Austin Journal of Plant Biology

Open Access

Austin Publishing Group

Short Communication

Ethidium Bromide-Free DNA in Chimeric Gene Construction Involving Multiple DNA Segments

Wun S Chao*
U.S. Department of Agricultural Research Service, Plant Science Research, Biosciences Research Laboratory, USA

***Corresponding author:** Wun S Chao, U.S. Department of Agricultural Research Service, Plant Science Research, Biosciences Research Laboratory, Sunflower and Plant Biology Research Unit, 1605 Albrecht Blvd, Fargo, ND 58102-2765, USA

Received: September 16, 2014; **Accepted:** October 20, 2014; **Published:** February 20, 2015

Abstract

This manuscript describes a method of extracting DNA from agarose gels without exposure to ethidium bromide (EtBr) and UV radiation. Although it is well known that DNA exposed to EtBr and UV is less effective for cloning work, EtBr is still the most widely used staining agent today. Another newer and well-liked staining agent, SYBR Green, also requires UV radiation. Here I describe a simple method that uses these highly sensitive fluorescent agents to locate DNA for extraction, but does not expose the DNA itself to either fluorescent dyes or UV. Thus, the purified DNA can be used effectively for multi-segment ligation and regular cloning work.

Keywords: Ethidium Bromide; Ligation; Cloning

Findings

Chimeric gene construction is one of the most important procedures for molecular manipulation of genes and gene products. Today, with the advance of enzymes and cloning vectors and the assistance of polymerase chain reaction (PCR), the DNA segment can be inserted easily into a cloning vector without much ingenuity. The challenging aspect of gene construction is inserting multiple fragments into one vector. These multi-segment constructs are desirable since it is well documented that transgenes with inverted repeats are more potent in gene suppression than those of simple sense or anti-sense construct (Waterhouse et al. 1998; Wesley et al.2001; Hannon 2002; Watson et al. 2005). Current methods of chimeric gene construction involve preparing insert and vector with compatible ends (blunt ends or overhangs) and gel-purification of insert and vector for ligation. The gel-purification step normally uses ethidium bromide (EtBr) and UV radiation to identify bands of interest, which causes transformation efficiency to drop considerably (Flores et al. 1992). The newer and well-liked staining agent, SYBR Green (Molecular Probe, Eugene, OR), also requires UV radiation. Thus, one either performs a series of ligation steps by cloning the fragments into a vector one at a time or implements a multi-segment ligation, at the risk of obtaining inconsistent results. Another staining agent, methylene blue, does not require UV light for detection of DNA bands. Plasmid DNA prepared from methylene blue-stained gels showed a 2-fold higher transformation rate than that from EtBr-stained gels (Flores et al. 1992). However, methylene blue is much less sensitive than EtBr and is not commonly used.

Here I outline a simple method to isolate DNA without exposure to ethidium bromide (EtBr) and UV radiation for multi-segment cloning. Using these DNA segments, we consistently generated constructs of inverted RNA structures of leafy spurge (*Euphorbia esula* L.) genes encoding cullin-like protein, chlorophyll a/b-binding protein, and an unknown protein using three different cloning vectors: pUC19, pBlueScript-SK, and pBI121. All constructs involved ligation of four DNA segments simultaneously. Figure 1 illustrates an example of a multi-segment construct encoding an inverted cullin gene sequence.

Two 400 bp cullin cDNA fragments and a 300 bp GUS fragment were generated by PCR. One of the cullin sequences was incorporated with an *Xba*I site at the 5' end and a *Bam*H1 site at the 3' end. The other cullin sequence was incorporated with an *Sst*I site at the 5' end and a *Not*I site at the 3' end. The GUS fragment was incorporated with a *Bam*HI site at the 5' end and a *Not*I site at the 3' end. Each PCR mixture was prepared in a 250-µL volume containing 0.5 µg template DNA, 25 µL 10X Pfu turbo buffer, 6 µL dNTP (10 mM), 3 µL each forward and reverse primers (20 mM), and 5 µL Pfu turbo polymerase (Stratagene, La Jolla, CA). The PCR mixture was aliquoted into 5 200-µL tubes for PCR. The Stratagene Robocycler was programmed for 30 cycles with denaturation at 94°C for 30 s, annealing at 60°C for 1 min, and elongation at 72°C for 2 min. The PCR product was pooled, extracted once with an equal volume of phenol/chloroform, chloroform, and precipitated in 70% ethanol.

The purified PCR product was then digested with the restriction enzymes. Since complete digestion is critical to the success of multi-fragment ligation, we perform serial digestions when the reaction buffer is not 100% compatible with both of the enzymes. Phenol/

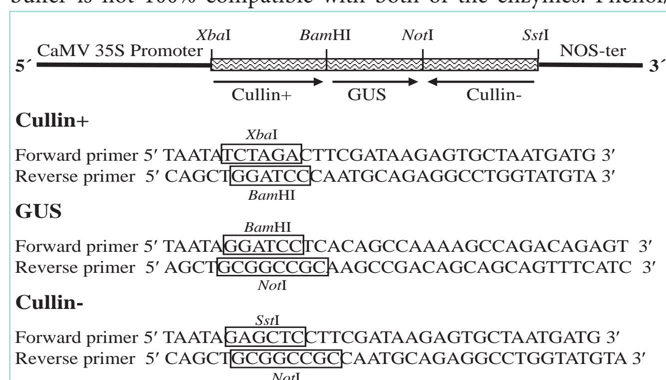

Figure 1: Multi-segment ligation to generate an inverted cullin gene sequence. The binary vector, pBI121, is digested with *Xba*I and *Sst*I to remove the GUS sequence and gel-purified according to Figure 2. This vector is then combined with 3 PCR-amplified segments for ligation (see text). The sets of PCR primers are shown. Arrows indicate the orientation of each PCR generated fragment.

ISSN: 2472-3738 | www.austinpublishinggroup.com
Chao. © All rights are reserved

Citation: Chao WS. Ethidium Bromide-Free DNA in Chimeric Gene Construction Involving Multiple DNA Segments. Austin J Plant Biol. 2015;1(1): 1002.

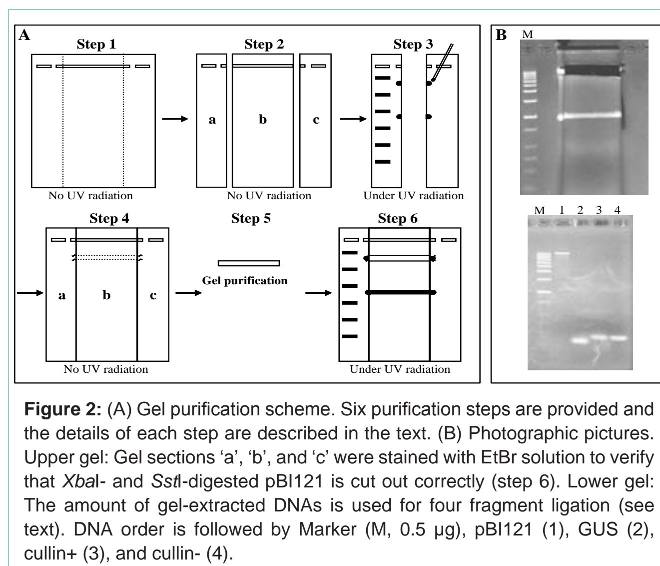

Figure 2: (A) Gel purification scheme. Six purification steps are provided and the details of each step are described in the text. (B) Photographic pictures. Upper gel: Gel sections 'a', 'b', and 'c' were stained with EtBr solution to verify that *Xba*I- and *Sst*I-digested pBI121 is cut out correctly (step 6). Lower gel: The amount of gel-extracted DNAs is used for four fragment ligation (see text). DNA order is followed by Marker (M, 0.5 μg), pBI121 (1), GUS (2), cullin+ (3), and cullin- (4).

chloroform and chloroform extractions were performed between the two serial digestions. The cloning vector (5 μg) was also digested in a similar fashion; depending upon the compatibility of reaction buffers, serial digestions may also be used. Following enzyme digestion, DNA fragments were gel-purified following non-UV-irradiation scheme according to Figure 2.

In Step 1 of Figure 2A, the middle, wide well is prepared by covering the small wells with self-adhering label tape before casting the gel. Marker DNA is loaded into the first well and the restriction enzyme digested DNA is loaded into the middle, wide well. After completing the run, the gel is cut vertically along the dashed lines (step 1). Gel sections 'a' and 'c' include a small portion of the middle well 'b'. Gel sections 'a' and 'c' are subjected to staining in EtBr solution (step 2). Gel section 'b' is wrapped and stored in a refrigerator. After staining and de-staining, the DNA is observed under UV light and the upper and lower boundaries of the desired band are marked with black India ink using the tip of a syringe (step 3). Gel segments (a, b, and c) are placed together on a glass plate, and the DNA band is cut from the gel 'b' with a razor blade based on the reference marks on gel sections 'a' and 'c' (step 4). The cut band is subject to gel purification (step 5). To confirm that the DNA band is cut correctly, gel section 'b' is subjected to staining and is then examined under UV-radiation (Figure 2A, step 6 and Figure 2B, upper gel).

GENECLEAN II Kit (Bio 101, Vista, CA) was used to extract DNA from gels. Twenty μL water was used to elute the PCR-amplified DNA, and 10 μL water was used to elute the vector DNA. Two μL of eluted DNA were run on a 1% agarose gel to confirm DNA quality (Figure 2B, lower gel). The ligation reaction was prepared in a 10 μL volume containing 2 μL 5X ligation buffer, 2 μL vector, 1.8 μL each of three PCR-generated fragments, and 0.5 μL T4 DNA ligase (Gibco BRL, Rockville, MD). The ligation mixture was incubated at 14°C overnight. Heat shock procedures were used for transformation. Each transformation requires 2-4 μL of ligation product in 100 μL of competent cells (XL1-Blue). Although the number of positive clones varies among different constructs, we have consistently obtained an ample amount of positive clones (10 to 30 clones) after a single transformation experiment. The accuracy of ligation constructs was confirmed by DNA sequencing and restriction enzyme digestion.

The major advantage of using EtBr-free DNA is consistency. For this reason, we also use this method to prepare DNA for other experiments such as making probes for DNA or RNA blot analysis.

References

1. Flores N, Valle F, Boliver F, and Merino E. Recovery of DNA from agarose gels stained with methylene blue. BioTechniques. 1992; 13: 203-205.
2. Hannon GJ. RNA interference. Nature. 2002; 418: 244-251.
3. Waterhouse PM, Graham MW, and Wang M-B. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc Natl Acad Sci USA. 1998; 95: 13959-13964.
4. Watson JM, Fusaro AF, Wang M-B, Waterhouse PM. RNA silencing platforms in plants. FEBS Letters. 2005; 579: 5982-5987.
5. Wesley SV, Helliwell CA, Smith NA, Wang M, Rouse DT, Liu Q, *et al.* Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J. 2001; 27: 581-590.

Austin J Plant Biol - Volume 1 Issue 1 - 2015
ISSN: 2472-3738 | www.austinpublishinggroup.com
Chao. © All rights are reserved

Citation: Chao WS. Ethidium Bromide-Free DNA in Chimeric Gene Construction Involving Multiple DNA Segments. Austin J Plant Biol. 2015;1(1): 1002.