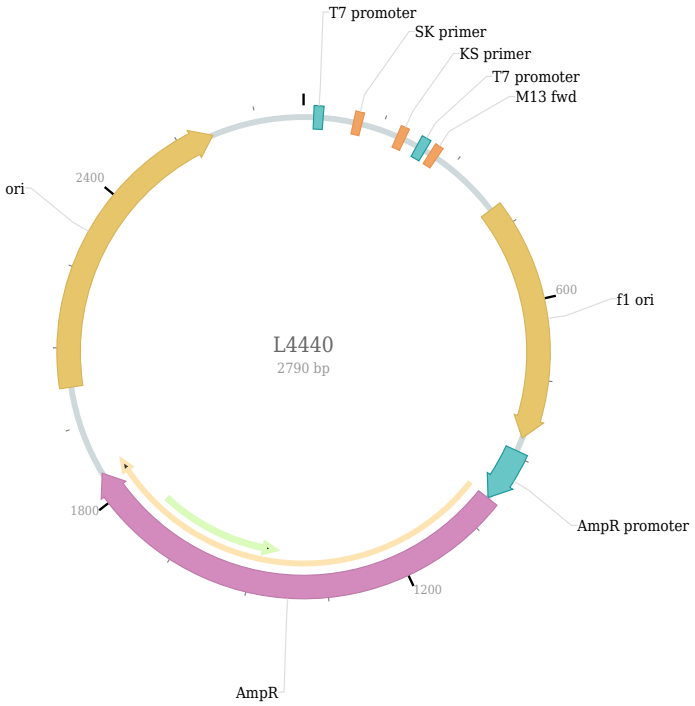
L4440

Vector ID	V012784
Antibiotic Resistance	Ampicillin
Length	2790 bp
Vector Type	RNAi

Product Information

Replication Origin	ori
Source/Author	Andrew Fire
Copy Number	High copy number
Promoter	T7
Cloning Method	Enzyme Cut
Growth Strain(s)	DH10B
Growth Temperature	37℃
Expression Method	IPTG induced
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.

Vector Map



Plasmid Resuspension Protocol

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 ℃.