Western Blot

DAY I
Streak strains for colonies

DAY II - Days II – IV can be condensed into one day's work
Grow cells to mid log
Take OD. ** This is especially important for quantitative work
Pellet 1mL
Pull of all sup with pipetman, not aspirator
If you do not have time to finish the next phase, pellets may be stored at -20C

DAY III
SDS gel: (1.5 – 2 hr)
0.75mm 12% SDS-PAGE
Resuspend pellet in 100mL GTE + 2 mg/mL lysozyme, and 1mL AEBSF
    Make a stock solution, and do not shake or vortex the lysozyme solution!
Incubate @ 37C for 15 minutes
Incubate on ice for 15 minutes
Check to see if cells have lysed
Add 100mL 2X sample buffer
Boil for 5 minutes
Load gel, normalizing to OD (use MV=MV, standardizing to 10mL of the lowest OD)
    Use 10mL BioRAD prestained ladder
    If probing different proteins, run a ladder for each set so you can cut the blot
Run gel

Western Transfer: (20 – 30 min)
Set up while the gel is running (putting all liquids in appropriate trays):
Cut PVDF membrane using template in box
Dip membrane in 100% methanol to activate; put only a thin layer of methanol (<5mL) in tray
Soak for 5 min in H_2O
Let equilibrate in transfer buffer 10+ minutes

	blot paper
	gel
	PVDF membrane
	blot paper

Cut two fat pads of blot paper to same size as membrane; this reduces waste and increases the efficiency of transfer
Soak blot paper in transfer buffer

When gel has finished running, cut off the combs and ease the gel into transfer buffer using the green paint-knife tool. Soak the gel in transfer buffer for 10-20 min; do not soak >20 min!

Set up gel sandwich! Mmm.
Press out bubbles by rolling a test tube over each layer.
Run for 20 min at 20V
    Use righthand power box (PowerPac 200)
Lift up corner of gel to check transfer

Cleanup:
Do not leave any transfer buffer on electrodes.
Wash promptly w/ ddH_2O and Kimwipe, let dry.
    Store with blot paper between metal electrodes!
Don't let the metal surfaces touch!

Block: (30 min – O/N)
Use 5% milk in 1X PBS (2.5g milk in 50mL 1X PBS)
Incubate 30 min – O/N
    ** If incubating O/N, cover gel in saran wrap and place on shifter in cold room

DAY IV
Probe: (3.5 hours – O/N) All of this can be done in ambient light, @ RT.
Primary antibody stain for 2-5 hrs (can run O/N if nonspecific binding isn't a problem)
    Make 1% milk (dilute the 5% 1:5, or just add some flecks to 1X PBS; not precise)
    10mL 1% milk/probe/blot (so 20mL/blot for primary and secondary)
    Label trays; have a tray for each probe
    Don't let the blot dry out
    Put blot in 10mL 1% milk
    Add probe at appropriate dilution (1:1000 for rabbit Spo0J, 1:5000 for rabbit FtsZ)
    Make sure you use the primary antibody that complements your secondary (rabbit, etc)
    Put on shaker for 2+ hours; chicken antibody usually needs more than rabbit
Wash 3 times in 1X PBS
    Pour on, pour off a few times
    For thorough final wash, you may place on shaker in 1X PBS for ~10 min
Secondary antibody stain for 1 hr – not longer than 1 hr!!
    Rabbit secondary is 1:5000
    Store at -20C, and do not let warm up; it is conjugated to enzyme HRP (horseradish peroxidase)
Wash 3 times in 1X PBS

Develop: (1 hr)
Pour off all remaining PBS
Developing HRP-conjugated secondary using Detection Reagents 1 and 2 (in 4C fridge)
DON'T LET THE REAGENTS MIX IN THEIR BOTTLES! They'll react and go bad.
Add 1mL of each reagent (2 mL total), mix in tray
Pipet mixture all over blot repeatedly
Pick up blot with tweezer, shake off, dab edge on Kimwipe
Place between sheets of transparency film or Saran Wrap

Take Pictures:

Sign up for Fuji Imager in Kranz Lab (sign up for an hour in case some steps run long)
Set shelf # based on gel size
Make sure settings are for "Chemiluminescent Samples"
Make sure tray is pushed all the way in
Program Settings – don't adjust temperature:

FUJIFILM LAS 1000

Operation Mode:	Precision	[x] Dark Frame Subtraction
Image Type:	Image	[x] Spotting
Exposure:	30 seconds	[x] Flat-Frame Correction
[x] Binning pixels		[x] Distortion Correction
[x] Visible Frame Grab	[ ] Invert Pixels	
		Image Data Offset: 256

Save As: file type "Fuji Film (Quantable)"