Construction of a cDNA fragment library from SH-SY5Y cells using restriction display PCR

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ABSTRACT

A complementary DNA (cDNA) fragment library from SH-SY5Y cells is constructed using a restriction display polymerase chain reaction (RD-PCR) technique. Messenger RNA (mRNA) is extracted from SH-SY5Y cells and single-strand cDNA synthesised using an anchored oligo primer (dT_{18}). The second strand is produced by nick translation. The double strands are cleaved with the restriction enzyme Sau3AI and the fragments ligated with universal linker. The products are amplified with universal primers and selected primers, ligated into the pMDI8-T vector, and then sequenced. The library constructed contained 136 subgroups, each comprising seven to 12 cDNA fragments. RD-PCR proved a simple, effective way to construct a cDNA library, and this will contribute to the investigation of gene expression in the neuron in future microarray studies.

KEY WORDS: Gene library.
Polymerase chain reaction.

Introduction

Following completion of the human genome project, a new post-genomics era has begun in which research focuses on the investigation and analysis of the function of genes and their transcripts. Already, techniques are available to study the pattern of gene expression on a large scale, such as serial analysis of gene expression (SAGE),[1] mini-fragment analysis of gene expression (MAGE),[2] rapid analysis of gene expression (RAGE)[3] and complementary DNA (cDNA) microarray – the DNA chip.[4]

cDNA microarray provides an easier but more sophisticated way to explore gene expression in a way that is both systematic and comprehensive. Using this technology, the first obstacle to success is the need for thousands of probes. The usual approach to constructing a cDNA library involves the random selection of clones, and thus repetitive or redundant ones cannot be avoided, and this increases research costs.

Here, we use an efficient and straightforward restriction display polymerase chain reaction (RD-PCR) method[5] to construct a cDNA gene fragment library from SH-SY5Y cells.

Materials and methods

Cell culture

The SH-SY5Y cell line was grown in a 5% CO_2 incubator in 1640 medium containing 15% fetal calf serum, 100 units/mL penicillin and 100 µg/mL streptomycin. Cells were passaged twice a week and seeded to grow until 80% confluency was achieved.

Gene fragment library construction

The construction protocol is shown in Figure 1. Total RNA was extracted from 80% confluent SH-SY5Y cells by acid guanidinium thiocyanate-phenol-chloroform extraction.[6] Messenger RNA (mRNA) was isolated from 100 µg total RNA using an mRNA enrichment kit (Omega Biotek, Doraville, USA). The first cDNA strand was synthesised by SuperScript™ II (RNaseH Reverse Transcriptase, Life Technologies, USA) with oligo(dT_{18}) as the primer. Double strand cDNA was produced by nick translation and digested with Sau3AI (Takara, Tokyo, Japan).

The fragments that showed 3′ excessive 5′-GATC were ligated with the linker designed to have a 5′-GATC overhang with T_4DNA ligase (Takara, Tokyo, Japan). After removing unligated linker fragments, the products were amplified with selective primer (^ 2 bp added onto 3′ universal primer, such as 5′-GTTTGGCTGGTGTGGATCN_1N_2-3′, abbreviated as PN_1N_2, where N_1 and N_2 represent one of the four bases [G, A, C or T]).

Reactions were performed in a 9700 thermocycler with an initial denaturation of 5 min at 95°C, followed by 30 cycles of 30 s at 95°C, 1 min at 60°C, 1 min at 72°C, and a final extension of 10 min at 72°C. One pair of selective primers was defined as one subgroup. The products of amplification were ligated into a pMD 18-T vector. Recombinant plasmid DNAs were transformed in *Escherichia coli* XL-1. Selected clones were identified with primer A (5′-GTAAAACG-ACGGCCAGT-3′) and primer B (5′-CAGGAAACAGC-TATGAC-3′) by electrophoresis on 2% agarose gel.

DNA bands were stained with ethidium bromide, viewed under ultraviolet light, and the fragment lengths calculated using Labworks software. Clones with a length of at least 30 bp less than their neighbours (clones were queued

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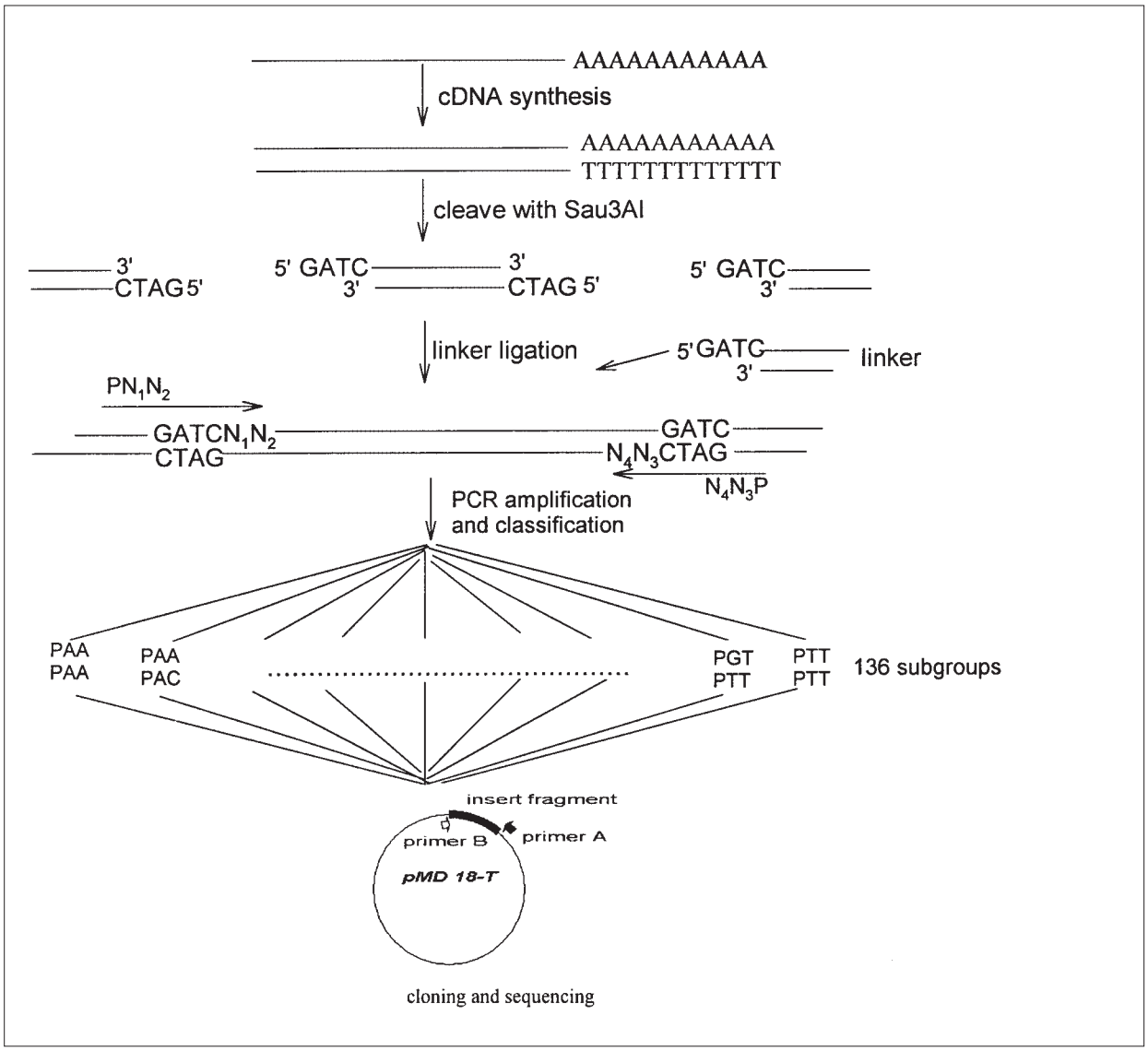
Fig. 1. Schematic representation of the construction of a cDNA gene library using RD-PCR.



according to length, from long to short) were chosen and sequenced with the *Taq*FS Dye terminator kit (Perkin Elmer) and analysed using an ABI 310 automated sequencer with primer A.

Linker
5′-GATCCACCACCAGCCAAACCCA-3′
3′-GTGTGGTCGGTTTGG-5′

Results

Following the procedure outlined in Figure 1, the number of DNA fragments distributed in each subgroup was analysed. Following PCR amplification, the products were analysed by electrophoresis on 5% polyacrylamide gel, and DNA bands were stained with a silver solution (Figure 2). Each subgroup produced seven to 12 clear DNA bands, with lengths ranging from 250 to 750 bp. The 30 *E.coli* XL-l recombinant clones were identified with primer A and primer B (Figure 3) In the PAA-PAC subgroup illustrated, there was only one clone that could not be amplified (Figure 3, clone 4). Clones 16, 1, 11, 19, 15, 25, 6, 23, 9, 30 and 7 were isolated according to their length, and these were confirmed as different gene fragments by sequencing. The study worked through 136 subgroups and collected 1248 clones that comprised the gene fragment library of SH-SY5Y cells.

Discussion

In this study, RD-PCR was used to identify differentially expressed genes. As most genes have two or more Sau3AI sites, unique and gene-specific target sequences of approximately 256 bp can be prepared from each cDNA by poly(A) selection and cleaved with Sua3AI. The selected primers contained a common region, derived from the linker, plus a 3′-specific region of 2 nt, allowing them to

Fig. 2. PCR products separated by electrophoresis and stained with silver. Lanes 1,2 and 3 show the primer products from PTA-PTA, PCG-PTA and PCG-PCG, respectively; and lane M is the marker.

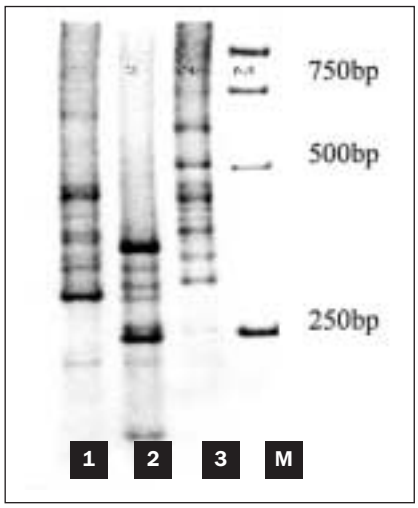
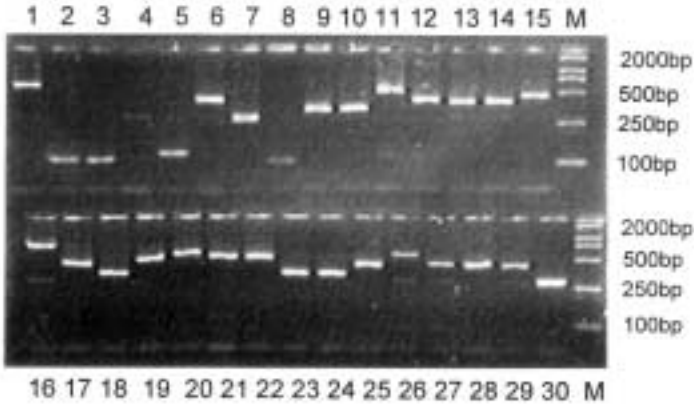


Fig. 3. Electrophoretic patterns produce by individual clones (1-30) and the marker (M).



selectively amplify cDNAs from a small number of genes from the gene fragment pool.

Here, we showed that some seven to 12 gene fragments can be isolated from each subgroup, and that when their products were cloned into the vector used we could collect recombinant plasmids comprising a similar number of gene fragments. Therefore, we believe that the RD-PCR method used in this study is a useful and efficient means to construct a gene fragment library.

The method described does have some disadvantages: for example, gene fragments that differ from others by less than 30 bp may be missed, due to the limit of resolution of the electrophoretic gel; and we have found that some repetition of clones still occurs, resulting from mismatching of primers. However, of the clones sequenced, more than 80% represented unique genes.

In contrast to conventional methods, RD-PCR has several advantages: it avoids repetitive cloning and sequencing, thus saving time and effort; and the length of the gene fragments in the library range from 250 to 750 bp, making them potentially suitable as cDNA microarray probes. □

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