

Successful Cloning Step By Step

1. PREPARATION OF THE INSERT BY PCR

- Absolute proof reader AND good yield: **Takara Primestar** or Takara Primestar with GC buffer
- Good Proof Reader for long and complex templates: **Takara LA Taq**
- **Choose your own PCR enzyme**
http://bio.takara.co.jp/bio_en/catalog_d.asp?C_ID=C1334

Request a Sample!

TAKARA PRIMESTAR

PrimeSTAR® HS DNA Polymerase, is a novel new high fidelity PCR enzyme which provides maximum fidelity as well as extended product length (8.5 kb for human genomic DNA; 22 kb for λ DNA). Targeted for demanding cloning (i.e. amplification of cDNA libraries) and sequencing applications, it offers extremely high accuracy, and is the only enzyme whose fidelity is calculated by direct sequence analysis. Sequence analysis provides a more realistic estimate of the actual frequency of errors than other commonly-used indirect phenotypic analysis (i.e. *lacI*.) used by other suppliers, which are prone to error due to lethal and silent mutations. It also offers excellent amplification efficiency and shortened reaction times. Finally, the antibody-mediated hot start formulation prevents false initiation events during reaction assembly due to mispriming or primer digestion, lowering background.

Read More: http://www.takaramirusbio.com/cgi/showGroup.cgi?tableName=PcrGroup&op=showGroup&Group_ID=GR010A

PrimeSTAR® HS with GC Buffer was developed for high-fidelity amplification of high GC ($\geq 75\%$) templates. The new GC buffer formulation facilitates robust extension through even very high GC regions efficiently and accurately. Inclusion of a monoclonal antibody suppresses both the DNA polymerase and 3'-5' exonuclease activities prior to the first denaturing step, preventing false initiation events during reaction assembly and primer digestion. PrimeSTAR® HS with GC buffer provides reliable amplification, high accuracy and high specificity in applications where amplification of high-GC DNA templates for cloning or library construction is required.

Read more: http://www.takaramirusbio.com/cgi/showGroup.cgi?tableName=PcrGroup&op=showGroup&Group_ID=GR044A

Features

- **High Accuracy:** A strong exonuclease activity and optimized GC Buffer result in an extremely low error rate of only 25 errors per 304,000 bp (high-GC DNA, determined by sequence analysis).
- **High Efficiency:** High efficiency amplification, even on GC-rich templates.
- **Longer Products:** Amplify high-GC human genomic DNA targets of up to 5 kb.
- **Fast Reaction Times:** Increased priming efficiency results in rapid annealing times and improved specificity.
- **High Specificity:** Antibody-mediated hot-start prevents false initiation events during reaction assembly due to mispriming or primer digestion.

TAKARA LA Taq

Takara *LA Taq*™ is a mixture of *Taq* Polymerase with a proofreading polymerase optimized for amplification of long DNA templates. Using *LA Taq*™, routine extension to 20 kb and up to 48 kb is possible on some templates, with less optimization than other long PCR systems. Because of the presence of the proofreading polymerase, the fidelity is significantly better (6.5X) than that of *Taq* Polymerase alone.

Robust amplification of large DNA templates (up to 48 kb possible) with minimal optimization
Longer and more accurate amplification of genomic PCR products

Request a Sample!

2. CLEANING OF INSERT AND VECTOR

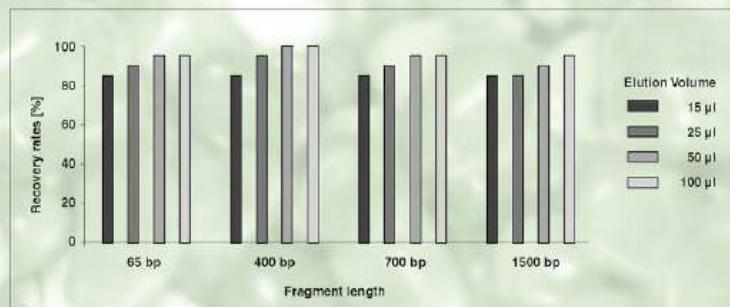
- **Nucleospin Extract from Macherey-Nagel:** 1 kit for PCR clean-up AND Gel Extraction AND MiniElute

Request a sample!!!

- ✓ 2 applications in one kit
- ✓ One buffer for PCR clean-up and Gel Extraction
- ✓ High recovery even for small fragments
- ✓ Reduced elution volume (15 µl)
- ✓ Effective removal of primers
- ✓ DNA is suitable for all common down stream applications
- ✓ Fast procedure

SUPERIOR

Purify fragments down to 65 bp with high recovery rates (90%)



PCR Fragments of different sizes were purified using **NucleoSpin® Extract II** and analyzed on a 1% TAE gel. After elution with different volumes the recovery was quantified by software ONE-Dscan from Scanalytics.

NucleoSpin® Extract II allows recovery rates of > 90% even for very small fragments.

QUANTITATIVE

Obtain high recovery with low elution volume



A PCR fragment with a size of 782 bp was purified from a 1% Agarose gel according to the standard protocol of **NucleoSpin® Extract II** using different elution volumes as shown. All eluates were adjusted to 25 µl plus 4.5 µl loading dye. For analysis the mixture was loaded onto a 1% TAE gel. Recovery rates were estimated against reference samples (lane 1-4).

Even with an elution volume down to 15 µl recovery rates of up to 75-100% can be achieved.

SPECIFIC

Complete removal of primers



PCR fragments of 645, 252 and 65 bp were spiked with additional primers. Elution was performed using 50 µl buffer NE. The same percentage of sample volume prior (lane 1) and after (lane 2 and 3) purification was analyzed on a 2% TAE-gel (M: 100 bp DNA size marker).

NucleoSpin® Extract II allows complete removal of primers even for PCR fragments down to 65 bp.

Remark: ssDNA smaller than 100 b will be removed completely.

Read more: <https://www.mn-net.com/web/MN-WEB-Biokatalog.nsf/Web/FramesE?Open>

3. RESTRICTION DIGESTION

- **Takara Restriction Enzymes** are native (thus more efficient) and come with universal and specific buffer, loading buffer and some are nearly half the price of competitor's enzymes.

Read more:

http://www.takaramirusbio.com/cgi/showGroup.cgi?tableName=MobioGroup&op=showGroup&Group_ID=G1112A

WE HAVE COMPARED TAKARA PRICES TO 3 OTHER WELL KNOWN BRANDS

*All Takara Restriction Enzymes are supplied with 10x reaction buffer and 10x loading buffer

ENZYME	Units	Takara +GST	Brand 1F +GST	Brand 2P +GST	Brand 3N +GST
Apal*	10 000	\$58.00	\$206.00	\$186.00	\$248.00
BamHI*	10 000	\$44.00	\$68.00	\$296.00	\$104.00
BglI*	2000	\$35.00	\$82.00	\$69.00	\$52.00
DraI*	4000	\$73.00	\$112.00	\$186.00	\$228.00
EcoRI*	10 000	\$20.00	\$60.00	\$96.00	\$104.00
HaeIII*	4000	\$35.00	\$173.00	\$148.80	\$138.60
HindIII*	10 000	\$35.00	\$60.00	\$106.00	\$104.00
HinfI*	3000	\$31.00	\$78.50	\$219.00	\$62.40
PstI*	10 000	\$41.00	\$90.66	\$190.00	\$114.00
SacI*	2000	\$47.00	\$90.00	\$126.00	\$104.00
SacII*	1000	\$35.00	\$36.00	\$146.00	\$52.00
Sall*	3000	\$35.00		\$241.50	\$156.00
Scal*	1500	\$41.00	\$96.00	\$133.50	\$171.00
SmaI*	2000	\$35.00	\$90.00	\$198.00	\$104.00
XbaI*	3000	\$47.00	\$68.00	\$127.50	\$124.00
XhoI*	5000	\$47.00	\$85.00	\$121.66	\$124.00

Note: prices were obtained directly from other suppliers' websites and adjusted to match Takara packaging size

4. LIGATION

Request a Sample!

- **Takara Ligation Kit Mighty Mix**

Takara's DNA Ligation Kit, Mighty Mix, is a one-solution premix ligation reagent that offers high efficiency ligations, particularly for blunt-ended ligation and TA cloning. The basic reaction can be performed quickly for either 5 minutes at 25°C, or 30 minutes at 16°C with equal results for both circular and linear DNA ligations. The 2X Ligation Mix allows small ligation reaction volumes (10 µL) when DNA is limiting, and sufficient reagent for 75-150 ligation reactions. The ligation reaction can be used directly in bacterial transformation

Read more:

http://www.takaramirusbio.com/cgi/showGroup.cgi?tableName=MobioGroup&op=showGroup&Group_ID=G6023
http://www.takaramirusbio.com/cgi/showGroup.cgi?tableName=MobioGroup&op=showGroup&Group_ID=G6027

- **Takara T4 DNA Ligase**

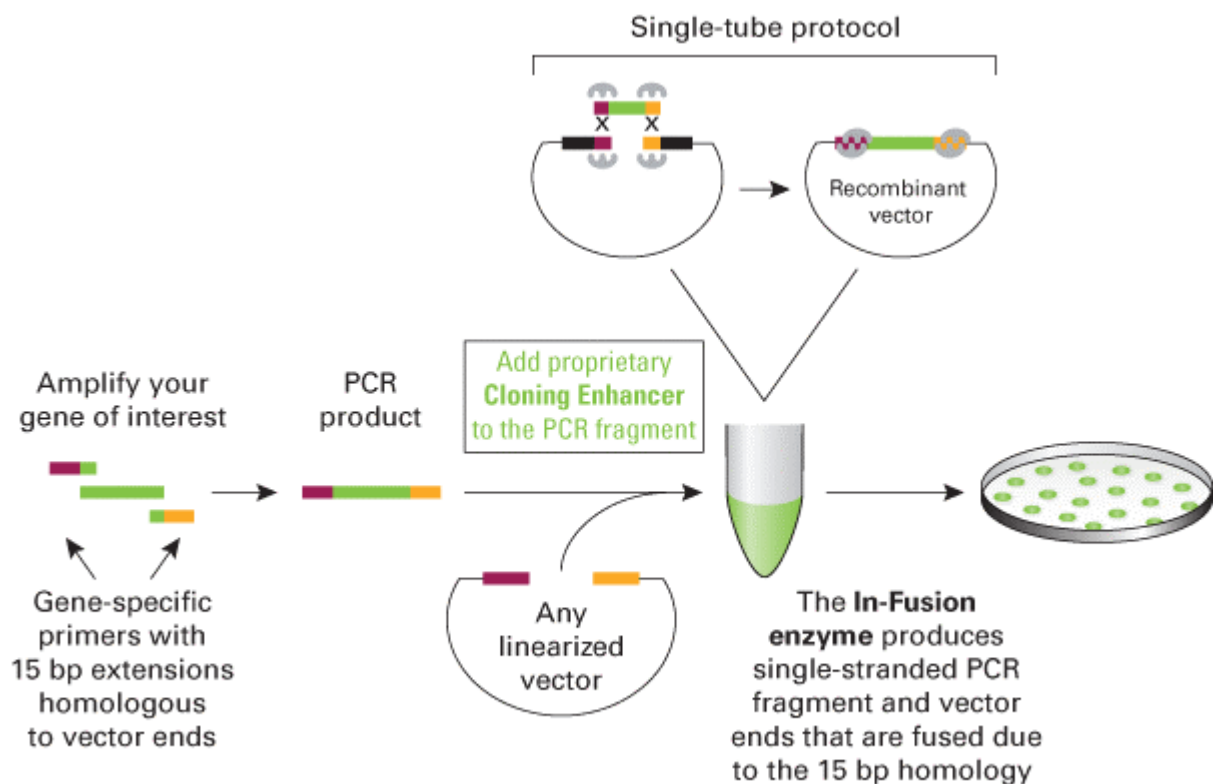
Read more:

http://www.takaramirusbio.com/cgi/showGroup.cgi?tableName=MobioGroup&op=showGroup&Group_ID=G2011S

- **Clontech In Fusion 2.0 PCR kit**

The In-Fusion 2.0 PCR cloning system allows you to clone **into your standard vectors, at any restriction site**, with ease and high efficiency. Precise clones can be generated **without PCR product purification**, restriction digestion, or blunt-end polishing, and the cloning is ligation-independent. In-Fusion 2.0 is validated for fragment lengths **less than 0.5 kb up to 12 kb**. This highly efficient cloning process minimizes the screening required to identify correct clones. The In-Fusion 2.0 kits come with **individual tubes of lyophilized dry-down reagents**. These convenient tubes can be stored right on the lab shelf. Performing a cloning reaction is very simple. You just generate a PCR insert using a DNA polymerase such as Advantage HD, then treat a portion of the PCR product with the Cloning Enhancer. After treatment, transfer the PCR insert and vector to the Dry-Down reaction tube in a total of 10 µl, and incubate. Then transform, and screen the resulting colonies for inserts.

The final product is the desired clone, **with the insert in the correct orientation and no additional bases added**. The ability to clone directly into any vector, including any vectors in your freezer, at any restriction site, eliminates the need for further subcloning or manipulation, and results in the exact clone required. http://www.clontech.com/products/detail.asp?product_family_id=1415&product_group_id=1433&product_id=162275



5. TRANSFORMATION

- **Takara E.coli Competent cells or Electro-cells**

Takara offers seven kinds of Competent Cells that have high transformation efficiency prepared with the modified Hanahan's method. (*E. coli* HB101, JM109, CJ236, BMH71-18 *mutS*, MV1184, TH2, DH5 α ; all of these are EK1 host cells.)

Read more: http://bio.takara.co.jp/BIO_EN/catalog_d.asp?C_ID=C0420

Takara also offers four kinds of Electro-Cells prepared with their original method that obtain high transformation efficiency. (*E. coli* HB101, JM109, MV1184, DH5 α)

Read more: http://bio.takara.co.jp/BIO_EN/catalog_d.asp?C_ID=C0427

- **Clontech Fusion Blue competent cells**

Fusion-Blue™ Cells are high-efficiency, chemically competent *E. coli* cells designed for blue/white screening when used with the appropriate plasmids. This K12 strain carries *recA* and *endA* mutations that make it an ideal host for obtaining high yields of plasmid DNA. They are recommended for use with the In-Fusion and Creator cloning systems.

Cells are available arrayed in a 96-well plate format for high-throughput transformation and in a 24 transformation size (50 μ l/tube). A Test Plasmid bearing the ampicillin resistance marker is provided as well as SOC Medium, 8-Cap Strips, Plate Lid, and Sterile Reagent Reservoir are also included with the 96-well plate size.

Read more:

http://www.clontech.com/products/detail.asp?product_family_id=1415&product_group_id=1431&product_id=10399

Please note that the price lists for Takara, Clontech and Macherey-Nagel products are available on the Scientifix website:

www.scientifix.com.au