Host Range Protocols

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Abstract

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Guidelines

Splitting Cells/Growing Up Your Host (cells)

1. Label flasks with the bacteria (host) name, the date, and the split ratio (ie. 1:20).

2. IN THE BIOLOGICAL SAFETY CABINET (HOOD): Add the correct amount of growth media to each flask (eg. for a 20mL grow up of WH7803 split at 1:20, I would add 18mL SN media).

3. IN THE BIOLOGICAL SAFETY CABINET (HOOD): Add the correct amount of bacteria to each flask and cap the flask (eg. for a 20mL grow up at WH7803 split at 1:20, I would add 2mL bacteria).

4. Put new flasks of cells in light chamber at the correct light level. Shake the cells at least 1x per week. Keep old cells in case new cells die.

	Split Ratio			
Bacteria	For optimal growth in 2-3 weeks	For optimal growth in 1-2 weeks	**Growth Media**	**Optimal Light Level**
WH7803	1:20	1:10	SN Media	15-20uE
CC9311	1:20	1:10	SN Media	10uE-20uE (Prefers 10uE on the diurnal cycle)
WH6501	1:20	1:10	SN Media	20uE
WH8102	1:20	1:10	SN Media	20-35uE
CC9605	1:20	1:10	SN Media	20uE
WH8020	1:20	1:10	SN Media	20uE
MIT9220	1:20	1:10	Pro99 Media or **50% AMP** Media	10-20uE
WH8101	1:10	1:5	SN Media	10-20uE
WH5701	1:10	1:5	SN Media / 50% AMP Media	10-20uE
UW105	1:10	1:5	SN Media / 50% AMP Media	10-20uE
UW106	1:10	1:5	**SN + NH4 Media**	10-20uE
UW179	1:10	1:5	SN Media / 50% AMP Media	10-20uE
UW92	1:10	1:5	SN Media / 50% AMP Media	10-20uE

UW69	1:10	1:5	**SN + NH4 Media**	10-20uE
UW140	1:10	1:5	SN Media / 50% AMP Media	10-20uE (Prefers 10uE on the diurnal cycle)
Med4	1:20	1:10	Pro99	20-40uE
NATL2A	1:20	1:10	Pro99	10-20uE
AS9601	1:30	1:15	Pro99	20-40uE
MIT9313	1:20	1:10	Pro99	10-20uE
MIT9312	1:20	1:10	Pro99	20-40uE
MIT9301	1:20	1:10	Pro99	10-20uE

Spot Test Protocol

5. Make bottom agar by following the media recipe and adding low melting point agarose.

6. IN THE BIOLOGICAL SAFETY CABINET (HOOD): Set up all square petri dishes (each has a 6x6 grid on the bottom dish) that you will be adding bottom agar to in stacks of 5. (Note: Save the sleeves the petri dishes came in).

7. Microwave bottom agar until the agarose is completely dissolved and it is now a "liquid."

8. IN THE BIOLOGICAL SAFETY CABINET (HOOD): While the bottom agar is HOT, pour 15mL bottom agar in a square petri dish that has a 6x6 grid. (Note: Make sure that the bottom agar has **completely coated** the bottom of the plate from edge to edge).

9. Let the plates cool overnight with the UV light OFF and the blower ON. Put a note on the hood to let the rest of the lab know that you are purposefully leaving on the blower and not to turn on the UV light.

10. The next day, carefully put your plates with bottom agar back in the sleeves they came in. Store in the 4°C cold room **right-side up**, NOT upside down. This means the agar filled part of the square petri dish is facing towards the ground.

11. Ensure you have enough host (cells) to create your spot test assays and that the host is growing well. If you do not have enough host, you must create your own grow up. Calculate how much host you will need and grow it up in advance. You will need 2-3mL host per square petri dish.

12. Once you know your host is growing well and ready to use in an assay, 2-3 days prior to adding your "spots" add your top agar + host.

a. Turn on the host water bath to 35°C and make sure you have the 50ml conical tube insert in the bath.

b. Ethanol your bench.

c. Take out your bottom agar square petri dishes and label the top and the side of the petri dish. Lay them out individually out on your bench.

d. For each plate that you will be adding top agar + host, create a 50mL conical with 2-3mL host (NOTE: Use your discretion...if the host is growing well, then only add 2mL...if the host is growing

poorly, then add 3mL).

e. Microwave your top agar until the agarose is completely dissolved and it is now a "liquid."

f. Aliquot enough hot agarose into 50mL conical tubes. You will need 15mL top agarose per square petri dish, so aliquot what you need + 10% more to account for pipetting error. (NOTE: Be careful! Media will be hot).

g. Allow the agarose to cool to 35°C. (NOTE: If the media is too hot, it will kill your cells).

h. Pour 15mL 35°C top agar into the 50mL conical tube containing the 2-3mL host. (NOTE: Only add the 15mL 35°C top agarose to 5 conical tubes at one time...otherwise your agarose will solidify and your lawn of bacteria will not be smooth).

i. Invert your 50mL conical tube with 15mL 35°C top agar + 2-3mL host only 1x. Pour over the bottom agar, ensuring proper coverage. Put lid on petri dish right away to prevent contamination. Let cool over night.

j. The next day, parafilm your plates and put in the light chamber to grow at the correct light level.

13. Two to three days after adding your top agar + host, create a grid map of where you will be adding your "spots" of phage on the plates. Add 5µl of phage per square in the grid based on the map you created. (NOTE: Be very careful not to move the plates while adding the "spots").

14. Let your spots absorb to the top agar for a least 1 hour, but no greater than 5 hours. Parafilm your plate carefully and put in the light chamber to grow at the correct light level.

15. Monitor your spot tests for the next 21 days. Take pictures on days 3, 7, 14, 21 and use ImageJ to measure the size of the "spots."

Plaque Assay Protocol

1. Make bottom agar by following the media recipe and adding low melting point agarose.

2. IN THE BIOLOGICAL SAFETY CABINET (HOOD): Set up all petri dishes that you will be adding bottom agar to in stacks of 5. (NOTE: Save the sleeves the petri dishes came in).

3. Microwave bottom agar until the agarose is completely dissolved and it is now a "liquid."

4. IN THE BIOLOGICAL SAFETY CABINET (HOOD): While the bottom agar is HOT, pour 1.3mL bottom agar in the tiny petri dishes or 3mL bottom agar in the medium size petri dishes. (NOTE: Make sure that the bottom agar has **completely coated** the bottom of the plate from edge to edge).

5. Let the plates cool overnight with the UV light OFF and the blower ON. Put a note on the hood to let the rest of the lab know that you are purposefully leaving on the blower and not to turn on the UV light.

6. The next day, carefully put your plates with bottom agar back in the sleeves they came in. Store in the 4°C cold room **right-side up**, Not upside down. This means the agar filled part of the square petri dish is facing towards the ground.

7. Ensure you have enough host (cells) to create your spot test assays and that the host is growing well. If you do not have enough host, you must create your own grow up.

Calculate how much host you will need and grow it up in advance. You will need 500µl-1000µl (1mL) per tiny petri dish and 1-2mL per medium petri dish.

8. Dilute your phages by performing a serial dilution.

9. For each tiny petri dish plaque assay you will be performing, add 500µL-1000µL (1mL) host to 5mL snap cap tubes. For each medium petri dish plaque assay you will be performing, add 1mL-2mL host to 15mL snap cap tubes.

10. Label these tues with the host, phage name, and the dilution of the phage.

11. For the tiny petri dish plaque assay, add 250µl of the correct dilution of the phage to the 5mL snap cap tube containing the host. For the medium petri dish plaque assay, add 500µL of the correct dilution of the phage to the 15mL snap cap tube containing the host.

12. Put the snap caps in the light chamber for 1 hour to overnight to let the phage find its host.

13. Turn on the host water bath to 35°C and make sure you have the 50mL conical tube insert in the bath.

14. Ethanol your bench.

15. Take out your bottom agar petri dishes and label the top of the petri dish. Lay them out individually out on your bench.

16. Microwave your top agar until the agarose is completely dissolved and it now a "liquid".

17. Aliquot enough hot agarose into 50mL conical tubes. You will need 2.5mL top agarose per tiny petri dish and 4.5mL top agarose per medium petri dish, so aliquot what you need +10% more to account for pipetting error. (NOTE: Be careful! The media will be hot).

18. Allow the agarose to cool to 35°C. (Note: If the media is too hot, it will kill your cells).

19. For the tiny petri dishes, pour 2.5mL 35°C top agar into the 5mL snap cap tubes contining the host and the phage. For the medium petri dishes, pour 4.5mL 35°C top agar into the 15mL snap cap tubes contining the host and the phage. (NOTE: Only add the 35°C top agarose to 5 conical tubes at one time...otherwise your agarose will solidify and yor lawn of bacteria will not be smooth).

20. Invert your conical tube with 35°C top agar + host + phage only 1x. Pour over the bottom agar, ensuring proper coverage. Put lid on petri dish right away to prevent contamination. Let cool over night.

21. The next day, parafilm your plates and put in the light chamber to grow at the correct light level.

22. Count plaques on plaque assays on days 10, 14, 17, and 21. (NOTE: If you use a sharpie to count, ethanol off the sharpie each time you count so that it does not make it difficult to count the next time).

Protocol