Instructions For Use

Agencourt CosMCPrep

High and Low Copy
Plasmid Purification

PN B47127AB
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BECKMAN COULTER

Agencourt CosMCPrep
High and Low Copy Plasmid Purification
Instructions for Use
PN B47127AB (January 2015)

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Revision History

Version AA, Initial Issue, 08/14

Issue Version AB, 01/2015
Changes or additions were made to Warnings and Precautions. Removed Appendix B, Frequently Asked Questions.

Note: Changes that are part of the most recent revision are indicated in text by a bar in the margin of the amended page.

Safety Notice

Read all product manuals before attempting to use this product. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact Beckman Coulter.

Alerts for Danger, Warning, Caution, Important, and Note

⚠ WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis.

⚠ CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the Important adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

Warnings and Precautions

	**DANGER**
N3 Solution: Acetic Acid 10 – 20%	
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves, protective clothing and eye/face protection.
P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor/physician.
SDS	Safety Data Sheet is available at *techdocs.beckmancoulter.com*.

	**DANGER**
L2 Solution: Sodium Lauryl Sulfate 1 – 5%	
Sodium Hydroxide 0.1% – 1%	
H314	Causes sever skin burns and eye damage.
P280	Wear protective gloves, protective clothing and eye/face protection.
P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor/physician.
SDS	Safety Data Sheet is available at *techdocs.beckmancoulter.com*.

WARNING	
RE1 Solution: Acetic Acid 0.1 – 0.5%	
H316	Causes mild skin irritation.
P332+P313	If skin irritation occurs: Get medical advice/attention.
SDS	Safety Data Sheet is available at *techdocs.beckmancoulter.com*.

Contents

Revision History, iii

Safety Notice, v

Alerts for Danger, Warning, Caution, Important, and Note, v

Warnings and Precautions, vi

CHAPTER 1: Overview, 1-1

Introduction, 1-1

Automation, 1-1

Process Overview, 1-2

Materials Supplied in the Agencourt CosMCPrep Kit, 1-3

Materials Supplied by the User, 1-3

- Consumables, 1-3
- Equipment, 1-4
- Reagents, 1-4

CHAPTER 2: Agencourt CosMCPrep Procedure, 2-1

Agencourt CosMCPrep Procedure, 2-1

CHAPTER 3: Growth of Glycerol Preculture Plates (Optional), 3-1

Growth of Glycerol Preculture Plates Procedure (Optional), 3-1

APPENDIX A: Troubleshooting Guide, A-1

Low Yield, A-1

RNA in Eluate, A-2

Flocculent Aspirated During Transfer, A-2

Abbreviations

Tables

1.1 Agencourt CosMCPrep Kit — Components, 1-3
A.1 Troubleshooting — Low Yield, A-1
A.2 Troubleshooting — RNA in Eluate, A-2
A.3 Troubleshooting — Flocculent Aspirated During Transfer, A-2

CHAPTER 1

Overview

Introduction

The Agencourt CosMCPrep 96-well plasmid purification system utilizes Beckman Coulter's patented SPRI paramagnetic bead technology for high-throughput preparation of high-copy or low-copy plasmid DNA from *E. coli* cells. Agencourt CosMCPrep can also be used with fosmid and BAC vector-based constructs. The system uses alkaline lysis followed by a SPRI purification to differentially bind plasmid DNA to paramagnetic beads. While the DNA is bound to the beads, contaminants can be rinsed away using a simple washing procedure. Because Agencourt CosMCPrep utilizes magnetic separation technology, the protocol does not require vacuum filtration. This makes Agencourt CosMCPrep extremely amenable to automation. Plasmid DNA purified with this system is most commonly used in:

- Sanger Sequencing
- PCR* amplification

NOTE This protocol is not intended for transfection quality DNA. For transfection applications, contact a Beckman Coulter representative.

NOTE Abbreviations and acronyms used in this document are defined in *Abbreviations*.

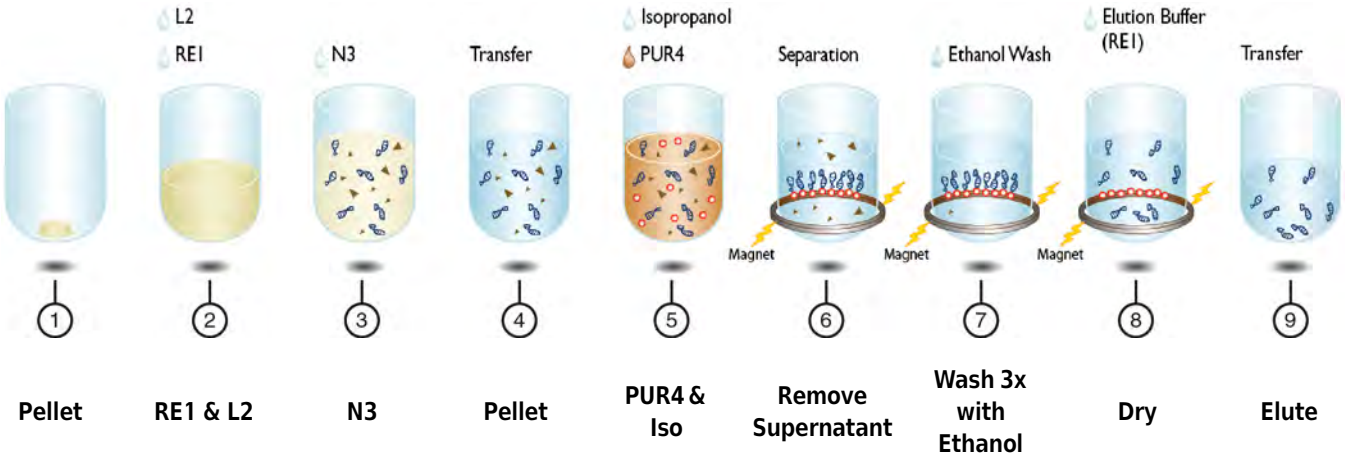
Automation

The Agencourt CosMCPrep plasmid purification system was designed for use with automated liquid handling robotics. The purification utilizes magnetic separation in place of vacuum filtration steps.

Beckman Coulter supports the Beckman Coulter Biomek FX/NX 96 MC Automated Laboratory Workstations for optimal automation of Agencourt CosMCPrep.

* The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.

Process Overview



The Agencourt CosMCPrep procedure is performed in the following stages:

A. Pelleted *E. coli* cells **(1)** are resuspended in RE1 solution, L2 solution is added to lyse the bacterial cultures **(2)**.

B. Addition of N3 solution neutralizes the high pH and maintains DNA integrity. During neutralization *E. coli* chromosomal DNA and cellular contaminants coagulate and form a flocculent in the lysate **(3)**.

C. Flocculent is pelleted via centrifugation and then the clear plasmid-enriched lysate is transferred into a fresh 96-well plate **(4)**.

D. Addition of isopropanol and PUR4 solution binds the DNA of interest to the magnetic beads **(5)**. A magnet plate is used to separate the beads from solution, so that the contaminants can be removed with the supernatant **(6)**.

E. Beads with captured plasmid are washed with ethanol to remove salts and other contaminants **(7)**.

F. Purified plasmid is eluted from the magnetic beads using RE1 solution **(8)** and transferred away from the beads **(9)**.

Materials Supplied in the Agencourt CosMCPrep Kit

Agencourt CosMCPrep reagents have a shelf life of up to 6 months if stored as directed. As part of good laboratory practice, gloves should be worn when using the Agencourt CosMCPrep kit (Table 1.1).

IMPORTANT See the bottle labels for the actual expiration date.

Table 1.1 Agencourt CosMCPrep Kit — Components

Kit Component/ Description	Reagent Volumes		Storage/Reuse
	A37064 (384 Prep Kit)	**A29174** (4,000 Prep Kit)	
Agencourt CosMCPrep RE1 Solution *Resuspension and elution solution*	100 mL	1 L	• Store at 2 – 8°C. • RE1 solution contains RNase A.
Agencourt CosMCPrep L2 Solution *Lysis solution*	50 mL	500 mL	• Store at room temperature (15 – 30°C) or, if the lab temperature is below 15°C, store in an incubator (at 37°C) to prevent precipitation. • If white precipitate forms due to cold lab conditions, warm the solution, and shake to re-dissolve the precipitate.
Agencourt CosMCPrep N3 Solution *Neutralization solution*	50 mL	500 mL	• Store at room temperature (15 – 30°C)
Agencourt CosMCPrep PUR4 Solution *Plasmid binding solution*	6 mL	50 mL	• Store at 2 – 8°C. • Shake well to resuspend the magnetic beads before using. The solution should be homogeneous prior to use.

Materials Supplied by the User

Consumables

- Magnetic Plate:
  - Agencourt SPRIPlate 96R Super Magnet Plate (PN A32782)

    OR
  - Agencourt SPRIPlate 96R Ring Magnet Plate (PN A29164)

- Overnight Culture/Source Plate:
  - 96-well 2.2 mL deep-well culture block, or equivalent
    (World Wide Medical Products, PN 99181000, www.wwmponline.com)
- Extraction/Destination Plate:
  - 1.2 mL Square Well Storage Plate, Low Profile (96-well) (Thermo, PN AB-1127), or equivalent

    OR
  - 300 µL round bottom 96-well microtiter plate (300 µL-well capacity)
    (Costar, PN 07-200-105; www.fishersci.com), or equivalent
- Gas Permeable Seals (Thermo Scientific AB-0718 www.thermoscientificbio.com, or equivalent) (*to cover plates during shaking*)

Equipment

- Liquid Handling Robotics – for example, Beckman Coulter Biomek Liquid Handling Workstation
- Centrifuge – for example, Beckman Coulter Avanti J-30I centrifuge (PN 363118) with JS-5.9 Rotor (PN 369331)
- Plate Shaker (Orbital or Linear) – for example, Phenix Research Products Microplate Shaker with 4 Position Microplate Platform (model no. S-1500)

  NOTE The Incubator (listed below) might also serve as the plate shaker.
- Incubator

Reagents

- Growth Media:
  - 2xYT bacterial growth media containing the appropriate antibiotic (American Bioanalytical, PN AB15063-01000, http://www.americanbio.com/), or equivalent

    OR
  - Teriffic Broth (TB) media (Sigma, PN T5574, www.sigmaaldrich.com), or equivalent
- (*Optional*) Luria Broth Media (LB) media with 10% Glycerol growth media containing the appropriate antibiotic
  (American Bioanalytical, CU08048-01000, http://www.americanbio.com/), or equivalent
- 100% Isopropanol, molecular biology grade
- 70% Ethanol (EtOH), molecular biology grade

  NOTE 70% EtOH is hygroscopic. Fresh 70% EtOH should be prepared regularly for optimal results.

CHAPTER 2

Agencourt CosMCPrep Procedure

Agencourt CosMCPrep Procedure

To perform the Agencourt CosMCPrep procedure:

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1 Pipette 1.7 mL 2xYT bacterial growth media containing the appropriate antibiotic (see *Recommendations* below) into each well of a 2.2 mL deep well culture block.

Final Concentration in Media or Glycerol:

- Chloramphenicol 12.5 µg/mL
- Ampicillin 50 µg/mL
- Kanamycin 35 µg/mL
- Carbenicillin 50 µg/mL
- Zeocin 25 µg/mL
- Tetracycline 2.5 µg/mL

Recommendations:

- TB media can be used to increase cell number and yield.
- Beckman Coulter strongly recommends reduced antibiotic concentrations for overnight cultures, as conventional concentrations can delay the propagation of cells.
- Regardless of the type of media, antibiotic, and other growth parameters used, growth times should be increased or decreased to ensure cells are pelleted before the death phase.

NOTE Very high cell numbers can lead to very viscous lysate that might stick to the pipette tip.

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2 Inoculate each well with a single plasmid containing *E. coli* bacterial colony.

Growth cultures can be inoculated directly from colony plates or from glycerol stocks prepared according to the step above.

3 Cover the plate with a gas permeable seal and shake at 300 RPM and 37°C.

Do not allow culture blocks to overgrow. If plates grow too long, the cells will begin to die and the Agencourt CosMCPrep purification process will not yield optimum results. Exceeding 19 hours will lead to overgrown culture blocks, but in some cases the lag phase starts earlier.

4 Pellet bacterial cultures by centrifuging culture plates at 2,500 × *g* for 10 minutes.

5 After centrifugation, remove the seal and invert the block to decant the media away from the cell pellets. Blot the inverted block on a paper towel to remove excess media.

Blot gently to avoid dislodging the cell pellet. Pelleted blocks can be sealed and frozen at -20°C or -80°C for later processing.

6 Add 100 µL of RE1 solution and thoroughly resuspend cell pellets by vortexing, shaking or pipette mixing.

Pipette mixing and shaking are most often used for automated processes. Pipette mix at least 20 times (if possible, pipette mix 5 times at 4 different locations in the well) for an even resuspension. Alternatively, shake 4 minutes at 600 to 1,200 RPM on a shaker. Shaking speeds will vary, depending on the orbit of the shaker. Vortexing will take 2 to 3 minutes on a high setting. The cell pellet should be completely resuspended so that the mixture appears homogeneous and has no cell clumps.

7 Add 100 µL of L2 solution, gently mix, and allow the samples to lyse for 5 minutes.

Shake 5 minutes at 300-600 RPM. Alternatively, gently pipette mix two times, and then allow the samples to sit for 5 minutes for a complete lysis. Vigorous pipette mixing is not recommended, as BACs can be easily sheared. Do not allow samples to lyse for longer than 10 minutes.

If a white precipitate is seen in the L2 solution prior to addition, warm the bottle in a 37°C water bath or under hot running water, shaking periodically, until the precipitate dissolves. It is recommended that gloves are worn when handling L2, as it is a basic solution.

8 Add 100 µL of N3 solution and shake 10 minutes on an orbital or linear shaker (for example, Phenix Research Products, model no. S-1500) to neutralize samples.

Addition of N3 neutralizes the solution and precipitates proteins and cellular debris, creating a white flocculent. BACs must be shaken to help release the large template from the flocculent; the same orbital shaker can be used for overnight culture (room temperature or 37°C are both fine). It is recommended that gloves are worn when handling N3.

9 Following neutralization, centrifuge samples at 4,700 × *g* for 20 minutes to pellet the flocculent.

Pelleting of the flocculent allows for transfer of a greater percentage of the lysate and cleaner plasmid. A 20 minute spin is usually sufficient to pellet the flocculent, but if the pellet is not tightly bound to the bottom of the well, the centrifugation time should be increased.

10 Transfer 175 µL to 240 µL of the clear lysate to a 1.2 mL deep well plate. **This transfer is the most critical step of the process.**

The transfer volume should be chosen so that the supernatant is free of cellular contaminants (flocculent) for optimal results. For best results, use slow aspiration speeds and remove the clear lysate from the top of the well. The aspiration height used for this transfer step will most likely need to be optimized to avoid disturbing the flocculent pellet.

11 Add 10 µL of Agencourt CosMCPrep PUR4 solution and the appropriate volume of 100% isopropanol. Pipette mix 10 times.

PUR4 contains magnetic particles in a plasmid binding buffer. As soon as both the magnetic particles and isopropanol are added, the beads may begin to fall out of solution if the sample is not pipette mixed immediately. The liquid should appear homogeneous after mixing. Shaking samples, instead of pipette mixing, may result in reduced yield.

The final concentration of isopropanol in the well should be 40% for optimal results. Use the following calculation to determine isopropanol volume:

$$Vol_{Iso} = 0.66 * (Vol_S + Vol_{PUR4})$$

Where:

Vol_{Iso} = volume of isopropanol to be added per well

Vol_S = volume of cleared lysate transferred

Vol_{PUR4} = volume of PUR4 solution (10 µL)

Isopropanol Volumes for Commonly Used Lysate Transfer Volumes:

Lysate Volume (µL)	Isopropanol Volume (µL)
100	80
150	106
175	122
200	139
225	155
240	165

12 Place the deep well plate on an Agencourt SPRIPlate 96R Super Magnet Plate (PN A32782) and allow beads to separate for 8 minutes. Alternatively, the separation can be on an Agencourt SPRIPlate 96R Ring Magnet Plate (PN A29164) for 15 minutes.

Once separation is complete, the supernatant should be clear, and the beads should form a ring around the bottom of the well. The supernatant may have a slight yellowish-brown tinge, but should otherwise be translucent. It should not be cloudy.

13 With the plate on the Agencourt SPRIPlate 96R Super Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.

Move the tips to the bottom of the well in the center of the ring without disturbing the beads. If the magnetic particles are disturbed during aspiration, more separation time may be required before removing the supernatant.

14 With the plate situated on the Agencourt SPRIPlate 96R Super Magnet Plate, dispense 200 µL of 70% ethanol into each well of the plate to wash the magnetic beads. Allow samples to incubate for 30 seconds, and then remove and discard the ethanol wash solution. Repeat the wash twice, for a total of three washes.

Allow the wash solution to remain in the wells of the plate for at least 30 seconds. For best results make fresh 70% ethanol each day. If the concentration of the ethanol is less than 70%, some of the plasmid may be washed away. Do not disturb the ring of beads during the wash step as beads have the target DNA bound to them.

15 Dry the BAC, Fosmid, or plasmid samples at 37°C for 5 minutes.

Plates can also be dried at room temperature for 5 to 10 minutes. For best results, do not over dry BACs.

16 Add 40 µL of RE1 solution (see note below) to each well of the plate, and then incubate for 5 minutes at 37°C.

NOTE RE1 solution is the optimal elution buffer for Agencourt CosMCPrep samples and is recommended for use in sequencing reactions. Other nuclease free aqueous low-salt buffers may also be used for elution.

Vortex or shake the plate for 30 seconds after incubating for 5 minutes at 37°C to fully elute the plasmid from the beads. For large templates, especially BACs or Fosmids, it is helpful to let the plates sit for 5 to 10 minutes after vortexing to allow the large templates extra time to dissociate from the beads. It is not necessary for the beads to go back into solution for complete elution to occur, however, it is extremely important for the elution buffer to completely cover the ring of beads for maximum recovery.

For long-term freezer storage, it is helpful to transfer the eluant away from the beads. Simply place the plate back on the magnet for 1 to 3 minutes, and then transfer the samples to a new clean plate.

CHAPTER 3

Growth of Glycerol Preculture Plates (Optional)

Growth of Glycerol Preculture Plates Procedure (Optional)

Beckman Coulter recommends picking colonies into glycerol stock plates so that clones can be retrieved for further analysis after DNA purification. If glycerol stock plates are not desired, bacterial cultures can be picked directly into deepwell growth blocks (for details, see CHAPTER 2, *Agencourt CosMCPrep Procedure*).

To make 96 and 384-well glycerol stocks:

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1 Determine the amount of glycerol stock required. For:

a. **96-Well Glycerols**, fill a 96-well round bottom microtiter plates (Costar, PN 3795) with 220 µL of LB / 10% Glycerol / appropriate antibiotic.

b. **384-Well Glycerols**, fill a 384-well flat bottom microtiter plates (Greiner, PN 781101 via World Wide Medical Products, PN 99031070, www.wwmponline.com) with 90 µL of LB / 10% Glycerol / appropriate antibiotic.

NOTE For low-copy plasmids, a much lower antibiotic concentration is required for healthy growth. Antibiotic concentrations for low-copy plasmids may be 2 to 5 fold less than for high-copy plasmids. Unusually high antibiotic concentrations may significantly hinder amplification during growth.*

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2 Inoculate each well with a single plasmid containing *E. coli* bacterial colony.

Use an automated picking machine to pick single colonies into each well of the plate.

NOTE Automated picking ensures accuracy and consistency; however, sterile toothpicks or thin pipette tips may also be used to manually pick colonies from an colony tray.

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* Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2001. Vol. 3: A2.6.

3 Cover plates with lids, wrap plates in plastic film and grow statically (no shaking) in a 37°C warm room for 12 hours.

If the plates do not have lids, an empty plate can be used in between the glycerol plates. Wrap each stack with plastic film to avoid evaporation. Do not allow glycerol plates to grow longer than 12 hours, as the cells will begin to die and will not yield healthy growth cultures. Overgrown glycerol pre-cultures often have visible cell mass clustered at the bottom of the affected wells. A thick milky consistency or web-like veils of cells can sometimes coat the sides of wells that have been overgrown. The quality of the pre-culture is very important for single- or low-copy purification success and any one of the aforementioned symptoms may inhibit optimum downstream results. If a culture grows poorly (uneven pellet sizes; large, loose pellets; wells with no growth), the pre-culture should be examined.

APPENDIX A

Troubleshooting Guide

Low Yield

TIP It may be possible to increase yield further by transferring more lysate. Determine how much of the clear lysate can be aspirated without disturbing the flocculent. Depending on the number of cells, it may be possible to transfer up to 240 µL. When the transfer volume is increased, the volume of isopropanol also must be increased per the provided formula. Be sure to transfer into a 1.2 mL Square Well Storage Plate, Low Profile (96-well) (Thermo AB-1127), if the transfer volume exceeds 175 µL.

Table A.1 Troubleshooting — Low Yield

Problem	Solution
Isopropanol concentration incorrect	Double check whether the volume of isopropanol added during the bind step is accurate, based on the volume of transferred lysate.
Not enough cells	If the cell pellets appear small or the measured cell density is low, adjust growth parameters to obtain a healthy culture: • Be sure to pellet cells while still on the log phase. • Do not add too much or too little antibiotic. • Grow cells in TB media or a different brand of media. • Ensure cultures are agitated enough during incubation and receive enough oxygen. • Optimize the amount of inoculum used • Ensure the pre-culture is healthy and not overgrown • Ensure all cells are pelleted during centrifugation step and that pellets are not dislodged during decanting.
Pelleted cells are in death phase	Cells in the death phase can lose their plasmids, leading to decreased yield. Use a growth curve to determine when cells are entering death phase and be sure to pellet them during the growth curve.
Dried on beads	Large BACs can irreversibly bind to the beads. Avoid drying beads completely when extracting BACs.
Poor mixing	Be sure samples are mixed well during the PUR4 bind step and the elution step.
Bead loss	Remove the supernatant only once the beads have settled and avoid aspirating any beads during the supernatant removal and the removal of the ethanol washes.

RNA in Eluate

Table A.2 Troubleshooting — RNA in Eluate

Problem	Solution
Excessive RNA in sample	The amount of RNase in the RE1 buffer may be insufficient to digest RNA completely if the cells produce an abundance of RNA. Additional RNase A can be added to the RE1 buffer used for elution at a relatively low concentration, for example, 3.5 µg/mL.
Digested RNA is still detected in eluate. (Eluate should be free of even *digested* RNA.)	Additional RNase can be added to RE1 for the elution step to digest RNA completely. **NOTE** If digested RNA in the eluate is a problem for downstream processing (for example, for quantitation using Spectrophotometry), the RNA can be removed by digesting it during the Resuspension step. Add 3.1 µL RNase A (Invitrogen Purelink 20 mg/mL, PN 12091-039) and 0.1 µL RNase T1 Sigma, PN R1003, 190 units/ µL) for each sample (per 100 µl RE1). If the activity of RNase T1 differs from the tested lot, adjust the volume added accordingly. Incubate 5 minutes after cells have been resuspended.
Pellets not completely resuspended	Clumps of cells during the 5 minute incubation time at resuspension will trap RNA that is inaccessible by the added RNase and the RNA will not be digested.

Flocculent Aspirated During Transfer

Table A.3 Troubleshooting — Flocculent Aspirated During Transfer

Problem	Solution
Aspiration too low or too fast	Aspirate the clear lysate very slowly and maintain distance to the flocculent pellet. The samples can be re-centrifuged if pellets have become dislodged. Try transferring a smaller volume if necessary.
Centrifugation speed insufficient	If a centrifuge capable of reaching 4,700 × *g* is not available, lower g forces may work in some cases. Depending on the cell number and flocculent consistency, centrifugal forces of as low as 3,500 × *g* have been effective at creating a tight flocculent pellet. Below 3,500 × *g*, pellets can become dislodged or flakes of flocculent might remain in the supernatant. Try increasing the separation time to 30 minutes if the flocculent is not compacted enough at the highest possible centrifugation speed. Minimize time between the end of the centrifugation and the transfer step as much as possible.
Overgrown cell cultures	Cell cultures pelleted in death phase tend to have flocculent pellets that don't stick well to the bottom of the plate. Perform a growth curve to determine when cells enter death phase and remove cultures from the incubator and pellet them before this time point.

Abbreviations

µL — microliter

BAC — Bacterial Artificial Chromosome

DNA — Deoxyribonucleic Acid

***E. coli* —** Escherichia Coli

EtOH — Ethanol

***g* —** gravitational force

LB — Luria Broth Media

MC — Multichannel

mL — Milliliter

PCR — Polymerase Chain Reaction

PN — Part Number

RPM — Revolutions per Minute

TB — Terrific Broth Media

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