PCR based λDNA library screening method

This is a very useful method for the one who wants to screen a phage library non-radioactive and do not need too many clones. Up to 5 clones can be obtained usually within one week. However a prerequisite of this method is some knowledge in λ phage handling and a specific and fast PCR method for the detection of the sequence you are searching for.

N_{ti} - total number of phages, used for each step of the screening, i - step number (1, 2, 3)

N_{wi} - number of phages in one well for each step of the screening, i - step number (1, 2, 3).

I. Determination of the lowest number of plaque forming units (pfu) necessary for the screening

- Prepare λ phage dilutions of 10^3, 10^4, 10^5 pfu/µl. Use 1 and 5 µl of each dilution as a template for a PCR reaction to determine the minimal amount of phages required for obtaining the PCR product (N_{t1}).

II. Primary screening (Figure)

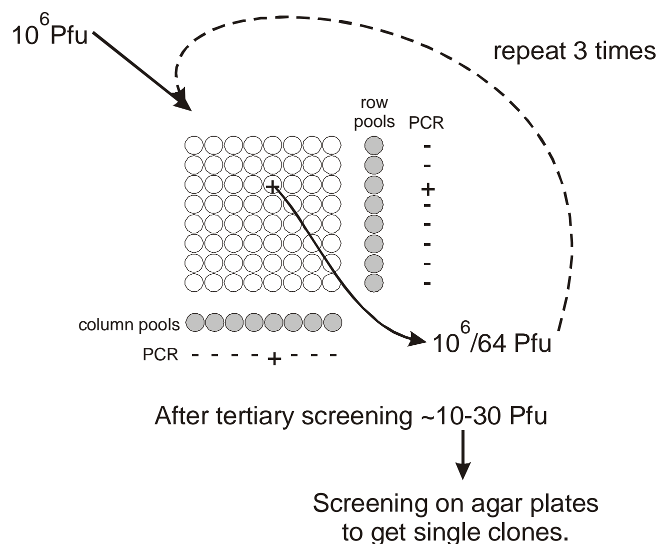

Figure. *PCR based screening of genomic library*

- prepare overnight culture of the bacteria host strain until OD_{600}=2.0.
- mix the overnight culture (1 ml) with the equal volume of λ dilution buffer, containing 3xN_{t1} phages.
- After 15 min incubation in a water bath at 37°C, add 18 ml of LB broth containing 10mM $MgSO_4$.
- Distribute 6.4 ml of the suspension in 8x8 microtiter plate wells, 100 µl/well.
- Seal the plate with a sealing tape to prevent cross-contamination and incubate at 37°C for 5-6 hours, with shaking at 200-300 rpm.
- Pool rows and columns by taking 20 µl from each well. Dilute pooled phage suspensions with distilled water 1:1.
- Perform PCR on pooled phage suspensions, using 1 µl of each suspension as a template source.
- Determine wells in the crossing of positive rows and columns.
- to confirm positive wells perform PCR with phages from individual wells.
- Determine phage titer.
- **Note:** Sometimes it's useful to determine phage titer from the positive well, but usually titer are almost equal in all wells on the plate and is approximately 1-6x10^9 pfu/ml.
- phage dilution for the second step of the screening is: N_{t2}=N_{w1}x2
- **Note:** only during primary screening different positive wells on one plate may contain different phage clones. During secondary and tertiary screening all positive wells on one plate contain the same phage clones.

III. Secondary screening

- Mix overnight culture of host bacteria (1 ml) with the equal volume of λ dilution buffer, containing 3xN_{t2} phages.
- After 15 min incubation in a water bath at 37°C add 18 ml of LB broth containing 10mM $MgSO_4$.
- Distribute 6.4 ml of the suspension in 8x8 microtiter plate wells, 100 µl/well.
- Seal the plate with a sealing tape to prevent cross-contamination and incubate at 37°C for 5-6 hours, with shaking at 200-300 rpm.
- Pool rows and columns by taking 20 µl from each well. Dilute pooled phage suspensions with distilled water 1:1.

- Perform PCR on pooled phage suspensions, using 1 µl of each suspension as a template source.
- Determine the wells in the crossing of positive rows and columns.
- to determine positive wells perform PCR with phages from individual wells.
- Select one positive well and determine phage titer.
- phage dilution for the next step of the screening is: $N_{t3}=N_{w2}x2$

IV. Tertiary screening.

- Repeat the same procedure as for the secondary screening using $3xN_{t3}$ phages.

V. Screening on agar plate.

- Plate 100 pfu of the phage obtained in tertiary screening on 90 mm plate and incubated overnight at 37°C
- Pick several plaques using Pasteur pipette
- Transfer picked plaques to the Eppendorf tube, containing 200 µl λ dilution buffer and a drop of chloroform, vortex briefly.
- Elute phage at 37°C with shaking for 4-6 hr.
- Remove debris by centrifugation at 10.000 x rpm for 2 min.
- Determine positive plaques by PCR.