User's Guide

MagListo™
5M Plasmid Extraction Kit

REF K-3600
K-3601

MagListo™ 5M Plasmid Extraction Kit

Kit for the extraction of plasmid from bacterial cultures using *MagListo*™

User's Guide

K-3600 Σ 500 **K-3601** Σ 100

Version No.: 1.1 (2016-04)

Please read all the information in booklet before using the unit

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Trademarks

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I. Overview

Description

MagListo™ 5M Plasmid Extraction Kit is designed to extract plasmid rapidly from bacterial cultures in 5 min for mini prep, 10 min for midi and 15 min for maxi, using Magnetic Nano Beads and *MagListo*™ Magnetic Separation Rack. For any scale of extraction (mini/midi/maxi prep), proper volume is added for each solution as described in this User's Guide. In addition, a key benefit of using *MagListo*™ Magnetic Separation Rack greatly reduces process time by not using centrifuge.

Features and Benefits

- Rapid extraction: mini-5 min, midi-10 min, maxi-15 min.
- No need of costly instruments except *MagListo*™ Magnetic Separation Rack.
- One kit serves mini, midi and maxi scale prep.

Applications

Gene Cloning, PCR, Sequencing, Transformation, Transfection, *In-vitro* Transcription/Translation

II. Kit Components

MagListo™ 5M Plasmid Extraction Kit Cat. no. 3601, 3600	*K-3601	**K-3600
Buffer ① (Resuspension buffer)	25 ml x 1 ea	110 ml x 1 ea
Buffer ② (Lysis buffer)	25 ml x 1 ea	110 ml x 1 ea
Buffer ③ (Neutralization buffer)	25 ml x 1 ea	110 ml x 1 ea
Buffer ④ (Binding buffer)	75 ml x 1 ea	180 ml x 2 ea
Buffer ⑤ (Elution buffer)	15 ml x 1 ea	50 ml x 1 ea
Magnetic Nano Bead	1.2 ml x 5 ea	1.2 ml x 25 ea
RNase A powder, lyophilized	3 mg x 1 ea	12 mg x 1 ea

*mini – 100 rxn, midi – 10 rxn, maxi – 4 rxn **mini – 500 rxn, midi – 50 rxn, maxi – 18 rxn

III. Storage

MagListo™ 5M Plasmid Extraction Kit should be stored at room temperature up to 2 years if it remains sealed. Buffers may form precipitates during storage. If this occurs, please warm the buffer to 37℃ until the precipitates are completely dissolved. After addition of RNase A powder, Buffer ① (Resuspension) is stable for 6 months when stored at 2–8℃. RNase A powder can be stored for two years at room temperature. A white precipitate may form in Buffer ② (Lysis) or Buffer ④ (Binding) after prolonged storage at low temperature. Incubating at 60℃ should dissolve any precipitate in Buffer ② (Lysis) or Buffer ④ (Binding).

IV. Intended Use

MagListo™ 5M Plasmid Extraction Kit is intended for research use only. This kit is not intended for human or veterinary diagnostics.

V. Safety Warnings and Precautions

Please inquire BIONEER's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.

Before, during and after use of this kit as described in this User's Guide, all potentially hazardous materials (i.e. materials that may have come in contact with genetically recombinant samples) including tubes, tips and materials should be processed and disposed of according to applicable and appropriate regulations of the municipality/government in which this product is being used. A user must also be equipped with basic experimental techniques required for correct execution of the experiments described in this User's Guide.

Some applications that may be performed with this kit may infringe upon existing patents in certain countries. The purchase of this kit does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on country and application. We do not condone nor recommend the unlicensed use of a patented application.

VI. Warranty and Liability

All BIONEER products are manufactured and tested under strict quality control protocols. BIONEER

guarantees the quality of all directly manufactured products during the warranty period of one (1) year from the date of purchase. If any issues are discovered relating to compromise in product quality, immediately contact BIONEER's Customer Service Center (sales@bioneer.com).

BIONEER does not assume liability for misuse of the product, i.e. usage of the product for any purposes other than its intended purpose as described in the User's Guide. BIONEER assumes liability under the condition that users disclose all information related to the problem to BIONEER in written form within 30 days of occurrence.

VII. Technical Assistance

At Bioneer, we pride ourselves on being responsive to your needs. If you have any questions or would like to find out more information about *MagListo*™ products, please contact us. We look forward to hearing from you!

Technical Support
For all technical questions and troubleshooting on Bioneer products and applications.
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VIII. Quality Management

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards. Each lot of *MagListo*™ 5M Plasmid Extraction Kit is carefully tested by the quality control team.

IX. Product Specifications

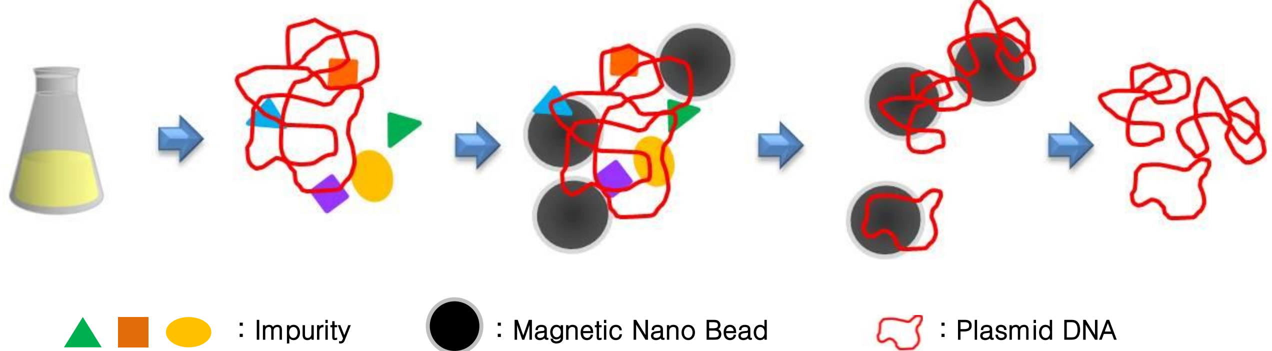
Scale	8-channel scale	Mini scale	Midi scale	Maxi scale
Starting culture volume	1~1.5 ml	1~5 ml	20~50 ml	100~200 ml
Preparation time	< 20 min	> 5 min	> 10 min	> 15 min
Elution volume	100 µl	100 µl	500 µl	1 ml
Expected DNA yield	Up to 10 µg	Up to 20 µg	Up to 200 µg	Up to 500 µg
Expected purity	$A_{260}/_{280} > 1.8$			

* The yield of low copy plasmid could be less than the figures shown in the table.

X. Principle

MagListo™ 5M Plasmid Extraction Kit is designed for the extraction of highly purified plasmid from cultured bacterial cells as fast as 5 min. The overall principle is based on the modified alkaline lysis method (Birnboim et al, 1979) and Bioneer's novel Nano-Technology (Patent pending).

Collected cells are re-suspended in Buffer ① (Resuspension buffer) that contains Nano beads, followed by a lysis step with the addition of Buffer ② (Lysis buffer). When Buffer ③ (Neutralization buffer) is added to the lysate, the chromosomal DNA and cell debris forms insoluble aggregates. While forming aggregates, Bioneer's Nano Beads effectively facilitate the process by binding to protein and cell debris aggregates and increasing the total weight of the complex. The aggregates are separated using a magnetic force from supernatant including plasmid, which is then transferred to a new tube for an ensuing process. Guanidine hydrochloride, as chaotropic agents in Buffer ④ (Binding buffer), removes water molecules around DNA and silica coated magnetic bead surface resulting in plasmid then being captured by silica coated magnetic beads. The magnetic bead and nucleic acid complexes are pulled and fixed on the tube wall using a magnetic force, followed by washing with 80% ethanol to remove debris and excessive salts. The captured nucleic acids are then eluted by Buffer ⑤ (Elution buffer), an aqueous solution with optimal pH.



XI. Materials and Equipment Needed But Not Provided

1. Table-top microcentrifuge, 16,000 x g (>13,000 rpm) (mini scale)
2. Centrifuge with rotor capable of 3,000 x g (midi & maxi scale)
3. 2 ml tube (mini scale), 15 ml tube (midi scale), 50 ml tube, (maxi scale), 1 ml tube (8-channel prep)
4. 96-well plate
5. Vortex mixer
6. 80% ethanol
7. *MagListo*™ Magnetic Separation Rack
8. Blow dryer or heat gun or dry oven

Magnetic Separation Rack Choice

Tube	*MagListo*™ Magnetic Separation Rack
1 ml tube with 8-cap strip	*MagListo*™-8Ch Magnetic Separation Rack (Cat. no. TM-1000)
1.5 ml or 2 ml microcentrifuge tube	*MagListo*™-2 Magnetic Separation Rack (Cat. no. TM-1010)
15 ml tube	*MagListo*™-15 Magnetic Separation Rack (Cat. no. TM-1020)
50 ml tube	*MagListo*™-50 Magnetic Separation Rack (Cat. no. TM-1030)

(Note) Please refer to the ordering information table on the latter part of the manual which contains the appropriate catalog number for specific size of tubes.

XII. Protocols

Before You Begin

1. Completely dissolve RNase A powder in Buffer ①. After addition of RNase A powder, Buffer ① (Resuspension) is stable for 6 months when stored at 2 ~ 8℃.
2. Buffer ④ (Binding) contains chaotropic salt. You should take the appropriate laboratory safety precautions and wear gloves when handling.
3. The relative centrifugal force (RCF) is calculated in g as follows:
   $RCF = 1.12 \times r \times (rpm/1,000)^2$
   Where 'r' is the radius of a rotor in cm, and 'rpm' is the speed of the rotor in revolutions per minute.

Bacterial Culture and Collection

- **For mini prep/ 8-channel prep**

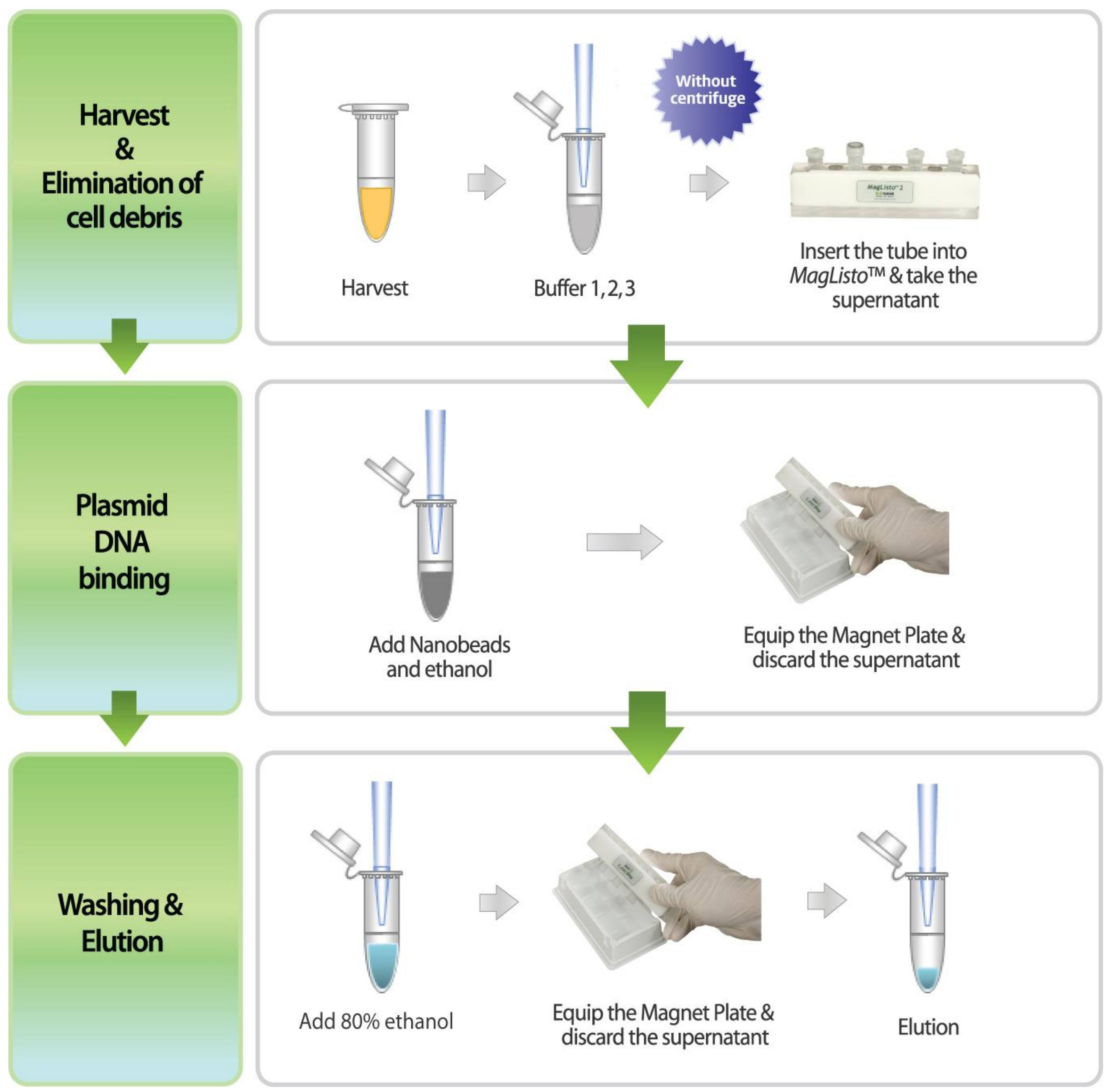
1. Pick up a single colony from fresh cultured LB (Luria-Bertani) agar plate containing appropriate antibiotics or your established media, and inoculate the cells into 1 ~ 5 ml of fresh LB liquid media containing appropriate antibiotics. Shake the container at 37℃ for 12 ~ 16 hours.
   (**Note**) Do not overgrow your *E. coli* cells. It will cause cell death, which may lead to lower yield from inefficient lysis in the following extraction procedure.
   For high copy number plasmid: 1 ~ 5 ml of *E. coli* cells.
   For low copy number plasmid: 2 ~ 10 ml of *E. coli* cells.
2. Collect the *E. coli* cells by centrifugation at 6000 x g for 15 min at 4℃. And completely remove the media by pipetting.
3. Go to page 10 "**Plasmid Extraction Protocol**" for the ensuing plasmid extraction.

- **For midi prep/ maxi prep**

1. Pick up a single colony from fresh cultured LB (Luria-Bertani) agar plate containing appropriate antibiotics or your established media, and inoculate the cells into 1 ~ 5 ml of fresh LB liquid media containing appropriate antibiotics. Shake the container at 37℃ for 12 ~ 16 hours.
2. After 12 ~ 16 hours incubation, take 50 µl of cultured cell and re-inoculate the cells into <u>20 ~ 50 ml (midi)/ 100 ~ 200 ml (maxi)</u> of fresh LB liquid media containing appropriate antibiotics or your established media. Shake the container at 37℃ for 12~16 hours.
3. Harvest the *E. coli* cells by centrifugation at 6,000 x g for 15 min at 4℃. And completely remove the media using paper towel by blotting.

Go to page 10 "**Plasmid Extraction Protocol**" for the ensuing plasmid extraction.

Overall Procedure



Plasmid Extraction Protocol for Mini/Midi/Maxi/8-channel

1. (Resuspension of *E. coli* pellet) Add 200 μl (mini)/ 2 ml (midi)/ 6 ml (maxi)/ 100 μl (8-channel) of Buffer ① to the harvested cell pellet and completely resuspend by vigorous vortexing or several pipetting.

   (Note) You must completely resuspend the cell pellet to achieve maximum lysis efficiency. Given that buffer ① contains Magnetic Nano Beads, please shake well the bottle before use.

   If you have harvested cell pellet in a 15 ml (mini)/ 50 ml (midi)/ 100~250 ml (maxi)/ 1.5 ml (8-channel) tube, please transfer the resuspended cells to a 2 ml or 1.5 ml (mini)/ 15 ml (midi)/ 50 ml (maxi)/ 1 ml (8-channel) tube.

   ***Caution!** Please check the magnetic bead completed suspended in solution. Incomplete suspension can lead to partial removal of aggregation.

2. (Lysis of cell) Add 200 μl (mini)/ 2 ml (midi)/ 6 ml (maxi)/ 100 μl (8-channel) of Buffer ② to the tube and mix by inverting the tube 3 ~ 4 times gently.

   (**Note**) Avoid vortexing! It may cause shearing of genomic DNA. Just invert gently.

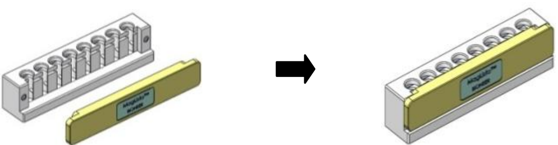
3. (Neutralization) Add 200 μl (mini)/ 2 ml (midi)/ 6 ml (maxi)/ 100 μl (8-channel) of Buffer ③ to the tube and immediately mix by inverting the tube 3~4 times gently.

   (**Note**) Genomic DNA and cell debris will form an insoluble complex with Nano Beads in this step. Again, be cautious not to shear genomic DNA.

4. (Debris removal: 4-6) Place the tube in *MagListo*™-2 (mini)/ *MagListo*™-15 (midi)/ *MagListo*™-50 (maxi)/ *MagListo*™-8Ch (8-channel) rack with the magnet plate attached and invert the tube 3~4 times gently until the beads tightly bind to magnet.

   (Optional) For better yield in mini scale, centrifuge the tube at >16,600 x g for 1 min in a micro-centrifuge. Transfer cleared lysate to a new 1.5 ml or 2 ml (mini) tube and go step 7.

- Attachment



Combine the magnet plate to the stand.

5. Without removing the tube from *MagListo*™ rack, carefully pour the supernatant out to a new 2 ml (or 1.5 ml) (mini)/ 15 ml (midi)/ 50 ml (maxi)/ 1 ml (8-channel) tube.

6. Repeat the above step 4 and 5 for clearer supernatant. We strongly recommend the repetition for better results.

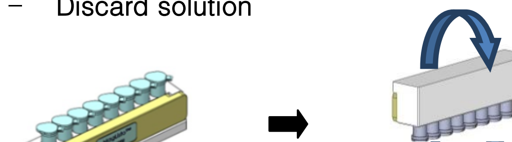
7. (Plasmid binding with Magnetic Nano Bead: 7-9) Add 600 µl (mini)/ 6 ml (midi)/ 18 ml (maxi)/ 300 µl (8-channel) of Buffer ④ and then 50 µl (mini)/ 300 µl (midi)/ 1 ml (maxi)/ 25 µl (8-channel) of evenly mixed Magnetic Nano Bead solution to the tube. Close the cap and mix by inverting the tube 3~4 times with the magnet plate detached.

   (Note) Magnetic Nano Bead solution contains magnetic nano beads. Please shake well before use.

8. Place the tube in *MagListo*™ rack with the magnet plate attached and invert the tube 3~4 times gently until the beads tightly bind to magnet.

9. Without removing the tube from *MagListo*™ rack, pour the supernatant out and completely remove the remaining supernatant using paper towel by blotting.

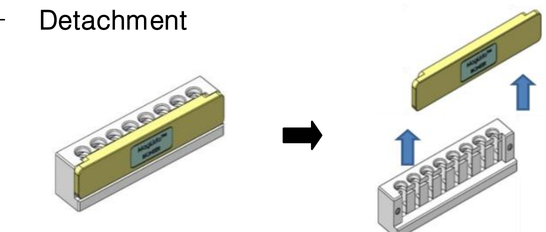
- Discard solution



Discard solution by inverting the *MagListo*™ rack. **The silicone immobilizer** inside the stand holds the tubes from falling.in an upside down position. When discard solution, invert the rack completely for the solution not to smear on the rack.

10. (Washing: 10–13) Detach the magnet plate from *MagListo*™ rack. Add 1 ml (mini)/ 10 ml (midi)/ 30 ml (maxi)/ 500 μl (8-channel) of 80% ethanol to the tube. Close the cap and mix by inverting until the beads are fully resuspended.

– Detachment



Push up the magnet plate gently.

11. Attach the magnet plate to *MagListo*™ rack and invert the tube 3~4 times gently until the beads tightly bind to magnet.

12. Without removing the tube from *MagListo*™ rack, pour the supernatant out and completely remove the remaining supernatant using paper towel by blotting.

13. Repeat the above step 13 ~ 15 by adding 1 ml (mini)/ 10 ml (midi)/ 30 ml (maxi)/ 500 μl (8-channel) of 80% ethanol for additional washing.

14. (Drying) Completely dry the beads with the tube open and the rack lying down in a dry oven at 60℃ for 10 minutes.

    (Note) For a faster procedure, use a heat gun or a blow dryer for 1 min (mini, 8-channel)/ 3 min (midi)/ 5 min (maxi) 3 cm away from the top of the tube.

15. (Elution: 15–19) Add 100 μl (mini, 8-channel)/ 500 μl (midi)/ 1 ml (maxi) of Buffer ⑤ (or distilled water) to the tube with the magnet plate detached and resuspend by tapping the tube wall.

16. Incubate the tube at 60℃ for 1 min.

17. Attach the magnet plate to *MagListo*™ rack and invert the tube 3~4 times gently until the beads tightly bind to magnet.

18. Without removing the tube from *MagListo*™ rack, carefully take the supernatant containing plasmid to a autoclaved microcentrifuge tube.

19. Discard the used Magnetic Nano Beads. Do not reuse the beads.

Summary of Reagents Volume in Each Step

Step	Buffer	8-channel	Mini	Midi	Maxi	Page
Sample collection	Culture volume	1~1.5 ml	1~10 ml	20~50 ml	100~200 ml	P. 6
Resuspension	Buffer ①	100 µl	200 µl	2 ml	6 ml	P. 8
Lysis	Buffer ②	100 µl	200 µl	2 ml	6 ml	P. 8
Neutralization	Buffer ③	100 µl	200 µl	2 ml	6 ml	P. 8
Plasmid Binding	Buffer ④	300 µl	600 µl	6 ml	18 ml	P. 9
	Magnetic Nano Bead	25 µl	50 µl	300 µl	1 ml	P. 9
Washing	80% Ethanol	500 µl	1 ml	10 ml	30 ml	P. 10
Elution	Buffer ⑤	100 µl	100 µl	500 µl	1 ml	P. 10

XIII. Troubleshooting guide

	Comments and suggestions
Low or no yield	Buffers or other reagents may have been exposed to conditions that reduce their effectiveness. Ensure that the reagents were stored at room temperature at all the times upon arrival and all reagent bottles were closed tightly after use to preserve pH and stability, and to avoid contamination.
	To obtain maximum yield for mini scale, we recommend centrifuge the tube at debris removal step. Refer to step 4 (optional) in page 8 for detail.
	The cells may not have been completely resuspended with Buffer ①. Incomplete resuspension decreases the efficiency of lysis.
Contamination of chromosomal DNA	During neutralization step, it is important that you do not vortex or shake sample tubes vigorously. Also, total lysis reaction should not be longer than 5 min. These conditions can shear chromosomal DNA. Handle the lysate gently!
Sample floating upon loading in an agarose gel	Floating is caused by leftover ethanol in samples. You must always completely remove the remaining supernatant and dry sample tubes in drying step.
Too many background bands in sequencing analysis	Did you check the endonuclease activity of your strain of host *E. coli*? HB101, JM series and normal wild-type hosts that have high endonuclease activity interrupt sequencing reaction by degrading plasmids. We recommend using EndA- strain instead of EndA+ strain.
Sample containing RNA	RNase A activity is fragile. If it has been over 6 months since adding RNase A powder to Buffer ①, the RNase A may not work properly. Add more RNase A powder, up to 100 ng/μl.

XIV. Ordering Information

Cat no.	Product Description	Size
K-3601SM	*MagListo*™ 5M Plamid Extraction Kit, 8 reactions (mini)	1 kit
K-3601	*MagListo*™ 5M Plamid Extraction Kit, 100 reactions (mini)	1 kit
K-3600	*MagListo*™ 5M Plamid Extraction Kit, 500 reactions (mini)	1 kit
K-3602	*MagListo*™ 5M Genomic DNA Extraction Kit, 8 reactions (mini)	1 kit
K-3603	*MagListo*™ 5M Genomic DNA Extraction Kit, 100 reactions (mini)	1 kit
K-3604	*MagListo*™ 5M Plant Genomic DNA Extraction Kit, 8 reactions (mini)	1 kit
K-3605	*MagListo*™ 5M Plant Genomic DNA Extraction Kit, 100 reactions (mini)	1 kit
K-3606	*MagListo*™ 5M Gel Extraction Kit, 8 reactions (mini)	1 kit
K-3607	*MagListo*™ 5M Gel Extraction Kit, 100 reactions (mini)	1 kit
K-3608	*MagListo*™ 5M PCR Purification Kit, 8 reactions (mini)	1 kit
K-3609	*MagListo*™ 5M PCR Purification Kit, 100 reactions (mini)	1 kit
K-3610	*MagListo*™ 5M Cell Total RNA Extraction Kit, 8 reactions (mini)	1 kit
K-3611	*MagListo*™ 5M Cell Total RNA Extraction Kit, 100 reactions (mini)	1 kit
K-3614	*MagListo*™ 5M Forensic Sample DNA Extraction Kit, 8 reactions (mini)	1 kit
K-3615	*MagListo*™ 5M Forensic Sample DNA Extraction Kit, 100 reactions (mini)	1 kit
TM-1000	*MagListo*™-8Ch Magnetic Separation Rack	1 ml tube x 8 holes
TM-1010	*MagListo*™-2 Magnetic Separation Rack	2 ml tube x 8 holes
TM-1020	*MagListo*™-15 Magnetic Separation Rack	15 ml tube x 6 holes
TM-1030	*MagListo*™-50 Magnetic Separation Rack	50 ml tube x 3 holes
TM-1040	*MagListo*™-96 Magnetic Separation Rack	96-well plate 1ea
TM-1100	*MagListo*™ Magnetic Separation Rack Bundle Set	*MagListo*™-2,-15,-50, and -96 (4 racks, 1 each)
K-3601-A	Blow Dryer	1 ea
HT-15-NG	1.5 ml microcentrifuge tube	500 ea / pk
HT-20-NG	2 ml microcentrifuge tube	500 ea / pk

XI. Explanation Symbols

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
REF	Catalog Number	Σ	Contains sufficient for (n) tests		USE BY	i	Consult Instruction For Use
LOT	Batch code		Caution, consult accompanying documents		Temperature Limitation	IVD	In Vitro Diagnostics Medical Device
	Manufacturer		Caution, Potential Biohazard		DO NOT REUSE	EC REP	Authorized Representative in the European Community

Bioneer Worldwide

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