PfuS Mix Purification prototol

Adapted from Görlich laboratory

I. Expression

	PfuS Polymerise	Pab-PPase	Pab-dTUPase
Overnight culture	pSF421 in BL21DE3Rosetta, 50 ml 2YT with Amp and CAM. 37 °C	pSF318 in BL21DE3Rosetta, 50 ml 2YT with Amp and CAM. 37 °C	pSF323 in BL21DE3Rosetta, 50 ml 2YT with 2% glycerin, Amp and CAM. 37 °C
Dilution	Add 20 ml of overnight culture in 1L prewarmed TB medium. grow until OD=0,5 at 37 ℃		Add 20 ml of overnight culture in 1L prewarmed 2YT medium with 0.4 M NaCl. grow until OD=0,5 at 37 ℃
Induction	add IPTG (final con. 0.2 mM), shake 4 h at 37 ℃	add IPTG (final con. 0.2 mM) shake 4 h at 37 ℃	add IPTG (final con. 1 mM) shake 4 h at 37 ℃
Harvest	add 1mM PMSF, centrifuge at 4k, 20 min The pellet could be freeze at -20 ℃.		

II. Purification

	PfuS Polymerise	Pab-PPase	Pab-dTUPase
Resuspension	thoroughly suspend the cells in HS-buffer with 5 mM DTT, 5 mM Imidazole, 1 mM PMSF and 1 mM Benzamidine. Use 1 ml for 100 OD, e. g. 10 ml for 1000ml culture of OD=1.	thoroughly suspend the cells in LS-buffer with 5 mM DTT, 5 mM Imidazole, 1 mM PMSF and 1 mM BenzamCidine. Use 1 ml for 100 OD, e. g. 10 ml for 1000ml culture of OD=1.	thoroughly suspend the cells in HS-buffer with 5 mM DTT, 5 mM Imidazole, 0.1% TX-100, 1 mM PMSF and 1 mM Benzamidine. Use 1 ml for 100 OD, e. g. 10 ml for 1000ml culture of OD=1.

Lysis	lyse cells with the microfluidizer		
Centrifuge	14K, 20 min, 2 times, transfer the clear supernatant to a new vial.		
Histrap column preparation	connect 3x1ml Histrap column tandemly on Äkta. wash with water and equilibrate with HS-buffer.	connect 3x1ml Histrap column tandemly on Äkta. wash with water and equilibrate with LS-buffer.	connect 3x1ml Histrap column tandemly on Äkta. wash with water and equilibrate with HS-buffer.
Column chromatography*	apply the clear supernatant to the column (1 ml/min), collect the flow through.		
	wash with HS-buffer with 5mM DTT and 5mM imidazole for 5 column volume and wash with LS-buffer with 5mM DTT and 5mM imidazole until the A280 does not change anymore	wash with LS-buffer with 5mM DTT and 5mM imidazole until the A280 does not change anymore	wash with HS-buffer with 5mM DTT and 5mM imidazole until the A280 does not change anymore
	elute with LS-buffer with 5 mM DTT and 400mM imidazole		elute with HS-buffer with 5 mM DTT and 600mM imidazole
	Pool the peak fractions.		
	$1OD_{280}$ =0.73 mg/ml	$1OD_{280}$ =0.70 mg/ml	$1OD_{280}$ =0.75 mg/ml

Note: The Histrap column shows purple color in the present of DTT.

III. Dilution.

Dilute and mix to the final concentration with dilution buffer.

PfuS : 100ng/ul
PPase: 15ng/ul
dUTPase: 5ng/ul

IV. Buffer:

HS-Buffer	LS-Buffer	Dilution Buffer
2 M NaCl 50 mM Tris-HCl pH 8.0 10 mM $MgCl_2$	290 mM NaCl 44 mM Tris-HCl pH 7.5 4.4 mM $MgCl_2$	20 mM Tris-HCl pH 7.5 100 mM KCl 1 mM DTT 0.1 mM EDTA 0.5% NP40 0.5% Tween20 100μg/ml BSA 50% Glycerol