


Intergenerational genetic tagging for hatchery evaluation, monitoring, fishery management, and ecological investigation of salmonids



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Expert Panel on The Future of the Coded Wire Tag Recovery Program for Pacific Salmon

- **RECOMMENDATION 12.** *We recommend support of a “proof-of-concept” empirical validation of the Full Parental Genotyping (FPG) method for use in management of Pacific salmon fisheries. This validation should occur in chinook salmon and should include support for further SNP development, a series of paired CWT and FPG tag recovery experiments, as well as thorough evaluation of relative costs of implementing these methods and the sampling necessary to provide equivalent tag recovery data.*



Emerging framework for genetic tagging of salmonids

- Tagging crucial to fishery management, population monitoring, ecological investigation, hatchery evaluation, etc.
 - Most tagging involves physical manipulation of juveniles
 - Tag recovery rates are dismally low ($<1\%$), because of high cumulative mortality
 - Intergenerational tagging would be a huge efficiency, since adults could be tagged and transmit tags to their offspring, removing need for inefficient manipulation of juveniles and consequent tag loss.
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Most salmonid tagging currently employs coded wire tags




- Mechanically inserted and manually extracted metal tags that are manually read under a microscope
- Since 1968, at least 71 agencies in 5 states and B.C. have inserted ~600 miles of wire and tagged ~ 1 billion salmon and steelhead
- Until 1996, only fish with CWTs generally received adipose fin clips
- Nearly 1 million heads analysed at Juneau head lab alone.



Current CWT tagging system

- Very useful tagging system over its 30+ year life
- Crucial to management objective of estimating multiple stock fishery mortality
- Provides stock of origin AND **cohort** of origin
- Large historical databases of tag recoveries provide comparative baseline


Challenges to CWT system

- Very low tag recovery rates (1.6 per 1,000 in chinook)
 - Tag loss rates are poorly known
 - CWT harvest may be underreported
 - Mass-marking - Not all Ad-clipped fish have CWTs
 - Assumption of equivalency of hatchery indicator stocks and genetically similar naturally spawning stocks (the gorilla assumption) - can be large areas.
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Parentage-based Tagging


(a.k.a. the method formerly known as FPG)

- Highly efficient, transgenerational, genetic tagging method
 - Genotype all hatchery parents
 - Create reference (parent) database of all possible parent pairs
 - Fishery sampling and genotyping in offspring generation
 - Query of reference (parent) database to determine if parents are present
 - Determine parental pair and, therefore, hatchery stock of origin and exact age
 - Information obtained for each tag recovery is the same as for a CWT (+more)
 - By genotyping two parents, you effectively tag all of their 1,000s of offspring
 - Requires no juvenile tagging, but MUCH higher tagging rates feasible.
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Parentage-based Tagging

(a.k.a. the method formerly known as FPG)

- Fundamentally different than genetic stock identification: matches fishery sample to pairs of parents in reference (parent) database that have Mendelian compatibility. GSI uses frequency based probability assessments
 - Can be done using either traditional exclusion or maximum likelihood
 - Power comes from number of loci, since each locus is an opportunity for incompatibility
 - Marking and sampling issues with other tagging systems don't entirely go away.
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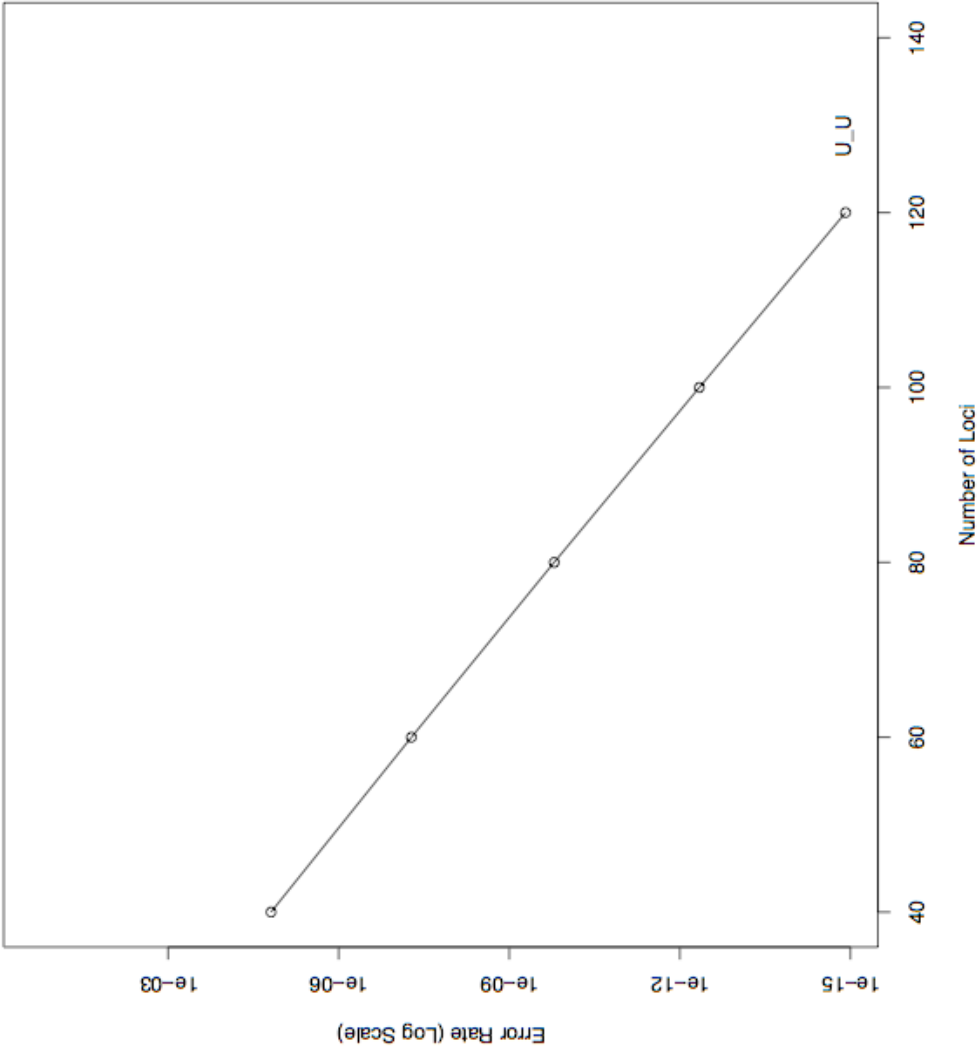
Power analysis for large scale parentage-inference

- Anderson and Garza (2006; Genetics) evaluated the plausibility of large scale PBT through evaluation of power of SNP markers to infer parentage
 - Determine false positive rates in large-scale parentage inference studies
 - Evaluate number of SNP loci necessary to correctly ID parent pairs
 - Describe new analytical method for fast ML parentage analysis
 - Evaluate effects of allele frequency, genotyping error and presence of kin
 - 100 SNP genotype can identify parental pairs with false positive rate less than 1 fish per 300,000 fishery samples.
 - False positive rates decrease exponentially with number of loci!
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Exponential decline in false positive rate with number of SNP loci

P = 0.2 GtypErrorRate = 0.01 POWER = 0.9

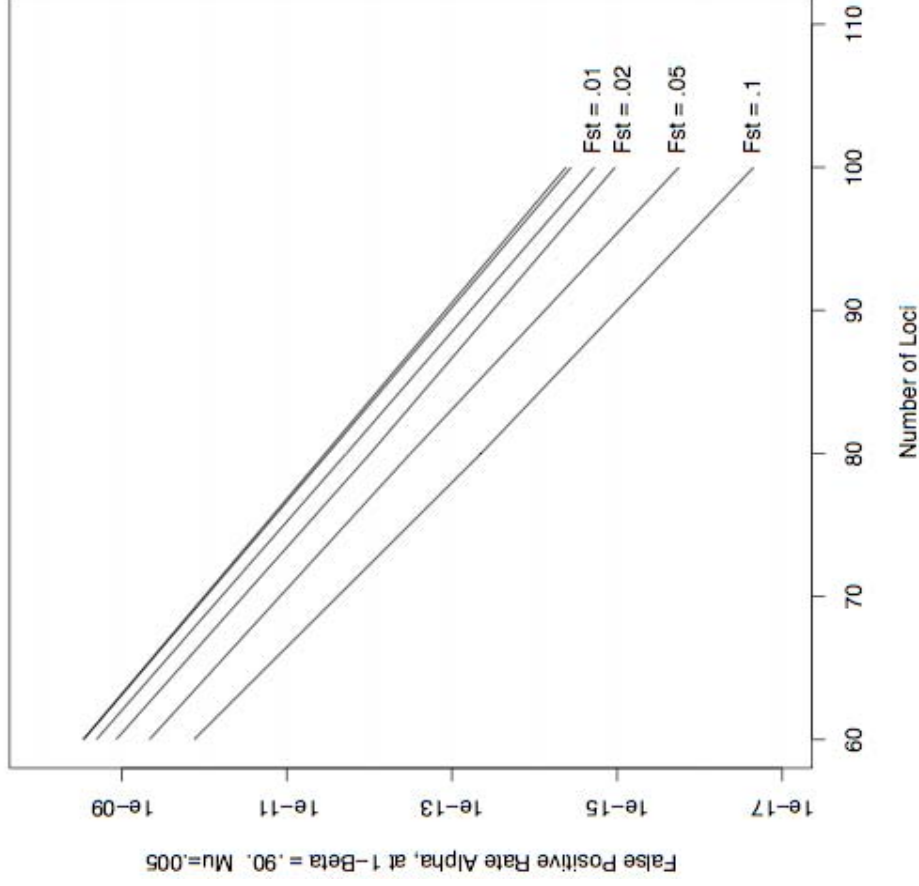


Per-trio false positive rates

80 loci = 4.6×10^{-10}

100 loci = 4.5×10^{-13}

Differentiation makes analyses conservative




-If unrelated individuals in parent database are from differentiated population, then probability of a false positive result with those fish decreases.

- F_{st} of 0.05 decreases chance of falsely concluding parentage by an order of magnitude.



Advantages of SNPs for large scale parentage-inference

- Low genotyping error rate
 - Allele calls (nomenclature) are easily standardized between labs
 - Minimal human interaction with the raw genotyping data
 - Amenable to high-throughput / low-cost genotyping- new technology brings material costs for 100 SNPs to same as 12-18 microsatellites, and labor costs are MUCH lower.
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Digital DNA Fingerprints

96 single nucleotide polymorphisms (SNPs) in separate genes

Paternal C,A,G,C,T,T,C,T,A.....T,A,G,A,T,C,A,C,T

Maternal C,A,A,T,T,G,C,A,A.....T,C,C,A,G,C,A,T,T

- Transform the way that we use population genetic data from allele frequency-based framework to an individual DNA fingerprint framework.
 - First line of analysis now considers all genotypes separately, as potential direct matches (recaptures) or as nodes in pedigrees involving other genotypes in the database(s).
 - Various options for second line of analysis: roll unidentified genotypes into frequency-based analyses, such as standard genetic stock identification.
- 




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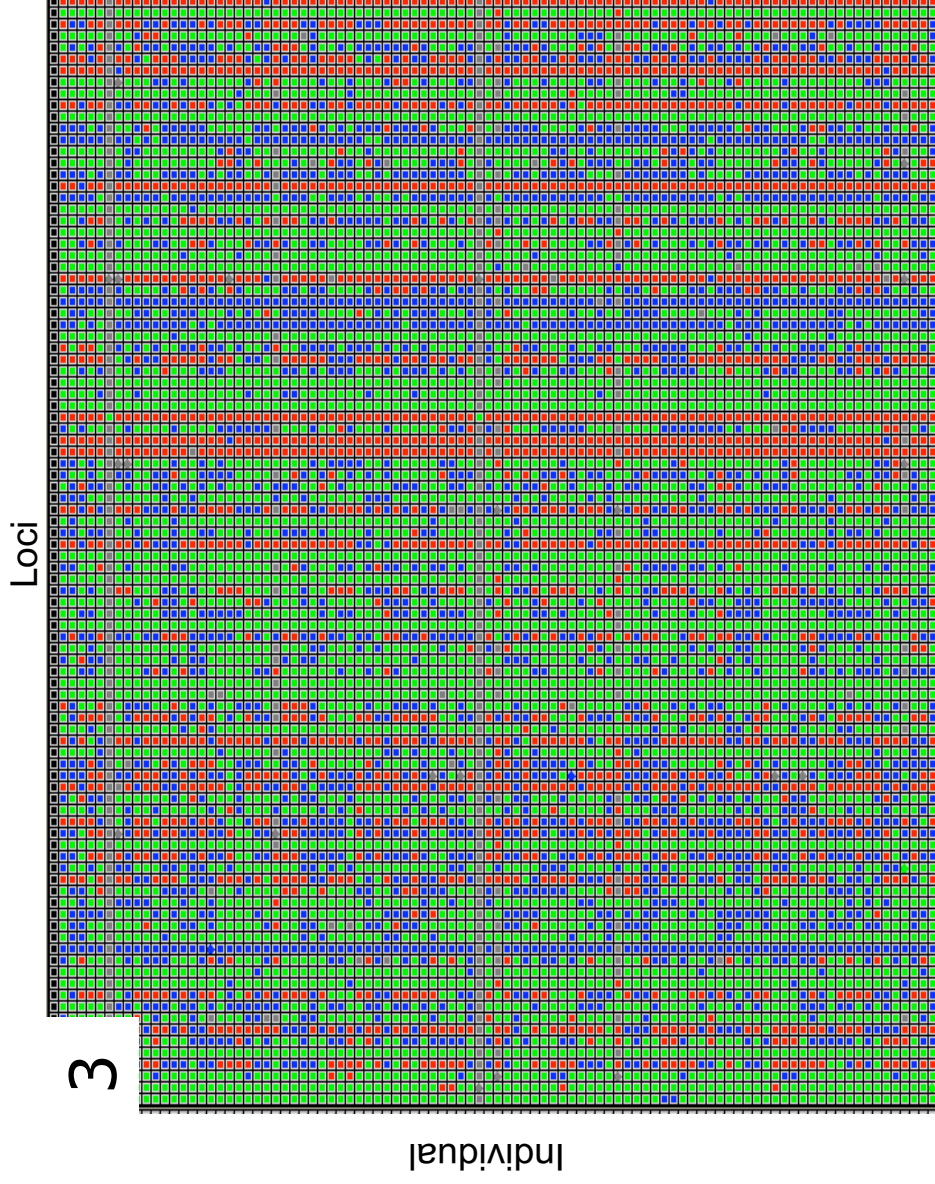
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Digital DNA Fingerprints

Fluidigm EP-1 w/ 96.96 dynamic arrays

Steelhead plate MID






Additional Information from PBT programs

- ecological and genetic applications


You get much more than stock-of-origin and cohort with PBT

- Reconstruction of large pedigrees
 - Map genes for phenotypic traits to locations in the genome
 - Near parametric estimates of variance in family size
 - Conduct large quantitative genetics studies of phenotypes
 - Evaluate different hatchery practices
 - Study differences in hatchery and naturally spawning fish by sampling at weirs, fish ladders or carcasses (w/ care)
 - Parentage analyses in marine fishes in realm of possible.
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Parentage-based tagging in salmonids

Where are we now?

- Marker development: **Done** for Chinook salmon and *O. mykiss*. Coho salmon and cutthroat trout in progress. MEGA projects have developed ~125 assays for Chinook salmon and ~175 for *O. mykiss*. Another ~75 assays available for Chinook and ~75 for *O. mykiss*.
 - Panel development: Panels of 96 SNPs will be selected for each species using multiple optimization criteria:
 - Chinook: Power for parentage inference at the Feather River, Trinity River and Livingston-Stone Hatcheries, power for GSI of stocks commonly harvested in coastal ocean of CA and OR.
 - O. mykiss*: Power for parentage inference at Warm Springs, Mad and Trinity River Hatcheries, power in Scott and Big Cks, power for GSI of hatchery rainbow trout.
 - Promulgation of panels as standardized genetic tags for California salmonids
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Parentage-based tagging in salmonids

Where are we now?

(cont.)


Chinook genetic tags are here!!!

Power analysis of 96 SNP loci

	False Positive Rate			
	False Negative Rate	0.1	0.05	0.01
FeatherSP		1.76E-13	9.64E-13	2.17E-11
KlamathIGH		3.19E-13	1.82E-12	3.97E-11
EelVA		2.51E-12	1.43E-11	2.82E-10
Kalama		1.60E-13	8.91E-13	1.91E-11
Cowlitz		2.61E-13	1.50E-12	3.22E-11

New standardized genetic tags provide more than sufficient power for PBT in the Central Valley, Klamath and (somewhat surprisingly) in the lower Columbia.





Parentage-based tagging in salmonids

Where are we now?


(cont.)

Chinook genetic tags are here!!!

Power analysis of 96 SNP loci

<u>Interbasin Assignment Accuracy</u>	
FeatherSP	0.9994
ButteSP	0.9996
MillSP	1
KlamathIGH	0.9976
EelVA	1
Spilus	1
Kalama	0.9986
Cowlitz	0.9952
Situk	1

The same genetic tags also provide accuracy of assignment to basin of more than 99% for all stocks. Similar or better than GAPS 13 microsatellites, in spite of small baseline.





Parentage-based tagging in salmonids

Where are we now?

Steelhead/rainbow trout tags too!!!

Power analysis of 96 SNP loci

False Negative Rate	False Positive Rate		
	0.1	0.05	0.01
BattleCreek	2.01E-13	1.20E-12	2.74E-11
Klamath	4.07E-12	2.32E-11	4.19E-10
Russian	2.31E-13	1.37E-12	3.38E-11
ScottCr-SC	2.06E-13	1.22E-12	3.22E-11
Eel	1.09E-12	6.41E-12	1.29E-10
Kamloops	7.33E-10	3.74E-09	5.53E-08
MtWhitney	8.28E-12	4.40E-11	8.73E-10
Eagle	6.96E-11	3.57E-10	8.25E-09
N.Zealand-Taupo	2.34E-12	1.09E-11	2.03E-10
Willamette	4.93E-09	2.34E-08	3.48E-07
Patagonia-SC	1.04E-11	5.52E-11	1.15E-09


New standardized genetic tags provide more than sufficient power for PBT with common CA steelhead and RBT stocks.




Parentage-based tagging in salmonids


Where are we now?

(cont.)

- Validation and implementation of PBT in Central Valley and Klamath hatcheries
 - Implement standardized SNP panels for upcoming ocean fishery work, both archival and future at-sea sampling
 - Construction of online database for deposition and accession of tags (genotypes)
 - Implementation of integrated PBT and GSI tagging/sampling program for determination of cohort & stock of origin for fish from PBT hatcheries and stock of origin for ALL fish.
 - Collaborative project to develop panels for Pacific Salmon Commission indicator hatcheries: transform international salmon fishery management.
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Parentage-based tagging in salmonids Alternative to mass marking and CWT?

- PBT provides an efficient manner to achieve 100% tagging of hatchery fish, which provides one of the main benefits of MM: the ability to ID all hatchery fish.
 - PBT does not enable mark selective fisheries-ID requires sampling and subsequent analysis.
 - PBT does not enable instantaneous manual segregation of hatchery and naturally-spawned fish at weirs or fish ladders-if desired, holding tanks would be needed. Analysis can be achieved in 24 hrs.
 - PBT would be cheaper than CWT system, particularly with added costs associated with MM.
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Parentage-based tagging in salmonids

Alternative 7?: 0% marking and 100%PBT

- Implementation of an integrated PBT/GSI program would allow total reformulation of sampling protocols, uncoupling them from mark status
 - PBT/GSI would provide “tag” recovery information for ALL fish: cohort & stock of origin for fish from PBT hatcheries and stock of origin for other fish.
 - Mark, ad-clip or other, could be reserved for special status release groups or study populations.
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