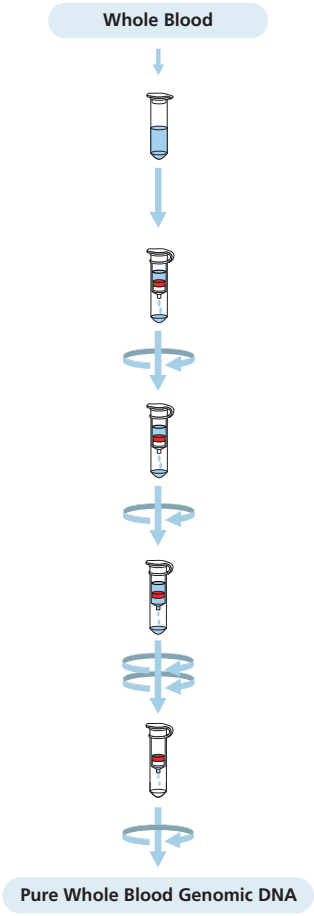
NA2100	NA2110	NA2120

GENELUTE BACTERIAL GENOMIC DNA KIT PROTOCOL

I. Gram Negative Bacteria



1 Harvest cells

- ❑ Pellet 1.5 ml of bacterial broth culture at 12,000-16,000 *x g* for 2 minutes, discard media. When using enriched media please refer to technical bulletin.

2 Resuspend cells

- ❑ Resuspend pellet in 180 µl Lysis Solution T. *Optional: Add 20 µl RNase A, incubate RT for 2 min.*

3 Lyse cells

- ❑ Add 20 µl Proteinase K to cell suspension, vortex or pipet to mix. Incubate at 55 °C for 30 min.
- ❑ Add 200 µl Lysis Solution C, vortex or pipet to mix. Incubate at 55 °C for 10 min.

4 Prepare column

- ❑ Add 500 µl of Column Preparation Solution to each binding column.
- ❑ *Spin at ≥12,000 x g, for 1 min.* Discard flow-through.

5 Bind DNA to column

- ❑ Add 200 µl ethanol to the lysed cells, vortex or invert to mix.
- ❑ Transfer EtOH mixture to binding column. *Spin at ≥6500 x g for 1 min.*

6 Wash column

- ❑ Transfer column to new collection tube. Add 500 µl Wash Solution O to column. *Spin at ≥6500 x g for 1 min.*
- ❑ Transfer column to new collection tube. Add 500 µl Wash Solution to column. *Spin at ≥12,000 x g for 3 min. to dry column.*

7 Elute DNA

- ❑ Transfer column to new collection tube. Add 200 µl of Elution Solution. *Spin at ≥6500 x g for 1 min. Optional: Repeat in new or same tube.*

Please refer to the technical bulletin for additional information.

SIGMA®

Order/Customer Service *Phone* 1-800-325-3010 *Fax* 1-800-325-5052 *Technical Service* 1-800-325-5832
World Headquarters 3050 Spruce St. St. Louis MO 63103 (314)771-5750 www.sigma-aldrich.com

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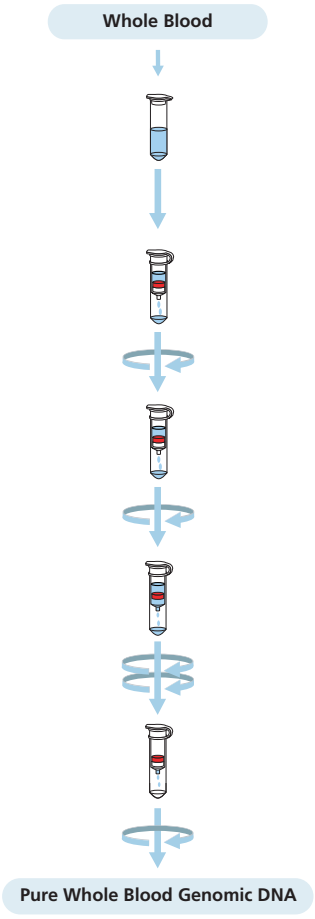

I. Gram Positive Bacteria

❑ **Prepare Lysozyme Solution**
(Lysozyme sold separately - Product Code L 7651)
Prepare a 2.115 x 10^6 unit/ml Lysozyme Solution using the included Gram Positive Lysis Buffer as the diluent. 200 µl of Lysozyme Solution is needed for each prep. Make extra to account for pipetting error.

1 Harvest cells

❑ Pellet 1.5 ml of bacterial broth culture at 12,000-16,000 *x g* for 2 minutes, discard media. When using enriched media please refer to technical bulletin.

2 Digest cell wall

❑ Resuspend pellet in 200 µl Lysozyme Solution and incubate at 37 °C for 30 min.
Optional: Add 20 µl RNase A, incubate RT for 2 min.

3 Lyse cells

❑ Add 20 µl Proteinase K and 200 µl Lysis Solution C to cell suspension, vortex or pipet to mix. Incubate at 55 °C for 10 min.

4 Prepare column

❑ Add 500 µl of Column Preparation Solution to each binding column.
❑ *Spin at ≥12,000 x g, for 1 min.* Discard flow-through.

5 Bind DNA to column

❑ Add 200 µl ethanol to the lysed cells, vortex or invert to mix.
❑ Transfer EtOH mixture to binding column. *Spin at ≥6500 x g for 1 min.*

6 Wash column

❑ Transfer column to new collection tube. Add 500 µl Wash Solution O to column. *Spin at ≥6500 x g for 1 min.*

7 Repeat wash

❑ Transfer column to new collection tube. Add 500 µl Wash Solution to column. *Spin at ≥12,000 x g for 3 min. to dry column.*

8 Elute DNA

❑ Transfer column to new collection tube. Add 200 µl of Elution Solution. *Spin at ≥6500 x g for 1 min.*
Optional: Repeat in new or same tube.

Please refer to the technical bulletin for additional information.

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