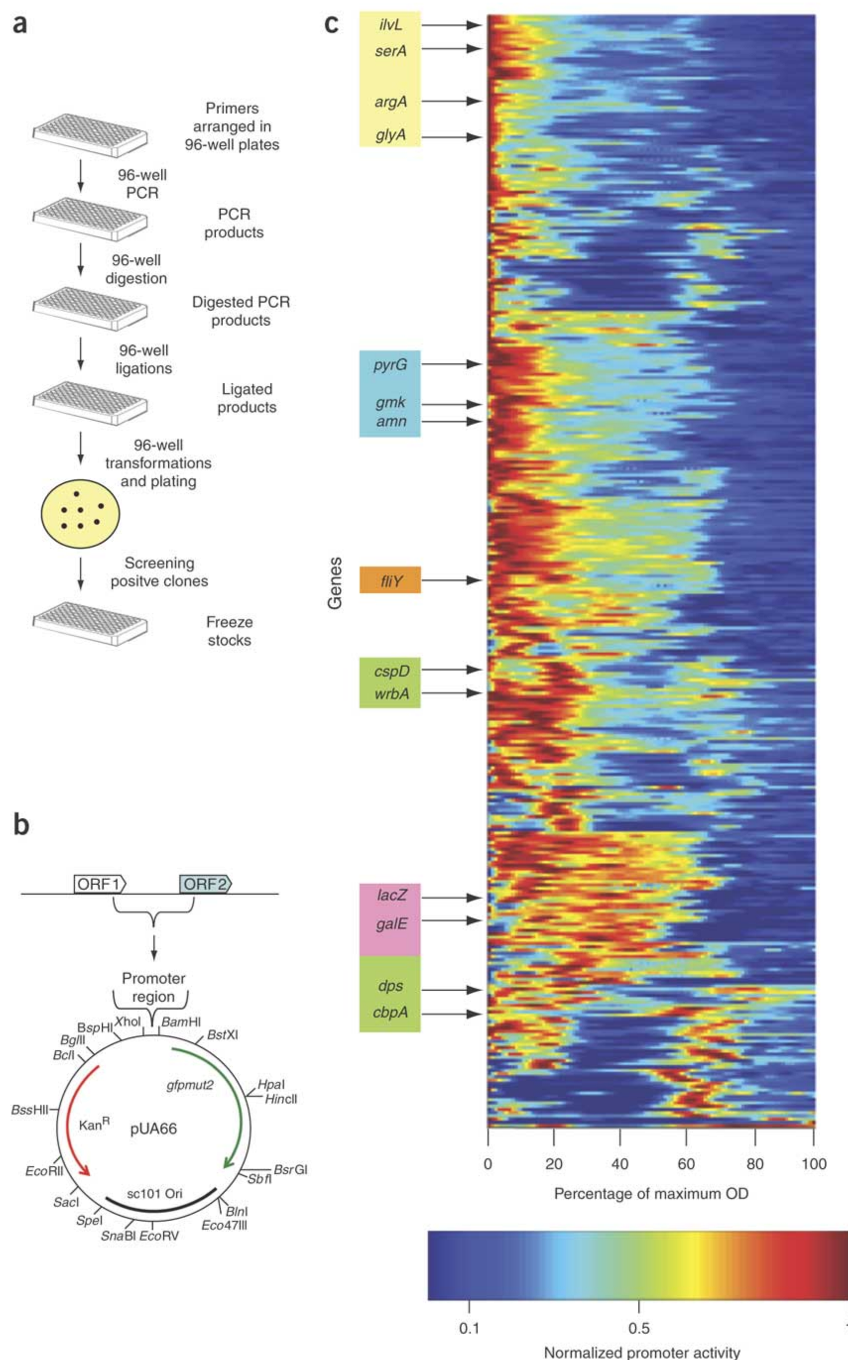
pUA66 Vector Information:



High-throughput cloning strategy used for the construction of the reporter-strain library, and promoter activity of the reporter strains during a diauxic shift experiment: (a) The cloning steps to construct the library were carried out in 96-well plates. (b) A map of the low copy-number plasmid, pUA66, used to construct the library. The plasmid contains a kanamycin resistance gene and a fast-folding gfpmut2 gene as a reporter gene with a strong ribosome binding site. The second plasmid used for the library construction, pUA139, is similar to pUA66, except that the restriction sites BamHI and XhoI are switched. (c) Promoter activity during growth in a diauxic shift condition. Shown are reporter strains showing detectable GFP signals above background levels. Promoter activity was determined by calculating dGFP/dt / OD. The normalized values of promoter activity are

shown.

Description: Total RNA was extracted from three different E. coli strains: MG1655, RP437, and RP437 ΔflhDC1,2. The strains were grown in M9 minimal medium (Sigma) supplemented with 0.2% casamino and 0.4% glucose. When cultures reached mid-exponential growth phase (OD ~ 0.4), RNA protect reagent (Qiagen) was added, and then total RNA was extracted (RNeasy Mini-kit, Qiagen). mRNA was reverse transcribed to cDNA using SuperScriptII reverse transcriptase (InVitrogene). Real time PCR was then used to quantify mRNA levels (ABI PRISM, Applied Biosystems). The primers used for the real time PCR were: for fliA, 5' CGCCACTCATCGTAGGAGAAGA and 5' CCAAATGTTGCTCGACACCAAT; for fliY, 5’ CCGGTTAATTTGCCGTCATCT and 5' AGGAACTTATCCGCCGTTCAG and for rpsB 5' GCTGACCACGAACACATTGCT and 5’ CCGGAATACCCAGGTTGTTTG. All mRNA measurements were normalized according to rpsB mRNA levels which were found to be constant in the three strains.

Use:

This bacterium was marked with green fluorescent protein (GFP) in plasmid pUA66. The bacterium was cultured with Kanamycin (30μg/ml). Green fluorescent protein was a marker for gene expression. GFP expression can be used to protein localization in living organisms. Thus this bacteria will be used in the biofilm observation without live/dead staining, which is very convenient.

Reference：

1. Nature Methods - **3**, 623 - 628 (2006)