C/P Lift® Membranes

Instruction Manual

Catalog Numbers
162-0162
162-0163

BIO-RAD

Table of Contents

Section 1
Introduction

C/P Lift membranes were developed for colony and plaque screening. When handling the membranes, always wear gloves or use forceps. After blotting, do not allow wet membranes to come in contact with each other. Contact may result in the transfer of blotted nucleic acids from one membrane to the other.

Section 2
Colony Screening

2.1 Direct Lifts

1. Plate cells containing recombinant plasmids onto the appropriate selective agar. Incubate plated cells until the colonies are 1-2 mm in diameter.
2. Place a circle of C/P Lift membrane onto the surface of the plate as follows. Hold the membrane at opposite edges with forceps, bending it slightly into a ‘U’-shape, and then lower the membrane until the fold makes contact with the center of the plate.

Lower the edges until the entire membrane is evenly wetted.

3. Orient the membrane and underlying agar by puncturing both with an 18-G needle containing black ink. Take care when removing the needle not to move the membrane.
4. After 2-5 minutes carefully peel the membrane off of the plate.
5. Store the plate at 4 °C. It may be desirable to first incubate the plate for a few hours to regenerate visible colonies.
6. Place the C/P Lift membrane, colony side up, onto a pad of 3MM filter paper saturated with 0.5 M NaOH for 5 min. Then blot the membrane briefly on dry 3MM filter paper.
7. Repeat step 6.
8. Rinse briefly in 2 x SSC, 0.2% SDS to remove cellular debris.
9. Air dry 30 minutes, bake 1 hour at 80 °C.
10. Hybridize (see Section 4).

2.2 Replica Lifts (with amplification)

1. Place a C/P Lift membrane disc on the appropriate selective agar. Plate cells containing recombinant plasmids onto this membrane, and incubate until colonies are 1-2 mm diameter.
2. Remove the disc and place it on two clean sheets of 3MM filter paper, colony side up.
3. Place a circle of C/P Lift membrane onto the surface of a selective agar plate for pre-wetting. Then transfer it onto the membrane disc with colonies. Place the surface of the C/P Lift membrane that did not come in contact with the agar onto the membrane disc with colonies. Hold the membrane at opposite edges with forceps, bending it slightly into a 'U'-shape, and then lower the membrane until the fold makes contact with the center of the nitrocellulose membrane. Lower the edges until the entire membrane is evenly wetted.
4. Place two clean sheets of 3MM filter paper on top of both membranes and apply firm, even pressure with a plate.

5 Remove the upper sheets of 3MM filter paper and orient by puncturing both membranes with an 18-G needle containing black ink. Take care when removing the needle not to move the membrane.

6. Carefully peel the membranes apart and place the C/P Lift membrane onto its selective agar plate with the colony side up. If desired, additional replicas can be prepared from the C/P Lift membrane master in the same manner.
7. Return the master filter to its original plate and store at 4 °C.
8. Incubate the C/P Lift membrane replica until the colonies are 0.2-0.5 mm diameter, then transfer the membrane (colony side up) onto a fresh plate containing chloramphenicol at 200 µg/ml and incubate for a further 6-18 h.
9. Place the C/P Lift membrane, colony side up, onto a pad of 3MM filter paper saturated with 0.5 M NaOH for 5 min. Blot the membrane disc briefly on dry 3MM filter paper.
10. Repeat step 9.

11. Rinse briefly in 2 x SSC, 0.2% SDS to remove cellular debris.
12. Air dry 30 minutes, bake 1 hour at 80 °C.
13. Hybridize (see Section 4).

Section 3
Plaque Screening

1. Cool plate containing plaques for 5 minutes.
2. Continue with steps 2 through 9 of Section 2.

Section 4
Hybrization Protocol

4.1 Prehybridization

1. Seal blotted membrane inside a heat sealable plastic bag.

2. Cut one corner of the plastic bag and pipet prehybridization solution in:

   1 mM EDTA

   0.5 M $NaHPO_4$ pH 7.2 *

   7% SDS

3. Reseal the plastic bag and incubate briefly at 65 °C for 5 minutes.

4.2 Hybridization

1. Cut one corner of the plastic bag, remove the prehybridization solution and replace it with same buffer.
2. Add denatured probe and remove all bubbles before resealing the bag. Hybridize for 4-24 hours at 65 °C with agitation.
3. Carefully remove the hybridization solution by cutting one corner. Remove hybridized membrane from plastic bag.

Note: At no stage before washing should the membranes be permitted to dry.

4.3 Washes

1. Wash membrane at 65 °C, 2 times, for 30-60 minutes each in the following:

   1 mM EDTA

   40 mM $NaHPO_4$ pH 7.2

   5% SDS

2. Wash membrane at 65 °C, 2 times, for 30-60 minutes each in the following:

   1 mM EDTA

   40 mM $NaHPO_4$ pH 7.2

   1% SDS

3. After washing, the blotted membranes are ready for autoradiography. Expose moist membranes between Saran Wrap or enclosed in a sealable plastic bag. Do not allow a wet membrane to come in contact with the film, because wet membrane will stick to the film.

*1 M $NaHPO_4$ pH ≈7.2	MW	g/l
1 M $Na_2HPO_4 \cdot 7H_2O$	268.07	134

Add 4 ml 85 % H_3PO_4 [1 M in Na^+]

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
LIT267 Rev B