

# Radioactive Hybridisation

## Prehybridisation:

min. 30' (better 1-2 hours) at 57°C in VIRCA-Solution (20-50 ml)

## Labelling:

with *Boehringer Mannheim Random Primed DNA Labeling Kit* or *Boehringer Mannheim High-Prime Kit* (see Datasheets)

## Cleaning of labelled probe:

with *Qiagen Nucleotide Removal Kit* (see Datasheet), determine activity of the cleaned probe in the Beta-Counter

## Hybridisation:

in VIRCA-Solution (5-10ml) containing min.  $10^6$  Counts/ml labelled probe at 57°C over night

## Wash:

with 5%SDS/1xSSC (each time approx. 20-30 ml)

- 10' at 20-25°C
- min 2-4x 20' at 57°C

pack membrane in plastic-foil, place it on *Kodak X-OMAT AR* film, expose at -80°C and develop film after 1-14 days

## Solutions:

### **1 M NaPO<sub>4</sub>:**

57,7 ml 1 M Na<sub>2</sub>HPO<sub>4</sub>  
42,3 ml 1 M NaH<sub>2</sub>PO<sub>4</sub>  
pH 7,0

### **20xSSC:**

3 M NaCl  
0,3 M Na-Citrat.2H<sub>2</sub>O  
pH 7,0

### **10%SDS:**

10 % SDS in H<sub>2</sub>O

### **VIRCA-Solution: (Hybridisation-Buffer)**

25 ml 1 M PIPES, pH 6,5  
10 ml 5 M NaCl  
25 ml 1 M NaPO<sub>4</sub>  
1 ml 0,5 M EDTA, pH 7,0  
250 ml 10%SDS  
190 ml H<sub>2</sub>O