Figure 33. *Spartina densiflora* Net Area

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Figure 37. 2010 Net Acres of *S. anglica*, *S. patens* and *S. densiflora x foliosa* hybrids.
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Figure 44. Field-identified *S. foliosa* with supporting genetic evidence (no genetic evidence of *S. alterniflora* ancestry).

Figure 45. Field-identified *S. alterniflora* hybrid with supporting genetic evidence (genetic evidence *S. alterniflora* ancestry).
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Figure 47. Field-identified *S. alterniflora/hybrid with contradicting genetic evidence (no genetic evidence of *S. alterniflora ancestry).

Figure 48. Field-identified *S. foliosa with contradicting genetic evidence (genetic evidence of *S. alterniflora ancestry).
Development of Scar Markers for use by the San Francisco Estuary Invasive Spartina Project

Emma Jack PhD
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Random amplified polymorphic DNA (RAPD) markers were initially used to gain provisional insight into the genetic variation among and within population of Spartina and their hybrids (Ayres et al., 1999). In contrast to simple sequence repeat (SSR) markers, RAPD does not involve high costs of time and money. The arbitrary decamer primer amplifies a set of unknown genomic sequences, which are run on an agarose gel and which can be scored as present or absent. However, a highly controlled and monitored setting is necessary to ensure its reproducibility (Ellsworth et al., 1993; Pan et al., 1997; Riedy et al., 1992; Scot et al., 1993). The ISP found that the variation in results between laboratories (University of California, Davis and STA Laboratories, Colorado) made this technique inappropriate to use for identifying hybrid spartina as it stood. However, if the RAPD analysis could be modified such that its error rate would be decreased, then this technique would become more predictable and more cost effective than SSR markers.

Converting the RAPD markers to sequence characterized amplified region (SCAR) markers could increase the efficiency and predictability of the RAPD assay. SCAR markers are created by using a longer primer that is developed by extending the sequence of the RAPD primer, and increasing the size of the primer to approximately 20 bases. By increasing the specificity (longer more specific primer, increasing the reaction temperature), the results are less sensitive to changes in the reaction conditions and are more reproducible. Ultimately, SCAR markers can provide robust genetic markers that are low cost and that can be used to identify hybrid spartina derived from interspecific hybridizations.

In 2010 the ISP addressed the issue of reducing genetic testing costs for identifying Spartina hybrid by developing a series of SCAR markers. The eleven (11) RAPD bands that have historically been used by the ISP were chosen for further analysis based on their repeated use for hybrid identification (Table 1). To convert the RAPD bands developed by Ayres et al., (1999); each unique band was cut from an agarose gel and eluted in TE buffer. The RAPD analysis was conducted by UCD to ensure reproducibility of scoring bands and to ensure the current bands were chosen for SCAR marker development (Ayres et al., 1999). The bands were then sent to the Genomics/Transcriptomics Analysis Core at San Francisco State University (SFSU), to Dr. Frank Cipriano for cloning and sequencing. In order to develop longer and more specific SCAR marker primers the sequencing of the RAPD bands was conducted. As RAPD primers often amplify a range of bands it was necessary to transfer the fragment of interest (i.e. B7 650 bp marker), into a self-replicating genetic element, a bacterial plasmid. The RAPD band can then be used to generate multiple copies to be used for further study without fear of contamination from similar but non-related bands produced in the RAPD reaction.

Results

The RAPD band sequencing that were obtained by SFSU are presented in Table 1. The fragments were compared to other known sequences in the gene bank database (http://www.ncbi.nlm.nih.gov) to obtain more information as to their reported function and/or location if any. More information will enable us to understand how these bands are segregating in the population that will inform us as to their usefulness as species-specific markers to identify hybrid plants. The results of the BLAST analysis are reported in Table 1.