

Mammalian SH2 Domain Collection

Catalog numbers: OHS4902 & OHS4903

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OPEN LABS PRODUCTS

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Because the available documentation for such products may be limited, it is useful to consult published articles for technical information, which will typically be listed on the corresponding product web page.

PRODUCT DESCRIPTION

The Mammalian SH2 Domain Collection contains 110 human and 21 mouse (total 130 unique) SH2 domains cloned into a modified pET28 bacterial expression vector. For protein capture and purification, a 6xHis tag is encoded for expression as an N-terminal fusion.

“SH2” (Src homology 2) is a 100-residue protein domain that specifically recognizes phosphorylated tyrosines and their surrounding structure. It was first identified as a conserved sequence in the oncoproteins Src and Fps.

PRODUCT APPLICATIONS

The Mammalian SH2 Domain Collection enables screening for phosphorylated tyrosines in a sample. This could be experimentally realized as a protein microarray or other *in vitro* assay. Applications might include discovering the identity of proteins that contain phosphorylated tyrosine residues (for example, finding the targets of a tyrosine kinase) or identifying which kinase cascade pathways are activated in a particular cellular process.

QUALITY CONTROL

Open Biosystems checks all cultures for growth prior to shipment. All strains have been sequence verified by Open Biosystems. Strains were also tested for protein expression.

VECTOR MAP

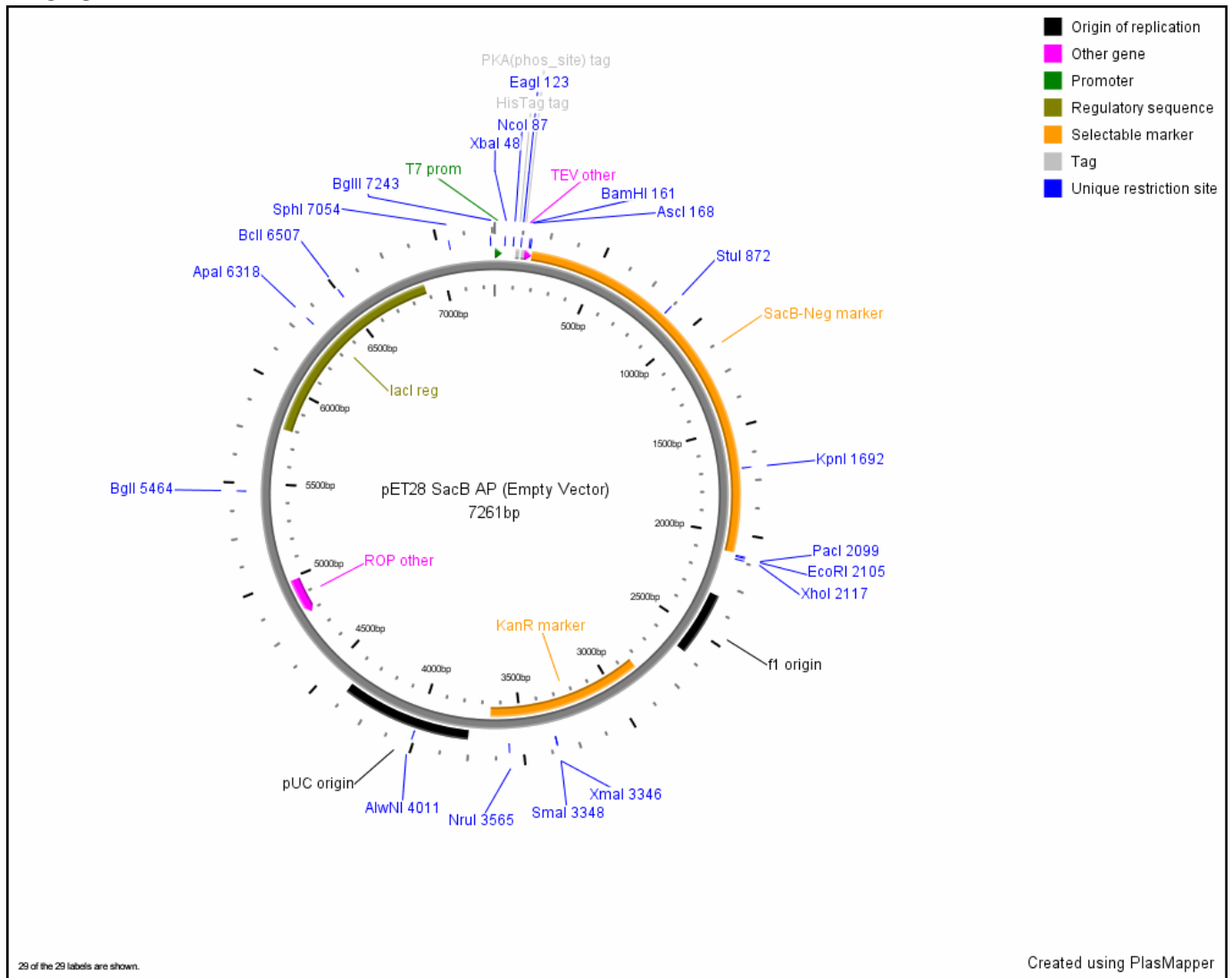


Figure 1. Vector map of pET28

PROTOCOL FOR PROTEIN TEST EXPRESSION

Day 1:

1. Thaw BL21- Codon Plus (DE3)-RIL competent cells (Stratagene) on ice.
2. Using a multichannel pipette transfer 1 μ L (~0.050 μ g) miniprep DNA from a 96 well microplate to the corresponding wells of the PCR plate kept on ice.
3. As soon as the competent cells are thawed, aliquot 10 μ L into each well of the sterile 96 well raised PCR plate containing the DNA and keep on ice for 30 minutes.
4. Heat shock the cells in a PCR machine at 42°C for 45 seconds.
5. Return the PCR plate to ice for 2 minutes.
6. Place the PCR plate at room temperature, and add 100 μ L of room temperature SOC with 34 μ g/mL chloramphenicol (final concentration) to each well.
7. Incubate a plate in a 37°C shaker at 250rpm for 1hour.
8. Transfer 8 μ L of the each transformation mixture to 800 μ L LB supplemented with 34 μ g/mL chloramphenicol (final concentration) in 96 well (2mL) block. Shake at 37°C at 250rpm overnight.

Day 2:

1. Prepare 300mL of TB small scale growth medium: add proper antibiotic, 300µL trace elements and 3mL MgSO₄ (100g/L).
2. Add 2mL of the TB growth medium to 4 X 24 well blocks.
3. Add 50µL of the overnight growth to the corresponding wells, shake at 37°C at 250rpm for 3 hours to reach OD₆₀₀ = 3.
4. Induce the samples with 20µL of 0.1M IPTG; incubate at 15°C overnight at 250rpm.
5. After inoculation, prepare glycerol stocks with 140µL of the overnight LB culture from the transformation by adding 60µL of 50% sterile glycerol to each well in a 96 well plate (Nunc). Seal the plate with aluminum sealing film. Store at -80°C

Day 3:

1. Centrifuge the 4 X 24 well blocks at 3000rpm for 10 minutes at 4°C.
2. Decant the supernatant. Completely drain the pellets of supernatant and freeze in -80°C for 10 minutes
3. Thaw the pellets.
4. Make suspension buffer: 30mL binding buffer and 0.3mL 100X protease inhibitor cocktail. Add 250µL of suspension buffer to the 4 blocks, and shake at speed 8 for 10 minutes, room temperature (titer plate shaker)
5. Make lysis buffer 100mL: 6 mL 10% CHAPS (0.6 % final), 93mL binding buffer, 1mL 100X protease inhibitor, 16.51µL benzonase (4128U total), 50mg lysozyme (0.5 mg/mL). Add 750µL of this buffer to a new 96 well plate (lysis plate)
6. Resuspend the cells and transfer 250µL of them to the lysis plate, shake at speed 8 for 30 minutes at room temperature (titer plate shaker)
7. Make Ni resin 1:5, add 7mL superflow Ni-NTA into a 50mL falcon tubes and wash 3X with 15mL lysis buffer (binding buffer and CHAPS only) by centrifuging 1000rpm for 5 minutes.
8. Add 150µL of Ni resin to each well of the binding plate using multipipette.
9. In a PCR plate add 10µL 5X SDS loading dye and 40µL of cell lysate, pipette to mix (total cell plate).
10. Centrifuge lysis plate at 3000rpm for 10 minutes at 4°C.
11. Remove 850µL of supernatant and put into Binding plate.
12. Shake the binding plate at 167rpm for 1 hour at room temperature (shaker, 25°C)
13. Centrifuge binding plate 1000rpm for 5 minutes.
14. Repeat 4X by adding 220µL wash buffer and centrifuging at 1500rpm for 5 minutes. The last wash step should be continued (last wash centrifuge for 10 minutes).
15. Elute by adding 40µL elution buffer and incubate for 5 minutes; put new PCR plate under filter and centrifuge 2000rpm for 10 minutes.
16. Add 10µL 5X dye to the 40µL purified protein samples
17. Heat samples (total lysate and purified) at 95°C for 10 minutes
18. Run 8X 26 well criterion gels (10-20% Tris HCl) using 5µL marker and 10µL sample, run 200V and 100W, 1.4A (initial current) for 1 hour and 20 minutes.
19. Wash gels 3X with water.
20. Add Simply Blue Safestain to cover gels; stain overnight.
21. Change to water to destain.

VALIDATION

Using the above protocol, protein expression was visualized on SDS-PAGE gels for 131 different SH2 constructs as seen in Figures 2, 3 (below).

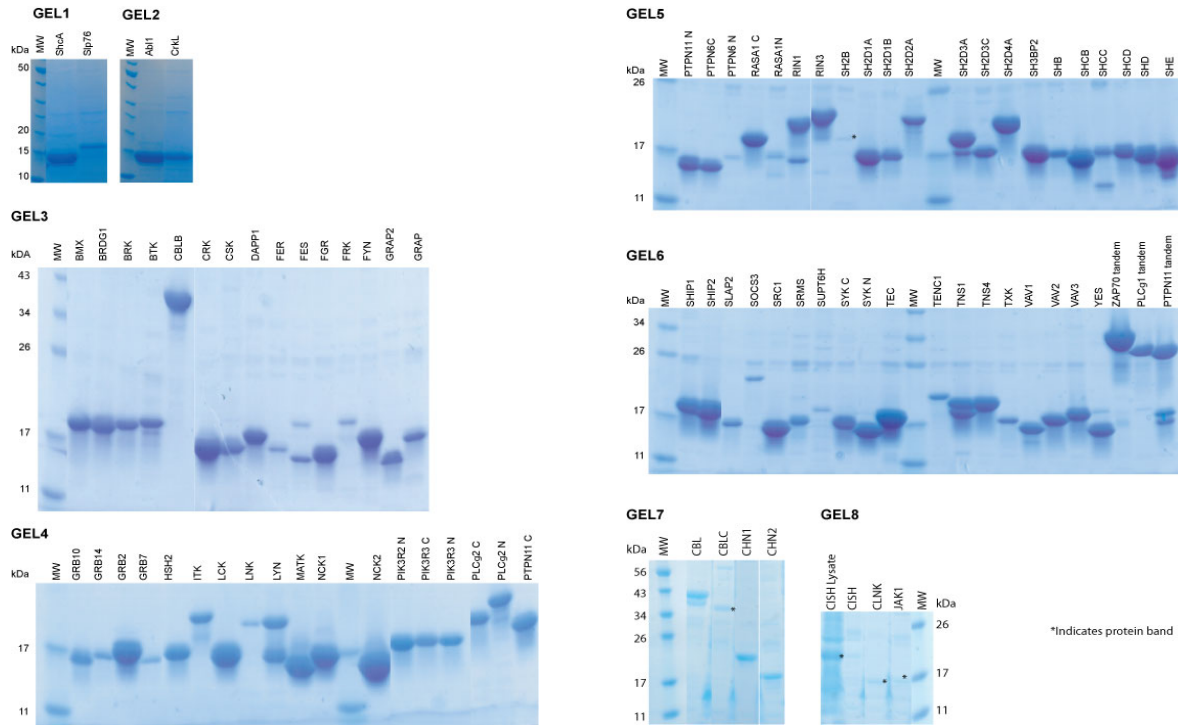


Figure 2. SDS-PAGE gels for protein expression. Of the 130 unique SH2 constructs tested, 123 produced soluble protein and seven produced little to no soluble protein.

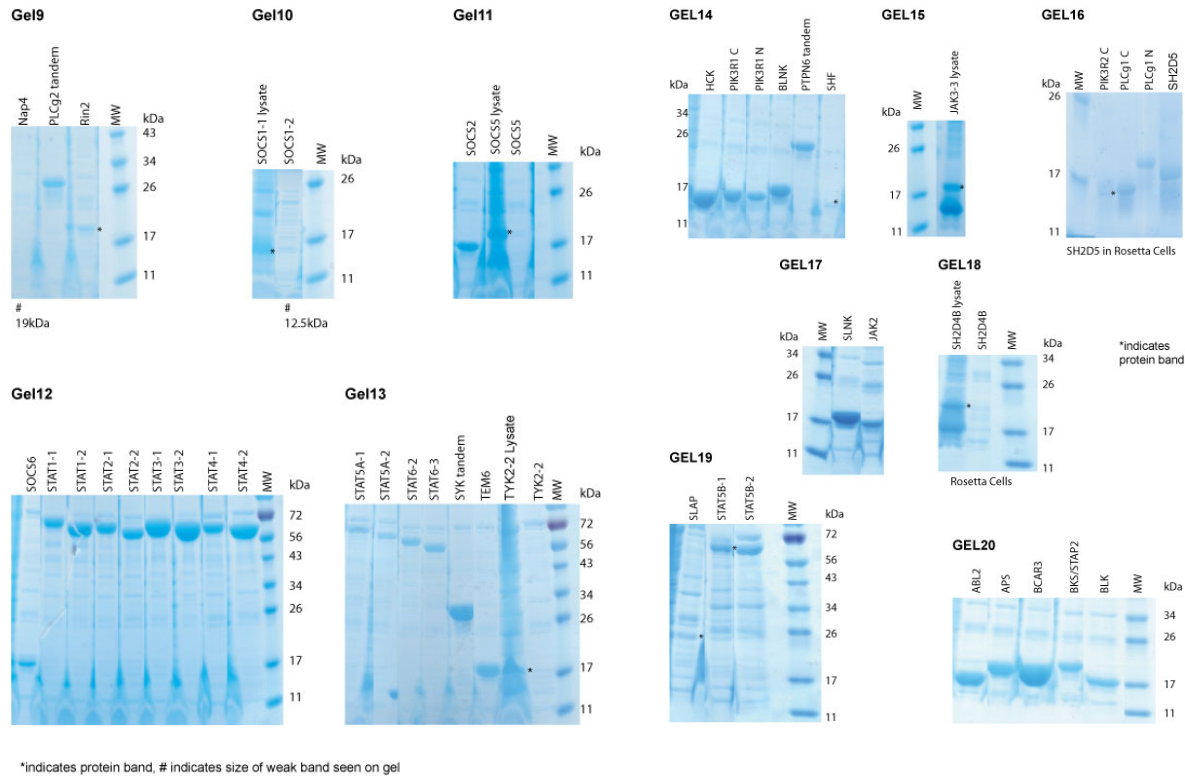


Figure 3. Continuation of SDS-PAGE gels for protein expression.

FAQS/TROUBLESHOOTING

For answers to questions about this collection, please mail technical support at info@openbiosystems.com with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

ACKNOWLEDGEMENTS

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Pawson Website SH2 Link:

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REFERENCES

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