TECHNICAL MANUAL

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INSPIRED CELL SOLUTIONS

E. coli SPA and TAP tag collection and strains

Cat #OEC4626, OEC4627, OEC4629, OEC4630

Product description

The Andrew Emili and Jack Greenblatt labs at the University of Toronto have released the Dharmacon™ Collection of SPA- and TAP-tagged *E. coli* strains intended to facilitate mapping of protein-protein interactions. Utilizing phage recombination system, sequence-specific PCR products encoding affinity-purication tags were inserted into discrete loci of the *E. coli* chromosome. 857 proteins, including 198 essential and conserved proteins, were successfully tagged. Over a quarter of these proteins were also successfully purified by the source lab.

Using affinity purification, proteins and their associated partners were purified and the components of the proteins in the complexes were further identified using LC-MS and MALDI-TOF mass spectrometry. An extensive network interaction resulting from the attempted tagging and purification of 1000 bait ORFs was published by Butland *et al.* (2005). Data generated from this study identified a reliable network of functionally diverse protein complexes.

These data offer insight into the function of uncharacterized proteins and outline the topological organization of the bacterial interactome. Knowledge of physical interactions mediated by conserved, essential bacterial proteins should facilitate the design of broad-range antimicrobials.

The strains are provided as bacterial cultures of *E. coli* (DY330) in Terrific Broth, 8% glycerol and kanamycin at a concentration of 50 µg/mL.

Shipping and storage

Individual clones are shipped at room temperature and may be stored for up to one week at +4 ºC. They may stored indefinitely at –80 °C. Plates are shipped on dry ice and should be stored at –80 °C.

To allow any CO_2 that may have dissolved into the media from the dry ice in shipping to dissipate, please store plates at –80 °C for at least 48 hours before thawing.

Glycerol stock replication

Culture clones in TB broth with 8% glycerol, and kanamycin at a concentration of 50 µg/mL.

Replication of individual clones

Once the clone has been streak isolated and the identity of the strain has been confirmed, we recommend making a stock of the pure culture. Grow the pure culture in LB broth + the appropriate antibiotic. Transfer 920 µL of culture into a polypropylene tube and add 80µL sterile glycerol to make an 8% glycerol freezing solution. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at –80 °C.

Prepare target plates

- Dispense ~160 µL of sterile LB media into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

Prepare source plates

- Remove the foil seals from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
- Thaw the source plates with the lids on. Wipe any condensation underneath the lid with a Kimwipe dampened with alcohol.

Replicate

- Gently place a disposable replicator into the thawed source plate and lightly move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the plate of the well.
- Gently remove the replicator from the source plate and gently place the replicator into the target plate. Gently move the replicator back and forth in the target plate to transfer cells.
- Discard the replicator.
- Place the lids back on the source plates and target plates.
- Seal the source plates, being mindful to avoid cross contamination.
- Repeat this process until all plates have been replicated.
- Return the source plates to the –80 °C freezer.
- Place the inoculated target plates in a 37 °C incubator. Incubate the plates for 12-24 hours.

Tag information

TAP-Tag

The exact sequence of the TAP-tag is shown in Rigaut *et al.*, 1999.

SPA-Tag

See Figure 1 for the schematic of the SPA-tag.

Name	Tag	Promoter	Expression	size (kb)
pMZ3F	SPA	Rpb1	ubiquitous	6.6
pMZS3F	SPA	CMV	strong	6.3
pMZI3F	SPA	Ecdysone	regulated	6
pMZI	TAP	Ecdysone	regulated	6.4

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TCCATGGAAAAGAGAAGATGGAAAAAGAATTTCATAGCCGTCTCA
S  M  E  K  R  R  W  K  K  N  F  I  A  V  S
          CBP
GCAGCCAACCGCTTTAAGAAAATCTCATCCTCCGGGGCACTTGAT
A  A  N  R  F  K  K  I  S  S  S  G  A  L  D

TATGATATTCCAACTACTGCTAGCGAGAATTTGTATTTTCAGGGT
Y  D  I  P  T  T  A  S  E  N  L  Y  F  Q  G
                       TEV site
GAGCTCGACTACAAAGACCATGACGGTGATTATAAAGATCATGAC
E  L  D  Y  K  D  H  D  G  D  Y  K  D  H  D
       3xFLAG
ATCGACTACAAGGATGACGATGACAAGTAG
I  D  Y  K  D  D  D  D  K  *
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References

1. Butland, G. *et al.* Interaction network containing conserved and essential protein complexes in *Escherichia coli*. *Nature*. **433**, 531-537 (2005).
2. Rigaut, G.; Shevchenko, A.; Rutz, B.; Wilm, M.; Mann, M.; Seraphin, B. A generic protein purification method for protein complex characterization and proteome exploration. Nat. Biotechnol. **17**, 1030-1032 (1999).
3. Zeghouf, M. *et al.* Sequential Peptide Affinity (SPA) system for the identification of mammalian and bacterial protein complexes. *J. Proteome Res.* **3,** 463-468 (2004).

If you have any questions, contact

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