8.APPENDIX

8.1. Creation of the double mutant *set1Δmog1Δ* from *set1Δ* strain

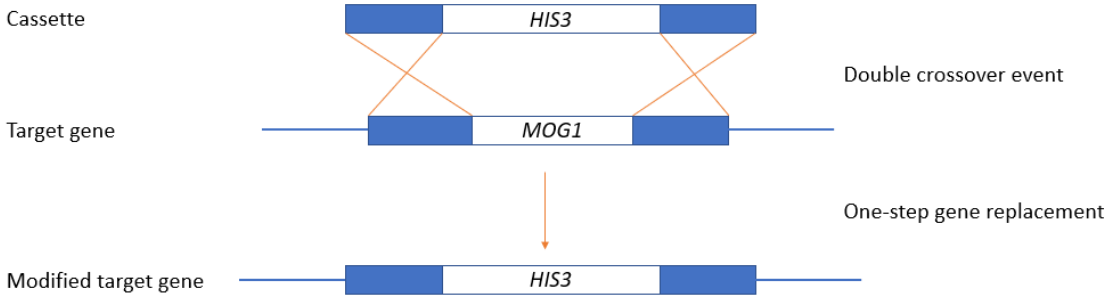

Figure 8.1. *MOG1* disruption by PCR amplified *HIS3* cassette. *MOG1* deletion takes place by a double crossover event during homologous recombination. The homologous regions flanking both *MOG1* and *HIS3* are 40nt length.

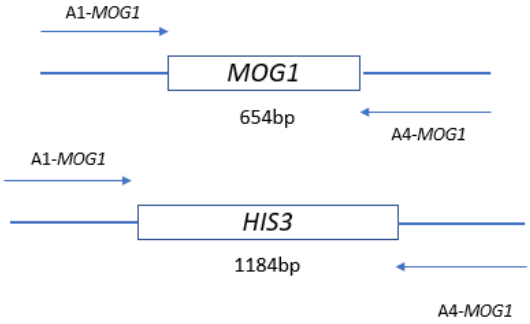

Figure 8.2. PCR amplification of the modified target gene. Primers designed to align to the flanking regions of the target gene allow the detection of the gene disruption due to difference in size between the target gene and the selection marker.

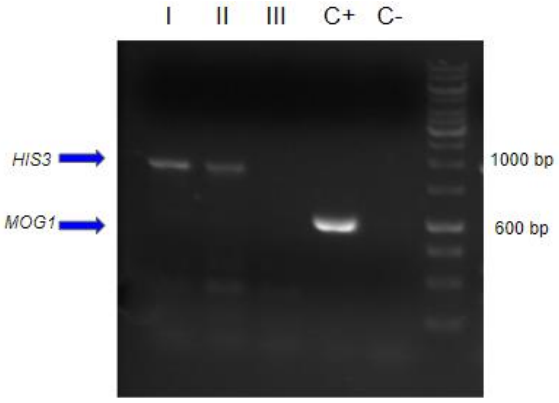

Figure 8.3. Confirmation of *MOG1* deletion in *set1Δ*. 1% agarose gel electrophoresis of the PCR products from the *HIS3/MOG1* amplification in the three clones transformed with *HIS3*::*MOG1*. As the positive control, the DNA from a non-transformed *set1Δ* colony was used while the negative control corresponded to the PCR mix without DNA.

8.2. Phenotypic effect of Mog1ΔK144-T153 and Mog1K189A mutations in *mog1Δ* and *set1Δmog1Δ S. cerevisiae* growth

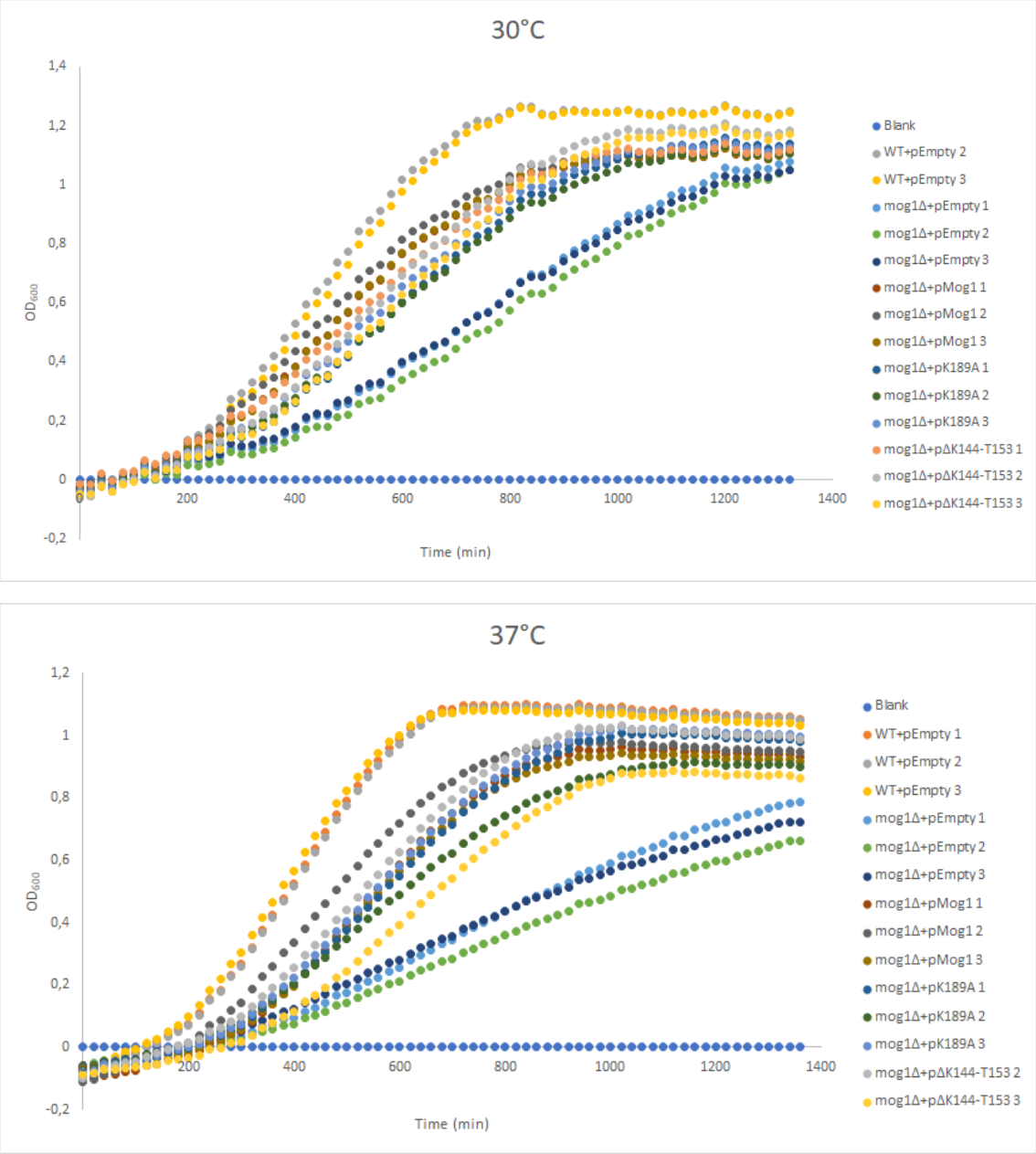

Figure 8.4. Growth curves of the WT/*mog1Δ* strains represented as OD_{600} vs. time. WT+pEmpty replicate 1 (30ºC) and *mog1Δ*+pΔK144-T153 replicate 1 (37ºC) were removed from the data set and not included in further analysis because of significant deviation from their replicates. Blank values corresponded to the OD_{600} measurements of the growth media SC-Leu and were used to normalise the OD_{600} values at each time point.

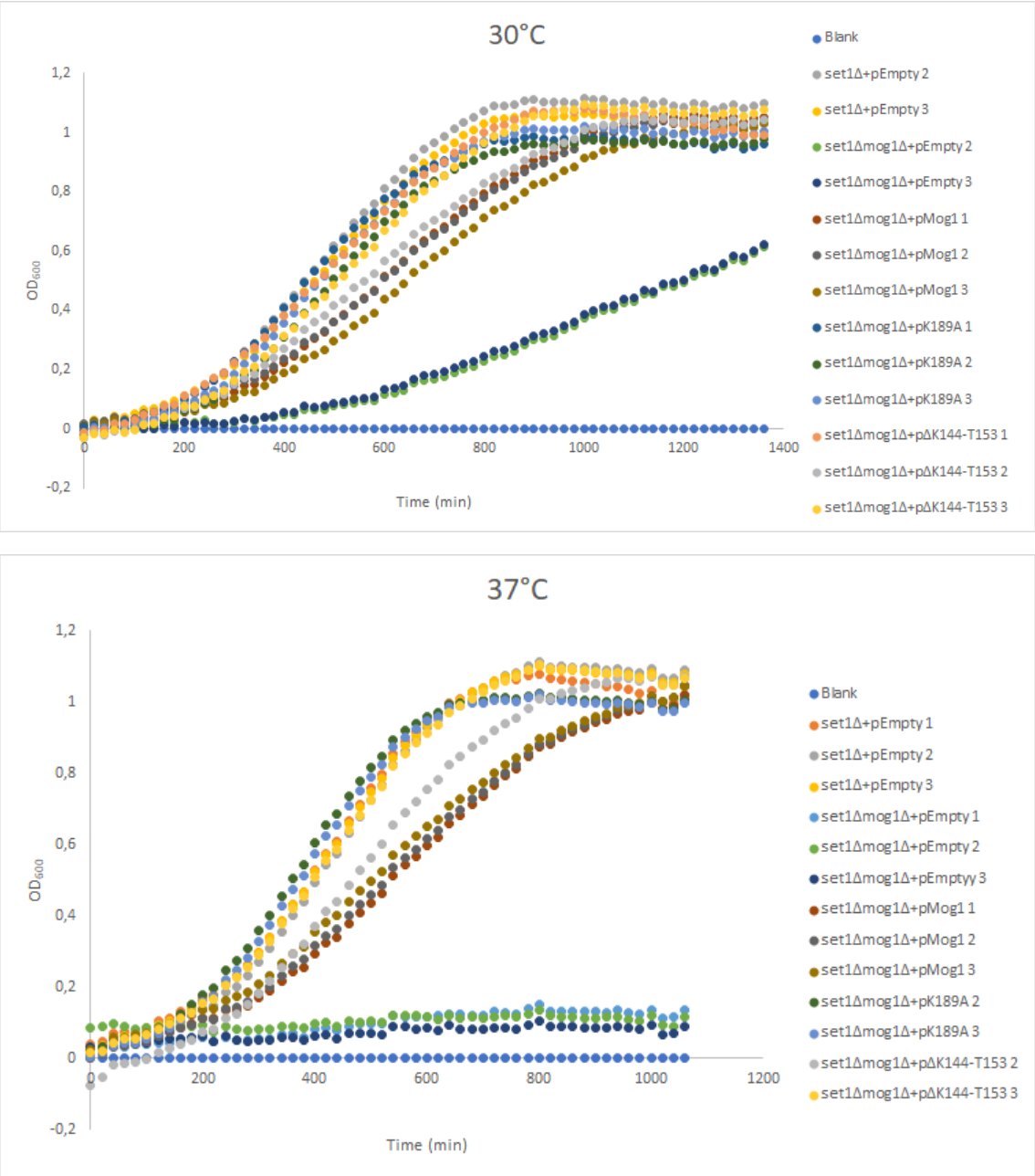

Figure 8.5. Growth curves of the *set1Δ*/*set1Δmog1Δ* strains represented as OD_{600} vs. time. *set1Δ*+pEmpty replicate 1 (30ºC), *set1Δmog1Δ*+pEmpty replicate 1 (30ºC), *set1Δmog1Δ*+pK189A replicate 1 (37ºC) and *set1Δmog1Δ*+pΔK144-T153 replicate 1 (37ºC) were removed from the data set and not included in further analysis because of significant deviation amongst their replicates. Blank values corresponded to the OD_{600} measurements of the growth media SC-Leu and were used to normalise the OD_{600} values at each time point.

Table 8.1. Growth rates and doubling times calculated from the exponential phase of the growth curves in Appendix, Figures 8.4 and 8.5. The values shown in the table correspond to the average of the growth rate and doubling time calculated for each of the replicates of the indicated strains

	GROWTH RATE (min^{-1})		DUPLICATION TIME (min)	
Strain	30°C	37°C	30°C	37°C
WT+pEmpty	0.0043	0.0043	161.28	163.12
mog1Δ+pEmpty	0.0020	0.0017	341.07	401.13
mog1Δ+pMog1	0.0036	0.0035	191.00	196.32
mog1Δ+pK189A	0.0032	0.0031	217.17	226.41
mog1Δ+pΔK144-T153	0.0034	0.0033	206.17	214.55
set1Δ+pEmpty	0.0032	0.0037	216.82	189.94
set1Δmog1Δ+pEmpty	0.0016	0.0000	447.66	0.00
set1Δmog1Δ+pMog1	0.0026	0.0028	263.30	244.71
set1Δmog1Δ+pK189A	0.0031	0.0038	223.75	182.53
set1Δmog1Δ+pΔK144-T153	0.0029	0.0035	236.55	201.97

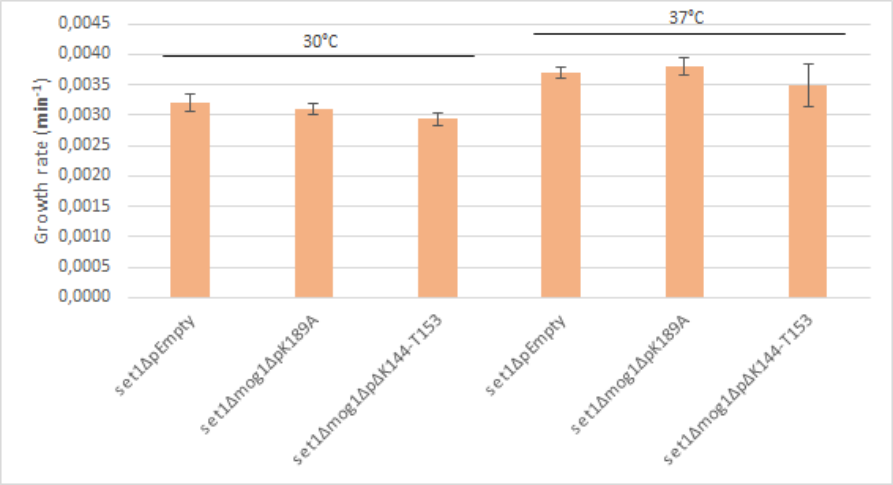

Figure 8.6 Growth rate values of *set1Δmog1Δ*+pK189A and *set1Δmog1Δ*+ pΔK144-T153 compared to *set1Δ*+pEmpty at 30ºC and 37ºC. The lines above the bars indicate the strains included in each ANOVA analysis which were then compared to *set1Δ*+pEmpty in pairs by t-test at each condition. None of the *set1Δmog1Δ*+pK189A and *set1Δmog1Δ*+ pΔK144-T153 showed significantly different growth rates compared to *set1Δ*+pEmpty.

8.3. IPTG induced expression of His-tagged H2B and purification by IMAC

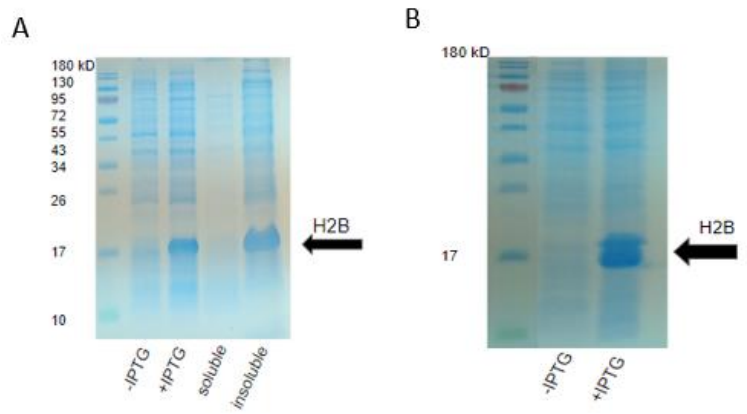

Figure 8.7. H2B induced expression test with 1mM IPTG at 4ºC for 3h. A) from 10ml LB+Chl+Kan. B) from 1L+Chl+Kan 15% SDS-PAGE gel for testing IPTG induced expression of H2B comparing samples before and after addition of 1mM IPTG together with soluble and insoluble fractions of the IPTG induced cell extract.

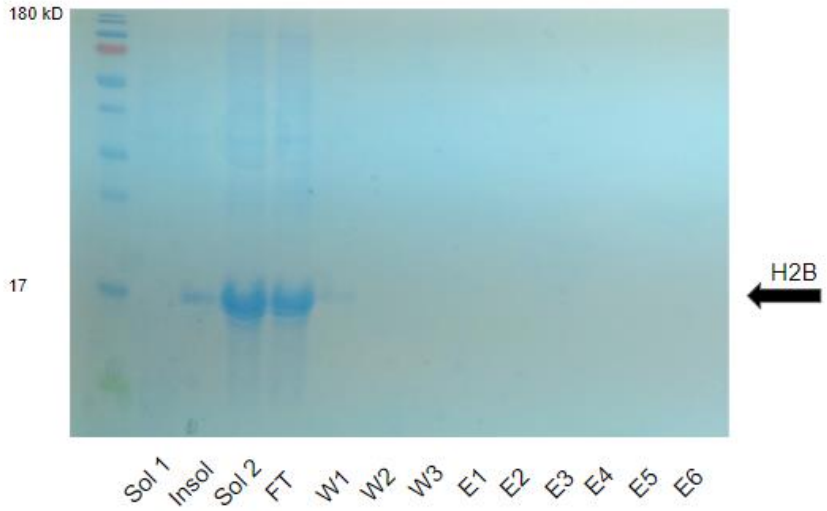

Figure 8.8. H2B purification with TALON column under denaturing conditions. The insoluble fraction of the cell extract was resuspended in washing buffer containing 8M urea and applied to the column containing TALON beads. Apart from the solubilised fraction (Sol 2), flow through (FW), washing (W1-3) and elution samples (E1-6), samples from the soluble fraction (Sol 1) and the insoluble fraction before solubilization in denaturing conditions (Insol) were also run in the gel to check solubilisation of H2B.

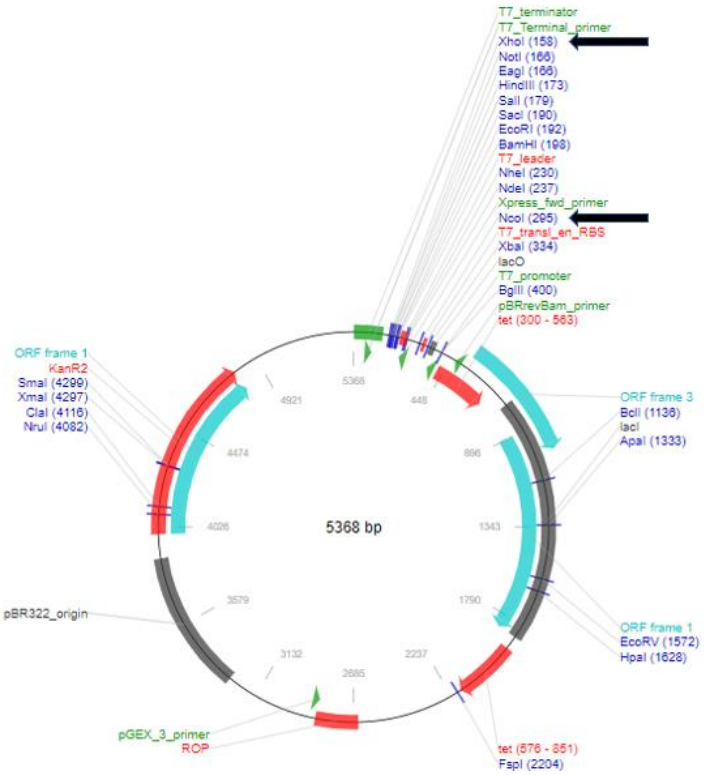

Figure 8.9. Map of the pET28b plasmid from ADDGENE (2019). The arrows indicate the cloning sites of *HTB1*.

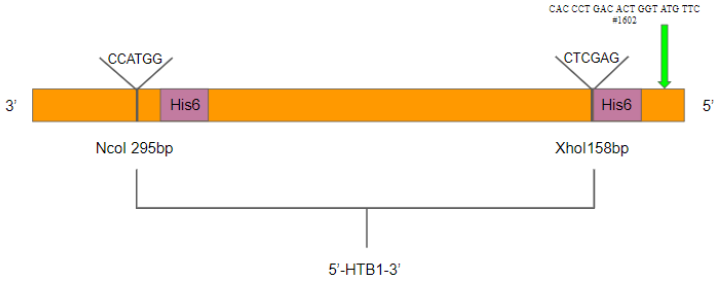

Figure 8.10. Cloning site of *HTB1* gene in pET28b plasmid. *A3-HTB1* 21bp primer designed to sequence from the 3' terminal of *HTB1* (Materials and Methods, Table 3.6), anneals 100bp away the 3' terminal.

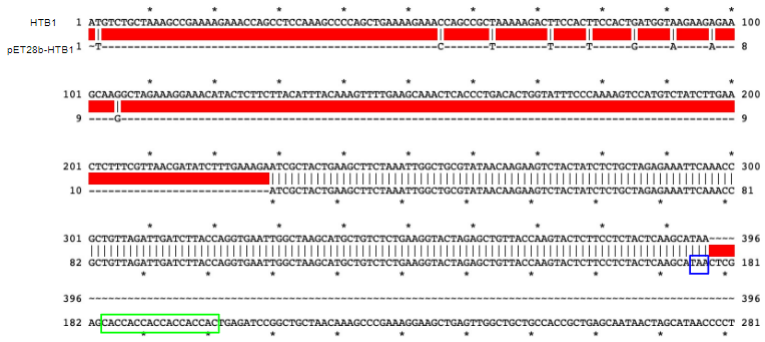

Figure 8.11. Local alignment of the *HTB1* gene sequence to the fragment of pET28b-*HTB1* sequenced using the primer named A3-*HTB1* (Materials and Methods, Table 3.6). The sequence on top corresponds to *HTB1* gene and the bottom sequence to the results of sequencing. Highlighted in blue the stop codon present in the *HTB1* gene cloned into pET28b and in green the His6-tag sequence at the 3' end of the gene.

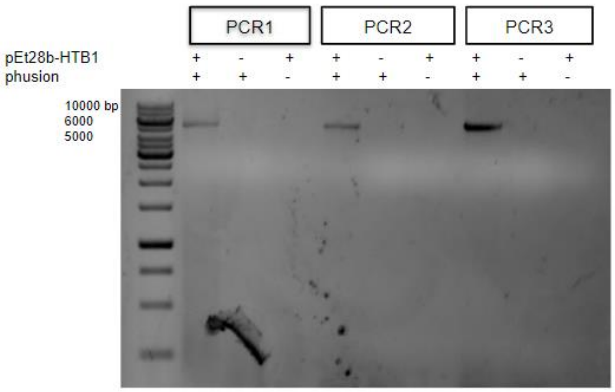

Figure 8.12. Deletion of the stop codon from the pET28b-*HTB1* plasmid by quick-change. PCR1: HF Phusion buffer® as the Taq polymerase buffer; PCR2: HF Phusion buffer® and 5% DMSO added to the transformation mix; PCR3: GC Phusion buffer® as the Taq polymerase buffer. Two negative controls for each condition: PCR negative control (without plasmid) and mutagenesis negative control (without Phusion polymerase). Samples were run in a 1% agarose gel with a 1kB marker.

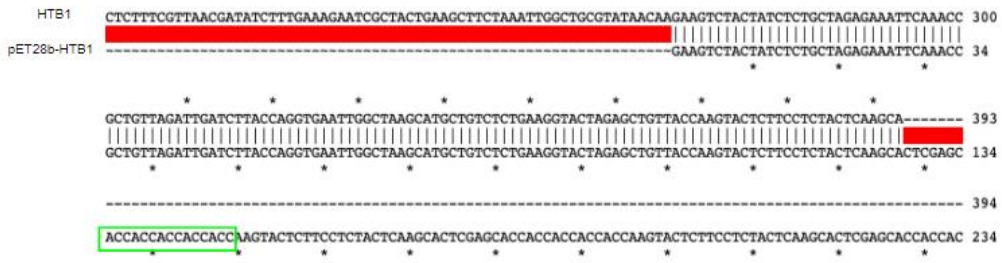

Figure 8.13. Local alignment of the *HTB1* gene sequence to the fragment of pET28b-*HTB1* sequenced using the primer named A4-pET28b (Materials and Methods, Table 3.6). The sequence on top corresponds to *HTB1* gene and the bottom sequence to the fragment of pET28b-*HTB1* Sequenced. Highlighted in green the His6-tag sequence at the 3' end of the gene. The stop codon has been successfully deleted.

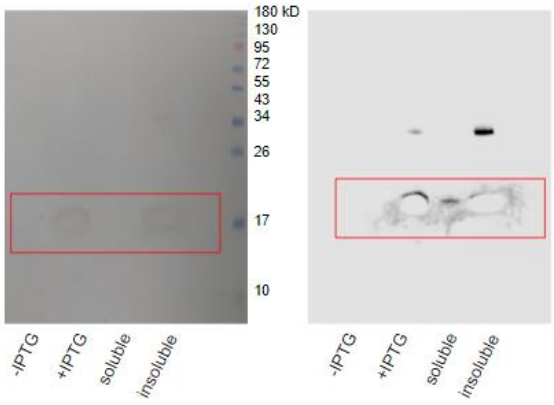

Figure 8.14. Expression of the His6 tag detected by transfer and immunoblotting of His6-tagged *HTB1*. The images show the membrane before (left) and after (right) being revealed in a luminescent image analyser. Membrane burnt because of high expression of the histidine tail.