

Random mutagenesis of gene-sized DNA molecules by use of PCR with Taq DNA polymerase

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Submitted August 20, 1991

Taq DNA polymerase lacks a 3′→5′ exonucleolytic editing activity (1–3). As such, *Taq* DNA polymerase is an error-prone DNA polymerase, with a measured error rate of 10^{-5} to 10^{-4} error per nucleotide synthesized (1–6).

Leung *et al.* (7) have reported random mutagenesis of short DNA molecules by use of PCR with *Taq* DNA polymerase in the presence of Mn^{++}. In this communication, we report extremely simple, extremely efficient, random mutagenesis of gene-sized DNA molecules by use of PCR with *Taq* DNA polymerase under essentially standard reaction conditions. Our procedure has four steps. In step one, a DNA molecule containing the DNA sequence of interest is subjected to 30 cycles of PCR DNA amplification using essentially standard reaction conditions (reaction volume: 200 μl; reaction composition: 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 50 μM each dNTP, 2 fmol template, 30 pmol each primer, and 5 units AmpliTaq DNA polymerase [Perkin Elmer Cetus]: cycle profile: 1 min 94°C, 2 min 59°C, 3 min 72°C). In step two, the amplified DNA is digested with a pair of restriction endonucleases that cut at each end of the DNA sequence of interest. In step three, the DNA fragment containing the DNA sequence of interest is ligated with restriction endonuclease digested vector DNA. In step four, the resulting recombinant DNA molecules are introduced into cells by transformation or electroporation.

We have used this procedure to mutagenize the 633-nucleotide *E. coli crp* gene, which encodes the catabolite gene activator protein (CAP). The steps involved are outlined in the figure. The resulting mutagenized recombinant DNA molecules were introduced into *E. coli* strain XE82 (*Δcrp strA lacPUV5-O*^{CAP} *thi*) by transformation.

To assess the phenotypic mutant frequency, mutagenized transformants were plated to ribose/tetrazolium/ampicillin indicator agar. Phenotypically wild-type clones (Crp^+ clones) produce white colonies on ribose/tetrazolium/ampicillin indicator agar; in contrast, phenotypically mutant clones (Crp^- clones) produce dark pink or red colonies on ribose/tetrazolium/ampicillin indicator agar. Approximately 11% of the mutagenized transformant clones were phenotypically mutant (Crp^-).

To assess the total mutant frequency, and to assess the mutagenesis spectrum, the DNA-nucleotide sequence of the entire *crp* gene was determined for each of 20 unscreened mutagenized transformant clones. Approximately 35% (7 of 20) of the unscreened mutagenized transformant clones contained a base substitution. This corresponds to a *Taq* DNA polymerase error rate of 3.7×10^{-5} error per nucleotide synthesized (formula in ref. 3), which is in good agreement with published estimates of the *Taq* DNA polymerase error rate under similar reaction conditions (1–6). All four transition substitutions (A:T→G:C, T:A→C:G, C:G→T:A, and G:C→A:T) and one transversion substitution (A:T→T:A) were recovered. There was no evidence for mutational hot spots.

The procedure described here is the simplest method available for high-frequency, broad-spectrum, random mutagenesis of gene-sized DNA sequences. The total mutant frequency obtained here, approximately 35%, is close to the ideal for most applications of random mutagenesis. The procedure described here can be used without modification for DNA sequences of approximately 100 nucleotides (expected total mutant frequency of ≈6%) to approximately 1,000 nucleotides (expected total mutant frequency of ≈60%). By reducing the number of cycles of PCR DNA amplification, the procedure described here can be used for DNA sequences up to approximately 5,000 nucleotides.

ACKNOWLEDGEMENTS

We thank Dr T.Kunkel for important discussions. This work was supported by National Institutes of Health grant GM41376 to R.H.E.

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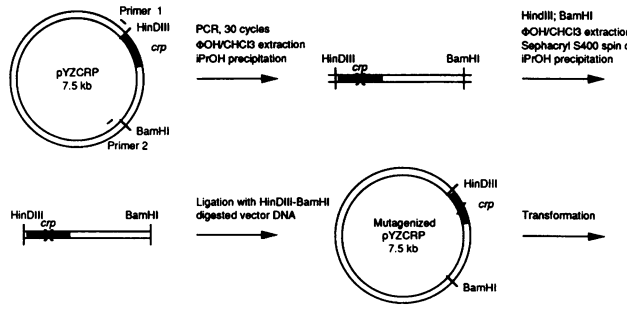

Mutagenesis of the 633-nucleotide *E. coli crp* gene, which encodes the catabolite gene activator protein (CAP). Plasmid pYZCRP has a unique *Hin*dIII site located between the *crp* promoter and structural gene, and has a unique *Bam*HI site located 1.1 kb after the *crp* structural gene.