Purification of chromatin-associated TSC RNA under mildly denaturing conditions, from I27 H54\*\* H32 H29 H21 I35

- For one 10cm plate of NFx1 TSCs, expect 20-25ug of RNA

- Wash cells with ice cold PBS, spin at 1300g for 2 mins

- R/S the plate in 2.5ml total of ice-cold, pre-chilled Dnase/Lysis buffer:

40 mM Tris 7.9

25mM NaCl

6mM MgCl2

1mM CaCl2

256mM Sucrose

0.5% Triton X-100

1U/ul RNAsin

450U/ml DNaseI

For 2.5ml:

250ul 10x Roche DNaseI buffer

7.5ul 5M NaCl

640ul 1M Sucrose

125ul 10% TritonX-100

1477.5ul H20

62.5ul 40U/ul RNasin

112.5ul 10U/ul DNaseI

- Rotate in cold room for 5 minutes

- spin out nuclei at 2250g for 2 minutes at 4C

- R/S nuclei in 2.5ml of 1x RT RNA prep buffer:

1.5% SDS

150ug/ml ProK

200mM NaCl

40mM Tris pH 7.9

For 2.5ml:

375ul 10% SDS

100ul 5M NaCl

100ul 1M Tris 7.9

125ul 10ug/ul ProK

1800ul H20

- rock for 45 minutes at RT

- Extract 2x with SHAPE-equilibrated Phenol Chloroform

100 mM HEPES, pH 8.0, 100 mM NaCl, 10 mM MgCl2

\* Back extract organic phase after first round extraction

- 2x chloroform extract

 - Precipitate or column purify RNA