Cilia preparation of MDCK cells for biochemical study

1) **MDCK cell growth:**
   The best way is to grow MDCK cells on transwell plate at 100% confluence for around 7-10 days (usually 10 days). Use DMEM+10% FBS at the outside membrane and DMEM/NO FBS at the inside membrane of transwell plate. Change media every 2-3 days. If using regular cell culture plate, not transwell plate, use low glucose DMEM+10% FBS (this medium makes a similar condition as serum starvation to cause longer cilia).

2) **Cilia preparation**
   The ciliary-conditioned MDCK cells were incubated in 30 mM ammonium sulfate for 3 h, which will induce shedding of intact cilia. Collect the medium supernatant containing the cilia. Centrifuge at 2000 × g for 30min to remove any floating cells. Centrifuge again at 10,000 × g for 30 min to remove the cell debris. Re-centrifuge at 16,000 × g for 30 min; the pellet contains the cilia population. Resuspend the cilia pellet in PBS for immunofluorescence or in lysis buffer (20 mM sodium phosphate, pH 7.2/ 150 mM NaCl/ 1 mM EDTA/ 10 % (vol/vol) glycerol/ 1 % Triton X-100/ protease inhibitors) for Western blot.