Project Title:
Development of allele ladders for 13 microsatellite loci approved by the PSC for Genetic Stock Identification of Chinook salmon

Proponents names:
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Performance Period:
April 1, 2006 through September 30, 2007

Objectives:
Our objective was to develop and test allele ladders for 13 microsatellite loci adopted by the Genetics Analysis of Pacific Salmon (GAPS) community for Chinook salmon standardization and to make them available to GAPS and non-GAPS laboratories allele ladder standards. Allele ladders are synthetic PCR templates created from pooled amplification products representing all or nearly all of the alleles that are sanctioned by the GAPS consortium. The ladders permit very simple and robust alignment of unknown fragments with recognized alleles in Chinook salmon. Our purpose was to support standardized allele identification in GSI throughout areas of relevance to the Pacific Salmon Treaty. In particular, these ladder references were intended to provide support for allele identification in conjunction with the GAPS Chinook salmon microsatellite database.

Project status:
The project was successfully completed. Our development and testing objectives were met and all allele ladders (representing each of the 13 Chinook salmon microsatellite GAPS loci) are now available upon request (Fig. 1). After ladder construction was completed, we successfully tested all ladders for consistent mobility under different laboratory conditions, and the results show complete concordance with expected mobility under four distinct laboratory conditions (Fig. 2, Table 1). Ladder development and testing went smoothly, though we had anticipated that some of the ladders would require extensive development, which was the case for Ots201b and Oki100. Development difficulties for these were overcome by partitioning each into two smaller and more manageable sub-ladder stocks. Successful testing of allele ladders was based on the ability of the experimenter to use the locus-specific allele ladder to identify “unknown” sample genotypes (i.e., blind, with no prior knowledge of sample genotype) or to correctly bin the ladders in cases where labs were previously self-equipped with the correct genotyping bins that represented each of the alleles contained in the ladders. Correct labeling of sample alleles or correct binning of alleles (Table 1) has demonstrated the necessary ladder mobility concordance across platforms, and therefore the ability of the ladder to be used as a sample reference to for labeling alleles concordant with GAPS

standards. Testing was completed after two phases: 1) in-house testing by the development laboratories, NMFS-Seattle, and USFWS-Anchorage, and 2) external testing at NMFS-Seattle, NMFS-Mukilteo, University of Washington in Seattle, and UW Friday Harbor Laboratories. Figure 1 illustrates the Ots213 ladder as it is actually put to use in converting lab–specific allele labels to standardized GAPS labels. Table 2 provides summary of results for the testing phase. The attached PowerPoint file provides a visual record and referencing guide for each allele ladder.

Two presentations of these data were given at regional scientific meetings: 1) North Pacific Chapter of the American Fisheries Society (Tacoma WA, May 2007), and 2) Six decades of fishery genetics: A retrospective view and a vision for the future (Seattle WA, September 2007).

Our results demonstrate the utility of allele ladders in providing definitive yet inexpensive microsatellite standardization among laboratories. This innovation, borrowed from human genetics, will be important both for ongoing work within and between GAPS laboratories, and especially for new laboratories that want to share standardized data but that were not part of the original GAPS standardization process (e.g., the University of Washington and Friday Harbor Laboratories). This study serves to validate the method of LaHood et al 2004 in the context of large-scale interagency collaboration with a realistic number of highly polymorphic microsatellites, and greatly improves the standardization of microsatellites by obviating exchange of large numbers of reference samples.

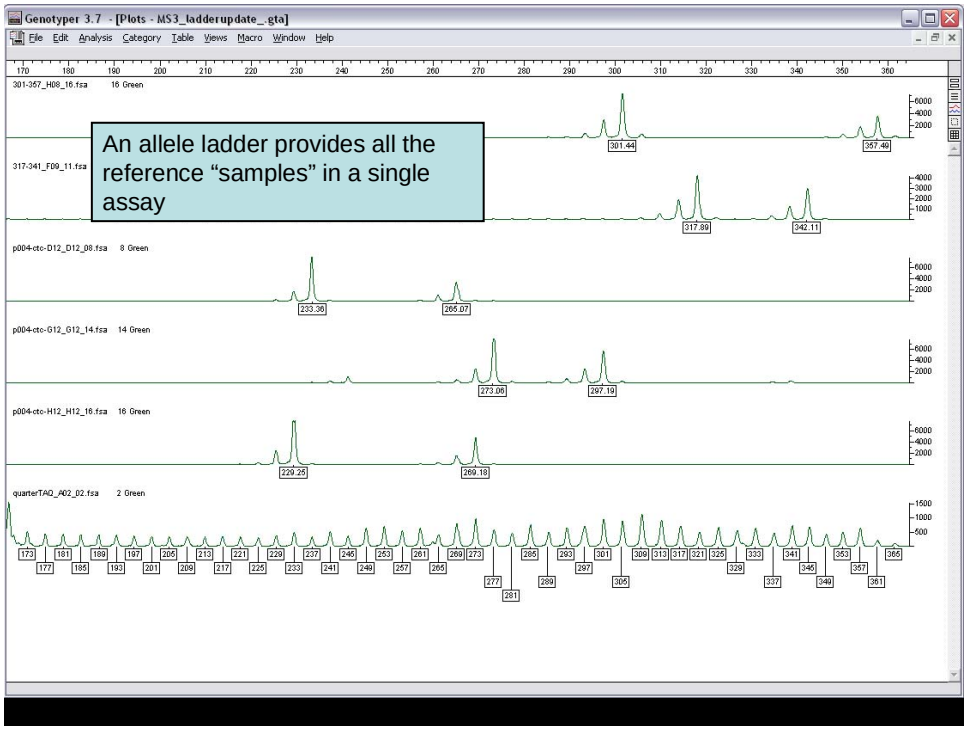

Figure 1. Example of GAPS-standardized allele ladder (Ots213 ladder) compared to non-standardized genotypes (i.e., raw estimated sizes).

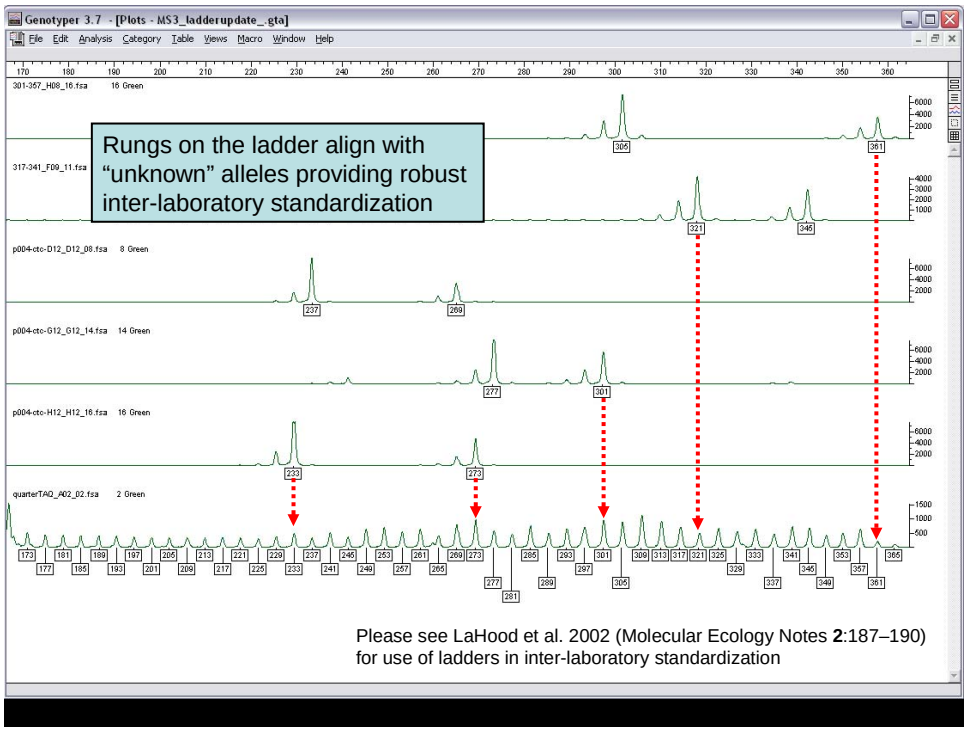

Figure 2. Ladder enables sample genotypes to be labeled according to GAPS-standardized allele nomenclature and also demonstrates concordant relative mobility of ladder alleles and the alleles in the unknown samples.

Table 1. Summary of blind genotyping tests among laboratories demonstrates complete concordance of genotyping and perfect inter-laboratory standardization, despite different electrophoresis platforms (cap=capillary array system, gel=slab gel system).

Agency/Lab	Platform	[a]Percent correct identification of unknown genotypes
USFWS Anchorage	ABI 3130/capillary(cap)	100
NMFS- Seattle	ABI 3100/cap	100
NMFS- Mukilteo	ABI 310/cap	100
University of Washington	Megabase/cap	100
Friday Harbor Labs, WA	ABI377, slab gel	100

[a]Concordant with GAPS-sanctioned allele designations