Calcium Chloride / BBS Transfection Protocol

Dear Dr. Robertson,
George told me that you need the protocol for transfection with CaCl2/BBS.
The recipe for 2x BBS is as follows:
50 mM Bes (Calbiochem)
280 mM NaCl
1.5 mM Na2HPO4
pH 6.95 (adjust with 1N NaOH)
The most crucial thing is the pH of the buffer.
Since no pH-meter is absolutely reliable, you will have to make 3X1 liter of buffer separately and measure the pH for the first one liter at 6.9 the second one at 6.95 and the third one at 7.00. Then you do pilot transfections (I do that with GFP vector) to measure the efficiency of each buffer. The one that gives you the best results is to be kept.
Now the transfection procedure is as follows:
For each well of a 6 well plate you add DNAs + 8.24λ 2.5M CaCl2 +distiledH2O up to 100λ. Then you add to each sample 100 λ BBS, shake briefly and incubate in RT for 20 min. You add the mix to 1ml medium/well (for a 6 well plate). You can scale the volumes up or down depending on the size of the plate you are using.
After 4 –6 hours you have to wash the cells 3 times with PBS and change medium.
For any questions don't hesitate to reach me.
Good luck with your experiments.
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Cheers Mario.