Constructing Genomic Libraries Using the pGEM®-T Vector

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An efficient method for constructing a genomic DNA library is presented using a thymidine (T)-tailed cloning vector. The method is based on ultrasonic cleavage of genomic DNA, blunting of the fragment ends with mung bean nuclease and addition of a single 3′-adenine nucleotide with Taq *DNA polymerase followed by ligation into the pGEM®-T Vector[(a)]. The method is especially useful for genomic DNA that is poorly digested with restriction enzymes due to, for example, DNA methylation, polysaccharides or tightly bound proteins.*

Introduction

Cloning of desired genes is dependent upon the quality of the genomic library. The quality of genomic libraries deteriorates when the prepared genomic DNA contains polysaccharides (1) or is methylated excessively (2). For example, the genomic DNA of some cyanobacterial species is poorly digested by most restriction enzymes, even with DNA purified using cesium chloride gradients and ultracentrifugation. Some restriction enzymes work, but often digestion across the whole span of the genomic DNA cannot be guaranteed due to interference by polysaccharides or excessive methylation. Hence a better strategy to construct genomic libraries not involving restriction enzyme digestion is needed (3). The method presented here consists of sonication of the genomic DNA, blunting of the fragment ends with mung bean nuclease, adding a single 3′-adenine nucleotide with *Taq* DNA polymerase and ligating into the pGEM®-T Vector System[(b)] (Cat.# A3600) (Figure 1). This article describes the details of our method, which we call the "T-vector method." To demonstrate the method, we used genomic DNA from *Spirulina platensis*, a typical filamentous cyanobacterium that is well known for the resistance of its DNA to restriction enzyme digestion (4).

Preparation of Genomic DNA

Genomic DNA was isolated from *S. platensis* (strain M-135, University of Tokyo) using the Wizard® Genomic DNA Purification Kit (Cat.# A1120).*

The protocol provided with the Wizard® Genomic DNA Kit (5) (**www.promega.com/tbs/**) was used with a minor modification. Cells were incubated with 10mg/ml lysozyme (Sigma) in 50mM EDTA at 37°C for one hour prior to the addition of Nuclei Lysis Solution, because *Spirulina* possesses a strong cell wall. This modification improved DNA yield by at least 30%. Table 1 shows DNA yields from various culture preparation volumes. The genomic DNA was then sonicated (Figure 2) and made blunt as previously described (3).

Table 1. Extraction of Total DNA from *Spirulina platensis* Using the Wizard® Genomic DNA Purification Kit.

Amount of Cell Pellet (µl)*	Yield of DNA (µg/µl pellet)	Purity (A_{260}/A_{280})
5	0.16	2.00
25	0.12	1.87
50	0.19	1.86
100	0.15	1.84

*A culture of 1ml with absorbance (560nm) of 1.0 yields about 21µl pellet.

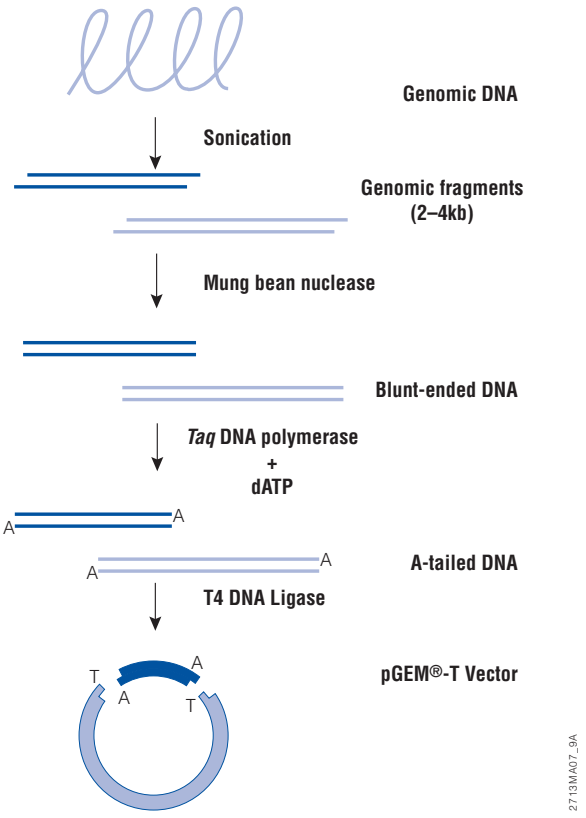

Figure 1. Overview of genomic DNA library construction using pGEM®-T Vector.

*We tested other commercially available genomic DNA purification kits, including SepaGen (Sanko Junyaku), Nucleon™ Phytopure™ (Scotlab Ltd.) and QIAamp Tissue Kit (Qiagen), as well as a conventional CsCl ultracentrifugation method. Only the Wizard® Genomic DNA Kit yielded DNA suitable for restriction enzyme analysis and as template for PCR[(c)].

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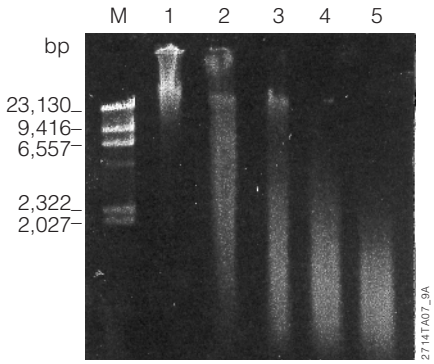

Figure 2. Ultrasonic cleavage of genomic DNA from *Spirulina platensis*. Each lane contains 100ng of genomic DNA. Lanes 1–5, sonication (50 watts) times of 0, 5, 15, 30 and 60 seconds. Lane M, Lambda DNA/*Hin*d III Markers (Cat.# G1711).

Modification of 3′ Ends with *Taq* DNA Polymerase

We created the adenine (A) overhangs at the 3′-blunt ends using the template-independent terminal transferase activity of *Taq* DNA polymerase. The reaction was performed with 1 unit of enzyme per microgram DNA in a total volume of 100µl at 70°C for one hour. The reaction buffer consisted of 2mM dATP, Tris-HCl (pH 8.3), 50mM KCl and 1.5mM $MgCl_2$. These modifications increase the transformation efficiency and greatly decrease the occurrence of self-ligation of the insert in the same manner that the T-tailed vectors are much more resistant to self-ligation.

Ligation with pGEM®-T Vector and Transformation

For ligation of DNA fragments, 50ng (about 25fmol) of pGEM®-T Vector were mixed with fragments at a 1:1 molar ratio and incubated at 15°C for 4 hours with T4 DNA Ligase. The ligation products were collected with silica beads and dissolved in 10µl TE buffer. One microliter (approximately 2.5fmol) of the solution was used for electrotransformation (Gene Pulser® II, Bio-Rad) using commercially available competent cells. Recombinants were identified by blue/white selection.

Summary

From this library we were able to clone the phyotene synthase gene, *crtB*, and part of the phyotene desaturase gene, *crtP*, from *S. platensis* (DDBJ Accession No. AB001284) (8). Ligation with blunt ends is less efficient than ligation with cohesive ends; therefore, most researchers prepare cohesive ends by addition of appropriate adapters to blunt ends of both inserts and vector. This process involves inefficient ligation with blunt ends and requires large amounts of adapters. We used the template-independent terminal transferase activity of *Taq* DNA polymerase to convert the 3′-blunt ends of the genomic DNA fragments into 3′-A overhang ends. This modification increased transformation efficiency (data not shown) and also protected the inserts against self-ligation. In our method, we added the 3′-A overhang to blunt ends, using only dATP as the substrate for *Taq* DNA polymerase.

This ligation method, which we call the T-vector method, is useful for constructing a genomic library when the genomic DNA is not amenable to restriction enzyme digestion. Blunting of the DNA fragments prior to addition of 3′-A with *Taq* DNA polymerase is necessary because ultrasonic cleavage of DNA yields fragments with staggered ends. This method should also be applicable to genomic library construction with phage vectors.

References

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Ordering Information

Product	Size	Cat.#	Price ($)
Wizard® Genomic DNA Purification Kit	100 isolations	A1120	
	500 isolations	A1125	
Taq DNA Polymerase[d]	100 units	M1661	
pGEM®-T Vector System I	20 reactions	A3600	
pGEM®-T Vector System II	20 reactions	A3610	
pGEM®-T Easy Vector System I*	20 reactions	A1360	
pGEM®-T Easy Vector System II*	20 reactions	A1380	

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