BIO 101 Protocol

web

G NOME®

2010-200	**10 preps**
2010-400	**25 preps**
2010-600	**100 preps**

- **Rapid 3 Step Procedure for Isolating Genomic DNA**
- **No Phenol or Chloroform Extractions**
- **DNA from Prokaryotic and Eukaryotic Cells and Tissues**
- **Ideally Suited to Multiple RFLP Assays or PCR and Cloning**
- **Most Preps can be Finished in Less than 1 Hour**

Shipping & Storage:

The kit is shipped at ambient temperature. However, upon arrival store **RNase Mixx** and **Protease Mixx** at 4°C. A few days at ambient temperature does not affect **RNase Mixx** or **Protease Mixx** activity. The **Protease Mixx** is a suspension. Cloudiness or visible aggregates may appear and can increase with time but do not affect activity.

***Revision #**: 2010-999-7H02P*

1070 Joshua Way, Vista CA 92083, USA
Tel: (760) 598-7299 • Fax (760)598-0116
E-mail: technical@bio101.com
Website: www.bio101.com

For technical support call (800) 424-6101

G NOME® Protocol

Kit Components 3
2010-200 (10 preps) 3
2010-400 (25 preps) 3
2010-600 (100 preps) 3
Introduction 4
Protocol 5
Suggested Cell Preparation Protocols 6
Bacteria 6
Plant Tissue 6
Blood Cells 6
Mammalian Tissue 6
Yeast Cells 7
How to Monitor Yeast Cell Lysis to Indicate Spheroplast Formation 7
Product Use Limitation & Warranty 8
General Information 8

Kit Components

2010-200 (10 preps)

Name	Volume	Cat #
Cell Lysis/Denaturing Solution	1 ml	2010-201
RNase Mixx	0.6 ml	2010-202
Protease Mixx	0.3 ml	2010-203
"Salt-Out" Mixture	6 ml	2010-204
Cell Suspension Solution	20 ml	2010-205

2010-400 (25 preps)

Name	Volume	Cat #
Cell Lysis/Denaturing Solution	2.5 ml	2010-401
RNase Mixx	1.4 ml	2010-402
Protease Mixx	0.8 ml	2010-403
"Salt-Out" Mixture	15 ml	2010-404
Cell Suspension Solution	50 ml	2010-405

2010-600 (100 preps)

Name	Volume	Cat #
Cell Lysis/Denaturing Solution	10 ml	2010-601
RNase Mixx	5.5 ml	2010-602
Protease Mixx	3 ml	2010-603
"Salt-Out" Mixture	60 ml	2010-604
Cell Suspension Solution	200 ml	2010-605

Introduction

The **G NOME®** kit is used to quickly and efficiently isolate high molecular weight genomic DNA from whole blood, cells, and tissues of any type, including bacteria, yeast, plant, and animal cells. Each preparation with the **G NOME®** kit yields up to 100 µg of genomic DNA. The DNA isolated by the **G NOME®** procedure is suitable for restriction enzyme digestion or PCR amplification in as little as 1 hour after cell lysis.

The **G NOME®** kit is available in 3 sizes to suit your needs: 10, 25, and 100 preparations/kit. The **G NOME®** kit utilizes **RNase Mixx** to eliminate RNA immediately after lysis, and **Protease Mixx** to rapidly degrade cellular proteins. This is followed by a proprietary “salting-out” technique which precludes the need for phenol, chloroform, or other organic extractions. The DNA can either be spooled, removed with forceps, or centrifuged and dissolved in TE.

The **G NOME®** kit contains the reagents to homogenize your cells or tissue and purify high molecular weight DNA. Ethanol is the only reagent not supplied in the kit. Lysing enzymes for yeast or bacterial cells are available separately, contact BIO 101 for information.

Protocol

1. Bring cells*/tissues to a final volume of 1.85ml in **Cell Suspension Solution**. (Use a 15 ml clear plastic tube for efficient mixing). Mix until the solution appears homogeneous.

2. Add 50µl of **RNase Mixx**, mix thoroughly.

3. Add 100µl of **Cell Lysis/Denaturing Solution****, mix well.

4. Incubate at 55°C for 15 minutes.

5. Add 25µl **Protease Mixx**, mix thoroughly. (Note: If precipitate is visible in **Protease Mixx** suspension, pulse spin and use 25 µl of supernatant.)

6. Incubate at 55°C for 30 to 120 minutes. (The longer time will result in minimal protein carry over and will also allow for substantial reduction in residual protease activity.)

7. Add 500µl **"Salt-Out" Mixture**, mix gently yet thoroughly. Divide sample into 1.5ml tubes. Refrigerate at 4°C for 10 minutes.

8. Spin for 10 minutes at maximum speed in a microcentrifuge (at least 10,000 x g). Carefully collect the supernatant, avoid the pellet. If a precipitate remains in the supernatant, spin again until it is clear. Pool the supernatants in a 15 ml (or larger) clear plastic tube.

9. To this supernatant, add 2 ml TE buffer and mix. Then add 8mls of 100% ethanol. If spooling the DNA, add the ethanol slowly and spool the DNA at the interphase with a clean glass rod. If centrifuging the DNA, add the ethanol and gently mix the solution by inverting the tube. Spin for 15 minutes at 1000-1500xg. Eliminate the excess ethanol by blotting or air drying the DNA.

10. Dissolve the genomic DNA in TE (10mM Tris pH 7.5, 1mM EDTA).

See attached protocols for preparing specific cell/tissue types for G NOME® protocol.

***If a precipitate forms in your stock solution, heat to 60°C to dissolve before using.*

Suggested Cell Preparation Protocols

The **G NOME®** kit is designed to yield 100ug of genomic DNA based on the following amounts of starting material and cellular prelysis methods. However, the **G NOME®** protocol is designed to accommodate a variety of cell preparation methods with the following provided as a guideline.

Bacteria

5 x 10^{-15}g DNA/*E. coli*, K12*

Inoculate a single colony of bacteria into 5ml of growth media (use antibiotic if appropriate). Grow overnight at 37°C with shaking. Pellet cells at 3000 rpm for 5 minutes. The wet pellet weight should be approximately 20 - 50 mg. Continue with step 1 of the general **G NOME®** protocol.

Plant Tissue

5.1 x 10^{-12}g DNA/tobacco plant cell*

Place 250mg of fresh tissue in a microcentrifuge tube and immerse in liquid nitrogen to freeze. Grind the tissue to a fine powder with a small conical-shaped pestle, adding liquid nitrogen as necessary to keep the tissue frozen. Add 500µl **Cell Suspension Solution** and continue to grind tissue with the tube on ice until it appears homogeneous. Add an additional 1.35 ml **Cell Suspension Solution**. Continue with step 2 of the general **G NOME®** protocol.

Blood Cells

Start with 2-5 x 10^7 nucleated white cells. Continue with step 1 of the general **G NOME®** protocol. A **G NOME®** Kit specifically designed for DNA isolation from whole blood is also available from BIO 101 (Cat #'s 2011-200, 2011-400, and 2011-600).

Mammalian Tissue

3.5 (human), 3.2 (rat) x 10^{-12}g DNA/cell*

Completely homogenize enough tissue to yield approximately 2-5 x 10^7 cells in 1 ml of **Cell Suspension Solution**. This will vary between 50 to 250mg of tissue depending upon the density of cells in the type of tissue. Continue with step 1 of the general **G NOME®** protocol.

**** Approximate DNA content per cell.***

Yeast Cells

1.6 x 10^{-14}g DNA/cell, *S. cerevisiae.**

Lysing reagents for yeast cells are available separately, call for information on the **Yeast Cell Lysis Kit** (BIO 101 Catalog # 2015-200, 2015-400 & 2015-600).

Brief Protocol of Yeast Cell Lysis Kit

Grow yeast in YPD (1% yeast extract, 2% neopeptone; 2% sterile glucose, added after autoclaving) to a cell density of 2 x 10^7 cells/ml. This usually takes 24-36 hours at 30^0 C for a 100 ml culture (**It is important to use the yeast culture before it reaches 10^8 cells/ml, at which point the yeast are much more difficult to lyse**). Pellet yeast at 600g for 5 minutes, pellet weight should be approximately 1g. Discard supernatant and resuspend in 1ml of **Yeast Suspension Buffer**. Transfer to a microcentrifuge tube and spin for 10 seconds. Discard supernatant and resuspend in 500µl **Yeast Enzyme Enhancer**. Add 10µl **Yeast Enzyme Salts** and 80µl **Spheroplasting Enzyme Mixx**. Incubate at 37°C until spheroplast formation is complete (usually 12-30 minutes). Start monitoring spheroplast formation after 12 minutes: Place 20µl of **spheroplast indicating solution** at one end of a glass slide and 20µl of **spheroplast control solution** at the other end. After 12 minutes mix 2µl of yeast cells with each solution, cover with cover slips, and observe under the microscope. Spheroplasting is sufficient when less than 5% of the control number of cells remain intact in the visual field with the indicating solution. Allowing the reaction to proceed after spheroplasting is complete is not beneficial to subsequent procedures.

Continue with step 1 of the general **G NOME®** protocol.

Approximate DNA content per cell.

Product Use Limitation & Warranty

Unless otherwise indicated, this product is for research use only. Purchase of BIO 101 products does not grant rights to reproduce, modify, repackage the products or any derivative thereof to third parties. BIO 101 makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and BIO 101's sole liability hereunder shall be limited to, at our option, product credits, refund of the purchase price of, or the replacement of all material(s) that does not meet our specification. By acceptance of the product, Buyer indemnifies and holds BIO 101 harmless against, and assumes all liability for the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refunds or replacement is conditioned on Buyer notifying BIO 101 within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (days) shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

General Information

BIO 101 is a pioneer in developing kits for molecular biology research. We introduced the **GENECLEAN**® Kits in 1986 and have since been manufacturing products to bring convenience into your research. Our goal is to make your life easier by simplifying the complexities of lab work.

Technical Support and Ordering Information

(800) 424-6101 (Toll Free - Continental USA)
(760) 598-7299 (Outside USA)
(760) 598-0116 (Fax line)
technical@bio101.com
order@bio101.com

Office Hours:

6:30 am - 6:00 pm P.S.T. (Mon - Fri)

Mailing Address:

BIO 101, Inc.
P.O. Box 2284
La Jolla, CA 92038-2284 USA