**NaPi/PEG hybridization buffer**

Prepare phosphate buffer as follows:
- approx. 500 mL demineralised water in 2 Liter beaker
- Dissolve 44.45 gram Na₂HPO₄ * 2H₂O (MW=177.99, Merck, Fluka)
- Dissolving takes 1 hour, heat a little bit, not more than 40°C
- 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 (5M)
- 4 mL 0.5 M EDTA (pH 8.0)
- 140 gram SDS, or 700 mL 20% SDS solution (for solid SDS use the hood (very toxic))
- 200 gram PEG-6000
- Add until almost 2 Liter with demineralised water
- Heat in water bath or microwave until 65°C, than stir until PEG and SDS are dissolved.
- Finally add demineralised water until exactly 2 Liter.

For hybridization:
- Heat until 65°C
- Add 100 ug/ml Salmon sperm DNA to hybridization buffer (preheated at 95°C) and start prehybridization (standard prehybridization at least 45 minutes)
- We do not replace the 'pre'-hybridization buffer by fresh hybridization buffer.

**2xNaPi hybridization buffer (for Formamide hyb mix)**

Prepare 2x phosphate buffer as follows:
- approx. 500 mL demineralised water in 2 Liter beaker
- Dissolve 44.45 gram Na₂HPO₄ * 2H₂O (MW=177.99, Merck, Fluka)
- Dissolving takes 1 hour, heat a little bit, not more than 40°C
- 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 (For solid SDS use the hood (very toxic))
- Add until almost 1 Liter with demineralised water
- Heat in water bath or microwave until 65°C, than stir until SDS is dissolved.

For hybridization:
- Heat until 65°C
- Add 1 volume of deionized Formamide
- Add 100 ug/ml Salmon sperm DNA to hybridization buffer (preheated at 95°C) and start prehybridization (standard prehybridization at least 45 minutes)
- We do not replace the 'pre'-hybridization buffer by fresh hybridization buffer.