Small scale expression using blocks (96-deep well)

DAY 1

1. Place PCR plate containing the plasmids (3 µl) on ice for 10 min.
2. Add 25 µl of competent cells.
3. Leave on ice for 10-30 min.
4. Heat shock at 42°C for 45 sec.
5. Place on ice for 2 min.
6. Recover in 850 µl of SOC for 1 h at 37°C with shaking. *Recovery can also be done in 125 µl of SOC and no centrifugation is needed.*
7. Spin down at 3000 rpm for 10 min.
8. Discard supernatant (flip plate only, do not tap, ~100 µl of media should remain at bottom). Use 55 µl to plate on LB + appropriate antibiotics (keep remaining cell suspension at 4°C in case the cells don't grow). Use glass beads to spread the sample.
9. Incubate plates overnight at 37°C. Remove beads.

NOTE: Plates with transformations should be used within two weeks, maximum.

DAY 2 *(gels can be cast on day 2 or 3)*

1. Take 3-5 colonies from a **fresh** transformation plate and aseptically transfer into 750 µl of ZYP-5052 rich medium (Studier) **without lactose**, containing antibiotics in a 96- deep well block and seal with an AirPore tape sheet. *(Use 10 µl tips and tape down the block to the shaker).*
2. Incubate at 37°C for 5-6 hours with shaking (230 rpm) until and OD_{600} of 1-1.5 is reached.
3. Switch temperature to 18°C and wait 15-30 min for the media to cool down.
4. Remove tips using tweezers *(sterilise with alcohol)* and add IPTG (10 µl/well from a 7.5 mg/ml solution)
5. Incubate overnight (12-14 h) with shaking.

DAY 3

1. Centrifuge plate at 3750 rpm for 15 min and discard the supernatant.
2. Freeze pellet at -80ºC (liquid N_2 will damage the block) for 15 min and thaw by leaving the plate at RTº.
3. Suspend pellets in 500 µl of Lysis Buffer (use multichannel in mixing mode) and incubate with gentle agitation at 5ºC for 1.5 h *(Use same incubator as before. Leave shaking at 230 rpm with the AirPore tape on)*.
4. Total Expression Sample: take 40 µl of lysate and mix to 10 µl of 5X Sample Buffer for SDS-PAGE in a PCR plate. Put tape on top and incubate plate for 10 min at 95ºC in PCR cycler. *Two possibilities for the layout of the samples in the plates: you can use one plate per fraction (one for total and one for soluble) or use one plate per set of samples tested (top lanes for total and bottom lanes for soluble or vice versa). There is no problem to reheat samples from a previous day.*
5. Spin lysate at 3750 rpm for 30 min.
6. Soluble Fraction: take 40 µl of supernatant *(Be careful not to touch the pellet. Stay as close to the surface as you can)* and add 10 µl of 5X Sample Buffer for SDS-PAGE in a PCR plate. Put a clear tape on top of plate and incubate for 10 min at 95ºC in PCR cycler.
7. Load 10 µl of sample per well with multichannel *(1 well will separate each tip)*, which gives the possibility to have Total Expression and Soluble fractions side by side for each clone.
8. Load 6 µl of ladder.
9. Run SDS-PAGE at 120 V for 75 min or until the migration front reaches the bottom.
10. Open cassette with «scraper». Remove stacking from top and «bulge» at bottom *(it will make it easier to scan and dry the gels)*.
11. Wash gel in water for 3 x 5 min at RTº with shaking (use about 100 ml per gel per wash.
12. Discard last wash and add Invitrogen SimplyBlue SafeStain (#LC6065) for overnight incubation at RTº with shaking. *If using Coomasie Blue staining solution, overnight might not be necessary. Destain with proper solution and leave in water for 15 min before taking scan to rehydrate the gel.*
13. The following morning, remove stain and rinse 5 min with water (can be left for longer with no problem).
14. Scan and label gel.

RECIPES

ZYP-5052 rich medium without lactose

Everything is sterile

dH_2O	828 ml
1000X Me mix	1 ml
1 M $MgSO_4$	1 ml
10X TY	100 ml
GG	20 ml
20X NPS	50 ml

Add Me Mix to water first. If not, it will precipitate. Add 1 ml of ampicillin stock and 0.5 ml of chloramphenicol stock. (Do not autoclave)

1000X trace Metal mixture (Me Mix)

(The final amount of Metal in the media is in parenthesis)

0.1 M $FeCl_3 \cdot 6H_2O$	50 ml (50 µM)
1 M $CaCl_2$	2 ml (20 µM)
1 M $MnCl_2 \cdot 4H_2O$	1 ml (10 µM)
1 M $ZnSO_4 \cdot 7H_2O$	1 ml (10 µM)
0.2 M $CoCl_2 \cdot 6H_2O$	1 ml (2 µM)
0.1 M $CuCl_2 \cdot 2H_2O$	2 ml (2 µM)
0.2 M $NiCl_2 \cdot 6H_2O$	1 ml (2 µM)
0.1 M $Na_2MoO_4 \cdot 5H_2O$	2 ml (2 µM)
0.1 M $Na_2SeO_3 \cdot 5H_2O$	2 ml (2 µM)
0.1 M H_3BO_3	2 ml (2 µM)

Add ~36 ml of dH_2O (to complete to 100 ml) and filter sterilize. Store at 4ºC until use. When making growth media, add the metal mix in the water first to avoid precipitation.

Stock solutions for Me Mix

Only the $FeCl_3$ solution is made in 50 mM HCl. All others are done in dH_2O. Everything is kept at RTº.

10X TY

Tryptone	100 g
Yeast extract	50 g

Complete to 1 L with dH_2O and autoclave. Keep at RTº.

GG

Glycerol	250 g
Glucose	25 g

Complete to 1 L with dH_2O and autoclave. Keep at RTº.

20X NPS

$(NH_4)_2SO_4$	66 g
KH_2PO_4	136 g
Na_2HPO_4	142 g

Complete to 1 L with dH_2O and autoclave. Keep at RTº.

Stock Ampicillin (100 mg/ml)

1 g of ampicillin in 10 ml dH_2O.

Filter sterilize (0.2 µm), aliquot and store at -20°C. Avoid freeze/thaw cycles.

Stock Chloramphenicol (50 mg/ml)

0.5 g of chloramphenicol in 10 ml of ethanol. Aliquot and store at -20ºC.

1X cell lysis buffer non-denaturing

1 M Tris-HCl pH 7.5	20 ml
5 M NaCl	30 ml
0.2 M Na_2EDTA	5 ml
0.8 M EGTA	1.25 ml
Triton X-100	10 ml
1 M Sodium pyrophosphate	2.5 ml
1 M β-glycerophosphate	1 ml
1 M Na_3VO_4	1 ml
Leupeptin	1 mg
dH_2O	

Complete to 1 L with dH_2O. Keep at 4°C until use.

Stock solution for cell-lysis buffer non-denaturing

All solutions are made in water (special protocol for Na_3VO_4) and kept at 4°C. Only leupeptin is kept at -20°C in its powder form.

1 M Na_3VO_4

sodium orthovanadate	4.6 g
dH_2O	15-20 ml

Adjust to pH 10, no less (can be a little above). Boil until translucent and readjust the pH. Repeat these steps until solution remains clear at pH 10. Complete to 25 ml with dH_2O. Aliquot and store at -20°C or keep at 4°C. Avoid freeze/thaw cycles.

Some solutions like the metal mix, and cell lysis buffer with *o*-vanadate are made by other members of our team in room 24. Please check with them before making new one.

5X Sample Buffer

0.5 M Tris-HCl pH 6.8	20 ml
Glycerol	20 ml
SDS	4 g
DTT	3.09 g
Coomassie Blue	0.2 g

Aliquot and keep at -20°C until use.

Resolving gel 15% (for 10 gels)

H_2O	35.7 ml
40% Acrylamide	37.3 ml
1.5 M Tris-HCl pH 8.8	25.3 ml
10% SDS	1 ml
TEMED	100 µl
10% APS	500 µl
	100 ml

Stacking gel 4% (for 10 gels)

H_2O	25.3 ml
40% Acrylamide	3.95 ml
0.5 M Tris-HCl pH 6.8	10.08 ml
10% SDS	400 µl
TEMED	40 µl
10% APS	200 µl
	40 ml

10X Running Buffer

Tris	60.4 g
Glycine	376 g
SDS	20 g
dH_2O	1.5 L

Complete to 2 L with dH_2O. Store at RT°. Use 1X for electrophoresis.

10% APS

1 g of ammonium persulfate in 10 ml of dH_2O.
Aliquot and store at -20°C until use. Do not refreeze thawed APS.

10% SDS

10 g of sodium dodecyl sulfate in 100 ml of dH_2O.
Store at RT°.

Tris-HCl

1.5 M, pH 8.8

Tris	181.71 g
dH_2O	700 ml

Adjust to desired pH with HCl and complete to 1 L with dH_2O. Keep at RT°.

0.5 M, pH 6.8

Tris	60.57 g
dH_2O	700 ml

Adjust to desired pH with HCl and complete to 1 L with dH_2O. Keep at RT°.

Gel Casting Instructions

1. Put the cassettes (Criterion Empty Cassettes, 1.0 mm thick with 26-well com, 10 sets/box; BioRad #345-9903) vertically on a tube rack.
2. Keep plastic bag for gel storage.
3. Remove comb (green) and keep it for later use.
4. Prepare the resolving gel acrylamide solution and add TEMED and APS last when ready to pour.
5. Fill the cassette to 1 cm below teeth of comb (about 10 ml/gel).
6. Immediately overlay the acrylamide mixture with water. The use of alcohol base solutions for overlay may discolour the cassette.
7. Let polymerize for 45 min.
8. Remove overlay water, gently tap on paper towel to remove extra water, and put upside down on the tube rack for 10 min.
9. Prepare the stacking gel acrylamide solution, except TEMED and APS, add when ready to pour.
10. Fill to the top of the glass and add comb.
11. Let polymerize for 45 min. *(If the gels are not used right way, keep them in wet paper towels in a plastic bag. The gels can last up to two months by keeping them humid in a horizontal position.)*
12. If used on the same day: add running buffer to tank, remove plastic sticker at the bottom of cassette and place it in the tank.
13. Fill the reservoir attached to the cassette with running buffer and remove combs.
14. Wash the wells with running buffer and make sure that the wells are undisturbed.
15. Load samples and ladder.

Big Gels (all volumes are in ml)

Resolving, 15%

Number of gels	**10**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**
Water	35.7	32.13	28.56	24.99	21.42	17.85	14.28	10.71	7.14	3.57
40% acrylamide	37.3	33.57	29.84	26.11	22.38	18.65	14.92	11.19	7.46	3.73
1.5 M Tris-HCl, pH 8.8	25.3	22.77	20.24	17.71	15.18	12.65	10.12	7.59	5.06	2.53
10% SDS	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
TEMED	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01
10% APS	0.5	0.45	0.4	0.35	0.3	0.25	0.2	0.15	0.1	0.05
Total volume	100	90	80	70	60	50	40	30	20	10

Stacking, 4%

Number of gels	**10**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**
Water	25.3	22.77	20.24	17.71	15.18	12.65	10.12	7.59	5.06	2.53
40% acrylamide	3.95	3.555	3.16	2.765	2.37	1.975	1.58	1.185	0.79	0.395
0.5 M Tris-HCl, pH 6.8	10.08	9.072	8.064	7.056	6.048	5.04	4.032	3.024	2.016	1.008
10% SDS	0.4	0.36	0.32	0.28	0.24	0.2	0.16	0.12	0.08	0.04
TEMED	0.04	0.036	0.032	0.028	0.024	0.02	0.016	0.012	0.008	0.004
10% APS	0.2	0.18	0.16	0.14	0.12	0.1	0.08	0.06	0.04	0.02
Total volume	40	36	32	28	24	20	16	12	8	4