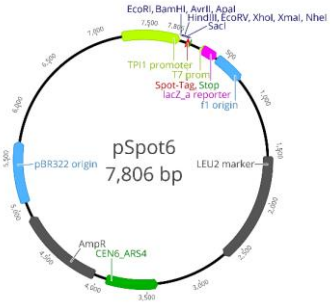
pSpot6 plasmid for expression of C-terminal Spot-Tag® fusion proteins in *S. cerevisiae*



For plasmid sequence, please visit www.chromotek.com.

Location of features

TPI1 promoter: 7232-9

MCS: 14-73

Spot-Tag®: 73-108

Stop codon (TAA): 109-111

T7 primer site: 223-241

Leucin marker (LEU2): 1557-2663

CEN replication origin: 3391-3909

Ampicillin resistance gene: 4041-4901

pRB322 replication origin: 5056-5675

Product	Code	Size
pSpot6	ev-6	1.25 µg

Vector type	yeast expression vector
Tag	Spot-Tag® (PDRVRAVSHWSS) C-terminal
MCS	BamHI, AvrII, ApaI, HindIII, EcoRV, XhoI, XmaI, NheI, SacI
Promoter	TPI1
Induction	constitutive
Host cells	*Saccharomyces cerevisiae* (*leuΔ*)
Selection	leucin (*S. cerevisiae*) ampicillin (*E. coli*)
Replication	CEN (*S. cerevisiae*) pBR322 (*E. coli*)
Use	Expression of a protein of interest fused to Spot-Tag® (C-terminal) in *S. cerevisiae*.

Vector description

The plasmid pSpot6 is an expression vector for Spot-Tag® fusion proteins in *S. cerevisiae*. After cloning the protein of interest (POI) into the multiple cloning site (MCS) provided by pSpot6, the Spot-Tag® (sequence: PDRVRAVSHWSS) will be fused to the C-terminus of the POI.

Expression in *S. cerevisiae*

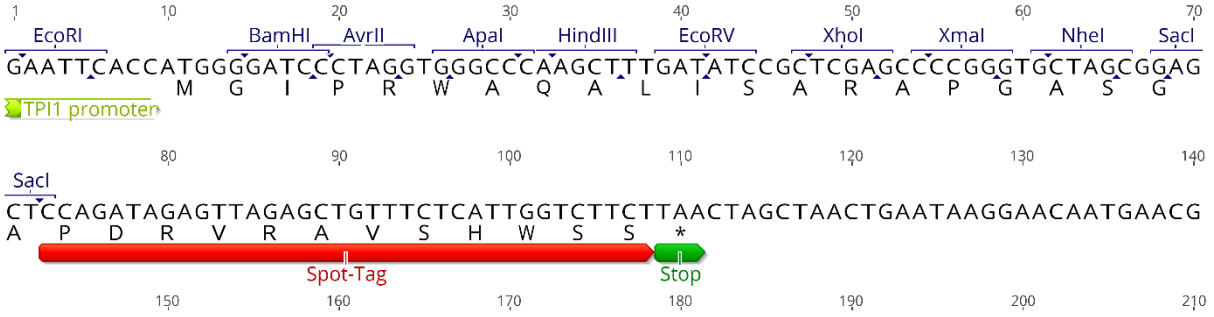
Transform a suitable *leu2* deletion strain of *S. cerevisiae* using pSpot6 and standard methods and grow on media lacking leucine. Protein expression is constitutive under the strong triosephosphate isomerase (TPI1) promoter. The resulting Spot-Tag® fusion protein can be purified using the ChromoTek Spot-Trap®.

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. The vector confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.

Note: The plasmid DNA was isolated from dam$^+$-methylated *E.coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites, you will need to transform the vector into a dam$^-$ host and make fresh DNA

Multiple cloning site (MCS)



Notice to Purchaser:
This plasmid was designed and generated by Dr. Philipp Kaiser from the Naturwissenschaftliches und Medizinisches Institut (NMI) at the University of Tübingen, Germany and is distributed by ChromoTek GmbH. Please acknowledge Dr. Philipp Kaiser (NMI, Tübingen, Germany) and ChromoTek GmbH (Martinsried, Germany) when using or redistributing this vector.
The development of this plasmid was supported by a grant "Zentrales Innovationsprogramm Mittelstand" (ZIM) from the Federal Ministry for Economic Affairs and Energy of Germany.