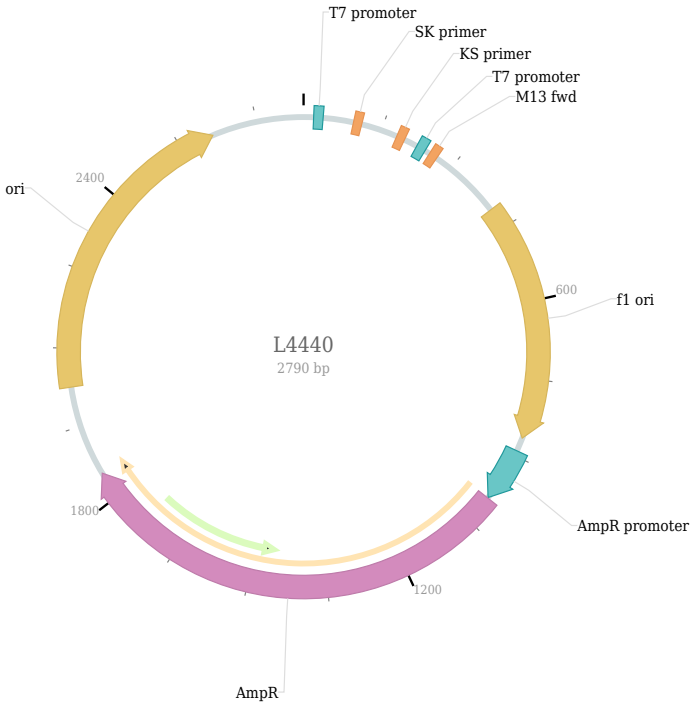
L4440

载体编号 Vector ID	V012784
抗性 Antibiotic Resistance	Ampicillin
长度 Length	2790 bp
载体类型 Vector Type	RNAi

产品信息

复制子 Replication Origin	ori
来源 Source/Author	Andrew Fire
拷贝数 Copy Number	High copy number
启动子 Promoter	T7
克隆方法 Cloning Method	Enzyme Cut
培养菌株 Growth Strain(s)	DH10B
培养温度 Growth Temperature	37℃
表达方式 Expression Method	IPTG induced
备注 Comment	E. coli bacteria carrying RNAi plasmids that produce the desired dsRNA can be fed to worms, and the dsRNA is transferred to the worm via the intestinal tract. RNAi plasmids typically consist of DNA coding sequence from the intended target gene cloned between two T7lac promoters. The plasmid also has a selectable marker that confers resistance to an antibiotic, in this case ampicillin. In 7.15, you will use the E. coli strain HT115 carrying various L4440 plasmids, each containing a different cloned gene sequence. HT115 is an RNase III-deficient E. coli strain with IPTG-inducible T7 polymerase activity. To induce dsRNA production from these plasmids, the HT115 bacteria is grown on special RNAi NGM feeding plates that contain IPTG and the ampicillin analog carbenicillin. Carbenicillin is preferred over ampicillin because it tends to be more stable.

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 ℃.