PfAGO (*Pfu Argonaute*)

Description

Prokaryotic Argonaute (pAGO) is the key protein in the host defense system by mediating nucleic acid molecules. As the DNA-guided endonuclease from hyperthermophilic archaeon *Pyrococcus furiosus*, pfAGO protein targets cognate DNA without the requirements of a PAM (protospacer adjacent motif) or PFS (protospacer flanking site) on the target sequence, which largely extends the application in selection of available target DNA sequence. Besides, compared to CRISPR/Cas systems, PfAGO endonuclease exploits guide DNA rather than guide RNA in stimulating target DNA cleavage, which is more convenient for in vitro use as guide DNA is more stable and easier, cost-saver to synthesis than guide RNA.

These properties enable pfAGO as a programmable DNA endonuclease guided by short guide DNAs in efficient, rapid and cost-effective Nucleic Acid Detection systems as well as in versatile Synthetic Biology platforms.

Features

High Biological Activity

Highly Specific

Low Endotoxin Level

Application

Nucleic Acid Detection, Cancer Diagnostics, Synthetic Biology, Genetic Screening

Source

PfAgo gene from *Pyrococcus furiosus* (NCBI-Protein ID: AAL80661) expressed in E. coli

Purity

Greater than 95% as determined by SDS-PAGE and FPLC

Activity

Linear pUC19 plasmid digested after 10 min reaction at 95°C.

The reaction is performed as follow:

System 20 µl, including 0.16 µM PfAGO Enzyme, 4 µM each of guide DNA, 600 ng linear pUC19 plasmid as target DNA and 2ul 10x reaction buffer.

Pre-incubate PfAGO-guide DNAs complex in reaction buffer at 75°C for 10 min. Then add target DNA, incubating at 95°C for 10 min, and slowly cooling down to 10°C.

Storage Condition

-20 °C

10 X Reaction Buffer

200 mM HEPES, pH 7.5,

2.5 M NaCl,

5 mM MnCl2

Storage Buffer

20 mM Tris-HCl, pH 8.0,

300 mM NaCl,

0.5 mM MnCl2,

50% (v/v) glycerol

Experimental Data

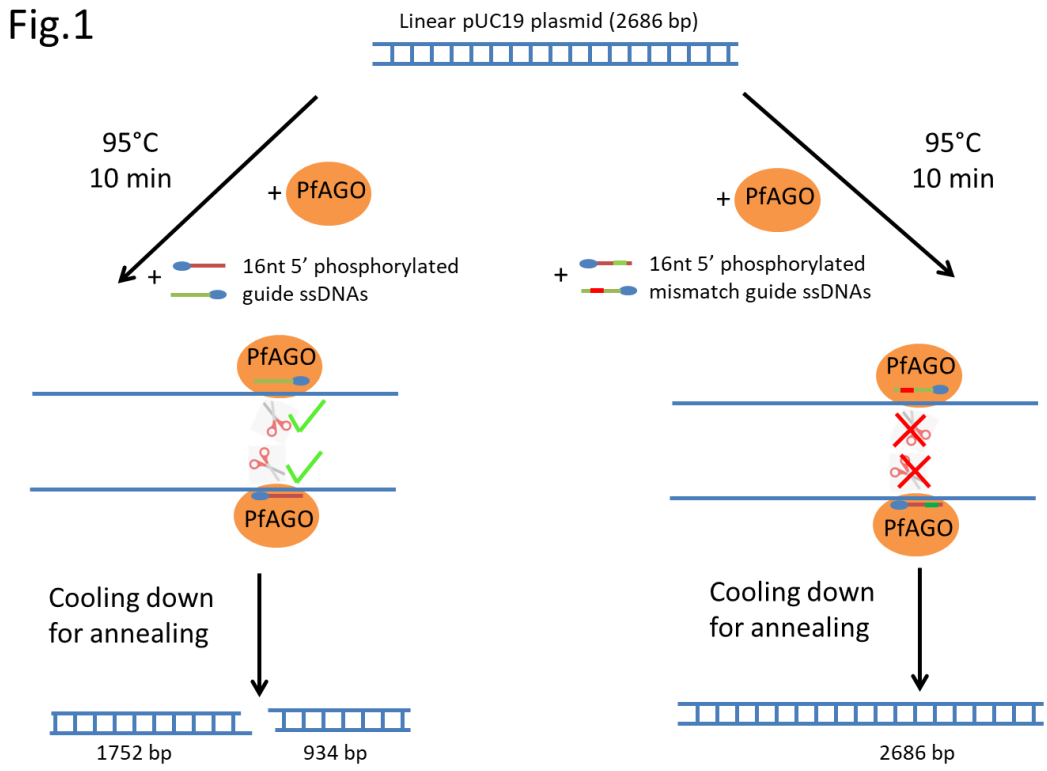

Figure 1. Schematic overview of the PfAGO-based ssDNA-guided DNA cleavage method.

Fig.2

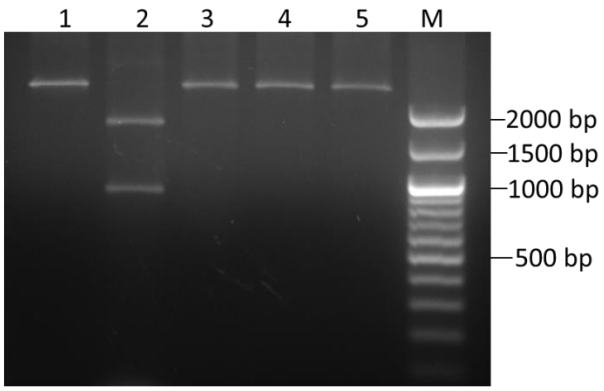

Figure 2. Agarose gel electrophoresis showing the PfAGO-based DNA cleavage results. DNA size showing as Figure 1.
Lane 1, 600 ng linear pUC19 plasmid;
Lane 2, 600 ng linear pUC19 plasmid +0.16 μM PfAGO +4 μM each of guide DNA.
Lane 3, 600 ng linear pUC19 plasmid +0.16 μM PfAGO +4 μM each of mismatch guide DNA; Mismatch site is on the position 11 of guide DNA.
Lane 4, 600 ng linear pUC19 plasmid +0.16 μM PfAGO +4 μM each of mismatch guide DNA; Mismatch site is on the position 12 of guide DNA.
Lane 5, 600 ng linear pUC19 plasmid +0.16 μM PfAGO +4 μM each of mismatch guide DNA; Mismatch sites are on the position 11 and 12 of guide DNA.
M, MCLAB 100 bp DNA ladder.

References

1. Ji-Joon Song et. al. (2004). Science. 305 (5689).

2. Daan C. Swarts et. al. (2015). Nucleic Acids Res. 43(10):5420-5129.

3. Yuqing Qin et. al. (2022). Trends Biotechnol. 40(8):910-914.