Expression and purification of recombinant isotope-labeled hCofilin2

MEDIA:

MMI medium – dissolve and pH Tris/HCl first, than add the other components (alternatively: use 1M Tris, pH 8.2 stock):

Final concentration		1L	4L
1.25%	Tryptone	12.5g	50g
2.5%	Yeast extract	25g	100g
125mM	NaCl	7.3g	29.2g
0.4%	Glycerol	4ml	16ml
50mM	Tris, pH 8.2	6.06g (50ml)	24.2g (200ml of 1M stock)

M9 medium – prepare fresh from sterile components (**1L**):

5x	5xM9 salts (autoclaved)	200ml
100x	100xBME Vitamins "Sigma" (sterile)	10ml
500x	1M $MgSO_4$ (autoclaved)	2ml
10,000x	1M $CaCl_2$ (autoclaved)	0.1ml
50x	20% D-glucose (filter sterilized)	20ml (4g/L)
100x	10% NH_4Cl (filter sterilized)	10ml (1g/L)

Autoclaved H_2O to **1L**

5x M9 salts (autoclaved):

KH_2PO_4 (0.11M)	15.0 g/L
$Na_2HPO_4x7H_2O$ (0.226M)	64.0 g/L
NaCl (0.043M)	2.5 g/L

pH to **7.2**
Autoclave

Glycerol stocks: 250ul 60% glycerol + 750ul of O/N culture.

EXPRESSION:

Note: Do not use pLys bacterial strains as lysozyme might interfere with purification of cofilin!!!

1. Several (10) freshly transformed colonies (with hCofilin2 plasmid – **Amp-R**) of BL21DE3 cells were tested for optimal level of expression in M9 minimal medium: O/N cultures grown in MMI were used to inoculate each of 10ml of MMI to OD_{600}=0.05-0.1 (leftovers of O/N cultures were used to prepare glycerol stocks@-80^{o}C) → cells were grown to OD_{600}=1-1.5, span down, washed in 1xM9 salts, and reconstituted in 2.5ml (1/4 of the initial culture volume) of complete M9 medium for each screened colony. Cultures in M9 medium were allowed to adjust to 25^{o}C for 1hr in new medium and 1mM IPTG was added. Expression was carried out O/N @25^{o}C. Cells were collected, lysed, and expression was analyzed on PAGE (compared to uninduced culture and purified hCofilin2).
2. The highest yield colony (glycerol stock) was used for a second round of selection: streak on plate, select several (10) colonies, repeat step 1 starting from O/N MMI cultures. Do not forget to prepare glycerol stocks@-80^{o}C.

3. Glycerol stock from the highest yield colony from the second round of selection was used to inoculate **100 ml MMI** and the culture was grown O/N at 37°C. OD_{600} was measured and cells were separated from old medium by centrifugation. Cells were resuspended in initial volume of fresh medium (100mls) and transferred to **4L of MMI** medium (8x0.5L in 2L flasks; 4L total) in a way to get OD_{600}=0.05. After that, the diluted cells were grown at 37°C until OD_{600} reached 1-1.5. Cells were span down, rinsed in **1xM9 salts w/o NH4Cl/Dextrose** and pelleted again. The pellet was resuspended in **1L M9 medium** with **0.4% (4g/L) C^{13}-dextrose (D-glucose)** and **0.1% (1g/L) N^{15}-NH4Cl** as the only carbon and nitrogen sources, respectively (Journal of Biomolecular NMR; 2001;20(1):71-5). Cells were incubated on shaker for 1hr @ 25°C and cofilin expression was initiated by adding **1mM IPTG**. After O/N incubation (14-16hours) @ 25°C, cells were pelleted, rinsed in 20mM Tris, pH 7.5, flash frozen in liquid nitrogen, and stored at -80°C until prepped.

Rosetta (DE3) cells give much higher yield! [Rosetta (DE3) cells with rare codon plasmid are Cm-R]

PURIFICATION:

Thaw the frozen cells in the Extraction buffer:20mM TRIS (pH7.5), 1mM DTT, 0.2mM PMSF supplemeted with a cocktail of protease inhibitors (PI; Roche). Disrupt cells thoroughly by using either microfluidizer (http://www.microfluidicscorp.com) or French press. Separate insoluble and soluble fractions by centrifugation at >20.000g at 4°C for 30-40 minutes. Apply supernatant, filtered via paper filters(Whatman), to Q-sepharose (15x300mm column). Wash the column with the extraction buffer w/o PI (only PMSF as a protease inhibitor). Elute cofilin by 0-4M NaCl gradient. Cofilin elutes normally at ~200-250 mM NaCl, ~16 mS/cm conductivity. Analyze fractions by 12.5 or 15% SDS PAGE. Concentrate the fractions containing cofilin (runs as ~17kDa protein, between 15 and 20 kDa mol weight PageRuler standarts; Fermentas) to ~1-2 mls on centricon/centriprep and apply on Superdex 75 16/600 gel filtration column equilibrated with the exptraction buffer containing 200 mM NaCl. Run at 0.8ml/min, collect 1ml fractions, analyze by SDS PAGE. Central fractions with pure cofilin combine and concentrate. Cofilin remains soluble at least until 20mg/ml. For NMR purposes, dialyze cofilin into 10mM PIPES (pH6.8), 1mM DTT, 0.05mM PMSF buffer. Aliquot if necessary and flash freeze in $N_{2\ liq}$.