



TA Cloning Kit with HIT DH5a Competent Cells

Catalog # RC001-C

Composition	RC001-C
pUCm-T vector (50ng/ul)	1 ug/20ul
10× Ligation Buffer	50 µl
50% PEG4000	50 µl
T4 DNA Ligase (5U/ul)	100U
Sterilized ddH ₂ O	1 ml
HIT DH5a Value 10 ⁷ Cells	20 x 100 ul

Storage: All contents of the kit should be kept at -20°C with the exception of competent cells which should be stored at -80°C. The Kit is stable for 9 months.

Introduction

The TA Cloning Kit is suitable for cloning of PCR products with an additional A at the 3' end. The unique ligation system provided with this kit enables the ligation of a PCR product in 1 hour.

The pUCm-T vector is specifically designed to simplify cloning of PCR products produced by DNA polymerase. To ensure full efficiency **UBI Recombinant Taq DNA Polymerase (Catalog # Taq-01)** is recommended, but successful ligation can be obtained when using any thermally stable DNA polymerase with no exonuclease activity.

The pUCm-T is a novel pUC derivative T vector, with multiple single site restriction sequences and an adjusted β-galactose reading frame to increase blue and white target clone screening. This vector contains two specially designed *Pst* I sites that flank the multiple cloning site (MCS) making it easy for screening the target clone by *Pst* I digestion. In addition other restriction sequences such as *EcoR* I and *Hind* III are present so cost saving yet efficient restriction enzymes can be used. M13 and T7 regions are also present in the pUCm-T vector so sequencing reaction can be conducted using the whole plasmid. In vitro transcription can also be induced utilizing the T7 RNA polymerase promoter present in the pUCm-T vector.

The complete sequence of the pUCm-T vector are identical to the pUC19 vector (GenBank Accession Number M77789) with the exception of differences at the multiple cloning site. For reference this sequence as well as a vector map are provided at the end of this protocol.

Preparation of ligation reaction

Purification of PCR products may or may not be required depending on the quality of the amplified product. If PCR products are specific, purification is not required. If plasmid sequence was used as a template, it is necessary to purify PCR product as plasmid template potentially may form white colonies after transformation resulting in false positives. PCR products should be separated electrophoretically, and

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fragment of interest excised from the gel. The excised fragment should subsequently be cleaned using the **E.Z.N.A. Gel Extraction Kit (Catalog # D2500-01/02)** for maximum efficiency.

PCR products amplified using *Taq*, *Tth*, *AmpliTaq*, *KlenTaq* DNA polymerase bear an additional A at 3' end. *Taq* DNA polymerase co-amplified using *Pfu*, *Pwo*, *Tli* or *Deep vent* DNA polymerase which possess 3→5' exonuclease activity are typically blunt ended and will require the addition of an additional A to the 3' end. This can be accomplished using UBI's A-Tailing Kit (Catalog # ATK-01).

Ligation Reaction

1. A standard 10µl ligation reaction will contain 50ng (1µl) pUCm-T vector, **0.2 pmol PCR product**, 1µl 10× Ligation Buffer, 1µl 50% PEG and 1µl T4 DNA Ligase, and ddH₂O to a final volume of 10 µl.

1µl	10× Ligation Buffer
1µl	50% PEG
1µl	pUCm-T vector
Xµl	0.2 pmol PCR Product
Yµl	Sterilized water
<u>1µl</u>	<u>T4 DNA Ligase</u>
Final Volume 10µl	

Note: Add T4 DNA ligase at after addition of all other components.

2. Incubate for a minimum of 1 hour to overnight at 16~23°C.

Note: Usually 1 hour is sufficient for most ligation reactions.

Transformation

We recommend using HIT DH5 alpha Competent Cells from UBI (Catalog # RH617) for maximum efficiency.

1. Place tube containing 100µl of HIT DH5a competent cells on ice, and allow to thaw to 1/3rd thawed.
2. Add 5µl of ligation mixture, and vortex to mix.
3. Incubate on ice for 1 to 10 minutes.
4. Plate cells on Amp⁺ plates which have had 25µl of 100 mM IPTG and 100µl of 20 mg/ml X-gal.
5. Place Glass Plating Beads (Catalog # RG001) on to plate and shake for 5 minutes to spread cells*.
6. Incubate plates in an inverted position overnight at 37°C.

* - Utilization of a standard spread bar is also acceptable except a decrease in plate coverage will be observed.

Appendix

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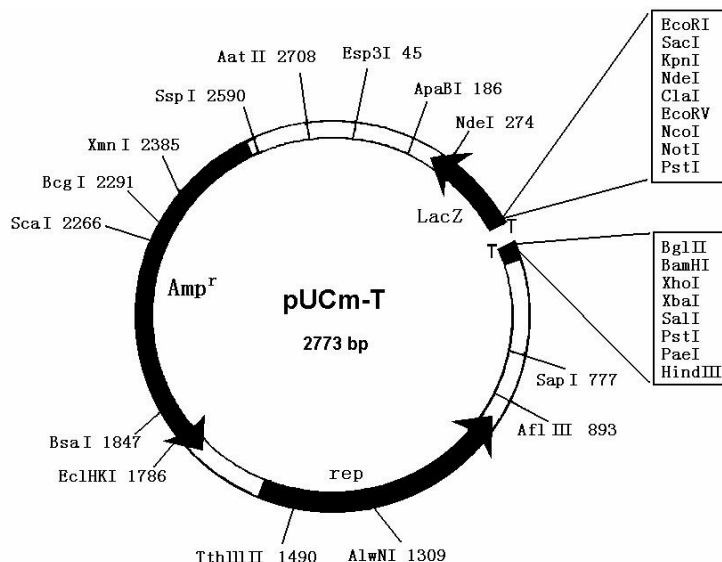
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Plasmid name: pUCm-T
 Plasmid size: 2773 bp
 Comments/References: Used for T/A cloning

Map of pUCm-T vector

