High Throughput Genotyping for Wild Isolate Identification

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This protocol is referenced in the wild_isolate_collection_protocol on the Andersen Lab google drive. It is intended to provide more detailed instructions for the lysis, PCR and visualization of PCR products.

Lysis

1. Ensure that S-plates are organized in alphanumerical order in proliferation boxes. Each proliferation box should contain up to 88 S-plates and at least one positive control plate (N2 worms).

2. Before beginning the lysis, prepare enough 12-well strip tubes for all of your samples (11 S-plates per 12-well strip tube).
    - Color the first cap and tube on one end of the strip. This will ensure that you always put the cap back on the correct sample and orient the strip (the colored tube is always position 1).
    - Label the opposite end of the strip tube with the unique 'strip_tube_number' assigned in the lysis worksheet. This label should be written on the cap strip and the tube strip to avoid confusion if they are separated.
    - Position 1 of each tube strip will always contain a control.
        - EVEN strip_tube_numbers will have a positive control (N2 worms) in position 1.
        - ODD strip_tube_numbers will have a negative control (no worms) in position 1.

3. Make up enough lysis buffer for all of your samples. For 100 samples you would need 800 µl. Mix together 16 µl of 20 mg/ml Proteinase K and 784 µl of dH_2O. Scale as necessary.

4. Work with one strip tube at a time:
    - Arrange the plates that will be used for that particular strip (use the printed lysis worksheet as a guide).
        - **Don't forget your control! These are not printed on the lysis worksheet. Color this cap/tube completely so we know it's position 1 (the control position).**
            - EVEN strip_tube_numbers will have a positive control (N2 worms) in position 1.
            - ODD strip_tube_numbers will have a negative control (no worms) in position 1.
    - Working with only one strip at a time, add 8 µl of lysis buffer to each cap with a repeat pipetor.
    - Pick 5-6 animals from the source plate (S-plate or N2) into the appropriate cap.
    - Work as quickly as possible to avoid evaporation of lysis buffer.
    - Place the strip caps on top of the tubes, making sure to match the colored cap with the colored tube.

5. When you have finished one strip, place the strip in a 96-well plate rack (or an empty 200 µl tip rack) and briefly spin down your samples so the material is now in the tube.

6. Place the strip in the -80ºC freezer until sample is frozen.
    - If you leave the strips in a rack during this, it will take a little longer for the samples to freeze.

7. Repeat steps 4-6 until all strips contain worms

8. Place the strips in a thermocycler and run the following lysis program below:

Time	Temperature	Number of Cycles
1 hour	60°C	1
15 min	95°C	1
Hold	4°C	1

9. When the lysis program is done, briefly spin down your samples. Keep lyses at -80°C until needed.

10. When you are ready to set up PCR, make up your PCR master mix on ice. First, some notes about the PCR:
    - The final volume for each individual reaction is 40 µl.
    - In the interest of saving money, we are using regular Taq, not ExTaq.
    - This is a PCR that amplifies a (2-1.5 kb ITS2 product).
    - The primer sequences are:

        - oECA202 - GCGGTATTTGCTACTACCAYYAMGATCTGC
        - oECA1687 - CTGCGTTACTTACCACGAATTGCARAC
        - * - Do not add Taq until you are ready to add Master Mix to the lyses.

11. Have a labeled 96-well plate(s) for PCR on ice.

12. To aliquot the master mix, we use the autoclaved troughs with V-bottoms. Add the master mix to the single, large trough. Use a 12-well multichannel pipette to add 39 µl of master mix to each well on ice.

	1X	**600X**
oECA1687, 4 µM	4 µl	2.4 ml
oECA202, 4 µM	4 µl	2.4 ml
PCR Buffer, 10X	4 µl	2.4 ml
dNTP mix, 2.5 µM	3.2 µl	1.92 ml
dH_2O	23.6 µl	14.16 ml
Taq*	0.2 µl	120 µl
lysis reaction	1 µl	-

13. Remove lyses from the freezer and spin down.

14. Carefully remove the lids of all the strip tubes. Place the caps in an empty tip wafer rack to keep them separated and in order.

15. Use a low-volume multichannel pipette (either 12-well or 8-well) to add 1 µl of lysis to the appropriate well.
    - Gently pipette the lysis up and down once before removing the 1 µl. I always check the tips to make sure that each has sample in it.
    - **Change tips before you pipette from the next row/column!**

16. Place caps back on lysis if needed.

17. If necessary, briefly spin down.

18. Place the 96-well plate in a thermocycler and run the following program:

Step	Time	Temperature
1	3 min	95°C
2	15 sec	95°C
3	15 sec	60°C
4	2 min	72°C
5	Go to step 2, repeat 36 times	
6	5 min	72°C
7	hold	15°C

19. During the PCR, pour a large agarose gel:
    - Add 4.5g agarose to 2 500ml bottles each.
    - Add 300ml TAE to each bottle.
    - Microwave each bottle for 2.5-3 minutes, taking the bottle out and shaking it at 1 and 2 minutes. (Careful, when boiling the bottles might overflow!)
    - Let the bottles cool to body temperature while stirring, this might take over 30 minutes. During this step also set up the large gel system.
    - Add 15µl EthidiumBromide to each bottle, stir very shortly after.
    - Pour gel, let it dry for 20 minutes.

20. Also during the PCR, add 6X loading dye to a trough. Use the 8-well multi-channel pipette to as 2 µl of 6X loading dye to each well of a 96-well plate. Each row in the plate can correspond to one of the strip tubes. Make sure to note which row on the plate is for which strip tube.

21. When the PCR is done, briefly spin down the samples.

22. Use a 12-well multi-channel pipette to add 5 µl of each sample to the appropriate well of the 96-well plate.

23. Use a 12-well multi-channel pipette to add the samples to the gel. Note that Row A will be interspersed with Row B, Row C will be interspersed with Row D, etc. **Also load the 1 KB plus ladder.**

24. Run the gel.

25. Visualize.

26. If only the rhabtidid-specific band amplified, send the PCR product in for sequencing using the primer oECA306 (sequence: CACTTTCAAGCAACCCGAC).