2-D Western: 7 cm strip (cup loading) for mini-gel

1. Sample preparation

- either lyse cells directly into IEF sample buffer, or perform precipitation, and resuspend pellet in sample buffer; keep salts and other ionic compounds to a minimum; load amount to get strong Western signal in 1D gel (if known)
- sample buffer should be supplemented with 20 mM DTT before use

2. Strip rehydration

- Thaw rehydration buffer stock
- prepare 125 µl rehydration buffer for each 7 cm strip:
- DeStreak reagent (kept in fridge): 12 µl / ml buffer
- IPG buffer of correct range (fridge): 1 % (v/v) final concentration
- Pipette complete buffer into slot of rehydration tray
- Take strip package from freezer
- take out strips one at a time with tweezers, remove thin plastic cover, place in tray on top of buffer, gel side down; avoid trapping bubbles
- cover with mineral oil (DryStrip Cover Fluid); no need to fill entire tray
- cover tray with lid, rehydrate strips for at least 6 to 8 hours at RT

3. Cup loading preparation

- get out and clean manifold ceramic tray, using only ‘Strip Holder Cleaning Solution’; dry completely using Kimwipes or air-dry
- put manifold onto IPGphor IEF machine, making sure that it is level
- place rehydrated IPG strips gel side up into adjacent slots, anodic (plus) end facing the back of the machine, and lining up with the guides printed on the side
- note strip numbers and slot positions to identify samples
- if necessary, cut appropriate number of sample cups from strip
- place cups over strips, typically on anodic (plus) end, pushing down with gloved fingers starting at one end and working towards the other; then use cup placement tool to push down cup feet
- get out two electrode wicks per strip, dampen with 150 µl water each, place in slots, one half of wick overlapping end of strip
- measure out 108 ml of DryStrip Cover Fluid (mineral oil), pour into manifold, making sure that nothing leaks into cups
- put electrode assemblies in place with electrodes placed where wicks overlap gel, close cams
- add some oil to cups (can be done after sample loading)
- pipet sample into cups, no more than 150 µl; note which sample is where
- close lid

4. IEF run

- turn on IPGphor unit (switch on back of instrument, by power cord)
- go to program from previous run and check parameters, or program new; please don't change programs #1-3; use 'Edit' button to look at or edit, down/up button from first digit in line to flip pages, right/left button to go to digit, down/up button to change or toggle values
- recommended conditions for 7 cm, pH 6-11, cup loading:

step	150 V	0:30 h (or longer)
step	300 V	0:30 h (or longer)
gradient	1000 V	3000 Vh
gradient	5000 V	4000 Vh
step	5000 V	500 – 2000 Vh
[step]	[500 V]	[12:00 h]*
		(total of 5000 – 6500 Vh)

  * optional step in case you are not around when the program ends; note total Vh when run finishes

- to log run, open 'IPGphor' program on Typhoon computer, check if communication to instrument is established
- start run by pushing 'Start' button on instrument; check that computer starts logging
- when run is complete, stop program by pushing 'Stop' three times if using 500 V step; remove electrode assemblies, cups, wicks; take out strips using tweezers, letting excess oil drip off, or blotting briefly on a fresh Kimwipe
- continue directly with second dimension, or store strips at -80˚C in 15 ml conical tubes or equilibration trays
- clean manifold carefully with 'Strip Holder Cleaning Solution'

5. Gels for second dimension

- cast or purchase mini-gels with straight top (one “well”), or use comb to create one small sample well, and one large strip well
- thickness at least 1 mm, to accommodate strip
- stacking gel is not required

6. Equilibration for SDS-PAGE

- thaw Equilibration buffer stock at room temperature
- prepare 2.5 ml each of Equilibration buffers 1 and 2 for each strip:
    - buffer 1: 2.5 ml stock plus 12.5 mg DTT (5 mg/ml)
    - buffer 2: 2.5 ml stock plus 112.5 mg iodoacetamide (45 mg/ml)
- equilibrate strip for 15 min in each buffer, using conical tubes or equilibration tray on horizontal shaker at RT; (frozen strips go directly into buffer, no extra thawing)
- during equilibration, prepare or melt agarose for sealing strips

7. Loading strips on gels

- for loading, leave gels outside gel tank
- rinse well(s) with running buffer
- [clip ends of strip if using sample well comb]
- take strip with tweezers, acidic (plus) end to the left, place plastic backing side on longer glass plate, push into well until flush with top of gel, without disturbing gel surface or trapping bubbles
- overlay with 0.5% low melt agarose in running buffer
- allow one minute for agarose to set
- put gels into tank, fill with running buffer, [load marker, if using well]

8. Running the second dimension gels

- recommended run conditions for 1 mm thick gels:
    - entry phase, 10 mA/gel, 15 min, then separation, 20 mA/gel, 1:30 h, or until BPB front has almost run out (GE 2D Handbook)
    - **or** 200 V constant, about 35 min (BioRad protocol)

Recipes

Sample Buffer (Cell lysis solution)

Reagent	Quantity		Final concentration
Urea (MW 60.06)	10.5g	4.2g	7M
Thiourea (MW 76.12)	3.8g	1.52g	2M
CHAPS (MW 614.89)	1g	0.4g	4% (w/v)
Tris (1M, not pH'ed)	750µl	300µl	30mM
H_2O MilliQ	to 25ml	to 10ml	

Rehydration Buffer Stock

Reagent	Quantity	Final concentration
Urea (MW 60.06)	10.5g	7M
Thiourea (MW 76.12)	3.8g	2M
CHAPS (MW 614.89)	1g	4% (w/v)
Bromophenol Blue (1% stock)	50µl	0.002% (w/v)
H_2O MilliQ	to 25ml	

SDS Equilibration Buffer Stock

Reagent	Quantity		Final concentration
Tris (1.0M, pH8.0)	20ml	40ml	100mM
Urea (MW 60.06)	72.07g	144.14g	6M
Glycerol (99.5% [v/v], MW92.09, density 1.26g/cm^3)	60ml/75.6g	120ml/151.2g	30% (v/v)
SDS (MW288.33)	4g	8g	2% (w/v)
Bromophenol Blue (1% stock)	400µl	800µl	0.002% (w/v)
H_2O MilliQ	to 200ml	to 400ml	

SDS-PAGE Running Buffer, 10x

Reagent	Quantity	Final concentration
Glycine (MW 75.07)	1152g	1.92M
Tris (MW 121.1)	242g	250mM
SDS (MW 288.38)	80g	1% (w/v)
H_2O, destilled/RO	to 8l	