T&A Expression Kit (C-terminal) Protocol

Cat. No	FYC201-10P
	FYC211-10P
	SYC201-3P
	SYC211-3P
Ver. No	QE1801

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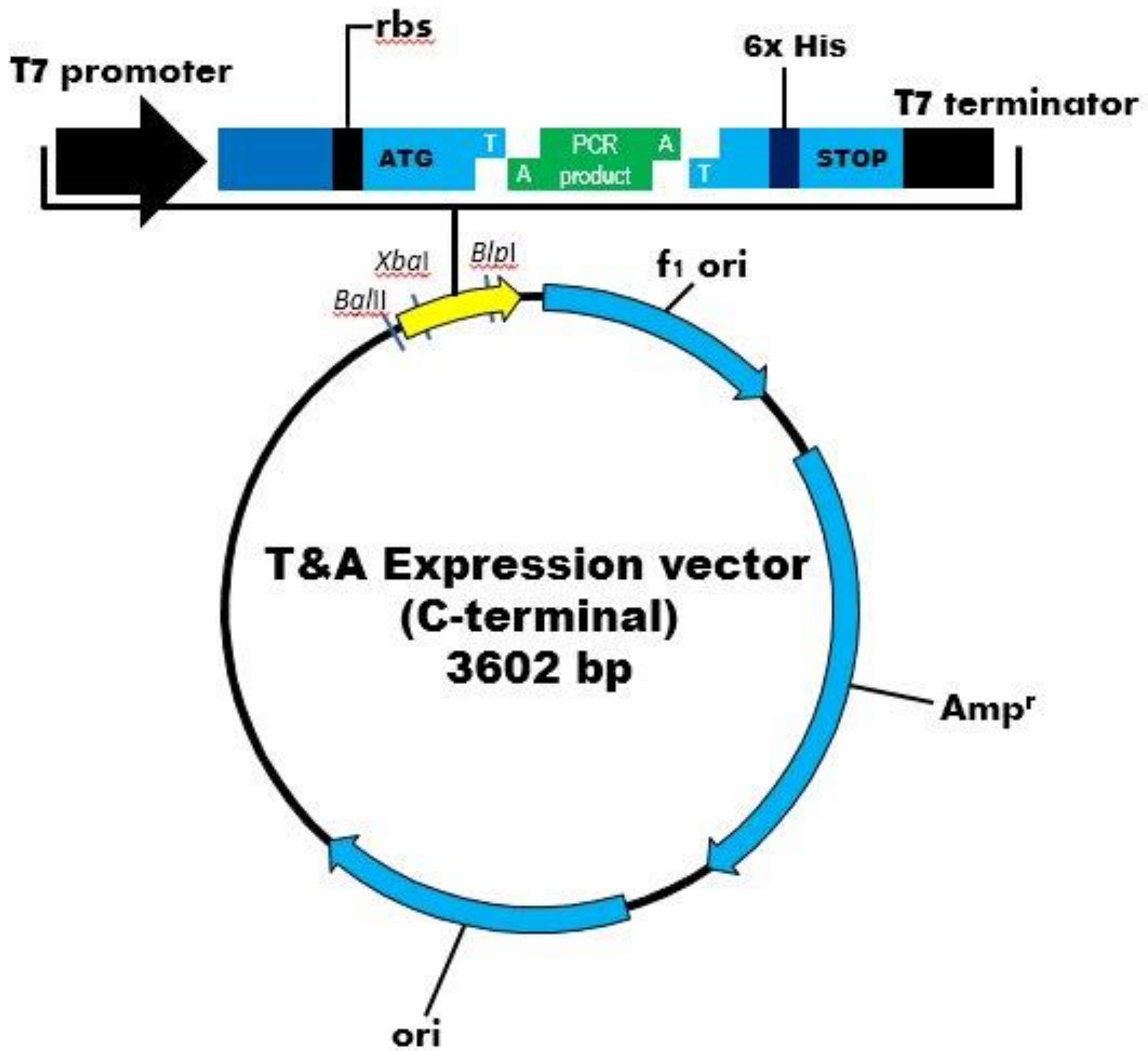
1 Vector map of T&A Expression Vector (C-terminal)



ATG start codon	**3428**
T7 promoter	**3348 ~ 3366**
T7 terminator	**3530 ~ 3577**
6x His (His Tag)	**3446 ~ 3463**
Stop codon	**3464**
Amp^r gene	**599 ~ 1459**
Ori	**1630 ~ 2218**
f1 ori	**12 ~ 467**

2 DNA sequence of multiple cloning sites in T&A Expression Vector (C-terminal)

```
                                            Bgl II                    T7 promoter
3301  CCCGTGGCCA GGACCCAACG CTGCCCGAGA TCTCGATCCC GCGAAATTAA
      GGGCACCGGT CCTGGGTTGC GACGGGCTCT AGAGCTAGGG CGCTTTAATT

                                                    Xba I
3351  TACGACTCAC TATAGGGAGA CCACAACGGT TTCCCTCTAG AAATAATTTT
      ATGCTGAGTG ATATCCCTCT GGTGTTGCCA AAGGGAGATC TTTATTAAAA

                         rbs
3401  GTTTAACTTT AAGAAGGAGA TATACCCATG AAT   Insert   ACCGCTG
      CAAATTGAAA TTCTTCCTCT ATATGGGTAC TTA    DNA     TGGCGAC
                                  Met Asn             ThrAlaG

                       His Tag
3441  GCGAGCACCA CCACCACCAC CACTGAGATC CGGCTGCTAA CAAAGCCCGA
      CGCTCGTGGT GGTGGTGGTG GTGACTCTAG GCCGACGATT GTTTCGGGCT
      lyGluHisHi sHisHisHis HisEnd

                                       Blp I  T7 terminator primer
3491  AAGGAAGCTG AGTTGGCTGC TGCCACCGCT GAGCAATAAC TAGCATAACC
      TTCCTTCGAC TCAACCGACG ACGGTGGCGA CTCGTTATTG ATCGTATTGG

                  T7 terminator
3541  CCTTGGGGCC TCTAAACGGG TCTTGAGGGG TTTTTTGCTG AAAGGAGGAA
      GGAACCCCGG AGATTTGCCC AGAACTCCCC AAAAAACGAC TTTCCTCCTT
```

3 Protocol for ligation using T&A Expression Vector (C-terminal)

3.1 Centrifuge T&A Expression Vector (C-terminal) and PCR DNA samples briefly to collect contents at the bottom of the tubes.

3.2 Vortex the ligation buffers vigorously before use.

3.3 Set up the following items as described below :

	Positive Control	Standard Test
T&A Expression Vector (C-terminal)	2 μl	2 μl
Control Insert DNA (C-terminal)	3 μl	---
PCR product (A-tail) (no stop codon)	---	X μl
10X Ligation Buffer A	1 μl	1 μl
10X Ligation Buffer B	1 μl	1 μl
yT_4 DNA ligase (2U)	1 μl	1 μl
Add deionized water to a final volume of 10 μl		

3.4 Mix the reactions by pipetting.

3.5 Incubate the reactions for 20~60 min at 22℃. Alternatively, if higher transformation efficiency is needed, incubate the reactions overnight at 4℃.

3.6 Transformation with appropriate competent cells according to downstream applications.

4 Protocol for colony PCR

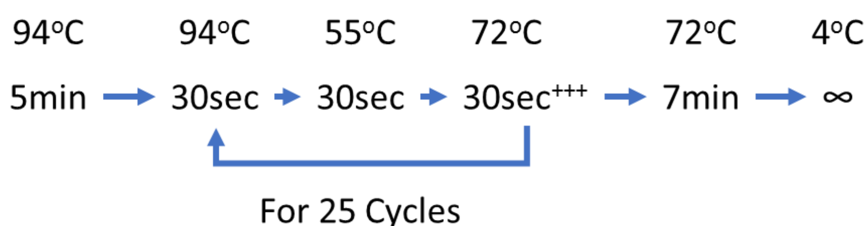
4.1 Prepare 25 μl of PCR reaction buffer in a microfuge tube as described below. Pick a single colony with a sterile toothpick and directly swirl it into PCR reaction buffer as the template.

PCR premixed buffer (O'in1 DNA polymerase premix, FYT201-100P)	23 μl
T7 promoter (10 μM)	1 μl
T7 terminator (10 μM)	1 μl

4.2 To confirm the clone which is correct orientation of the insert, prepare 25 μl of PCR reaction buffer in a microfuge tube as described below. Pick a single colony with a sterile toothpick and directly swirl it into PCR reaction buffer as the template.

PCR premixed buffer (O'in1 DNA polymerase premix, FYT201-100P)	23 μl
T7 promoter (10 μM)	1 μl
Insert Reverse Primer (10 μM)	1 μl

4.3 Set up PCR program

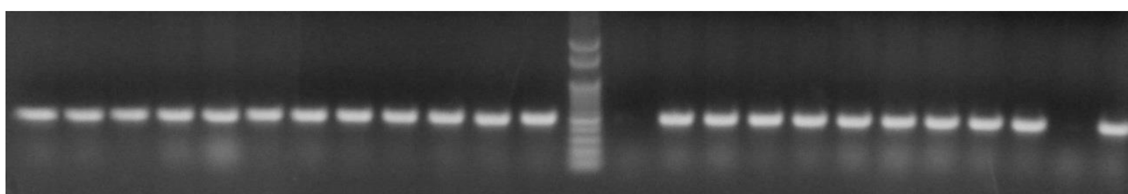


+++The time duration at 72°C is set according to the length of insert DNA.

(In general, DNA polymerase can synthesize 1 Kb of DNA in 1 minute.)

4.4 Run on 1% agarose gel

Example : Using the control Insert DNA provided in the T&A Expression vector kit as the insert DNA. The colony PCR result is shown as below.



* According to different primer designs, the size of colony PCR product seen on the agarose gel will change.

* In this colony PCR, the size of the PCR product on the gel is larger than the insert DNA size by about 190 bp.

Product components

T&A Expression Kit (C-terminal)		Cat. No. FYC201-10P	
Components		Concentration	Volume
HYC201-A01	T&A Expression Vector (C-terminal)	25 ng/μl	20 μl
HYC201-A02	Control Insert DNA (C-terminal)	10 ng/μl	10 μl
HYC201-A03	yT4 DNA Ligase (2U)	2 unit/μl	10 μl
HYC201-A04	10X Ligation Buffer A	10X	50 μl
HYC201-A05	10X Ligation Buffer B	10X	50 μl
HYC201-A06	Primer T7 promoter	10 μM	50 μl
HYC201-A07	Primer T7 terminator	10 μM	50 μl
Storage Condition: -20°C			

T&A Expression Vector (C-terminal)		Cat. No. FYC211-10P	
Components		Concentration	Volume
HYC201-A01	T&A Expression Vector (C-terminal)	25 ng/μl	20 μl
HYC201-A02	Control Insert DNA (C-terminal)	10 ng/μl	10 μl
HYC201-A06	Primer T7 promoter	10 μM	50 μl
HYC201-A07	Primer T7 terminator	10 μM	50 μl
Storage Condition: -20°C			

4.5 Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes by preparing single-use aliquots of the ligation buffers.

4.6 Pfu DNA polymerase possesses proofreading activity; it does not have the terminal transferase-like activity like Taq DNA polymerase. Ligation reactions using Pfu amplified DNA containing no A-tails will result in no positive colonies.

4.7 Methods for increasing the ligation efficiency:

4.7.1 A-tailing

<table>
<tr><td>Purified PCR product</td><td>X μl</td></tr>
<tr><td>10X PCR reaction buffer</td><td>10 μl</td></tr>
<tr><td>10 mM dATP</td><td>2 μl</td></tr>
<tr><td>Taq DNA Polymerase (2.5U/μl)</td><td>1 μl</td></tr>
<tr><td colspan="2">● Add deionized water to a final volume of 100 μl.
● Incubate at 72°C for 1~2 hr.
● Purify the A-tailed DNA and use in the ligation reaction.</td></tr>
</table>

4.7.2 If the maximum of transformants is required, incubate the reaction overnight at 4℃.

4.7.3 The optimal efficiency can be achieved by using a 1:3 molar ratio of vector DNA to the insert DNA.

4.7.4 Use competent cells with higher such as ECOS™ (>10^8 cfu/μg DNA) series for transformation.

5 Restriction enzyme sites of T&A Expression Vector (C-terminal)

Restriction enzymes that ONE Cut T&A Expression Vector (C-terminal)

Enzyme	Position	Enzyme	Position	Enzyme	Position	Enzyme	Position	Enzyme	Position
AccI	2511	Bbr7I	3155	BseYI	1976	HaeIV	1392	PstI	1146
AfeI	3028	BbsI	3155	BsgI	3105	MscI	3311	PvuI	1020
AflIII	2280	BglI	1273	BsmBI	2633	NaeI	143	PvuII	2691
AhdI	1392	BglII	3334	BstZ17I	2511	NgoMIV	143	SapI	2403
AloI	302	BlpI	3525	BtgI	3308	Nli3877I	3330	ScaI	909
AlwNI	1869	Bpu10I	3175	DraIII	249	PciI	2280	StyI	3547
AvaI	3330	BsaHI	851	FspAI	3302	PfoI	2638	Tth111I	2538
BanII	173	BsaXI	300	GdiII	999	PsiI	374	XbaI	3392

Restriction enzymes that DO NOT Cut T&A Expression Vector (C-terminal)

AarI	BbeI	BssHII	FseI	NruI	SacII	SrfI
AatII	BbvCI	BstAPI	HincII	NsiI	SalI	Sse232I
Acc65I	BclI	BstBI	HindIII	PacI	SanDI	Sse8647I
AflII	BfrBI	BstEII	HpaI	PflMI	SbfI	StuI
AgeI	BfrBI	BstXI	KasI	PmeI	SciI	SwaI
AleI	BmgBI	Bsu36I	KpnI	PmlI	SexAI	UthSI
ApaBI	BmtI	ClaI	MfeI	Ppu10I	SfiI	XcmI
ApaI	BplI	EagI	MluI	PshAI	SfoI	XhoI
AscI	BseRI	EcoICRI	NarI	PspOMI	SgrAI	XmaI
AsiSI	BsiWI	EcoNI	NcoI	PsrI	SmaI	ZraI
AvrII	BsmI	EcoRI	NdeI	RleAI	SnaBI	
BaeI	BspMI	EcoRV	NheI	RsrII	SpeI	
BamHI	BsrGI	FalI	NotI	SacI	SphI	

Restriction enzymes that cut T&A Expression Vector (C-terminal) more than 2 times

Name	Position	Name	Position	Name	Position
AceIII	5 sites	BstUI	15 sites	MboI	18 sites
AclI	4 sites	BstYI	9 sites	MboII	8 sites
AcuI	705, 1753	BtsI	966, 994	MlyI	6 sites
AluI	16 sites	Cac8I	15 sites	MmeI	292, 1906, 2090
AlwI	11 sites	CdiI	5 sites	MnlI	19 sites
ApaLI	720, 1966, 2466	ChaI	18 sites	MslI	6 sites
ApoI	431, 442	CjeI	5 sites	MspA1I	7 sites
AseI	1216, 3352	CjePI	6 sites	MwoI	17 sites
AvaII	5 sites	Csp6I	908, 2475	NciI	7 sites
BanI	207, 1439	CviAII	15 sites	NlaIII	15 sites
BbvI	18 sites	DdeI	10 sites	NlaIV	14 sites
BccI	5 sites	DpnI	18 sites	NspI	2280, 2647, 2939
BceAI	204, 1794	DraI	812, 1504, 1523	PleI	6 sites
BcefI	204, 1794	DrdI	295, 2178, 2593	PpiI	746, 302, 1589
BcgI	2692, 871	EaeI	999, 3311	PpuMI	3275, 3317
BciVI	544, 2071	EarI	598, 2402	Psp03I	5 sites
BfaI	7 sites	EciI	1234, 2062, 2208	PspGI	4 sites
Bme1580I	720, 1966, 2466	Eco57MI	4 sites	PssI	3275, 3317, 3552
BmrI	1342, 2536	EcoHI	7 sites	RsaI	908, 2475
BpmI	1302, 2774	EcoO109I	3275, 3317, 3552	Sau96I	10 sites
BpuEI	5 sites	EsaBC3I	4 sites	ScrFI	11 sites
BsaAI	246, 2530	FatI	15 sites	SelI	15 sites
BsaBI	3087, 3339	FauI	8 sites	SfaNI	13 sites
BsaI	1320, 3373	FmuI	10 sites	SfcI	5 sites
BsaJI	2120, 3308, 3547	FokI	8 sites	SimI	9 sites
BsaWI	5 sites	FspI	1167, 3301	SmlI	5 sites
BscAI	13 sites	HaeI	4 sites	SspD5I	9 sites
BseMII	10 sites	HaeII	6 sites	SspI	454, 585
BsiEI	4 sites	HaeIII	12 sites	StsI	8 sites
BsiHKAI	6 sites	HgaI	6 sites	StyD4I	11 sites
BslI	12 sites	Hin4I	1392, 1318, 1392	TaiI	12 sites
BsmAI	4 sites	HinfI	9 sites	TaqI	4 sites
BsmFI	14, 2994	HpaII	17 sites	TaqII	4 sites
Bsp1286I	7 sites	HphI	9 sites	TatI	909, 2476
Bsp24I	1600, 1778	Hpy188I	12 sites	TauI	10 sites
BspCNI	10 sites	Hpy188III	17 sites	TfiI	2305, 2726, 3230
BspEI	3091, 3602	Hpy8I	10 sites	TseI	17 sites
BspHI	552, 1560	Hpy99I	1122, 1385, 2179	Tsp45I	6 sites
BsrBI	102, 548, 2349	HpyAV	8 sites	Tsp509I	8 sites
BsrDI	1151, 1333	HpyCH4I	15 sites	TspDTI	10 sites
BsrFI	143, 1307	HpyCH4III	9 sites	TspGWI	4 sites
BsrI	14 sites	HpyCH4IV	12 sites	TspRI	10 sites
BssSI	723, 2107	HpyCH4V	13 sites	Tth111II	4 sites
BstF5I	8 sites	HpyF10VI	17 sites	UnbI	10 sites
BstKTI	18 sites	LpnI	6 sites	VpaK11AI	5 sites
BstNI	4 sites	MaeIII	15 sites	XmnI	792, 2726

6 Suggestions and notes

6.1 By using the colony PCR technique, clones can be screened easily and precisely.

6.2 Multiple freeze-thawing DOES NOT affect the quality of T&A Expression Vector, but exposure to frequent temperature changes will degrade ATP resulting in poor ligation.

6.3 For questions not addressed here, please visit our web site for details. http://www.yeastern.com