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# *Enterococcus faecalis* protoplast generation

Elizabeth Fozo<sup>1</sup><sup>1</sup>In-house protocol**1** Works for me

This protocol is published without a DOI.

 Eadewunm

## PROTOCOL CITATION

Elizabeth Fozo 2020. *Enterococcus faecalis* protoplast generation. **protocols.io**  
<https://protocols.io/view/enterococcus-faecalis-protoplast-generation-bp24mqgw>

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## CREATED

Nov 24, 2020

## LAST MODIFIED

Nov 25, 2020

## PROTOCOL INTEGER ID

44860

## MATERIALS TEXT

### Required media

- BHI
- Isotonic solution – 20% sucrose, 0.145M NaCl, 50mM Tris-HCl

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## BEFORE STARTING

### Required media

- BHI
- Isotonic solution – 20% sucrose, 0.145M NaCl, 50mM Tris-HCl

## Protocol

- 1 Grow OG1RF in BHI + condition until OD<sub>600</sub> 0.3. This works for as little as 10mls of culture.

Growing in 2% glycine will reduce “gluing” of cells, but massively increase generation time.

- 2 Spin cells down and resuspend in half volume isotonic solution.
- 3 Add 2mg/ml lysozyme and incubate for 60 minutes. More time may be required.
- 4 Check for wall removal using Gram stain. (Do not move on until protoplast confirmation)
- 5 Spin cells down and wash with isotonic solution, resuspending again in isotonic solution in half the volume of the original culture.

When resuspending cells after lysozyme treatment, pipette mix and refrain from over-vortexing as this will kill protoplast cells.