RESEARCH REPORT

SUPPLEMENTARY MATERIAL FOR:

Random gene dissection: a tool for the investigation of protein structural organization

Rimantas Sapranauskas and Arvydas Lubys

Table S1. Plasmids Used in this Work

<table>
<thead>
<tr>
<th>Plasmid Name</th>
<th>Relevant Characteristics</th>
<th>Drug Resistance</th>
<th>Reference, Source, or Short Description/Construction</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC57</td>
<td>N.A.</td>
<td>Ap</td>
<td>Fermentas UAB</td>
</tr>
<tr>
<td>pACYC184</td>
<td>N.A.</td>
<td>Cm, Tc</td>
<td>(1)</td>
</tr>
<tr>
<td>pBR322</td>
<td>N.A.</td>
<td>Ap, Tc</td>
<td>(2)</td>
</tr>
<tr>
<td>pBfilIRM14</td>
<td>bfiIR, bfiMC1, bfiMC2</td>
<td>Ap</td>
<td>(3)</td>
</tr>
</tbody>
</table>

The plasmid was constructed in two steps. First, a DNA fragment of 4.57 kb, obtained after the cleavage of λ 1105 (4) with EcoRI and carrying both Tn10 transposon derivative “mini-kan” and Tn10 transposase gene, which is located outside the transposon under the control of the P_{tac} promoter, was inserted into EcoRI-digested pBR322. After that, the self-complementary octanucleotide 5’-GCGGCCGC-3’ with NotI target was inserted into the Eco147I site within the transposon for cloning purposes.

The plasmid was constructed in two steps. First, the 5’ region of the bfiIR gene was PCR-amplified using Taq DNA polymerase (Fermentas UAB), pBfilIRM14 DNA as a template, and primers 5’-GTCCGGAGTTTTTTAATGATTATTCTCTTCAACCA-3’ (the Shine-Dalgarno sequence is underlined, and the translation initiation codon ATG that replaced the original TTG codon is bolded) and 5’-CTACTAAAGATTCTCCTAAG-3’ (Eco88I-generated cohesive DNA ends were blunted).

The plasmid was constructed in two steps. First, the KspAI-Eco72I DNA fragment of 3.18 kb from pBfilIRM14 encoding BfiI methyltransferases was ligated with the PvuII-Eco88I DNA fragment of 2.4 kb from pACYC184 (Eco88I-generated cohesive DNA ends were blunted).

The plasmid was constructed in two steps. First, the KspAI-Eco72I DNA fragment of 3.18 kb from pBfilIRM14 encoding BfiI methyltransferases was cloned into Eco32I-cleaved pACYC184. The resulting plasmid was then digested with Acc65I-Cfr42I, and a 3.98-kb DNA fragment was inserted into NotI-cleaved pBrmTn10-dKmNotI (sticky DNA ends produced by Acc65I, Cfr42, and NotI were blunted).

The plasmid was constructed in two steps. First, the KspAI-Eco72I DNA fragment of 3.18 kb from pBfilIRM14 encoding BfiI methyltransferases was cloned into Eco32I-cleaved pACYC184. The resulting plasmid was then digested with Acc65I-Cfr42I, and a 3.98-kb DNA fragment was inserted into NotI-cleaved pBrmTn10-dKmNotI (sticky DNA ends produced by Acc65I, Cfr42, and NotI were blunted).

Ap, ampicillin; Cm, chloramphenicol; Tc, tetracycline; Km, kanamycin; N.A., not applicable.

Institute of Biotechnology, Vilnius, Lithuania

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REFERENCES


SUPPLEMENTARY METHODS

Construction of the RR1-Bfi Strain

RR1-Bfi is an Escherichia coli RR1 derivative that produces F' encoded BfiI methyltransferases. The strain was constructed using the following scheme. First, genes for both BfiI methyltransferases were inserted into the Tn10 derivative, so-called “mini-kan” (KmR) transposon (1), resulting in plasmid pTn-BfiIM. Next, strain XL1-Blue (pTn-BfiIM) was grown to mid-log phase, and transcription of the transposase gene (which is located outside the transposon under the control of P_tac) was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. The strain was allowed to grow overnight and, after that, F' plasmid conjugative DNA transfer into recipient strain RR1 (F SmR) was carried out to select for those F' variants that acquired a copy of modified mini-kan transposon. The liquid mating assay protocol was as follows. The donor strain was diluted 1:50 into Luria-Bertani (LB) from saturated overnight culture grown under selection and allowed to grow to an A600 of approximately 0.1 in the absence of selection at 37°C. Then, 200 μL of donor culture were mixed with 10 μL of overnight culture of the recipient and incubated at 37°C. After 30 min, 10-fold serial dilutions of mating mixture were prepared using LB and plated onto LB agar-containing streptomycin, tetracycline, and kanamycin to counter-select donor and unmated recipient cells while selecting for transconjugants that contain F' plasmid (TcR) with inserted transposon (KmR). A few resulting transconjugants carrying the expected recombinant plasmid F'::mTn10 (bfilmC1+ bfilmC2+) were colony purified. DNA methylation properties of individual transconjugants were then tested by their transformation with pUCBfiiIR, which expresses BfiI restriction endonuclease. One strain that tolerates the introduction of pUCBfiiIR was called RR1-Bfi.

REFERENCES