

***Dictyostelium* Full-length enriched cDNA library (FL cDNA library)**

Source

Dictyostelium discoideum strain **Ax4** was used for RNA preparation. Total RNA was isolated from axenically growing cells (designated as **V series**), cells developed on nitrocellulose filter to at aggregation (8 hrs; **A series**), slug (16 hrs; **S series**) and early culmination stage (20 hrs; **C series**).

Preparation of FL-cDNA

From the total RNA of each developmental stage, cDNA was synthesized using **oligo-capping method** (Maruyama and Sugano *Gene* **138**, 171-174, 1994, Suzuki *et al.*, *Gene* **200**, 149-156, 1997). In principle, the method consists of the following four steps.

- 1) Treatment of the total RNA with bacterial alkaline phosphatase followed by tobacco acid pyrophosphatase to remove phosphate group at the 5'-end of truncated mRNA and cleave the cap structure to expose phosphate group at the 5'-end of capped (full-length) mRNA.
- 2) Ligation of oligo RNA to the 5'-end of mRNA. Because only full-length mRNA has the phosphate group exposed at the 5'-end, the oligo RNA can be ligated to only full-length mRNA, not truncated mRNA.
- 3) First strand cDNA synthesis primed by oligo-dT with adapter sequence.
- 4) Amplification of cDNA by PCR with primers complementary to the the 5'-oligo RNA and 3'-adapter sequences.

The amplified cDNA was size-fractionated by agarose electrophoresis. One fraction (**fraction F**) contains cDNA longer than 0.8 kb and the other (**fraction H**) contains longer than 2 kb.

Vector and host strain

The cDNA fraction was directionally ligated to the *Dra*III site of plasmid vector

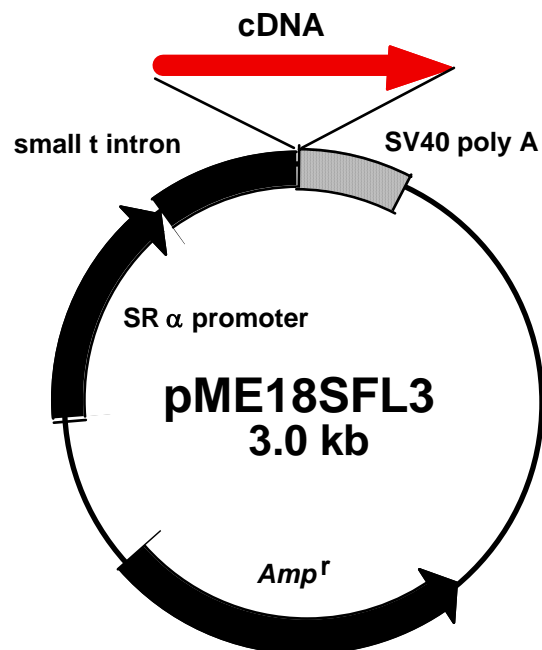


Fig. 1. Plasmid vector pME18SFL3

pME18SFL3 (Acc. No. AB009864, Fig. 1). Because the vector is originally for expression in mammalian cells, the promoter, intron and terminator may not work in *Dictyostelium* cells.

The sequence around the cDNA insert is shown in Fig. 2. Sequencing primer site used for test sequencing (not conventional M13 primer sequences) are shown in bold blue. Restriction sites available for excision of cDNA insert are also indicated as underlined letters. Note that the *DraIII* sites are no longer available.

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TACGGAAGTGTTACTTCTGCTCTAAAAGCTGCGGAATTC CTCGAG  
5'-sequencing primer                      EcoRI  XhoI  
  
CACTGTTGGCCTACTGG (5' -cDNA- 3' ) GGCCACATGTG CTCGAG  
(DraIII)                               (DraIII)  XhoI  
  
CTGCAGGTCGCGGCCGCTAGACTAGTCTAGAGAAAAACCTCCCACA  
PstI      NotI                               3'-sequencing primer
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Fig. 2. Sequences around the cDNA insertion site.

The ligated cDNA was introduced in *E. coli* DH10B cells.

Sequencing and database

We have constructed eight cDNA libraries in combination with **four developmental stages (V, A, S and C series) and two fractions by size (fractions F and H)**. The names of the libraries are defined as the combination of two letters, each of which indicates the developmental stage and fraction. For example, library AF shows the one prepared from >0.8 kb fraction of aggregation stage cDNA.

We are currently sequencing randomly picked clones of each library from the both ends. The number of sequenced clones is as follows;

Library VF, AF, SF, and CF: 8,000 clones/each (32,000 clones in total)

Library VH, AH, SH and CH: 3,000 clones/each (12,000 clones in total).

Sequence data are available on the following site (*Dicty_cDB*).

<http://www.csm.biol.tsukuba.ac.jp/cDNA/database.html>