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FLEX(+) DNA Extraction Protocol for Nanopore Sequencing V.1

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Disclaimer

Please note, this protocol has only been validated for the following organisms (despite potential utility in all Gram-negative bacterial extractions): MRSA, Groups A,B, C and G Streptococci, Streptococcus pneumoniae, Enterococcus faecalis, and Enterococcus faecium.

Abstract

To rapidly extract high-quality, long-fragment genomic DNA from Gram-positive bacteria (e.g., *Staphylococcus*, *Enterococcus*, β-hemolytic *Streptococcus*, viridans group streptococci) suitable for downstream molecular workflows (e.g., long-read sequencing, qPCR, library prep, and particularly *Nanopore*). This protocol has been validated by Mount Sinai, Department of Clinical Microbiology, and is currently in use prior to Nanopore sequencing workflows.

Attachments

PDF

Flex(+) DNA Extracti...

147KB

Guidelines

5. **Definitions and Abbreviations:**
   - **FLEX(+)**: Fast, Long-fragment EXtraction for Gram-positive bacteria.
   - **SPRI**: Solid-Phase Reversible Immobilization paramagnetic bead chemistry.
   - **RT**: Room temperature (20–25 °C).

Materials

Reagents

- FLEX TE buffer (10 mM Tris, 1 mM EDTA), pH 8.00, filtered
- Lysozyme, 100 mg/mL stock. (in ddH2O or TE Buffer)
- Proteinase K, 20 mg/mL stock. (in ddH2O or TE Buffer)
- SPRI-Select paramagnetic beads (Beckman Coulter)
- Molecular-grade water (ddH2O)
- Fresh 80% ethanol (v/v) for bead washes.

Consumables

- Qiagen 0.5 mm glass **PowerBead** tubes (or equivalent) with screw caps.
- Sterile 1 µL loop (or 10 µL loop).
- 1.5 mL low-bind microcentrifuge tubes or 0.8 mL midi 96-well plate.
- Filter pipette tips (20–1000 µL).

Equipment

- Vortex with **Qiagen PowerBead vortex adapter** top
- Temperature blocks or water baths at 37 °C and 56 °C (calibrated).
- Magnetic racks:
  - Thermo **DynaMag** (for 1.5 mL tubes) OR Illumina-style **96-well magnet** (for midi plates).
- Qubit Fluorometer (HS dsDNA assay) and Nanodrop (or equivalent spectrophotometer).
- Fragment analysis system (e.g., TapeStation, Femto Pulse) as available.

Before start

Specimen Collection

- **Specimen**: Single pure isolate from a confluent purity plate (18–24 h typical, organism-appropriate conditions).
- **Transport/Storage**: Room temperature (RT) bench-top during setup
- **Rejection criteria**: Mixed culture, plate age under 72 h (discretionary), or visible contamination.

FLEX(+) Extraction Preparation

1 Label PowerBead tube(s), Lo-Bind Tubes, plate wells or 1.5ml Eppendorf tubes, keeping each set in the same format/order.

2 Pre-equilibrate temperature blocks/water baths to 37 °C and 56 °C.

3 Attach Qiagen Vortex Adapter top to Vortex Genie.

4 Calculate how much of each enzyme is required for given batch size (See Table 1).

5 Table 1:

	10% Sarkosyl Solution	Proteinase K 20mg/mL
For All Species	8ul per sample	5ul per sample

6 Calculate how much of each reagent is required for given batch size (See Table 2).

7 Table 2:

A	B	C	D
TE Buffer (10 mM Tris, 1 mM EDTA), pH 8.00)	SPRI Select Beads (Beckman Coulter)	Fresh 80% Ethanol	Molecular Grade Water (ddH2O)
300ul per sample	225ul per sample 1200ul per sample 100ul per sample	1200ul per sample	100ul per sample

8 Remove enzymes from the freezer and allow them to thaw. Keep at 4 °C until ready for use.

9 Aliquot 300 µL of FLEX TE into each 0.5mm Glass Powerbead Tube.

10 Inoculate each Powerbead tube with a heaping 1 µL loop, or half of a 10 µL loop, avoiding agar carryover.

11 Mix by spinning the loop inside the tube.

12 If beads or inoculum have stuck to the tube's side wall, tap several times until it settles to the bottom of the tube.

Enzymatic Lysis

13 Pipette 4 µL of Lysozyme (L100) (Using a P10) into each Powerbead tube, directly into the solution.

14 Slowly and gently pipette mix up and down 2-3 times.

15 Place Powerbead tube into a 37 °C dry incubator or a 37 °C water bath, maintaining the original layout of the tubes on their respective racks.

16 Incubate for 15 min (This is a good time to begin thawing Proteinase K).

17 Remove from the incubator.

Mechanical Lysis

18 Insert Powerbead tubes into a Qiagen Vortex Adapter Top, with the cap side facing inward.

19 Place the top onto a Vortex Genie.

20 Set the vortex speed to 9, and allow the top to spin for 5 minutes.

21 Turn off the vortex and remove the adapter top.

22 Place the tubes back onto a rack, maintaining the original format.

Denaturation of Interfering Proteins

23 Pipette 5 µL of Proteinase K (20 mg/ml) into each Powerbead tube, directly into solution.

24 Slowly and gently pipette mix up and down 2-3 times.

25 Place Powerbead tube into a 56 °C dry incubator or a 56 °C water bath, maintaining the original layout of the tubes on their respective racks.

26 Incubate for 15 min.

27 Remove from incubator.

Removal of Contaminants/DNA Binding

28 Transfer 225 µL of solution from Powerbead tubes into either a 0.8 ml, 96 well MIDI Plate (for e36 samples) OR a 1.5 ml Eppendorf tube (for c36 samples).

29 Add 225 µL of SPRI Select Beads into each well/tube.

30 Mix by pipetting up and down 3-5 times.

31 Place onto appropriate magnet (96 well Illumina Magnet for MIDI plates, Thermo DYNAMag Magnet for Eppendorfs).

32 Leave on magnet until lysate appears clear (3-5 min), and a pellet has formed on the well/tube side wall.

33 With the plate/tube STILL on the magnet, carefully remove the supernatant with a P1000 set to 1000 µL. Avoid touching the pellet. If any pellet is sucked into the pipette, put it back into the well/tube, towards the magnet side, and leave for another 2 minutes before removal of supernatant.

Ethanol Washes x 2

34 Remove the tube/plate from the magnet.

35 Pipette 600 µL of 80% ethanol into each well/tube directly onto the pellet.

36 Resuspend pellet in the ethanol by pipetting up and down until homogenous.

37 Place tube/plate back onto the magnet.

38 Allow pellet to reform (2-3 min).

39 Remove supernatant with a P1000 set to 1000 µL.

40 Remove tube/plate from magnet again.

41 Pipette 600 µL of 80% ethanol into each well/tube directly onto the pellet.

42 This time, do not resuspend the pellet fully by mixing.

43 Place the tube/plate back onto the magnet.

44 Wait 2-3 min.

45 Remove supernatant with a P1000 set to 1000 µL, avoiding the pellet.

46 Allow pellet to air dry for 10 min, monitoring and wicking away any residual pooling; do not over-dry to cracking.

47 Once dry, take the tube/plate off the magnet.

Final DNA Elution

48 Add 100 µL of Molecular Grade Water (ddH2O) directly onto the pellet.

49 Resuspend pellet fully, by mixing up and down with a pipette.

50 Place tube/plate back onto the magnet.

51 Wait 2-3 min for pellet to reform.

52 Transfer all 100 µL of the supernatant into a labelled Lo-Bind Eppendorf tube, this is your final DNA solution, which can now be stored at -80 °C for up to 10 years.

53 Discard the remaining tubes/plate with the pellet.

Eluate Quality Control

54 Quantify DNA with a Qubit Fluorometer, using an eluate input of 4 µL, and High Sensitivity Reagent input of 196 µL. (See Table 3 for acceptable concentration values)

55 Check for contamination using a Nanodrop or equivalent device. Using an eluate input of 1 µL (See Table 3 for acceptable 260/230 and 260/280 values)

56 Optionally: Check for high fragment length retention using a Bioanalyzer or Fragment Analyzer (See Table 3 for acceptable fragment lengths)

57 If QC fails, discard eluate and redo the process. Perform SPRI Cleanup again, but use 100 µL of SPRI beads instead of original 225 µL.

58 Discard eluate, repeat full FLEX protocol with 3 min of bead beating instead of 5 min.