

AT2G35300 (AtLEA18) recombinant protein induction optimization

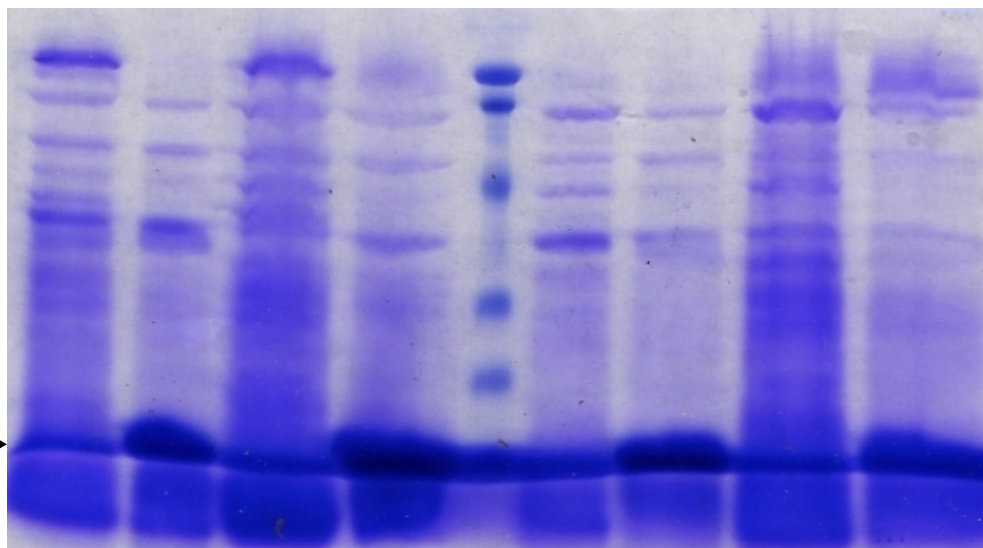
Sequencing Results

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCAT**ATGG**GCTAGCATG
ACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCATGCAGTCGGCGAAGGAAAAGATCAGTGACATGGCC
AGTACGGCCAAGGAGAAACTCAACATCGGTGGCGCAAAGGCACAAGGTCATGCGGAGAAGACGATGGCAAGG
ACCAAAAAAGAGAAGAAGTTGGCCCAAGAGCGAGAGAAGTCTAAGGAGGCGCAGGCCAAAAGCTGACCTCCAT
CAATCCAAGGCTGAGCATGCTGCGGACGCTCAGGTTACAGGCCACCATCTTCCCGGTCACTCCACCTACCCT
ACCCGAGCCACCGGAGCTAATTACCCGCCGGGACAGATCTAA

M G S S H H H H H S S G L V P R G S H **M** A S M T G G Q Q M G R G
S E F M Q S A K E K I S D M A S T A K E K L N I G G A K A Q G H A
E K T M A R T K K E K K L A Q E R E K S K E A Q A K A D L H Q S K
A E H A A D A Q V H G H H L P G H S T Y P T R A T G A N Y P P G Q
I Stop

1. 25 °C (4hrs) Uninduced
 2. 25 °C (4hrs) Induced
 3. 25 °C (o/n) Uninduced
 4. 25 °C (o/n) Induced
 5. 37 °C (4hrs) Uninduced
 6. 37 °C (4hrs) Induced
 7. 37 °C (o/n) Uninduced
 8. 37 °C (o/n) Induced
- M Protein Marker

1 2 3 4 M 5 6 7 8



pET28 containing the coding sequence of *AT2G35300*, including its stop codon, was placed in BL21(DE3)RIL. Cells were grown to an OD_{600} of 0.8 at 37 °C at which point the culture was split into 4 tubes. Two tubes remained at 37 °C while 2 were removed to 25 °C. One tube at each temperature was induced by making it 1 mM with respect to IPTG. Aliquots were retrieved at the indicated times and the bacterial pellet boiled in SDS-loading buffer and electrophoresed through a 15% total, SDS-polyacrylamide gel.