GET™ DNA Template-Mag

Genomic Efficient Technology for DNA Template based on purification with silica magnetic beads

(Cat. # 786-1725, 786-1726)

INTRODUCTION

Genomic Efficient Technology (GET) for purification of DNA templates from diverse sample types, including cells, tissues, mouse tails, plant, blood, nucleated blood cells, body fluid, fixed and embedded tissues, stained specimen, bacteria, yeast, fungal tissues, and so forth.

GET™ is based on a highly efficient Genomic lysis buffer that liberates nucleic acid from cellular protein complexes, making nucleic acids free and available for purification in pure form. Free nucleic acids, DNA templates, are immobilized, in the presence of high concentration of chaotropic agents, on silica surface. Following the capture of DNA template on the silica membrane or beads, a series of washing steps removes interfering impurities. In the final step, pure DNA template is eluted in concentrated form with elution buffer.

The GET™ DNA Template- Mag is based upon the principle of binding of nucleic acids on silica beads with magnetic core in presence of chaotropic salts. Nucleic acids are bound to the silica magnetic beads under high concentrations of chaotropic salts and the impurities are removed during the wash steps (Fig:1). The DNA extraction is rapid with less than 15-30 minutes hands- on time.

The eluted DNA template is highly pure and does not require any further processing, making it suitable for a wide variety of applications including PCR, library construction, southern blotting, SNP analysis and molecular diagnostic assays.

The kit is supplied in two sizes 50 preps (Cat. #786-1725) and 100 preps (Cat. #786-1726) based on number of samples purified for DNA.

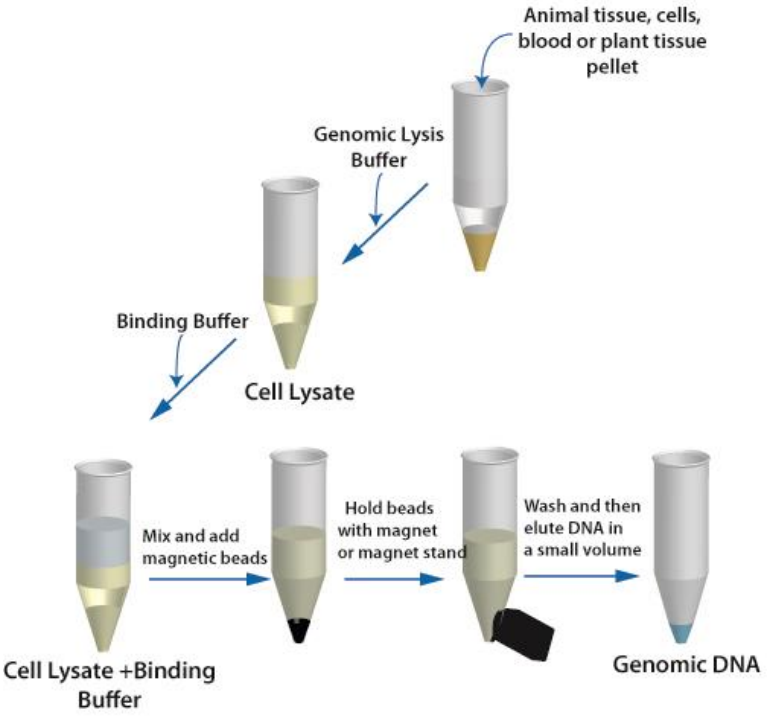

Fig.1

ITEM(S) SUPPLIED

Description	Cat. # 786-1725 50 preps	Cat. # 786-1726 100 preps
Genomic Lysis Buffer	30 ml	2 x 30 ml
GET Binding Buffer	50 ml	2 x 50 ml
Longlife™ Proteinase K	0.5 ml	0.5 ml
Silica Magnetic Beads	2.5 ml	2 x 2.5 ml
GET Wash I	30 ml	2 x 30ml
GET Wash II	20ml	2 x 20ml
GET Elution Buffer	10ml	10ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store the kit components as recommended on the label. The kit components are stable for 1 year, if stored as directed.

ADDITIONAL ITEM(S) REQUIRED

- Magnetic stand (Cat. # 786-888) or a magnet
- Absolute ethanol or molecular grade ethanol
- Nuclease-free 1.5 ml microfuge tube, nuclease free tips for pipettes
- ***Optional***: LongLife™ RNase (Cat. # 786-040)
- *Longlife*™ Lysozyme (Cat. # 786-037) for bacterial culture
- LongLife™ Zymolyase® (Cat # 786-036) for yeast culture

IMPORTANT INFORMATION

- Bring the buffers to room temperature if stored at 4°C. Check the GET Binding Buffer for any precipitate. Warm the buffer at 37°C to dissolve any precipitate.
- Thaw and keep the enzymes Longlife™ Proteinase K on ice when performing DNA isolation. Avoid repeated thawing of enzymes. Make small aliquots and store at -20°C for long term storage.

PREPARATION BEFORE USE

1. Add 18 ml of molecular grade ethanol to 30 ml GET Wash I bottle and check the box on the bottle label to indicate ethanol has been added.
2. Add 80ml molecular grade ethanol to the GET Wash II bottle (20 ml) and check the box on the bottle label to indicate ethanol has been added.
3. Equilibrate Elution Buffer to 70°C.

PROTOCOL

From Animal Tissue

1. Transfer 10-50mg tissue to a microcentrifuge tube. Add 200µl Genomic Lysis Buffer. Homogenize the tissue with a microfuge pestle.
2. Add 5 µl Longlife™ Proteinase K suspension into the sample, mix and incubate at 55°C-60°C for 1 hr.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
3. Centrifuge the tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer and vortex to mix.
4. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
5. Place the sample tube on magnetic stand or use a magnet to immobilize the beads at one end of the tube.
6. Gently remove and discard the supernatant without disturbing the beads.
7. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.

8. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
9. Gently remove and discard the supernatant without disturbing the beads.
10. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
11. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
12. Gently remove and discard the supernatant without disturbing the beads.
13. Repeat step 11-13.
14. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
15. Add 50 µl of prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by brief vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
16. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
17. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From Cultured Cells

1. Transfer 1-5x10^6 cells, suspension or trypsinized cells into a 1.5ml microfuge tube.
2. Centrifuge the cells at 1000 x g for 5 minutes. Discard the supernatant.
3. Add 200µl Genomic Lysis Buffer to the pellet. Gently pipette up and down several times to release nuclei from the cells. Transfer lysate to a clean tube.
4. Add 5 µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.
   NOTE: *Invert the Longlife™ Proteinase K tube 3-4 times to get uniform suspension before opening.*
5. Centrifuge the tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer and vortex to mix.
6. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously*
7. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
8. Gently remove and discard the supernatant without disturbing the beads.
9. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.

10. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
11. Gently remove and discard the supernatant without disturbing the beads.
12. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
13. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
14. Gently remove and discard the supernatant without disturbing the beads.
15. Repeat step 12-14.
16. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
17. Add 50 µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
18. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
19. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From paraffin embedded tissue

1. Finely chop 0.5-2mg paraffin-embedded tissue and place in 1.5ml microfuge tube with 100µl xylene or safe xylene substitute and incubate at room temperature for 5 minutes with constant mixing.
2. Centrifuge at 14,000xg for 2 minutes and discard xylene or xylene substitute. Repeat steps 1-2 twice to achieve a total of three washes.
3. Add 100µl 100% ethanol and incubate for 5 minutes at room temperature with constant mixing.
4. Centrifuge at 14,000xg for 2 minutes and discard ethanol. Repeat steps 3-4 once to achieve a total of two washes.
5. Add 100µl Genomic Lysis Buffer and homogenize the sample with a microfuge pestle until a homogenous suspension is acquired, approximately 30-60 strokes.
6. Add 2.5 µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
7. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 200 µl of GET Binding Buffer and vortex to mix.
8. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.

NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*

9. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
10. Gently remove and discard the supernatant without disturbing the beads.
11. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
12. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
13. Gently remove and discard the supernatant without disturbing the beads.
14. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
15. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
16. Gently remove and discard the supernatant without disturbing the beads.
17. Repeat step 14-16.
18. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
19. Add 50 µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
20. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
21. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From ethanol or formalin fixed tissue

1. Blot excess fixative from tissue with clean absorbent paper.
2. Transfer 10-50mg ground frozen tissue or fresh tissue to a microcentrifuge tube containing 200µl Genomic Lysis Buffer and incubate for 15 minutes at 55-65°C to soften tissue.
3. Homogenize the sample with a microfuge pestle until a homogenous suspension is acquired, approximately 30-60 strokes.
4. Add 5µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.

   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
5. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer to the sample and vortex to mix.

6. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
7. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
8. Gently remove and discard the supernatant without disturbing the beads.
9. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
10. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
11. Gently remove and discard the supernatant without disturbing the beads.
12. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
13. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
14. Gently remove and discard the supernatant without disturbing the beads.
15. Repeat step 12-14.
16. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
17. Add 50 µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
18. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
19. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From nucleated blood cells from bird, fish & frog

1. Add 10µl nucleated blood to a 1.5ml microfuge tube containing 200µl Genomic Lysis Buffer.
2. Add 5 µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
3. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer to the sample and vortex to mix.

4. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
5. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
6. Gently remove and discard the supernatant without disturbing the beads.
7. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
8. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
9. Gently remove and discard the supernatant without disturbing the beads.
10. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
11. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
12. Gently remove and discard the supernatant without disturbing the beads.
13. Repeat step 10-12.
14. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
15. Add 50µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
16. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
17. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From gram negative bacteria

1. Add 0.5 ml of an overnight culture to a 1.5ml microfuge tube.
2. Centrifuge at 16,000xg for 2-3 minutes to pellet the cells. Remove and discard the supernatant. Vortex the tube to re-suspend the cells in residual supernatant.
3. Add 200µl Genomic Lysis Buffer and mix by inverting a few times.
4. Add 5 µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*

5. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400µl of GET Binding Buffer to the sample and vortex to mix.
6. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
7. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
8. Gently remove and discard the supernatant without disturbing the beads.
9. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
10. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
11. Gently remove and discard the supernatant without disturbing the beads.
12. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
13. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
14. Gently remove and discard the supernatant without disturbing the beads.
15. Repeat step 12-14.
16. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
17. Add 50µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
18. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
19. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From gram positive bacteria

1. Aliquot 0.5ml Gram positive bacteria overnight culture into a 1.5ml microfuge tube and centrifuge at 14,000xg for 30 seconds. Discard the supernatant.
2. Add 450µl sterile water and 50µl EDTA to the pellet and gently vortex to resuspend.
3. Add 50µl *Longlife*™ Lysozyme, invert to mix and incubate at 37°C for 45 minutes with periodic mixing.

NOTE: *Before use, Invert the Longlife™ Lysozyme tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*

4. Centrifuge for 5 minutes at 14,000xg and discard the supernatant. Gently vortex the tube to resuspend the pellet in the residual liquid.
5. Add 200µl Genomic Lysis Buffer and mix by inverting the tube several times.
6. Add 5 µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.

   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use*
7. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer to the sample and vortex to mix.
8. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.

   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
9. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
10. Gently remove and discard the supernatant without disturbing the beads.
11. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
12. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
13. Gently remove and discard the supernatant without disturbing the beads.
14. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
15. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
16. Gently remove and discard the supernatant without disturbing the beads.
17. Repeat step 14- 16.
18. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
19. Add 50 µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
20. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
21. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA.

Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From plant tissue (fresh or frozen)

1. Transfer 10-50mg finely ground dried tissue, frozen tissue or fresh leave tissue to a microcentrifuge tube.
   NOTE: *Most plant tissues are best prepared by freezing in liquid nitrogen. Grinding samples in liquid nitrogen to a fine powder and quickly add to an appropriate volume of Genomic Lysis Buffer.*
2. Add 200µl Genomic Lysis Buffer.
3. If ground, vortex for 5 seconds; if unground, homogenize the sample with a microfuge pestle until a homogenous suspension is acquired, approximately 15-30 strokes.
4. Add 5µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
5. Centrifuge the tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer and vortex to mix.
6. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
7. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
8. Gently remove and discard the supernatant without disturbing the beads.
9. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
10. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
11. Gently remove and discard the supernatant without disturbing the beads.
12. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
13. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
14. Gently remove and discard the supernatant without disturbing the beads.
15. Repeat step 12-14.
16. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
17. Add 50µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.

18. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
19. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From mouse tail tissue

1. Add 0.25-0.5cm, approximately 20-50mg, mouse tail in to a 1.5ml microcentrifuge tube with 200µl Genomic Lysis Buffer.
2. Add 5µl Proteinase K solution and incubate at 60°C for 3-4 hours to overnight. Invert the tube periodically if possible.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
3. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer and vortex to mix.
4. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
5. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
6. Gently remove and discard the supernatant without disturbing the beads.
7. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
8. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
9. Gently remove and discard the supernatant without disturbing the beads.
10. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
11. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
12. Gently remove and discard the supernatant without disturbing the beads.
13. Repeat step 10-12 once.
14. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
15. Add 50 µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.

16. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
17. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From Yeast

1. Aliquot 1.5ml yeast overnight culture into a 1.5ml microfuge tube and centrifuge at 14,000xg for 30 seconds. Discard the supernatant.
2. Add 150µl PBS, 5µl *LongLife™ Zymolyase®* and 1µl β-mercaptoethanol to the pellet and gently vortex to resuspend.
   NOTE: *Before use, Invert the Longlife™ Zymolyase® tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
3. Incubate at 37°C for 30 minutes with periodic mixing.
4. Centrifuge for 5 minutes at 14,000xg and pour off the supernatant. Gently vortex the tube to resuspend the pellet in the residual liquid.
5. Add 200µl Genomic Lysis Buffer and mix by inverting the tube several times.
6. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer to the sample and vortex to mix.
7. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
8. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
9. Gently remove and discard the supernatant without disturbing the beads.
10. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
11. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
12. Gently remove and discard the supernatant without disturbing the beads.
13. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
14. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
15. Gently remove and discard the supernatant without disturbing the beads.
16. Repeat step 13-15 once.
17. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.

18. Add 50µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
19. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
20. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From fungal tissue

1. Collect fungal tissue from liquid culture and wash 2-3 times in sterile water.
2. Fungal mycelia are best prepared by grinding samples using Molecular Grinding Resin™ in Genomic Lysis Buffer. For fungal teliospores, grinding samples in liquid nitrogen to a fine powder and quickly add to an appropriate volume of Genomic Lysis Buffer is recommended.
3. Add 10-20mg fungal mycelia to a microcentrifuge tube containing 200µl Genomic Lysis Buffer. Add 30µl Molecular Grinding Resin™ using a wide bore pipette tips and grind with a microcentrifuge pestle. For teliospores, add ground powder to 200µl Genomic Lysis Buffer and vortex to wet sample.
4. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer to the sample and vortex to mix.
5. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously*.
6. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
7. Gently remove and discard the supernatant without disturbing the beads.
8. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
9. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
10. Gently remove and discard the supernatant without disturbing the beads.
11. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
12. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
13. Gently remove and discard the supernatant without disturbing the beads.
14. Repeat step 11-13.

15. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
16. Add 50µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15minutes with gentle mixing on rotor at room temperature.
17. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
18. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From blood (≤0.2ml)

1. To ≤200µl whole blood, buffy coat, bone marrow or packed cells in a 2ml microfuge add 200 µl Genomic Lysis Buffer.
2. Add 10 µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.
3. Cool the sample on ice for 1 minute.
4. Add 800 µl of GET Binding Buffer to the sample and vortex to mix. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
5. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
6. Gently remove and discard the supernatant without disturbing the beads.
7. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
8. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
9. Gently remove and discard the supernatant without disturbing the beads.
10. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
11. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
12. Gently remove and discard the supernatant without disturbing the beads.
13. Repeat step 10-12.
14. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
15. Add 50µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.

16. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
17. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From blood stained & body fluid stained material

1. To 10-30mm^2 section of stained material in a 2ml microfuge tube, add 500µl Genomic Lysis Buffer and 10µl Proteinase K. Invert to mix.
2. Incubate the sample at 65°C for 4 hours with periodic inversions.
3. Allow to cool to room temperature, remove the stained material and remove excess buffer from the material with a pipette and return to the tube.
4. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 1ml of GET Binding Buffer and vortex to mix.
5. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
6. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
7. Gently remove and discard the supernatant without disturbing the beads.
8. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
9. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
10. Gently remove and discard the supernatant without disturbing the beads.
11. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
12. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
13. Gently remove and discard the supernatant without disturbing the beads.
14. Repeat step 11-13.
15. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
16. Add 50µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
17. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.

18. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

From body fluids

This includes CSF, plasma, saliva, serum, sputum, synovial fluid, urine and whole blood

1. Add 50µl body fluid to a 1.5ml microfuge tube.
   NOTE*: For body fluids with a low cell number, concentrate the cells by centrifuging 5-40ml sample at 2,000xg for 10 minutes*.
2. For samples with a normal protein concentration, add 200µl Genomic Lysis Buffer and mix by pipetting up and down.
3. Add 5 µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
4. Add 500µl of GET Binding Buffer to the sample and vortex to mix.
5. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   NOTE: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
6. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
7. Gently remove and discard the supernatant without disturbing the beads.
8. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
9. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
10. Gently remove and discard the supernatant without disturbing the beads.
11. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
12. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
13. Gently remove and discard the supernatant without disturbing the beads.
14. Repeat step 11-13.
15. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
16. Add 150-200µl prewarmed Elution Buffer to the magnetic beads and resuspend the beads complex by vortex or shaking. Incubate for 3 minutes with gentle mixing on rotor at room temperature.

17. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the Elute to a 1.5 ml nuclease free tube. Store the DNA at 4°C or -20°C until use.
18. ***Optional***: Removal of RNA. Invert the LongLife™ RNase vial 3-4 times to uniformly suspend the enzyme mix. Add 1µl LongLife™ RNase per 50 µl of eluted DNA. Incubate at room temperature for 15 minutes. Store the DNA at 4°C or -20°C until use.

TROUBLESHOOTING

Issue	Suggested reason	Possible solution
Low nucleic acid yield or purity	Kit components are not stored properly	Store kit components as indicated in the label.
	Ethanol not added to the Wash Buffers	Add absolute ethanol to the Wash buffers before using
	Reagent and samples not properly mixed	Mix the sample tube well after addition of each reagent.
Low yield of Tissue DNA	Incomplete Proteinase K digestion	Homogenize tissue sample in Genomic Lysis buffers completely before adding Proteinase K
		Incubate tissue with proteinase K for longer period 2-4 hours
Low yield from bacteria or yeast	Bacteria and Yeast not lysed efficiently with lysozyme and zymolyase respectively	Lyse bacteria and Yeast as mentioned in the protocol
Eluted DNA is degraded	Nuclease activity in un-lysed tissue	Tissue should be frozen from the time of extraction to lysis
		Use small pieces of tissue or homogenize in Genomic Lysis buffer
	Nuclease contamination from tips, tubes, or regents	Use nuclease free tip and reagents. Avoid touching mouth of regent bottles.
		Is suspect contamination coming from reagent bottle, discard the bottle and use fresh bottle or order new

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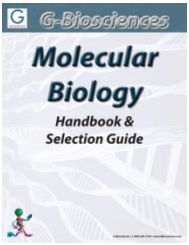

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