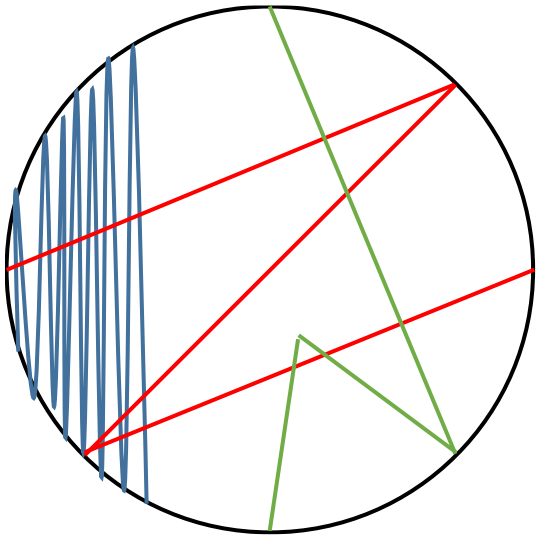
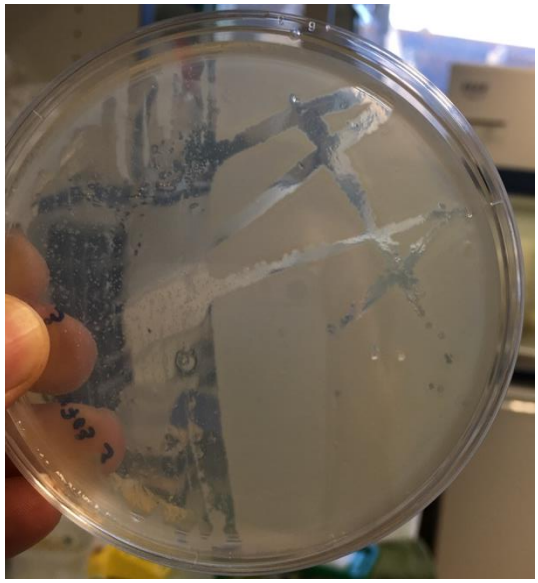
# Serial purification of phages by streaking

(Adapted from Kauffman and Polz 2018)

Introduction

After isolating a phage from a plate and before producing a high titre stock, it is necessary to re-isolate it at least twice.





**Materials (example for V. crassostreae)**

- Marine broth (MB)
- Marine agar (MA)
- Top agar (entre 0.4 et 0.2% selon les phages)

**Protocol:**

1. Boil heavily the top agar bottle and check that the media is completely liquid and homogeneous. Keep at 55ºC with stir.

2. Dispense 100 µl of host overnight culture on dry MA plates with a 2,5 ml fresh agar overlay (if top agar has "clumps" discard it or boil again). **Next steps have to be done quickly since top agar has to be molten.**

3. Quickly, insert yellow tip into the plaque or liquid phage source and spread it (from the border of the plate to the centre) on the still-molten agar overlay (blue line on figure).

4. Use a second yellow tip to make three separate strokes (Z) from the initial zone through the top agar **(never come back when you are doing the Z!)** (red line on figure).

5. Finally, use a third yellow tip to make a new Z through the strokes done in the step 4 (green line on figure).

6. Incubate the plates for 24h. Once plaques appear, use this plate as phage source and repeat the process from step 2 twice.
7. Collect a single plaque using 1 ml tip (previously, cut a little bit the 1 ml tip) from the third serial purification. Eject the agar plugs of plaque in Eppendorf with 750 µl of MB and allow the elution of phage particles into the media overnight at 4ºC.

8. After soaking, use a syringe to filter the phages by 0,2 μm in a new tube (you will lose volume due to the filtration, approx. your final volume will be 300 μl). Purified phage stock could be stored at 4ºC (or -80ºC with 25% glycerol) or use for preparation of high titer stocks.

**Important tip!**
Most of the phages in our collection (Caudovirale) are isolated using 0.4% TOP agar. Schizotequatroviruses require 0.2% TOP agar made with VWR agar J637-500G.

## Reference

Kauffman KM, Polz MF. Streamlining standard bacteriophage methods for higher throughput. MethodsX. 2018 Jan 31:5:159-172. doi: 10.1016/j.mex.2018.01.007.