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Plaque PCR

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Protocol status: Working

We use this protocol and it's working

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- 1 Mark independent plaques on the back of the Petri dish with a black pen. Take 6 points in a plate and try to take points randomly and evenly.
- 2 Take 24 1.5 ml EP tubes.
 - A) Add 200 μ l SM Buffer per tube
 - B) Add 2 μ l chloroform to each tube

Sampling

- 3 Suck out the plaque just spotted with the pipette
- 4 Put into the prepared EP tube.
- 5 Open the metal bath to 95 degrees for lysis of the phage
- 6 Place the EP tube at room temperature for one hour to dissolve the sample sufficiently and mix several times during the period.

PCR system

- 7 KOD FX 20ul system
- 8 Add 18.4 μ l to each tube (1.6 μ l template is needed)
- 9 After an hour, put the EP tube in a metal bath and heat it for 10 minutes.
- 10 Centrifugation for 5 min, 12,000 rpm
- 11 Take 1.6ul supernatant from EP tube and add it to new EP tube.
- 12 Centrifuge the EP tube briefly with a centrifuge.



13 Place in PCR instrument.