**Reagents, Media, Solutions and Particular Equipment – Supplementary Information**

**Post-translational-selective intracellular silencing of acetylated proteins with de novo selected intrabodies**

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# Reagents

* Yeast Nitrogen Base without amino acids and ammonium sulfate (SIGMA #Y-1251)
* Bacto Agar (BD # 214010)
* NaOH (Riedel-deHaen #30620)
* Adenine hemisulfate salts (SIGMA #A-9126)
* L-arginine HCL (SIGMA #A-5131)
* L-cysteine (SIGMA #C-6852)
* L-threonine (SIGMA #T-8652)
* L-aspartic acid (SIGMA #A-4534)
* L-isoleucine (SIGMA #I-2752)
* L-methionine (SIGMA #M-9625)
* L-phenylalanine (SIGMA #P-2126)
* L-proline (SIGMA #P-0380)
* L-serine (SIGMA #S-4500)
* L-tyrosine (SIGMA #T-3754)
* L-histidine (SIGMA #H-8125)
* UraciL (SIGMA #U-0750)
* L-leucine (SIGMA #L-8912)
* L-lysine HCL (SIGMA #L-5626)
* L-tryptophan (SIGMA #T-0254)
* Ammonium sulfate (Fluka #09980)
* Polyethylene glycoL 4’000 (Fluka #95904)
* D-(+) Glucose anhydrous (SIGMA #G-7021)
* Lithium Acetate dihydrate (SIGMA #L-6883)
* Succinic acid (SIGMA #S-7501)
* DimethyL sulfoxide –DMSO (Fluka #41639)
* Glass beads 425-600 microns acid washed (SIGMA #G-8772)
* Yeast extract (BD # 211931)
* Bacto peptone (BD #211840)
* 3-amino-1,2,4-triazole (3AT) (SIGMA #8056)
* 5-bromo-4-chloro-3 indolyl-β -D-galactosidase –X-gaL (Eppendorf #0032006.400)
* Carrier DNA from salmon sperm (SIGMA #d1626)
* β-mercaptoethanoL (BMe) (SIGMA # M6250)
* D-SorbitoL (SIGMA #S1876)
* Trizma base (SIGMA # T1503)
* Whatman qualitative filter paper (WHA10010155|ALDRICH)
* Nitrocellulose filter circles (Scheicher and SchuelL BA85)
* Lyticase (SIGMA #L2524)
* Protease Inhibitor CocktaiL (SIGMA #P8340)
* Triton X 100 (SIGMA #T9284)

# Media

**SD (Synthetic minimal) medium** is prepared by adding to autoclaved YNB (Yeast Nitrogen Base) a “salt and glucose solution”, an “aminoacidic mix”, and desired “omitted aminoacid” solutions.

## YNB w/o aa and ammonium sulfate (1L) for SD medium:

1.2 g yeast nitrogen base, w/o amino acids and ammonium sulfate

For plates, add 20 g bacto-agar

*Add H2O to 800 ml, pH to 5.8 and autoclave 121°C for 15 min.*

### *Salt and glucose solution*

5.4g NaOH

10g succinic acid

5g ammonium sulfate

22g D-glucose

*Add H2O to 100 mL and dissolve alL components one by one to obtain a finaL volume of150mL. Store at 4°C.*

### *Amino acids (aa) MIX*

5.8 g NaOH

1 g Adenine hemisulfate salts, L-Arginine HCl, L-Cysteine, L-Threonine (each)

0.5 g L-Aspartic acid, L-Isoleucine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine

*Dissolve in 80 mL H2O*

L-Tyrosine: 0.2 g NaOH, 0.5 g L-Tyrosine

*Dissolve in 10 mL by heating*

*Add to aa MIX the L-Tyrosine solution and H2O to make a finaL volume of 100 ml. Filter –sterilize, aliquot and store at -20°C for up to 1 year.*

### *Omitted aminoacid solutions*

L-Histidine : 5 g/L

L-Leucine : 10 g/L

L-Tryptophan : 10g/L

*Filter-sterilize and aliquot omitted amino acids (aa) solutions (H, L, W) and store at -20°C for up to 1 year.*

**YPD medium** is a rich medium used to culture untransformed L40 or for recovery after transformation. Variants without glucose and/or with adenine are also used in the screening protocol.

## YPD medium

10 g yeast extract

20 g bacto peptone

2% glucose

For plates add 20 g bacto-agar

*Add H2O to 950 ml. Adjust pH to 5.8 then adjust to 1 liter. Autoclave 121°C for 15 min*

## YPA medium

10 g yeast extract (BD # 211931)

20 g bacto peptone (BD # 211840 )

0.1g Adenine hemisulfate salt (SIGMA # A-9126)

For plates add 20g bacto-agar (BD # 214010)

*Add H2O to 950 ml. Adjust pH to 5.8 then adjust to 1 liter. Autoclave 121°C for 15 min*

## YPAD medium

10 g yeast extract (BD # 211931)

20 g bacto peptone (BD # 211840 )

0.1g Adenine hemisulfate salt (SIGMA # A-9126)

*Add H2O to 950 ml. Adjust pH to 5.8 then adjust to 1 liter. Autoclave 121°C for 15 min*

# Solutions

* 10X LiAc buffer: 1M LiAc, pH 7.5 adjusted with diluted glaciaL acetic acid autoclave-sterilized
* 50% (w/v) autoclave-sterilized PEG 4000 (Solution must be kept in a tightly sealed glass bottle to avoid evaporation)
* 10X TE buffer: 100mM Tris, 10mM EDTA, pH7.5, autoclave-sterilized
* 10 mg/mL denatured carrier DNA from salmon sperm
* Lyticase stock solution (330 U/mL in H2O)
* 3 AT stock 2M : 168.2g in 1L of H2O, filter sterilize, store at 4°C. Preferably freshly prepared each time. Light sensible.
* Z -buffer : 60 mM Na2HPO4, 40 mM NaH2PO4, 10 mM KCl, 1mM MgSO4, pH 7.0. Store at room temperature for up 1 year)
* X-gaL 20 mg/mL : Dissolve in N,N-dimethylformamide (DMF) at a concentration of 20 mg/ml. Store in dark at -20°C.
* Z-buffer / X-Gal/BMe solution (add 167uL of 20mg/mL X-gaL and 27uL of BMe to 9.8mL of Z-buffer)
* Lyticase solution : 20mM Tris PH=7.5, Lyticase stock (330 U/ml) in equaL volume

# Particular Equipment and Materials Needed for PISA Screening protocol

* 15 mL Falcon tubes (Corning, cat. no. 430790)
* 50 mL Falcon tubes (Corning, cat. no. 430828)
* 250mL Falcon conicaL tubes (BD, cat. no. 352075)
* 0.2 μm filter (Millipore, cat. no. SLGP033RS)
* 10 cm (SD-WL/SD-WHL) and 15 cm or 25cm large petri plastic plates (SD-WHL)
* Bench-top centrifuge
* Centrifuge (Eppendorf 5810R or similar, able to spin 250mL Falcon conicaL tubes at a setting of 2,500g; Eppendorf)
* Stationary incubators at 30 and 37 °C
* Shaker incubators at 30 and 37 °C (able to shake 2.5-literflasks at a setting of 230 r.p.m.)
* Water bath equilibrated at 42 °C
* Established bait linere covered from GS
* 500ug of Antibody library
* 1 aliquot of ssDNA 10 mg/ml, denatured 10min at 100°C
* 200 mL SD-W
* 2 L YPAD
* 1 L YPA
* 1 L SD-WL plates (100mm)
* 1 L SD-WHL plates (100mm)
* 2 L SD-WHL plates (15 or 25 cm)
* 1L SD-WL
* 1L SD-WHL
* 2M 3AT (if needed)
* 100 mL 10X TE
* 20 mL 10X LiAc
* 150 mL 50% PEG 4000
* 20 mL DMSO