

# Mycobacteria DNA extraction using bead disruption - Manual method

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## Abstract

This method is best used on colonies grown on solid media and subcultures in 7H9 liquid media. This method is high yielding and is very efficient for short read NGS. It is not appropriate for direct tissue or fecal extraction. It is also not appropriate for liquid media containing high amounts of egg yolk.

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**Citation:** Patrick Camp,David Farrell,Suelee Robbe-Austerman Mycobacteria DNA extraction using bead disruption - Manual method. **protocols.io**

[dx.doi.org/10.17504/protocols.io.nsgdebw](https://dx.doi.org/10.17504/protocols.io.nsgdebw)

**Published:** 12 Mar 2018

## Protocol

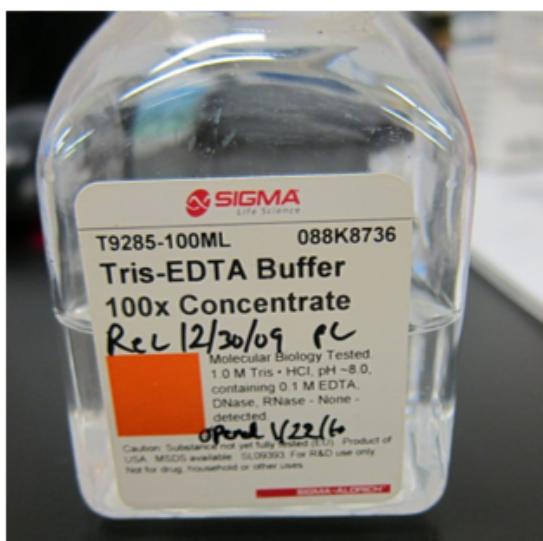
Prepare 2ml Sealed screw cap bead beater tubes by adding  $200\mu\text{l} \pm 100\mu\text{l}$  of 0.1mm silica beads.

### Step 1.



Make 1X TE: Add 500 $\mu$ l of Tris-EDTA Buffer 100X Concentrate + 45.5ml of H<sub>2</sub>O.

### Step 2.



Add 400 $\mu$ l of 1X TE into the bead beater tubes with silica beads

### Step 3.

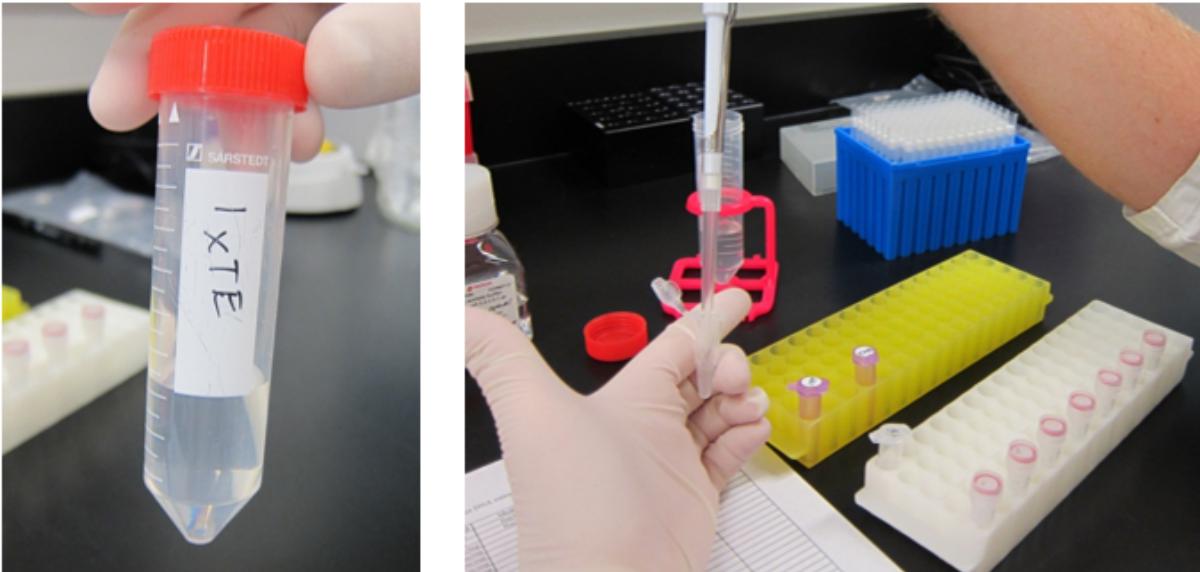
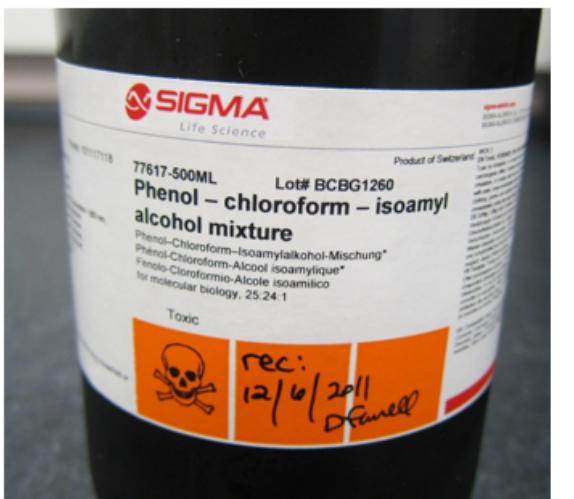


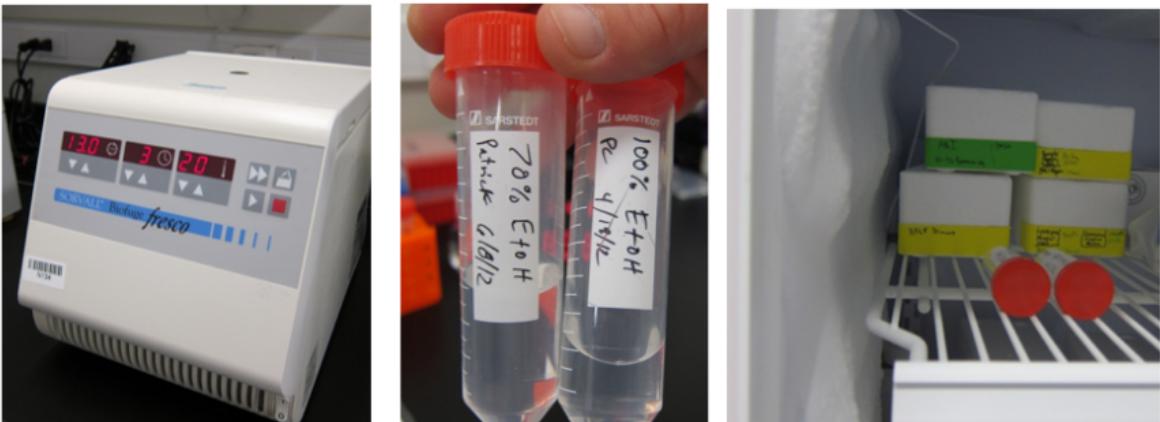
Figure the amount of Phenol/Chloroform/Isoamyl alchol (PCI) 25:24:1 (v:v:v) needed per sample (400 $\mu$ l/sample). Place the amount needed in a 50ml conical tube inside a fume hood. Use the 50ml conical tube to add 400 $\mu$ l into the bead beater tubes from step 3.

#### Step 4.



Turn on centrifuge to cool down to 4°C and place 70% & 100% Ethanol in the -20°C freezer to use at a later step.

### Step 5.



Move to appropriate Biological Safety Cabinet and add 2-3 loopfuls of growth from solid media to prepared beat beater tubes with 1X TE and PCI.

### Step 6.



Beat the samples at full speed for 2 minutes (depending on machine) using the Beadbeater machine.

**Step 7.**



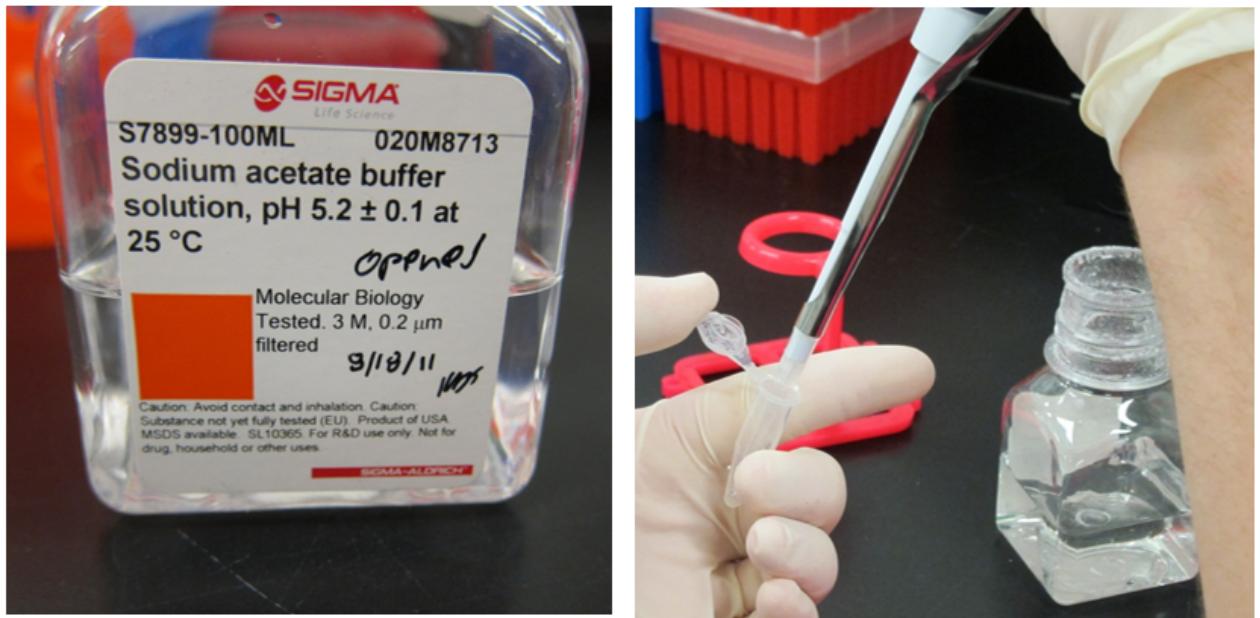
Spin samples at  $16,000 \pm 500 \times g$  for  $5 \pm 1$  minute.

**Step 8.**



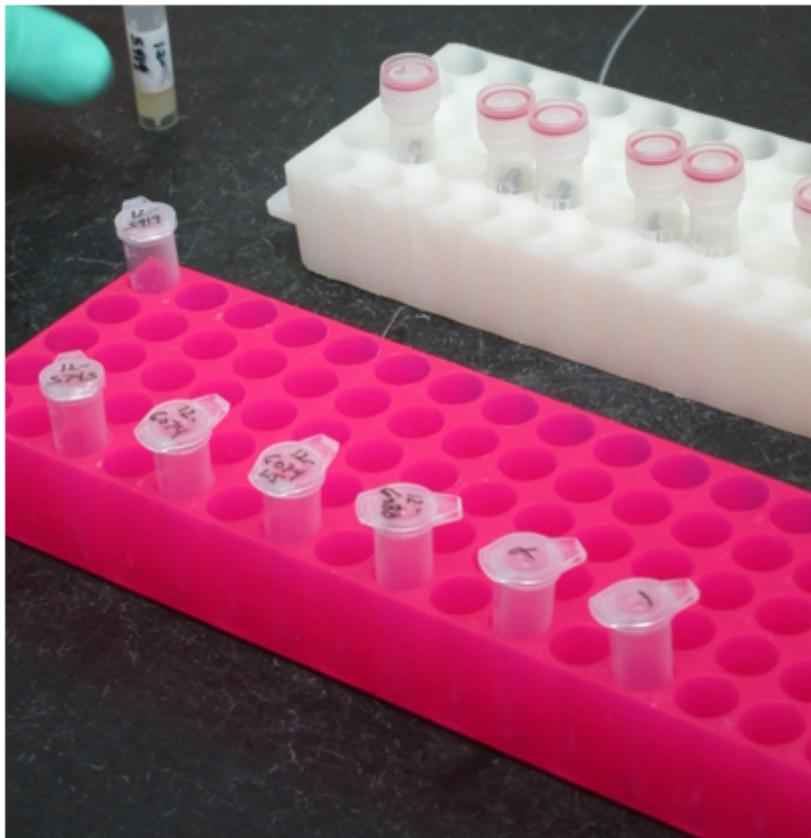
While samples spin add 30 $\mu$ l 3 M sodium acetate buffer solution to a labeled 1.5ml microcentrifuge tube.

**Step 9.**



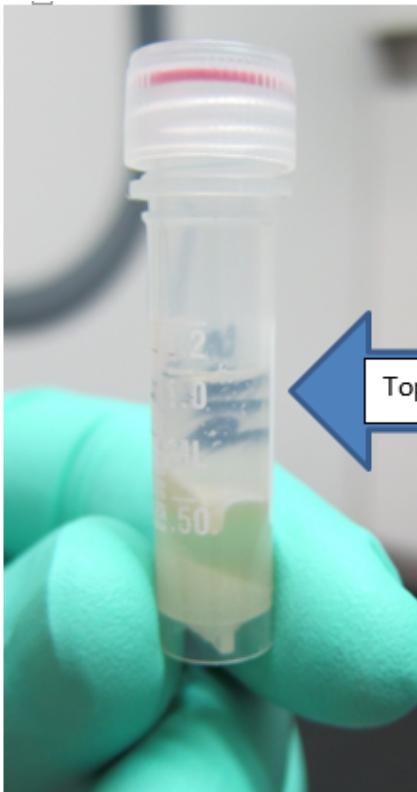
Pull samples from the centrifuge.

**Step 10.**



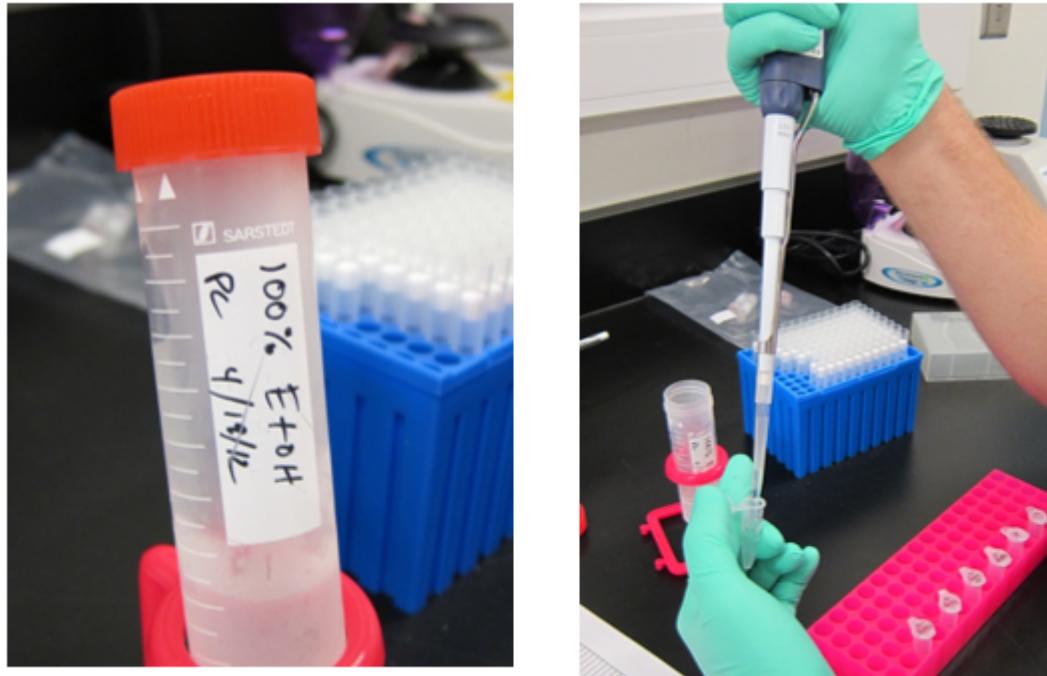
DNA is contained in the top aqueous layer. Remove this layer, approximately 300 $\mu$ l, by pipetting and add it to the 1.5 ml microcentrifuge tube with 3 M sodium acetate buffer solution.

**Step 11.**



Add 700 $\mu$ l of 100% ice-cold ethanol to the samples. Invert the tubes 10 times and vortex full speed for < 2 seconds.

**Step 12.**



Cool samples at -80°C for 10-15 minutes (or put in -20°C freezer for 30 min or indefinitely).

**Step 13.**



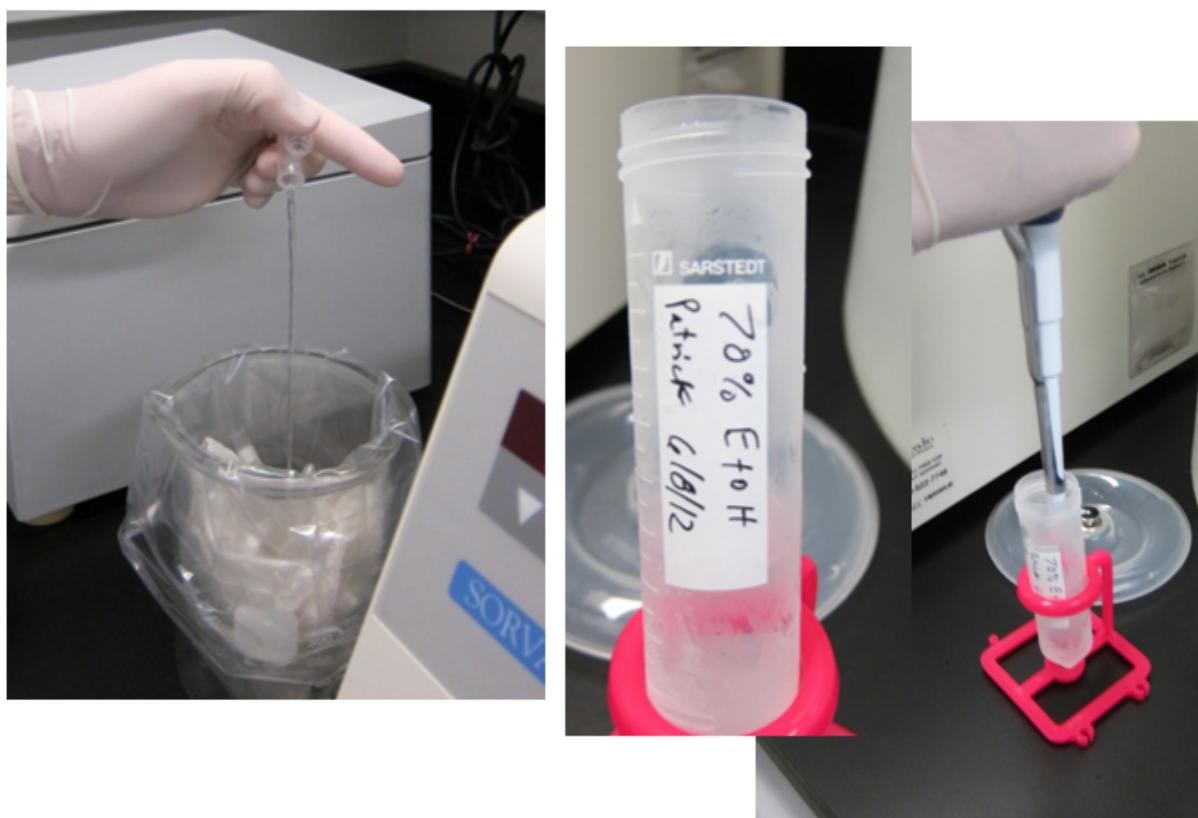
Spin at  $4 \pm 2^\circ\text{C}$ ,  $16,000 \pm 500 \times g$  for  $15 \pm 1$  minute.

**Step 14.**



Pour off the ethanol and add 1 ml ice-cold 70% ethanol.

**Step 15.**



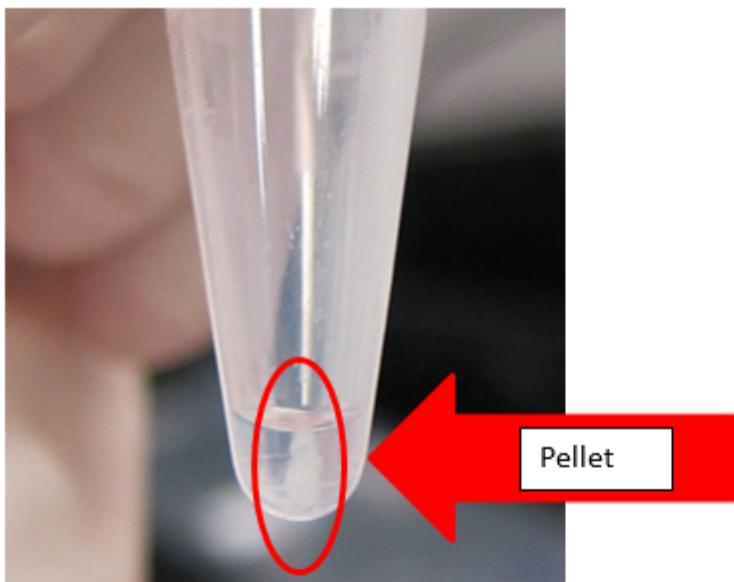
Spin at  $4 \pm 2^\circ\text{C}$ ,  $16,000 \pm 500 \times g$  for  $5 \pm 1$  minute and pour off ethanol.

**Step 16.**



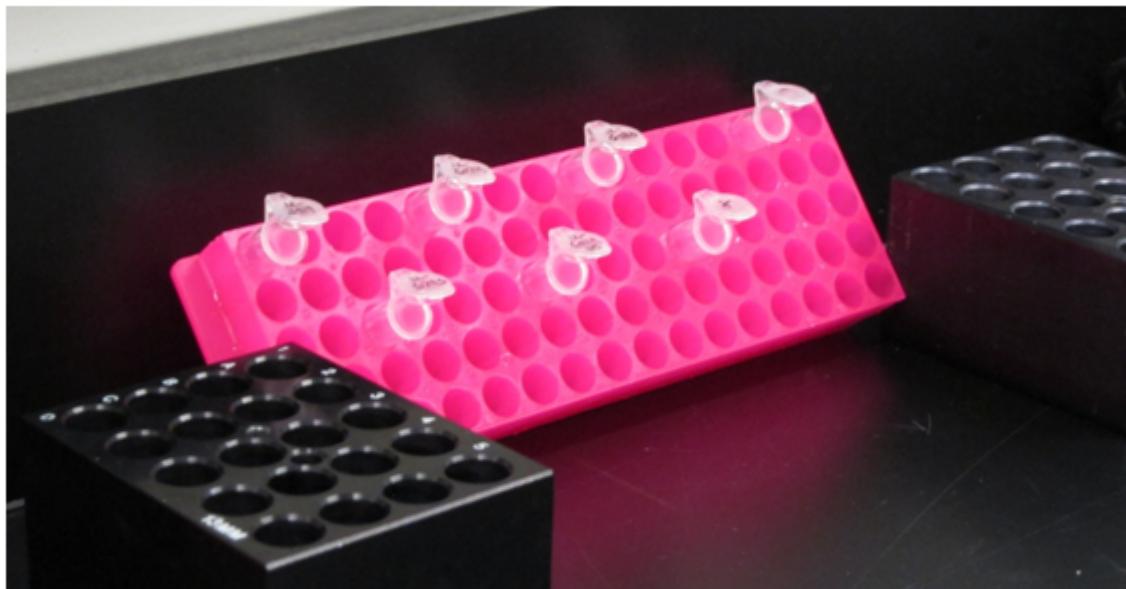
Spin at  $8,000 \pm 500 \times g$  for  $15 \pm 5$  seconds, and immediately remove remaining ethanol with a  $100 - 200\mu\text{l}$  pipette. Place tip of pipette on the opposite side from the pellet.

**Step 17.**



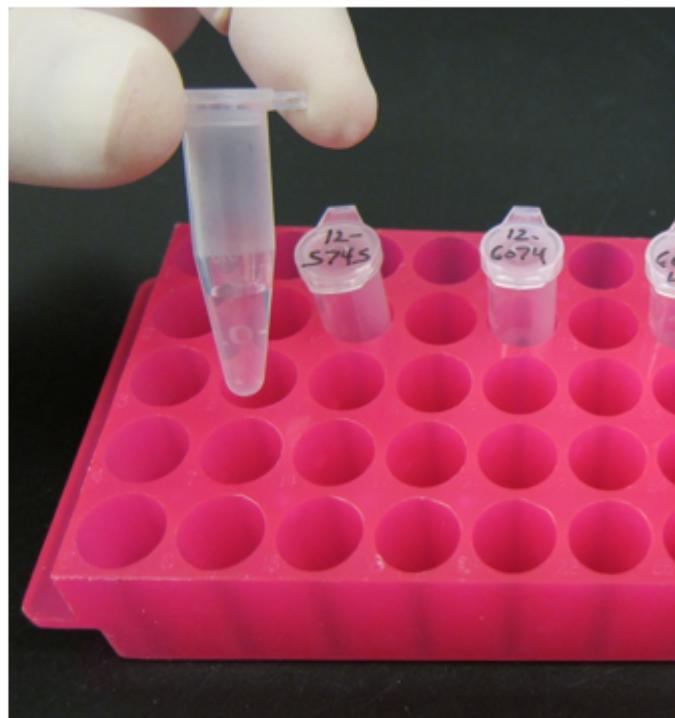
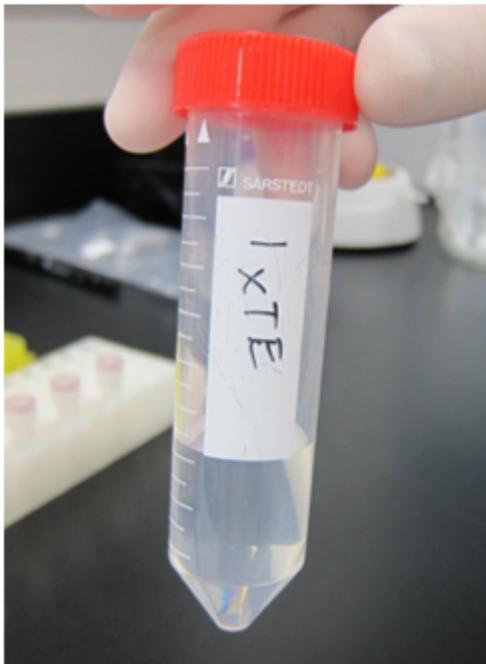
Dry the samples at room temperature with tubes tilted and caps open for 15-20 minutes or until remaining ethanol has evaporated.

**Step 18.**



Add 300 $\mu$ l of 1X TE buffer. If a small amount of DNA is expected a smaller volume of 1X TE buffer can be used. Scrape the inside of the tube with the pipette tip when resuspending the DNA.

**Step 19.**



Keep DNA at 4°C for short term storage or -80°C for long term storage.

**Step 20.**