TaKaRa LABIOTECH.eu

THE LANDSCAPE OF NEXT GENERATION SEAMLESS CLONING TECHNOLOGIES

Gene cloning or DNA cloning can be defined as the production of exact copies of a particular gene or DNA sequence using genetic engineering techniques.

Unlike traditional ligation-based cloning, seamless cloning methods enable researchers to perform sequence-independent cloning, without retaining undesired nucleotides or scar sequences between vector and insert.

These scarless methods are powerful tools for various molecular biology needs, including cloning multiple inserts in a specific order, creating fusion proteins, and preserving reading frames.

MILESTONES IN GENE CLONING HISTORY

1953
Molecular structure of DNA
Francis Crick, Rosalind Franklin & James Watson

1972
First recombinant DNA molecules created through gene-splicing
Paul Berg

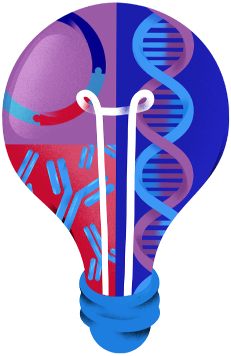
1975
First DNA sequencing method, the "Sanger method"
Frederick Sanger

1985
Invention of the Polymerase Chain Reaction (PCR)
Kary B.Mullis

1995
First complete genome sequenced of a free-living organism
J. Craig Venter & Robert D. Fleischmann et al.

2007
In-Fusion® Cloning technology developed
Michael D. Hamilton et al

ALL ABOUT SEAMLESS CLONING



Application Areas:

- High-throughput (HTP) antibody fragment cloning
- CRISPR/Cas9 cloning
- Synthetic gene assembly

Advantages of Seamless Cloning Technologies:

- Sequence independent cloning of any gene insert

2009 **Gibson Assembly® developed**
Daniel Gibson

2014 **Type IIS RE Cloning method developed**
Eszter Tóth et al.

into any vector at any locus

- Scarless cloning of gene inserts
- Facilitation of assembly & cloning of multiple gene inserts in a single step

SEAMLESS CLONING METHODS

GIBSON ASSEMBLY

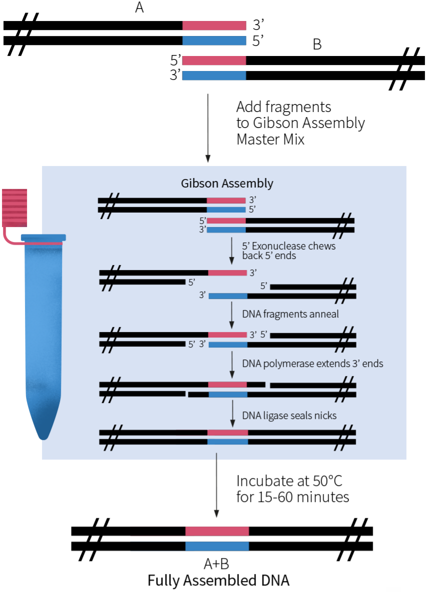
Key Characteristics

- Seamless sequence-independent cloning method
- Features a three-enzyme mix
- Requires 25 bp homology between ends of insert & linearized vector

Challenges

- Potential sequence errors within cloning junctions
- Possibility of high level of background colonies due to *in vitro* ligation
- Possibility of lower cloning efficiency at HTP

How Does it Work?


A
3'
5'
B
5'
3'
Add fragments to Gibson Assembly Master Mix
Gibson Assembly
3'
5'
5'
3'
5' Exonuclease chews back 5' ends
3'
5'
5'
3'
DNA fragments anneal
5' 3'
3' 5'
DNA polymerase extends 3' ends
DNA ligase seals nicks
Incubate at 50°C for 15-60 minutes
A+B
Fully Assembled DNA

Source: Adapted from New England BioLabs Inc.

TYPE IIS RE CLONING FOR EXAMPLE GOLDEN GATE ASSEMBLY

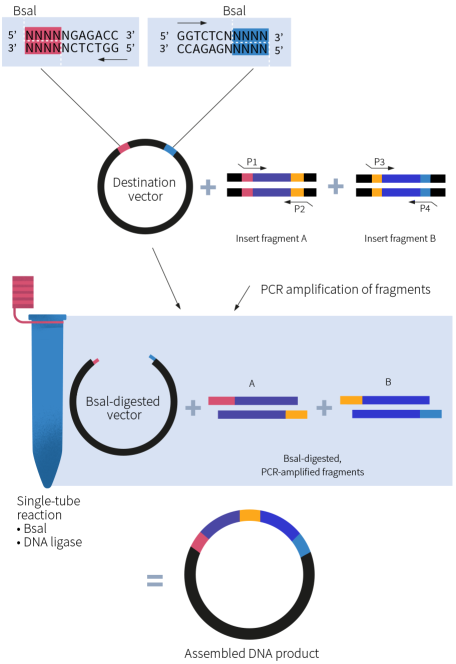
Key Characteristics

- Type IIS restriction endonuleases can cleave DNA outside of their recognition sequence
- Features Type IIS RE & T4 DNA Ligase cloning mix
- Similar to traditional digestion/ligation based cloning

Challenges

- Not completely sequence-independent
- Requires specific design of inserts & vectors for enzymatic removal of recognition site
- Lower cloning efficiency at HTP
- Difficult to scale up reactions

How Does it Work?


BsaI
5' NNNNNGAGACC 3'
3' NNNNNCTCTGG 5'
BsaI
5' GGTCTCNNNNN 3'
3' CCAGAGNNNNN 5'
Destination vector
P1
P2
P3
P4
Insert fragment A
Insert fragment B
PCR amplification of fragments
BsaI-digested vector
A
B
BsaI-digested, PCR-amplified fragments
Single-tube reaction
• BsaI
• DNA ligase
=
Assembled DNA product

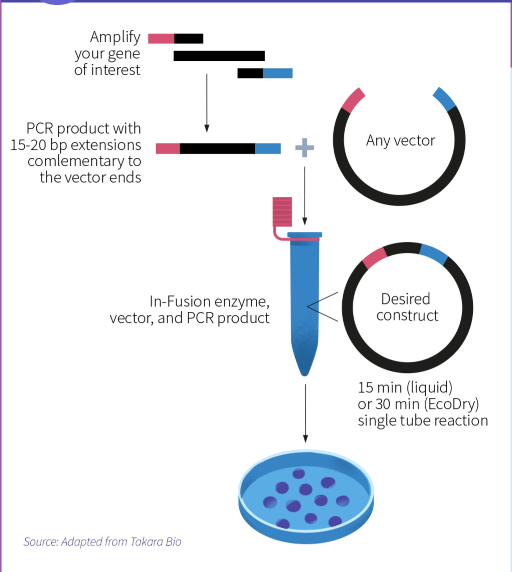
Source: Adapted from New England BioLabs Inc.

WHAT IS THE SEAMLESS CLONING TECHNIQUE WITH THE HIGHEST SEQUENCE ACCURACY?

Meet In-Fusion

In-Fusion HD Cloning Plus products enable directional, seamless cloning of any PCR fragment—or multiple fragments—into any linearized vector in a single 15-30 minute reaction.

How Does it Work?


Amplify your gene of interest
PCR product with 15-20 bp extensions comlementary to the vector ends
+
Any vector
In-Fusion enzyme, vector, and PCR product
Desired construct
15 min (liquid) or 30 min (EcoDry) single tube reaction

Source: Adapted from Takara Bio

1. Requires 15-20 bp homology between insert & linearized vector.
2. 3'exonuclease chew-back activity creates compatible ends, allowing *in vitro* annealing.
3. Gap repair & cohesive bond formation occurs *in vivo* in the bacterial cell.

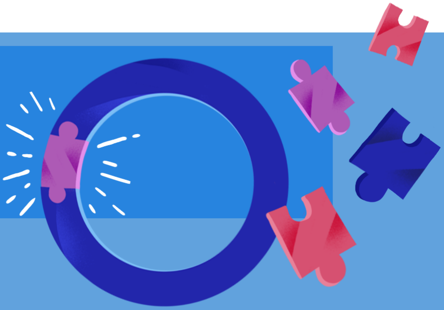
Benefits for Researchers

- ✓ **90-100%** sequence accuracy
- ✓ Cohesive bonds protect against sequence errors & nucleotide mismatches
- ✓ Over **95%** cloning efficiency
- ✓ Sequence independence
- ✓ High sequence accuracy, ideal for HTP cloning workflows
- ✓ Seamless construction
- ✓ Almost no background colonies
- ✓ Flexibility to clone single or multiple fragments

Interested in In-Fusion?

Takara Bio has a brand new In-Fusion Cloning Primer Design Tool that allows for single- or multiple-insert cloning, accommodates vector linearization by inverse PCR or restriction digest, and enables site-directed mutagenesis.

Find out more by visiting Takara Bio at www.takarabio.com/In-Fusion!



Sources
Takara Bio Inc.
Sciencehistory.org
Nobelprize.org
Whatisbiotechnology.org
New England Biolabs Inc.

Want to work with us?
Reach out to contact@labiotech.eu

Text: Larissa Warneck
Design and images: Elena Resko

Created with love at labiotech.eu