Colony PCR with ready-to-load PCR master mix (similar to GoTaq)

Stephen Floor

Abstract

This protocol makes a master mix for PCR that is ready to load into an agarose gel. Useful for colony PCR and related.

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Protocol

Prepare the master mix

Step 1.

First make these:

40% sucrose with 0.25% xylene cyanol and 0.25% orange G
(this is a 5X stock)

To make 10 ml:

4g sucrose

25 mg xylene cyanol

25 mg orange G

bring to 10 ml with ddH2O

Master mix ingredients for 2X:

Reagent	1ml 2X master mix	10ml 2X master mix	50ml 2X master mix
Water	138 ul	1.38 ml	6.9 ml
HF buffer	400 ul	4 ml	20 ml
dNTPs	40 ul	400 ul	2 ml
Phusion polymerase (or another polymerase)	20 ul	200 ul	1 ml
Sucrose dye mix (above)	400 ul	4 ml	20 ml

It can also be useful to make a 1.25X master mix where you only need to add primers and no water, for colony PCR.

For 1.25X master mix:

Reagent	1ml 1.25X master mix	10ml 1.25X master mix	50ml 1.25X master mix
Water	462.5 ul	4.62 ml	23.1 ml
HF buffer	250 ul	2.5 ml	12.5 ml
dNTPs	25 ul	250 ul	1.25 ml
Phusion polymerase (or another polymerase)	12.5 ul	125 ul	625 ul
Sucrose dye mix (above)	250 ul	2.5 ml	12.5 ml

Aliquot to 1ml or less.

Colony PCR with master mix

Step 2.

Mark the colonies you would like to do PCR from by circling and numbering the plate. Colonies must be far enough apart from others that you are able to pick a single colony.

Colony PCR with master mix

Step 3.

Prepare forward and reverse primers at 2.5 micromolar each in water. Design primers to amplify across the region being tested - typically looking for an insertion by the length of the PCR products.

e.g. 1 ml primer mix

25 ul forward primer (100 uM)

25 ul reverse primer (100 uM)

950 ul water

Colony PCR with master mix

Step 4.

Combine 2 volumes of 2.5 micromolar primer mix with 8 volumes of 1.25X master mix from above

for example, add 200 ul primer mix to 800 ul master mix

Colony PCR with master mix

Step 5.

Put 10 ul of PCR master mix with primers into as many PCR tubes as needed

Colony PCR with master mix

Step 6.

Put a tip on a 10 microliter pipet set at 7 microliters and touch the tip to a marked colony. Move the tip into the PCR tube and pipet up and down at least 5 times while swirling the tip to move the colony into the PCR tube

Colony PCR with master mix

Step 7.

After picking all colonies, run a standard PCR with an elongation time sufficient to extend across your amplicon and load directly onto a gel.