SPLICING ASSAYS (20 ul volume)

2.0 ul	20 mM MgCl2
2.0 ul	ATP/CrPO4
1.0 ul	RNAsin
1.0 ul	100 mM DTT
1.0 ul	[32P]pre-mRNA
7.0 ul	Nuclear Extract
6.0 ul	dH2O (or buffer)

Start reaction by adding the nuclear extract (2 hours at 30C)
Quick 5 min spin (to get the solution off the sides)
Add to tube containing 3 ul stop solution and 3.5 ul proteinase K/tRNA
Spin to get solution off sides
Heat at 45C for 45 min.

Add 210 ul of EtOH/NH4Acetate
Precipitate on ice 15 min
Spin 10 min
Rinse with 500 ul of 70% EtOH
Spin 5 min
Speedivac 5 min
Add loading buffer (5 ul formamide LB)
Incubate 65C for 10 min
Run on 10% PA (1:30)/8 M Urea gel in 1X TBE at 30W for 70 min.
Run markers and pre-mRNA along with samples

Dry gel and expose overnight

SPLICING ASSAYS (20 ul volume)

2.0 ul	20 mM MgCl2
2.0 ul	ATP/CrPO4
1.0 ul	RNAsin
1.0 ul	100 mM DTT
1.0 ul	[32P]pre-mRNA
7.0 ul	Nuclear Extract
6.0 ul	dH2O (or buffer)

Sample	**Addition (ul)**	**dH2O (ul)**
1	-	6
2	-	6
3	-	6
4	-	6
5	-	6
6	-	6
7	-	6
8	-	6
9	3 ul GST-NIPP	3
10	3 ul GST-NIPP	3
11	3 ul GST-NIPP + 3 ul p99	-
12	6 ul MQ19	-
13	6 ul S100 (6/9/94)	-
14	3 ul GST-NIPP	3
15	3 ul GST-NIPP + 3 ul p99	-
16	6 ul MQ19	-
17	6 ul S100 (6/9/94)	-
18	4 ul p99	2

Start reaction by adding the nuclear extract (2 hours at 30C)
Quick 5 min spin (to get the solution off the sides)
Add to tube containing 3 ul stop solution and 3.5 ul proteinase K/tRNA
Spin to get solution off sides
Heat at 45C for 45 min.

Add 210 ul of EtOH/NH4Acetate and recipitate on ice 15 min

Spin 10 min
Rinse with 500 ul of 70% EtOH and spin 5 min
Speedivac 5 min
Add loading buffer (5 ul formamide LB) and incubate 65C for 10 min
Run on 10% PA (1:30)/8 M Urea gel in 1X TBE at 30W for 70 min.
Dry gel and expose overnight