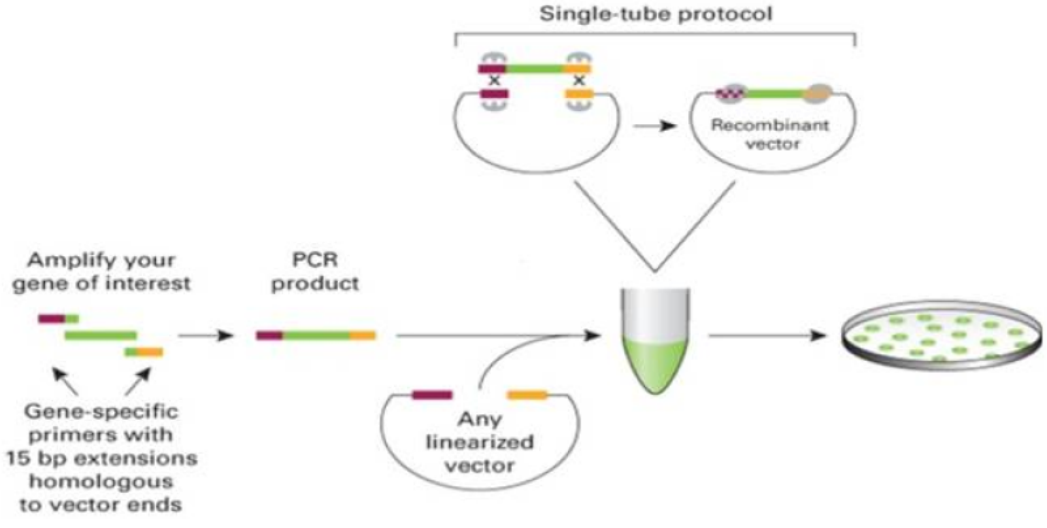
Product Datasheets

Product Name:	FuseIn™ Easy Cloning System
Product Code:	5700000001
Document Version:	1040522
Accessories:	FuseIn™ Easy Cloning 10X Buffer (P/C: 5700000002)
Storage Temp.:	-20℃
Description:	Engineering DNA molecules by homologous recombination presents an alternative to traditional methods using restriction endonucleases and ligases. The FuseIn™ Easy Cloning System is a highly efficient, vector-independent system for the seamless assembly of DNA fragments that share terminal end-homology and allows the seamless assembly of DNA inserts up to 12 kb and virtually any linearized E. coli vector. This System relies on homologous recombination to assemble adjacent DNA inserts sharing end-terminal homology. The DNA fragments must share an end-terminal homology of 8-30 bp. Homology of 15 bp overlap shows the best performance.



This System eliminates restriction enzyme digestion, dephosphorylation, blunting sticky ends, nick ligation, terminal adenylation and intermediate vectors from PCR product cloning. This System takes only 20-30 minutes to fuse DNA fragments to one DNA molecule.

This System is recommended for the following application, but not limited to:

1. Cloning PCR products

2. Transferring gene from one vector to another
3. *In vitro* joining of DNA fragments
4. Site-Directed Mutagenesis
5. Gene Engineering
6. ….

Procedures Outline:

- Vector linearization by restriction enzyme digestion or PCR
  - ✔ It is very important to have a complete digest (i.e., very low background of uncut vector). Therefore, an increased enzyme digestion time (2–3 hours to overnight) and reaction volume is recommend.
- Preparation of DNA insert fragment by PCR or chemical synthesis
  - ✔ If DNA fragments are prepared by PCR, purify the DNA fragments through a PCR cleanup kit. After preparing your DNA fragments by PCR, verify the PCR products by gel electrophoresis. If multiple bands are obtained, gel purify your DNA insert fragment. Be sure to elute the DNA fragments from column using water.
  - ✔ When gel purifying DNA fragments, employ extra caution to minimize any potential damage to the ends of the DNA fragments by leaving the gel on the gel tray when exposing it to UV light, using low UV power, and minimizing the time the gel is exposed to UV light. An additional isopropanol precipitation after gel solubilization might be required to obtain the best results.
- *In vitro* FuseIn™ cloning and assembly reaction
- Competent cell transformation
- Analysis of positive colonies by restriction analysis and/or sequencing

FuseIn™ Reaction: Reaction Mixture Setup

Component	Amount(ng)	Volume(uL)
Insert DNA Fragment	100~200*	X
Linearized Vector	100~200*	Y
FuseIn™ Easy Cloning 10X Buffer		2
FuseIn™ Easy Cloning System†		1
Deionized, Nuclease Free Water		20-(X+Y+3)
Total		20

*For maximum cloning efficiency, use a 1:1~5 molar ratio of vector:insert. If vector and insert share a similar size, 1:1 molar ratio is recommended.

†Be sure to add the FuseIn™ Easy Cloning System at the end after you have mixed all the other reaction components. It is crucial to add the FuseIn™ Easy Cloning System as the last component.

- Mix the reaction components by gently tapping the sides of tubes and incubate it at room temperature(best performance at 25℃, this system will not work at

temperature higher than 30℃) for 20-40 minutes. For larger inserts, incubation time could be slightly extended, but one hour is the maximum.

- Proceed to the transformation step immediately after incubation or store the reaction mixture at -20℃ for later transformation.

Trouble Shooting:

Symptom	Cause	Solution
No colonies after transformation	Low transformation efficiency	Check transformation efficiency. Competent cells with transformation efficiency > 10^8 cfu/ug are recommended.
	Too much FuseIn™ reaction mixture inhibits transformation.	Do not add more than 10uL of FuseIn™ reaction mixture into 100uL of competent cells.
	Insufficient amount of transformed cells plated	Increase the amount of transformed cells plated.
	Inhibitors in FuseIn™ reaction mixture	Purify PCR products and linearized vectors before FuseIn™ reaction.
	Incorrect ratio of DNA insert fragment to vector	Be sure to use the correct amounts of DNA fragment and vector for FuseIn™ reaction.
	DNA fragments do not share the required end-terminal homology	Ensure that DNA insert fragments and the linearized- cloning vector share the required 8-30 bp end-terminal homology.
	Ends of the DNA fragments generated by PCR were damaged.	Employ extra caution to minimize any potential damage to the ends of your DNA fragments by leaving the gel on the gel tray when exposing to UV light, using low UV power, and minimizing the time the gel is exposed to UV light. Note that an additional isopropanol precipitation after gel purification might be required to obtain the best results.
Large number of the transformants contain no insert	Cloning vector incompletely linearized	Remove uncut vector prior to FuseIn™ reaction by gel purification.
	Contamination by plasmids with the same selection antibiotic	Remove template plasmids by gel purifying PCR products.
Large number of the transformants contain incorrect insert	PCR products not pure enough	If your PCR product is not a single distinct band, then it is necessary to gel purify the PCR product to ensure cloning of the correct insert.