p13E-11 hybridizations

Dextrane Hybridization buffer (1 Liter):

500 mL H2O
200 mL 5M NaCl
25 mL 2M Tris-HCl (pH 7.5)
50 mL 20% SDS
100 g Dextran sodium sulfate (→add slowly, while mixing the solution)
Add until 1 Liter with demineralized water
Heat in water bath until 65°C, then stir until Dextran sulfate is dissolved

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.

D4Z4 hybridizations

2xNaPi hybridization buffer (1 liter), use in combination with an equal volume of deionized Formamide)

Prepare 2x phosphate buffer as follows:
approx. 500 mL demineralized water in 2 Liter beaker
Dissolve 44,45 gram Na_2HPO_4 * $2H_2O$ (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 prior 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 1 Liter with demineralized water
Heat in water bath until 65°C, then 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.

For 4qA+4qB and all other hybridizations:

NaPi/PEG hybridization buffer (2 liter):

Prepare phosphate buffer (0.5 M) as follows:
approx. 500 mL demineralized water in 2 Liter beaker
Dissolve 44,45 gram $Na_2HPO_4 * 2H_2O$ (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 prior 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 demineralized water
End concentration:
0.125 M phosphate buffer, 0,25M NaCl, 1mM EDTA, 7% SDS, 10% PEG6000
Heat in water bath until 65°C, then stir until PEG and SDS are dissolved.
Finally add demineralized water until 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.