I. Introduction

Purpose. 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)**

Principle. FLEX(+) combines brief lysozyme digestion, bead-beating in 0.5 mm PowerBead tubes, and Proteinase K treatment, followed by SPRI-based purification to remove inhibitors (salts, proteins, residual detergents) while preserving long fragments.

II. 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 >72 h (discretionary), or visible contamination.

III. Reagents / Materials / Media

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.

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).

FLEX(+) Extraction Preparation

For MRSA, VRE, GNB, GAS, GBS

Step 1 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. Calculate how much of each reagent is required for given batch size (See Table 2).

TABLE 1: LYSIS ENZYME TABLE

	Lysozyme **L100** (100mg/mL)	Proteinase K 20mg/mL
For All Species	4ul per sample	5ul per sample

TABLE 2: REAGENT TABLE

	TE Buffer (10 mM Tris, 1 mM EDTA), pH 8.00)	SPRI Select Beads (Beckman Coulter)	Fresh 80% Ethanol	Molecular Grade Water (ddH2O)

For All Species	300ul per sample	225ul per sample	1200ul per sample	100ul per sample

6. Remove enzymes from the freezer and allow them to thaw. Keep @4 ºC until ready for use.
7. Aliquot 300ul of FLEX TE into each 0.5mm Glass Powerbead Tube
8. Inoculate each Powerbead tube with a heaping 1ul loop, or half of a 10ul loop, avoiding agar carryover
9. Mix by spinning the loop inside the tube
10. If beads or inoculum have stuck to the tube's side wall, tap several times until it settles to the bottom of the tube

Step 2 Enzymatic Lysis:

11. Pipette 4µL of Lysozyme **(L100)** (Using a P10) into each Powerbead tube, directly into the solution
12. Slowly and gently pipette mix up and down 2-3 times
13. 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
14. **Incubate for 15min** (This is a good time to begin thawing Proteinase K)
15. Remove from the incubator

Step 3 Mechanical Lysis:

16. Insert Powerbead tubes into a Qiagen Vortex Adapter Top, with the cap side facing inward.
17. Place the top onto a Vortex Genie
18. Set the vortex **speed to 9**, and allow the top to spin for **5 minutes**
19. Turn off the vortex and remove the adapter top
20. Place the tubes back onto a rack, maintaining the original format

Step 4 Denaturation of Interfering Proteins:

21. Pipette **5µL** of Proteinase K **(20mg/ml)** into each Powerbead tube, directly into solution.
22. Slowly and gently pipette mix up and down 2-3 times
23. 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
24. **Incubate for 15min**
25. Remove from incubator

Step 5 Removal of Contaminants/DNA Binding:

26. Transfer **225µl** of solution from Powerbead tubes into either a 0.8ml, 96 well MIDI Plate (for >36 samples) OR a 1.5ml Eppendorf tube (for <36 samples)
27. Add **225µl** of SPRI Select Beads into each well/tube
28. Mix by pipetting up and down 3-5 times
29. Place onto appropriate magnet (96 well Illumina Magnet for MIDI plates, Thermo DYNAMag Magnet for Eppendorfs)
30**. Leave on magnet** until lysate appears clear (3-5 min), and a pellet has formed on the well/tube side wall
31. With the plate/tube **STILL on the magnet,** carefully remove the **supernatant** with a P1000 set to 1000µl. **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

Step 6 Ethanol Washes x 2:

32. **Remove the tube/plate from the magnet**
33. Pipette 600µl of 80% ethanol into each well/tube directly onto the pellet
34. Resuspend pellet in the ethanol by pipetting up and down until homogenous
35. **Place tube/plate back onto the magnet**
36. Allow pellet to reform (2-3min)
37. Remove supernatant with a P1000 set to 1000µl
38. **Remove tube/plate from magnet again**
39. Pipette 600ul of 80% ethanol into each well/tube directly onto the pellet
40. This time, **do not** resuspend the pellet fully by mixing
41. **Place the tube/plate back onto the magnet**
42. Wait 2-3min
42. Remove supernatant with a P1000 set to 1000µl, avoiding the pellet
43. Allow pellet to air dry for **10min,** monitoring and wicking away any residual pooling; do not over-dry to cracking.
44. Once dry, take the tube/plate **off the magnet**

Step 7 Final DNA Elution:

45. Add 100µl of Molecular Grade Water (ddH2O) directly onto the pellet
46. Resuspend pellet fully, by mixing up and down with a pipette
47. Place tube/plate **back onto the magnet**
48. Wait 2-3min for pellet to reform
49. Transfer **all 100µl** 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
50. Discard the remaining tubes/plate with the pellet

Step 8 Eluate Quality Control:

For the following steps, follow manufacturer's guidelines for each device

51. Quantify DNA with a Qubit Fluorometer, using an eluate input of 4µl, and High Sensitivity Reagent input of 196µl. (See Table 3 for acceptable concentration values)
52. Check for contamination using a Nanodrop or equivalent device. Using an eluate input of 1µl (See Table 3 for acceptable 260/230 and 260/280 values)
53. Optionally: Check for high fragment length retention using a Bioanalyzer or Fragment Analyzer (See Table 3 for acceptable fragment lengths)

TABLE 3: ACCEPTABLE ELUATE QC VALUES

	Qubit Results (ng/µl)	**Nanodrop Results**	**Fragment Length Results** (Requires specialized training for interpretation)
Acceptable Range:	>5ng/ul	260/230: >1.3 260/280: >1.4	Majority of fragments greater than 500bp (or nucleotides)
Next step if QC fails:	Discard Eluate (Redo)	Perform SPRI Cleanup again, but **use 100µl** of SPRI beads instead of original 225µl	Discard eluate, repeat full FLEX protocol **with 3min** of bead beating instead of 5min