B24101

Baran Biotech

pUC-T Vector

For T/A Cloning

Overview

T/A cloning is a subcloning technique that avoids the use of restriction enzymes and is easier and quicker than traditional subcloning. The technique relies on the ability of adenine (A) and thymine (T) (complementary basepairs) on different DNA fragments to hybridize and, in the presence of ligase, become ligated together. This technique takes advantage of the terminal transferase activity of Taq DNA polymerase and other non-proofreading thermostable DNA polymerases. Such enzymes add a single 3'-A overhang to both ends of the PCR product. Such PCR amplified inserts are cloned into linearized vectors that have complementary 3' thymine overhangs (T vector). After the ligation mixture is transformed into competent E. coli cells, recombinant clones can be selected by methods such as blue/white screening.

Baran pUC-T vector is a kind of pUC derivative T vector that is ready to use for efficient ligation with PCR products. The vector contains a LacZα sequence that facilitates the selection of target clones through blue and white colony screening. Multiple restriction sites beside inserted fragments make it easy for screening target clone by double digestion. Inserted fragments also could be sequenced using universal primers M13 forward and reverse. The map and the multiple cloning site of pUC-T vector are presented in Fig.1 and Fig.2.

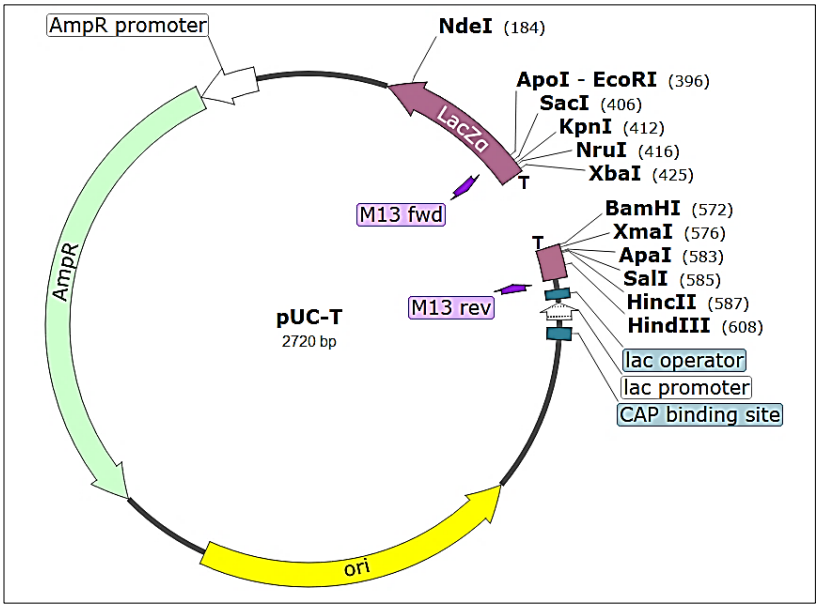

Fig.1 Map of pUC-T vector. Some unique common restriction sites in MCS are indicated.

Restriction enzymes, that do not cut pUC-T vevtor:

AfeI, AflII, AgeI, AleI, AscI ,AsiSI ,AvrII, BaeI ,BbsI, BbvCI, BclI, BfuAI, BglII, BlpI, BmgBI, BmtI, Bpu10I, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BspDI, BspEI, BspMI, BsrGI, BssHII, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FseI, HpaI, I-CeuI, I-SceI, MfeI, MluI, MscI, NaeI, Nb.BbvCI, NcoI, NgoMIV, NheI, NotI, Nt.BbvCI, PacI, PaeR7I, PaqCI, PflFI, PI-PspI, PI-SceI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspXI, RsrII, SacII, SbfI, SexAI, SfiI, SgrAI, SnaBI, SpeI, SrfI, StyI, SwaI, Tth111I, XcmI, XhoI

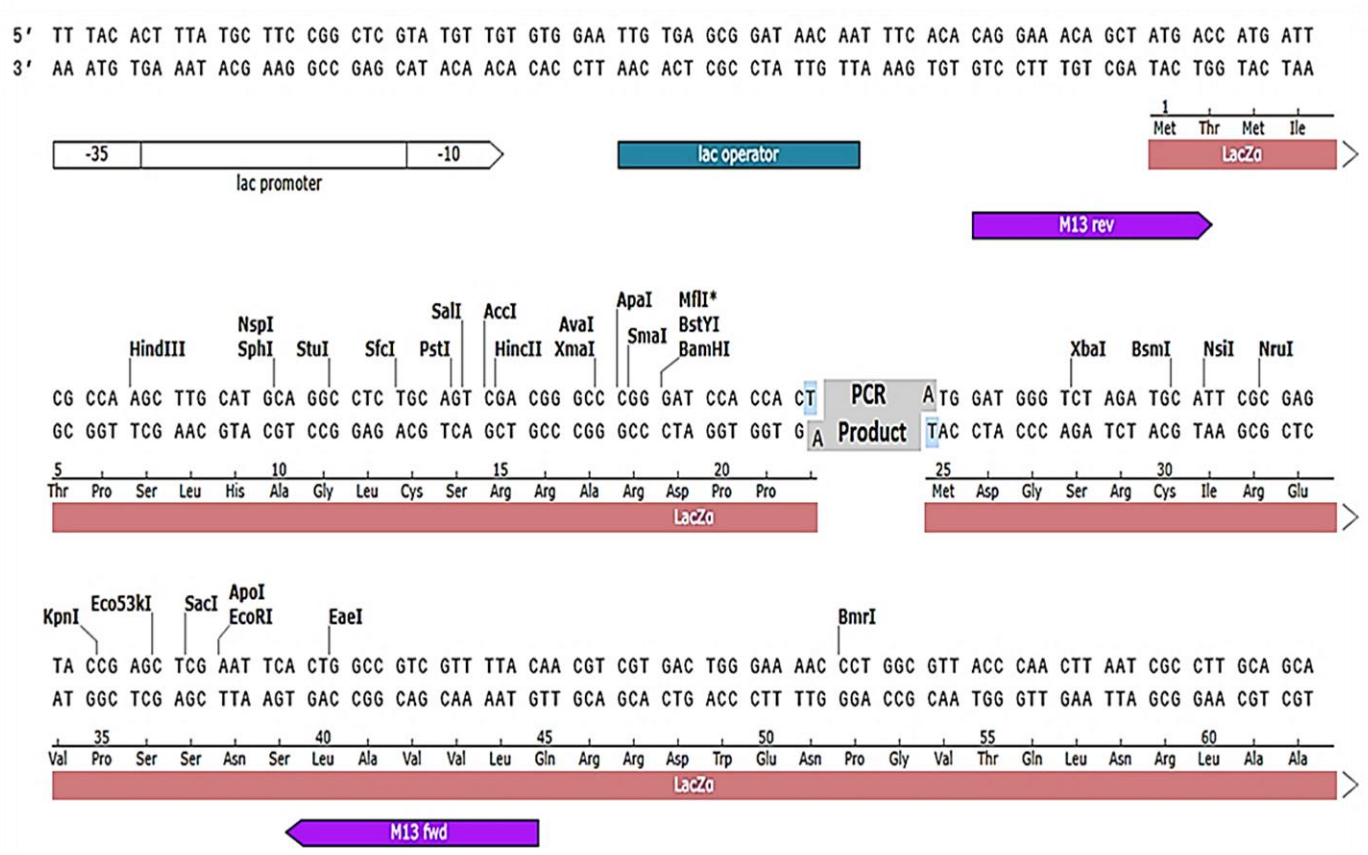

Fig.2 Promoter and multiple cloning site of pUC-T vector

Restriction enzymes, that cut pUC-T once:

	Enzyme	Cut position		Enzyme	Cut position
1	AatII	2654/2650	27	KpnI	412/408
2	Acc65I	408/412	28	NarI	236/238
3	AccI	458/460	29	NdeI	184/186
4	AflIII	839/843	30	NmeAIII	1881/1879
5	AhdI	1732/1731	31	NruI	416
6	AlwNI	1255/1252	32	NsiI	424/420
7	ApaI	455/451	33	PciI	839/843
8	ApoI	396/400	34	PflMI	438/435
9	AvaI	448/452	35	PluTI	239/235
10	BamHI	444/448	36	PspOMI	451/455
11	BcgI	2237/2235+2271/2269	37	PstI	466/462
12	BpmI	1802/1800	38	SacI	406/402
13	BsaI	1793/1797	39	SalI	457/461
14	BsaXI	684/681+714/711	40	SapI	723/726
15	BseYI	1143/1147	41	ScaI	2212
16	BsmI	424/422	42	SfoI	237
17	BsoBI	448/452	43	SmaI	450
18	BspQI	723/726	44	SphI	478/474
19	BsrFI	1812/1816	45	SspI	2536
20	BstAPI	185/182	46	StuI	470
21	Eco53kI	404	47	TspMI	448/452
22	EcoO109I	2708/2711	48	XbaI	425/429
23	EcoRI	396/400	49	XmaI	448/452
24	HincII	459	50	XmnI	2331
25	HindIII	480/484	51	ZraI	2652
26	KasI	235/239			

Kit Components Item Quantity

Item	Quantity
pUC-T linear vector	30 µL

Store at -20°C. Do not freeze and thaw frequently

Important Notes Before Ligation Reaction

- The final extension step in PCR should be prolonged to 20-30 minutes to ensure efficient 3'-dA tailing of the PCR product.
- Analyze the PCR products by electrophoresis for specificity and yield before cloning. If PCR products are very specific and appear as one discrete band on the gel, purification step is not necessary.
- If the PCR product is contaminated by non-specific PCR products or primer dimers or if a plasmid with ampicillin resistance marker is used as a template, it is necessary to gel purify PCR product.
- The optimal insert/vector molar ratio is 3:1. The recommended amounts of inserted DNA (usually a purified PCR product) for the ligation reaction based on its length in base pairs (bp) are shown in the table below, which can be calculated by the following equation:

$$mass\ of\ insert = \frac{mass\ of\ vector \times length\ of\ insert(bp) \times 3}{length\ of\ vector\ (bp)}$$

Length of Insert DNA (bp)	Optimal Insert quantity
100	13 ng
200	26 ng
300	38 ng
400	52 ng
500	65 ng
750	98 ng
1000	130 ng
1500	194 ng
2000	257 ng

- If using the unpurified PCR product, do not use more than 3 µL of it in the 20 µl ligation mixture.
- Before opening, gently tap the pUC-T vial and then spin it.

Recommended T/A Cloning Protocol Using pUC-T Vector:

1. Set up the ligation reaction:

Component	**Volume**
pUC-T Vector (38 ng/µL)	3 µL
10X Ligation Buffer	2 µL
50% PEG4000	2 µL
Insert DNA*	Variable (3:1 molar ratio to vector)
Water, nuclease-free	Up to 20 µL
T4 DNA Ligase	1 µL
Total volume	**20 µL**

2. Mix and spin briefly.

3. Incubate the ligation mixture for overnight at 4-8°C.

4. Transform the ligation mixture into chemically competent E.coli cells (Heat shock method).

5. Do blue/white screening using LB-ampicillin X-Gal/IPTG agar plates.

The entry of the foreign DNA fragment into the vector causes a shift in the LacZα gene reading frame. Therefore, when the bacterium containing the recombinant vector grows on the plate containing X-Gal and IPTG, because it is not able to break X-Gal, it forms a white colony.