

AxyPrep Multisource Genomic DNA Miniprep Kit

*For the purification of genomic DNA from animal tissues,
plant tissue, cultured cells and yeast*

Kit contents, storage and stability

Cat. No.	AP-MN-MS-GDNA-50	AP-MN-MS-GDNA-250
Kit size	50 preps	250 preps
AxyPrep column	50	250
RNase A	70 µl	350 µl
Spin-filter	50	250
2 ml microfuge tube	150	750
1.5 ml microfuge tube	50	250
Buffer G-A	45 ml	2 × 112 ml
Buffer G-B	24 ml	120 ml
Buffer DV (empty)	1	1
Buffer DV-A	5 ml	10 ml
Buffer BV	24 ml	120 ml
Buffer W1	28 ml	135 ml
Buffer W2 concentrate	24 ml	2 × 72 ml
Eluent	12 ml	60 ml
Protocol Manual	1	1

Except for the RNase A, all buffers are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature. To preserve RNase activity, the RNase A is suspended in a solution containing a high concentration of ammonium sulfate. On occasion, a precipitate may form. If this occurs, the precipitate is easily dissolved in Buffer S1 and the RNase activity is unaffected.

RNase A: 50 mg/ml. Store at room temperature.

Buffer G-A: Lysis buffer. Store at room temperature.

Buffer G-B: Protein-removal buffer. Store at room temperature.

Buffer DV-A: Buffer DV Additive. Used for preparation of Buffer DV (refer to Preparation before experiment on page 2 for details). Store at room temperature.

Buffer DV: Phase-partition buffer. Store at room temperature.

Buffer BV: DNA Binding buffer. Store at room temperature.

Buffer W1: Wash buffer. Store at room temperature.

Buffer W2 concentrate: Desalting buffer. Before using the kit, add the amount of ethanol specified on the bottle label to the Buffer W2 concentrate. Either 100% or 95% denatured ethanol can be used. Store at room temperature.

Eluent: 2.5 mM Tris-HCl, pH 8.5. Store at room temperature.

Introduction

The AxyPrep Multisource Genomic DNA Miniprep Kit is designed to purify genomic DNA from animal and plant tissues, cultured cells and yeast. This system employs a special lysis buffer, G-A to efficiently release genomic DNA from the biologic starting material. Proteins, pigments, carbohydrates and lipids are then efficiently segregated from the genomic DNA by a unique two-phase partition. The lower phase is aspirated off and free genomic DNA is bound to an AxyPrep Genomic DNA column, where residual impurities and salt are removed. The purified DNA is then eluted in either a Tris buffer or deionized water. Genomic DNA prepared by this method is approximately 30 Kb in length and is suitable for a variety of applications, such as PCR amplification, Southern blot analysis, RAPD, AFLP and RFLP, etc. Each AxyPrep column will bind and purify up to 20 µg of genomic DNA.

For the purification of genomic DNA from whole blood, we recommend the AxyPrep Whole Blood Genomic DNA Kits: Mini #AP-MN-BL-50/250; Midi (#AP-MD-BL-GDNA-10 or -25); (Maxi #AP-MX-BL-GDNA-10 or -25). For the purification of bacterial genomic DNA, please refer to the AxyPrep Bacterial Genomic DNA Miniprep Kit (#AP-MN-BT-GDNA/50 or -250).

Caution

Buffer G-A, Buffer G-B, Buffer BV and Buffer W1 contain chemical irritants. When working with the buffers, always wear suitable protective clothing such as safety glasses, laboratory coat and gloves. Be careful and avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek for medical assistance.

Equipment and consumables required

- Microcentrifuge capable of 12,000 × g
- Mortar and pestle
- Heated water bath
- Vacuum manifold (#AP-VAC)
- Vacuum regulator
- Vacuum source (-25-30 inches Hg required)
- Isobutanol and isopropanol.

Preparation before experiment

- 1) Before using the kit, add the amount of ethanol specified on the Buffer W2 label and mix well. Either 100% or 95% (denatured) ethanol can be used.

- 2) Prepare Buffer DV: Add 2 ml of Buffer DV-A, 125 ml of isopropanol and 75 ml of isobutanol to the 250 ml bottle provided with kit and mix well.
- 3) Chill Buffer DV at 4°C before proceeding.
- 4) Adjust a water bath to 65°C.
- 5) Check Buffer G-A and Buffer G-B for precipitation before each use. If precipitation occurs, incubate at 65°C to dissolve the precipitate.
- 6) Pre-warming the Eluent at 65°C will improve elution efficiency.

Protocols

- I. Purification of Genomic DNA from Animal Tissues
- II. Purification of Genomic DNA from Plant Tissues
- III. Purification of Genomic DNA from Cultured Cells
- IV. Purification of Genomic DNA from Yeast

Each type of starting material has different requirements for the method(s) used to achieve efficient lysis/homogenization. This necessitates that the protocol be organized into groups of steps, with each group associated with a particular type of function or process within the overall protocol. Before starting the protocol, carefully read the entire procedure, including the various “Notes” in each step. Determine the correct protocol path in advance and prepare all required reagents, buffers and equipments.

Groups:

- Steps 1-3 Lysis and homogenization of sample
- Steps 4-5 Phase-partitioning to remove proteins and other impurities
- Steps 6-7 Spin-filter clarification of the aqueous phase
- Steps 8-12 Binding, washing and elution on the AxyPrep column
 - A. Vacuum procedure
 - B. Centrifuge procedure

I. Purification of Genomic DNA from Animal Tissues

[Animal Tissue vacuum protocol]

This procedure requires the use of Axygen's vacuum manifold or other manifold with complimentary luer fittings which can accommodate the AxyPrep columns. The use of a vacuum regulator is also recommended. A negative pressure of -25-30 inches Hg is required. This is equivalent to approximately -850-1,000 mbar and -12-15 psi.

Lysis and homogenization of sample

Using a mortar and pestle for homogenization

1. Select 1-20 mg of tissue from animal or human and transfer to a mortar, pre-chilled on ice. Grind rapidly and vigorously to form a homogenate.

Note: the following tissue-types should be completely frozen in liquid nitrogen before grinding:

- DNase-rich tissues, such as pancreas, thymus, lymphoid tissue, etc.
- Collagen-rich tissues, such as skin, connective tissue, etc.

- Keratoprotein-rich tissues or hard tissues, such as bone.

2. Add 650 μ l of Buffer G-A and 0.9 μ l of RNase A. Gently grind for 30 seconds to homogenously mix the Buffer G-A with the ground tissue.

Note: For those tissue types in Step 2A (above) requiring freezing in liquid nitrogen, please perform the following steps after pulverization: Add 650 μ l of Buffer G-A and 0.9 μ l of RNase A. Warm the mortar to 65°C in water bath until the Buffer G-A just melts. Gently grind for 1 minute. Then proceed to Step 3, below.

3. Collect 650 μ l of the homogenate and transfer to a 2 ml microfuge tube (provided). If the volume of the homogenate is less than 650 μ l, make it up to 650 μ l with Buffer G-A. Incubate for 5 min at 65°C in a water bath.

Phase-partition to removal of protein and other impurities

4. Add 400 μ l of Buffer G-B and 1 ml of Buffer DV (pre-chilled to 4°C) in that order. Mix vigorously and centrifuge at 12,000 \times g for 2 minutes.

Note: Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.

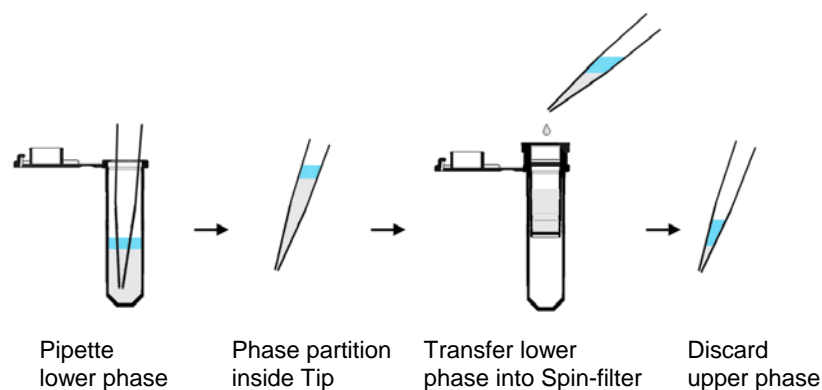
5. Discard the upper-phase as much as possible, keep the interface precipitate and the lower-phase in the tube. Add 1 ml of Buffer DV pre-chilled at 4°C, mix well and centrifuge at 12,000 \times g for 2 minutes.

Spin-filter clarification of the aqueous phase

6. Discard the upper phase, transfer the lower phase to a Spin-filter (placed in a 2 ml microfuge tube), and centrifuge at 12,000 \times g for 1 minute.

Note: When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to figure below).

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.



Note: Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 8, below).

7. Discard the spin-filter. Add 400 μ l of Buffer BV to the flow-through and mix well by brisk, repeated inversion.

Binding, washing and elution on the AxyPrep column

8. Attach the vacuum manifold base to a vacuum source. Firmly position the AxyPrep column(s) into the complimentary fittings on the manifold top. Transfer the binding mix from Step 7 to the AxyPrep column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the AxyPrep column.
9. Add 500 μ l of Buffer W1 and draw all of the solution through the column.
10. Add 700 μ l of Buffer W2 along the wall of AxyPrep column to wash off residual Buffer W1 and draw all of the solution through the column. Repeat this wash with a second 700 μ l aliquot of Buffer W2.
Note: Make sure that ethanol has been added into Buffer W2 concentrate.
Note: Add Buffer W2 along the tube wall to wash off any residual salt.
Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.
11. Transfer the AxyPrep column to a 2 ml microfuge tube and centrifuge at 12,000 \times g for 1 minute.
12. Transfer the AxyPrep column into a clean 1.5 ml microfuge tube. To elute the genomic DNA, add 100-200 μ l of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000 \times g for 1 minute.
Note: Pre-warming water or Eluent at 65°C will often improve elution efficiency.

[Animal Tissue spin protocol]

Lysis and homogenization of sample

Using a mortar and pestle for homogenization

1. Select 1-20 mg of tissue from animal or human and transfer to a mortar, pre-chilled on ice. Grind rapidly and vigorously to form a homogenate.
Note: the following tissue-types should be completely frozen in liquid nitrogen before grinding:
 - DNase-rich tissues, such as pancreas, thymus, lymphoid tissue, etc.
 - Collagen-rich tissues, such as skin, connective tissue, etc.
 - Keratoprotein-rich tissues or hard tissues, such as bone.
2. Add 650 μ l of Buffer G-A and 0.9 μ l of RNase A. Gently grind for 30 seconds to homogenously mix the Buffer G-A with the ground tissue.
Note: For those tissue types in Step 2A (above) requiring freezing in liquid nitrogen, please perform the following steps after pulverization: Add 650 μ l of Buffer G-A and 0.9 μ l of RNase A. Warm the mortar to 65°C in water bath until the Buffer G-A just melts. Gently grind for 1 minute. Then proceed to Step 3, below.
3. Collect 650 μ l of the homogenate and transfer to a 2 ml microfuge tube (provided). If the volume of the homogenate is less than 650 μ l, make it up to 650 μ l with Buffer G-A. Incubate for 5 min at 65°C in a water bath.

Phase-partition to removal of protein and other impurities

4. Add 400 μ l of Buffer G-B and 1 ml of Buffer DV (pre-chilled to 4°C) in that order. Mix vigorously and centrifuge at 12,000 \times g for 2 minutes.

Note: Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.

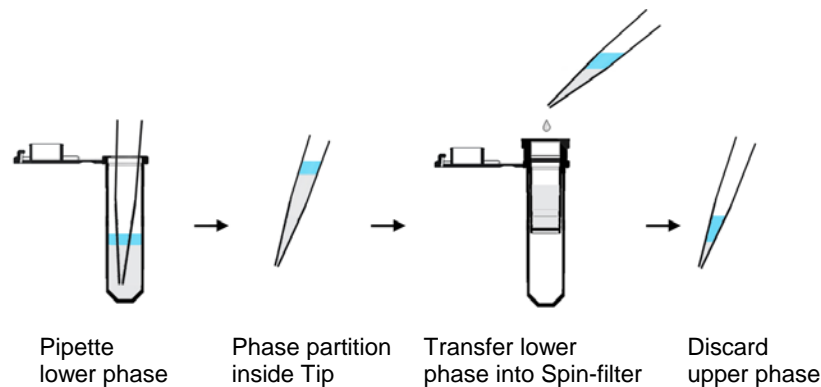
5. Discard the upper-phase as much as possible, keep the interface precipitate and the lower-phase in the tube. Add 1 ml of Buffer DV pre-chilled at 4°C, mix well and centrifuge at 12,000 \times g for 2 minutes.

Spin-filter clarification of the aqueous phase

6. Discard the upper phase, transfer the lower phase to a Spin-filter (placed in a 2 ml microfuge tube), and centrifuge at 12,000 \times g for 1 minute.

Note: When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to figure below).

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.



Note: Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 8, below).

7. Discard the spin-filter. Add 400 μ l of Buffer BV to the flow-through and mix well by brisk, repeated inversion.

Binding, washing and elution on the AxyPrep column

8. Place a AxyPrep column to a 2 ml microfuge tube (provided). Transfer the binding mix from Step 7 to the AxyPrep column. Centrifuge at 12,000 \times g for 1 minute.
9. Discard the filtrate in the 2 ml microfuge tube. Place the AxyPrep column back to the 2 ml microfuge tube. Add 500 μ l of Buffer W1 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.
10. Discard the filtrate and place the AxyPrep column back to the 2 ml microfuge tube. Add 700 μ l of Buffer W2 and centrifuge at 12,000 \times g for 1 minute.

Note: Make sure that ethanol has been added into Buffer W2 concentrate.

11. **Optional Step:** Discard the filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add 700 μ l of Buffer W2 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.

Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

12. Discard filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Centrifuge at $12,000 \times g$ for 1 minute.
13. Transfer the AxyPrep column into a clean 1.5 ml microfuge tube (provide). To elute the genomic DNA, add 100-200 μ l of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at $12,000 \times g$ for 1 minute.

Note: Pre-warming water or Eluent at 65°C will often improve elution efficiency.

II. Purification of Genomic DNA from Plant Tissues

[Plant Tissue Vacuum Protocol]

This procedure requires the use of Axygen's vacuum manifold or other manifold with complimentary luer fittings which can accommodate the AxyPrep columns. The use of a vacuum regulator is also recommended. A negative pressure of -25-30 inches Hg is required. This is equivalent to approximately -850-1,000 mbar and -12-15 psi.

Lysis and homogenization of sample

Using a mortar and pestle for homogenization

1. Using Table 1(below), weigh out the appropriate amount of fresh plant tissue (the amount should be reduced by half if lyophilized, dehydrated, or dry tissues are used) and transfer to the mortar. Carefully add liquid nitrogen directly to the sample until it is completely frozen. Use the pestle to pulverize it quickly and vigorously until it is reduced to a fine powder.

Table 1. Types of fresh plant tissues used for genomic DNA preparation

Flower or leaves	10-100 mg
Plant stem	≤ 240 mg
Plant root	≤ 240 mg
Plant seed	≤ 240 mg

Note: If cultured plant cells are used, collect $2 \times 10^3 \sim 1 \times 10^7$ plant cells and spin for 1 minute at $10,000 \times g$ to pellet the cells. Resuspend the plant cells in 150 μ l of deionized water and transfer to the mortar. Carefully add liquid nitrogen directly to the sample until it is completely frozen. Use the pestle to pulverize it quickly and vigorously until it is reduced to a fine powder. Add liquid nitrogen as required to prevent the material from thawing during pulverization. After pulverization, warm the mortar at 65°C in a water bath until the pulverized material just melts. Proceed to Step 2, below.

2. Add 700 μ l of Buffer G-A and 1.2 μ l of RNase A. Quickly grind the sample for 30 seconds.

Note: Incomplete grinding will reduce the yield of genomic DNA.

Note: When the weight of the fresh plant tissue is >120 mg or the dried plant tissue is >60 mg, add 1.3 ml of Buffer G-A. After Step 2 has been completed, divide the sample evenly between two 2 ml microfuge tubes. Steps 4 -7 will proceed in two parallel 2 ml microfuge tubes. In Step 8, the contents of the two tubes will be consolidated into a single AxyPrep column.

3. Transfer the tissue homogenate into a 2 ml microfuge tube. Determine the approximate volume. If the volume of the homogenate is less than 650 μ l, add additional Buffer G-A up to 650 μ l. Incubate it for 15 minutes at 65°C in a water bath.

Note: If fibrous samples such as plant stem and root, or starch- and protein-rich samples such as seeds are used, increase the incubation time to 60 minutes in the water bath.

Phase-partition to removal of protein and other impurities

4. Add 400 μ l of Buffer G-B and 1 ml of Buffer DV (pre-chilled to 4°C) in that order. Mix vigorously and centrifuge at 12,000 \times g for 2 minutes.

Note: Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.

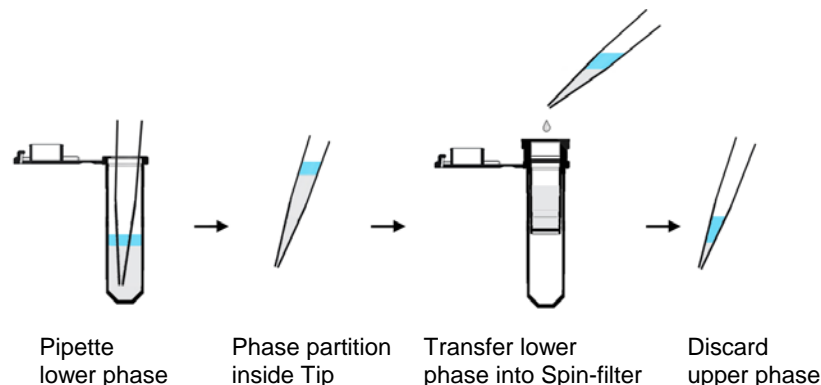
5. Discard the upper-phase as much as possible, keep the interface precipitate and the lower-phase in the tube. Add 1 ml of Buffer DV pre-chilled at 4°C, mix well and centrifuge at 12,000 \times g for 2 minutes.

Spin-filter clarification of the aqueous phase

6. Discard the upper phase, transfer the lower phase to a Spin-filter (placed in a 2 ml microfuge tube), and centrifuge at 12,000 \times g for 1 minute.

Note: When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to figure below).

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.



Note: Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 8, below).

7. Discard the spin-filter. Add 400 μ l of Buffer BV to the flow-through and mix well by brisk, repeated inversion.

Binding, washing and elution on the AxyPrep column

8. Attach the vacuum manifold base to a vacuum source. Firmly position the AxyPrep column(s) into the complimentary fittings on the manifold top. Transfer the binding mix from Step 7 to the AxyPrep column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the AxyPrep column.

9. Add 500 μ l of Buffer W1. Draw all of the solution through the column.
10. Add 700 μ l of Buffer W2 along the wall of AxyPrep column to wash off residual Buffer W1, draw all of the solution through the column. Repeat this wash with a second 700 μ l aliquot of Buffer W2.
 - Note:** Make sure that ethanol has been added into Buffer W2 concentrate.
 - Note:** Add Buffer W2 along the tube wall to wash off any residual salt.
 - Note:** Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.
11. Transfer the AxyPrep column to a 2 ml microfuge tube (provided) and centrifuge at 12,000 \times g for 1 minute.
12. Transfer the AxyPrep column into a clean 1.5 ml microfuge tube (provided). To elute the genomic DNA, add 100-200 μ l of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000 \times g for 1 minute.
 - Note:** Pre-warming water or Eluent at 65°C will often improve elution efficiency.

[Plant Tissue Spin Protocol]

Lysis and homogenization of sample

Using a mortar and pestle for homogenization

1. Using Table 1 (below), weigh out the appropriate amount of fresh plant tissue (the amount should be reduced by half if lyophilized, dehydrated, or dry tissues are used) and transfer to the mortar. Carefully add liquid nitrogen directly to the sample until it is completely frozen. Use the pestle to pulverize it quickly and vigorously until it is reduced to a fine powder.

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Flower or leaves	10-100 mg
Plant stem	\leq 240 mg
Plant root	\leq 240 mg
Plant seed	\leq 240 mg

Note: If cultured plant cells are used, collect $2 \times 10^3 \sim 1 \times 10^7$ plant cells and spin for 1 minute at 10,000 \times g to pellet the cells. Resuspend the plant cells in 150 μ l of deionized water and transfer to the mortar. Carefully add liquid nitrogen directly to the sample until it is completely frozen. Use the pestle to pulverize it quickly and vigorously until it is reduced to a fine powder. Add liquid nitrogen as required to prevent the material from thawing during pulverization. After pulverization, warm the mortar at 65°C in a water bath until the pulverized material just melts. Proceed to Step 2, below.

2. Add 700 μ l of Buffer G-A and 1.2 μ l of RNase A. Quickly grind the sample for 30 seconds.

Note: Incomplete grinding will reduce the yield of genomic DNA.

Note: When the weight of the fresh plant tissue is >120 mg or the dried plant tissue is >60 mg, add 1.3 ml of Buffer G-A. After Step 2 has been completed, divide the sample evenly between two 2 ml microfuge tubes. Steps 4 -7 will proceed in two parallel 2 ml microfuge tubes. In Step 8, the contents of the two tubes will be consolidated into a single AxyPrep column.

3. Transfer the tissue homogenate into a 2 ml microfuge tube. Determine the approximate volume. If the volume of the homogenate is less than 650 μ l, add additional Buffer G-A up to 650 μ l. Incubate it for 15 minutes at 65°C in a water bath.

Note: If fibrous samples such as plant stem and root, or starch- and protein-rich samples such as seeds are used, increase the incubation time to 60 minutes in the water bath.

Phase-partition to removal of protein and other impurities

4. Add 400 μ l of Buffer G-B and 1 ml of Buffer DV (pre-chilled to 4°C) in that order. Mix vigorously and centrifuge at 12,000 \times g for 2 minutes.

Note: Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.

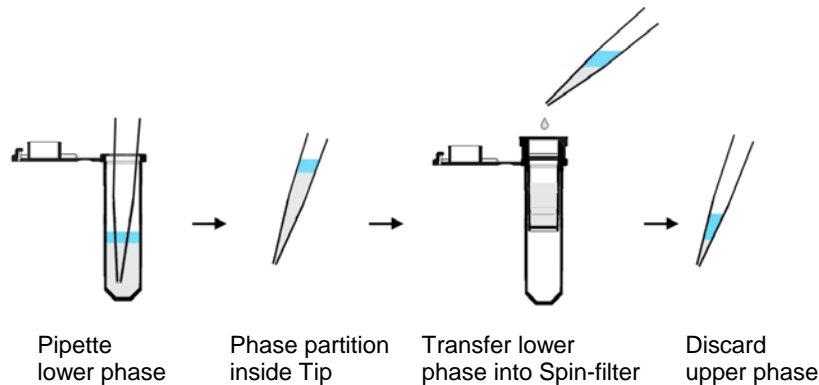
5. Discard the upper-phase as much as possible, keep the interface precipitate and the lower-phase in the tube. Add 1 ml of Buffer DV pre-chilled at 4°C, mix well and centrifuge at 12,000 \times g for 2 minutes.

Spin-filter clarification of the aqueous phase

6. Discard the upper phase, transfer the lower phase to a Spin-filter (placed in a 2 ml microfuge tube), and centrifuge at 12,000 \times g for 1 minute.

Note: When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to figure below).

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.



Note: Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 8, below).

7. Discard the spin-filter. Add 400 μ l of Buffer BV to the flow-through and mix well by brisk, repeated inversion.

Binding, washing and elution on the AxyPrep column

8. Place a AxyPrep column to a 2 ml microfuge tube (provided). Transfer the binding mix from Step 7 to the AxyPrep column. Centrifuge at 12,000 \times g for 1 minute.

9. Discard the filtrate in the 2 ml microfuge tube. Place the AxyPrep column back to the 2 ml microfuge tube. Add 500 μ l of Buffer W1 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.
10. Discard the filtrate and place the AxyPrep column back to the 2 ml microfuge tube. Add 700 μ l of Buffer W2 and centrifuge at 12,000 \times g for 1 minute.
Note: Make sure that ethanol has been added into Buffer W2 concentrate.
11. **Optional Step:** Discard the filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add 700 μ l of Buffer W2 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.
Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.
12. Discard filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Centrifuge at 12,000 \times g for 1 minute.
13. Transfer the AxyPrep column into a clean 1.5 ml microfuge tube (provide). To elute the genomic DNA, add 100-200 μ l of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000 \times g for 1 minute.
Note: Pre-warming water or Eluent at 65°C will often improve elution efficiency.

III. Purification of Genomic DNA from Cultured Animal Cells

[Cultured Animal Cells Vacuum Protocol]

This procedure requires the use of AxyGen's vacuum manifold or other manifold with complimentary luer fittings which can accommodate the AxyPrep columns. The use of a vacuum regulator is also recommended. A negative pressure of -25-30 inches Hg is required. This is equivalent to approximately -850-1,000 mbar and -12-15 psi.

Lysis and homogenization of sample

Select homogenization method A or B, depending upon the type of cultured animal cell used. If genomic DNA is extracted from plant cells, please follow the previous protocol "Purification of Genomic DNA from Plant Tissues" (above) to homogenize the plant cells.

A. Cells grown in suspension or cell suspension freshly-isolated from animal or human tissues:

- 1A. Collect 1×10^3 - 2×10^6 cells in suspension and transfer into a 2 ml microfuge tube. Centrifuge at 2,000 \times g for 5 minutes to pellet the cells. Discard the supernatant.
- 2A. Add 150 μ l of deionized water or PBS to resuspend the cells and then add 500 μ l of Buffer G-A. Let the tube stand for 1 minute at room temperature.

Proceed to Step 3, below.

B. Cells grown in a monolayer in a 96-well, 24-well, 12-well or 6-well plate:

- 1B. Discard as much of the supernatant as possible, then add 650 μ l of Buffer G-A into each well. Let the plate stand for 1 minute at room temperature.
 - 2B. Pipette up and down several times, then transfer 650 μ l of the cell homogenate into a 2 ml microfuge tube.
3. Add 0.8 μ l of RNase A. Vortex for 15 seconds and let the tube stand for 1 minute at room temperature.

Phase-partition to removal of protein and other impurities

4. Add 400 μ l of Buffer G-B and 1 ml of Buffer DV (pre-chilled to 4°C) in that order. Mix vigorously and centrifuge at 12,000 \times g for 2 minutes.

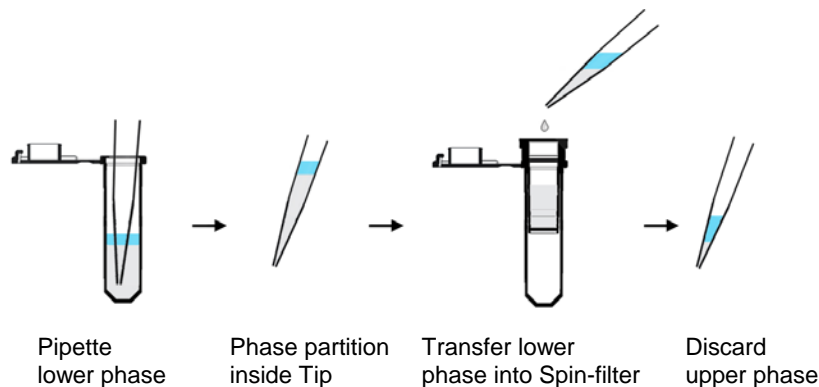
Note: Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.
5. Discard the upper-phase as much as possible, keep the interface precipitate and the lower-phase in the tube. Add 1 ml of Buffer DV pre-chilled at 4°C, mix well and centrifuge at 12,000 \times g for 2 minutes.

Spin-filter clarification of the aqueous phase

6. Discard the upper phase, transfer the lower phase to a Spin-filter (placed in a 2 ml microfuge tube), and centrifuge at 12,000 \times g for 1 minute.

Note: When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to figure below).

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.



Note: Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 8, below).

7. Discard the spin-filter. Add 400 μ l of Buffer BV to the flow-through and mix well by brisk, repeated inversion.

Binding, washing and elution on the AxyPrep column

8. Attach the vacuum manifold base to a vacuum source. Firmly position the AxyPrep column(s) into the complimentary fittings on the manifold top. Transfer the binding mix from Step 7 to the

AxyPrep column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the AxyPrep column.

9. Add 500 μ l of Buffer W1. Draw all of the solution through the column.
10. Add 700 μ l of Buffer W2 along the wall of AxyPrep column to wash off residual Buffer W1, draw all of the solution through the column. Repeat this wash with a second 700 μ l aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate.

Note: Add Buffer W2 along the tube wall to wash off any residual salt.

Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

11. Transfer the AxyPrep column to a 2 ml microfuge tube (provided) and centrifuge at $12,000 \times g$ for 1 minute.
12. Transfer the AxyPrep column into a clean 1.5 ml microfuge tube. To elute the genomic DNA, add 100-200 μ l of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at $12,000 \times g$ for 1 minute.

Note: Pre-warming water or Eluent at 65°C will often improve elution efficiency.

[Cultured Animal Cells Spin Protocol]

Lysis and homogenization of sample

Select homogenization method A or B, depending upon the type of cultured animal cell used. If genomic DNA is extracted from plant cells, please follow the previous protocol "Purification of Genomic DNA from Plant Tissues" (above) to homogenize the plant cells.

A. Cells grown in suspension or cell suspension freshly-isolated from animal or human tissues:

- 1A. Collect 1×10^3 - 2×10^6 cells in suspension and transfer into a 2 ml microfuge tube. Centrifuge at $2,000 \times g$ for 5 minutes to pellet the cells. Discard the supernatant.
- 2A. Add 150 μ l of deionized water or PBS to resuspend the cells and then add 500 μ l of Buffer G-A. Let the tube stand for 1 minute at room temperature.

Proceed to Step 3, below.

B. Cells grown in a monolayer in a 96-well, 24-well, 12-well or 6-well plate:

- 1B. Discard as much of the supernatant as possible, then add 650 μ l of Buffer G-A into each well. Let the plate stand for 1 minute at room temperature.
- 2B. Pipette up and down several times, then transfer 650 μ l of the cell homogenate into a 2 ml microfuge tube.

3. Add 0.8 μ l of RNase A. Vortex for 15 seconds and let the tube stand for 1 minute at room temperature.

Phase-partition to removal of protein and other impurities

4. Add 400 μ l of Buffer G-B and 1 ml of Buffer DV (pre-chilled to 4°C) in that order. Mix vigorously and centrifuge at 12,000 \times g for 2 minutes.

Note: Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.

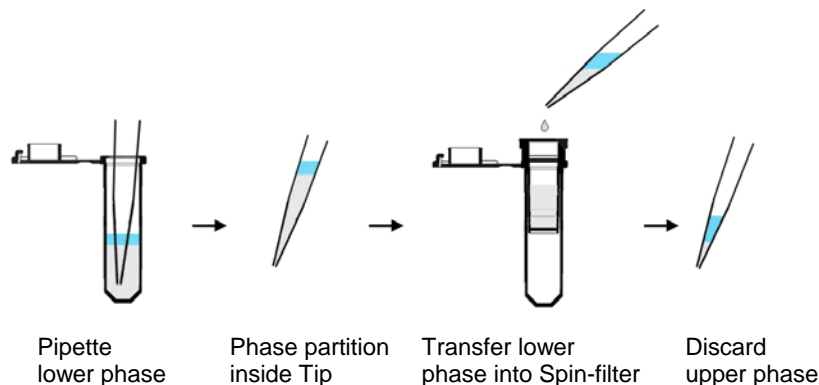
5. Discard the upper-phase as much as possible, keep the interface precipitate and the lower-phase in the tube. Add 1 ml of Buffer DV pre-chilled at 4°C, mix well and centrifuge at 12,000 \times g for 2 minutes.

Spin-filter clarification of the aqueous phase

6. Discard the upper phase, transfer the lower phase to a Spin-filter (placed in a 2 ml microfuge tube), and centrifuge at 12,000 \times g for 1 minute.

Note: When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to figure below).

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.



Note: Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 8, below).

7. Discard the spin-filter. Add 400 μ l of Buffer BV to the flow-through and mix well by brisk, repeated inversion.

Binding, washing and elution on the AxyPrep column

8. Place a AxyPrep column to a 2 ml microfuge tube (provided). Transfer the binding mix from Step 7 to the AxyPrep column. Centrifuge at 12,000 \times g for 1 minute.

9. Discard the filtrate in the 2 ml microfuge tube. Place the AxyPrep column back to the 2 ml microfuge tube. Add 500 μ l of Buffer W1 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.

10. Discard the filtrate and place the AxyPrep column back to the 2 ml microfuge tube. Add 700 μ l of Buffer W2 and centrifuge at 12,000 \times g for 1 minute.

Note: Make sure that ethanol has been added into Buffer W2 concentrate.

11. Optional Step: Discard the filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add 700 μ l of Buffer W2 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.

Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

12. Discard filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Centrifuge at $12,000 \times g$ for 1 minute.
13. Transfer the AxyPrep column into a clean 1.5 ml microfuge tube (provide). To elute the genomic DNA, add 100-200 μ l of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at $12,000 \times g$ for 1 minute.

Note: Pre-warming water or Eluent at 65°C will often improve elution efficiency.

IV. Purification of Genomic DNA from Yeast

[Yeast Vacuum Protocol]

This procedure requires the use of AxyGen's vacuum manifold or other manifold with complimentary luer fittings which can accommodate the AxyPrep columns. The use of a vacuum regulator is also recommended. A negative pressure of -25-30 inches Hg is required. This is equivalent to approximately -850-1,000 mbar and -12-15 psi.

Lysis and homogenization of sample

Using a mortar and pestle for homogenization

1. Collect 2×10^6 - 5×10^7 yeast cells and centrifuge for 1 minute at $10,000 \times g$ to pellet the cells. Resuspend the yeast cells in 150 μ l of water and transfer to a mortar.

Note: For yeast, an OD_{600} of 1 $\cong 3 \times 10^7$ cells/ml.

2. Gradually add liquid nitrogen until the yeast suspension is completely frozen. Using the pestle, quickly and forcefully reduce it to a fine powder. Add liquid nitrogen to prevent the sample from thawing during pulverization. After grinding is complete, warm the mortar at 65°C in water bath until it just begins to melt.
3. Add 600 μ l of Buffer G-A and 1.2 μ l of RNase A. Quickly grind the sample for 30 seconds. Transfer 650 μ l of the yeast homogenate into a 2 ml microfuge tube. If the volume of the homogenate is less than 650 μ l, add additional Buffer G-A up to 650 μ l. Incubate it for 10 minutes at 65°C in a water bath.

Proceed to Step 4 (below)

Phase-partition to removal of protein and other impurities

4. Add 400 μ l of Buffer G-B and 1 ml of Buffer DV (pre-chilled to 4°C) in that order. Mix vigorously and centrifuge at $12,000 \times g$ for 2 minutes.

Note: Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.

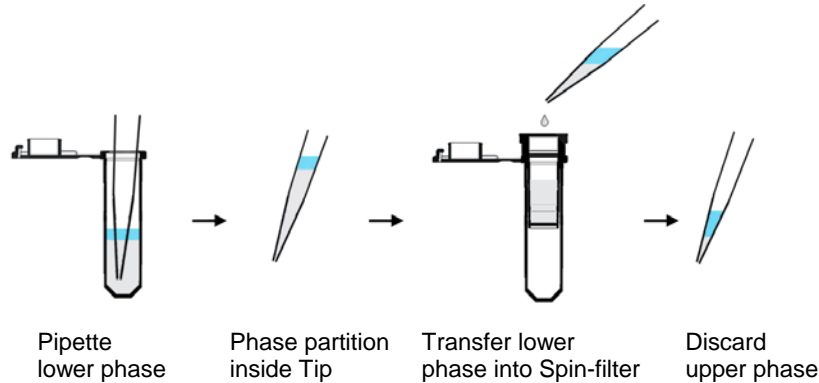
5. Discard the upper-phase as much as possible, keep the interface precipitate and the lower-phase in the tube. Add 1 ml of Buffer DV pre-chilled at 4°C, mix well and centrifuge at $12,000 \times g$ for 2 minutes.

Spin-filter clarification of the aqueous phase

6. Discard the upper phase, transfer the lower phase to a Spin-filter (placed in a 2 ml microfuge tube), and centrifuge at $12,000 \times g$ for 1 minute.

Note: When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to figure below).

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.



Note: Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 8, below).

7. Discard the spin-filter. Add 400 μl of Buffer BV to the flow-through and mix well by brisk, repeated inversion.

Binding, washing and elution on the AxyPrep column

8. Attach the vacuum manifold base to a vacuum source. Firmly position the AxyPrep column(s) into the complimentary fittings on the manifold top. Transfer the binding mix from Step 7 to the AxyPrep column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the AxyPrep column.

9. Add 500 μl of Buffer W1. Draw all of the solution through the column.

10. Add 700 μl of Buffer W2 along the wall of AxyPrep column to wash off residual Buffer W1, draw all of the solution through the column. Repeat this wash with a second 700 μl aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate.

Note: Add Buffer W2 along the tube wall to wash off any residual salt.

Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

11. Transfer the AxyPrep column to a 2 ml microfuge tube (provided) and centrifuge at $12,000 \times g$ for 1 minute.

12. Transfer the AxyPrep column into a clean 1.5 ml microfuge tube. To elute the genomic DNA, add 100-200 μl of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at $12,000 \times g$ for 1 minute.

Note: Pre-warming water or Eluent at 65°C will often improve elution efficiency.

[Yeast Spin Protocol]

Lysis and homogenization of sample

Using a mortar and pestle for homogenization

1. Collect 2×10^6 - 5×10^7 yeast cells and centrifuge for 1 minute at $10,000 \times g$ to pellet the cells. Resuspend the yeast cells in 150 μ l of water and transfer to a mortar.

Note: For yeast, an OD_{600} of 1 $\cong 3 \times 10^7$ cells/ml.

2. Gradually add liquid nitrogen until the yeast suspension is completely frozen. Using the pestle, quickly and forcefully reduce it to a fine powder. Add liquid nitrogen to prevent the sample from thawing during pulverization. After grinding is complete, warm the mortar at 65°C in water bath until it just begins to melt.

3. Add 600 μ l of Buffer G-A and 1.2 μ l of RNase A. Quickly grind the sample for 30 seconds. Transfer 650 μ l of the yeast homogenate into a 2 ml microfuge tube. If the volume of the homogenate is less than 650 μ l, add additional Buffer G-A up to 650 μ l. Incubate it for 10 minutes at 65°C in a water bath.

Proceed to Step 4 (below)

Phase-partition to removal of protein and other impurities

4. Add 400 μ l of Buffer G-B and 1 ml of Buffer DV (pre-chilled to 4°C) in that order. Mix vigorously and centrifuge at $12,000 \times g$ for 2 minutes.

Note: Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.

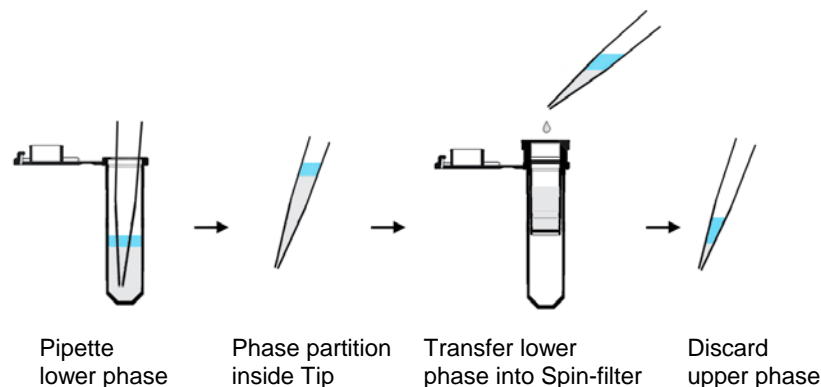
5. Discard the upper-phase as much as possible, keep the interface precipitate and the lower-phase in the tube. Add 1 ml of Buffer DV pre-chilled at 4°C, mix well and centrifuge at $12,000 \times g$ for 2 minutes.

Spin-filter clarification of the aqueous phase

6. Discard the upper phase, transfer the lower phase to a Spin-filter (placed in a 2 ml microfuge tube), and centrifuge at $12,000 \times g$ for 1 minute.

Note: When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to figure below).

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.



Note: Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 8, below).

7. Discard the spin-filter. Add 400 μ l of Buffer BV to the flow-through and mix well by brisk, repeated inversion.

Binding, washing and elution on the AxyPrep column

8. Place a AxyPrep column to a 2 ml microfuge tube (provided). Transfer the binding mix from Step 7 to the AxyPrep column. Centrifuge at 12,000 \times g for 1 minute.

9. Discard the filtrate in the 2 ml microfuge tube. Place the AxyPrep column back to the 2 ml microfuge tube. Add 500 μ l of Buffer W1 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.

10. Discard the filtrate and place the AxyPrep column back to the 2 ml microfuge tube. Add 700 μ l of Buffer W2 and centrifuge at 12,000 \times g for 1 minute.

Note: Make sure that ethanol has been added into Buffer W2 concentrate.

11. **Optional Step:** Discard the filtrate from the 2 ml Microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add 700 μ l of Buffer W2 to the AxyPrep column and centrifuge at 12,000 \times g for 1 minute.

Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

12. Discard filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Centrifuge at 12,000 \times g for 1 minute.

13. Transfer the AxyPrep column into a clean 1.5 ml microfuge tube (provide). To elute the genomic DNA, add 100-200 μ l of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000 \times g for 1 minute.

Note: Pre-warming water or Eluent at 65°C will often improve elution efficiency.

Overview

Make up to 650 μ l with Buffer G-A
Add RNase A and heat

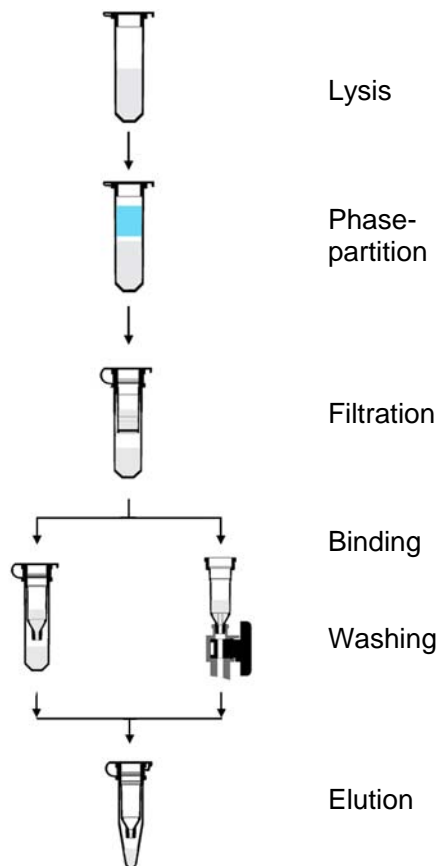
Add 400 μ l of Buffer G-B

Add 1 ml of Buffer DV
Repeat extraction with Buffer DV

Add 400 μ l of Buffer BV

Add 500 μ l of Buffer W1
Add 700 μ l of Buffer W2
Repeat wash with Buffer W2

Add 100-200 μ l of water or the
Eluent



Troubleshooting:

1. Low or no yield

- Insufficient bacteria processed
- Inefficient lysis
- Bring the upper phase liquid into the lower phase.
- DNA not efficiently eluted
- AxyPrep column membrane overdried during vacuum removal of W2

2. Low $A_{260/280}$

- Too many material processed
- Inefficient lysis
- Contamination with interphase material

3. RNA present (elevated $A_{260/280}$)

- Failure to add RNase A to Buffer S
- Buffer S dated

4. Genomic DNA appears to be degraded

Depending upon the completeness of degradation, the genomic DNA will either appear as a smear or as a smear trailing in front of a high molecular weight band on an agarose gel. Since no physical measure used during the purification process is sufficient to cause any visually discernable degradation, the most likely source is enzymatic. Many strains of bacteria exhibit high levels of endonuclease activity. These endA+ strains must be lysed rapidly and completely to prevent substantial enzymatic degradation of the intrinsic genomic DNA.

5. Genomic DNA performs poorly in enzymatic reactions

- Low DNA concentration
- Salt contamination: insufficient Buffer W1 removal
- Ethanol contamination: insufficient centrifugation to remove residual Buffer W2

6. Clogged spin-filter

- Too much material processed
- Inefficient lysis

7. Clogged AxyPrep column

- Too many bacteria processed
- Inefficient lysis