TECHNICAL MANUAL

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Yeast Kinase YFP Fusion Collection

Cat #YSC5108

Product description

The Dharmacon™ Yeast Kinase YFP Fusion Collection was developed by Anuj Kumar's research laboratory at the University of Michigan. It is a mini-collection of low-copy yeast plasmids bearing gene-YFP fusions. The genes are cloned along with 1 kb upstream sequence to encompass native promoters using the Gateway Cloning system. The fusion is at the carboxy-terminus of the encoded protein (Figure 1). This plasmid-based collection of fluorescent protein fusions is a valuable and versatile resource, facilitating systematic studies of protein localization (Ma *et al*. 2008).

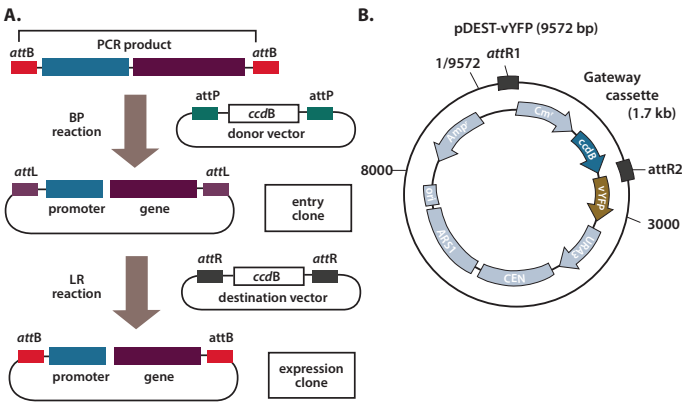

Figure 1. A suite of destination vectors for recombination-based cloning of yeast genes as fluorescent protein fusions. A. Overview of Gateway cloning. By the approach employed here, each amplified PCR product was cloned into the donor vector pDONR221, generating an "entry" clone. A subset of the promoter-gene cassettes were subsequently introduced into a destination vector, generating an "expression" clone by the LR reaction indicated. The LR reaction is technically simpler than the initial cloning process; accordingly, the entry clone collection represents a useful resource for recombination-based subcloning, even without extensive experience in Gateway-based techniques. **B.** Plasmid map of the destination vector pDEST-vYFP, derived from the centromeric yeast shuttle vector YCp50. Arrows indicate gene-coding sequences.

Antibiotic resistance

Table 1. Antibiotic resistances conveyed by pDEST-vYFP.

Antibiotic	Concentration	Utility
Ampicillin (carbenicillin)	100 µg/mL	Bacterial selection marker

Validation

This collection of Yeast Kinase YFP Fusions was used to investigate the subcellular distribution of kinases in response to cellular cues, screening for differential localization during filamentous growth. Six cytoplasmic kinases (Bcy1p, Fus3p, Ksp1p, Kss1p, Sks1p, and Tpk2p) were identified that localize predominantly to the nucleus during filamentous growth (Bharucha, Ma *et al.* 2008).

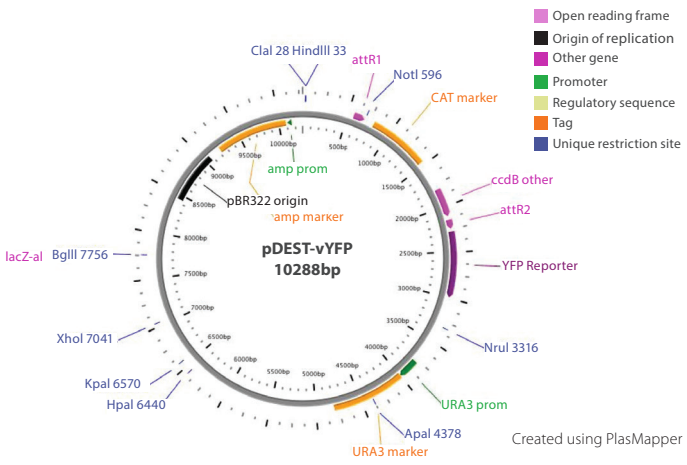

Figure 2. Vector map of pDEST-vYFP

Protocol I—replication

Table 2. Materials for replication.

Item	Vendor	Cat #
2X LB-Lennox broth (low salt)	Fisher Scientific	BP1427500
Peptone, granulated, 2 kg - Difco	Fisher Scientific	BP9725-2
Yeast Extract, 500 g, granulated	Fisher Scientific	BP1422-500
Glycerol	Fisher Scientific	BP2291
Carbenicillin	Fisher Scientific	BP2648-250

2X LB Broth (low salt) Medium Preparation

LB-Broth-Lennox	20 g/L
Peptone	10 g/L
Yeast Extract	5 g/L

For archive replication, grow all Yeast Kinase YFP Fusion clones at 37 °C in 2X LB-Lennox (low salt) medium plus 100 µg/mL carbenicillin. Prepare medium with 8% glycerol* and the appropriate antibiotics.

**Glycerol should be omitted from the medium if you are culturing for plasmid preparation. If making copies of the constructs for long term storage at –80 °C, 8% glycerol is required.*

Freeze at –80 °C for long term storage. Avoid long periods of storage at room temperature or higher in order to control background recombination products.

Protocol II—plasmid preparation

For plasmid preparation, grow all Yeast Kinase YFP Fusion clones at 37 °C in 2X LB-Lennox (low salt) medium plus 100 µg/mL carbenicillin. Most plasmid mini-prep kits recommend a culture volume of 1–10 mL for good yield. For these constructs, 5 mL of culture can be used for one plasmid mini-prep generally producing 5–10 µg of plasmid DNA.

1. Upon receiving your glycerol stock(s) store at -80 °C until ready to begin.
2. To prepare plasmid DNA first thaw your glycerol stock culture and pulse vortex to resuspend any *E. coli* that may have settled to the bottom of the tube.
3. Take a 10 µL inoculum from the glycerol stock into 3–5 mL of 2X LB (low salt) with 100 µg/mL carbenicillin. Return the glycerol stock(s) to –80 °C. **Note: If a larger culture volume is desired, incubate the 3–5 mL culture for 8 hours at 37 °C with shaking and use as a starter inoculum. Dilute the starter culture 1:500-1:1000 into the larger volume.**
4. Incubate at 37 °C for 18–19 hours with vigorous shaking.
5. Pellet the 3–5 mL culture and begin preparation of plasmid DNA.
6. Run 3–5 µL of the plasmid DNA on a 1% agarose gel. pDEST-vYFP without ORF is 10288 bp.

FAQs/troubleshooting

What Clones Are Part Of My Collection?

A USB containing the data for this collection will be shipped with each collection. This data file can be downloaded from the Yeast Gene YFP Fusion product page: https://horizondiscovery.com/en/gene-modulation/overexpression/non-mammalian/products/yeast-yfp-fusion-kinase-collection

What Antibiotic Should I Use?

You should grow all Yeast Gene YFP Fusion clones in LB-Lennox low salt with 100 µg/mL carbenicillin.

What Is The Host Strain For This Collection?

The Yeast Gene YFP Fusion clones are in *E. coli* Top10 Cells (Invitrogen).

References

1. J. Ma *et al.*, Localization of autophagy-related proteins in yeast using a versatile plasmid-based resource of fluorescent protein fusions. *Autophagy*. **4**(6), 792-800 (August 2008).
2. N. Bharucha *et al.*, Analysis of the Yeast Kinome Reveals a Network of Regulated Protein Localization during Filamentous Growth. *Mol. Biol. Cell.* **19**(7), 2708-2717 (July 2008).

If you have any questions, contact

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