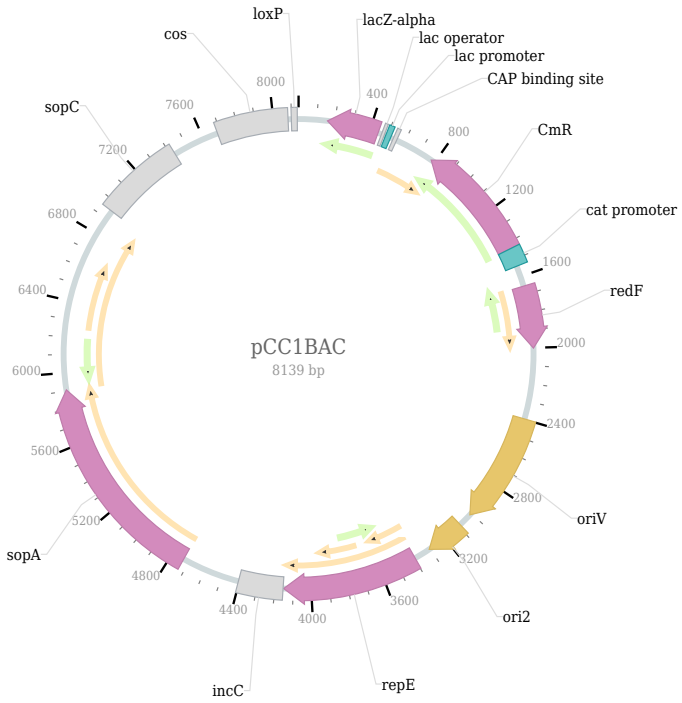
pCC1BAC

载体编号 Vector ID	V008675
抗性 Antibiotic Resistance	Chloramphenicol
长度 Length	8139 bp
载体类型 Vector Type	Cloning vector

产品信息

复制子 Replication Origin	ori2
来源 Source/Author	EPICENTRE Biotechnologies.
拷贝数 Copy Number	Single copy
培养菌株 Growth Strain(s)	EPI400
培养温度 Growth Temperature	37℃
备注 Comment	The pCC1BAC is a single copy vector that is inducible with 0.04% Arabinose or 1x CopyControl Induction Solution (CopyControl Fosmid clones grown in TransforMax EPI300 cells can be amplified to 10-50 copies per cell). An EPI400/EPI300 strain is recommended. The protocol is as below: a) Add 4 ml LB media into each test tube. Inoculate each tube with bacterial culture with antibiotic at the proper concentration. b) Incubate the tubes at 37°C, shaking overnight. c) Dilute the starting culture (from step b) 1:10 into antibiotic-supplemented fresh media. d) Supplement induction solution with a ratio of 1:1000, and grow the culture at 37°C for 4 h with vigorous shaking (approx. 250 rpm). e) Isolate DNA from the induced culture cells as per the protocol provided.The pCC1BAC vector has two origins of replication – a single copy E. coli F-factor replicon (ori2 or oriS) and a high-copy origin of replication called "oriV". Initially, replication of CopyControl clones can be controlled by the F-factor replicon so the vector is present at one copy per cell. Maintaining clones at single copy ensures insert stability and allows cloning of toxic gene products. Initiation of replication from oriV requires the trfA gene product. CopyControl Vectors use a specifically engineered E. coli host strain, EPI300/EPI400, which contains a mutant trfA gene under tight control of an inducible promoter (araBAD promoter). Addition of the CopyControl Induction Solution to the growth medium induces expression of trfA and subsequent amplification of the clone to high-copy number. Induction of CopyControl BAC clones from singlecopy up to 10-20 copies per cell greatly improves the yield and purity of BAC DNA for sequencing, fingerprinting and other applications. The ParA ATPase of F plasmid, SopA, is stimulated by SopB that assembles into a partition complex on the centromere-like locus, sopC, on the plasmid cargo. Mini-F plasmid has the trans-acting genes sopA and sopB and the cis-acting site sopC which are essential for accurate partitioning of plasmid DNA molecules into both daughter cells.

质粒图谱



复溶方法

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.