User-developed protocol

User-Developed Protocol:

Isolation of very low-copy plasmids from *Streptomyces* spp. using the QIAGEN® Plasmid Maxi Kit

This procedure has been adapted by customers from the Very Low-Copy Plasmids/Cosmids Protocol for plasmid/cosmid purification and is for use with the QIAGEN Plasmid Maxi Kit and the QIAGEN-tip 500. **It has not been thoroughly tested and optimized by QIAGEN.**

The procedure has been used successfully for isolation of plasmids SCP2 and SCP2* as well as the plasmids listed in Table 1 (see next page) from *S. coelciolor* and *S. lividans* strains.

Please be sure to read the *QIAGEN Plasmid Purification Handbook* and the detailed Very Low-Copy Plasmids/Cosmids Protocol carefully before beginning this procedure.

Procedure

1. **Inoculate 200 ml YEME medium with 10^8 *Streptomyces* spores and incubate for 48–62 h at 29°C and shake at 200 rpm.**

   Spores can be stored in glycerol at –20°C.

   Better results were obtained with YEME rather than TSB medium. YEME should be supplemented on the basis of the strain's auxotrophic markers. Antibiotic selection does not increase plasmid yield as SCP2 and SCP2* are segregationally very stable. To prevent bacterial clumping during growth, cultures should be grown in Erlenmeyer flasks with baffles to which have been added a stainless-steel spring (available in standard hardware stores) with the ends hooked to form a circle (1).

2. **Harvest the bacterial cells by centrifugation at 3000 x *g* for 10 min at 4°C (without brake).**

   A yield of 3 g wet weight is obtained from 200 ml if the strain grows well. If less is obtained, pool cultures to obtain 3 g.

   The cells should be harvested with the brake turned off since the bacterial pellet is very loose.

3. **Wash the pellet in 20–30 ml 10.3% sucrose. Freeze at –20°C.**

4. **Add 10 ml Buffer P1 containing RNase A and 4 mg/ml lysozyme, and transfer to a 15 ml polypropylene centrifuge tube. Incubate for 60 min at 37°C in a cell mixer with moderate rotation and mix by inversion every 15 min. Transfer to a sterile 50 ml polypropylene centrifuge tube.**

   Note: lysozyme is absolutely required for lysis.

5. **Add 10 ml of Buffer P2, mix gently but thoroughly by inverting 4–6 times, and incubate at room temperature for at least 15 min.**

   Incubating for 15 min was found to increase yield relative to the standard protocol.

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6. **Add 10 ml of chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 1 h.**
   Incubating for 1 h was found to increase yield relative to the standard protocol.
7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**
8. **Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow.**
   Please refer to the handbook for more detailed information.
9. **Apply the supernatant from step 7 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**
10. **Wash the QIAGEN-tip with 2 x 30 ml Buffer QC.**
11. **Elute the DNA with 15 ml Buffer QF.**
12. **Precipitate the DNA with 0.7 volumes of room temperature isopropanol and 2 µl glycogen for 1 min.**
13. **Centrifuge at ≥15,000 x *g* for 30 min at 4°C.**
14. **Wash the DNA pellet twice by adding 1 ml ice-cold 70% ethanol, centrifuging at ≥15,000 x *g* for 10 min, and carefully decant the supernatant without disturbing the pellet.**
15. **Air-dry the pellet for 5 min and redissolve the DNA in 50 µl TE, pH 8.0 by incubation at 4°C overnight.**

Table 1. Yield and purity of different plasmid preparations from *Streptomyces* strains

Plasmid	Size (kb)	Copy number (no. per chromosome)	Replicon	Host strain	Yield (plasmid DNA per gram wet weight)	A_{260}/A_{280}
SCP2	31.4	Very low (~1)	SCP2	*S. coelicolor* A3(2) subsp.	4 µg	1.91
SCP2*	31.4	Very low (~1)	SCP2*	*S. coelicolor* A3(2) subsp.	4 µg	1.83
pIJ903	25.8	Very low (~1)	SCP2*	*S. coelicolor* A3(2) subsp.	4 µg	1.79
pIJ903	25.8	Medium (~20)	pMB1	*E. coli* DH5α, XL1-Blue	50 µg	1.81
pIJ702	5.8	High (~300)	pIJ101	*S. lividans* TK23	30 µg	1.74
pIJ6021	7.8	High (~300)	pIJ101	*S. lividans* 1326	27 µg	1.83

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YEME medium composition

Yeast Extract Malt Extract (YEME) (per liter, Ref. 1)		
	Difco yeast extract	3 g
	Difco bacto-peptone	5 g
	Oxoid malt extract	3 g
	Glucose	10 g
	Sucrose	340 g

After autoclaving, add 2 ml $MgCl_2 \cdot 6H_2O$ (2.5 M) and 25 ml glycine (20%).

Suppliers

Erlenmeyer flasks with baffles are available from Nalge Nunc International.

Lysozyme can be obtained from SERVA, Heidelberg, Germany.

Glycogen is available from Boehringer Mannheim, Germany.

Reference

1. *Hopwood, D.A., et al. (1985) Genetic manipulation of Streptomyces: A laboratory manual. The John Innes Foundation, F. Crowe & Sons Ltd., Norwich, England.*

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