Affinity purification of MS2-tagged 30S

(4L prep of pop2136-pLK35.16S.ms2)

Prepare 4L cell culture

1. Streak stock cell on plate. Grow 2.5ml LB +amp overnight culture at 30°C.
2. Inoculate 200ml LB of 2ml o/n culture. Grow at 30°C until OD_{600}=0.6(~3h). Pre-warmed LB
3. Inoculate 20~25ml of this mid-log phase starter into 8x500ml LB at 42°C.
4. Shake at 225rpm until OD_{600}=0.6
5. Place flasks on ice for 30min. From here everything must be done on ice.
6. Spin cultures using the JLA10.5 rotor for 10min at 6000rpm at 4°C.
7. Discard supernatant and resuspend pellets in 100ml (total volume) of buffer A (ice-cold).
8. Repeat spin, discard supernatant and freeze pellet at –80°C.

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Prepare crude ribosome

9. Resuspend pellet in 45ml of buffer A
10. Lyse cells using the French press (two passes at 15-20000psi). Keep lysate on ice.
11. Add 12ul of RQ1 Rnase free Dnase I
12. Prepare four 11ml of sucrose cushions in red-capped Ti70 bottles and keep at 4°C until use.
13. Spin the lysate from step 11 in JA17 rotor, 15000rpm for 30min.
14. Transfer cleared lysate to new bottles and repeat spin .
15. Layer the cleared lysate (~12ml) onto the sucrose cushions.
16. Spin in Ti70 rotor for 17hr, 36000rpm at 4°C.

Salt wash

17. Wash pellet with 5ml of salt wash buffer.
18. Resuspend each pellet in 10ml of the same buffer on ice by using baked small stirring bars.
19. Pool all together . Spin in JA17 rotor at 15000rpm for 15min at 4°C. Discard pellet.
20. Transfer supernatant to two clean red-capped bottles and fill tube with salt wash buffer.
21. Spin in Ti70 rotor for 1h20min at 56000rpm at 4°C.
22. Wash the pellet with 5ml of loading buffer (10mM Mg^{2+}).
23. Resuspend in 1ml loading buffer (10mM Mg^{2+}).

If you are not going to do the purification on the same day. Store ribosomes at –80C in loading buffer (10mM Mg^{2+}). Dilute down to 1mM with loading buffer(0mM) on the day of purification

(Save a small aliquot (50pmol) for PE analysis and determine the expression level of your tagged ribosome. You can assume your percentage expression. It's tends to be ~40-50% for wt and 25-30% for lethal mutants)

Purification

1. Filter with 0.2um PES membrane (Millipore).
2. Check concentration and calculate the total yield.
3. Wash 5ml GSTrap FF column (Amersham) with 10VC water. Flow rate=5ml/min
4. Equilibrate with 3CV loading buffer (1mM Mg^{2+}). Flow rate=5ml/min
5. Load 4x MS2-GST protein into 10ml loop.(1mg MS2-GST =25nmol,MW=39kDa). Flow rate=1ml/min
6. Wash with 3CV loading buffer. Flow rate=2.0ml/min
7. Load 10ml crude ribosome into 10ml loop. Inject sample at flow rate 0.2ml/min.
8. Wash column with 10CV loading buffer. Flow rate=2ml/min
9. Elute 30S with elution buffer. Flow rate=1ml/min. collect <15ml
10. Using a15ml Amicon Ultra concentrator (100,000MWCO, Millipore), concentrate in IEC central CL2 centrifuge.
11. Add storage buffer and spin again.
12. Check concentration and make a small aliquot
13. Quickly freeze in liquid nitrogen. Store at -80°C.

Regenerate column

14. Wash column with water. Flow rate=5ml/min
15. Regenerate column by washing with 2cv of 6M guanidine hydrochloride.
16. Wash column with water.
17. Wash with 2CV 20% ethanol. Store column at 4°C.

Typical yield of wt is about 3-4nmol of tagged ribosome from 4L culture. For mutant ribosome, yield is 1.5-1.8nmol from 4L culture.

Buffers For MS2-tagged 30S Preparation

Make buffers a day in advance and store them at 4C. Add 2-mercaptothanol(BME) to the buffers just before use.

Stock solutions (filter sterilized)

1M Tris-HCl, pH 8.0
1M Tris-HCl, pH 7.5
1M Tris-HCl, pH 7.0
1M $MgCl_2$
5M NH_4Cl
0.5M EDTA, pH8.0

Buffers

Buffer A	250ml
20mM Tris-HCl, pH7.5	5ml
100mM NH_4Cl	5ml
10mM $MgCl_2$	2.5ml
0.5mM EDTA	0.25ml
6mM BME	100ul
H_2O	237.25ml

Sucrose cushion	100ml
1.1M(w/v) sucrose (FW=342)	37.7g
20mM Tris-HCl, pH7.5	2ml
500mM NH_4Cl	10ml
10mM $MgCl_2$	1ml
0.5mM EDTA	0.1ml
6mM BME	40ul

Salt wash buffer	50ml x 2
20mM Tris-HCl, pH7.5	1ml
500mM NH_4Cl	5ml
10mM $MgCl_2$	0.5ml
0.5mM EDTA	0.05ml
6mM BME	20ul
H_2O	43.45ml

Loading buffer(10mM Mg^{2+})	15ml
20mM Tris-HCl, pH7.5	0.3ml
100mM NH_4Cl	0.3ml
10mM $MgCl_2$	0.15ml
6mM BME	6ul
H_2O	14.25ml

H_2O (degas)	500ml
6M Gunidine Hydrochloride (filter,degas)	50ml
20% ethanol in H_2O(degas)	50ml

Loading buffer(1mM Mg^{2+})	400ml (filter,degas)
20mM Tris-HCl, pH7.5	8ml
100mM NH_4Cl	8ml
1mM $MgCl_2$	0.4ml
6mM BME	160ul
H_2O	383.6ml

Loading buffer(no Mg^{2+})	10ml
20mM Tris-HCl, pH7.5	0.2ml
100mM NH_4Cl	0.2ml
6mM BME	4ul
H_2O	9.6ml

Elution buffer	100ml (filter,degas)
50mM Tris-HCl, pH8.0	5ml
100mM NH_4Cl	2ml
1mM $MgCl_2$	0.1ml
10mM reduced gluthatione	307mg
6mM BME	40ul
H_2O	~92ml

Storage Buffer (Buffer C)	20ml
50mM Tris-HCl, pH7.5	1.0ml
100mM NH_4Cl	0.4ml
10 mM $MgCl_2$	0.2ml
6mM BME	8ul
H_2O	18.4ml