Molecular Biology Protocols /a /l

Bacterial Genomic DNA Purification via Qiagen colu

Cultures should be grown in LB for best results. To avoid overloading the Qiagen colu number of cells/volume of culture should be determined. Qiagen recommends not more a mini-column, 2x10E10 for the midi, and 8x10E10 for the maxi. For this is ~0.4-0.8 n to 20.0 ml for overnight cultures in LB.

1. Prepare buffers according to recipe at end. Equilibrate all to room temp. before use.

Amount/prep	Mini	Midi	Maxi
B1	1 ml	3.5 ml	11 ml
B2	0.35 ml	1.2 ml	4 ml
QBT	2 ml	4 ml	10 ml
QC	3 ml	15 ml	30 ml
QF	2 ml	5 ml	15 ml
RNaseA	0.2 mg	0.7 mg	2.2 mg
lysozyme	2 mg	8 mg	30 mg
Qiagen protease*	0.9 mg	2 mg	10 mg

*or proteinase K

2. Dissolve Rnase A in buffer B1 to concentration of 200 ug/ml. Stock solutions of lys K can be made in dH2O to concentrations of 100 mg/ml and 20 mg/ml respectively.

3. Pellet cells by centrifugation at 3000-5000x g for 5-10 min. Remove supernatant.

4. Resuspend the bacterial pellet in 1/3.5/11 ml (mini/midi/maxi columns) of Buffer B1 speed.

5. Add 20/80/300 ul of lysozyme stock solution and 45/100/500 ul of proteinase K. Inc least 30 min.

6. Add 0.35/1.2/4 ml of Buffer B2 and mix by inversion several times. Incubate at 50 C this step is important for efficient deproteinization. It is also important that the lysate be

mns

mns, the correct
e than 4x10E9 cells for
nl, 2.0-4.0 ml, and 10.0

ozyme and proteinase

by vortexing at top

ubate at 37 C for at

C for 30 min. Mix well;
comes clear at this

stage.

7. Equilibrate Qiagen genomic tip with 2x1/4/10 ml Buffer QBT, and allow the tip to e

8. Vortex the lysate for 5-10 sec and apply onto the equilibrated column. Again, allow
column by gravity flow. Flow can be assisted with gentle positive pressure (a plunger f
fits the midi columns) but it is also OK to dilute the lysate with an equal volume of Buf
loading. The latter is preferable by virtue of personal experience.

9. Wash the column with 3x1 ml/2x7.5ml/2x15 ml of Buffer QC. Allow buffer to pass
flow. Two washes should be enough.

10. Elute the genomic DNA with 2x1 ml/5 ml/15 ml of Buffer QF. Precipitate with 0.7
ml) isopropanol, equilibrated to room temperature. The DNA should be spoolable; if nc
by centrifugation at 5000+ x g. Wash the precipitate with 70% EtOH and dry. Resuspe
solvent (TE, water).

Solutions:

Buffer	Composition (Storage)
B1	50 mM EDTA, 50 mM Tris/HCl, 0.5% Tween 20, 0.5% Triton X-100 (room [4 C after addition of RNase])
B2	3 M GuHCl, 20% Tween 20 (room temp)
QBT	750 mM NaCl, 50 mM MOPS, 15 % ethanol, 0.15% triton X-100, pH 7.0 (room temp.)
QC	1.0 M NaCl, 50 mM MOPS, 15% ethanol, pH 7.0 (room t
QF	1.25 M NaCl, 50 mM Tris/HCl, 15 % ethanol, pH 8.5 (r

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mpty by gravity flow.

it to pass through
rom a 10 ml syringe
fer QBT prior to

through by gravity

volumes (1.4/3.5/10.5
ot, pellet the precipitate
nd in the appropriate

temp.)

room temp.)

NA--they own the

/font