Cloning Differential Display-PCR Products with pGEM®-T Easy Vector System

Article

Figures & Tables

Comments & Ratings

ABSTRACT

PCR products were cloned using the pGEM®-T Easy Vector System and the LigaFast™ Rapid DNA Ligation System.

Rosalia Sirchia, Valentina Ciacciofera and Claudio Luparello.
Dipartimento di Biologia Cellulare e dello Svliuppo, Universita di Palermo, Italy

Publication Date: 2001

Introduction

The identification of differentially expressed genes to investigate a specific developmental system or the genetic changes associated with a given pathology is one of the most commonly pursued tasks in molecular and cellular biology. Diverse techniques allow the study of the modulation of gene expression including differential display (DD)-PCR. DD-PCR permits the isolation of cDNA reverse transcribed from mRNA, allowing comparison of gene expression of differentially treated samples. The cDNA fragments are cloned and identified by sequencing and homology searches. The differential expression of the cDNA can then be confirmed using more stringent techniques for the evaluation of steady-state mRNA such as Northern blots and semi-quantitative PCR.

We are currently analyzing gene expression in cultured breast cancer cells in response to microenvironmental modulators such as parathyroid hormone-related peptide (PTHrP), which is known to affect cell proliferation and invasivity(1) (2) . Using mRNA DD-electrophoretograms and re-amplifying and purifying selected cDNA fragments(3) , we have obtained a number of DNA preparations suitable for sequencing and subsequent identification of the parental transcript.

Here we report the successful use of LigaFast™ Rapid DNA Ligation System and pGEM®-T Easy Vector System (Cat.# A1360) for cloning PCR products. This procedure yields enough recombinant plasmid for sequence analysis.

Results and Discussion

To identify genes selectively regulated in a breast cancer cell line after treatment with a midregion fragment of PTHrP, we isolated cDNA fragments by DD-PCR(3) . The cDNA fragments were ligated into pGEM®-T Easy Vector. When working with DD-PCR insert-containing vectors, we found that plating 300–500µl of transformed bacterial cells generated a higher number of colonies with a consequent higher percentage of white colonies. Additionally, the 6:1 insert:plasmid ratio yielded a 50% increase of colony number. On the other hand, when the control DNA fragment was used for transformation, an insert:plasmid

© Promega Corporation, Updated 2001.

ratio of 3:1 and 100µl of transformation culture gave optimal results, (positive control yielded more than 90% white colonies). The number of colonies grown in the background control was negligible.

Figure 1 shows the electrophoretic analysis of recombinant plasmids purified from the cell lysates; we were able to obtain 1-5µg of recombinant plasmid from 5ml cultures, an amount suitable for automated sequencing. Figure 2 shows the restriction analysis of recombinant plasmids digested with EcoR I, whose recognition site closely flanks the insertion site. We found that after enzymatic treatment, DNA fragments corresponded in size to those of the DD-PCR bands plus the remnant region of the polylinker.

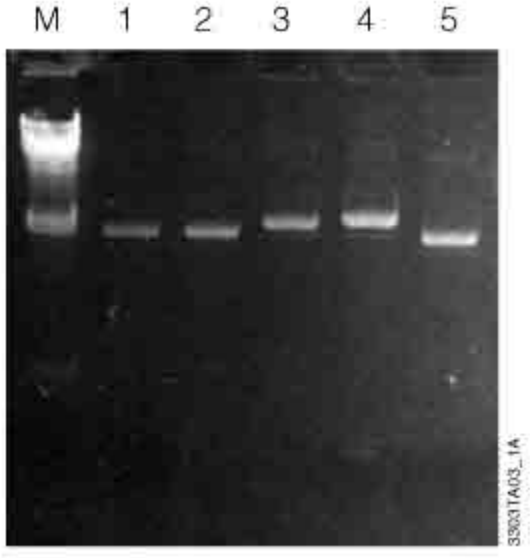

Figure 1. Gel electrophoresis of five different recombinant plasmids (A–E) extracted from JM109 lysates. Lane M, Lambda DNA/Hind III size marker. 1% agarose gel, ethidium bromide-stained.

We compared the colony-forming efficiency of three different bacterial strains, JM109, HB101 and XL1 Blue. JM109 cells yielded 20% more colonies, both blue and white, than the other two cell types. We have also successfully cloned DNA fragments extracted and purified from agarose gel bands.

In conclusion, we can recommend the pGEM®-T Easy Vector System, in conjunction with LigaFast™ Rapid DNA Ligation System, as a versatile tool for the quick cloning of DD-PCR products and the production of recombinant plasmids for subsequent sequencing.

Materials and Methods

Preparation of cDNA fragments: We amplified cDNA fragments (180-750bp) obtained from DD-PCR using PCR Master Mix (Cat.# M7501). Annealing temperatures and cycling profiles were empirically determined for each reaction. For the A-tailing reaction, the elongation time of the last cycle was prolonged to 20 minutes. We determined the purity and the amount of the PCR products by agarose gel electrophoresis.

Ligation and transformation reactions: The ligation reaction was set up using pGEM®-T Easy vector and LigaFast™ Rapid DNA Ligation System using a PCR product:vector molar ratio of 3:1 or 6:1. Positive and background controls were prepared in

parallel as recommended by the manufacturer(4) . The reactions were incubated overnight at 4°C, after which they were quickly centrifuged and 2µl added to 50µl of either JM109, HB101 (Cat.# L2011) or XL1 Blue (kind gift of Prof. Ida Albanese and Mario La Farina) competent cells. Transformation was performed according to manufacturer's instructions(4) onto LB/Ampicillin/IPTG/X-Gal plates.

Plasmid extraction: White bacterial colonies were picked and grown overnight at 37°C with agitation in 5ml Luria broth supplemented with 25µl Ampicillin (100µg/ml). Polypropylene tubes were used for colony incubation. Recombinant plasmids were purified using a commercial miniprep kit and quantitated by gel electrophoresis. Insertion of the PCR product of interest into the plasmid was verified by EcoR I digestion of 200–400ng of recombinant plasmid and analysis by agarose gel electrophoresis.

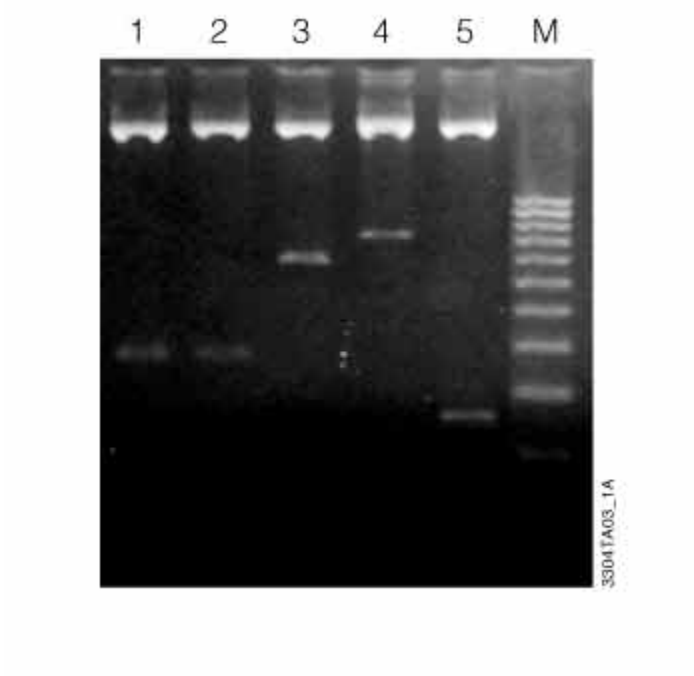

Figure 2. EcoR I restriction analysis of the recombinant plasmids. The DNA fragments obtained show the expected sizes: lanes 1 and 2, 290bp; lane 3, 600bp; lane 4, 750bp; lane 5, 180bp. Lane M, 100bp ladder. 2% agarose gel, ethidium bromide-stained.

Acknowledgments

Work partially supported by Italian MURST (Cofin and ex 60%).

REFERENCES

1. Luparello, C. *et al.* (1995) Parathyroid hormone-related peptide and 8701-BC breast cancer cell growth and invasion in vitro. Evidence for growth-inhibiting and invasion-promoting effect. *Mol. Cell. Endocrinol.* **111**, 225–32.
2. Luparello, C. *et al.* (1997) Clonal heterogeneity of the growth and invasive response of a human breast carcinoma cell line to parathyroid hormone-related peptide fragments. *Carcinogenesis* **18**, 23–9.

3. Luparello, C. *et al.* (2000) Use of M-MLV RT, RNase H-, Point Mutant, for mRNA-differential display analysis of parathyroid hormone-related peptide (PTHrP)-treated breast carcinoma cells. *eNotes*
4. *pGEM®-T and pGEM®-T Easy Vector Systems* Technical Manual, TM042, Promega Corporation.

HOW TO CITE THIS ARTICLE

Scientific Style and Format, 7th edition, 2006

Sirchia, R., Ciacciofera, V. and Luparello, C. Cloning Differential Display-PCR Products with pGEM®-T Easy Vector System. [Internet] 2001. [cited: year, month, date]. Available from: http://www.promega.com/resources/pubhub/enotes/cloning-differential-display-pcr-products-with-pgemt-easy-vector-system/

American Medical Association, Manual of Style, 10th edition, 2007

Sirchia, R., Ciacciofera, V. and Luparello, C. Cloning Differential Display-PCR Products with pGEM®-T Easy Vector System. Promega Corporation Web site. http://www.promega.com/resources/pubhub/enotes/cloning-differential-display-pcr-products-with-pgemt-easy-vector-system/ Updated 2001. Accessed Month Day, Year.

Products may be covered by pending or issued patents. Please visit our patent and trademark web page for more information.

FIGURES

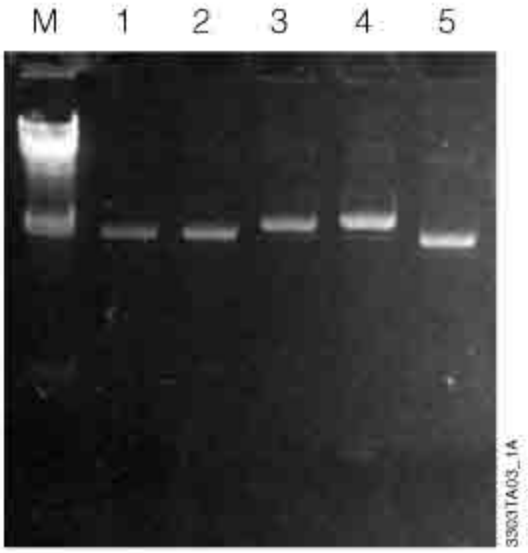

Figure 1. Gel electrophoresis of five different recombinant plasmids (A–E) extracted from JM109 lysates. Lane M, Lambda DNA/Hind III size marker. 1% agarose gel, ethidium bromide-stained.

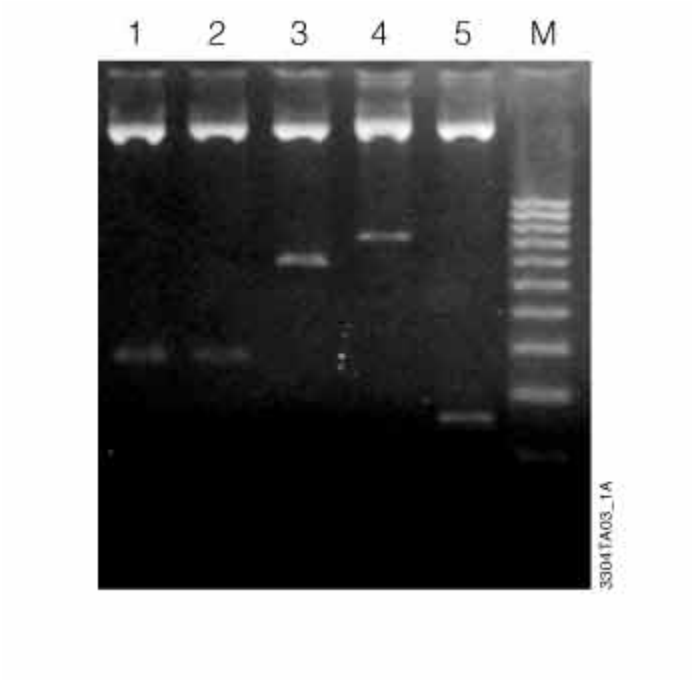

Figure 2. EcoR I restriction analysis of the recombinant plasmids. The DNA fragments obtained show the expected sizes: lanes 1 and 2, 290bp; lane 3, 600bp; lane 4, 750bp; lane 5, 180bp. Lane M, 100bp ladder. 2% agarose gel, ethidium bromide-stained.

It appears that you have Javascript disabled. Our website requires Javascript to function correctly. For the best browsing experience, please enable Javascript.