RBM22: Expression and Purification

RBM22 fragment (146-420) was cloned in BL21 pET-15b by GenScript. Cells were incubated at 37 ℃ until OD_{600} ~ 0.6 and at 17 ℃ for induction with 1mM IPTG then harvested after 24 hours. Cell pellets were first suspended in lysis buffer (50 mM HEPES, pH 7.5, 2mM β-mecaptoethanol) containing RNase I; sonicated with a Sonic Dismembrator at Set 3 (10-11 Watts) and then centrifuged at 10000 rpm at 4℃ for 1hour. Supernatant containing soluble RBM22 was purified by Ion-EXchange chromatography (IEX), followed by Immobilized Metal Affinity Chromatography (IMAC). Lysate supernatant was loaded on HiTrap SP 5ml column and eluted with a gradient of NaCl (from 0 M to 1 M concentraion). The fractions containing most of RBM22 from IEX were purified further by IMAC in an ÄKTA Purifier FPLC (GE Healthcare) with HisTrap HP 5 ml column and eluted with an Imidazole gradient (from 10 mM to 1 M). The eluent was dialyzed against phosphate buffer containing 20 mM NaP*i*, 100 mM NaCl, 1 mM DTT, 5% glycerol, pH 6.5 using a Float-A-Lyzer with molecular weight cut off (MWCO) of 20 kDa and then concentrated down to 200-400 μl using Amicon Ultra centrifugal device (Millipore) with MWCO of 3kDa. Purify and integrity of RBM22 was confirmed by migration on a SDS-PAGE gel and a non-denaturing PAGE gel which were visualized by staining with Commasie® Blue. RBM22 concentration was determined using Bradford Assay using the Coomassie® Protein Assay Reagent Kit from Pierce Biotechnology with BSA as standard.