Protocol: Acid Urea Gel for Electrophoresis of Aminoacylated tRNA

2X Sample Buffer: 0.1M sodium acetate, pH 5.0, 8M urea, 0.05% bromophenol blue and 0.05% xylene cyanol

Gel: Small gel size, 0.75 mm thick 6.5% polyacrylamide gel (19:1 acrylamide : bisacrylamide) containing 8M urea in 0.1M sodium acetate buffer, pH 5.0. Add TWICE the amount of 10% APS and TEMED to speed-up polymerization.

Electrophoresis buffer: 0.1M sodium acetate buffer, pH 5.0

tRNA: Load about 100 picomoles of amnioacylated tRNA per lane. Include appropriate control tRNA (deacylated, N-acetylated, aminoacylated, etc)

Power supply: Run at 10W (500 volts?) for 12 hours in the cold room (4 C inhibits deacylation of tRNA)

Staining Solution: 0.06% Methylene Blue in 0.1M sodium acetate, pH 5.0. Stain for 30-60 min. Destain with water. Change water frequently. May take several hours to destain.

Expected pattern of mobility on gel:
Top (Runs slower): Aminoacylated-tRNA
Middle: N-Ac-aminoacylated-tRNA
Bottom: Deacylated-tRNA

Reference: See U.L. RajBhandary papers