AT2G35300 (AtLEA18) large scale recombinant protein production and purification

1. One liter bacterial culture having an absorbance at 600 nm of 0.8 was induced by making it 1mM with respect to IPTG and placing it at 25°C overnight.
2. Next day it was centrifuged at 7500Xg for 10 min.
3. The pellet was washed with buffer (50mM Tris HCl, pH 7.4, 0.5 M NaCl).
4. 10mL Lysis buffer was added {(50mM Tris HCl, pH 7.4, 0.5 M NaCl) + 1mL of lysozyme stock (25mg/mL)} the pellet resuspended, and the suspension kept on ice for 1 hour before commencing 3 freeze-thaw cycles of; -80°C for 1 hour; placed in ice until completely thawed.
5. 50 units of Benzonase nuclease were added + 20µl 1M $MgCl_2$ for 4 hours on ice.
6. Next, the lysate was sonicated thrice for 20 sec each time with cooling intervals of 1 minute between bouts.
7. The whole was centrifuged at 10,000Xg for 45 minutes and filtered through a 0.45µm filter and the filtrate loaded on a nickel-equilibrated, washed, HiTrap column.
8. Elution buffer was 1M imidazole + 50mM Tris HCl pH 7.4, 0.5 M NaCl which was gradually added to the Tris HCl pH 7.4, 0.5 mM NaCl wash as 1 mL fractions were collected.

M. Protein marker
1. Load
2. Flow through
3. Wash
4. Before
5. After
6. Fraction 10
7. Fraction 12
8. Fraction 14
9. Fraction 16
10. Fraction 18
11. Fraction 20
12. Fraction 22
13. Fraction 25
14. Fraction 28
15. Fraction 30
16. Fraction 31
17. Fraction 32
18. Fraction33