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San Diego R&D Project Report

SE50416 Strain Sequencing

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Purpose

The purpose of this sequencing effort was to determine whether the culture of SE50416 is mixed algal isolates or if the culture is unialgal but possessing Intragenomic diversity

Background

A culture of *Tetraselmis sp.* (SE50416) was obtained from Dr. Palenick at SIO. In routine sequencing of the culture to determine the ITS 1+2 sequence for strain identification it was discovered that there were mixed ITS sequences in the results. Multiple positions in the consensus contained overlapping peaks, however all sequences returned had a BLAST result of *Tetraselmis marina.* In previous experience this would indicate a mixed culture and require sorting, however after a round of sorting into liquid sequencing of the individual wells returned the same pattern of overlapping peaks. This might indicate that sorting was ineffective so two random cultures were serially diluted to isolate a clonal source and perform a more in-depth analysis of variation in the ITS sequence.

Hypothesis

SE50416 is a unialgal culture, however this strain is unique in that it possesses a high degree of intragenomic variation in the ITS sequence.

Experimental Design

SE50416 culture was sorted to liquid. Two randomly selected liquid sorts were then serially diluted to agar plates. From the serial dilutions of these two initial sort events three clones each were isolated, lysed, and the ITS region was amplified. Purified fragments were TOPO cloned and 48 *E. coli* clones were sequenced for each clonal isolate from each sort event.

Results

SE50416 was sorted to liquid, two sort events were serially diluted, and from each serial dilution 3 clones were isolated. This was done to assure that, within reason, a clone that was the result of a single

cell was isolated and analyzed. 48 TOPO clones were analyzed for each subclone. Contigs were assembled in SeqMan and contigs that were 100% identical were grouped together. Any variation in the ITS sequence, even by a single base pair, resulted in a contig being considered a variant. The results of this process are found in Table 1.

All subclones share the same contig as the dominant fragment from the TOPO clone counts. However, within each subclone there is a significant number of other sequence variants. This is the first time this phenomenon has been observed at Sapphire that this level of variant ITS sequences have been cloned from a single clonal isolate. It is also interesting that similar level of variant ITS sequences occurred in six clonal isolates from two different sorting events from a single culture of algae.

Conclusions

Routine ITS sequencing of the SE50416 culture indicated a mixed population of ITS fragments. Historically this data indicates a mixed culture of algae, and the algae is sorted and re-sequenced for clonal isolates. After sorting, and then dilution and selecting single colonies, mixed ITS sequences were again confirmed. All subclones shared the same dominant contig as well as the second most common contig. All the ITS sequencing on SE50416 has returned *Tetraselmis marina* as the top BLAST result. Overall, the sequencing results indicate that SE50416 is most likely a unialgal strain, but one that possesses a degree of intragenomic genetic diversity not observed before at Sapphire.

Table 1: Contig Counts for individual TOPO clones. Results of sequencing 48 TOPO clones for each subclone. All the clones share the same most common two contigs and each clone has a subset of their own unique variants.

Clone	*3_1*	*3_2*	*3_3*	*4_1*	*4_2*	*4_3*
Contig 1	35	19	32	32	37	28
Contig 2	3	5	7	9	4	9
Contig 3		5				2
Contig 4	2			3	1	
Contig 5		2		1	1	
Contig 6					1	
Contig 7	1					1
Contig 8	2					
Contig 9	1					
Contig 10	1					
Contig 11	1					
Contig 12		2				
Contig 13		2				
Contig 14		1				
Contig 15		1				
Contig 16		1				
Contig 17		1				
Contig 18		1				
Contig 19		1				
Contig 20		1				
Contig 21		1				
Contig 22			1			
Contig 23				1		
Contig 24				1		
Contig 25				1		
Contig 26					1	
Contig 27						1
Contig 28						1
Contig 29						1
Contig 30						1
Contig 31						1
Contig 32						1