

## Two liter hybridization buffer Leiden:

Prepare phosphate buffer as follows:

- approx. 500 mL demineralised water in 2 Liter beaker
- dissolve 44,45 gram  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  (MW=177,99, we have used this salt of several companies (Merck, Fluka) with same good results) (dissolving takes 1 hour, heat a little bit, not more than 40C)
- Set pH with 85% phosphoric acid (2-3 mL) to exactly pH = 7.2
- Do not sterilize, prepare previous to preparation hybridization buffer.

Add to phosphate buffer:

- 100 mL NaCl
- 4 mL 0.5 M EDTA (pH 8.0)
- 140 gram SDS, or 700 mL 20% SDS solution (If you use solid SDS, use the hood (very toxic))
- 200 gram PEG-6000

Add demineralised water until almost 2 Liter

Heat in water bath or microwave until 65C, than stir until PEG and SDS are dissolved.

Add demineralised water until exactly 2 Liter.

Buffer is now ready to use (You do not have to check the pH again)

- Heat to 65 C
- Add 100 ug/ml Salmon sperm DNA to hybridization buffer and start prehybridization.
- Prehybridize for at least 45 minutes, Then add probe

**Note:** I do not replace the 'pre'-hybridization buffer with fresh buffer. Instead I use the same buffer for prehybridization and hybridization