Hybridization Protocol

1] Clean hybridization bottles with RNAse Zap as per instructions
2] Heat Ultrahyb to ~65C to dissolve precipitant
3] Carefully place blot in hybridization bottle with RNA side facing inward
4] Add 10ml Ultrahyb/100cm^2 blot area to a clean hybridization bottle
5] Close tightly (make sure that no part of the blot overhangs the edge of the bottle and gets into the threaded areas ….this will end up in wicking of Ultrahy out of bottle and you will have a mess)
6] Pre-Hybridize at 42C in Hybridization Chamber for at 30-60min.
7] Add Radioactive probe directly to Ultrahy buffer in the Hybridization Bottle (no need to change buffer)
8] Close tightly again
9] Place back in hybridization chamber at 42C for 14-24 hrs.
10] Next day, wash 2x5min with Low Stringency Wash Buffer
11] Wash 2x15min with High Stringency Wash Buffer
12] Carefully! take the blot out of bottle after last wash and wrap in Saran
13] Place blot in Saran into Phosphorimaging cassette (make sure the screen has been erased)
14] Let the blot expose in cassette at least 3-4 hours (longer if small signal expected)