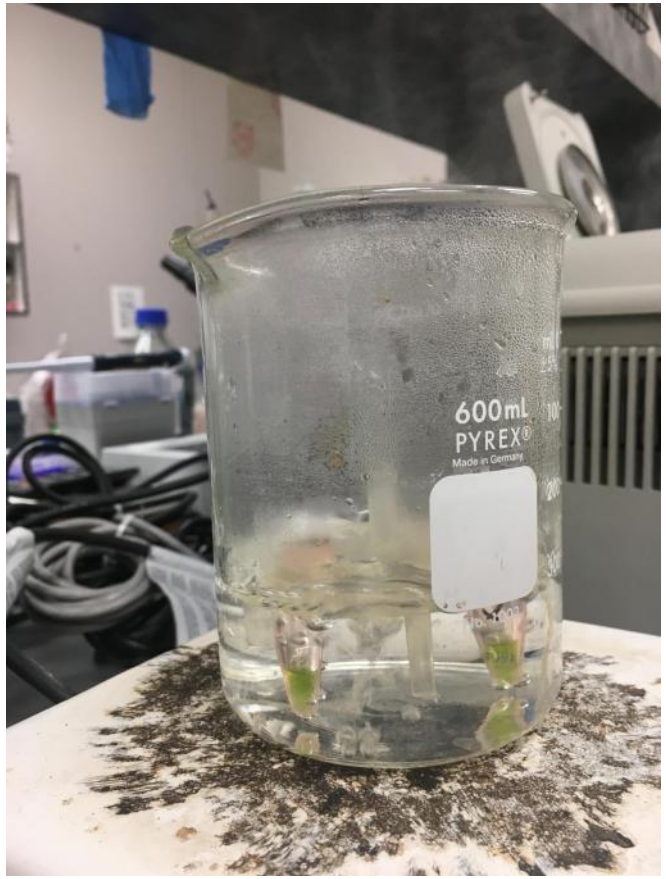
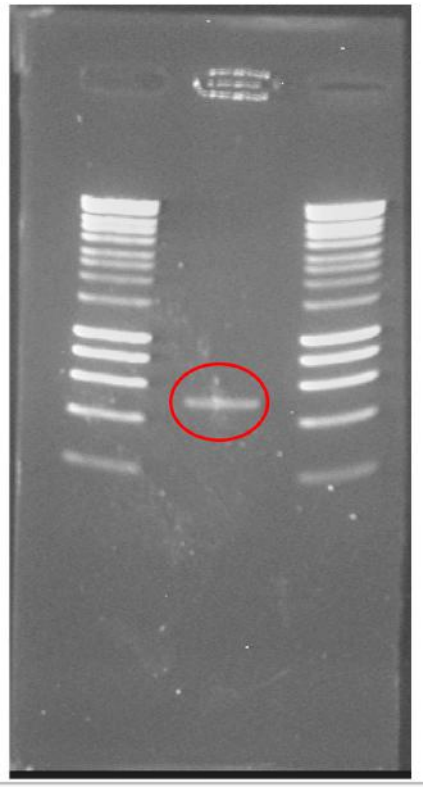
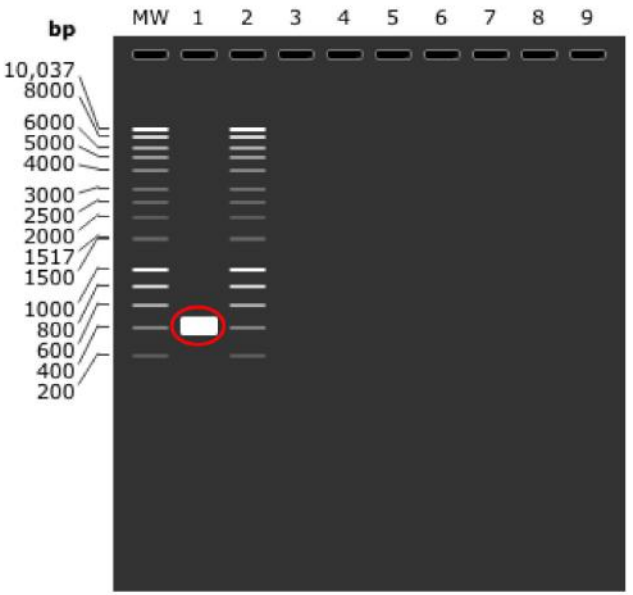
Confirmation of Proinsulin gene in chlamydomonas

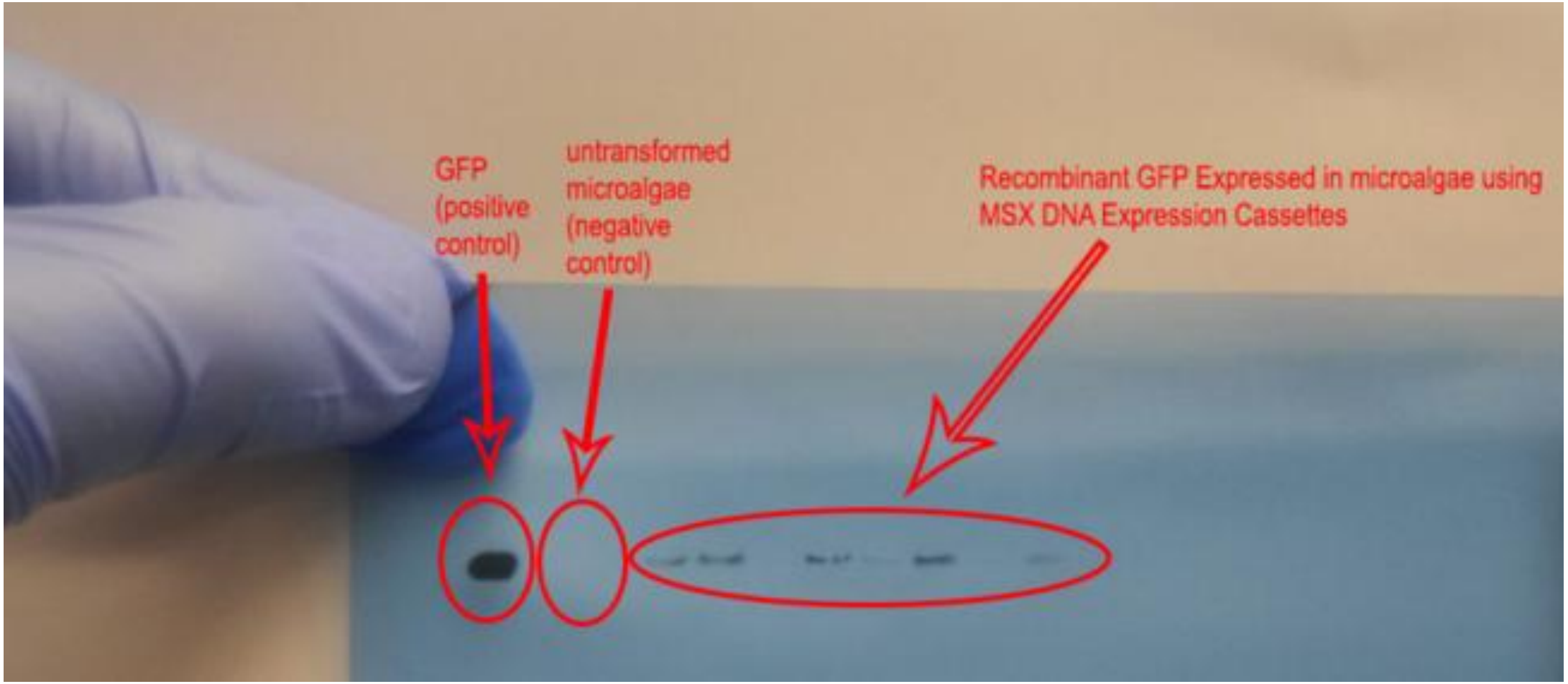






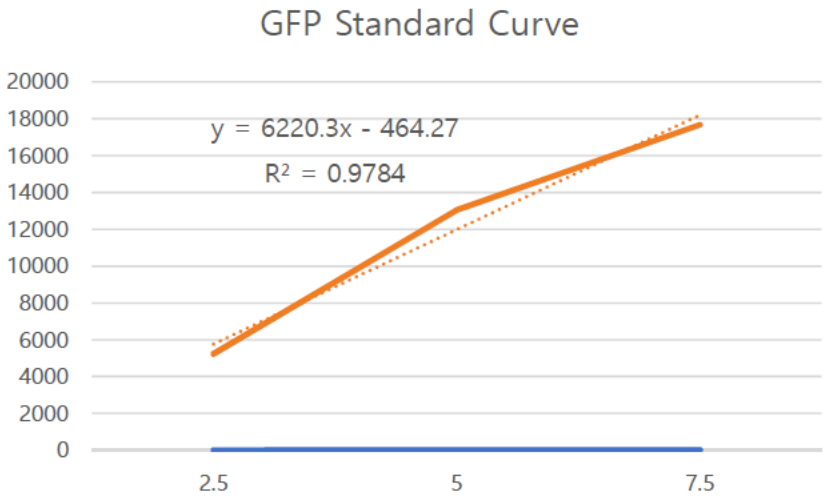
Primers complementary to the proinsulin insert
(Forward primer 5’-tatgacaagggtgaaccattacttt -3’,
Reverse primer 5’-accatttacaagctgaacgaattac3’)
Were used on the putative transformed algal biomass after the chloroplast DNA was isolated and purified to confirm the presence of the synthetic proinsulin construct integrated within the microalgal chloroplast genome.

Western Analysis of GFP Expressed in Chlamydomonas



Western Analysis confirmed the expression of GFP within the microalgal biomass after the cell lysate from the transformed chlamydomonas was run on a gel, stained with anti-GFP antibodies, and imaged using an X-ray film exposed for 10 seconds.

GFP Quantification in chlamydomonas



Expression from pASapI construct reached 0.002% Total Soluble protein

			ng/20ug	% TP
GFP standard	2.5	5222.598		
	5	13043.23		
	7.5	17663.22		
TN72 transformants	219-1	2354.335	0.4531	**0.0023**
	219-2	1615.335	0.3343	**0.0017**
	376-1	422.092	0.1425	**0.0007**
	376-2	2229.406	0.4330	**0.0022**
	376-3	ND		

% TP : percentage of total protein

A Bradford Assay was used to generate a standard curve using a GFP standard protein to estimate the % of Total Soluble Protein that was GFP from the transgenic microalgae.