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TEDA Cloning of ACTR5 Overexpression Vector

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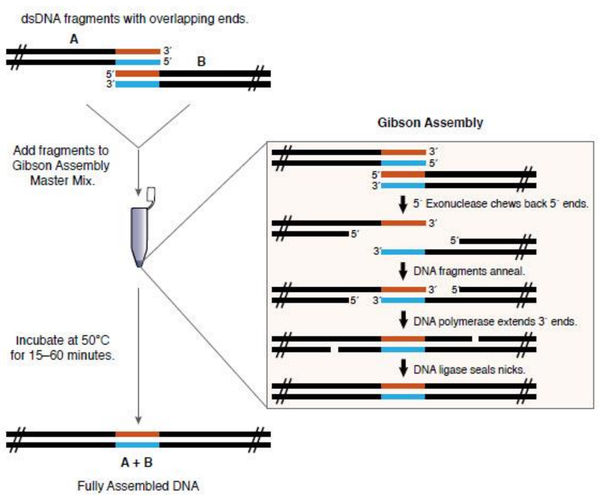
Abstract

ACTR5 is a core component of INO80 chromatin remodeler. It is crucial for the complex's function in histone exchange and sliding.[1] Previous experiments suggest it is oncogenic in liver cancer, possibly regulating histone acetylation and transcription activation[1]. However, the detailed mechanism is unknown. Here, an overexpression plasmid of ACTR5 is constructed to facilitate future studies. In the cloning procedure, improvements were made on reverse transcription and vector-insert assembly. Specifically, priming the mRNA extract with oligo-dT, rather than random primers, allows successful cDNA generation. For the assembly, the original system depending on the 5' exonuclease activity of T4 DNA Polymerase is replaced by TEDA (T5 exonuclease DNA assembly) cloning[2]. The latter is a modification based on Gibson Assembly and SLiCE[3]. This cloning provides a protocol that is more stable, convenient, and cheaper[2], which may be applied in the construction other expression vectors.

Introduction

Gibson Assembly, SLiCE and TEDA Cloning



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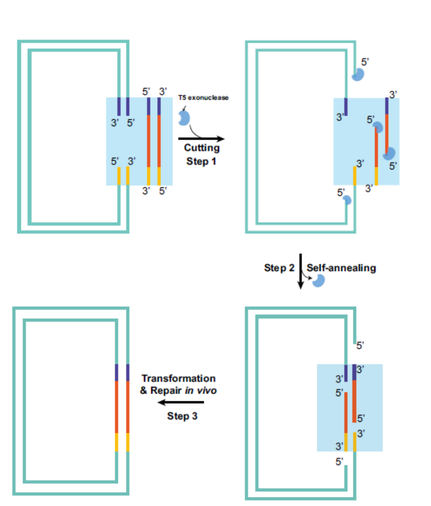
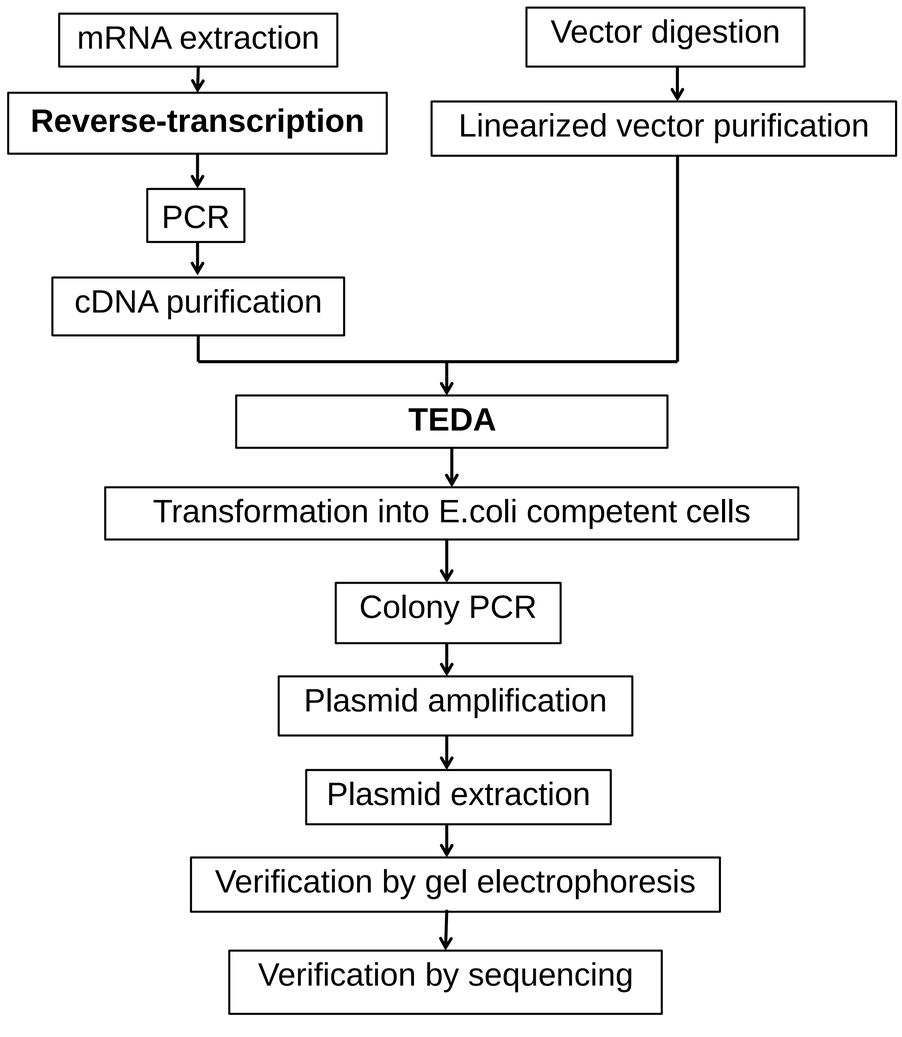


Retrieved from: Figure 1, Xia, Y.; Li, K.; Li, J.; Wang, T.; Gu, L.; Xun, L. T5 Exonuclease-Dependent Assembly Offers a Low-Cost Method for Efficient Cloning and Site-Directed Mutagenesis. *Nucleic Acids Research* **2018**, *47* (3).

	Gibson Assembly	SLiCE	TEDA
5' exonuclease digestion	In vitro	In vitro	In vitro
Gap-filling	In vitro	In vitro	In vivo
Ligation	In vitro	In vitro	In vivo
Note	All 3 enzymes required in vitro	Enzymes extracted from bacterial lysate	Use only 5' exonuclease

Materials and methods



Results

1. PCR to obtain ACTR5 cDNA

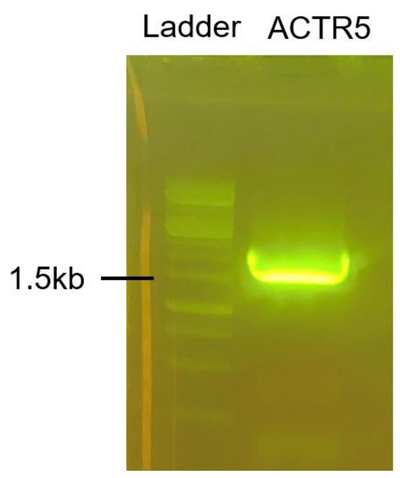


Figure 1

2. Colony PCR

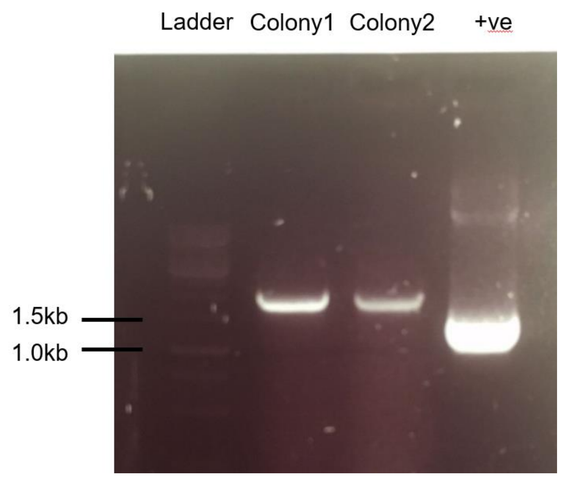


Figure 2

3. Verification on plasmid size

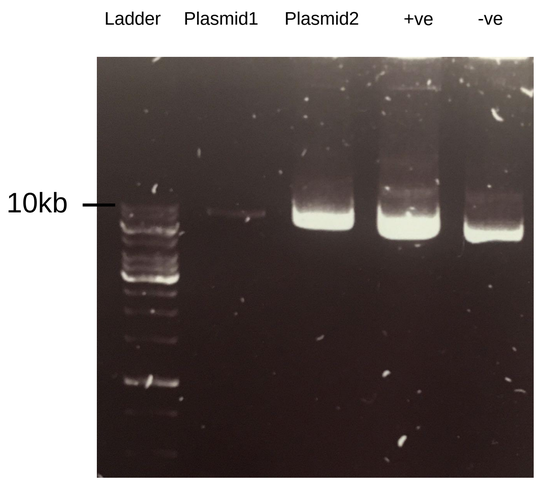


Figure 3

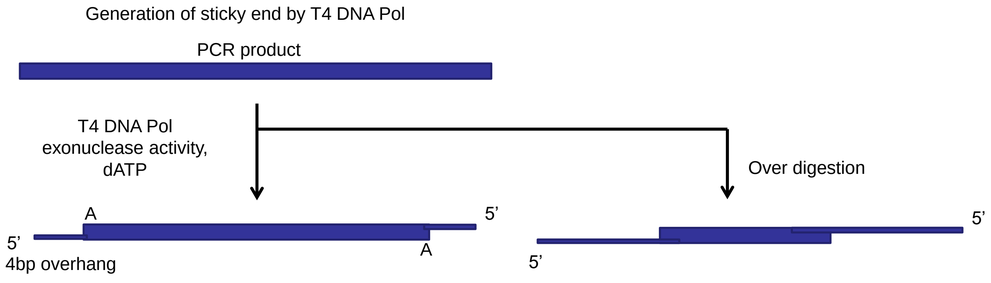
Figure1: PCR product. cDNA is ~1.5kb.

Figure 2: Colony PCR screening for successful insertion. Primers flank the insertion site. Negative colonies (not shown) would have band sizes of 250bp, positive colonies have bands similar to insert sizes. +ve: positive control, previously cloned vector with an insert of 1kb

Figure 3: plasmids were run on agarose gel. –ve: negative control, empty vector, which is 10kb. +ve: positive control, previously cloned vector with 1kb insert.

Discussion

1. Improvement on reverse transcription: randomly primed mRNA may result in truncated cDNA because the primed position may occur within the target gene.
2. Improvement on assembly: generation of sticky end in insert by T4 DNA Pol.



Limitation: requires accurate control of T4 DNA Pol activity.

Advantage of TEDA cloning:
-More stable compared to T4 DNA Pol.
-Cheaper than Gibson assembly because only 1 enzyme (T5 exonuclease) is used at low concentration in vitro. Gap-filling and ligation is done in E.coli cells.
-Easier to prepare and longer storage than the SLiCE extract.

References

1. Tosi, A.; Haas, C.; Herzog, F.; Gilmozzi, A.; Berninghausen, O.; Ungewickell, C.; Gerhold, C. B.; Lakomek, K.; Aebersold, R.; Beckmann, R.; Hopfner, K.-P. Structure and Subunit Topology of the INO80 Chromatin Remodeler and Its Nucleosome Complex. *Cell* **2013**, *154* (6), 1207–1219.

2. Xia, Y.; Li, K.; Li, J.; Wang, T.; Gu, L.; Xun, L. T5 Exonuclease-Dependent Assembly Offers a Low-Cost Method for Efficient Cloning and Site-Directed Mutagenesis. *Nucleic Acids Research* **2018**, *47* (3).

3. Zhang, Y.; Werling, U.; Edelmann, W. SLiCE: a Novel Bacterial Cell Extract-Based DNA Cloning Method. *Nucleic Acids Research* **2012**, *40* (8).