

Jun 26, 2025

Isolation of genomic DNA from Gram-positive cocci using laboratory reagents

DOI

<https://dx.doi.org/10.17504/protocols.io.eq2lyqm5wvx9/v1>

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Protocol Citation: Matthew Sullivan 2025. Isolation of genomic DNA from Gram-positive cocci using laboratory reagents. protocols.io <https://dx.doi.org/10.17504/protocols.io.eq2lyqm5wvx9/v1>

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Protocol status: Working

We use this protocol and it's working

Created: June 25, 2025



Last Modified: June 26, 2025

Protocol Integer ID: 221023

Keywords: isolation of genomic dna, genomic dna from gram, positive cocci, genomic dna, positive bacteria, bacterial culture, dna, high molecular weight dna, using laboratory reagent, supernatant by isopropanol precipitation, isopropanol precipitation

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Abstract

A method used to isolate the genomic DNA from Gram-positive bacteria. The bacterial culture is lysed, proteins degraded and removed with CTAB, and high molecular weight DNA is recovered from the supernatant by isopropanol precipitation.



Materials

Equipment:

- 37°C shaking incubators
- Shaker
- 10mL Falcon tubes
- Microcentrifuge
- Autoclave
- 1.5ml sterile Eppendorf tubes
- Fume hood

Reagents:

- TE Buffer
- 10% SDS
- 20mg/mL proteinase K
- 5M NaCl
- CTAB/NaCl solution
- 24:1 chloroform/isoamyl alcohol
- 25:24:1 phenol/chloroform/isoamyl alcohol
- Isopropanol
- 70% ethanol
- RNase A 100mg/ml
- Mutanolysin or Lysostaphin

Preparation

- 1 TE Buffer: 10mM Tris, 1mM EDTA. Made from 1M Tris (pH 7.5) and 250mM EDTA (pH 8.0).
- 2 CTAB/NaCl Solution: 10% CTAB in 0.7M NaCl. Dissolve 4.1g NaCl in 80 ml H₂O. Slowly add 10g CTAB while heating and stirring at 65°C to dissolve. Make up to 100ml and keep at 65°C.
- 3 Lysis buffer: 50 mM Tris-HCL, 0.145 M NaCl, pH 7.5

Procedure

- 4 Grow a pure culture of the isolate in the preferred medium to stationary phase overnight at 37°C.
- 5 Spin up to 10 ml of the culture in a centrifuge at max speed for 2 min so that there is a compact pellet. Discard supernatant.
- 6 Resuspend pelleted bacteria in 395 µl Lysis buffer by pipetting up and down to suspend the cells in solution
 - For *Streptococcus*, add 10 uL of mutanolysin (1U/uL) and mix well.
 - For *Staphylococcus*, add 5 uL of lysostaphin (2 mg/mL) and mix well.
- 7 Incubate for 1.5 hours at 37°C to weaken the Gram-positive cell wall.
- 8 Add the following to the enzyme-treated cells:
 - 154 µl of Lysis buffer
 - 30 µl of 10% SDS
 - 6 µl of 20 mg/ml proteinase K
 - 2 µl of RNase A 100 mg/ml.Mix thoroughly and incubate 1 h at 37°C. Lyses the cell wall and degrades protein and RNA.
- 9 Add 100 µl of 5 M NaCl and mix thoroughly. Mix using repeated pipetting for about 3-4 min. Very important step and needs to be mixed correctly.
- 10 Add 80 µl CTAB/NaCl solution. Mix thoroughly and then incubate 10 min at 65°C. Every 2-3 minutes, mix thoroughly by inverting the tubes up and down for 30 seconds. This



step removes cell wall debris, denatured protein, and polysaccharides by complexing them with CTAB.

- 11 Add an equal volume (700 μ l) of chloroform (in a fume hood), mix thoroughly and spin 5 min at max speed in a microcentrifuge (e.g. 13,000 x rpm). This step removes the CTAB-protein precipitates. There will be three layers: chloroform on the bottom, a white interface, and the supernatant containing DNA on top.
- 12 Remove the top, aqueous, viscous supernatant to a new, labelled 1.5 mL Eppendorf tube, leaving the white interface and bottom layer behind (~800 μ L). Discard the contents of the old tube into a phenol/chloroform liquid waste container and the contaminated plastic in the phenol/chloroform solid waste container
- 13 Add an equal volume of phenol/chloroform/isoamyl alcohol using the fume-hood, check the lids are firmly closed and mix the samples thoroughly by vigorous inversion or vortexing for 10-30s. Spin at max speed in microcentrifuge for 5 min. Repeat if necessary.
- 14 Transfer the top aqueous supernatant to a new, labelled 1.5 mL Eppendorf tube. Precipitate DNA by adding 0.6 volumes of isopropanol (eg. volume of sample * 0.6 = volume of isopropanol to add). Mix by gently swirling the tube to mix the solvent. As you mix the isopropanol, you should be able to visibly see the DNA precipitate as a white stringy substance.
- 15 Place the tubes in the micro centrifuge with the hinges facing vertically upward, and then spin the precipitate for 5 min at max speed in a microcentrifuge. The DNA should form a small, slightly opaque pellet directly below the hinge of the tube if oriented correctly.
- 16 Gently remove all supernatant with a P1000 pipette. Add 500 μ l 70% ethanol, mix gently by inversion. You should be able to see the DNA pellet dislodge and become floating in the solution. Orient the tubes in the centrifuge as in step 12 and then spin 5 min at max speed.
- 17 Carefully remove the tubes from the centrifuge without disturbing them too much. Remove supernatant, first by using a P1000 to take 90% of the liquid, then switch to a P200, then a P20, so that you can remove 99.9% of the remaining liquid, BUT, be careful not to disturb the DNA pellet. Air-dry the DNA pellet in the tube by incubating at 37°C in an incubator with tubes slightly open for 5 minutes.
- 18 Redissolve the pellet in 200 μ l H₂O. Dissolve by mixing well and heating at 50°C for 30 minutes. Alternatively, store at 4°C overnight in the fridge to aid solubilisation of DNA.
- 19 Quantify genomic DNA by UV-vis spectrophotometric (e.g. Nanodrop) or fluorescence (e.g. Qubit) methods and store the remaining DNA at -20°C.



- 20 If using DNA for PCR, make a 1:10 dilution (e.g. 5 µl in 45 µl H₂O) and use as a working solution. This avoids freeze-thawing your main DNA stock too often. B

Acknowledgements

Glen Ulett and Deepak Ipe, Griffith University