

SHH-N Conditioned Media (CM):

COS cells can be purchased from ATCC or obtained from your colleagues. They are typically cultured in DMEM-high glucose / 10% FBS / pen-strep.

1. Seed 10cm plates with ~ 50% confluency of COS cells the night before.
2. Wait until cells reach ~80% confluency the following day (usually in the afternoon).
3. Transfection (for each 10cm plate):

Dilute: 10ug of pRK5-SHH-N in 500ul of OPTI-MEMI
: 30ul of Lipofectamine in 500ul of OPTI-MEMI

Mix the two (1ml total) in a 15 ml tube, vortex very briefly (when applicable, please scale up accordingly).

Wait for 20min, RT.

4. At the end of mixture incubation, add 4ml of OPTI-MEMI to the mixture, vortex briefly.
5. Rinse COS cells twice with 5ml of OPTI-MEMI. Put the final transfection mix on COS cells. Swirl the plates to mix, O/N in the incubator.
6. Next day morning, rinse plates with 5ml culture media once, then switch to the media you wish to use in your experiment.

We typically use 2% serum in the media so Western can be performed without too much serum interference – you can supplement serum to the required percentage when you use the CM on your experiment.

48hrs later, collect the media, filter through 0.2um filter, aliquot, snap freeze in Lig. N2 or Dry ice-EtOH bath.

Make sure you do a Gli-reporter assay and run a Western to assess the activity and protein concentration before you use the CM. Typically, we get between 0.5-1ug/ml SHH-N.