SUPPLEMENTARY METHODS

DNA Isolation

1. Spike in 5 µL of each diluted plasmid into individual donor cell pellets for controls. Gently flick tube to mix.
2. Add 100 µL of suspended Qiagen Proteinase K into the 15 mL conical tubes.
3. Transfer cell pellets and plasmids using 1 mL of PBS into the 15 mL conical tubes.
4. Add 1.2 mL of Qiagen Lysis buffer to each tube.
5. Pulse vortex the tubes 5 times and place them in a 70°C water bath for 10 minutes.
6. After the bath, wipe the tubes with 70% ethanol and place tubes in a centrifuge. Spin for 1 minute at 1850xg.
7. Add 1.2 mL of 100% Ethanol to each sample.
8. Pulse vortex the tubes 5 times and spin for 1 minute at 1850xg.
9. Transfer samples from the 15 mL conical tubes into the Qiagen spin columns.
10. Spin for 3 minutes at 1850xg.
11. Discard collected liquid waste.
12. Add 2 mL of Qiagen Wash Buffer 1 to the spin columns.
13. Spin for 1 minute at 4500xg.
14. Without discarding the liquid waste, add 2 mL of Qiagen Wash Buffer 2 to the spin columns.
15. Spin for 15 minutes at 4500xg
16. When the spin is complete, spin again for 1 minute at 4500xg
17. Dispose of liquid waste and transfer the spin columns into a new collection tube.
18. Add 200 µL of Qiagen AE buffer to each column. Let buffer sit for 2 minutes.

19. Spin tubes for 1 minute at 4500xg
20. Take tubes out of the centrifuge and add 200 µL and let sit for 1 minute
21. Repeat steps 21 and 22 until a total of 1 mL has been eluted from the columns.
22. In 2 respectively labeled 1.5 mL tubes for each sample, add 1 µL of glycogen to each tube.
23. Split 500 µL of the eluents between two tubes.
24. Add 50 µL of 3M Sodium Acetate to each sample.
25. Dispense 1 mL of -20°C ethanol into each 1.5 mL tube.
26. Invert each tube and place in the freezer overnight.
27. Spin samples for 30 minutes at -20°C at 20000xg.
28. After the spin, discard the ethanol from the tubes into waste.
29. Combine the pellets from the two respectively labeled samples into one tube using 1 mL of 70% ethanol.
30. Spin samples for 2 minute at 18000xg.
31. Discard the ethanol.
32. Rinse pellets with 1 mL of 70% ethanol.
33. Repeat spin.
34. Discard ethanol from tubes.
35. Spin tubes for 1 minute at 18000xg.
36. Discard the remaining ethanol air dry for 15 minutes.
37. Add 50 µL of Qiagen AE buffer to each pellet.
38. Vortex samples, spin down, and measure OD and record A260/280.

ddPCR

1. Create a restriction digest mixture with XbaI for all samples using the chart below. The number of reactions can be calculated from the number of samples being processed and number of replicates, plus excess for pipetting error.

   Replicates of 8:

Reagent	Volume (µL)	No. of Rxns	Per sample (µL)
CutSmart Buffer	0.7	10	7
XbaI Restriction Enzyme	0.3	10	3
Water	0.9	10	9
Total	1.9		19

   Replicates of 4:

Reagent	Volume	No. of Rxns	Per sample (µL)
CutSmart Buffer	0.7	5	3.5
XbaI Restriction Enzyme	0.3	5	1.5
Water	0.9	5	4.5
Total	1.9		9.5

2. In a 24 well plate, dispense 19 µL of the digestion mix for replicates of eight and 9.5 µL for replicates of four.
3. Normalize DNA to 200/µL with DNA/RNase-Free water. Each well should have a 1000ng DNA input.
4. Vortex and spin down plate.
5. Digest samples for 37°C for 1 hour, 65°C for 20 minutes, and end with a 12°C hold.

6. Create 2 separate 20x primer probe mix using GAGLTR and RPP30 primers and probes, Bio-Rad ddPCR Supermix and water using the charts below:

Replicates of 8:

Reagent	Volume (µL)	No. Rxns	Per sample (µL)
ddPCR Supermix for Probes	10	8.5	85
GAGLTR 20x Primer Probe Mix	1	8.5	8.5
Water	2.1	8.5	17.85
Total	13.1		111.4
DNA			58.7

Reagent	Volume (µL)	No. Rxns	Per sample (µL)
ddPCR Supermix for Probes	10	1.5	15
RPP30 20x Primer Probe Mix	1	1.5	1.5
Water	2.1	1.5	3.15
Total	13.1		19.7
DNA			10.4

Replicates of 4:

Reagent	Volume (µL)	No. Rxns	Per sample (µL)
ddPCR Supermix for Probes	10	4.5	45
GAGLTR 20x Primer Probe Mix	1	4.5	4.5
Water	2.1	4.5	9.45
Total	13.1		59
DNA			31.1

Reagent	Volume (µL)	No. Rxns	Per sample (µL)
ddPCR Supermix for Probes	10	1.5	15
RPP30 20x Primer Probe Mix	1	1.5	1.5
Water	2.1	1.5	3.15
Total	13.1		19.7
DNA			10.4

7. In a new 24 well plate, dispense 111.4 µL of the GAGLTR probe mix for 8x reactions and 59 µL of the GAGLTR primer probe mix for the 4x reactions. In a separate new plate, dispense 19.7 µL of the RPP30 mix for both 8x and 4x reactions.
8. In a new 24 well plate, dispense 27 µL of water for each sample. This will be a dilution plate for RPP30
9. When digestion is complete, take the samples, vortex, and spin down.
10. Take 3 µL of digested DNA and place into the dilution plate. Vortex and spin.
11. Take 58.7 µL of the 8x digested samples and mix into the respective GAGLTR primer probe wells.
12. Take 31.1 µL of the 4x digested samples and mix into the respective GAGLTR primer probe wells.
13. Take 10.4 µL of the diluted DNA and mix into the RPP30 primer plate for both 8x and 4x reactions.
14. Vortex and spin down the GAGLTR and RPP30 plate.
15. Pour a bottle of Bio-Rad generator oil in a clean reservoir.
16. Plug in ddPCR droplet generator machine.
17. Place a Bio-Rad 24 well droplet cartridge into the cartridge plate holder.

18. Take 20 µL of the GAGLTR primer probe mix and place into the middle row of the cartridge. It is important that the entire row has sample in the wells. If a row cannot be filled, use 20 µL of 1x buffer (1 part supermix, 1 part water)
19. Take 70 µL of generator oil and slowly dispense in the designated oil wells.
20. Place a rubber droplet gasket on the cartridge holder.
21. Open up the manual ddPCR oil generator machine. Place the cartridge holder inside. Close the lid. Droplet generation will begin once the lid is closed.
22. After the machine finishes running, open the machine and take out the gasket holder.
23. Slowly take 44 µL of the newly generated droplets and slowly dispense into a 96 well plate. Do not disrupt the droplet-oil separation layer.
24. Repeat steps 16-20 until there are 8 replicates of GAGLTR reactions for each sample and NTC and 1 run of RPP30 for each sample and NTC.
25. Turn on plate sealer and seal the plate using ddPCR seals for 5 seconds at 180°C.
26. Place the plate into thermal cycler for the following conditions:

95°C	10 minutes	
94°C	30 seconds	60 cycles
60°C	60 seconds	
98°C	10 minutes	
12°C	Hold	

27. Leave samples in thermal cycler overnight.

ddPCR Analysis

1. The next day, turn on the Bio-Rad ddPCR droplet reader.

2. Open up QuantaSoft program on the computer.

3. Assign all wells used in the plate to the following:

a. Fill in sample name along with target

b. Select Rare Event Detection (RED) as the experiment type

c. Select “ddPCR Supermix for Probes (No dUTPs)” for supermix type

d. For GAGLTR samples: Channel 1s Target is “None” with target type set to “Ch1 Unknown”. Channel 2s Target is “GAGLTR” with target type set to “Ch2 Unknown”.

e. For RPP30 samples: Channel 1s Target is “None” with target type set to “Ch1 Unknown”. Channel 2s Target is “RPP30” with target type set to “Ch2 Unknown”.

f. NTC wells target type is set to “NTC”

4. Transfer the plate from the thermal cycler to the reader and press run.

5. Once the run is finished, switch to the droplet tab and observe the total number of droplets for each well. Anything below 6,000 droplets should be excluded from analysis.