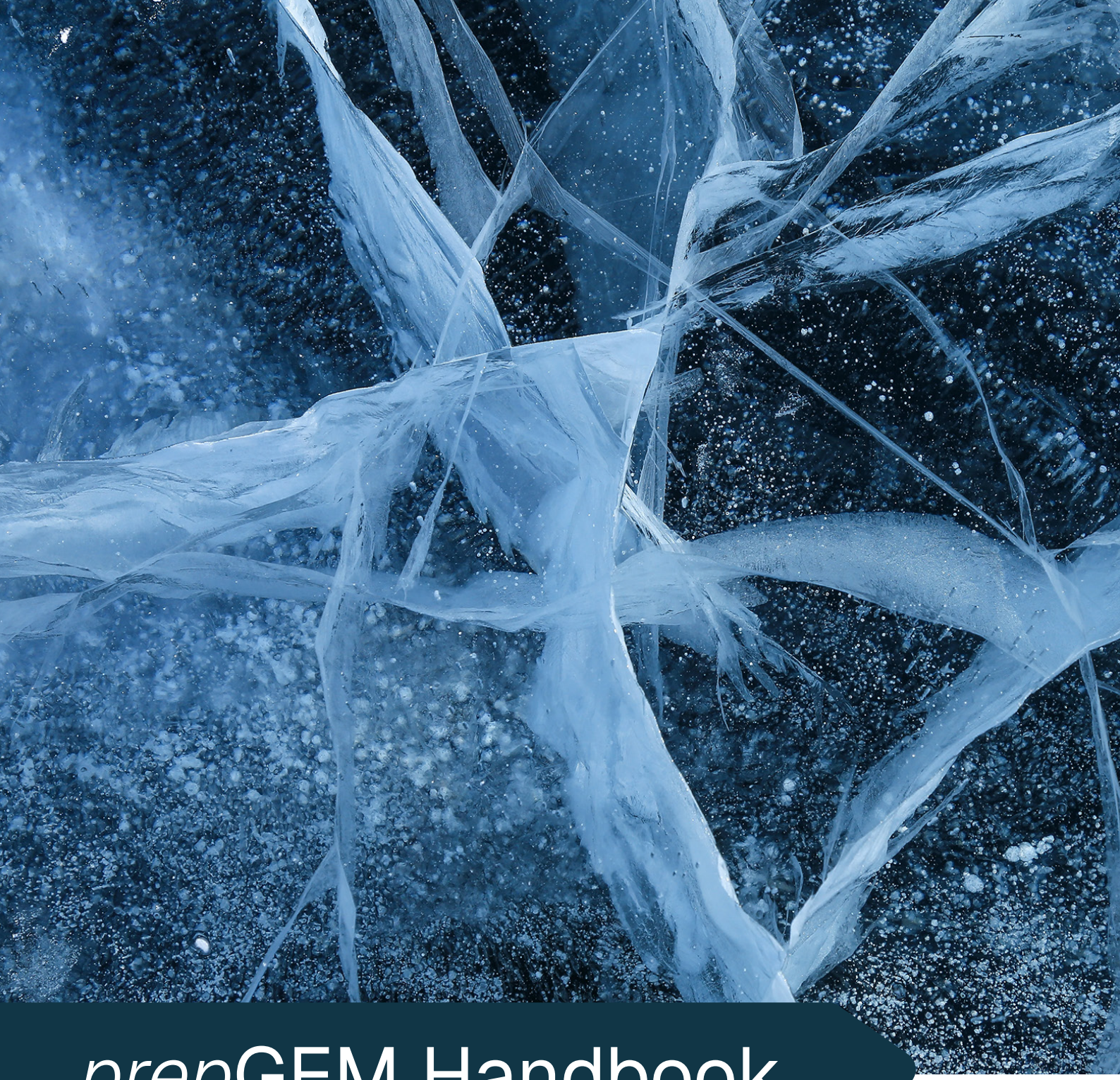


*prep*GEM Handbook

*prep*GEM Universal | *prep*GEM Bacteria

Exymes

C0083 V1

CONTENTS

KIT CONTENTS AND STORAGE

Exymes kits come with proprietary buffers and enzymes required for nucleic acid extraction, listed in Table 1 and Table 2. Kits contain excess volume to allow for minor pipetting errors and overage while preparing Master Mix for extractions ensuring customers get the full number of reactions out of their kits.

*prep*GEM Universal Kit Contents

Kit contains: prepGEM, Histosolv, 10X BLUE buffer, 10X RED+ buffer, 10X ORANGE+ buffer

Table 1. Kit components for *prep*GEM Universal.

Component	Volumes				Temperature (shipping)	Temperature (storage)
	50 rxn	100 rxn	500 rxn	1,000 rxn		
Catalogue no.	PUN0050	PUN0100	PUN0500	PUN1000		
*prep*GEM*	50 µl	100 µl	500 µl	1000 µl	RT	-20°C
10X BLUE buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
10X RED+ buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
10X ORANGE+ buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
Histosolv**	50 rxn	100 rxn	500 rxn	1,000 rxn	RT	-20°C

*NOTE: After tubes have been opened, the prepGEM enzyme should be placed at -20°C. To minimize the number of freeze/ thaw cycles, prepGEM can be aliquoted into smaller volumes.

**NOTE: Once Histosolv has been rehydrated, it is stable 7-12 months at -20°C. If you do not plan to use all the Histosolv immediately, it is recommended that you aliquot Histosolv into smaller volumes and store at -20°C to minimize the number of freeze/thaw cycles.

*prep*GEM Bacteria Kit Contents

Kit contains: prepGEM, Lysozyme, 10X GREEN+ buffer, Enhancer, 5X WASH+ buffer

Table 2. Kit components for *prep*GEM Bacteria.

Component	Volumes				Temperature (shipping)	Temperature (storage)
	50 rxn	100 rxn	500 rxn	1000 rxn		
Catalogue no.	PBA0050	PBA0100	PBA0500	PBA1000		
*prep*GEM*	50 µl	100 µl	500 µl	1000 µl	RT	-20°C
10X GREEN+ buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
5X WASH+ buffer**	1 ml	1 ml	5 ml	10 ml	RT	4°C
1X WASH+ buffer	Prepared by customer				4°C	
Enhancer	1 ml	1 ml	5 ml	10 ml	RT	4°C
Lysozyme***	50 rxn	100 rxn	500 rxn	1,000 rxn	RT	-20°C

*NOTE: After tubes have been opened, the prepGEM enzyme should be placed at -20°C. To minimize the number of freeze/ thaw cycles, prepGEM can be aliquoted into smaller volumes.

**NOTE: 5X WASH+ buffer may arrive with some floating crystals, if this occurs please warm the buffer to ~35°C to dissolve before diluting to 1X.

***NOTE: Once Lysozyme has been rehydrated, it is stable for several weeks at 4°C. If you do not plan to use all the Lysozyme immediately, it is recommended that you aliquot Lysozyme into smaller volumes and store at -20°C.

Storage

Once received Exymes Kit buffers and reagents should be stored dry at the temperatures indicated on page 3 in Table 1, and Table 2. prepGEM must be stored at -20°C, potentially in aliquots to reduce the number of freeze/thaw cycles. Histosolv and lysozyme once hydrated, should also be stored at -20°C in aliquots if not planning on using immediately to minimize the number of freeze/thaw cycles. Buffers and Enhancer should be stored at 4°C.

PRODUCT INFORMATION

Product Use Limitations

Exymes kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products. We recommend all users of Exymes products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines. NIH Office of Science Policy:

https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/

Product Warranty and Satisfaction Guarantee

Exymes guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Exymes will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a Exymes product does not meet your expectations, simply contact:

Technical Service (techsupport@exymesplc.com) or their distributor.

We will credit your account or exchange the product. A copy of Exymes terms and conditions can be obtained on request and is also provided on the back of our invoices. If you have questions about product specifications or performance, please contact Technical Service (techsupport@exymesplc.com) or your distributor.

Technical Assistance

At Exymes, we pride ourselves on the quality and availability of our technical support. Our Technical Support Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of Exymes products. If you have any questions or experience any difficulties regarding prepGEM Kits or Exymes products in general, please do not hesitate to contact us at:

techsupport@exymesplc.com

Exymes customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at Exymes. We therefore encourage you to contact us if you have any suggestions about product performance, publications or new applications and techniques.

Quality Control

Exymes reagents are made from certified DNA-free chemicals and solutions and all buffers and enzymes are treated with DNase and UV before shipment. Be aware however, that we have no control over the reagents of other vendors.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective eyewear. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in the resource section of the product pages or by emailling techsupport@exymesplc.com.

Product Principle/Product Overview

Exymes's extraction process relies on temperature-driven extraction utilizing a thermostable proteinase, which functions at temperatures ideal for nucleic acid extraction – inactive at low temperatures, becoming active at 75°C, lysing cells, and removing nucleoproteins from the DNA, and inactivating again at 95°C. This 95°C inactivation step results in DNA that is largely single-stranded, thus still suitable for many applications such as: genotyping including SNP and STR analysis, as well as PCR, and qPCR. This process reduces the number of steps required by traditional extraction methods (i.e., silica columns, magnetic beads, or organic extractions) reducing time and increasing sample processing.

The Exymes prepGEM kits are designed for purification of DNA from a variety of sample types. This includes, but is not limited to blood (liquid and on storage cards), saliva, buccal swabs, cells, animal tissue, human tissue, and insects using the prepGEM Universal kit (PUN). For bacteria samples,Exymes's prepGEM kit Bacteria (PBA) can extract DNA from both Gram-positive (Gram +) and Gram-negative (Gram -) bacteria in liquid culture, colonies, biofilms, mucus, environmental samples (e.g., stool or soil), biofilms along with protozoa and archaea.

Procedure Overview (*prep*GEM Universal)

Sample to DNA < 15 min / 1 - 96 samples.

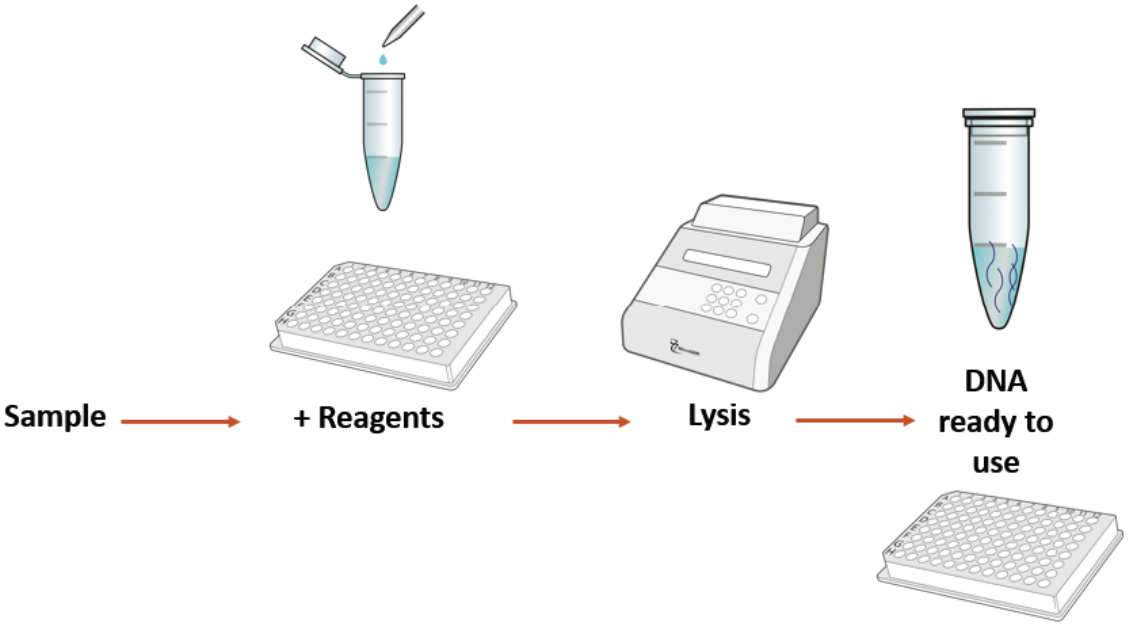


Figure 1. Generalized workflow for sample processing (1-96 samples) using Exymes' s temperature driven extraction (TDE) process.

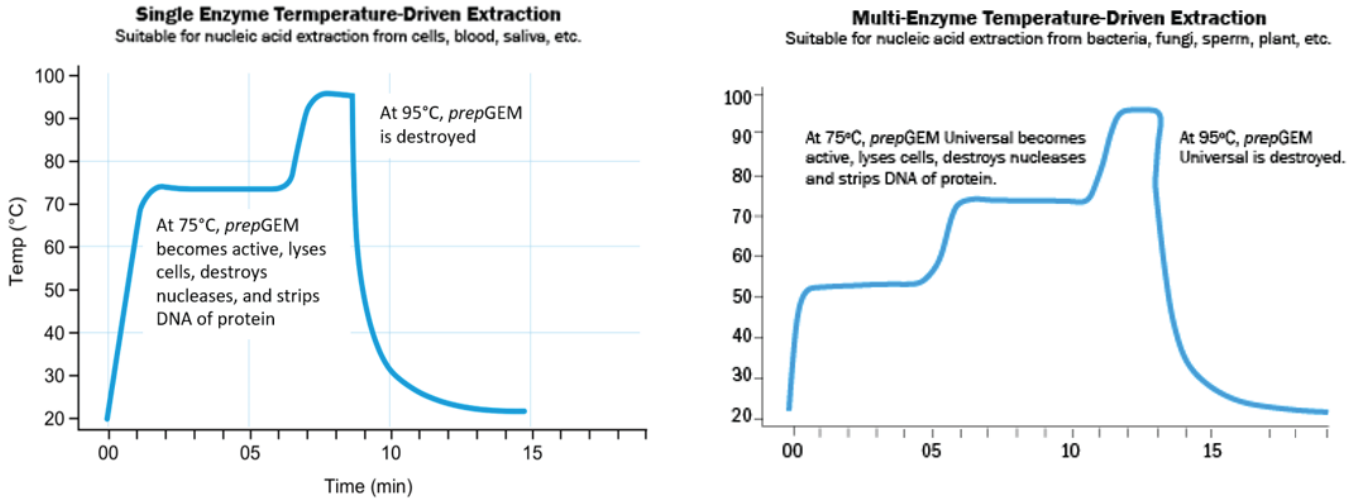


Figure 2. (Left) for single enzyme Temperature Driven Extraction (TDE), *prep*GEM is the only enzyme in the reaction. Simple temperature changes activate the enzyme to extract DNA, free of proteins. (Right) Multi-enzyme TDE utilizes multiple enzymes (e.g., Lysozyme or Histosolv) to extract DNA from more challenging sample types, such as tissue samples (*prep*GEM Universal) or Gram + bacteria (*prep*GEM Bacteria). The low activity of the prepGEM enzyme below 75°C allows for mesophilic cell-wall degrading enzymes to be used when needed for specific sample types. Both reactions can be carried out in a single tube or scaled up to a 96-well plate and can be programmed for a thermocycler or Exymes's PDQeX Nucleic Acid Extractor.

Equipment and Reagents to be Supplied by User

For all protocols:

Equipment

Vortexer
Pipettes
Thermocycler or heat block

Consumables

Pipette tips
Nuclease-free water
Nuclease-free (RNase and DNase Free) microfuge tubes/plates
100 mM Tris (tris(hydroxymethyl) aminomethane) pH 8.0 (not provided)
(*prep*GEM Bacteria only).
TE buffer (for long term DNA storage)

Important Notes

Reagent Scaling for Cell Culture

With cultured cells, you can expect linear yields for 5 to approximately 100,000 cells, making Exymes' s *prep*GEM Universal kit ideal for low-cell number work. For low numbers of cells we recommend reducing the extraction volume. The minimum volume possible will depend on evaporation with the equipment you are using. The recommended amounts of *prep*GEM to use for the different extraction volume are shown in below. Use 1/10th volume of 10X BLUE buffer.

Table 3. Extraction volumes by cell number.

Extraction Volume (µl)	Cell Numbers	*prep*GEM Volume (µl)	10X BLUE buffer Volume (1/10 of total volume)
50-100	50,000-500,000	1 µl	5-10 µl
20-50	5,000-50,000	1 µl	2-5 µl
5-20	100-5,000	0.5 µl	0.5-2 µl
1-15	1-500	0.2 µl	0.1-1.5 µl

TECHNICAL TIPS

- The method, enzyme formulation and buffer have been carefully optimized for extracting DNA. Using the enzyme with other methods or buffers is not recommended. If you need to modify the method in any way, please email: techsupport@exymesplc.com.
- *prep*GEM is a preparative method for DNA extraction. This method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can then be used for many molecular biology applications including SNP and STR analysis as well as quantitative, multiplex and end-point PCR.
- There is no concentration step in the procedure and so the concentration of the extract is dependent on: 1) the quality of the sample input; 2) in the case of swabs, the type of swab and the volume of water used to wash the swab; 3) the extraction volume (which in some cases can be scaled).
- DNA extracted using *prep*GEM is largely single stranded because of the 95°C heat step.
- For accurate quantification, a quantitative PCR (qPCR) is recommended. If standard fluorescent chelating dyes are to be used for normalizing samples, then we recommend taking a sample of the extract before the 95°C step. Alternatively, you can generate a standard curve using a previously made extract that has been quantified. Additional quantification information can be found on the Exymes website.
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are fresh, and handled at 4°C, or on ice, before and after extraction.
- For long term storage of the extracted DNA, add TE buffer and store at -20°C.
- The removal of RNA from DNA preparations with RNase is unnecessary for many applications.

PRE-EXTRACTION STEPS

Pre-Extraction (*prep*GEM Universal): Resuspend the Histosolv

Histosolv is a mixture of reagents that weaken tissue cell walls. It is delivered as a dry powder. Before it is ready to be used, the powder should be dissolved in Nuclease-free water. Different kit sizes contain tubes with different amounts of enzyme (ensure you follow the directions on the Histosolv label in your kit). Be sure to add the correct amount of water (see the table below).

1. In a clean environment, open the tube and add the following and vortex to mix:

Kit reaction size	Product Code	Nuclease-free water
50 rxn	FUN0050	0.55 ml
100 rxn	FUN0100	1.1 ml
500 rxn	FUN0500	5.5 ml
1000 rxn	FUN1000	11.0 ml

2. Once Histosolv has been rehydrated, it is stable for 7-12 months at -20°C. If you do not plan to use all of the Histosolv immmediately, it is recommended that you aliquot Histosolv into smaller volumes and store at -20°C, to minimize freeze/thaw cycles.

Pre-Extraction (*prep*GEM Bacteria): Dilute WASH+ buffer

The WASH+ buffer is provided at a 5X concentration and may appear yellow. This solution needs to be diluted to 1X using nuclease-free water prior to use in protocols. You may find that the 5X WASH+ buffer contains some floating crystals, if this is the case, you should warm the 5X WASH+ buffer to ~35°C to fully dissolve these crystals prior to diluting to 1X. Aliquots of 1X WASH+ buffer can be made and stored at either RT or 4°C for long term storage.

Pre-Extraction (*prep*GEM Bacteria): Resuspend the Lysozyme

Lysozyme is provided as a lyophilized powder, to use, resuspend in 100 mM Tris (tris(hydroxymethyl) aminomethane) pH 8.0 to the volume specified on the label. Once Lysozyme has been rehydrated, it is stable for several weeks at 4°C. If you need longer term storage, we recommend aliquoting into smaller volumes and storing at -20°C.

Prepare Master Mix

In order to ensure that the yields are uniform amongst samples, it is recommended that an extraction Master Mix be prepared prior to performing extractions. The Master Mix can then be either added to sample in tubes or put into tubes prior to adding sample. We recommend using the *prep*GEM reagents within one hour of preparation. For longer periods, reagents should be frozen.

An example of a Master Mix preparation is shown below:

Table 4. Example calculation for preparing Master Mix for 10 buccal swab (eluate) samples.

Component	Volume per single extraction (µl)	Number of Reactions +1 (overage)	Total volume added (µl)
10X BLUE buffer	10	11	110
Nuclease-free water	69	11	759
*prep*GEM	1	11	11

DNA Extraction from Cells

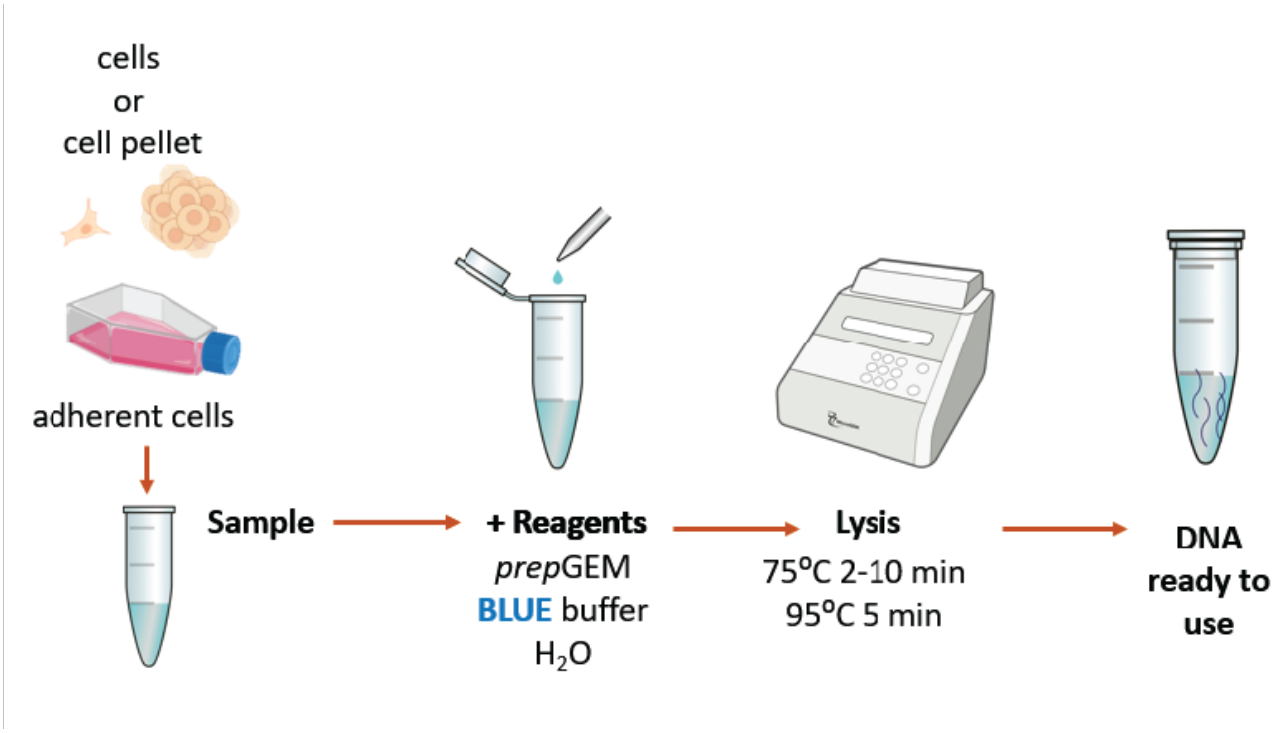


Figure 3. Workflow for DNA extraction from cells.

*prep*GEM DNA extraction is ideal for low cell numbers. Because the buffers used for prepGEM are compatible with most downstream processes, it means that the entire sample can be used. In addition, because *prep*GEM does not need purification steps, extractions can be performed in sub-microliter volumes.

*prep*GEM reagents are sensitive to EDTA and other chelating agents. If cells are presented in an EDTA-containing solution (such as Trypsin-EDTA used to detach adherent cells), cells should be centrifuged at 200 RCF and washed in 1X BLUE buffer before extraction.

With cultured cells, you can expect linear yields for 5 to approximately 100,000 cells. This method is ideal for low-cell number work. For low numbers of cells, we recommend reducing the extraction volume. The minimum volume possible will depend on evaporation with the equipment and workflow you are using.

The recommended amounts of prepGEM to use for different extraction volumes are shown in the table below. Use 1/10 volume of 10X BLUE buffer.

Cell Numbers Pellet	Extraction Volume Total (µl)	*prep*GEM Volume (µl)	10X BLUE buffer Volume (1/10 of total volume)	Nuclease-free water
50,000 - 500,000	50-100	1 µl	5-10 µl	44-89 µl
5,000 - 50,000	20-50	1 µl	2-5 µl	17-44 µl
100 - 5,000	5-20	0.5 µl	0.5-2 µl	4-17.5 µl
1 - 500	1-15	0.2 µl	0.1-1.5 µl	0.7-13.3 µl

Sample Preparation

Sample prep - Cells in Suspension

1. Centrifuge the suspension at 200 RCF for 5 minutes.
2. Remove all the liquid.
3. Resuspend the pellet in *prep*GEM extraction solution.

Sample prep - Adherent Cells

1. If the cells are in flasks, dislodge cells by preferred method (Trypsin or cell scraper) and centrifuge suspension at at 200 RCF for 5 minutes. Otherwise, the *prep*GEM extraction solution can be added directly to the cell monolayer. *prep*GEM reagents are sensitive to EDTA and other chelating agents. If cells are presented in an EDTA-containing solution (such as Trypsin-EDTA used to detach adherent cells), they should be centrifuged at 200 RCF and washed in 1X BLUE buffer before extraction.
2. Remove all the liquid.
3. Add *prep*GEM extraction solution.

Sample prep - Cell pellets:

Up to 5 x 10^6 cells can be extracted using the recommended method. Linear extraction efficiency is best achieved within the range of <10 cells to approximately 10^6 cells. Cell pellets can be used directly. Alternatively, the pellet can be resuspended in 1X BLUE buffer, and an appropriate quantity added to the extraction mixture.

Sample prep - FACS and LCM:

Cells can be collected directly into the extraction solution or extraction solution added directly to a capillary from LCM. If cells are collected in a different buffer, it may be necessary to add 1/10 volume of the *prep*GEM 10X BLUE buffer after collection. We recommend using the *prep*GEM reagents within one hour of preparation. For longer periods, reagents should be frozen.

Sample prep - RNAlater™

RNAlater is a somewhat viscous solution and contains inhibitory compounds that need to be removed before extraction (this is required for most extraction processes).

1. Centrifuge suspension at 3,000 RCF for 5 minutes.

2. Remove all of the liquid (a quick spin on the benchtop microcentrifuge can help gather the last few drops).

3. Resuspend the pellet in the *prep*GEM extraction solution.

Extraction Procedure

Extractions (this example is for a 50 µl reaction, volumes can be scaled based on cell number, use the table above to scale reagents correctly).

1. Add:
    a. Cell suspension or pellet
    b. 5 µl 10X BLUE buffer
    c. 1 µl *prep*GEM
    d. Water to a final volume of 50 µl

2. Vortex and incubate:
    a. 75°C for
        i. >50,0000 cells 10 min
        ii. 1,000-5,000 cells 5 min
        iii. <1,000 cells 2 mins
    b. 95°C for 5 minutes

3. The sample is now ready for analysis. Vortex and spin the extract before using.

DNA extraction from Buccal Swabs

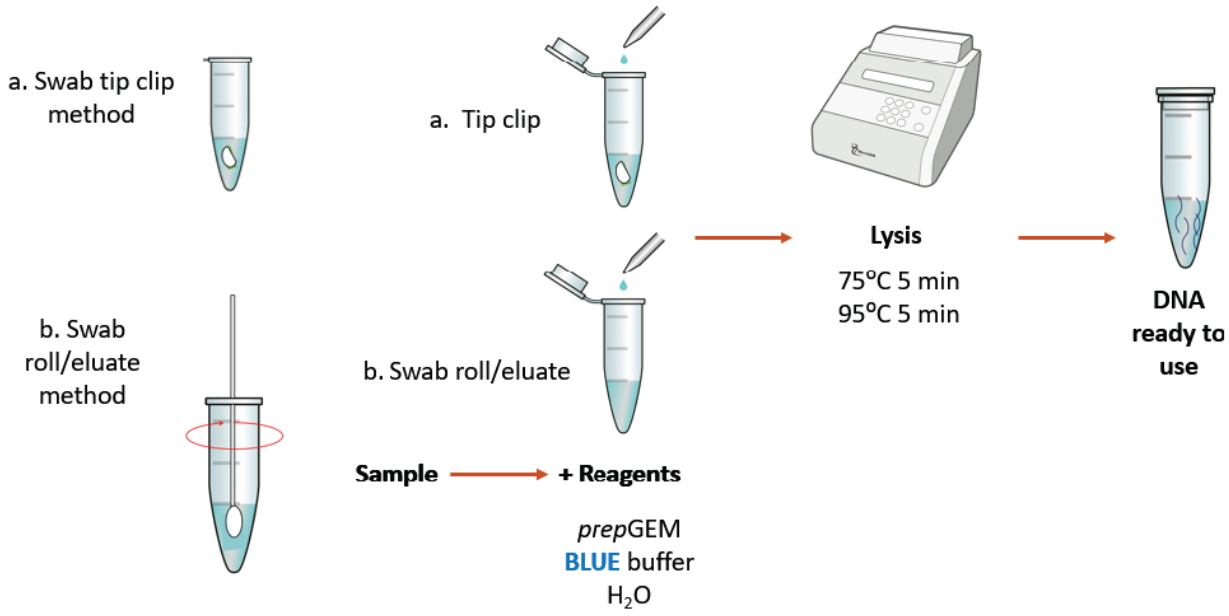


Figure 4. Workflow for DNA extraction from buccal swabs.

1. Wash the buccal swab in a minimum amount of Nuclease-free water to cover the swab head. Typically, a cotton swab requires 400-500 µl. Use a rolling action against the tube sides and press the swab against the side to squeeze as much of the liquid out as possible. An alternative approach it to cut off a portion of the swab.

2. In a thin-walled PCR tube or 96-well plate (0.2 ml) add:
    a. 20 µl of the eluate
    b. 10 µl of the 10X BLUE buffer
    c. 1 µl *prep*GEM
    d. 69 µl Nuclease-free water

3. In a thermocycler, incubate:
    a. 75°C for 5 min
    b. 95°C for 5 min

4. The sample is now ready for analysis. Vortex and spin the extract before using.

DNA extraction from Liquid Saliva

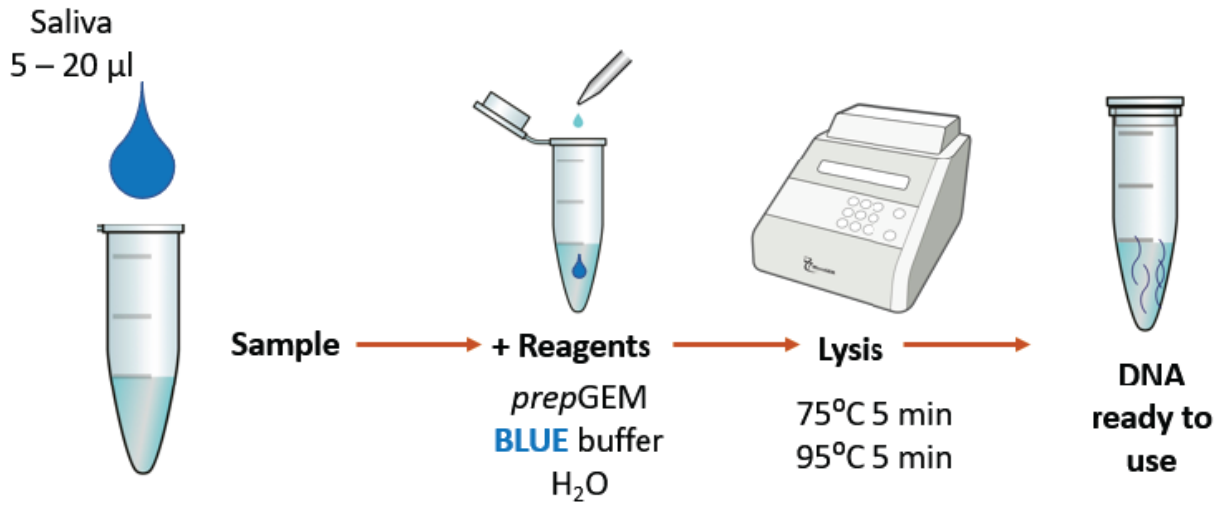


Figure 5. Workflow for DNA extraction from saliva.

1. Volume of liquid saliva, 5-20 µl, should be added to a thin-walled PCR tube or 96-well plate.
2. To the PCR tube containing the saliva add:
    a. 10 µl of the 10X BLUE buffer
    b. 1 µl *prep*GEM
    c. Volume of Nuclease-free water to total 100 µl
3. In a thermocycler, incubate:
    a. 75°C for 5 min
    b. 95°C for 5 min
4. The sample is now ready for analysis. Vortex and spin the extract before using.

DNA extraction from Saliva on storage cards

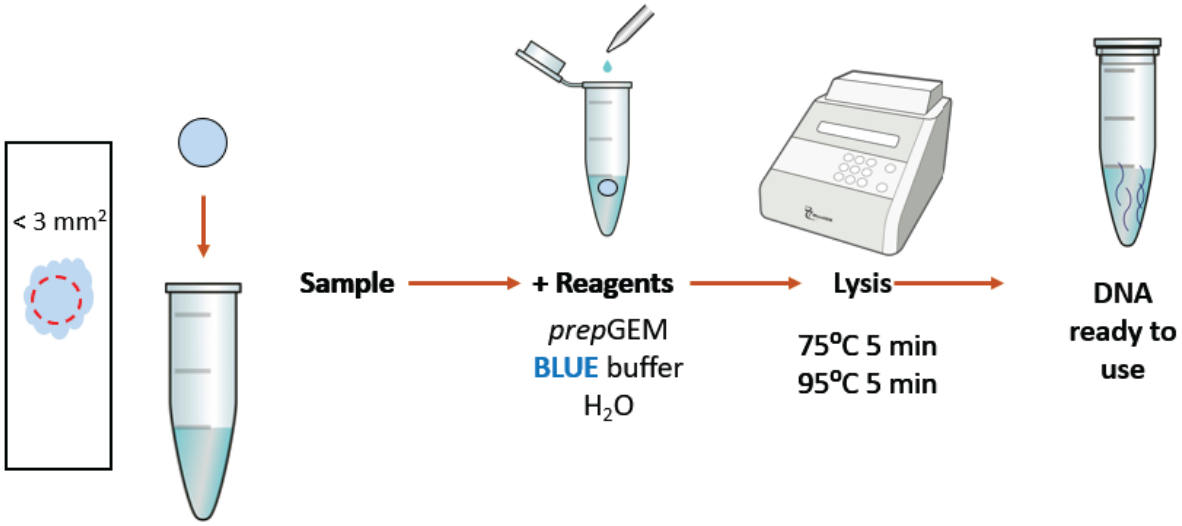


Figure 6. Workflow for DNA extraction from saliva on storage cards (FTA).

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to some DNA polymerases and so a pre-wash is recommended prior to DNA extraction.

1. Remove one 3 mm disc from the card-stored sample and place into a thin-walled PCR tube or a 96-well plate.

2. Wash the disc in 100 µl of Nuclease-free water by incubating at room temperature for 15 minutes. Aspirate the water from the disc and discard the water.

3. To the tube add:
    a. 5 µl of the 10X BLUE buffer
    b. 1 µl *prep*GEM
    c. 44 µl Nuclease-free water

4. In a thermocycler, incubate:
    a. 75°C for 5 min
    b. 95°C for 5 min

5. Pipette the solution to a new tube.

6. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. The disc can be discarded. The extract is now ready for analysis. Vortex and spin the extract before using. Typically, 2-5 µl should be used in PCR.

DNA extraction from insects or mouse tails/ear cuts

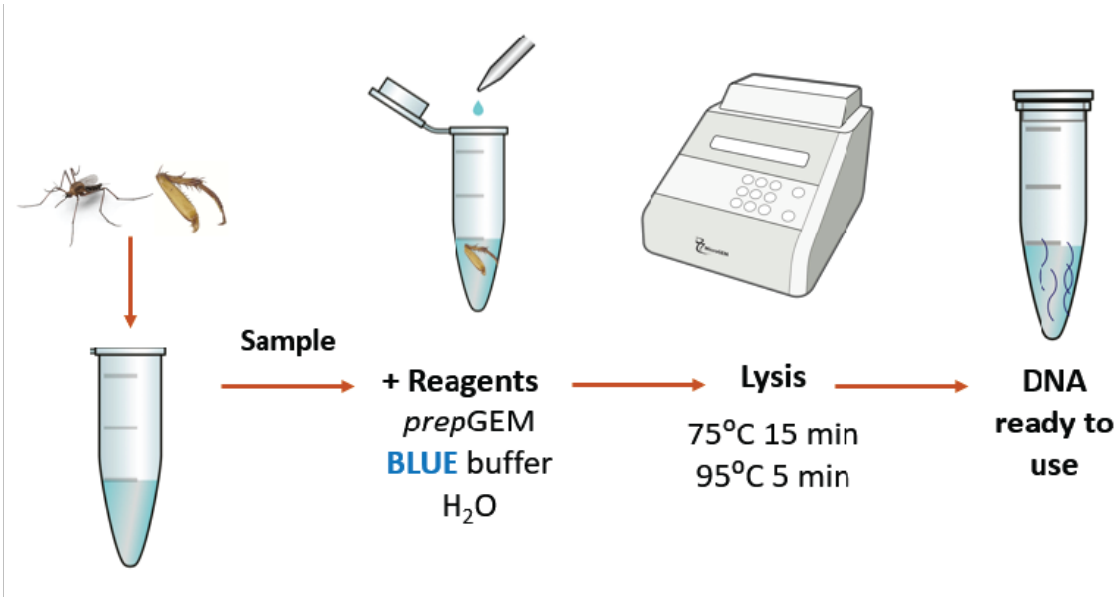


Figure 7. Workflow for DNA extraction insects (intact).

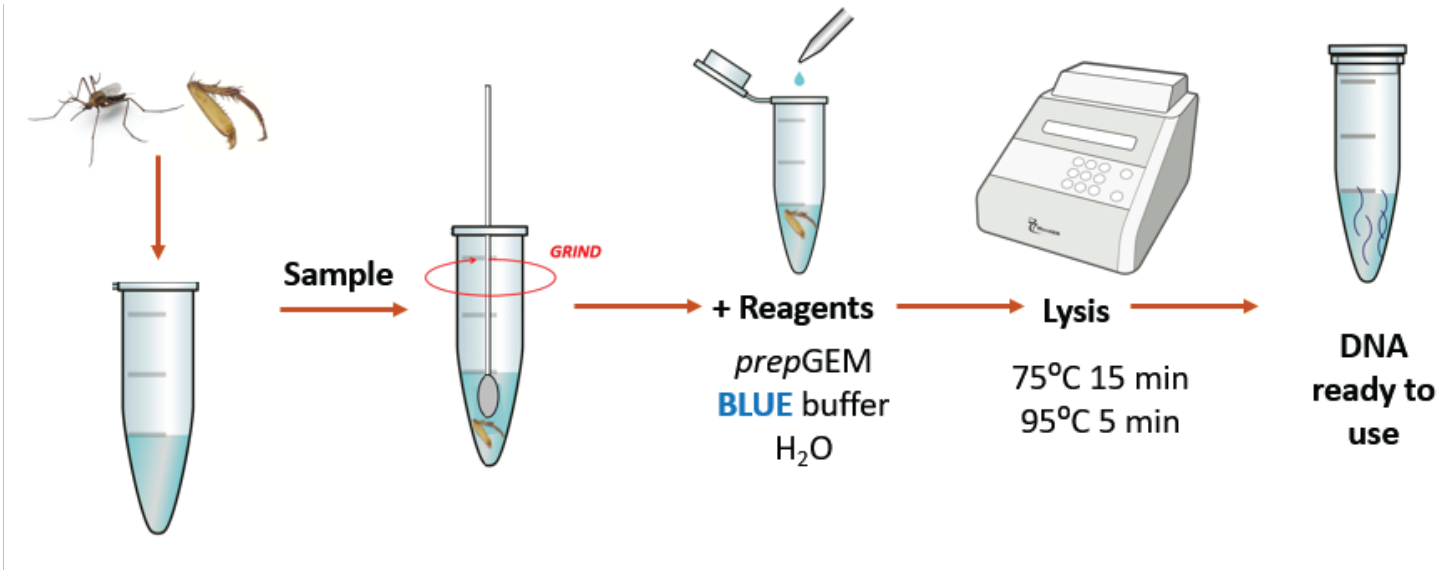


Figure 8. Workflow for DNA extraction insects (homogenized).

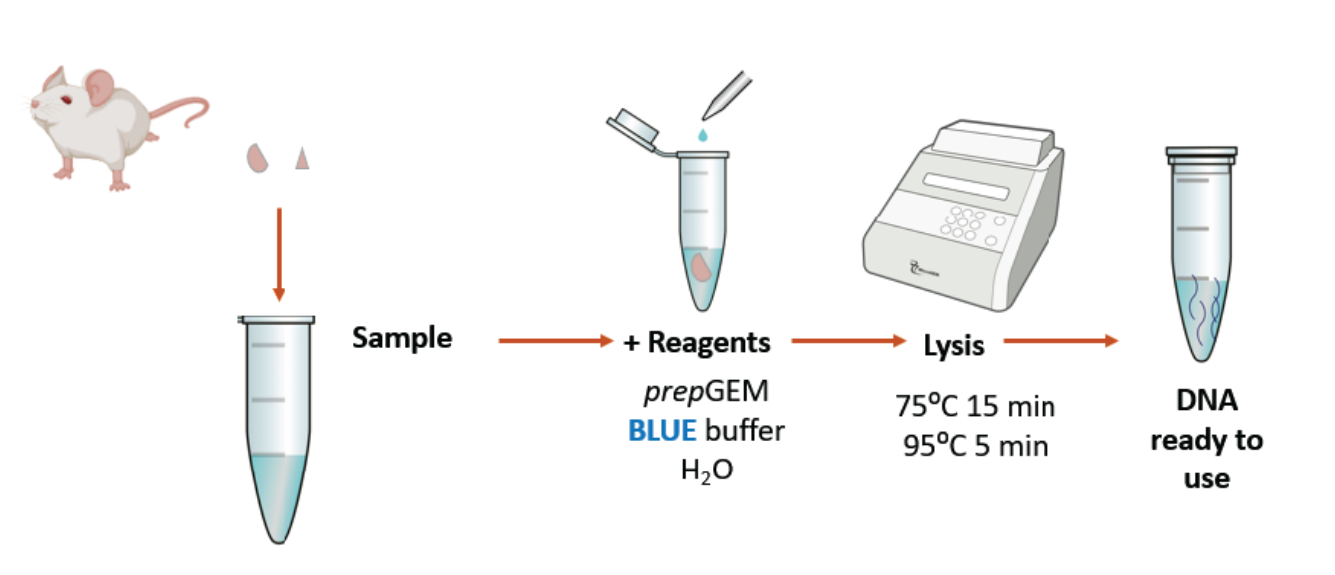


Figure 9. Workflow for DNA extraction from mouse/rat tail tips or ear cuts.

1. Put the sample into a thin-walled PCR tube or 96-well plate.
    a. Mouse tail/ears: < 5 mg or 2 mm²
    b. Insect: whole insect, insect leg

2. Add extraction solution: (volumes can be scaled up or down depending on sample size), then grind with pipette tip.
    a. 44 µl Nuclease-free water
    b. 10 µl 10X BLUE buffer
    c. 1 µl *prep*GEM

3. Incubate at:
    a. 75°C for 15 min
    b. 95°C for 5 min

4. Transfer supernatant to a new tube. The DNA is in the solution (DO NOT DISCARD). The sample is now ready for analysis. Vortex and spin the extract before using.

DNA extraction from liquid whole blood

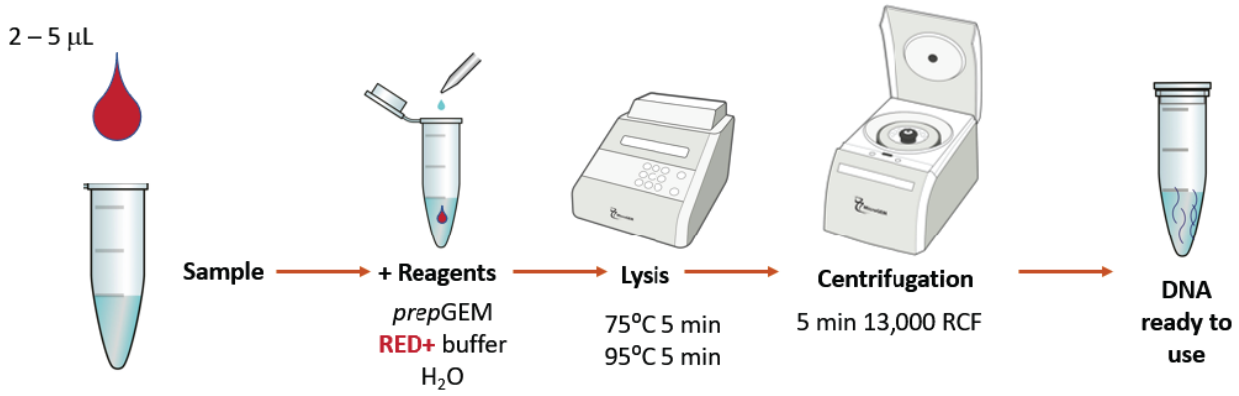


Figure 10. Workflow for DNA extraction from blood.

The RED+ buffer is a proprietary formulation that precipitates PCR inhibitors. The solid material should not be disturbed when removing the supernatant (Step 4).

Typically, 5 minutes at 13,000 RCF is sufficient to give a well-packed pellet. Longer spins should be used for lower RCF centrifugations. For example, a typical 96-well plate, swing out rotor rated at 3,000 RCF should be spun 10 minutes. Centrifugation should be performed immediately after extraction.

Yields will vary depending on the White Blood Cell (WBC) count of the sample.

1. Add 2-5 µl liquid blood to a thin-walled PCR tube or 96-well plate.

2. To the PCR tube add:
    a. 5 µl of the 10X RED+ buffer
    b. 1 µl *prep*GEM
    c. Nuclease-free water up to a total volume of 100 µl

3. In a thermocycler, incubate:
    a. 75°C for 5 min
    b. 95°C for 5 min

4. Centrifuge for 5 minutes at maximum speed (~13,000 RCF). Centrifugation should be performed immediately after extraction).

5. Pipette the supernatant to a new tube without disturbing the pellet. Make sure to not disturb the solid material when removing the supernatant).

6. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and spin the extract before using.

*__NOTE__: There may be some discoloration (yellow-pink-brown) of the solution due to heme from the blood.

DNA extraction from whole blood (storage cards)

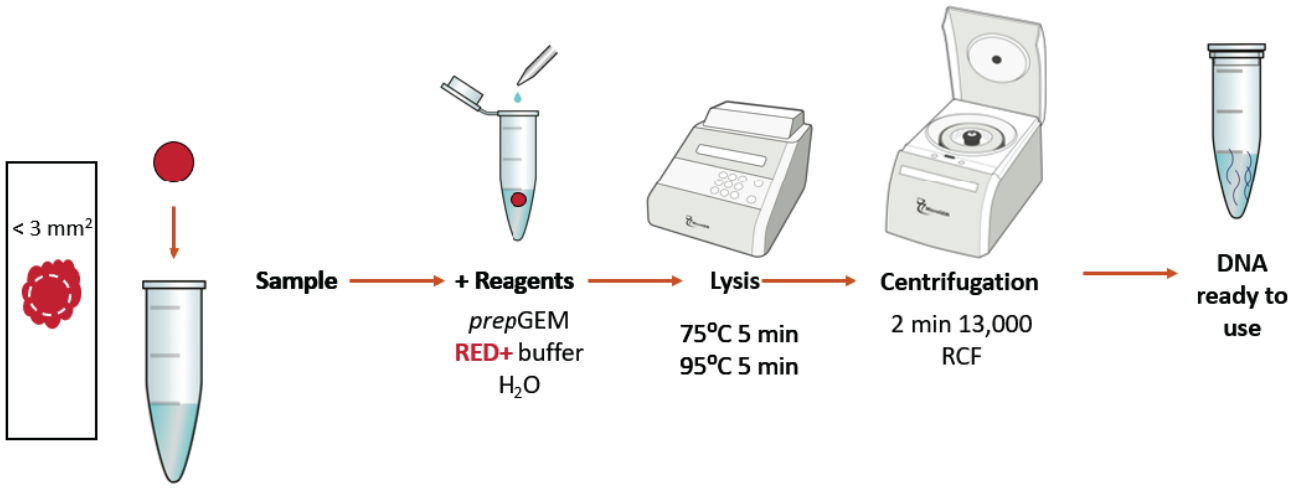


Figure 11. Workflow for DNA extraction from blood on storage cards (FTA).

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to Taq DNA polymerase and so a prewash is recommended prior to DNA extraction.

1. Remove one 3 mm² disc from the card-stored blood sample and place into a thin-walled PCR tube or a 96-well plate. For the best results, punch in the center of the area where the blood was applied.

2. Wash the disc in 100 µl of Nuclease-free water by incubating at room temperature for 15 minutes. Aspirate the water from the disc(s) and discard.

3. Place the washed disc into a thin-walled PCR tube or a 96-well plate.

4. In a thin-walled PCR tube add:
    a. 5 µl of the 10X RED+ buffer
    b. 1 µl *prep*GEM
    c. Nuclease-free water

5. In a thermocycler, incubate:
    a. 75°C for 5 min
    b. 95°C for 5 min

6. Centrifuge for 2 minutes at maximum speed and transfer the supernatant to a fresh tube.

7. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and spin the extract before using.

***NOTE:** There may be some discoloration (yellow-pink-brown) of the solution due to heme from the blood. Typically, 2-5 µl should be used in PCR.

DNA extraction from human tissue

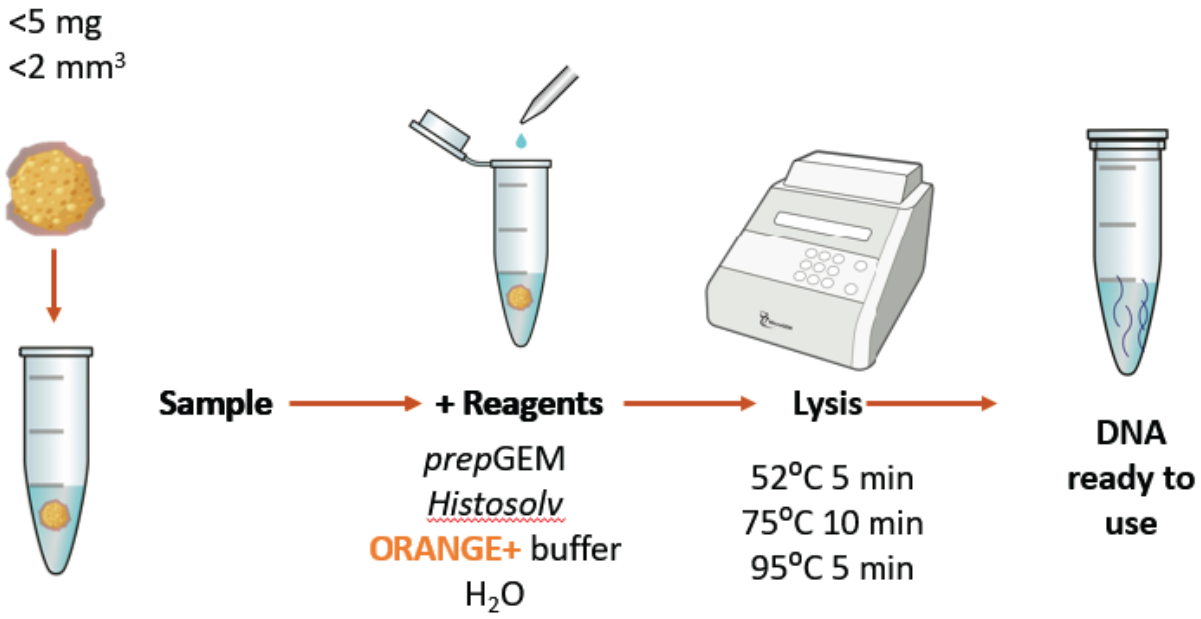


Figure 12. Workflow for DNA extraction from tissue.

1. Cut the tissue into pieces of approximately 1-2 mm³. With hair follicles, use 1-3 hairs. Cut off the shaft 4 mm above the follicle.

2. In a thin-walled PCR tube or 96-well plate add:
   a. 10 µl of the 10X ORANGE+ buffer
   b. 1 µl prepGEM
   c. 10 µl Histosolv
   d. 79 µl Nuclease-free water
3. Add the sample.

4. Mash the sample with a pipette tip and disperse by vortexing.

5. In a thermocycler, incubate:
   a. 52°C for 5 min
   b. 75°C for 10 min
   c. 95°C for 5 min

6. Aspirate the extract away from the residual material.

7. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and spin the extract before using.

DNA extraction from ONLY Gram - bacteria or Mycoplasma pellets

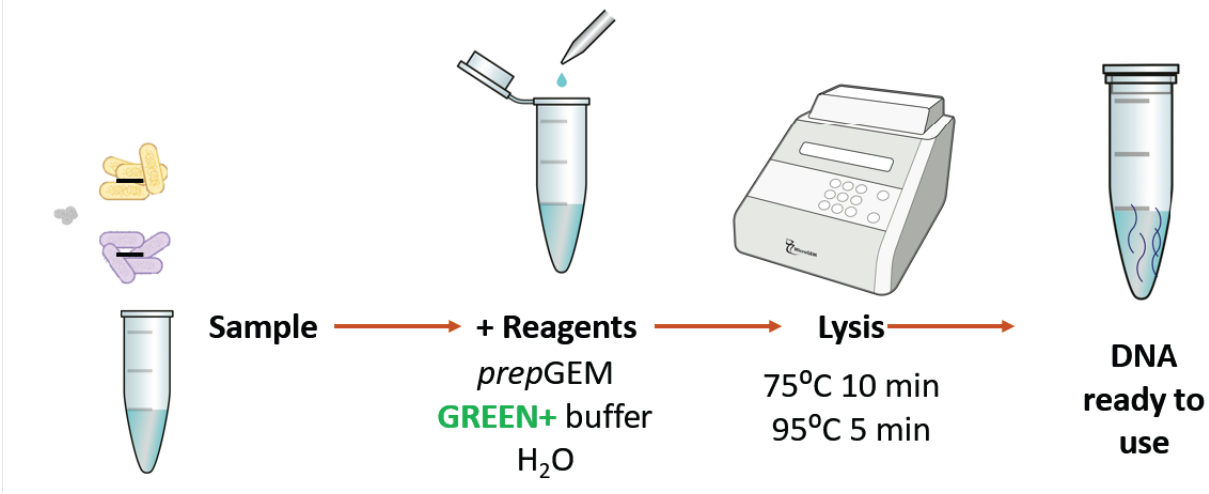


Figure 13. Workflow for DNA extraction from ONLY Gram - bacteria or Mycoplasma pellets.

1. Place bacteria/mycoplasma pellet or colony in thin-walled PCR tube or 96-well plate.

2. Resuspend the pellet in the extraction mixture.
    a. 1 µl *prep*GEM
    b. 10 µl 10X GREEN+ buffer
    c. 89 µl Nuclease-free water

3. Incubate:
    a. 75°C for 10 min
    b. 95°C for 5 min

4. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and spin the extract before using.

DNA extraction from samples that may include BOTH Gram- and Gram+ bacteria

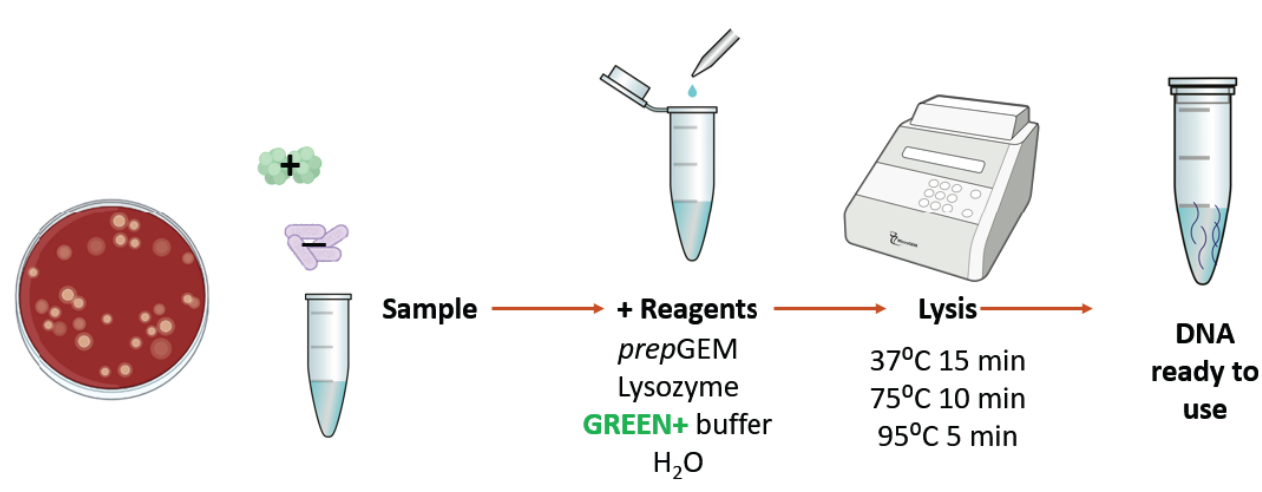


Figure 14. Workflow for DNA extraction from samples that may include BOTH Gram- and Gram+ bacteria

1. Place bacteria colony or pellet into a thin-walled PCR tube or 96-well plate.
2. Resuspend the pellet in the extraction mixture.
    a. 1 µl *prep*GEM
    b. 1 µl Lysozyme
    c. 10 µl 10X GREEN+ buffer
    d. 88 µl Nuclease-free water
3. Incubate:
    a. 37°C for 15 min
    b. 75°C for 10 min
    c. 95°C for 5 min
4. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and spin the extract before using.

DNA extraction from capsulated bacteria/colonies/biofilms

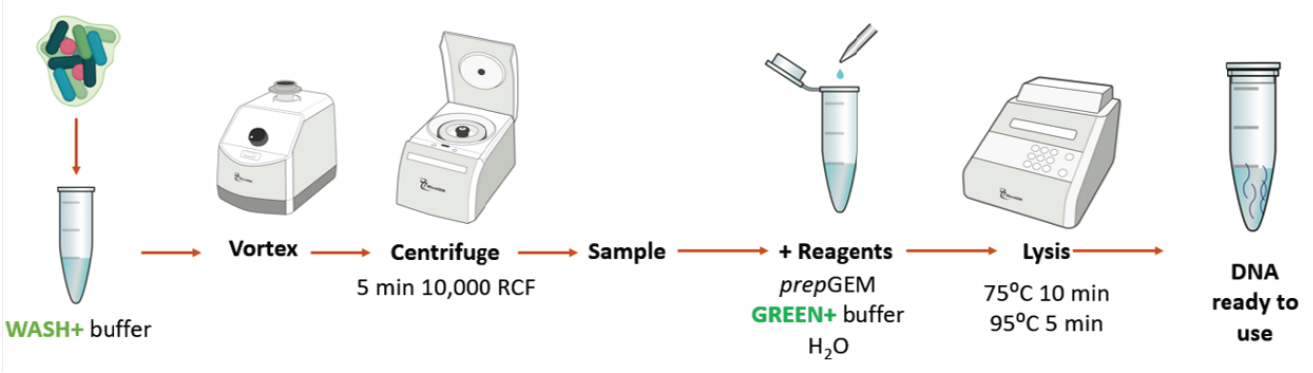


Figure 15. Workflow for DNA extraction from capsulated bacteria, colonies or biofilms

1. Pipette 400 µl of 1X WASH+ buffer into a 1.5 ml microfuge tube.

2. Lift a small amount of bacterial colony/biofilm with a sterile loop or pipette tip (up to 2 mm²) and resuspend in 1X WASH+ buffer.

3. Vortex vigorously to disperse the cells.

4. Centrifuge the cells at >10,000 RCF for 5 minutes.

5. Remove ALL of the supernatant and discard.

6. Resuspend the pellet in the appropriate extraction mixture below.
    a. Extraction mixture for **ONLY** Gram - samples
        i. 1 µl *prep*GEM
        ii. 10 µl 10X GREEN+ buffer
        iii. 89 µl Nuclease-free water
    b. Extraction mixture for samples that may include **BOTH** Gram - and Gram + bacteria
        i. 1 µl *prep*GEM
        ii. 1 µl Lysozyme
        iii. 10 µl 10X GREEN+ buffer
        iv. 88 µl Nuclease-free water

7. Incubate:
    a. Temperatures and times for **ONLY** Gram - samples
        i. 75°C for 10 min
        ii. 95°C for 5 min
    b. Temperatures and times for samples that may include **BOTH** Gram -ve and Gram +ve bacteria
        i. 37°C for 15 min
        ii. 75°C for 10 min
        iii. 95°C for 5 min

8. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and spin the extract before using.

DNA extraction from mucosal swabs and swabbed surfaces

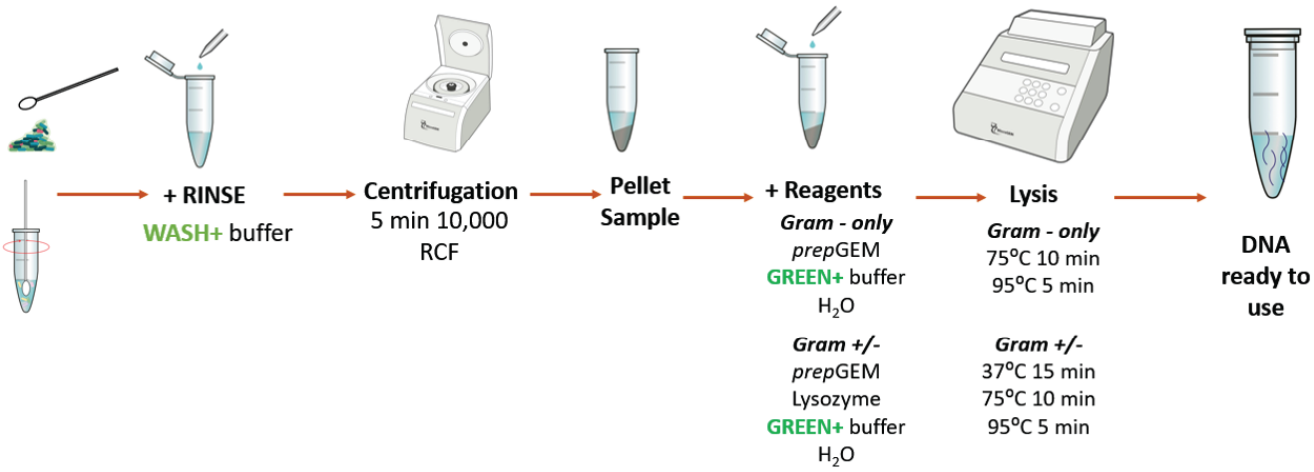


Figure 16. Workflow for DNA extraction from mucosal swabs or swabbed surfaces

1. Wash the swab for 30 seconds in 400 µl of 1X WASH+ buffer in a 1.5 ml microfuge tube using a rolling action. Squeeze the swab head against the wall of the tube to extract as much liquid as possible before discarding.
2. Vortex vigorously to disperse the cells.
3. Centrifuge the cells at > 10,000 RCF for 5 minutes.
4. Remove ALL of the supernatant and discard.
5. Resuspend the pellet in 100 µl extraction mixture.
    a. Extraction mixture for **ONLY** Gram - samples
        i. 1 µl *prep*GEM
        ii. 10 µl 10X GREEN+ buffer
        iii. 89 µl Nuclease-free water
    b. Extraction mixture for samples that may include **BOTH** Gram - and Gram + bacteria
        ii. 1 µl *prepG*EM
        ii. 1 µl Lysozyme
        iii. 10 µl 10X GREEN+ buffer
        iv. 88 µl Nuclease-free water
6. Incubate:
    a. Temperatures and times for **ONLY** Gram - samples
        i. 75°C for 10 min
        ii. 95°C for 5 min
    b. Temperatures and times for samples that may include **BOTH** Gram - and Gram + bacteria
        i. 37°C for 15 min
        ii. 75°C for 10 min
        iii. 95°C for 5 min
7. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and spin the extract before using.

DNA extraction from environmental samples (soil and stool)

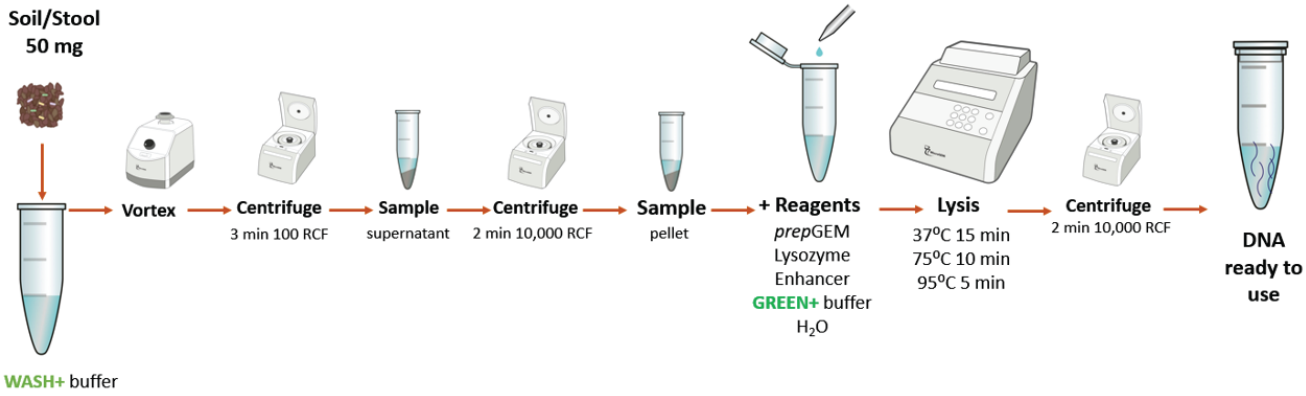


Figure 17. Workflow for DNA extraction from environmental samples (soil or stool)

Extracting DNA from soil samples is complicated due to the release of humic substances. This method relies on a short differential sedimentation of solids in a proprietary buffer.

1. Add up to 50 mg of soil or stool to a 1.5 ml microfuge tube.
2. Resuspend in 500 µl 1X WASH+ buffer.
3. Vortex vigorously for 1 minute to disperse cells.
4. Centrifuge at 100 RCF for 3 minutes.
5. Transfer the supernatant to a new tube.
6. Centrifuge at full speed > 10,000 RCF for 2 minutes.
7. Carefully pipette away all the WASH+ buffer.
8. Resuspend the pellet in 100 µl water.
9. Prepare extraction solution:
    a. 50 µl cell suspension
    b. 1 µl *prep*GEM
    c. 1 µl Lysozyme
    d. 1 Enhancer
    e. 10 µl 10X GREEN+ buffer
    f. 78 µl Nuclease-free water
10. Incubate:
    a. 37°C for 15 min
    b. 75°C for 10 min
    c. 95°C for 5 min
11. Centrifuge at full speed >10,000 RCF for 2 minutes.
12. The DNA is in the supernatant (DO NOT DISCARD) and ready for analysis. Transfer to a new tube before using. Use 1 µl or less for PCR/qPCR. In some cases, diluting your extract 1:10 can improve results.

E. Coli Rapid Plasmid Prep culture (prepGEM Bacteria kit, GREEN+ buffer)

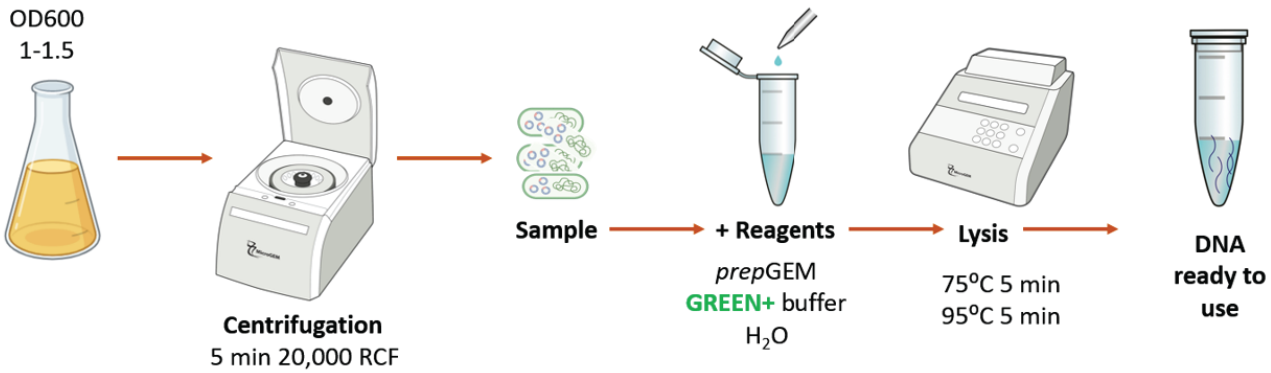


Figure 18. Workflow for DNA extraction from *E. Coli* plasmid prep.

1. Grow overnight cultures to OD600 of approximately 1-1.5.
2. Transfer 200 µl of the culture to a thin-walled PCR tube or 96-well plate.
3. Centrifuge at full speed (10,000 RCF) for 2 min and pipette away the medium from the pellet.
4. Resuspend the pellet with:
    a. 5 µl 10X GREEN+ buffer
    b. 44 µl Nuclease-free water
    c. 1 µl *prep*GEM
5. In a thermocycler, incubate:
    a. 75°C 5 min
    b. 95°C 5 min
    c. 4°C 2 min (Rapid cooling step should be included)
6. Centrifuge for 5 min at 20,000 RCF.
7. Discard the pellet. Transfer the supernatant containing the plasmid DNA to a fresh tube.
8. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and spin the extract before using. Use 1 µl or less for PCR/qPCR. In some cases, diluting your extract 1:10 can improve results.

TROUBLESHOOTING

This troubleshooting guide may be helpful in solving any problems that may arise, we have also included some protocol modifications to aid in extraction from samples not specifically addressed in the handbook, for further help please reach out to techsupport@exymesplc.com.

Troubleshooting post extraction application issues:

	Issue	Possible Causes	Potential Solutions
1	Inaccurate nucleic acid quantification	Exymes' s extracts are not compatible with Nanodrop, or UV based quantification methods.	As an alternative, use fluorometric quantification methods, such as Quantas, Qubit, PicoGreen-DNA etc., or quantitative PCR (qPCR).
2	Observed PCR or qPCR inhibition	Co-extraction of inhibitors from too much starting material or extracting longer than recommended.	Perform a new PCR with a lower amount of DNA (1-10 ng) as a template. 1 ng of DNA is usually sufficient for most PCR/qPCR applications.
		Too much DNA template added into PCR mix.	Add BSA to your PCR mix (5-20%, or 1 µl of a 10 mg/ml solution in 25 µl reaction).
		PCR/qPCR primers are not working effectively.	Carry out a serial dilution of the extracted RNA or DNA to allow for less DNA to be used as a template for the PCR.
			Ensure the problem is due to inhibition and not due to a low concentration of DNA (this can be done by looking at the slope and endpoint of a qPCR plot compared to a positive control). Running PCR controls will inform you as to whether the PCR mix and/or primers are working as intended.
			Centrifuge extract and aspirate supernatant into a new tube (this step is not normally needed) but if there is obvious solid material in extract this can reduce PCR efficiency.
			Carry out a downstream purification step such as using magnetic beads, silica columns, or centrifugal filters to further purify the DNA extract.
3	Observed DNA degredation after long-term storage	Exymes' s storage recommendations were not followed correctly.	If you do not intend to analyze the extracted DNA immediately, then add to the extract 10% of DNA extract volume as TE buffer (100 mM Tris [pH 7.5], 10 mM EDTA). Stability studies show that samples stored in this way can be kept at 4°C for a month. For long-term storage, we recommend storage at -20°C.

	Issue	Possible Causes	Potential Solutions
4	Concentration of extract too low for downstream application	Exymes' s temperature driven extraction method does not include concentration steps in the procedure. The concentration of the extract is dependent on the quality of the sample and the extraction volume used.	Scale up/down the protocol (use more or less than the suggested volume of extraction reagents) according to your concentration requirements. Lower total volume of extraction reagents will likely lead to an increased final extraction concentration. Alternatively, you could use a downstream concentration method such as magnetic beads or silica columns to concentrate your extract.
5	Observed low yield of extracted nucleic acids	Incorrect storage of starting material.	DNA yield is dependent on type, size, age and storage of starting material, lower yields can be expected from older improperly stored samples.
		Buffer incorrectly prepared. Sample/extraction buffer not mixed properly.	Make sure to vortex the buffer completely before use, if you see any precipitates, incubate at 37°C until dissolved, then use.
		Improper storage of extraction reagents. Incompatible quantification method used.	Mix your sample with the extraction buffer by pulse vortexing for 15 sec before heating.
		Extract still "hot" when adding DNase I reagents.	Ensure all of your sample is immersed in the extraction buffer. This may require increasing the volume of the extraction buffer.
			Increase the amount of sample input and/or the volume of the extraction reagents.
			For larger samples be sure to vortex and/or homogenize the sample prior to extraction.
			Ensure you are using a compatible quantification method such as fluorometric dyes, qPCR. (See #1 of the troubleshooting section above).
6	Extracted DNA is sheared	Exymes' s lysis method should result in minimal fragmentation of the DNA due to a gentle lysis method and the lack of bead or column-based purification.	
		The likely cause of fragmentation is due to improper sample handling such as the sample being too old and/or the sample undergoing multiple freeze/thaw cycles.	Try to use fresher samples. Avoid repeated freeze/thaw cycles of your starting material.
7	Observed interference with downstream enzymes	Exymes's proteinase insufficiently inactivated	Ensure the extraction mix, not just the heating apparatus, reaches 95°C for specified time in protocol (5 min).

	Issue	Possible Causes	Potential Solutions
8	Observed low yield of extracted nucleic acids from blood samples	Incorrect storage of starting material. Buffer incorrectly prepared. Sample/extraction buffer not mixed properly.	Make sure to closely follow the Exymes extraction protocols.
		EDTA present in blood sample.	If chelating agents like EDTA are present, add 10 mM CaCl˜ to the extraction (2 µl in a 100 µl reaction volume).
		Low white blood count (WBC) in blood sample.	May need to separate WBC from blood sample prior to extraction.
9	Observed background signal when using universal primers (16S rRNA gene primers)	Interference from PCR reagents	Please refer to literature for best practices to minimize interference from contaminating DNA in PCR reagents1

Troubleshooting for atypical sample input extraction issues:

	Sample Type	Sample Issue	Potential Solutions
1	Dried tissue	Dried tissue/sample absorbs too much of the extraction mix and results in an inadequate extract volume.	Rehydrate the tissue in water or extraction buffer (without enzyme) at 4°C overnight prior to extraction to limit the amount of extraction mix absorption.
2	Tough tissue	Tougher tissue types (i.e., lung, kidney) give lower DNA yield.	Increase volume of enzyme in the extraction mix (e.g., 2X).
			Increase the 75°C incubation time to extract more DNA. A 30-minute 75°C incubation should be sufficient.
3	Hair follicles	Unsure how many hair follicles should be used for extraction.	1 - 3 hair follicles should be sufficient to extract enough DNA extraction for PCR. We do not recommend the addition of more than 10 hair follicles in the extraction mix.
4	Tissue extraction	Unsure how much tissue to use as starting material in the extraction.	We recommend <5 mg or <2 mm² of solid tissue (such as mouse tails). It is generally better to work with a smaller amount of solid tissue to limit the co-extract of too many PCR inhibitors.

	Sample Type	Sample Issue	Potential Solutions
5	Blood	Are all blood storage (anti-coagulants) compatible with Exymes extractions?	Heparin (green top tubes) and sodium citrate (light blue top tubes) work without modification to the protocol. EDTA is a chelating agent, so if using EDTA (purple top tubes) add 2 µL CaCl2 [10 mM CaCl2] per 100 µl extraction.
6	Insects	Would like intact/non-homog-enized insect.	Increase 75°C incubation time to 120 min[1].
7	Swabs	Some swabs have binding agents or fine particles that can affect PCR.	Test swab for inhibition by washing in water and adding to PCR control.
		Buccal cells sediment rapidly in wash solution.	Mix suspension immediately before transferring to extraction buffer.
8	Avian blood	Avian blood cells are nucleat-ed.	Increase 75°C step to 15 min[2].
9	Tough tissue type (animal)	Low yield from certain animal tissue types (pancreas, spleen, lung).	Add 0.5% non-ionic detergent such as Triton X-100.
10	Fish tissue or fins	Fish tissue can be processed.	2 mm^3 fragment or 2 mm^2 punch Lysis: 2.5 l 10X RED+ buffer 2.5 µl Histosolv 1 µl prepGEM 19 µl Nucle-ase-free water 52°C 5 min, 75°C 10 min, 95°C 5 min
11	Bird feather	Bird feathers can be pro-cessed.	2-5 mm fragment of calamus (quill) increase prepGEM to 2 µl (50 µl total extraction volume).
12	Blood volume 200 µl	Blood samples of 200 µl mix with 2 ml RBC Lysis buffer and incubate for 5 min, centrifuge 3 min 3,000g, resuspend pel-let in extraction buffer.	10 µl 10X RED+ buffer 10 µl Histo-solv 3 µl prepGEM Nuclease-free water (to 100 µl) 52°C 5 min 75°C 10 min 85°C 2 min (lower temp used to prevent coagulation)
13	Saliva volume 300 µl	Saliva was collected in 50 ml tubes, 200 µl PBS was added, centrifuged 3 min 16,000 RCF. Resuspend pellet in extraction mixture.	10 µl 10X BLUE buffer 1.5 µl prepGEM 38.5 µl saline solution (0.9% NaCl) 75°C 10 min 95°C 5 min

ORDERING / DISTRIBUTION

Information regarding ordering is provided below. To contact our Commercial Team, please email commercial@exymesplc.com.

Kit Type	Kit Reaction Number	Product Code
*prep*GEM Universal 100 reactions	*prep*GEM 10X BLUE buffer 10X ORANGE+ buffer 10X RED+ buffer Histosolv	PUN0100
*prep*GEM Universal 500 reactions	*prep*GEM 10X BLUE buffer 10X ORANGE+ buffer 10X RED+ buffer Histosolv	PUN0500
*prep*GEM Universal 1000 reactions	*prep*GEM 10X BLUE buffer 10X ORANGE+ buffer 10X RED+ buffer Histosolv	PUN1000
*prep*GEM Bacteria 100 reactions	*prep*GEM 5X WASH+ buffer 10X GREEN+ buffer Enhancer Lysozyme	PBA0100
*prep*GEM Bacteria 500 reactions	*prep*GEM 5X WASH+ buffer 10X GREEN+ buffer Enhancer Lysozyme	PBA0500
*prep*GEM Bacteria 1000 reactions	*prep*GEM 5X WASH+ buffer 10X GREEN+ buffer Enhancer Lysozyme	PBA1000

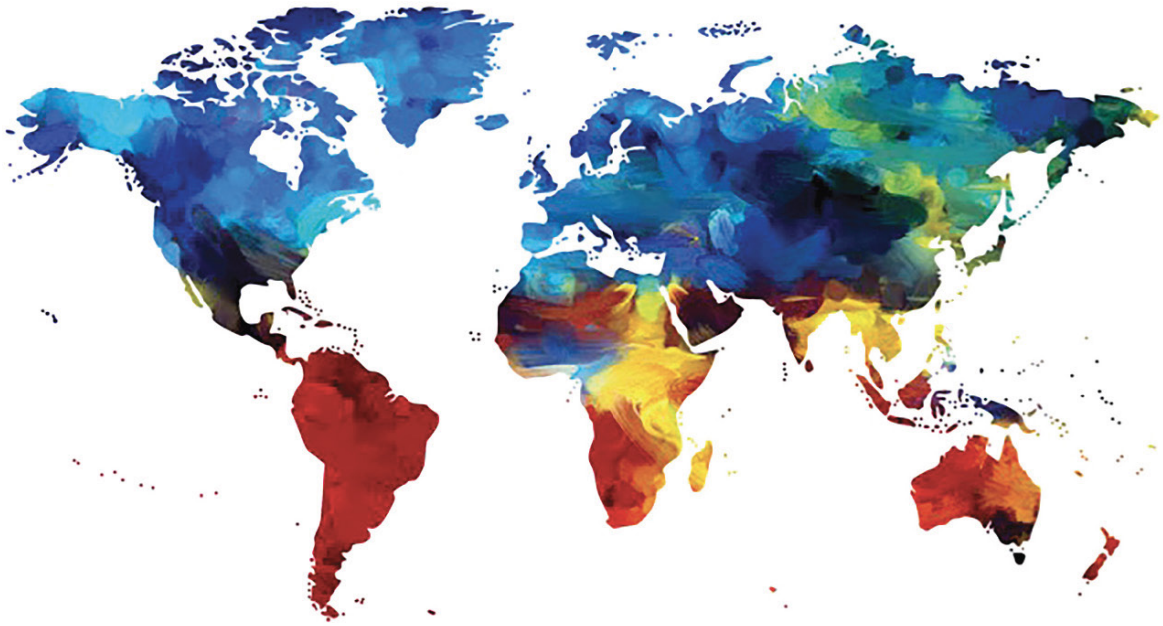
Distribution

Information about Exymes' s distributors can be found on the Exymes Website: www.exymesplc.com

References

(1) Amouroux, P.; Crochard, D.; Correa, M.; Groussier, G.; Kreiter, P.; Roman, C.; Guerrieri, E.; Garonna, A.; Malausa, T.; Zaviezo, T. Natural Enemies of Armored Scales (Hemiptera: Diaspididae) and Soft Scales (Hemiptera: Coccidae) in Chile: Molecular and Morphological Identification. PLOS ONE 2019, 14 (3), e0205475. https://doi.org/10.1371/journal.pone.0205475.

(2) Patel, S.; Millar, C. A Rapid DNA Extraction Method for Avian Blood Samples. N. Z. 1991, 2. Crime/ Universal Kit and Qiagen QIAamp Investigator Kit: A Comparison and Optimization Study of DNA.



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At Exymes, our goal is to provide ultra fast nucleic extraction enabling a broader spectrum of users to both employ and benefit from molecular techniques. The first step is the simplification of sample preparation. Our temperature-driven, single-tube process simplifies and reduces the number of steps for traditional nucleic acid extraction, resulting in high-quality extracts with reduced contamination and high yields - all in minutes, not hours.

Committed to minimal packaging, reduced plastic use and a sustainable world

Exymes