Probing the mutation spectrum in *E. coli*

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ABSTRACT

The mutation spectrum, together with mutation frequency, is decisive for the size and nature of genetic diversity. We constructed plasmid-encoded probes for specific detection of each and all of the six base substitutions. Using the set of the probes, we analyzed the mutation spectrum on the plasmids caused by different types of mutagens and mutator enzymes/alleles.

INTRODUCTION

Directed evolution has become a widely applied method to develop catalysts, sensor modules, pathways and logic circuits. A directed evolution comprises two major steps: generating diverse mutant libraries and screening for improved variants. A variety of methods for random mutagenesis, including chemical\(^1\),\(^2\), enzymatic\(^3\),\(^4\), and biological\(^5\),\(^6\), ones, have been developed. These methods differ significantly in the mutation spectrum and frequency. Due to the redundant and biased nature of the genetic code\(^7\),\(^8\), base substitutions are not evolutionarily equivalent. In general, transversions result in more dramatic changes in amino acids than transitions. Some base substitutions (such as G:C \(\rightarrow\) T:A) have high probability to generate stop codons, while others (such as A:T \(\rightarrow\) G:C) do not. Such traits strongly affect the quality of the library\(^9\). For each of the mutagenesis techniques, it is important to know what type of mutations occurs to what extent.

There have been developed various genetic methods to detect the mutations occurring in the cell. Cupples and Miller created a series of *E. coli* strains with different mutations at the active site (Glu 461) in the lacZ gene\(^10\). Foster and coworkers have reported the quick and specific detection of G:C \(\rightarrow\) T:A transversion by measuring the frequency of mutations at Ser 70 in plasmid-encoded TEM1-beta lactamase (bLA) in *E. coli*\(^11\).

In this paper, we report the set of plasmids (pBLA\(_{X}\)\(_{r}\)) encoding bLA with different mutations at position 70. Each strain is designed to revert penicillin\(\beta\) phenotype only when the certain base substitution occurs at the position. By simply monitoring/comparing the reversion frequency of the six different plasmids, we could evaluate the mutation specificity of various mutagenesis methods.

RESULTS AND DISCUSSION

Beta lactamase (bLA) catalyzes the hydrolysis of beta-lactam rings of the penam and cepham antibiotics via acyl-enzyme intermediate formed between the hydroxyl group of Ser 70 and the carbonyl-carbon of the beta-lactam ring\(^12\),\(^13\). Foster et al., inactivated bLA by the introduction by substituting AGC (Ser 70) with CGC (Arg)\(^11\). Among the nine mutations that could occur at this codon, only the C to A mutation at the first position restore the Ser thereby conferring *E. coli* ampicillin\(\beta\).

We randomized Ser 70 residue of bLA gene on the derivative of pGPS3 (New England Biolabs.) by placing NNN (N stands for the equimolar mixture of dA, dG, dT, and dC) at this position. The resultant plasmid library was transformed into *E. coli* XL2 (strategene) and plated both on Kanamycin and kanamycin/ carbencillin plates.

We sequenced 24 variants from both plates. A variety of amino acids were observed at residue 70 among the non-

<table>
<thead>
<tr>
<th>Plasmid name</th>
<th>Codon at 70 (probe for)</th>
<th>Number of revertants sequenced</th>
<th>Sequence found</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBLA(_a)</td>
<td>AAC (A:T (\rightarrow) G:C)</td>
<td>12</td>
<td>AGC (12)</td>
</tr>
<tr>
<td>pBLA(_b)</td>
<td>CCA (G:C (\rightarrow) A:T)</td>
<td>8</td>
<td>AGC (8)</td>
</tr>
<tr>
<td>pBLA(_c)</td>
<td>GCT (G:C (\rightarrow) T:A)</td>
<td>5</td>
<td>TCT (5)</td>
</tr>
<tr>
<td>pBLA(_d)</td>
<td>TAA (A:T (\rightarrow) C:G)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pBLA(_e)</td>
<td>ACA (A:T (\rightarrow) T:A)</td>
<td>6</td>
<td>TCA (6)</td>
</tr>
<tr>
<td>pBLA(_f)</td>
<td>TGA (G:C (\rightarrow) C:G)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(\beta\) No colonies obtained for pBLA\(_d\) and pBLA\(_f\) for this experiment due to the low frequency of the A:T \(\rightarrow\) C:G / G:C \(\rightarrow\) C:G mutations.
selected variants (from carbenicillin free plate), but all 24 variants from carbenicillin plate had Serine at the residue (not shown). This is in spite that cysteine can substitute for Ser 70 in the reaction, resulting in the very weak but detectable level (ca. 3% of the original) of activity. Probably, activity of bLaCys70 is not enough to support the growth of E. coli on carbenicillin plate. Systematic mutation of BLA showed that Ser 70 was intolerant to the amino acid substitutions when selected with ampicillin plate. Next, we co-transformed each of pBLA with error-prone E. coli pol I into JS200 strains (polI strain). After in vivo mutagenesis (culturing at 37°C for 20h), revertants from each batch were subjected to the sequence analysis (Table 1). All revertants had expected sequence at position 70.

Using the system, we have measured the mutation spectrum of four different mutator systems. In each cases, pBLA were independently transformed into the mutator strains and then cultured in mutagenic conditions. The resultant cultures were spotted and diluted to appropriate cell density, and plated both on Kanamycin plates and on Kanamycin + Carbenicillin plates. For each of the plasmid, the reversion frequency was calculated by counting the number of Carbenicillin8 colonies appeared per 108 viable cells. XL1 red (knockout of MutS, together with mutD/ mutJ) showed significant difference in the mutation spectrum given by error-prone polymerase6 (Figure 1). Deminase (human AID)-driving mutagenesis revealed the significant mutation bias on C/G → A/T mutations, reflecting the chemistry AID catalyzes (oxidative deamination of C in DNA)17. Consistent to the previous report18, dP-assisted mutagenesis showed strong and characteristic bias to A:T → G:C mutations.

CONCLUSION

We report the set of plasmids (pBLA) to specifically detect all of the base substitutions. For all tested systems, our method gave consistent mutation spectrum. This method is applicable to any types of mutagenesis methods, both in vivo and in vitro, as well as the combination of them.

REFERENCES


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