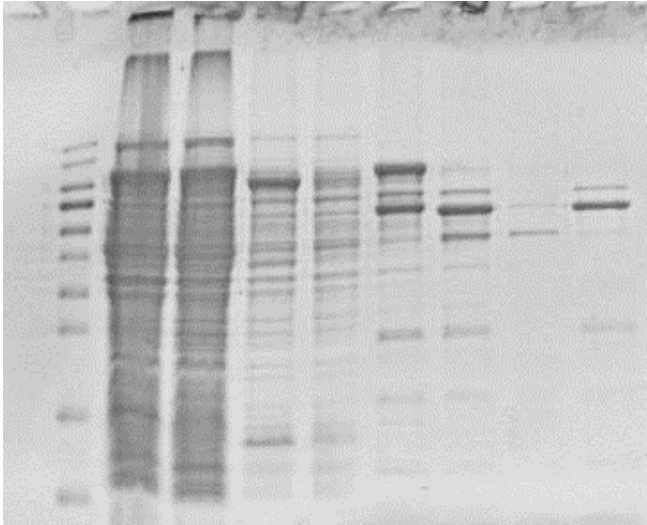According to this paper "Protein Expression and Purification 77 (2011) 185–192", mouse RI is not quite soluble just with His tag. I subcloned the RI into my own vectors. MBP-His-SUMO-RI (pS) and MBP-His-TEV-RI (pT). TEV and SUMO are two cleavage sites.

This is the first try. It seems like the solubility of MBP-His-TEV-RI is not that good.

1. pS expressed in BL21 at 18C O/N
2. supernant of lysate
3. pT expressed in BL21 at 18C O/N
4. supernant of lysate
5. Ni-NTA elution of pS (500 mM imidazole)
6. Overnight digestion by Ulp1 to remove MBP-SUMO (dialysis to buffer without imidazole)
7. Flow through from second Ni-NTA (RI)
8. Elution from Ni-NTA (MBP-SUMO)

It seems that there is some degradation with the MBP-His-SUMO-RI after first Ni-NTA (no change with or without PMSF). After the second Ni-NTA elution, the yield of RI (Lane 7) is around 3 mg per liter LB. The RI also flow through with some free MBP-SUMO tag. It is far away from the results indicated in that paper. So, I move the vector to Rosetta2. The final yield goes to ~10 mg per liter LB. There is still some free tag.
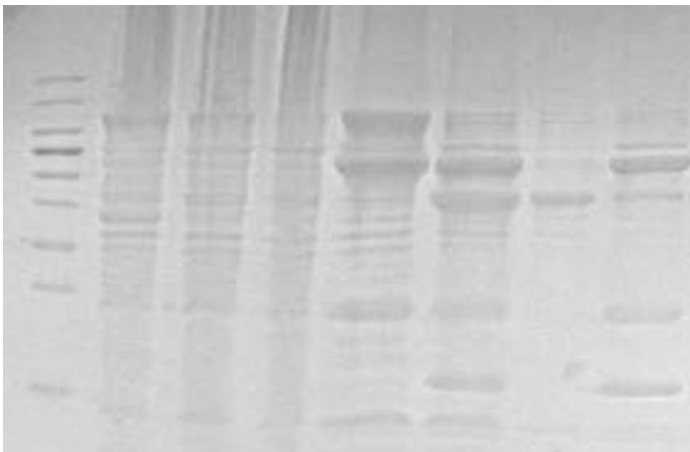
1. pS expressed in Rosetta 2 at 18C O/N
2. supernant of lysate
3. flow through from first Ni-NTA
4. Ni-NTA elution of pS (500 mM imidazole)
5. Overnight digestion by Ulp1 to remove MBP-SUMO (dialysis to buffer without imidazole)
6. Flow through from second Ni-NTA (RI)
7. Elution from Ni-NTA (MBP-SUMO)

I didn't do the unit define. I just buffer exchange the RI to Hepes 7.5 20mM, KCl 50mM, BME 14 mM and Glycerol 50%. The final concentration was determined by nanodrop, reading is 0.5 mg/ml (it assume ecoefficiency is 1). The ecoefficiency of RI is 0.82. According to that paper, it is around 70,000 unit/mg. I just assume my prep is around 40 U/ul, like all the commercial one. I did a inhibition test as shown on Sigma (Roche product)
https://www.sigmaaldrich.com/catalog/product/roche/rnainhro?lang=en&region=US.

No DNase and RNase activity detected. No inhibition on 2-step RT-qPCR using Mashup with 20 U/20 ul reaction.