From left, 0.6ng, 1.2ng or 2.4ng ivt-MRP (lanes 1–3), and 2μg or 4μg total RNA from HEK293T cells (lanes 4, 5) were loaded on a 7M Urea, 1X TBE, 6% polyacrylamide sequencing gel, transferred to a positively charged membrane (GE Healthcare), and crosslinked in place. A 5’ end labeled oligonucleotide probe complementary MRP RNA (5'-GAGTCCTCAGTGTGTAGCCTAGGA-3') was hybridized to the membrane for 1h at 50°C with 15mL of Rapid Hyb solution (GE Healthcare). Blot was rinsed once and washed three times for 10 min at 50°C, each with 60mL Wash buffer (0.1X SSC, 0.1% SDS). Hybridized bands were visualized by autoradiography on a Phosphoimager. MRP RNA comprises approximately 300 pg per μg of total RNA, or around 0.03% of total RNA by mass. This corresponds to approximately 12,000 molecules of MRP RNA per HEK293T cell.