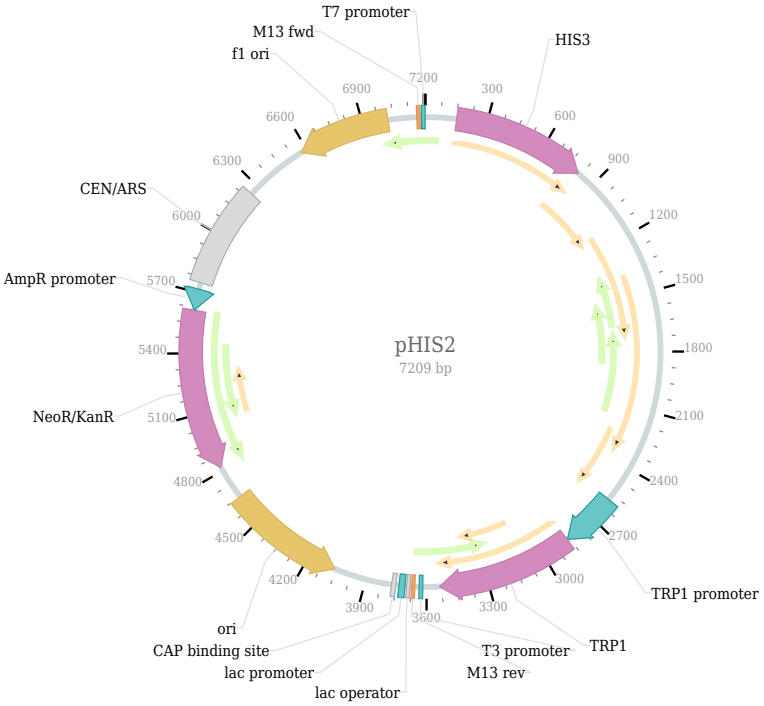
pHIS2

Vector ID	V001297
Antibiotic Resistance	Kanamycin
Length	7209 bp
Vector Type	Yeast one hybrid systems

Product Information

Replication Origin	ori
Promoter	TRP1
Cloning Method	Enzyme digestion and ligation
Growth Strain(s)	DH10B
Growth Temperature	37℃
Comment	pHIS2 is a reporter vector that can be used in yeast one-hybrid assays to identify and characterize DNA-binding proteins. The vector was specifically designed for use with the BD Matchmaker One- Hybrid Library Construction & Screening Kit (#K1617-1). It contains a HIS3 nutritional reporter gene, located downstream of a multiple cloning site (MCS) and the minimal promoter of the HIS3 locus (PminHIS3). Cis-acting DNA sequences, or DNA target elements, can be inserted into the MCS and used as baits to screen GAL4 AD/cDNA fusion libraries for proteins that interact with the target sequence. A protein-DNA (or one-hybrid) interaction can be detected by performing the assay in a yeast strain such as Y187 that is auxotrophic for histidine. Positive one-hybrid interactions drive expression of the HIS3 reporter gene, which enables the host cell to grow on histidine-deficient media.In the absence of activation, the constitutive HIS3 expression from PminHIS3 is very low. During library screening, the leaky expression of HIS3 is controlled by adding 3-amino-1,2,4-triazole (3-AT) to the medium. The concentration of 3-AT needed to fully suppress leaky HIS3 expression must be determined empirically for each DNA target element.pHIS2 can be maintained in both yeast and bacteria. It contains an autonomous replication sequence (ARS4) and TRP1 nutritional marker for replication and selection in yeast (1, 2); it contains a Col E1 origin and a kanamycin resistance gene (Kanr) for propagation and selection in E. coli. The centromeric sequence CEN6 ensures proper segregation of the plasmid during cell division in yeast (1, 2).To use pHIS2 in a one-hybrid assay, clone one or more copies of a cis-acting DNA target sequence into the MCS. Then introduce the plasmid into competent yeast cells using the transformation protocol in the BD Matchmaker Library Construction & Screening Kits User Manual (PT3529-1). In contrast to the original BD Matchmaker One- Hybrid System, this reporter vector does not need to be integrated into the yeast genome. Instead, it is maintained as an episome throughout the assay. Inserting your target element may alter the level of background HIS3 expression. Therefore, constructs should be tested for background (leaky) HIS3 expression before you start a one-hybrid analysis. Background growth due to leaky HIS3 expression is controlled by adding 3-AT to the selection medium, as described in the User Manual (PT3529-1).

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 ℃.