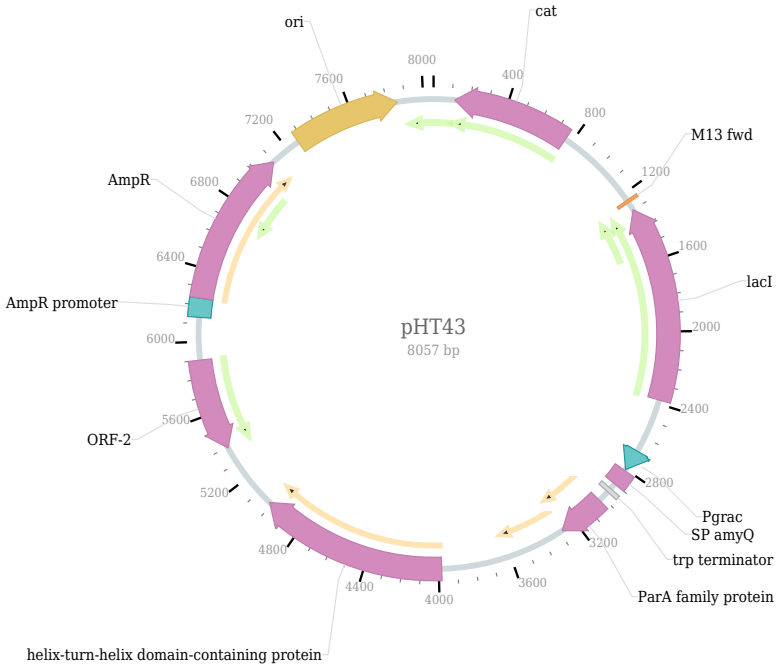
pHT43

Vector ID	V011875
Antibiotic Resistance	Ampicillin, Chloramphenicol
Length	8057 bp
Vector Type	Bacillus subtilis expression vectors

Product Information

Replication Origin	ori
Copy Number	High copy number
Promoter	Pgrac
Cloning Method	Enzyme digestion and ligation
5' Primer	LacI-R□GGCATACTCTGCGACATCGT
Fusion Tag	N-amyQ
Growth Strain(s)	DH10B
Growth Temperature	37℃
Comment	Introducing an inducible expression system into the groESL operon allows for co-expression of a pre-aggregation chaperonin (groESL) and a target protein (bgaB). This system utilizes IPTG to induce co-expression of 80% chaperonin and 20% target protein. Combining this system with the chaperonin-encoding genes htpG and pbpE resulted in 10-13% induced chaperonin. The method was further extended to co-express and identify the amyQα-amylase and the A and B subunits of glutamate synthase using Western blotting. To enhance expression, the chaperonin-protein complex was inserted into a high-copy SD plasmid with multiple cloning sites. The amyQ gene region of α-amylase was inserted into the SD plasmid cloning region, yielding pHT01, which facilitated the expression of a soluble chaperonin-protein complex.

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 ℃.