

Making Master Filters for colony screening

Cut nitrocellulose to fit plate.
label each filter w/pencil or ball point pen
float in tray of water until wet
sandwich between two 3MM pieces
wrap stack of filters in foil and sterilize by autoclaving
Lay sterile filter on plate until wet on each side

Plating transformation onto filter

Lay wet filter on plate
spread cells onto filter leaving edge clear.
Let liquid absorb a bit, face up
Place in incubator inverted O/N

Freezing master filter

Transfer filter to plate containing 25% glycerol
Incubate 37°C for 2 hrs.
Seal with parafilm. Store inverted at -80
Thaw at room temp before making replica

Making replica filters

Peel NC off plate and lay on damp pad of 3MM
Lay damp sterile filter on master filter (no bubbles)
Press down with glass plate
Poke 3 holes to orient later
Lay new replica on plate and incubate at 37 until colonies appear
Put master filter on fresh plate w/ 25% glycerol put at 37°C for 1 hr.
Store master filter at -80.