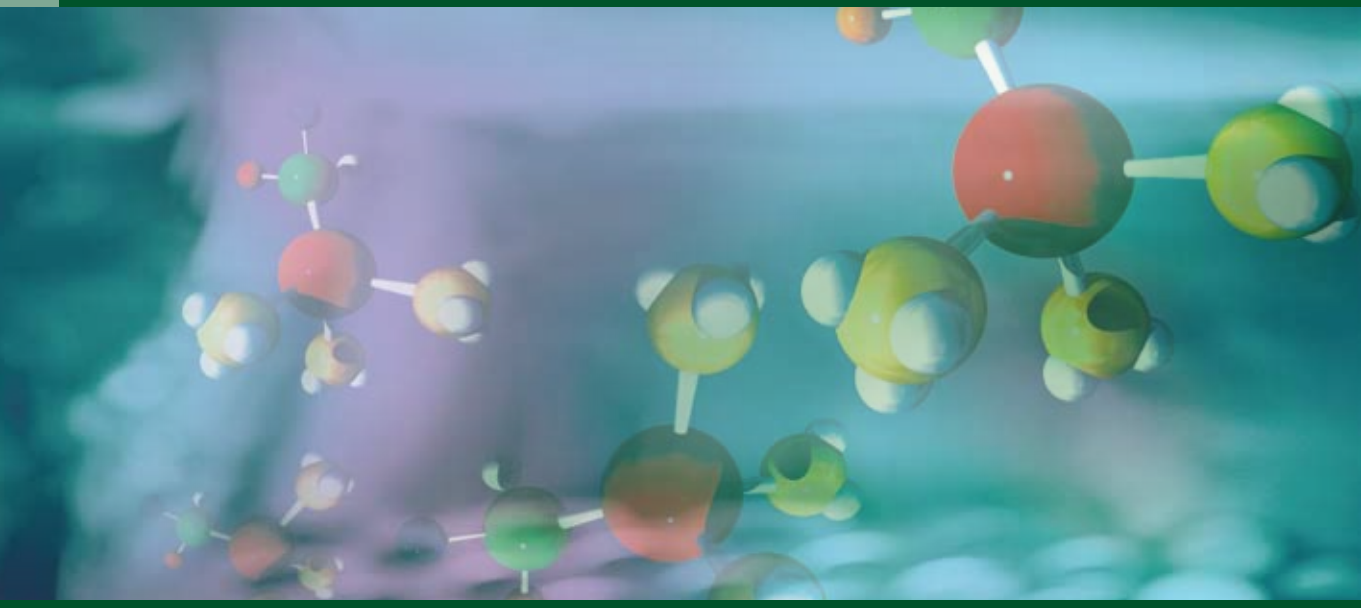


RBC Cloning Systems

Cloning and Transformation = only 6 Minutes

Introduction

Making cloning easier with high quality cloning reagents from Real Biotech Corporation. From plasmid and gel purification kits, polymerases, vectors, ligases and unique competent cells, we provide rapid and economical cloning solutions.

RBC Rapid Ligation Kit

For blunt and sticky end cloning, utilizing the scientists own cloning vector and RBC Rapid Ligation kit is very convenient. Ligation using the high quality T4 DNA ligase and optimized buffers could be achieved within 5 minutes, although 20-30 minutes may be optimal for certain sequences. Ligation product may be used instantly for transformation or purified to achieve increased efficiency.

RBC T&A Cloning System

RBC cloning system is ideal for cloning amplified products generated using a thermostable DNA polymerase, such as RealTaq DNA Polymerase, which adds a single terminal 3'-dA nucleotide overhang. The kit contains all the components needed such as T4 DNA ligase, vector and buffers. Optimal ligation time is 30 minutes, ligation can proceed within 5-15 minutes or up to 1 hour according to user. It is recommended to check ligation on an agarose gel. The vector confers ampicillin resistance and blue/white screening capability. The highly purified vector decreases possibility of background cloning. Real Biotech Corporation also provides the T&A cloning vector as a stand alone product.

Benefits of RBC Cloning Kits

Economical : Unbeatable value for regular cloning.
Convenient : Accept amplified, blunt and sticky end DNA.
Stable : T&A Vector backbone is stable and regularly sequenced.
Rapid: Ligation can be achieved within 5 minutes for RBC Rapid Ligation.
Versatile: Accepts a wide size range of inserts.
Downstream : Ideal for rapid transformation into HIT Competent Cells™.

Applications

	RBC T&A Cloning Kit	RBC T&A Vector	RBC Rapid Ligation Kit*
PCR amplified DNA (Taq)	Yes	Yes	No
Terminal dA Tailed (Taq)	Yes	Yes	No
PCR amplified DNA (Pfu)	No	No	Yes
Blunt end-dephosphorylated	No	No	Yes
Sticky end	No	No	Yes
Linker Ligation	No	No	Yes

* Vector not included (T4 DNA ligase and buffers only)

Note: The use of the PCR process is covered by various patents obtained by Hoffman-La Roche. PCR is a trademark of Hoffman-La Roche.

RBC Rapid Ligation Kit

Rapid and Economical Ligation

Cat. No. RC011 (100 reactions)
T4 DNA Ligase (3U/µl): 100 µl
10X Ligation Buffer A: 200 µl
10X Ligation Buffer B: 200 µl

Store at -20°C

Description

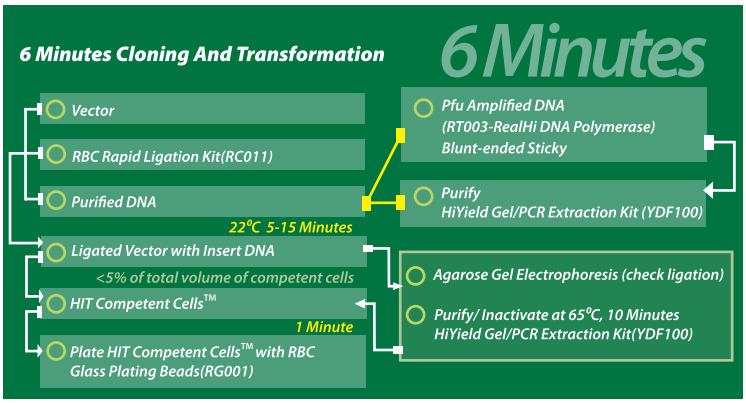
T4 DNA Ligase catalyzes the joining of two strands of DNA between the 5'-phosphate and the 3'-hydroxyl groups of adjacent nucleotides in either a cohesive-ended or blunt-ended termini. RBC Rapid DNA Ligation Kit is designed for efficient ligation of DNA inserts into plasmid vectors in just 5 minutes.

Unit Definition

1. *One unit of enzyme catalyzes the conversion of 1 nanomole of [^{32}PPi] into Norit-adsorbable form in 20 mins at 37°C(Weiss unit).*
2. *We recommend using a 1:3 molar ratio of vector : insert DNA when cloning a fragment into a plasmid vector. These ratios will vary with other types of vectors.*
3. *In a microcentrifuge tube prepare 5-10 µl mix in water or TE buffer of digested vector DNA (50-400 ng) and foreign DNA to be inserted.*
4. *Add the following components to the same tube:*
   - *i. 10X Ligase Buffer A 2 µl*
   - *ii. 10X Ligase Buffer B 2 µl*
   - *iii. T4 DNA Ligase 1 µl*
   - *iv. Nuclease-Free Water to final volume of 20 µl*
5. *Vortex the tube and spin down in microcentrifuge for 3-5 secs.*
6. *Incubate the mixture for 5 -20 mins at 22°C.*
7. *Inactivate T4 DNA Ligase by heating the reaction mixture at 65°C for 10 mins. Use the mixture for transformation.*

Applications

Joining double-stranded DNA with sticky or blunt termini.
Joining of oligonucleotide linkers to blunt-ended DNA.
Repairing nicks in duplex DNA, RNA or DNA-RNA hybrids.



Storage Conditions

RBC Rapid Ligation Kit should be stored immediately upon receipt at -20°C in a constant temperature freezer. RBC Rapid Ligation Kit can be stored for up to 12 months without showing any deduction in performance and quality with proper storage.

Notes:

i) T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 0.2M.
ii) 10X Ligation Buffer B greatly increases the rate of ligation of blunt-ended DNA.
iii) The inactivation of T4 DNA Ligase by heating at 65 °C for 10 minutes is recommended as a standard procedure prior to transformation of cells with DNA. In some cases, this simple step can increase the number of transformants by two orders of magnitude.
iv) Transformation efficiency is increased if DNA is extracted prior to transformation, Use equal or higher (up to 3-fold) molar concentration of insert DNA termini over vector DNA. If the yield of ligation product is insufficient, prolong the reaction time (overnight), Ligation reactions performed at lower temperatures require longer incubation times.

RBC T&A Cloning Kit

Rapid and Economical Cloning

Cat. No. RC001 (20 reactions)
T&A Cloning Vector (25 ng/μl): 40 μl
Control Insert DNA (10 ng/μl): 10 μl
T4 DNA Ligase (3 U/μl): 20 μl
10X Ligation Buffer A : 100 μl
10X Ligation Buffer B : 100 μl
Forward Primer(M13-F) (10 μM): 50 μl
Reverse Primer(M13-R) (10 μM): 50 μl

Store at -20°C

Cat. No. RC013 (20 reactions)
T&A Cloning Vector (25 ng/μl) : 40 μl

Description

RBC T&A cloning system is ideal for cloning PCR products generated using thermostable DNA polymerases which add a single terminal 3'-dA nucleotide overhang (Taq). It is extremely economical and convenient for rapid cloning. Following ligation, the mixture may be used directly to transform HIT Competent Cells™ or other competent cells or purified to achieve a higher efficiency of transformation. The vector is highly purified to reduce background cloning.

Ligation Conditions

	User Sample	Positive Control
Ligation Buffer A	1 μl	1 μl
Ligation Buffer B	1 μl	1 μl
T&A cloning vector	2 μl	2 μl
PCR product	X μl	****
T4 DNA Ligase	1 μl	1 μl
Control DNA	****	3 μl

Add deionized water to a final volume of 10 μl. Incubate the reactions for 5 to 15 mins at 22°C.

Applications

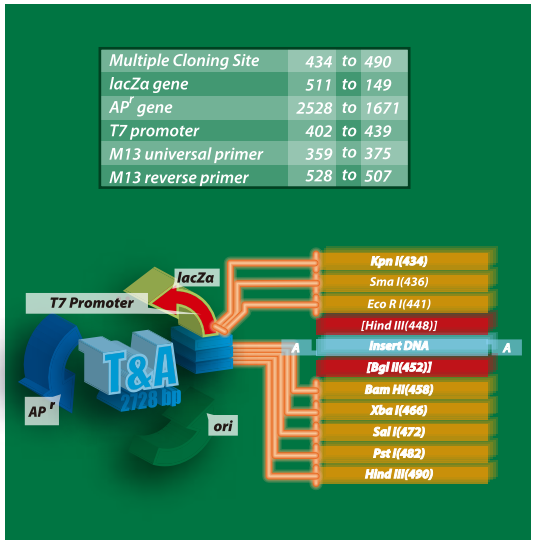
Accepts terminal 3'-dA nucleotide overhang PCR products.
Transform ligation product (purified/unpurified) into HIT Competent Cells™.
LacZ complementation for blue/white screening.
Ampicillin marker for antibiotic selection.
Universal primer for easy transformation screening.

Storage Conditions

RBC T&A Cloning Kit should be stored immediately upon receipt at -20°C in a constant temperature freezer. RBC T&A Cloning Kit can be stored for up to 12 months without showing any deduction in performance and quality with proper storage.

Notes: Following are comparison results from RBC Labs.
i) RBC T&A cloning system is very efficient at LIGATION with higher resulting transformation efficiency compared to company P's transformation system. RBC T&A cloning system provides two types of ligation buffer for your convenience.
ii) RBC T&A cloning system high efficiency ligation takes only 20 minutes versus 1 hour to overnight for company P's system.
iii) RBC T&A clonng system resulted in higher ACCURACY compared to company P's in colony PCR pickings.
iv) RBC T&A cloning system shows very good efficiency of cloning up to 5 kb INSERTS, while company P's system may show slightly higher efficiency > 5 kb.

Figure 1. Map and Sequence reference points of the RBC T&A Cloning Vector



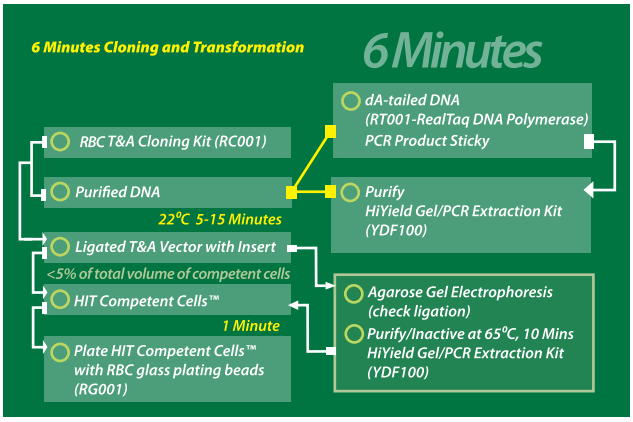
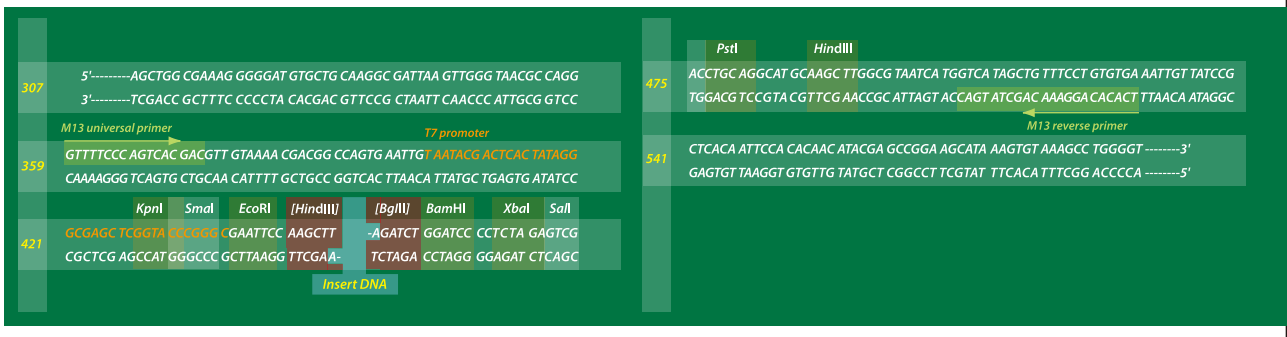


Figure 2. Multiple cloning site sequence of the RBC T&A Cloning Vector



RBC T&A Cloning Vector restriction enzyme sites

Unique restriction enzyme cut sites of RBC T&A Cloning Vector

Name	Position	Name	Position	Name	Position	Name	Position	Name	Position
AatII	2664	AspEI	1742	Cfr10I	1822	MamI	457	SspI	2546
Acc65I	430	AvaI	434	DraII	2718	NarI	237	XbaI	466
AccI	473	BanII	428	Eam1105I	1742	NdeI	185	XmaI	434
AcsI	441	BamHI	458	Ecl136II	426	PstI	482	XmnI	2341
AflIII	849	BcgI	2281	EcoO109I	2718	SacI	428		
AhdI	1742	BpmI	1812	EcoRI	441	SalI	472		
AlwNI	1265	BsaBI	457	HincII	474	SapI	733		
ApoI	441	BsaI	1803	HindII	474	ScaI	2222		
Asp700	2341	BspMI	485	KasI	236	SmaI	436		
Asp718	430	BsrFI	1822	KpnI	434	SphI	488		

For quick assurance of correct orientation of insert and reduction of cloning with self-ligated plasmid, colony screening is recommended.

Note: *The PCR process is covered by patents obtained by Hoffmann-La Roche. PCR is a trademark of Hoffmann-La Roche.*