Fis purification

~~Martin~~ grew cultures in Amp+Kan but strain he gave me is Amp+Tet?! => Had given me wrong strain, replaced with correct one.

- spermidine S5559-1G £18.80 sigma
- lysozyme L7651 10g £134.00 Sigma
- Heparin sepharose 17-0998-01 £270.00 GE Healthcare
- Heparin sepharose columns 5×5ml 17-0407-03 £379.00 GE Healthcare
- Resource S column 17-1180-01 £1,050.00 GE Healthcare

↳ (in the end used a High trap SP-XL cation exchange column) 22/7/08

10/7/08

- Fis test overexpression.
- Inoculate 2× 10ml of LB (+amp+kan) with 200µl of O/N RJ4529 culture.
- When cultures get to an OD_{650} of ~0.6 add 12.5µl of 1M IPTG to and grow both cultures for a further 60 minutes.

14/7/08

- Use 15ml of O/N LB culture of RJ4529 (with Amp+Kan) to innoculate 750ml of pre-warmed LB.
- Grow to and OD_{650} of ~0.6
- Add 940µl of 1M IPTG. Continue growing @ 37°C for a further 1 hour.
- Spin down cells and resuspend in 15ml 50mM Tris-HCl (pH8) +10% Sucrose
- Freeze cells in -80°C then thaw.
- Add:

71µl	0.5M	EDTA
19µl	0.1M	PMSF
94µl	1M	DTT
94µl	1g/ml	spermidine
1.9µl	3M	Ammonium SO_4^{2-}
38µl	100mg/ml	lysozyme

=> Incubate on ice for 90 minutes, then sonicate 3×30 sec

=> Spin down debris at high speed and keep supernatant (can freeze O/N

FB buffer : 4L of ...

- of 20mM Tris-HCl pH7.5
  1.0 mM EDTA
  10% Glycerol
  1.5M NaCl

20mM Tris-HCl pH7.5
1.0mM EDTA
10% Glycerol
0.3M NaCl

15/7/08

- Thaw supernatant from yesterday and transfer to dialysis membrane.
- ~~Dialyse against 1L FB @ 4°C for 3hrs.~~
- ~~Change to a fresh 1L FB buffer and dialyse for further 40min.~~

=> Didn't do this, dialysed against 2L FB O/N instead.

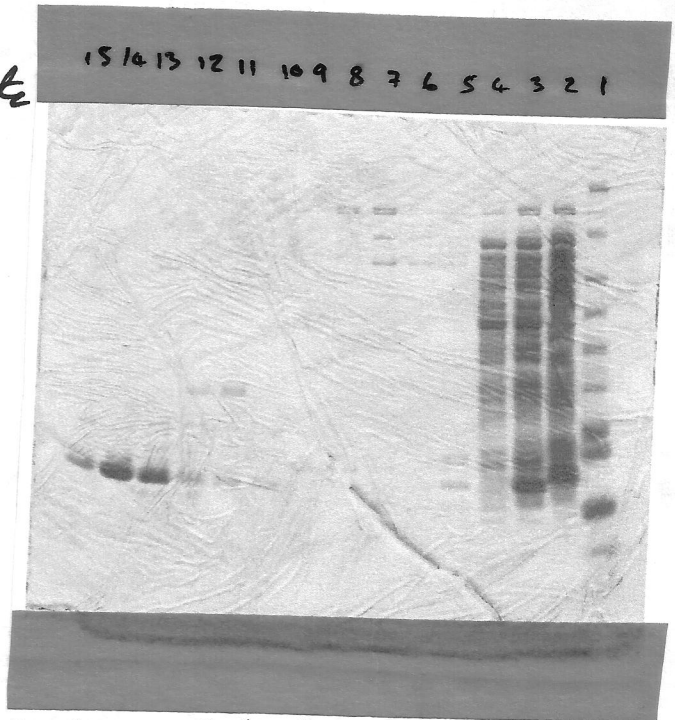
16/7/08

Column ① : 5ml Heparin Sepharose equilibrate with buffer FB.

- Load crude extract.
- Elute in gradient upto 1.5M NaCl. Should come off ~0.75M NaCl.

Gel (loaded 6µl blue + 4µl protein)

1 Marker
2 Crude lysate
3 ~~Flow through (F3)~~ Dialysed lysate
4 ~~F23~~ F3
5 F25
6 F27
7 F29
8 F31
9 F33
10 F35
11 F37
12 F39
13 F41
14 F43
15 F45



=> FRACTIONS 40→46 STORED @ -80°C

rification re-run

Overexpress etc as previously done.

- After O/N dialysis centrifuge to remove any precipitate.

Prepare Akta

- Remove any column if attached.
- Set to load and run 100% B through line B
- Run 100% A (also on load) through line A.
- Set to inject, switch lines on superloop, and wash out superloop.
- Switch loop lines again and wash out in opposite direction.
- Turn down flow rate (2ml/min), attach column, and wash to with A.
- While still on load, add sample to superloop. (pause?
- Switch to inject and start collecting 4ml fractions.
- When protein is loaded, switch back to load.
- Start collecting 2ml samples.
- Set gradient to run over 180ml up to 100% B.
- Pool peak fractions 60 → 64 (10ml total in ~ 864mM NaCl)
- Make 1L buffer FB with 0.2M NaCl to use as buffer A with cation exchange column. (5ml HiTrap SP-XL column) (20mM Tris, 1.0mM EDTA, 10% Glycerol, 0.2M NaCl)
- Make 50ml FB with no salt to dilute peak fracs. → dilute 1:4 to give ~ 200mM NaCl

ıel

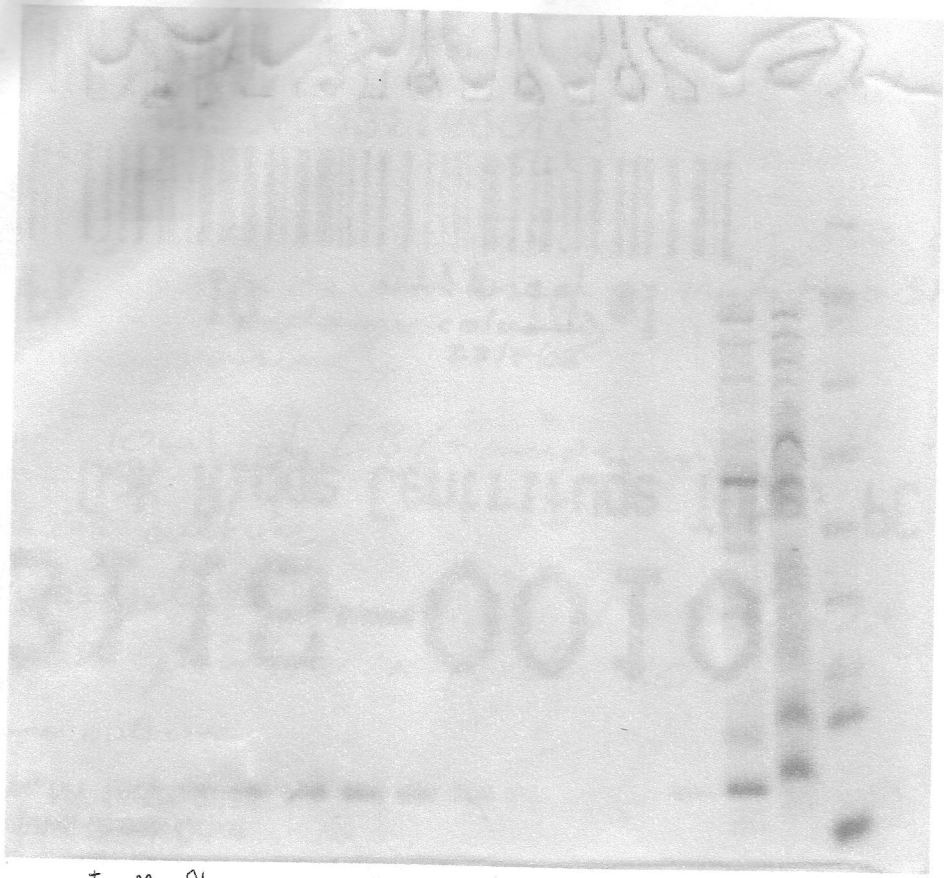
1 M
2 Crude extract
3 Dialysed
4 Pooled Hep-Seph frac's
5 F 24
6 F 25
7 F 26
8 26 F 27
9 27 F 28
10 28 F 29
11 29 F 30
12 30 F 31
13 31 F 32
14 32 F 33
15 33 F 34

From M. Trap SP-XL (1ml) column

Fis from HiTrap SP-XL column

22/7/08

• Each lane 4µl protein + 6µl Hee. Loaded 5µl of this.



• Used 5µl for each frac's in a Biorad assay.

	OD_{595}	[Protein]
F28	0.040	10.2µM
F29	0.065	16.6µM*
F30	0.052	13.3µM
F31	0.030	7.7µM

⇒ Molecular weight of Fis is 11.24 kD.

* Tested in EMSA @ dps vs cbpA. Behaves as previous prep from Martin