Day 1

- Streak out G509 (IHFβ, IHFά operon in pRSETb, *via* pRSUB, derivative) from frozen glycerol stock onto fresh LB+agar+Amp100 plate. 37° C overnight.

Day 2

- Innoculate 2 x 5 ml LB+Amp100 cultures. 37° C overnight

Day 3

- Perform standard alkaline lysis phenol/chloroform plasmid prep
- Dialyze DNA against nanopure water for 30 minutes
- Transform into electrocompetent BL21 cells (contains pLysS plasmid)
- Plate on LB+agar+Amp100/Cam30. 37° C overnight.

Day 4

- Pick a colony and inoculate 100 ml of LB+Amp100/Cam30

Day 5

- Subculture 1:100 in LB+Amp100/Cam30
- 6 x 1 liter in 2.8 liter flasks, 250 rpm, 37° C
- Grow until OD_{600} = 0.5 *(this took 4.5 hours)*
- Induce with IPTG to final of 0.5 mM *(0.5 ml of 1 M)*
- Grow at 37° C for 3 hours *(OD_{600} = 1)*
- Transfer to 500 ml centrifuge bottles
- Centrifuge at 8,000 rpm for 15 minutes, decant supernatant
- Store pellets at -80° C *(15 g of cells)*

Day 6

- Remove bottles from freezer and combine frozen pellets
- Weigh cells *(15 g of cells)*
- Add 48 ml of buffer A:

	Stock	**For 50 ml**
50 mM Tris-HCl pH 8.0	1 M	2.5 ml
10% Sucrose		5 g
12.5 mM EDTA	0.5 M	1.25 ml
2.5 mM DTT	1 M	125 µl
Water		Up to 50 ml

- Buffers made fresh before use
- Use 30 ml homogenizer (mortar and pestle type) to resuspend cells in buffer
- Add protease inhibitors:
  - 450 µg leupeptin
  - 450 µg pepstatin A
  - 900 µg soybean trypsin inhibitor
  - 45 mg PMSF (in 1 ml EtOH)
- Add lysozyme ($1/20^{th}$ volume @ 10 mg/ml in 0.25 M Tris-HCl pH 8.0)
- Homogenize several times to mix up protease inhibitors and lysozyme

- Add 12 ml buffer B:

	Stock	**For 15 ml**
200 mM Tris-HCl pH 8.0	1 M	3 ml
1 M NaCl		0.875 g
200 mM spermidine		0.775 g
Water		Up to 15 ml

- Add 20 µl Benzonase nuclease (Novagen)
- Rotate in coldroom (4° C) for 2 hours
- Place in 37° C shaker for 5 minutes
- Split into 2 centrifuge bottles
- Spin in Ti55.2 rotor at 40,000 rpm for 1 hour
- Remove supernatant, add same amount of protease inhibitors from above and keep on ice
- Re-extract pellet in 8 ml buffer A and 2 ml buffer B and proceed as above
- Combine both supernatant and filter through steritop filter
- Freeze clarified extract in liquid nitrogen

Day 7

- Prepare 1 liter IHF buffer – LS

	Stock	**For 1 liter**
20 mM HEPES pH 7.0	1M	20 ml
1 mM EDTA	0.5 M	2 ml
400 mM NaCl	5 M	80 ml
10% glycerol	50%	200 ml
Water		Up to 1 liter

- Chill buffer to 4° C
- Remove IHF supernatant from -80° C, thaw on ice
- Ammonium sulfate to give 50% in 40 ml = 12.52 g
- Slowly add ammonium sulfate whilst stirring at 4° C
- Once all is in solution, let stir an additional 30 minutes
- Transfer to 50 ml centrifuge bottle, spin at 15,000 rpm for 40 minutes
- Transfer supernatant to new beaker, add ammonium sulfate to 80% saturation (8.56 g). Add slowly and stir as above
- Spin at 15,000 rpm for 40 minutes
- Remove supernatant, redissolve pellet in 1-2 pellet volumes of buffer
- Use 2,000 MWCO dialysis tubing, dialyze overnight against LS buffer

Day 8

- Thaw samples on ice (~ 2 hours)
- Set up column (10 mm ID x 10 cm length with flow adapter)
- Resuspend heparin, transfer 11 ml to falcon tube
    - Centrifuge at setting 3 for 1 minute (clinical ‘fuge)
    - Remove supernatant, add equal amount of IHF buffer – LS and resuspend
    - Repeat wash 4 times
    - Pour resin in pre-wetted column

- Set up column with gradient peristaltic pump. Wash 10 volumes (50 ml) at 1 ml/min @ 100 % IHF buffer – LS
- Load sample at 1 ml/min (~ 15 ml)
- Wash with 10 bed volumes at 1 ml/min with 100% IHF buffer – LS
- Elute at 1 ml/min from 0% to 100% IHF buffer – HS over 50 ml with 1 ml fractions
- Quickfreeze fractions in liquid nitrogen

Day 9

- Determine IHF fractions by dotting 1 µl of each fraction onto nitrocellulose and doing a western
- Run a 10 – 20% protein gel on IHF containing fractions then coomassie stain
- Perform nuclease test on fractions
- Pool appropriate fractions (22 – 30 in my case = 9 ml), quantitate by OD_{276}
- The 9 ml pool ended at 1.2 mg/ml which is fine for us so we didn't concentrate the sample