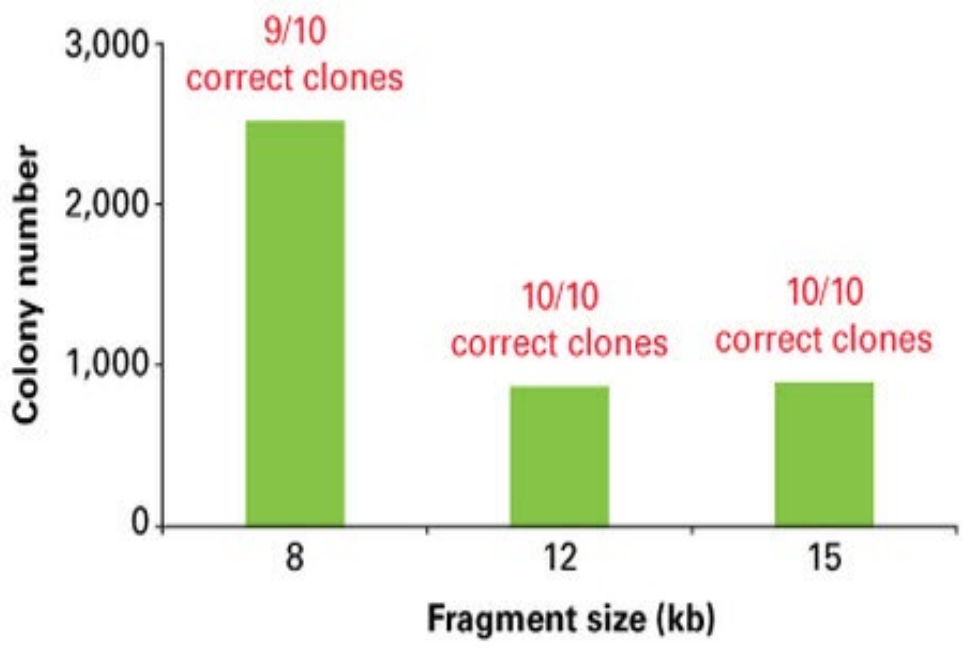

95% positive recombinants with In-Fusion Cloning

## Excellent performance

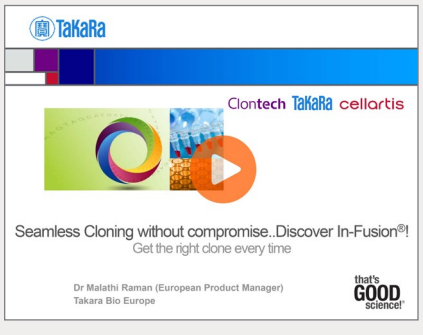
- 95% positive recombinant clones
- Fast (15-30 mins) and accurate

## Simple & powerful

- Single tube 15-30 minute reaction
- 20 bp oligos to 15 kb inserts, large cosmid vectors
- One system with multiple applications




Read the tech note:
Choosing a seamless cloning method




Webinar:
Seamless Cloning without compromise…Discover In-Fusion! »

Watch our technology webinar to learn how to build any construct without compromise using In-Fusion HD Cloning Plus.

# One cloning system for multiple applications

## Site-directed mutagenesis »

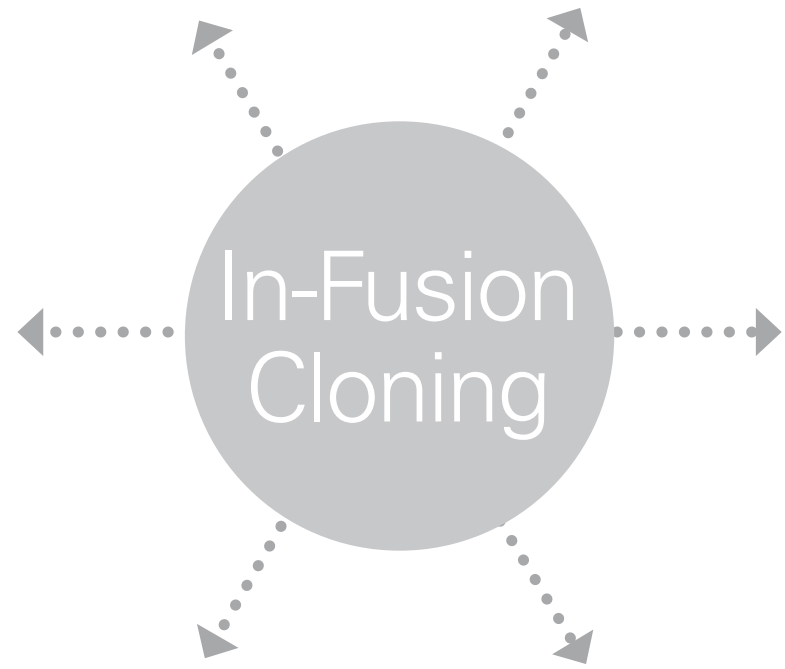
Create deletion constructs, base substitutions, or add small tags to your gene of interest in one cloning step.

## Multi-fragment cloning »

Directional, ligase-free, and one-step cloning of two or more inserts into a vector.

## Synthetic gene assembly »

Seamless cloning for synthetic gene assembly. No restriction enzymes needed.




## High-throughput cloning »

Efficient, rapid, and scalable cloning using In-Fusion Cloning in high-throughput formats.

## Cloning small tags »

Tag your protein with a streamlined, single-step reaction.

## Lentiviral cloning »

Overcome restriction-site limitations and difficult-to-clone lentiviral sequences with In-Fusion Cloning.

# Multi-fragment cloning

## 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.

## Highlighted citations

In-Fusion Cloning has been used to efficiently and accurately assemble constructs with more than one insert.

In-Fusion assembly: seamless engineering of multidomain fusion proteins, modular vectors, and mutations. Read now »

Substrate promiscuity: AglB, the archaeal oligosaccharyltransferase, can process a variety of lipid-linked glycans. Read now »

Multi-homologous recombination-based gene manipulation in the rice pathogen *Fusarium fujikuroi*. Read now »

Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. Read now »

Structure-function studies of an engineered scaffold protein derived from Stefin A. II: Development and applications of the SQT variant. Read now »

Regulating prospero mRNA stability determines when neural stem cells stop dividing. Read now »

Identification of the N-terminal domain of the influenza virus PA responsible for the suppression of host protein synthesis. Read now »

Root developmental programs shape the *Medicago truncatula* nodule meristem. Read now »

Towards a systems approach in the genetic analysis of archaea: accelerating mutant construction and phenotypic analysis in *Haloferax volcanii*. Read now »

Potential pitfalls and solutions for use of fluorescent fusion proteins to study the lysosome. Read now »

# Multi-fragment cloning

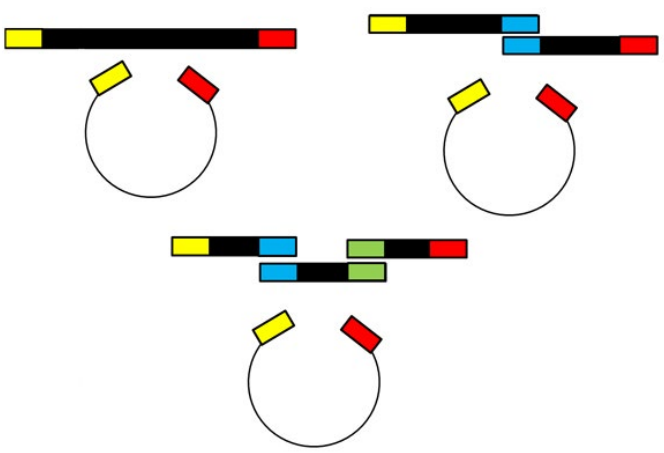
## Clone multiple fragments with ease!

## Customer success story

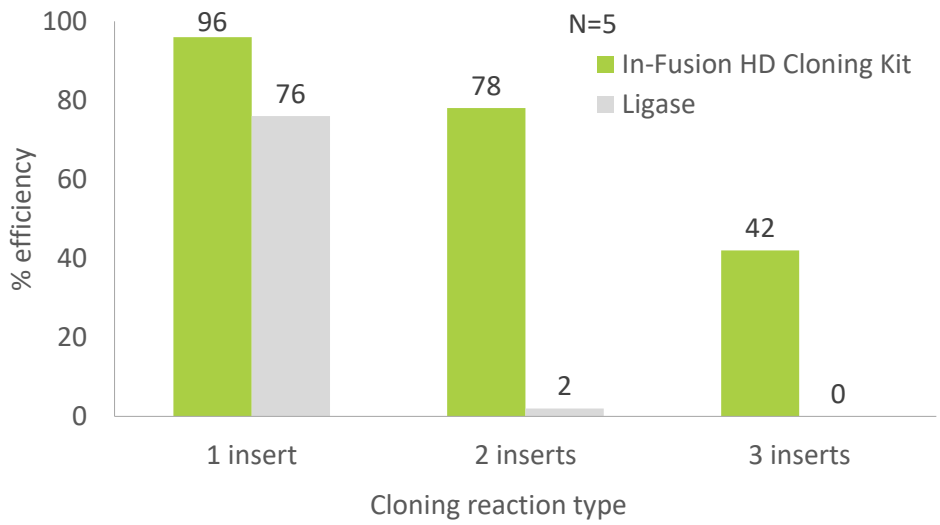
> *In conclusion, the In-Fusion HD Cloning Kit provided us with a higher cloning efficiency and faster results compared to traditional ligation based cloning for both single and multiple insert cloning.*
>
> —Dr. Samuel Bru
> Postdoc, Orphan Cyclins Group
> Universitat Internacional de Catalunya

Dr. Samuel Bru and his colleagues in Dr. Josep Clotet's research group routinely purify recombinant chimeric proteins from *S. cerevisiae*; the group was looking for a fast, easy way to successfully clone the chimeric proteins their work requires. In this study, two methods were tested side-by-side for cloning single and multiple inserts: In-Fusion Cloning and ligation-based cloning. Cloning experiments using each method were set up for one, two, or three inserts, respectively. Colonies were screened by restriction digest, and cloning efficiency was determined as the number of positive clones obtained from ten randomly chosen colonies, averaged across five independent experiments.

In-Fusion technology outperformed traditional ligation-based cloning methods in direct comparisons of both single- and multiple-insert cloning experiments.



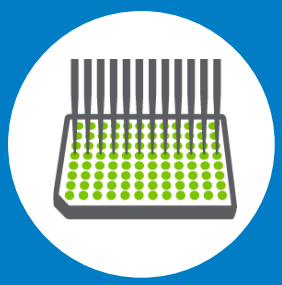
**Schematic representation of single- and multiple-fragment cloning reactions using In-Fusion Cloning and ligation-based cloning with T4 DNA ligase.**




**Quantitative comparison of cloning efficiencies obtained with In-Fusion Cloning and ligation-based cloning methods for single- and multiple-fragment cloning reactions.**

Read the full tech note

## In-Fusion applications collection



# High-throughput cloning

## Efficient, rapid, and scalable!

Efficient and rapid HTP cloning is essential for the development of therapeutic antibodies, as part of a lead identification and optimization program. The majority of current cloning methods are impossible to scale up for this type of workflow, as they suffer from lengthy protocols, low efficiency, and reading frame complications. Therefore, HTP cloning often becomes outsourced. However, seamless In-Fusion cloning technology can bring high-throughput cloning back into your own lab, with an efficient, highly accurate, one-step method that streamlines ambitious workflows.

- One-enzyme, 15-min reaction creates hundreds to thousands of clones in parallel
- Formats for automated liquid handling systems (e.g., 96-well plates or custom dispenses)
- Ligase-free mechanism reduces background and sequence errors at cloning junctions

## Highlighted citations

High-throughput cloning in antibody engineering workflows In-Fusion Cloning has been used for streamlined, high-throughput cloning in antibody engineering workflows.

Rapid high-throughput cloning and stable expression of antibodies in HEK293 cells. Read now »

Creation of antigen-dependent β-lactamase fusion protein tethered by circularly permuted antibody variable domains. Read now »

Efficient generation of monoclonal antibodies from single rhesus macaque antibody secreting cells. Read now »

# High-throughput cloning

## Efficient, rapid, and scalable!

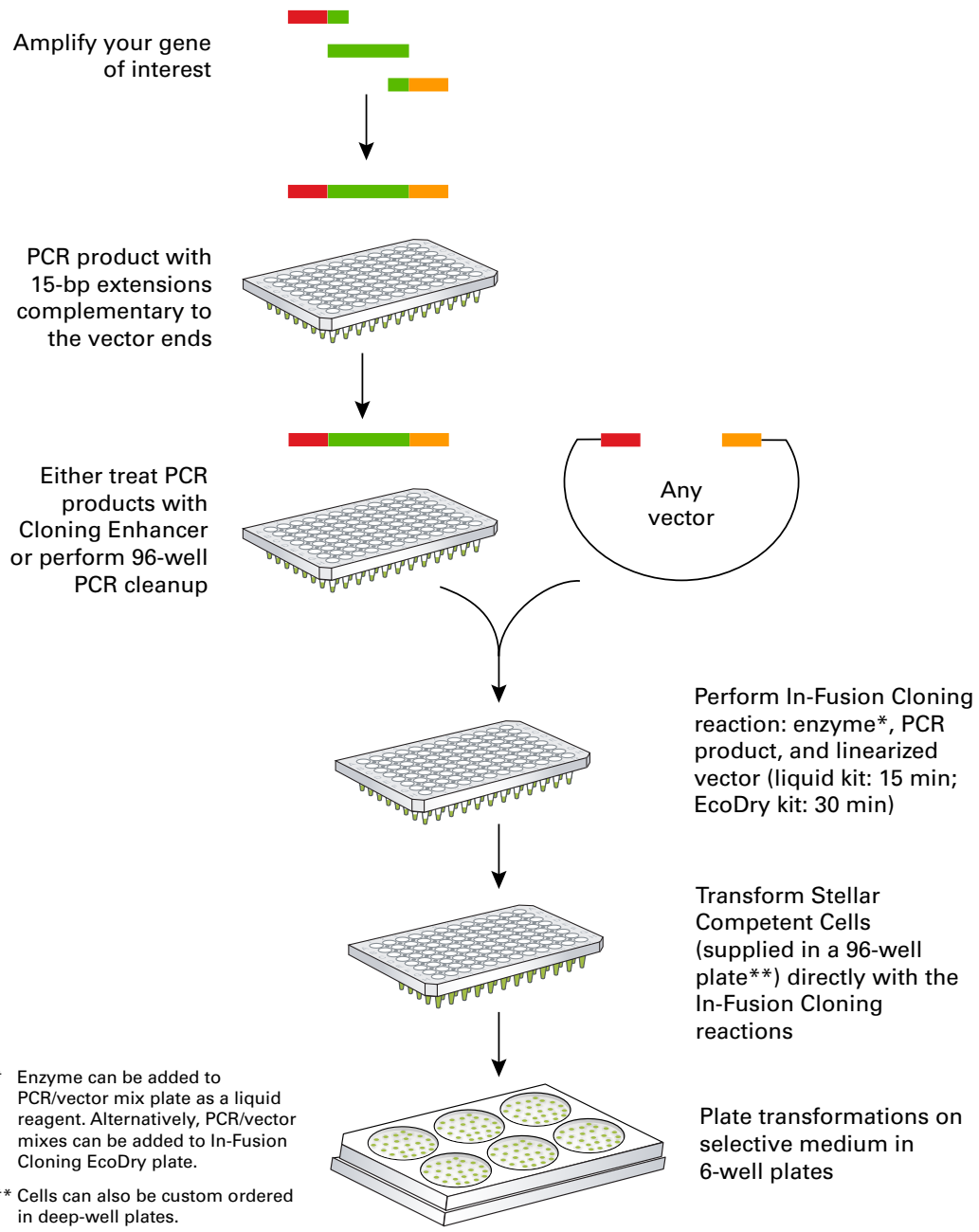
## Customer success story

Having previously evaluated other cloning methods, Jared L. Spidel *et al.* at Morphotek, Inc.chose In-Fusion Cloning for its simplicity and high percentage of positive clones, a clear advantage over traditional restriction digests and ligation.

With the high accuracy of In-Fusion Cloning at their disposal, they developed their own high-throughput process for the rapid generation of antibodies, further streamlining the workflow with an alternative outgrowth method (Spidel, J.L. *et al.* 2016).

> *The percentage of positive clones was consistently higher than we see when using traditional restriction digestion/ligation. That allowed us to streamline our process and increase our throughput by eliminating the plating step following transformation. Since most all plasmids contain the insert, we can grow the transformed bacteria as a pool and directly miniprep.*
>
> —Dr. Jared L. Spidel
> Morphotek, Inc.

Read the full tech note




**HTP In-Fusion Cloning Protocol**

In-Fusion applications collection

# Lentiviral cloning

## Overcoming restriction site limitations in lentiviral vectors

Working with lentiviral plasmids can often prove challenging. Limited restriction site availability is a major hurdle, and their size alone can be problematic for traditional cloning methods, with long terminal repeats making them prone to instability. What if you could get around all of that without the hassle of introducing new restriction sites or subcloning into an intermediary vector?

In-Fusion technology offers a sequence-independent cloning strategy which allows you to create your final lentiviral vector with just one streamlined cloning reaction.

## Highlighted citations

In-Fusion Cloning has been used for cloning inserts directly into large, complex lentiviral vectors.

Gene expression profiling reveals U1 snRNA regulates cancer gene expression. Read now »

Single cell resolution *in vivo* imaging of DNA damage following PARP inhibition. Read now »

*Lrig1* is a haploinsufficient tumor suppressor gene in malignant glioma. Read now »

Self-targeting of TNF-releasing cancer cells in preclinical models of primary and metastatic tumors. Read now »

# Lentiviral cloning

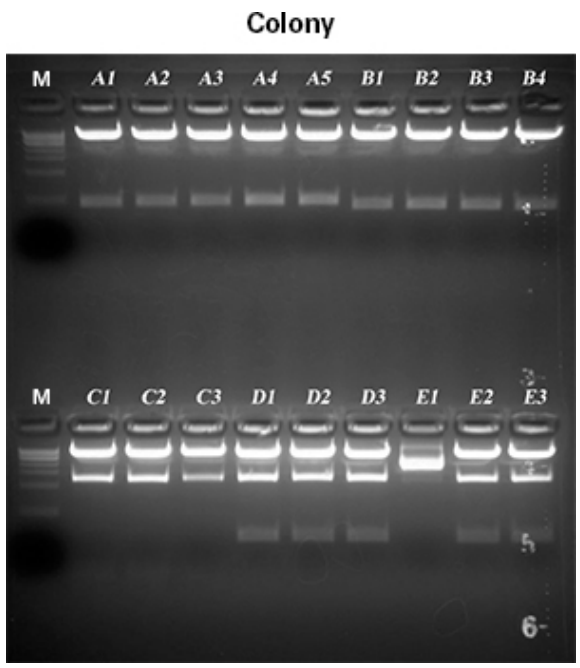
## Overcoming restriction site limitations in lentiviral vectors

## Customer success story

> *I have not tried ligation-based cloning for any of my current cloning experiments, but I have done a lot before we tested the In-Fusion kit. The major differences between these two cloning methods in our lab are the additional TA cloning, transformation, minipreps, digestion, and ligation needed for ligation-based cloning, which means at least three more working days. Typically we needed to screen at least nine colonies for each cloning experiment, with 70–80% positive confirmed by restriction digest. In my experience, the In-Fusion method is very convenient, reliable, and time-saving.*
>
> —Dr. Jun Yang
> Instructor
> University of Texas Medical Branch

In the following experiment, In-Fusion Cloning was used to clone five different PCR fragments into a lentiviral vector for eukaryotic expression. This vector had only one restriction site suitable for subcloning, which made it difficult and time-consuming to perform PCR cloning using traditional ligation methods.

In-Fusion Cloning generated five positive lentiviral clones with a success rate of nearly 100%. PCR conditions did not need to be modified for each insert, and direct cloning into a large vector was highly efficient. Final vectors were complete in just three days, with only two to three hours of hands-on time required per day.




**Restriction digest of clones.**
Screening of transformant colonies was performed via XbaI/EcoRI restriction digest. Results show only one negative colony (E1) out of all 18 colonies resulting from five individual cloning reactions.

Read the full tech note

# Synthetic gene assembly

## Seamless sequence independent cloning for synthetic gene assembly

The synthesis of long (above 2.5 kb in size) and complex (GC or AT-rich) genes can often be difficult due to the formation of inhibitory secondary structures. However, In-Fusion cloning technology can help researchers get around these secondary structure issues. The first step involves splitting the long gene into a series of smaller overlapping synthetic inserts, split in such a way as to avoid the formation of these secondary structures, but containing the overlaps required for In-Fusion cloning . The second step then simply involves performing a multiple insert cloning reaction with In-Fusion to seamlessly assemble the synthetic inserts into the desired vector.

## Highlighted citations

In-Fusion Cloning has been used to efficiently assemble and clone synthetic DNA fragments, such as gBlocks.

Initiation of translation in bacteria by a structured eukaryotic IRES RNA. Read now »

Targeted mutagenesis of guinea pig *Cytomegalovirus* using CRISPR/Cas9-mediated gene editing. Read now »

Engineering fatty acid synthases for directed polyketide production. Read now »

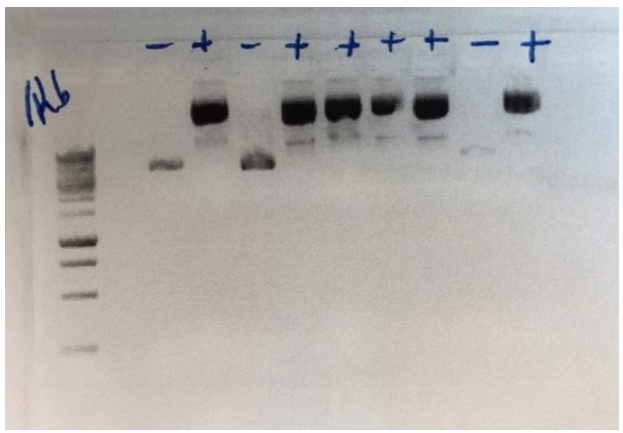
# Synthetic gene assembly

## Seamless sequence independent cloning for synthetic gene assembly

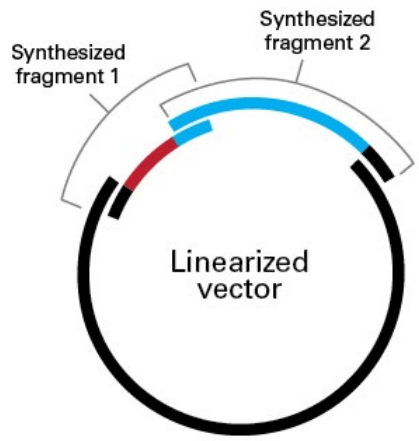
## Customer success story

> *I hadn't tried any other cloning methods for this experiment, but directly tested In-Fusion [Cloning]. Restriction site availability was definitely an issue with this experiment and I was happy not to depend on it...Speed and accuracy as well as easiness of this application have convinced me to purchase this product again.*
>
> —Dr. Christian Joerg Braun
> Postdoc, MIT

In this study, In-Fusion Cloning was used to quickly clone multiple, overlapping synthetic fragments of a transcriptional activation domain into a preexisting Cas9-dead viral expression vector. The secondary structure of the activation domain prevented synthesis of the full sequence, and building the domain from separate pieces with traditional ligation-based methods was difficult due to limited restriction-site availability. Instead, In-Fusion technology was used to insert two synthesized portions of the domain in a single cloning reaction without any need to worry about compatible restriction sites. The full-length sequence was seamlessly cloned directly into the expression vector, and positive clones were identified by restriction digest and Sanger sequencing. The final vector was complete in three days, with hands-on time totaling just over two hours.



**Restriction digest of clones.** Screening of transformant colonies was performed via EcoRI restriction digest. Results show six positive colonies out of the nine colonies tested. The three negative colonies are religated vector backbone.




**Approximation of cloning reaction setup with multiple inserts.** Each synthesized fragment has 15-bp overlapping sequences required for In Fusion Cloning. The overlaps with the vector are represented by the black segments. The blue segment on Synthesized fragment 1 represents the overlap with Synthesized fragment 2. The red and blue parts of both fragments together make up the full-length transcriptional activation domain.

Read the full tech note

# Site-directed mutagenesis

## A single system for deletions, base substitutions, or additions

In-Fusion Cloning makes it easy to perform site-directed mutagenesis by combining the power of the In-Fusion HD enzyme with inverse PCR. To perform mutagenesis with In-Fusion, design your PCR primers so that they have a 15-bp overlap with each other at their 5' ends and incorporate the mutation of interest (Figure 1 below). The In-Fusion Cloning reaction is then performed with the linear inverse PCR product, to re-circularize the linear DNA at the 15-bp overlaps, that also contain the mutagenic changes.

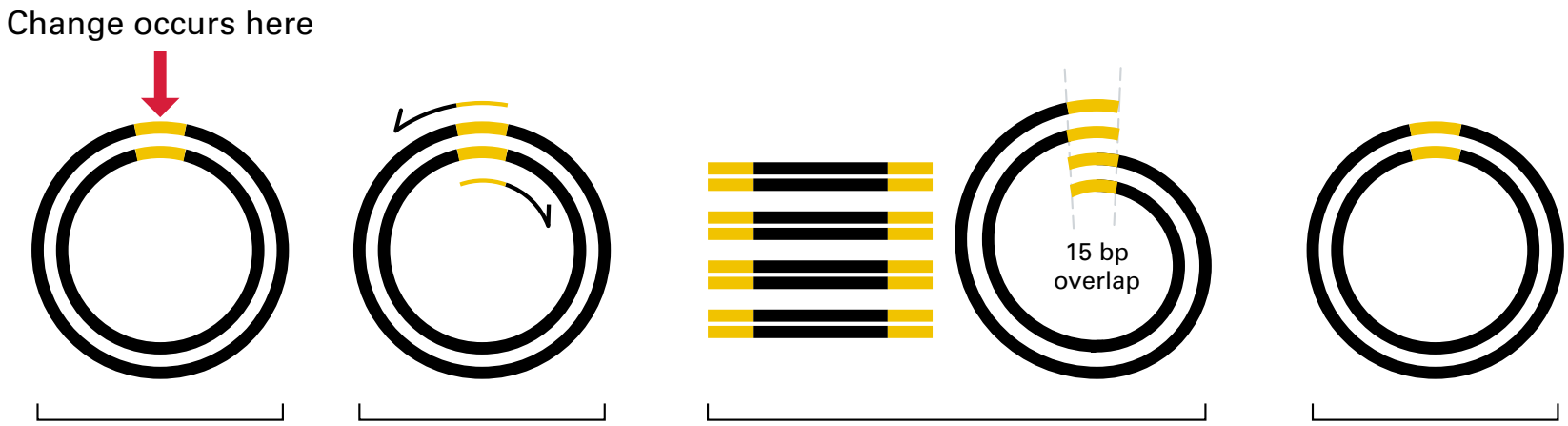



**Figure 1.** Procedure for performing mutagenesis with In-Fusion technology. The area where mutagenesis occurs is shown in yellow. After designing your experiment, perform the protocol (Step 3 above) on Day 1, and recover your final construct (Step 4 above) on Day 2.

Read the full tech note


Video: Learn how In-Fusion Cloning makes mutagenesis easy »

See how In-Fusion technology can be used for your mutagenesis experiments.

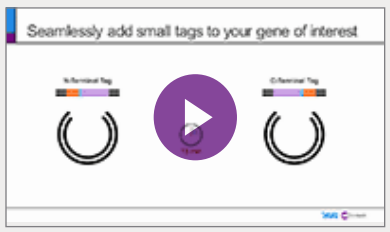
In-Fusion applications collection

# Cloning small tags

## Streamlined, single-step reaction

Traditionally, the engineering of tagged recombinant proteins has been performed by restriction-dependent cloning. This method requires a donor template to amplify the selected tag sequence and several cloning steps. The advent of restriction-independent, seamless In-Fusion Cloning has greatly simplified this process, allowing the engineering of recombinant proteins with small tags (<10 aa) in a single step and without the need for a tag template (de novo).

- Eliminating reliance on available restriction sites
- Removing the need for a tag template
- Allowing the incorporation of multiple inserts and tags with a single reaction



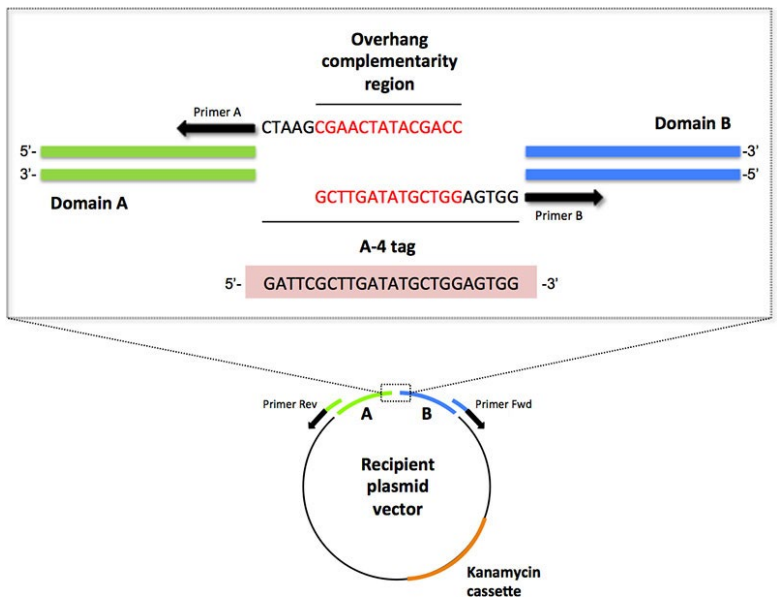

Video: Easily add tags to your plasmids with In-Fusion Cloning »

## Customer success story

> *In-Fusion cloning really helped organize the time-frame of my research. I had a nearly 100% success rate by designing primers and performing the cloning according to the recommended specifications.*
>
> —Fabio Antenucci, Post-doctoral research fellow
> Veterinary Clinical Microbiology, University of Copenhagen

In the experiment described here, In-Fusion HD Cloning Plus was used for the construction of a recombinant chimera harboring domains from two different template proteins (A and B) and the de novo inserted fusion tag A-4 (Zhou *et al.* 2008; Figure 1).

Generating tagged fusion proteins can be time-intensive and require multiple subcloning steps for each vector to generate targeted inserts in turn. With a relative efficiency of 100% (3/3 true positive clones), the In-Fusion Cloning protocol demonstrated a fast and reliable method for the de novo inclusion of small fusion tags using a single-step cloning reaction.

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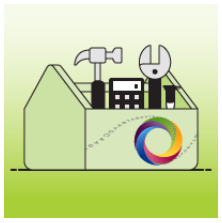
In-Fusion applications collection

# In-Fusion Cloning resources

## In-Fusion Cloning online tools



### Primer design tool »

Design your In-Fusion primers with our step-by-step design tool, or access the molar ratio calculator and construct simulator.

### Construct simulation software »

SnapGene software allows easy planning and visualization of molecular biology procedures, including In-Fusion Cloning.

### Molar ratio calculator

Calculate the optimal amounts of vector and insert for an In-Fusion Cloning reaction. Just enter the sizes of both vector and insert(s), in base pairs.

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## Seamless cloning tips and tricks

### FAQs »

Learn more about In-Fusion Cloning, including applications, tips, primer design, and vector and insert requirements.

### Tips »

Explore new and interesting tips and solutions for seamless PCR cloning with In-Fusion technology.

### Seamless cloning primer design »

Useful tips to keep in mind when designing your seamless cloning projects.

### In-Fusion Cloning tech notes »

View application data on using In-Fusion for all of your cloning needs.

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## Cloning kit formats

### In-Fusion Cloning selection guide »

Select the right In-Fusion Cloning kit for fast, efficient, and accurate ligation-free cloning for your application.

### High-throughput In-Fusion Cloning kits »

High-throughput formats for projects that require a large number of clones, e.g., expression screening studies & library construction.


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