Store at -20°C

HIT™ Competent Cells
Real Biotech Corporation

For high efficiency transformation of T&A Cloning Vector in 1-10 minutes, utilise unique RBC HIT Non-Heat Shock Competent Cells!

HIT Competent Cells™-DH5a: Value 10^8, High 10^8 and Super 10^9.
HIT Competent Cells™-JM109: Value 10^8 and High 10^8.
HIT Competent Cells™-Blue: Value 10^8, High 10^8 and Super 10^9.
HIT Competent Cells™-BL21: Value 10^7.

RBC
It's the Joy of Discovery

Real Biotech Corporation

Real Biotech Corporation is a leading R&D based life science solutions provider.
For more information on our extensive and innovative life science range, please visit our website.

RBC Cloning Systems

RBC T&A Cloning Kit Protocol Book

Rapid and Economical Cloning PCR Product

RC001 RBC T&A Cloning Kit / RC013 RBC T&A Cloning Vector

Contents

Product Description

Introduction

Real Biotech Corporation T&A Cloning System is ideal for rapid cloning PCR product generated using a thermostable DNA polymerase, such as RealTaq Polymerase, which adds a single terminal 3'-dA nucleotide overhang.

Contents

- *Cat. No. RC001 RBC T&A Cloning Kit (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.*
  - *Protocol Book.*

— Cat. No. RC013 RBC T&A Cloning Vector (20 reactions)
- ✻ T&A Cloning Vector (25 ng/μl): 40 μl.
- ✻ Protocol Book.

Shipping Conditions

RBC T&A Cloning Kits have been monitored to ensure -20°C AT ALL TIMES during batch processing, shipping and storage. RBC T&A Cloning Kits shall be shipped with dry ice.

Storage Conditions

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

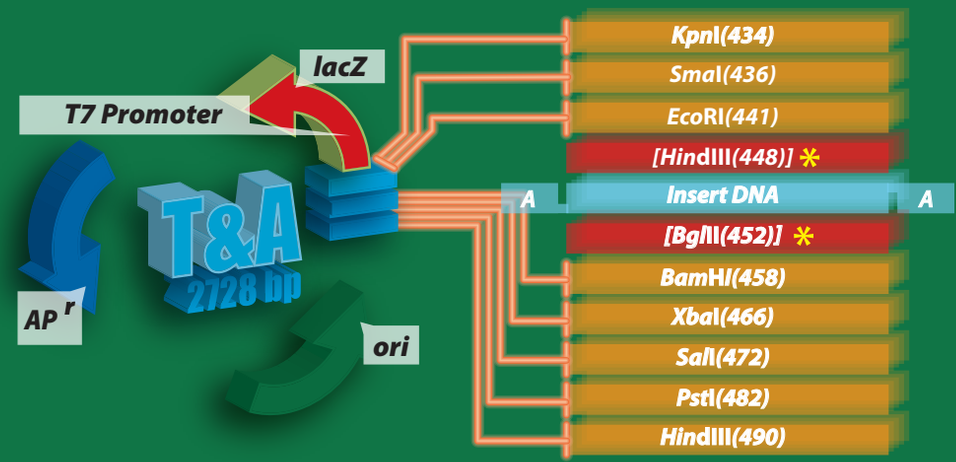
Application Table

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

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

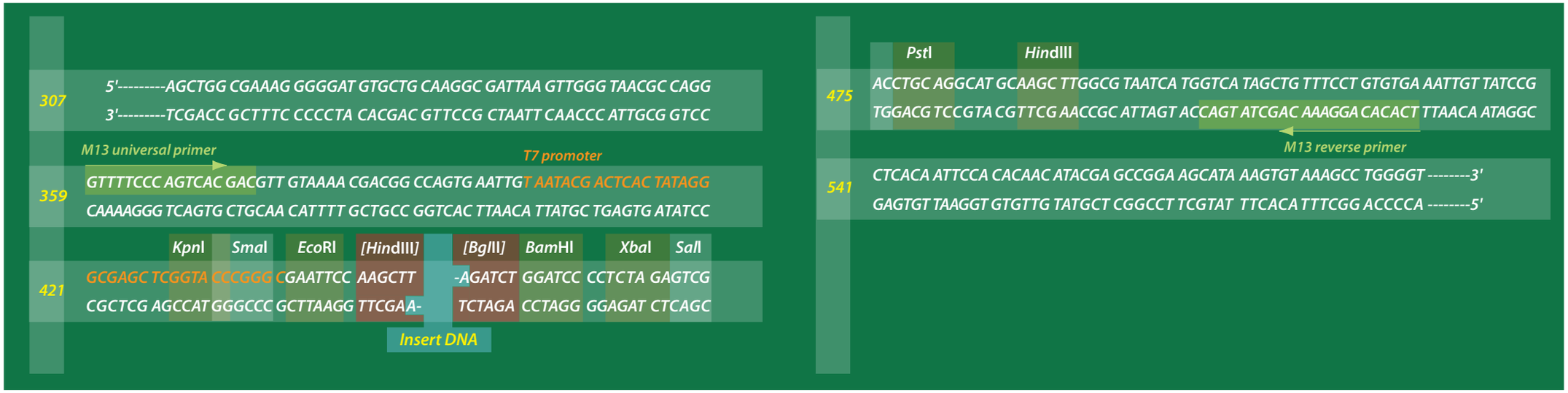
Vector Map of RBC T&A Cloning Vector

Multiple Cloning Site	434	to	490
lacZ gene	511	to	149
AP^r gene	2528	to	1671
T7 promoter	402	to	439
M13 universal primer	359	to	375
M13 reverse primer	528	to	507



✻ Before the insert incorporated into the RBC T&A Cloning Vector, there is only 1 HindIII site and no BglII site. When the incorporation is done, the T and A nucleotides on the insert will complement the sequence on the vector and generate two new sites.

DNA Sequence of RBC T&A Cloning Vector Multiple Cloning Site



Restriction Enzymes and RBC T&A Cloning Vector

1. No restriction sites to cut RBC T&A Cloning Vector for the following enzymes:

AatI	BbsI	BsiWI	BstBI	Eco47III	MroI	NsiI	Ppu10I	SpeI
AccIII	BclI	BsmFI	BstEII	EcoNI	MscI	NspV	PpuMI	SspBI
AflII	BfrI	BsmI	Bsu36I	EcoRV	MunI	PacI	RsrII	StyI
AgeI	BlnI	Bsp120I	CelII	EspI	NaeI	PaeR7I	SacII	SwaI
ApaI	Bpu1102I	BspDI	ClaI	HpaI	NcoI	PflMI	SexAI	Tth111I
AspI	BpuAI	BspEI	Csp45I	KspI	NgoMI	PinAI	SfiI	Van91I
AsuII	BsaAI	BsrGI	DraIII	MfeI	NheI	PmaCI	SfuI	XcmI
AvrII	BseAI	BssHII	EagI	MluI	NotI	PmeI	SgrAI	XhoI
BbrPI	BsgI	Bst1107I	EclXI	MluNI	NruI	PmlI	SnaBI	XmaIII

2. Single restriction site to cut RBC T&A Cloning Vector for the following enzymes:

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		

3. More than one restriction site to cut RBC T&A Cloning Vector for the following enzymes:

Name	Position	Name	Position	Name	Position
AciI	34sites	CfoI	17 sites	MboI	16 sites
AluI	17sites	Csp61	169,431,2221	MboII	7 sites
Alw44I	178,1163,2409	DdeI	6 sites	McrI	5 sites
AlwI	10 sites	DpnI	16 sites	MnlI	13 sites
AosI	259,1964	DpnII	16 sites	MseI	13 sites
ApaLI	178,1163,2409	DraI	1608,1627,2319	MslI	1994,2153,2512
AseI	620,679,1914	DrdI	98,957	MspA1I	6 sites
AsnI	620,679,1914	DsaV	12 sites	MspI	13 sites
AspHI	5 sites	EaeI	389,688,2130	MvaI	5 sites
AvaII	1880,2102	EarI	297,733,2537	MvnI	10 sites
AviII	259,1964	Eco57I	1397,2409	MwoI	13 sites
		EcoRII	5 sites	NciI	7 sites
BanI	4 sites	Esp3I	4,46	NdeII	16 sites
BbvI	12 sites	Fnu4HI	19 sites	NlaIII	11 sites
BfaI	4 sites	FnuDII	10 sites	NlaIV	11 sites
BglI	252,1862	FokI	5 sites	NspI	42,488,853

Name	Position	Name	Position	Name	Position
BmyI	5 sites	FspI	259,1964	PleI	5 sites
BsaHI	237,2279,2661	HaeII	240,727,1097	Psp1406I	1968,2341
BsaJI	4 sites	HaeIII	11 sites	PvuI	280,2112
BsaWI	1055,1202,2033	HgaI	4 sites	PvuII	309,673
BsiEI	5 sites	HgiAI	5 sites	RcaI	1569,2577,2682
BsiHKAI	5 sites	HhaI	17 sites	RsaI	170,432,222
BsiYI	6 sites	HindIII	(448),490	Sau3AI	16 sites
BslI	6 sites	HinfI	7 sites	Sau96I	6 sites
BsmAI	4 sites	HinP1I	17 sites	ScrFI	12 sites
Bsp1286I	5sites	HpaII	13 sites	SfaNI	8 sites
BspHI	1569,2577,2682	HphI	7 sites	SfcI	5 sites
BspWI	13 sites	HPY188I	10 sites	SnoI	178,1163,2409
BsrBI	541,782,2583	HtaI	19 sites	TaqI	473,949,2393
BsrDI	1803,1977	Ksp632I	297,733,2537	TfiI	684,824
BsrI	11 sites	MaeI	4 sites	ThaI	10 sites
BstNI	5 sites	MaeII	5 sites	Tru9I	13 sites
BstUI	10 sites	MaeIII	11 sites	Tsp509I	8 sites
BstYI	7 sites			XhoII	7 sites

Full Length of RBC T&A Cloning Vector

```
   1  TCGCGC GTTTCG GTGATG ACGGTG AAAACC TCTGAC ACATGC AGCTCC CGGAGA CGGTCA
      AGCGCG CAAAGC CACTAC TGCCAC TTTTGG AGACTG TGTACG TCGAGG GCCTCT GCCAGT

  61  CAGCTT GTCTGT AAGCGG ATGCCG GGAGCA GACAAG CCCGTC AGGGCG CGTCAG CGGGTG
      GTCGAA CAGACA TTCGCC TACGGC CCTCGT CTGTTC GGGCAG TCCCGC GCAGTC GCCCAC

 121  TTGGCG GGTGTC GGGGCT GGCTTA ACTATG CGGCAT CAGAGC AGATTG TACTGA GAGTGC
      AACCGC CCACAG CCCCGA CCGAAT TGATAC GCCGTA GTCTCG TCTAAC ATGACT CTCACG

 181  ACCATA TGCGGT GTGAAA TACCGC ACAGAT GCGTAA GGAGAA AATACC GCATCA GGCGCC
      TGGTAT ACGCCA CACTTT ATGGCG TGTCTA CGCATT CCTCTT TTATGG CGTAGT CCGCGG

 241  ATTCGC CATTCA GGCTGC GCAACT GTTGGG AAGGGC GATCGG TGCGGG CCTCTT CGCTAT
      TAAGCG GTAAGT CCGACG CGTTGA CAACCC TTCCCG CTAGCC ACGCCC GGAGAA GCGATA

 301  TACGCC AGCTGG CGAAAG GGGGAT GTGCTG CAAGGC GATTAA GTTGGG TAACGC CAGGGT
      ATGCGG TCGACC GCTTTC CCCCTA CACGAC GTTCCG CTAATT CAACCC ATTGCG GTCCCA

      M13 universal primer                                  T7 promoter
 361  TTTCCC AGTCAC GACGTT GTAAAA CGACGG CCAGTG AATTGT AATACG ACTCAC TATAGG
      AAAGGG TCAGTG CTGCAA CATTTT GCTGCC GGTCAC TTAACA TTATGC TGAGTG ATATCC

                                          Insert DNA
 421  GCGAGC TCGGTA CCCGGG CGAATT CCAAGC TT  IN  AGATCT GGATCC CCTCTA GAGTCG ACCTGC
      CGCTCG AGCCAT GGGCCC GCTTAA GGTTCG AA  IN  TCTAGA CCTAGG GGAGAT CTCAGC TGGACG

 481  AGGCAT GCAAGC TTGGCG TAATCA TGGTCA TAGCTG TTTCCT GTGTGA AATTGT TATCCG
      TCCGTA CGTTCG AACCGC ATTAGT ACCAGT ATCGAC AAAGGA CACACT TTAACA ATAGGC
                                 M13 reverse primer

 541  CTCACA ATTCCA CACAAC ATACGA GCCGGA AGCATA AAGTGT AAAGCC TGGGGT GCCTAA
      GAGTGT TAAGGT GTGTTG TATGCT CGGCCT TCGTAT TTCACA TTTCGG ACCCCA CGGATT

 601  TGAGTG AGCTAA CTCACA TTAATT GCGTTG CGCTCA CTGCCC GCTTTC CAGTCG GGAAAC
      ACTCAC TCGATT GAGTGT AATTAA CGCAAC GCGAGT GACGGG CGAAAG GTCAGC CCTTTG
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 661  CTGTCG TGCCAG CTGCAT TAATGA ATCGGC CAACGC GCGGGG AGAGGC GGTTTG CGTATT
      GACAGC ACGGTC GACGTA ATTACT TAGCCG GTTGCG CGCCCC TCTCCG CCAAAC GCATAA

 721  GGGCGC TCTTCC GCTTCC TCGCTC ACTGAC TCGCTG CGCTCG GTCGTT CGGCTG CGGCGA
      CCCGCG AGAAGG CGAAGG AGCGAG TGACTG AGCGAC GCGAGC CAGCAA GCCGAC GCCGCT

 781  GCGGTA TCAGCT CACTCA AAGGCG GTAATA CGGTTA TCCACA GAATCA GGGGAT AACGCA
      CGCCAT AGTCGA GTGAGT TTCCGC CATTAT GCCAAT AGGTGT CTTAGT CCCCTA TTGCGT

 841  GGAAAG AACATG TGAGCA AAAGGC CAGCAA AAGGCC AGGAAC CGTAAA AAGGCC GCGTTG
      CCTTTC TTGTAC ACTCGT TTTCCG GTCGTT TTCCGG TCCTTG GCATTT TTCCGG CGCAAC

 901  CTGGCG TTTTTC CATAGG CTCCGC CCCCCT GACGAG CATCAC AAAAAT CGACGC TCAAGT
      GACCGC AAAAAG GTATCC GAGGCG GGGGGA CTGCTC GTAGTG TTTTTA GCTGCG AGTTCA

 961  CAGAGG TGGCGA AACCCG ACAGGA CTATAA AGATAC CAGGCG TTTCCC CCTGGA AGCTCC
      GTCTCC ACCGCT TTGGGC TGTCCT GATATT TCTATG GTCCGC AAAGGG GGACCT TCGAGG

1021  CTCGTG CGCTCT CCTGTT CCGACC CTGCCG CTTACC GGATAC CTGTCC GCCTTT CTCCCT
      GAGCAC GCGAGA GGACAA GGCTGG GACGGC GAATGG CCTATG GACAGG CGGAAA GAGGGA

1081  TCGGGA AGCGTG GCGCTT TCTCAT AGCTCA CGCTGT AGGTAT CTCAGT TCGGTG TAGGTC
      AGCCCT TCGCAC CGCGAA AGAGTA TCGAGT GCGACA TCCATA GAGTCA AGCCAC ATCCAG

1141  GTTCGC TCCAAG CTGGGC TGTGTG CACGAA CCCCCC GTTCAG CCCGAC CGCTGC GCCTTA
      CAAGCG AGGTTC GACCCG ACACAC GTGCTT GGGGGG CAAGTC GGGCTG GCGACG CGGAAT

1201  TCCGGT AACTAT CGTCTT GAGTCC AACCCG GTAAGA CACGAC TTATCG CCACTG GCAGCA
      AGGCCA TTGATA GCAGAA CTCAGG TTGGGC CATTCT GTGCTG AATAGC GGTGAC CGTCGT

1261  GCCACT GGTAAC AGGATT AGCAGA GCGAGG TATGTA GGCGGT GCTACA GAGTTC TTGAAG
      CGGTGA CCATTG TCCTAA TCGTCT CGCTCC ATACAT CCGCCA CGATGT CTCAAG AACTTC

1321  TGGTGG CCTAAC TACGGC TACACT AGAAGG ACAGTA TTTGGT ATCTGC GCTCTG CTGAAG
      ACCACC GGATTG ATGCCG ATGTGA TCTTCC TGTCAT AAACCA TAGACG CGAGAC GACTTC
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1381  CCAGTT ACCTTC GGAAAA AGAGTT GGTAGC TCTTGA TCCGGC AAACAA ACCACC GCTGGT
      GGTCAA TGGAAG CCTTTT TCTCAA CCATCG AGAACT AGGCCG TTTGTT TGGTGG CGACCA

1441  AGCGGT GGTTTT TTTGTT TGCAAG CAGCAG ATTACG CGCAGA AAAAAA GGATCT CAAGAA
      TCGCCA CCAAAA AAACAA ACGTTC GTCGTC TAATGC GCGTCT TTTTTT CCTAGA GTTCTT

1501  GATCCT TTGATC TTTTCT ACGGGG TCTGAC GCTCAG TGGAAC GAAAAC TCACGT TAAGGG
      CTAGGA AACTAG AAAAGA TGCCCC AGACTG CGAGTC ACCTTG CTTTTG AGTGCA ATTCCC

1561  ATTTTG GTCATG AGATTA TCAAAA AGGATC TTCACC TAGATC CTTTTA AATTAA AAATGA
      TAAAAC CAGTAC TCTAAT AGTTTT TCCTAG AAGTGG ATCTAG GAAAAT TTAATT TTTACT

1621  AGTTTT AAATCA ATCTAA AGTATA TATGAG TAAACT TGGTCT GACAGT TACCAA TGCTTA
      TCAAAA TTTAGT TAGATT TCATAT ATACTC ATTTGA ACCAGA CTGTCA ATGGTT ACGAAT

1681  ATCAGT GAGGCA CCTATC TCAGCG ATCTGT CTATTT CGTTCA TCCATA GTTGCC TGACTC
      TAGTCA CTCCGT GGATAG AGTCGC TAGACA GATAAA GCAAGT AGGTAT CAACGG ACTGAG

1741  CCCGTC GTGTAG ATAACT ACGATA CGGGAG GGCTTA CCATCT GGCCCC AGTGCT GCAATG
      GGGCAG CACATC TATTGA TGCTAT GCCCTC CCGAAT GGTAGA CCGGGG TCACGA CGTTAC

1801  ATACCG CGAGAC CCACGC TCACCG GCTCCA GATTTA TCAGCA ATAAAC CAGCCA GCCGGA
      TATGGC GCTCTG GGTGCG AGTGGC CGAGGT CTAAAT AGTCGT TATTTG GTCGGT CGGCCT

1861  AGGGCC GAGCGC AGAAGT GGTCCT GCAACT TTATCC GCCTCC ATCCAG TCTATT AATTGT
      TCCCGG CTCGCG TCTTCA CCAGGA CGTTGA AATAGG CGGAGG TAGGTC AGATAA TTAACA

1921  TGCCGG GAAGCT AGAGTA AGTAGT TCGCCA GTTAAT AGTTTG CGCAAC GTTGTT GCCATT
      ACGGCC CTTCGA TCTCAT TCATCA AGCGGT CAATTA TCAAAC GCGTTG CAACAA CGGTAA

1981  GCTACA GGCATC GTGGTG TCACGC TCGTCG TTTGGT ATGGCT TCATTC AGCTCC GGTTCC
      CGATGT CCGTAG CACCAC AGTGCG AGCAGC AAACCA TACCGA AGTAAG TCGAGG CCAAGG

2041  CAACGA TCAAGG CGAGTT ACATGA TCCCCC ATGTTG TGCAAA AAAGCG GTTAGC TCCTTC
      GTTGCT AGTTCC GCTCAA TGTACT AGGGGG TACAAC ACGTTT TTTCGC CAATCG AGGAAG
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2101  GGTCCT CCGATC GTTGTC AGAAGT AAGTTG GCCGCA GTGTTA TCACTC ATGGTT ATGGCA
      CCAGGA GGCTAG CAACAG TCTTCA TTCAAC CGGCGT CACAAT AGTGAG TACCAA TACCGT

2161  GCACTG CATAAT TCTCTT ACTGTC ATGCCA TCCGTA AGATGC TTTTCT GTGACT GGTGAG
      CGTGAC GTATTA AGAGAA TGACAG TACGGT AGGCAT TCTACG AAAAGA CACTGA CCACTC

2221  TACTCA ACCAAG TCATTC TGAGAA TAGTGT ATGCGG CGACCG AGTTGC TCTTGC CCGGCG
      ATGAGT TGGTTC AGTAAG ACTCTT ATCACA TACGCC GCTGGC TCAACG AGAACG GGCCGC

2281  TCAATA CGGGAT AATACC GCGCCA CATAGC AGAACT TTAAAA GTGCTC ATCATT GGAAAA
      AGTTAT GCCCTA TTATGG CGCGGT GTATCG TCTTGA AATTTT CACGAG TAGTAA CCTTTT

2341  CGTTCT TCGGGG CGAAAA CTCTCA AGGATC TTACCG CTGTTG AGATCC AGTTCG ATGTAA
      GCAAGA AGCCCC GCTTTT GAGAGT TCCTAG AATGGC GACAAC TCTAGG TCAAGC TACATT

2401  CCCACT CGTGCA CCCAAC TGATCT TCAGCA TCTTTT ACTTTC ACCAGC GTTTCT GGGTGA
      GGGTGA GCACGT GGGTTG ACTAGA AGTCGT AGAAAA TGAAAG TGGTCG CAAAGA CCCACT

2461  GCAAAA ACAGGA AGGCAA AATGCC GCAAAA AAGGGA ATAAGG GCGACA CGGAAA TGTTGA
      CGTTTT TGTCCT TCCGTT TTACGG CGTTTT TTCCCT TATTCC CGCTGT GCCTTT ACAACT

2521  ATACTC ATACTC TTCCTT TTTCAA TATTAT TGAAGC ATTTAT CAGGGT TATTGT CTCATG
      TATGAG TATGAG AAGGAA AAAGTT ATAATA ACTTCG TAAATA GTCCCA ATAACA GAGTAC

2581  AGCGGA TACATA TTTGAA TGTATT TAGAAA AATAAA CAAATA GGGGTT CCGCGC ACATTT
      TCGCCT ATGTAT AAACTT ACATAA ATCTTT TTATTT GTTTAT CCCCAA GGCGCG TGTAAA

2641  CCCCGA AAAGTG CCACCT GACGTC TAAGAA ACCATT ATTATC ATGACA TTAACC TATAAA
      GGGGCT TTTCAC GGTGGA CTGCAG ATTCTT TGGTAA TAATAG TACTGT AATTGG ATATTT

2701  AATAGG CGTATC ACGAGG CCCTTT CGTC
      TTATCC GCATAG TGCTCC GGGAAA GCAG
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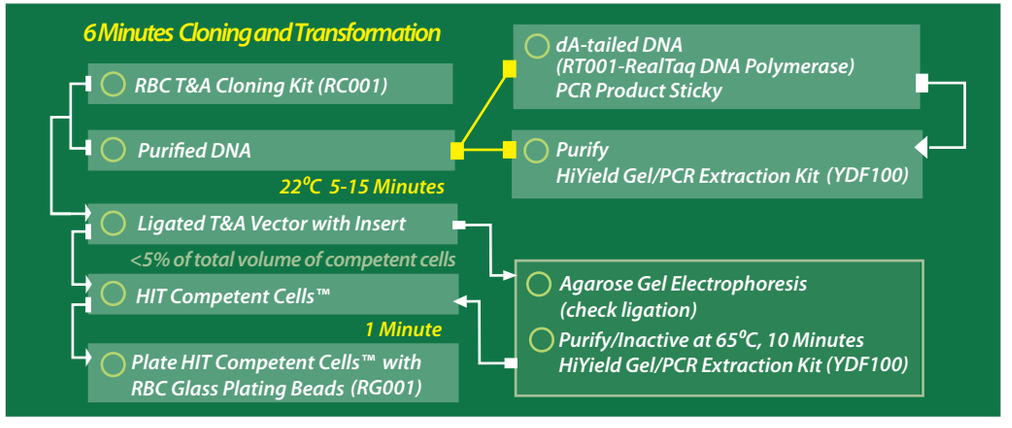
Protocol for Ligation Using RBC T&A Cloning Vector

1. Centrifuge RBC T&A Cloning Vector and/or PCR DNA tubes to collect contents at the bottom of the tubes.
2. Vortex the ligation buffer vigorously before use.
3. Set up the following items as described below:

Component	User Sample	Positive Control
10X Ligation Buffer A	1µl	1µl
10X Ligation Buffer B	1µl	1µl
T&A Cloning Vector	2 µl	2 µl
PCR Product	X µl	****
Control Insert DNA	****	3µl
T4 DNA Ligase	1µl	1µl
Deionized Water	to 10 µl	to 10 µl

4. Mix the reactions by pipetting.
5. Incubate the reactions for 5 to 15 minutes at 22°C. And, if the maximum of transformants is required, incubate the reactions overnight at 4°C.



Notes

1. For blue-white selection, plating should be done onto plates containing 50 µg/ml ampicillin plus 100 µl 100 mM IPTG and 20 µl 50 mg/ml X-gal. Spread the mixture evenly onto a plate at least 2 hours prior to plating transformed cells. After colony growth, the plates can be kept at 4°C and the blue color will continue to develop.
2. Using Colony PCR, clones can be precisely and easily screened.
3. Multiple freeze-thawing DOES NOT affect the quality of RBC T&A Cloning Vector, but exposure to frequent temperature changes will degrade ATP activity in ligase buffer. Always making single-use aliquots of ligase buffers.
4. Pfu DNA polymerase possesses proofreading activity, it does not have the terminal transferase-like activity demonstrated by Taq DNA Polymerase. Ligation reactions using non-tailed amplified DNA will result in no positive colonies. When using non-tailed amplified DNA, refer to methods indicated in note 5 for increasing the ligation efficiency

5. Methods for increasing the ligation efficiency:

A. A-tailing

Purified PCR product	X µl
10X PCR buffer	10 µl
10 mM dATP	2 µl
Taq	1 µl

i. Add deionized water to a final volume of 100 µl.
ii. 72°C for 3 hours.
iii. Purify the A-tailed DNA and use in the ligation reaction.

B. If the maximum of transformants is required, incubate the reactions overnight at 4°C.

C. The optimized efficiency is using a 1:3 molar ratio of vector DNA to the insert DNA.

D. Use higher efficiency competent cells e.g. HIT (>10^8 cfu/µ g DNA) series.

RBC T&A Cloning Kit

Troubleshooting

Problem	No Colonies	Less Than 100 Colonies	High Colony Count But High % of Blue with Insert DNA.	No Blue Colonies Are Present on the Plate
Comments	1. Problems in transformation. 2. Low efficiency competent cells.	1. Improper molar ratio of vector DNA to the insert DNA. 2. Bad A-tailed insert DNA.	1. Improper ligation reaction. 2. DNA is inserted, but it's not disrupting the LacZ gene.	1. Ampicillin is inactive. 2. IPTG/X-Gal is insufficient.
Suggestions	Background vector only should yield < 100 colonies, and with A-tailed insert DNA should yield > 500 colonies. If there are no colonies observed on the plate, use high efficiency competent cells to overcome the problem*. *RBC HIT Non-Heat Shock Competent Cells: 1. HIT Competent Cells™-DH5a. 2. HIT Competent Cells™-JM109. 3. HIT Competent Cells™-Blue. 4. HIT Competent Cells™-BL21.	1. Check the quality of A-tailed insert DNA. 2. Use a 1:3 to 1:6 molar ratio of vector DNA to the insert DNA.	1. PCR products should be gel-purified. 2. Avoid muliple freeze-thaw and exposure to frequent temperature changes by making single-use aliquots of Ligase Buffer. 3. If the maximum of transformants is required, incubate the ligation reactions overninght at 4°C. 4. Multiple PCR product, target DNA should be gel-purifed before ligation. 5. Use colony PCR to screen the clones.	1. Make sure ampicillin plates are made properly and used within 1 month. 2. Make sure IPTG/X-Gal are fresh. 3. Make sure strain is with blue-screen genotype.