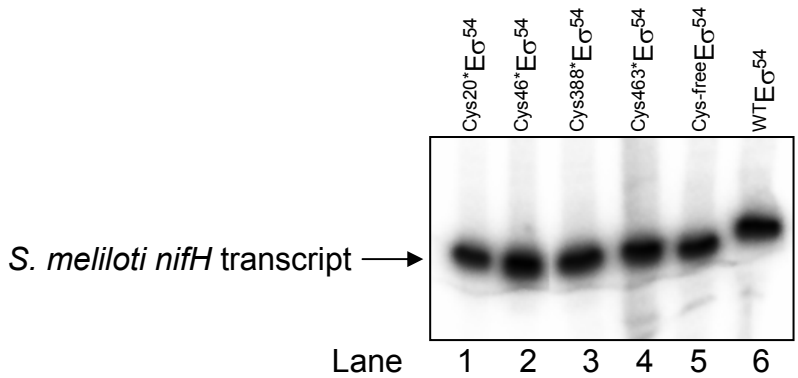

In vitro single-round transcription activity of RNAP reconstituted with the FeBABE modified σ^{54} variants. Assays (10µl reactions) were conducted from the *S. meliloti nifH* promoter on the supercoiled plasmid pMKC28. Mutant Eσ^{54} (100nM, reconstituted with 1:4 molar ratio of core RNAP to σ^{54}) was incubated with 20nM pMKC28 in STA buffer (see Material and Methods) at 37°C for 5 minutes. Activator $PspF_{1\text{-}275}$ (5µM) and ATP (4mM) were added to initiate open complex formation. Following a further 5 minutes incubation at 37°C, transcript formation was initiated by adding 1mM ATP, CTP and GTP, 0.5mM UTP, 3 µCi of [α-^{32}P] UTP (20 µCi/µl), and 100 µg/ml of heparin. Following a further 5 minutes incubation at 37°C, the reaction was stopped by adding 4µl of formamide-loading dye (3% w/v xylene, 3% w/v bromophenol blue and 800 µl of 250 mM EDTA in 10 ml of deionised formamide). The reaction was heated at 90°C for 1 min and transferred to ice. Half (7µl) of the reaction is used for electrophoresis on a 6% w/v polyacrylamide-6 M urea gel. Following electrophoresis, the gel was dried and analyzed by PhosphorImager analysis. As shown above, all Eσ^{54} reconstituted with the σ^{54} variants (lanes 1-5) displayed 85-100% wild-type (lane 6) levels of transcription activity.