Sugden Lab.

SI Nuclease Mapping Protocol

Kinase of Oligonucleotides

γ32P ATP ~6,000Ci/mM, 150 mCi/ml CAT#NEG-035C (DuPont NEN)

1ul Oligo @ 10pm/ul
2ul 10xNEB Kinase Buffer
15ul H2O
1ul 32P ATP
1ul PNK

32P ATP comes at ~20pm/µl
Use at 2:1 ratio of [ATP] / [Oligo]

On ice o/n @ 4°C

Qiagen Nucleotide Removal Kit (50% recovery in 50ul) or a G25 column
Oligonucleotide will be at 100 fm/ul final concntration

Annealing and SI Digestion

50 µg of RNA for Luciferase Assays
5 or 25 µg of RNA for GAPDH
100 fm of each oligonucltide

	Final
20 µl Formamide	50%
3.2 µl 5M NaCl	0.4M
1.6 µl 1M PIPES (pH 6.4)	0.04M
4 µl 1% SDS	0.1%
1 µl labeled Oligonucleotide DNA	2.5fm/µl
x µl RNA	
up to 40 µl with H_2O	

SI Buffer (2mL)
1 µl SI Nuclease (400Units)
100 µl 5M NaCl
20 µl 3M NaOAc
20 µl 100mM $ZnSO_4$
1µl 10 mg/ml Salmon Spern

1860µl H_2O

Anneal at 42°C >12 hours

400 µl SI Buffer Mix

50°C 75-90min

Stop reaction by adding 80µl 4M NH_3OAc w/20mM EDTA and 3.2µg of tRNA
(400 µl 4M NH_3OAc + 16 µl 0.5M EDTA + 1 µl 15mg/ml tRNA+4 µl Glycogen)

EtOH PPT with 1ml 100% EtOH and resuspend in 5-10µl loading dye

Run on a 10% Dneanturing PAGE (23g UREA, 5ml 10xTBE, 12.5ml 40% 19:1 Acrylamide, 10 ml H_2O- dissolve and add 25 µl TEMED and 250 µl 10% APS)

Last Modified 06/05/05

10x Poly Nuclotide Kinase Buffer (PNK)	1M PIPES (pH 6.4)	Loading Dye (1ml)
7 µl 1M TRIS pH7.6 1 µl 1M $MgCl_2$ 1 µl 0.5M DTT 91 µl H_2O	3g PIPES 9ml H_2O pH w/HCl Use dipotassium of PIPES	950 µl Formamide 40 µl 0.5M EDTA 10 µl of 5%B-Blue & Xylene Cyanol FF