TZ May 2026 Fischer Lab

Extract DNA:

1) Suspend colonies in 150 mL of distilled water
2) Boil in water bath for 10 mins
3) Ice bath for 10 mins immediately after boiling
4) Centrifuge at 12700 rpm for 2 mins a room temperature
5) Transfer 50 mL of the supernatant to an autoclaved tube, store at −20 °C

Or use DNA extraction protocol using phenol-chloroform extraction.

Primers:

1) To lyophilized primers from IDT, add listed amount of water to achieve 100 µM conc.
2) Before diluting primers in water to achieve a 1 µM primer conc., calculate the total volume required for the number of reactions (+1 extra) + controls.
    a. For 20 isolates (+1) & 4 controls; 25 total reactions = 250 µL Primer Mix
    b. For 250 µL Primer Mix @ 1 µM primer concentration =
    2.5 µL of FW primer + 2.5 µL of RV primer + 245 µL ddH20
    c. For 100 µL Primer Mix @ 1 µM primer concentration =
    1 µL of FW primer + 1 µL of RV primer + 98 µL ddH20
    d. For multiplex PCR, decrease the amount of water added while keeping the volume of each primer the same.
        i. Example: 250 µL Primer Mix using **Two** Primer Sets =
        2.5 µL of FW Primer **X** + 2.5 µL of RV Primer **X** +
        2.5 µL of FW Primer **Y** + 2.5 µL of RV Primer **Y** +
        240 µL ddH20
3) Make 1X Taq MasterMix by diluting your 1 µM Primers 1:1 with 2x Taq MasterMix
    a. Ex: For 250 µL of 1µM Primer Mix, add 250 µL of 2x Taq MasterMix
4) Transfer 20 µL of 1x MM to each PCR tube
5) Add 1.5 µL of Isolate DNA extraction to its labeled PCR tube
6) Transfer tubes to thermocycler & follow next steps

Thermocycler Conditions for PCR using NEB Taq 2x MasterMix:

Step	Temperature	Time
Initial Denaturation	95 °C	30s
30 Cycles	95 °C	20s
T_a of Primers ->	45-68 °C	15-60s
	68 °C	1min per KB of PCR Prod.
Final Extension	68 °C	5mins
Hold @	4 °C	

Thermocycler

1) Set thermocycler conditions for primer conditions
2) Make sure it runs for 30 cycles
3) For Primer Treatment #1:
    a. Set Cyclic Annealing Temperature (T_a) to 50 °C for 30s
    b. Set Cyclic Extension time to **30s**
4) For Primer Treatment #2:
    a. Set Cyclic Annealing Temperature (T_a) to 50 °C for 30s
    b. Set Cyclic Extension time to **90s**

PCR Genotyping:

Gene Amplified	Annealing Temp (°C)	Product Lengths	Identified Strains:
Lanthionine synthetase C family protei n	50	308 bp	Primer Treatment #1: 1, 5, 8, 30
efflux RND transporter subunit		144 bp / 501bp	
mcrC		211 bp	
aldehyde reductase	50	395 bp	Primer Treatment #2: 59, 398, 582, Other
mtlD		235 bp	
sucD-lytN		~600 bp / ~1500 bp	

*Intergenic space between sucD-lytN adds bases, that's why it is ~ product length

Gel Electrophoresis – Casting the Gel:

1) Make a 1.5% agarose gel:
    a. To 100 mL of 1x TAE buffer in a flat-bottom glass flask, add 1.5g of agarose.
    b. Heat in microwave 45s x2, and 30s once. Carefully swirl to mix using a rubber glove between heating times.
2) Once melted, let cool for 5 mins, then add 10 µL of SYBR Safe Gel Stain and swirl until fully mixed
3) Prepare agarose gel casting tray with combs making enough wells for your # of reactions + positive & negative controls + DNA ladders
    a. Ex: 20 reactions + 4 strain controls + 2 ladders = Minimum 26 wells on the gel
    b. Make sure your casting tray is level!!
4) Ensure that gel combs are in place and tray is level, carefully pour the mixture into the tray.
5) Let gel cool for 15-30 minutes undisturbed before using.

Loading the Gel:

1) Load PCR tube samples with NEB Purple 6X Gel Loading Dye
    a. 4 µL of Gel Loading Dye per 20 µL reaction
2) Carefully remove gel combs by slowly pulling them straight up and out of the gel
3) Remove casting tray and arrange it so the wells face the negative electrode of the gel buffer tank
4) Fill gel buffer tank with 1X TAE to fill line, nearing the white electrodes on both ends of the gel (should be over the top of the gel)
5) Load first well with 10 µL 1kb plus DNA ladder
6) Load remaining wells with controls & samples
    a. Larger wells load 20 µL
    b. Smaller wells load 10 to 15 µL
    c. More sample is better – just don't overfill
7) Place gel lid with negative electrode at top of well and positive electrode at bottom of gel on the gel buffer tray; plug in to correct places on power supply
8) Run ~100V, checking every 30 minutes for how far the lanes have progressed down the gel
9) When lanes have progressed over halfway down the gel, turn off power supply and remove lid
10) Carefully transfer gel to Bio-Rad gel imaging machine

11) Adjust contrast/brightness on the machine to maximize band visibility, save to flash-drive, toss gel into biohazard bin and wipe down gel imaging machine platform