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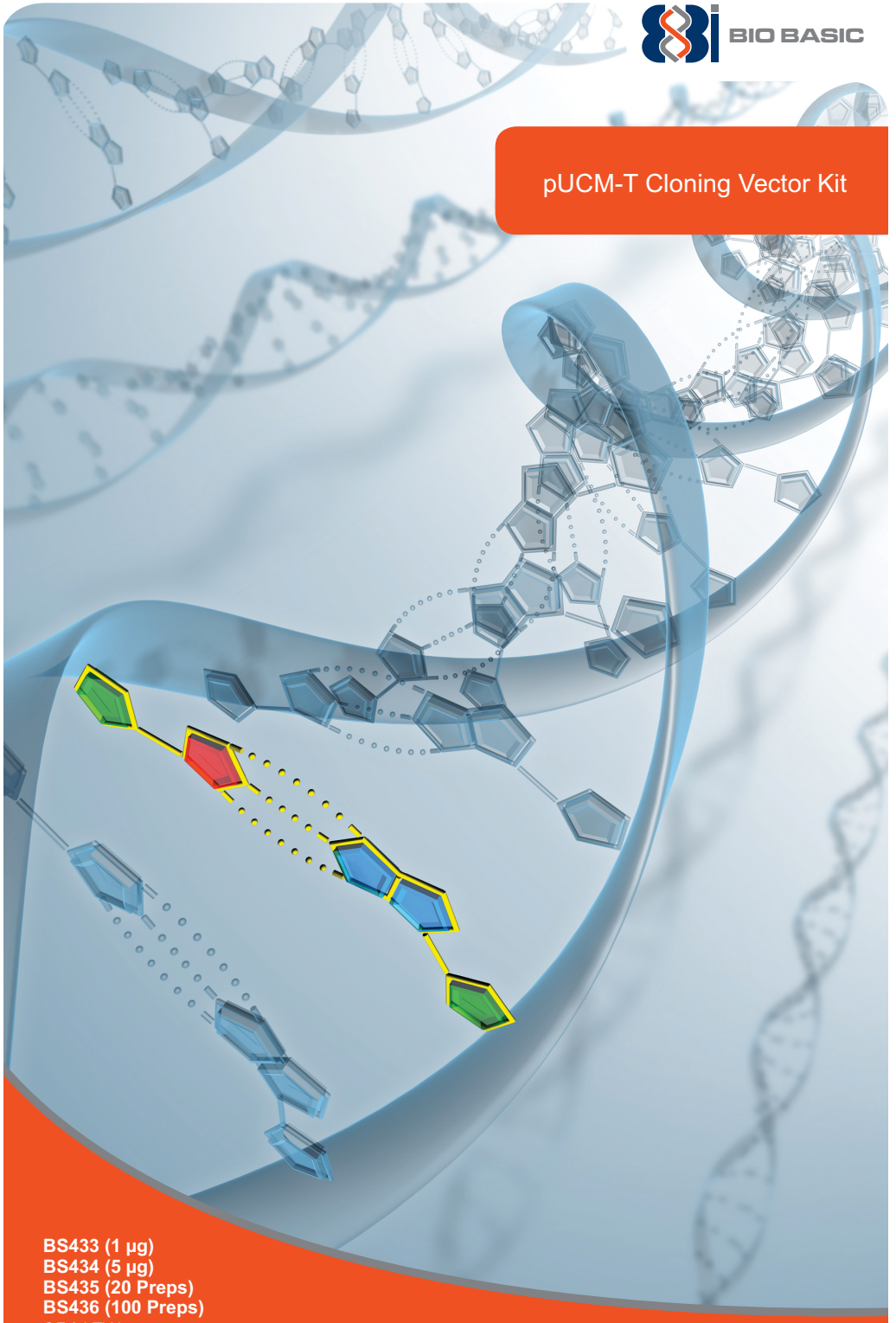
# pUCM-T Cloning Vector Kit



**BS433 (1 µg)**
**BS434 (5 µg)**
**BS435 (20 Preps)**
**BS436 (100 Preps)**


*QF 24 TV4*
*CV1 2020*


***For Research Use Only***

# *pUCM-T Cloning Vector Kit*

## *Code: BS433 (1 μg)*
## *BS434 (5 μg)*
## *BS435 (20 Preps)*
## *BS436 (100 Preps)*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The T-Vector PCR Product Cloning Kit is suitable for cloning of PCR products with additional A at 3' end. The special ligation system provided by the kit enables customs to finish ligation in 1-2 hours.

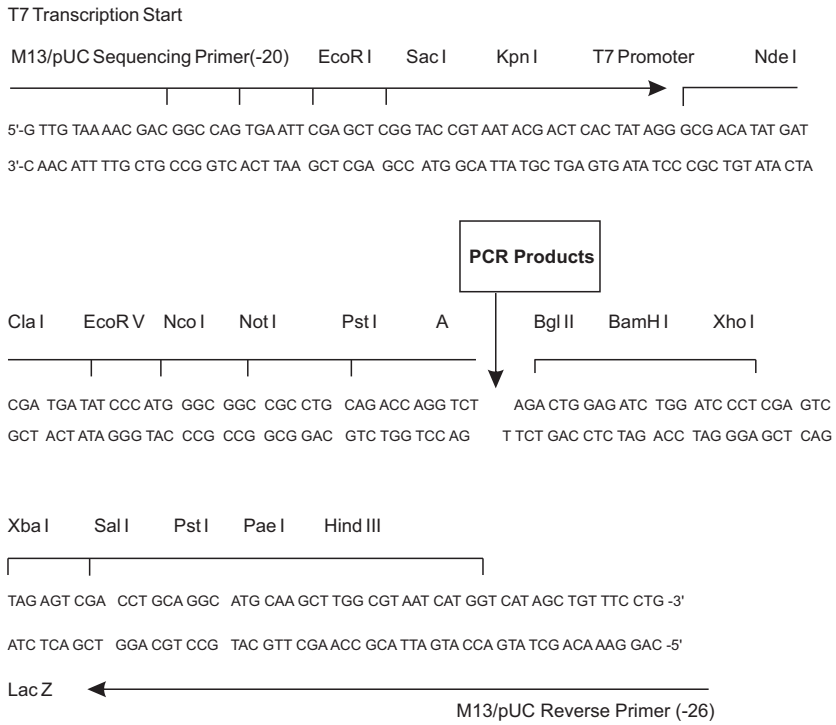
pUCm-Tvector of our company isdesign for simplifing cloning of PCR products. Many thermal stable DNA polymerase, PCR products amplified by DNApolymerase such as Taq, Tth DNA produce additional A at 3' end which could be easily ligated to T vector with additional T.

pUCm-T of our company is a kind of noval pUC derivative T vector, the mutiple restrict sits with most of them single site and adjusted b-galatose reading frame make it easily for screening target clone through blue and white plaque. Specially designed two Pst I sites beside inserted fragments make it easy for screening target clone by Pst I digestion, at same time inserted fragments also could be screened by cheap and efficient restrict enzymes such as EcoR I and Hind III double digestion. Inserted fragmentsalso could be sequencedusing universal primers M13 and T7 promoterprimer. In vitro transcription could be processed through site of T7 RNA polymerase promotor in pUCm-T.

Technical materials of our product are displayed at the end of protocol, the complete sequence of our T vector are same as pUC19 (GenBank Accession Number M77789) except difference at multiple cloning site.

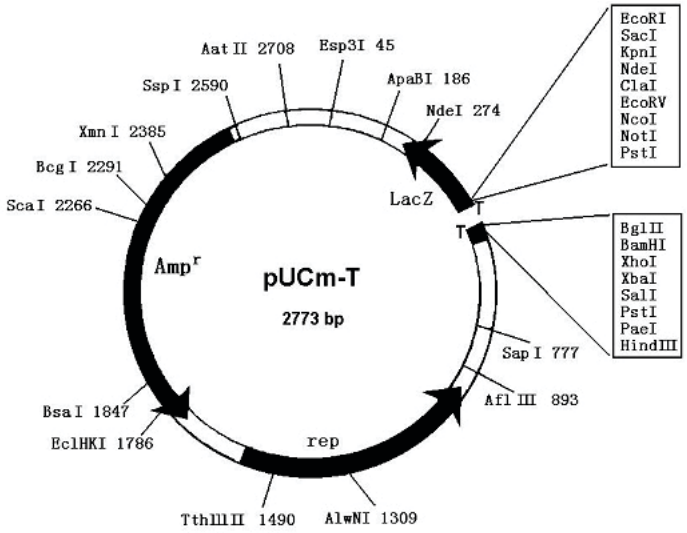


## B. Promoter and multiple cloning sites of pUCm-T vector



## Appendix

### A. Map of pUCm-T vector




**Plasmid name: pUCm-T**
**Plasmid size: 2773 bp**
**Comments/References: Used for T/A cloning**



## Kit Contents

| Components | BS433 | BS434 | BS435 | BS436 |
|---|---|---|---|---|
| pUCm-T vector (50 ng/ul) | 1 μg | 5 μg | 1 μg | 5 μg |
| 10x Ligation Buffer | - | - | 50 μl | 200 μl |
| 50% PEG4000 | - | - | 50 μl | 200 μl |
| T4 DNA ligase, 5U/ul | - | - | 100 U | 500 U |
| Sterilized dd$H_2O$ | - | - | 1 ml | 1 ml |

## Storage

All contents of the kit should be kept in freezer after usage. The kit is stable for 9 months.

## Preparation of Ligation Reaction

Purification of PCR products or not depends on qulity of amplified product. If PCR products are very specific, purification of PCR products is not necessary. But if plasmids were used as template, it is necessary to purify PCR product, because plasmid template could form white colony after transformation. PCR products could be separated by argarose electrophoresis. PCR product gel extraction kit of our company (BS353/BS354) could recover >60 bp DNAfragment efficiently.

PCR products amplified by *Taq, Tth*, *AmpliTaq*, *KlenTaq* DNA polymerase bear additional A at 3'end. *Taq* DNA polymerase co-amplify with *Pfu, Pwo, Tli* or *Deep vent DNA polymerase which posses* 3→5 exonuclease activity may bear a additional A at 3' end. PCR products posses additional A at 3' end could be ligated to pUCm-T. PCR products amplified by DNA polymerase with 3'→5 exonuclease activity is blunt end, cloning of this kind of fragments need to add additional A to blunt end.

A-Tailing Kit (BS513) of our company could be used for efficiently to add additional A to 3' end.

## Ligation Reaction

1. In a standand ligation 10 µl reaction system, 50ng (1 µl) pUCm-T vector, 0.2 pmol PCR product, 1 µl 10 x ligation Buffer and 1 µl T4 DNAligase, add dd$H_2O$ to 10 µl final volume, usually it is not necessary to quantify PCR products. Ligation reaction could be manipulated as followings:

| | |
|---|---|
| **1µl** | **10x Ligation Buffer** |
| **1µl** | **50%PEG** |
| **1µl** | **pUCm-T vector** |
| **Xµl** | **purified PCR product** |
| **Yµl** | **Sterilized water** |
| **1µl** | **T4 DNA Ligase** |
| **Final Volume 10µl** | |

**NOTE:** Add T4 DNA ligase at last step.

2. Ligation for 1 hour to overnight at 16~23ºC.

**NOTE:** Usually 1 hour is sufficient for normal experiment

## Transformation

Suggest using Competent Cell Preparation Kit (BS523/524 or BS525/526)

1. Put 100 µl competent cell thawed on ice, gently mix well after complete thawed.
2. Add 5 µl ligation mixture, gently mix well. Put on ice for 30 minutes.
3. Heat shock at 42ºC on water bath for 1 minute. Put on ice for 2 minutes.
4. Add 400 µl SOC culture shake at 37ºC, 200-250 rpm for 1 hour.
5. Centrifuge at 4000 rpm for 5 minutes, discard 400 µl supernatant using pipette, resuspend the cells with rest of culture.



6. Plate bacteria at Amp+ plater which had been plated with 20 µl 100 mM IPTG and 100 µl 20 mg/ml X-gal.

**NOTE:** Quantity of bacteria used depends on efficiency of ligation and transformation

7. Discard surplus liquid after put the plate upward for 1 hour at 37 ºC, put downward and culture overnight.

## Screening

Screened transformants by blue/white colony.

When foreign DNA fragment were cloned into pUCm-T, reading frame of LacZ gene coding sequence was changed by insertion of foreign DNA, thus the activity of a-fragment was affected, recombinant clone on X-gal/IPTG plater display which colony at same time non recombinant display blue colony. Select which colony on IPTG/X-gal plater, transfer colony to Amp+ liquid with toothstick and culture overnight at 37ºC.

## Identification of Transformants

1. Extract plasmid from liquid culture of white colony, digest plasmid by PstI or other suitable restriction enzyme, and check the size of inserted fragment in order to decide whether the clone contains target fragment.

2. The sequence of target clone is determined by M13 universal primer or other suitable primer to further confirm the inserted fragment.