# Rapid Prep for PCR Applications Protocol 2

Lyse & Purify (16 Tubes)
Cell Suspensions and Cell Pellets

Rapidly lyse and purify DNA from cell suspensions or cell pellets. This kit contains materials for processing 16 samples.

**Additional Items Needed: (Not included; see Suggested Supplier list below)**

1. Additional Items **Required** for Lyse and Purify
    a. 8-strip 0.2 mL PCR tubes w/individual domed caps for sample lysis and purification
    b. Sterile Laboratory Grade Water
    c. Fresh 70% ethanol
    d. TE Buffer - 10mM Tris, 1mM EDTA, pH 8.0
    e. 0.4M KOH Solution
    f. Magnetic Rack ('bar' or 'post' suggested for higher DNA yield)
2. Suggested items for rapid processing
    a. Repeater Pipette with multi-volume tips
    b. Single Channel Manual Pipettes
    c. Multichannel Pipette 0.5-10 µL working volume
    d. Mini Centrifuge for 200 µL 8-strip tubes

**Kit Contents for Shoreline Biome Lyse and Purify**

1. Two 8-tube strips of dried Lysis-1
2. Purification Buffer (two 1.5 mL tubes with **green** cap)

## Shoreline Biome Lyse and Purify Protocol

1. **Make 0.4M KOH**. Measure 0.135g KOH into 15 mL tube, then add 6 mL $H_2O$. 0.4M KOH expires after 7 days.
2. **Make 70% Ethanol**. Add 17.5 mL 100% ethanol to 7.5 mL sterile laboratory grade water
3. Bring Purification Buffer (**green** capped tube) to room temperature.
4. **Label a clean 200 µL tube for each sample** (e.g., for 16 samples, label two 8-strip 200 µL tubes)
5. **Sample Type:**
    5.1. **Cell suspension:**
        5.1.1. **Transfer 50 µL of cell suspension** (between $10^6$ and $10^8$ cells) to Lysis-1 tube. Do not mix.
    5.2. **Cell pellets:**
        5.2.1. Reconstitute the cell pellet by adding 50 µL Molecular Biology Grade water, TE, or PBS and pipetting up and down to suspend cells.
        5.2.2. **Transfer 50 µL** of the cell suspension (between $10^6$ and $10^8$ cells) to Lysis-1 tube. Do not mix.
6. **Add 50 µL 0.4M KOH** to each tube. Do not mix. (A precipitate will form)
7. Cap samples tightly; set PCR machine for proper tube height, close and lock the lid and **heat samples to 95°C for 5 minutes**. Precipitate will dissolve. After incubation, **allow sample to cool to RT before unlocking PCR lid** – failure to do so may result in lids popping off tubes.
8. **While samples are cooling**: Vortex Purification Buffer tubes (Green Cap) to re-suspend any brown magnetic beads that may have settled
9. Add **100 µL of Purification Buffer** (Green Cap) to each sample. If precipitate formed, it should re-dissolve. Pipette up and down to mix.

10. Cap samples tightly; set PCR machine for proper tube height, close and lock the lid, and **heat samples to 50°C for 3 minutes** to allow DNA to bind to beads.
11. **After 50°C incubation, place samples on magnetic rack** to pellet beads with bound DNA to side of the tube (~60 seconds)
12. Once all beads are pelleted, **remove all supernatant SLOWLY and discard**. Avoid aspirating beads.
13. **Remove tubes from magnet, add 100 µL 70% ethanol** (at room temperature) to each tube, and re-suspend beads by pipetting gently up and down.
14. **Return tubes to magnet and allow beads to pellet** (~30 seconds)
15. **Pipette off residual ethanol and let air dry for 2 - 3 minutes**. Over-drying will make eluting DNA more difficult, so watch to ensure ethanol is mostly gone but bead pellet does not begin to shrink and crack.
16. **Remove tubes from magnet.**
17. Add **20 µL of 1X TE buffer** to tubes and briefly pipette up and down to re-suspend beads. Solution should turn brown in color to reflect beads mixed homogeneously throughout.
18. **Let solution incubate at room temperature for 2 minutes.**
19. **Place tubes on magnetic rack** for 30 - 60 seconds to pellet magnetic beads. **DNA is now in solution.**
20. **Transfer supernatant containing eluted DNA** into **clean,** labeled microcentrifuge tubes on ice, avoiding pelleted beads.
21. **Remove tubes with pelleted beads from rack**.
22. **Add another 20 µL 1X TE buffer** onto bead pellets, gently pipette up and down to re-suspend beads.
23. **Incubate tubes for 2 minutes at room temperature**.
24. Return tubes to magnetic rack. Allow beads to pellet for 30 - 60 seconds.
25. **Remove supernatant containing DNA** and combine with first eluate in clean, labeled tubes, avoiding pelleted beads.
26. **Store eluted DNA at +4°C**.

**NOTES:** DNA is **denatured**, due to KOH treatment during lysis, which is optimal for PCR. Store Lysis Mix at +4°C for up to 10 days, or frozen at -20°C for 3 months. Avoid repeated freeze/thaws.

## Suggested Suppliers

Dot Scientific Inc.: 8-strip PCR tubes w/individual domed caps**,** #503-8PCR
Fisher Scientific: Biotech Grade Water BP24854; Potassium Hydroxide P250; TE BP24731
Permagen: 0.2 mL PCR Strip Magnetic Separation Rack 8 or 12 Strip, Cat #MSR812

This document and its contents are proprietary to Shoreline Biome, LLC and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Shoreline Biome. Shoreline Biome does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

SHORELINE BIOME DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE) OR ANY USE OF SUCH PRODUCT(S) OUTSIDE THE SCOPE OF THE EXPRESS WRITTEN LICENSES OR PERMISSIONS GRANTED BY SHORELINE BIOME IN CONNECTION WITH CUSTOMER'S ACQUISITION OF SUCH PRODUCT(S).

All names, logos, and trademarks are the property of their respective owners.