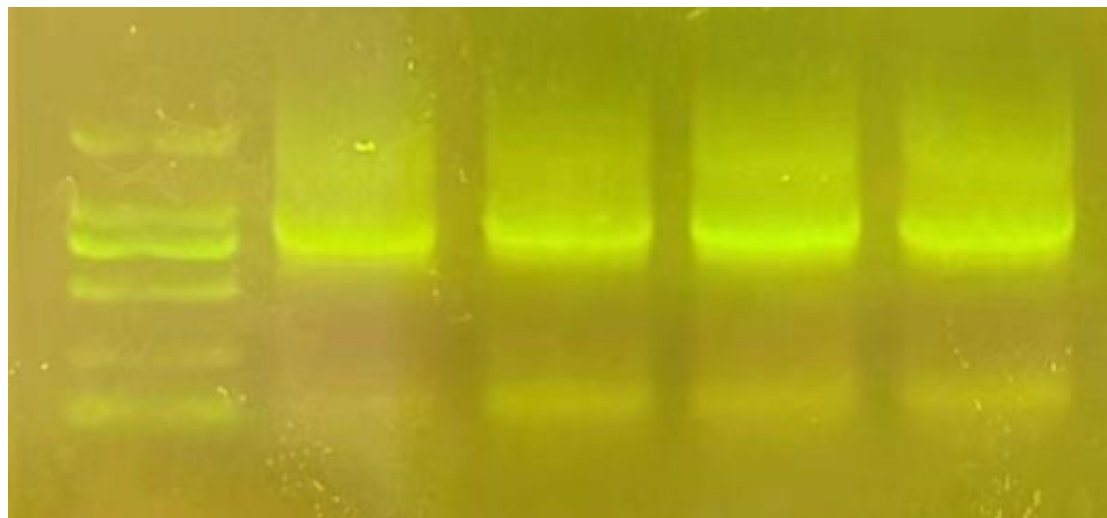


## Supplementary data



**Supplementary figure S1. Error-prone PCR product electrophoresis**

### Materials

E.Z.N.A. Plasmid DNA Mini Kit I ,V spin and E.Z.N.A. Gel Extraction Kit V spin were purchased from Omega Bio-Tek. Restriction enzyme BamH I (1010S)、restriction enzyme Xho I (1094S)、T4 DNA Ligase (2011A)、DL2000 DNA Marker (3427Q)、DL10000 DNA Marker (3584Q) and 10×Loading Buffer (9157 Takara) were purchased from Takara. 4S Green Plus Nucleic Acid Stain and Bacterial Activity-Keeping Lysis Buffer were purchased from Sangon Biotech. Controlled Error-prone PCR Kit (ZY-160903) was purchased from Zeye Biotech. 96-well Clear Flat Bottom UV-Transparent Microplate was purchased from corning. Other common biochemicals and chemicals were purchased from standard sources for laboratory use.

### AlyAYO sequence

```
MAQLDPSKAPSENFDSLKWKIVIPMEDTKPERKGGKVMESKAELNKGYPHAPWFYTDKE
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SEVPITRSFAIDLAKGHYLGNKYDAGYQNEWFFFKAGAYNQCNTGVVRCCKNEGIEAGDY
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```

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### **pET32a-Precession sequence**

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### **Enzymatic activity analysis of expressed proteins**

The strain	OD <sub>235</sub> at the beginning of the reaction	OD <sub>235</sub> at the end of the reaction	Reaction time	Enzyme activity	Enzyme dilution rate
The mutant strain G3	0.505	0.998	3	41.083	5
The mutant strain E3	0.404	1.043	4	39.938	5
The mutant strain H2	0.280	0.921	4	40.063	5
Original strain	0.412	0.659	5	12.350	5