THE SAINSBURY LABORATORY
STANDARD OPERATING PROCEDURE

The Sainsbury Laboratory TSL

TITLE: Yeast-two-Hybrid assay (Summer School Protocol)

APPLIES TO STAFF IN: Sainsbury Laboratory

HEALTH & SAFETY INFORMATION INCLUDED: YES

REFERENCE No: TSL039	**VERSION No: 1.0**
DATE EFFECTIVE: August 2017	**REVIEW DATE: August 2019**
AUTHOR: Joe Win	**APPROVED BY: Simon Foster**

1 PURPOSE OF PROCEDURE/METHOD AND ITS SCOPE

To test protein-protein interaction in yeast.

2 EQUIPMENT NEEDED

Pipettes
Pipette tips
Water bath
Shaking incubator
Y2H Gold cells from Clontech
Cuvettes
1M lithium acetate (LiAc) solution
50% PEG (MW. 3350) solution
1M TE-buffer pH 8
0.2% adenine solution
Dimethyl sulfoxide (DMSO)
Carrier-DNA (for transformation)
Plasmid DNA
Yeast Dropout Medium (-leu/-trp and –leu/-trp/-ade/-his)
YPD-Medium

3 STEPS IN PROCEDURE

This yeast two hybrid (Y2H) protocol is based on the user manual of Clontech MatchMaker GAL4 Two Hybrid System 3. Please consult this manual for further details and trouble-shooting.

- **Wear lab coat and gloves. Observe local safety rules. Do not eat or drink in the laboratory.**
- **Dispose of all biologically contaminated waste according to local safety rules, e. g., use the 'kill box' for re-useable items and blue box for disposable items. Sharp items must be enclosed in yellow-cap plastic bottles.**

<u>Yeast Transformation</u>

1. Grow Y2H Gold cells in 50 ml YPD (+Adenine) liquid medium in a shaking incubator at 28 °C at 220 RPM overnight up to OD_{600} 1.0
2. Spin cells at 1100 x *g* for 5 min in a centrifuge at room temperature

3. Wash the cells with H_2O twice
4. Resuspend the cells in 5 ml sterile 100 mM LiAc, 100mM TE-buffer pH 8.0 solution
5. Mix 100 ng each of two plasmid DNAs (in 1-5 µl volume) with 10 µl of carrier DNA
6. Add 100 µl cells to the DNA mix
7. Add 500 µl sterile LiAc/PEG solution (100 mM LiAc; 40% PEG, 100 mM TE-buffer pH 8.0), mix well and incubate in a shaking heat block at 28 ºC for 30 min
8. Add 70 µl sterile DMSO, mix gently
9. Incubate at 42 ºC for 15 min in a water bath, cool on ice for 2 min
10. Spin cells at 16000 x *g*) for 30 sec at room temperature
11. Resuspend cells in 250 µl 100 mM TE-buffer, plate on SD (-leu/-trp) agar plates

Y2H assay

1. Inoculate 3 ml SD -leu/-trp liquid culture with a single transformant (single colony) from the SD –leu/-trp selection plate, and place at 28 °C shaking incubator at 220 RPM overnight
2. Measure the OD_{600} of the overnight culture (dilute culture 1:10 in SD medium for measurement)
3. Adjust the OD_{600} of all test strains to 1.0
4. Prepare serial 1:10 dilutions (1:10 / 1:100 / 1:1000 i.e. OD_{600} 1 / 0.1 / 0.01 / 0.001). Mix well by pipetting
5. Prepare replica drop plates of all dilutions on SD –leu/-trp and SD –leu/-trp/-ade/-his+α-X-gal by placing 3 µl drops from each dilution. Let the droplets dry on the plate
6. Incubate the plates at in an incubator at 28 °C for 4 days
7. Record the results by taking a photograph/scan of each plate

4 RISK STATEMENT

This activity is low risk and does not involve handling of any hazardous chemicals except adenine which is used in growing media at trace amount.

All individuals using this procedure will be shown the risk assessment and given appropriate information, instruction and training in the risks and

precautions necessary, including the use of any personal protective equipment required.

SOP HEALTH RISK ASSESSMENT

[1] Activity:	**Yeast-two-hybrid assay to test protein-protein interaction**		
[2] Location of activity:	The Sainsbury Laboratory and Chris Lamb Training Suite		
[3] Who is involved:	Science staff in The Sainsbury Laboratory and participants to the TSL Summer School		
[4] Frequency of activity:	Variable		
[5] Duration of Activity:	3 hours		
[6] Chemical Hazard Name:	**Hazard Statements**	**Route of Exposure***	**Quantity Used**
Lithium acetete	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.	N/A	Less than 1 ml
Polyethylene glycol	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.	N/A	Less than 1 ml
Dimethyl sulfoxide	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.	N/A	Less than 1 ml
Adenine 	H301- Toxic if swallowed.	I	Less than 1 ml
[7] Details of biological agents of risk to human health:			**GMRA Number**
None			

[8] Other Hazards:	Please ■ as necessary				
Hot or Cold Burns	☐	Ionising Radiation**	☐	Ultra Violet or Infra Red	☐

Dust	☐	Noise	☐	Pollen Sensitizer	☐
Repetitive Action	☐	Extreme Cold Environment (< 0°C)	☐	Lifting / Manual Handling	☐
Asphyxiation	☐	Cuts	☐	Electrical	☐
Slips / trips / falls	☐	Display Screen Equipment	☐	Other (give details)	☐

[9] Control Measures: Please ■ as necessary

Fume Cupboard	☐	Microbiological Safety Cabinet	☐	Total Containment Cabinet	☐
Ventilated Bench	☐	Spill Tray	☐	Trained personnel only	☒
Signs	☐	Reduce frequency/alternate activity	☐	Reduce duration of activity	☐
Sub divide a load	☐	2 man lift of equipment	☐	Not for more than 1 hour	☐
Regular, short breaks	☐	Alternate activities	☐	Other (give details)	☐

[10] Personal Protection: Please ■ as necessary

Lab coat	☒	Safety Glasses	☐	Face Shield	☐
Goggles	☐	Gloves	☒	Thermal Protective Gloves	☐
Ear defenders	☐	Other (give details)	☐		

[11] Is personal monitoring and/or health surveillance required? Yes ☐ No ☒

Details:

[12] Restrictions: Please ■ as necessary

No lone working	☐	Not to be left unattended	☐	Named persons only	☐
In restricted area	☐	Not by new or expectant mothers	☐	Under constant supervision	☐
Not by under 18's	☒	Other (give details)	☐		

[13] Level of Residual Risk: Please ■ as necessary

Low	☒	Medium	☐	High	☐

Name of Assessor:	Date:
Simon Foster	21st July 2017

* Route of exposure; S = skin, I = ingestion, B = inhalation

Activities involving new or expectant mothers and young persons also require additional risk assessment.

5 DOCUMENTATION

Links to relevant H&S information on intranet or internet

Reference any relevant manuals

Link to: JIC Chemical Tables:
http://intranet/infoserv/support/QualityAssurance/Chemical_Tables_SOPs.htm

Link to: Good Laboratory Practice in the Use of Chemicals:
http://intranet/infoserv/support/Safety/Chemical/GLP_Chems.htm

Link to Biological and GM Safety:
http://intranet/infoserv/Support/Safety/Biological/index.htm

Link to Laboratory Waste Disposal:
http://intranet/infoserv/Support/Safety/Waste/index.htm

6 RELATED PROCEDURES

Other relevant SOPs

7 NOTES

8 APPENDICES

Chemical	**Lithium acetate dihydrate**	Sigma L6883 CAS: 6108-17-4
Hazard Statement(s)	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008. This substance is not classified as dangerous according to Directive 67/548/EEC.	
Precautionary Statement(s)	N/A	
Handling	Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.	
Storage	Store in cool place. Keep container tightly closed in a dry and well-ventilated place.	
Disposal	Dispose of in a chemically compatible container; ensure liquids are placed in a container designed for liquids. Label and place in the collection tray for “Waste Not Suitable for Bulking-Up” in the Chemical Waste Store. Where possible, use the container in which the chemical was supplied. Follow the user instructions for Using the Chemical Waste Store. Consult the Chemical Safety Officer or your Lab Manager for more information.	
Spillage	Wear appropriate personal safety equipment; lab coat, gloves and safety glasses and clean up spills with blue roll or spill tamer kit. Put in suitable container and dispose of in Chemical Waste Store.	

Chemical	**Dimethyl Sulphoxide** (DMSO)	Sigma D2650 CAS:67-68-5
Hazard Statement(s)	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.	
Precautionary Statement(s)	N/A.	
Handling	Avoid inhalation of vapour or mist. Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.	
Storage	Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Store under inert gas. Hygroscopic	
Disposal	Dispose of in a chemically compatible container; ensure liquids are placed in a container designed for liquids. Label and place in the collection tray for “Waste Not Suitable for Bulking-Up” in the Chemical Waste Store. Where possible, use the container in which the chemical was supplied. Follow the user instructions for Using the Chemical Waste Store. Consult the Chemical Safety Officer or your Lab Manager for more information.	
Spillage	Wear appropriate personal safety equipment; lab coat, gloves and safety glasses and clean up spills with blue roll or spill tamer kit. Put in suitable container and dispose of in Chemical Waste Store.	