质粒大提实验报告

1 实验器材及试剂

1.1 实验器材

名称	厂家	型号
离心机	Heal Force	台式高速冷冻离心机 Neofuge 15R
分光光度计	Thermo	nanodrop 2000
标准试剂型纯水仪	青岛富勒姆科技有限公司	FBZ2001-up-p
超净工作台	苏净集团苏州安泰空气技术有限公司	SW-CJ-1FD
恒温振荡摇床	常州澳华仪器有限公司	SHZ-82A

1.2 主要实验试剂及耗材

试剂	厂家	货号
无内毒素质粒大提试剂盒	Tiangen Biotech(Beijing) CO.,LTD	DP117
无水乙醇	国药集团化学试剂有限公司	10009218
LB 固体培养基（干粉）	Wanwu	G3101
氨苄青霉素	Wanwu	G5053
硫酸卡那霉素	Wanwu	G5054-1G
异丙醇	国药集团化学试剂有限公司	80109218
离心管	Wanwu	
TIP 头	Wanwu	

2 质粒大提实验步骤

2.1 质粒大提

2.1.1 于超净工作台上，吸取 1ul 菌液，接种于含千分之一抗生素的 LB 液体培养基中，37℃下 220rpm 振荡培养过夜。

2.1.2 于超净工作台上，按 1/1000 的比例，转接 1）中的菌液于含千分之一抗生素的 LB 液体培养基中，37℃下 220rpm 振荡培养过夜。

2.1.3 4℃下 8000rpm 离心收集菌体，用 TIANGEN 无内毒素质粒大提试剂盒提取质粒，步骤如下：

2.1.3.1 柱平衡，向吸附柱中加入 2.5ml 平衡液，8000rpm 离心 2min，倒掉废液，将吸附柱放回收集管。

2.1.3.2 取 100ml 过夜培养的菌液，室温 8000rpm 离心 3min 收集细菌。

2.1.3.3 倒掉废液。

2.1.3.4 加入 8ml 溶液 P1，重悬菌体。

2.1.3.5 加入 8ml 溶液 P2，立即温和翻转 6-8 次，室温放置 5min。

2.1.3.6 加入 8 ml 溶液 P4，立即温和翻转 6-8 次，室温放置 10min。

2.1.3.7 将溶液转入过滤器中，过滤，保留滤液。

2.1.3.8 向滤液中加入 0.3 倍体积的异丙醇，颠倒混匀。

2.1.3.9 转移到吸附柱中。

2.1.3.10 8000rpm 离心 2min，倒掉收集管中的废液。

2.1.3.11 向吸附柱中加入 10ml 漂洗液 PW，室温 8000rpm 离心 2min，倒掉废液，将吸附柱重新放回收集管中。

2.1.3.12 向吸附柱中加入 3ml 无水乙醇，室温 8000rpm 离心 2min，倒掉废液，将吸附柱重新放回收集管中。

2.1.3.13 室温 8000rpm 离心 5min，将吸附柱放置于一个干净的收集管中。

2.1.3.14 向吸附膜中央加入 0.75ml ddH_2O，室温放置 5min。

2.1.3.15 室温 8000rpm 离心 2min，所得溶液即为质粒。

2.2 质粒浓度测定 ： 见 excel 表

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1 Laboratory Equipment and Reagents

1.1 Laboratory equipment

Equipment	Manufacturers	Model
Centrifuge	Heal Force	Neofuge 15R
Spectrophotometer	Thermo	NANODROP 2000
Standard reagent type pure water meter	Qingdao Fulum Technology Co., Ltd	FBZ2001-up-p
Clean bench	Suzhou Antai Air Tech Co., Ltd	SW-CJ-1FD
Constant temperature oscillation shaker	Changzhou Aohua Instrument Co ., Ltd	SHZ-82A

1.2 Reagents

Reagents	Manufacturers	Order
EndoFree Maxi Plasmid Kit	Tiangen Biotech(Beijing) CO.,LTD	DP117
Anhydrous ethanol	Sinopharm Group Chemical Reagent Co., Ltd.	10009218
Luria-Bertani Liquid Medium(powder)	Wanwu	G3102
Luria-Bertani Solid Medium(powder)	Wanwu	G3101
Ampicillin	Wanwu	G5053
Kanamycin	Wanwu	G5054-1G
Isopropyl alcohol	Sinopharm Group Chemical Reagent Co., Ltd.	80109218
Centrifuge tube	Wanwu	
TIP	Wanwu	

2 EndoFree Maxi Plasmid Experimental steps

2.1 To cultivate 200 ml bacterial culture，with 37℃、220rpm、16h.

2.2 Column equilibration: place a Spin Column CP6 into a 50 ml collection tube (supplied in the kit) and add 2.5 ml Buffer BL to Spin Column CP6. Centrifuge for 2 min at 8,000 rpm (~8,228 × g). Discard the flow-through, and place Spin Column CP6 into the same collection tube.

2.3 Harvest 100 ml (for low copy plasmid, please harvest 200 ml) overnight cultured bacterial cells by centrifuging at 8,000 rpm (~8,228 × g) for 3 min at room temperature (15-25°C), and then remove all traces of supernatant.

2.4 Try to remove all traces of supernatant, use clean filter paper to absorb the fluids inside the tube wall.

2.5 Resuspend pelleted bacterial cells in 8 ml Buffer P1 (Ensure that RNase A has been added). The bacteria should be resuspended completely by vortex or pipetting up and down until no cell clumps remain.

2.6 Add 8 ml Buffer P2 and mix thoroughly by inverting the tube 6- 8 times, then incubate at room temperature for 5 min.

2.7 Add 8 ml Buffer P4, and mix immediately and thoroughly by gently inverting 6-8 times, until the whole solution become cloudy. Incubate at room temperature for 10 min. Centrifuge for 5-10 min at 8,000 rpm (~8,228 × g), the white material should be in the bottom of the centrifuge tube (prolong centrifugation time properly). Transfer the supernatant into a Filtration CS1 (avoid transferring large clump into the Filtration CS1, which will clog the filtration membrane). Gently insert the plunger into the Filtration CS1 and filter the cell lysate into a new 50 ml tube (not supplied in the kit).

2.8 contamination), mix completely by reverting upside and down and then transfer all solution to the Spin Column CP6 (put Spin Column CP6 into 50 ml collection tube).

2.9 Centrifuge for 2 min at 8,000 rpm (~8,228 × g). Discard the flow-through and place the Spin Column CP6 back into the same collection tube.

2.10 Add 10 ml Buffer PW to the Spin Column CP6 and centrifuge at 8,000 rpm (~8,228 × g) for 2 min. Discard the flow-through and place the Spin Column CP6 back into the same collection tube.

2.11 Repeat step 9.

2.12 Add 3ml 96-100% ethanol to the Spin Column CP6 (put the CP6 in a collection tube). Centrifuge for 2 min at 8,000 rpm (~8,228 × g), discard the flow-through.

2.13 Put Spin Column CP6 back to collection tube, centrifuge at 8,000 rpm (~8,228 × g) for 5 min for removing residual ethanol.

2.14 To elute DNA, place the Spin Column CP6 in a clean 50 ml collection tube (supplied in the kit) and add 1-2 ml Buffer TB to the center of the membrane and incubate 5 min at room temperature, centrifuge at 8,000 rpm (~8,228 × g) for 2 min. Transfer the eluate from 50 ml centrifuge tube to a clean 1.5 C for storage.°ml centrifuge tube and put at -20 for storage.

2.2 Determination of plasmid concentration ： see Excel.