Parentage Based Tagging in the Snake River basin: From conception to implementation

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Idaho Department of Fish and Game
LSRCP Annual Meeting
2015
Conception:

Salmon Hatchery Management
Initiation of PBT in the Snake River basin

• Parentage Based Tagging was initiated in the Snake River basin following requests by IDFG managers to investigate alternative tagging methods to increase tagging rates and recoveries to estimate stock contributions of hatchery stocks returning over Lower Granite Dam back to Idaho and in-State fisheries.

While between 500-800 coded-wire tags have been recovered annually (2005-2007), ~ten times that many (~6,000) adults have been physically examined each year (Hansen 2007)

500-800 < 6,000
Initiation of PBT in the Snake River basin

• In addition, during this same period, several committees and science review groups specifically recommended that large-scale evaluations of the technology be performed (PFMC 2008; PSC 2008; ISRP/ISAB 2009).


Special Report of the GSI Steering Committee and the Pacific Salmon Commission’s Committee on Scientific Cooperation.

January 2008

Pacific Salmon Commission
Technical Report No. 23

INDEPENDENT SCIENTIFIC REVIEW PANEL
INDEPENDENT SCIENTIFIC ADVISORY BOARD

TAGGING REPORT

A comprehensive review of Columbia River Basin fish tagging technologies and programs

March 17, 2009
ISRP/ISAB-2009-1
Why PBT?

Parentage-based genetic tagging - PBT
(Anderson and Garza 2005)

Parentage-based tagging is based on the same techniques as those used in human parentage testing.
Each year all broodstock at each hatchery are genotyped, creating a database of parental genotypes.
Offspring from any of these parents (either collected as juveniles or returning adults) could be assigned back to their parents, thus identifying their origin and age.
Benefits of PBT

• Provides same information as CWTs (stock and cohort)
  ✓ Run reconstruction (age, sex, stock of returning adults)
  ✓ Harvest composition estimates

• Additionally, many issues associated with tagging studies all but go away
  ✓ Tag loss
  ✓ Tag detectability
  ✓ Differential mortality
Additional benefits of PBT

**PROS:**

• It is a passive mark (no handling of juveniles needed)

• “Tagged” fish can be non-lethally interrogated

✓ Fin-clip

✓ Scales

✓ Biopsy hook

“The hollow stainless steel tip of the hook collects a small amount of tissue as the fish strikes, and a special rasp holds the sample in place while the hook is in the water”
Versatility of PBT

**PROS:**
• Can potentially provide much more information than stock and cohort of origin

• Host of other life history, ecological and quantitative genetic questions
Initiation of PBT in the Snake River basin

• In 2010, IDFG and CRITFC received funding from the Bonneville Power Administration to initiate and evaluate PBT technology in the Snake River basin.

Snake River Chinook and Steelhead Parentage Based Tagging-Proposal #201003100

Proposal 201003100: Snake River Chinook and Steelhead Parental Based Tagging

Jump to:

1. Administrative 6. Objectives  Reviews
2. Location 7. Work elements
3. Species 8. Budget
5. Relationships 10. Narrative

• We also get additional funding for sampling and inventory from:
  ✓ LSRCP
  ✓ Idaho Power Company
  ✓ Pacific Coast Salmon Recovery Fund
Major objectives of this project (BPA# 2010-031-00)

1. Demonstrate that a set of 96 SNP loci (including a sex marker) for each species can provide robust genotyping and sufficient power for accurate parentage assignment.

2. Demonstrate that the SNP loci used for PBT can be integrated within a set of SNP loci used for GSI.

3. Demonstrate that multiple labs can adopt similar protocols and procedures and demonstrate concordance in producing SNP genotype data for PBT
Major objectives of this project (BPA# 2010-031-00)

4. Demonstrate the feasibility of PBT sampling and inventorying all hatchery broodstock spawned in the Snake River basin.

5. Demonstrate parental baselines can be constructed each year and that high genotyping and tagging rates can be obtained for each species.

6. Demonstrate the application and versatility of this technology through the summary of multiple back end projects that use completed PBT baselines to assign parentage to samples of unknown origin.
Implementation:
1. Demonstrate that a set of 96 SNP loci (including a sex marker) for each species can provide robust genotyping and sufficient power for accurate parentage assignment.

Steele et al (2013), empirically demonstrated that fewer than 100 SNPs are needed to accurately conduct steelhead PBT in the Snake River basin. He also demonstrated that the 95 SNP set provides accurate parental assignment with low false negative and false positive rates, and that stock assignments made with this SNP panel matched those made using CWTs.
1. Demonstrate that a set of 96 SNP loci (including a sex marker) for each species can provide robust genotyping and sufficient power for accurate parentage assignment.

Steele et al (2013) has demonstrated high concordance between sex-specific markers used for steelhead and Chinook salmon and known phenotypic sex of broodstock.

In the most recent comparison, the sex-specific assay for steelhead matched phenotypic sex in 99.6% of the samples (4,727 comparisons) and the sex-specific assay for Chinook Salmon matched phenotypic sex in 97.3% of the samples (8,888 comparisons) (Steele et al 2014).
2. Demonstrate that the SNP loci used for PBT can be integrated within a set of SNP loci used for GSI.

The utility of a 192 SNPs (2 panels) to characterize genetic variability throughout the Snake and Columbia rivers and to perform GSI analyses at Lower Granite Dam, Bonneville Dam, and in lower Columbia River mixed fisheries has been demonstrated (Ackerman et al 2012, Hess et al 2012; Steele et al 2012). This 192 SNP set includes the 96 SNP loci used for PBT and allows dual interrogation of both wild and hatchery fish.
2. Demonstrate that the SNP loci used for PBT can be integrated within a set of SNP loci used for GSI.

- Importantly, the screening of all unmarked adults against the Snake River PBT baseline, allows for improved estimation of wild abundance.

- For example, sampling at Bonneville Dam has demonstrated that approximately 16% of the returning steelhead adults phenotypically identified as “wild” (unmarked) were unclipped hatchery fish using PBT. Similar sampling has been done at Lower Granite Dam, where approximately 12% of the “wild” steelhead returning to the Snake River basin in Idaho were identified as unclipped hatchery fish.
3. Demonstrate that multiple labs can adopt similar protocols and procedures and demonstrate concordance in producing SNP genotype data for PBT

This project has demonstrated that multiple labs can adopt similar protocols and procedures for inventorying and genotyping samples, conducting QA/QC procedures, storing standardized meta- and genetic data in secure in-house databases, and combine PBT data into single, secure databases that make PBT baselines available to agencies throughout the Columbia River Basin.

- To produce Snake River PBT and GSI baselines for Chinook salmon and steelhead, CRITFC and IDFG labs each genotype 50% of the broodstock annually.

- Each lab follows similar laboratory methods/protocols for genotyping samples and performing QA/QC procedures. These protocols are published on Monitoring Methods.org (https://www.monitoringmethods.org/)
3. Demonstrate that multiple labs can adopt similar protocols and procedures and demonstrate concordance in producing SNP genotype data for PBT

- In addition to SNP genotyping concordance between IDFG and CRITFC, this project has also demonstrated >99% SNP genotyping concordance with both PBT and GSI SNP panels among five labs (Washington Department of Fish and Wildlife, Northwest Fisheries Science Center, Columbia River Inter-Tribal Fish Commission, Idaho Department of Fish and Game, and Abernathy Fish Technology Center).

✓ This confirms that SNP genotype data is accurately reproducible among labs.
3. Demonstrate that multiple labs can adopt similar protocols and procedures and demonstrate concordance in producing SNP genotype data for PBT

Standardized genotypes are stored on Progeny database servers housed at IDFG and CRITFC. Progeny software (http://www.progenygenetics.com/) is currently in use by a large number of GAPS and SPAN labs throughout the Pacific Northwest: CRITFC, IDFG, WDFW, UW and USFWS. The commonality of database software promotes seamless sharing of genetic data among labs.

Snake River PBT baselines are stored on a publicly available database repository (www.FishGen.net).

http://www.fishgen.net/WebPages/Dataset/Dataset.aspx
4. Demonstrate the feasibility of PBT sampling and inventorying all hatchery broodstock spawned in the Snake River basin.

5. Demonstrate parental baselines can be constructed each year and that high genotyping and tagging rates can be obtained for each species.
Snake River Chinook

- All Spring/Summer Chinook broodstock sampled since 2008
- All Fall Chinook (Lyons Ferry/NPT) since 2011
## Spring/Summer Chinook salmon: How many are we tagging?

<table>
<thead>
<tr>
<th></th>
<th>Spawn Year</th>
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<tbody>
<tr>
<td></td>
<td>2008</td>
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<tr>
<td>Genotyped</td>
<td>10,630</td>
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<td>“Tagging” Rate of Offspring</td>
<td>96.2%</td>
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<tr>
<td>Smolts Produced *</td>
<td>~18.96 mil</td>
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</tbody>
</table>

* Assuming 3,500 smolts produced per broodstock pair, ± Numbers not final

This project genetically tags ~34% of spring/summer hatchery Chinook salmon released in the Columbia River basin each year
Snake River Steelhead

- Majority sampled in 2008
- All broodstock sampled since 2009
Steelhead: How many are we tagging?

<table>
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<tbody>
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<td></td>
<td>2008</td>
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<td>Broodstock sampled</td>
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<tr>
<td>Genotyped</td>
<td>5,070</td>
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<tr>
<td>“Tagging” Rate of Offspring</td>
<td>96.9%</td>
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<tr>
<td>Smolts Produced *</td>
<td>~9.01 mil</td>
</tr>
<tr>
<td>Smolts “Tagged”</td>
<td>~8.74 mil</td>
</tr>
</tbody>
</table>

* Assuming 3,500 smolts produced per broodstock pair

This project genetically tags ~61% of hatchery steelhead released in the Columbia River basin each year
High PBT tagging rates have been realized at the hatchery stock, rearing stock, and release site levels.

Huge thanks to Carl Stiefel for his vision and leadership for developing family tracking protocols for LSRCP hatcheries!!!!!
PBT tagging rates at the hatchery stock level:

<table>
<thead>
<tr>
<th>Hatchery Stock</th>
<th>Total Unique Families Genotyped</th>
<th>Total Females Genotyped</th>
<th>Total Females Spawned</th>
<th>Total Males Genotyped</th>
<th>Total Males Spawned</th>
<th>Number of smolts Released</th>
<th>Number of PBT tagged smolts tracked to release group</th>
<th>PBT tagging Rate</th>
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<tbody>
<tr>
<td>Dworshak</td>
<td>646</td>
<td>667</td>
<td>670</td>
<td>491</td>
<td>500</td>
<td>2,953,935</td>
<td>2,848,122</td>
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<td>11</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>59,209</td>
<td>54,275</td>
<td>0.917</td>
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<td>Dyeber</td>
<td>100</td>
<td>103</td>
<td>103</td>
<td>100</td>
<td>102</td>
<td>578,380</td>
<td>561,534</td>
<td>0.971</td>
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<td>Pahsimeroi</td>
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<td>355</td>
<td>357</td>
<td>357</td>
<td>357</td>
<td>1,740,352</td>
<td>1,730,602</td>
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<td>Sawtooth</td>
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<td>286</td>
<td>286</td>
<td>285</td>
<td>286</td>
<td>1,381,958</td>
<td>1,377,126</td>
<td>0.997</td>
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<tr>
<td>Upper Salmon B-run</td>
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<td>69</td>
<td>69</td>
<td>62</td>
<td>62</td>
<td>258,674</td>
<td>258,674</td>
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<td>S.F. Clearwater</td>
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<td>214</td>
<td>214</td>
<td>125</td>
<td>131</td>
<td>931,522</td>
<td>892,346</td>
<td>0.958</td>
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<td>Pahsimeroi-Egg Box</td>
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<td>143</td>
<td>144</td>
<td>138</td>
<td>144</td>
<td>N/A</td>
<td>N/A</td>
<td>0.951</td>
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<td>Total/Average</td>
<td>1808</td>
<td>1848</td>
<td>1855</td>
<td>1571</td>
<td>1595</td>
<td>7,904,031</td>
<td>7,722,679</td>
<td>0.969</td>
</tr>
</tbody>
</table>

Average: 96.9%
PBT tagging rates at the rearing stock level:

| Hatchery Stock | Rearing Stock | Total Unique Families Genotyped | Total Females Genotyped | Total Females Spawned | Total Males Genotyped | Total Males Spawned | Number of smolts Released | Number of PBT tagged smolts tracked to release group | PBT tagging Rate |
|----------------|---------------|---------------------------------|-------------------------|-----------------------|----------------------|----------------------|----------------------------|--------------------------------------------------|----------------|-------|
| Dworshak       | Clearwater    | 130                             | 141                     | 142                   | 105                  | 111                  | 587,719                    | 538,053                           | 0.915         |
| S.F. Clearwater| Clearwater    | 69                              | 69                      | 69                    | 62                   | 62                   | 258,674                    | 258,674                           | 1.000         |
| Dworshak       | Dworshak      | 470                             | 479                     | 481                   | 345                  | 347                  | 2,228,021                   | 2,177,068                          | 0.977         |
| EFSR Natural   | Hagerman      | 11                              | 11                      | 12                    | 13                   | 13                   | 59,209                     | 54,275                            | 0.917         |
| Sawtooth       | Hagerman      | 285                             | 286                     | 286                   | 285                  | 286                  | 1,381,958                   | 1,377,126                          | 0.997         |
| Dworshak       | Magic         | 46                              | 47                      | 47                    | 41                   | 42                   | 138,195                     | 135,255                           | 0.979         |
| Pahsimerol     | Magic         | 93                              | 93                      | 93                    | 93                   | 93                   | 480,493                     | 480,493                           | 1.000         |
| Upper Salmon   | Magic         | 205                             | 214                     | 214                   | 125                  | 131                  | 931,522                     | 892,346                           | 0.958         |
| Oxbow          | Niagara       | 100                             | 103                     | 103                   | 100                  | 102                  | 578,380                     | 561,534                           | 0.971         |
| Pahsimerol     | Niagara       | 262                             | 262                     | 264                   | 262                  | 264                  | 1,259,859                   | 1,250,315                          | 0.992         |
| Pahsimerol     | SBT Egg Box   | 137                             | 143                     | 144                   | 138                  | 144                  | N/A                        | N/A                               | 0.951         |
| Total/Average  |               | 1808                            | 1848                    | 1855                  | 1569                 | 1595                 | 7,904,031                   | 7,725,138                          | 0.969         |

Average: 96.9%
### PBT Tagging Rates at the Release Group Level:

<table>
<thead>
<tr>
<th>Hatchery Stock</th>
<th>Rearing Stock</th>
<th>Release Site</th>
<th>Mark/Tag</th>
<th>Total Unique Families Genotyped</th>
<th>Total Females Genotyped</th>
<th>Total Males Genotyped</th>
<th>Total Females Spawned</th>
<th>Total Males Spawned</th>
<th>Number of Smolts Released</th>
<th>Number of PBT Tagged Smolts Tracked to Release Group</th>
<th>PBT Tagging Rate</th>
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</thead>
<tbody>
<tr>
<td>DWOR</td>
<td>Meadow Cr.</td>
<td>2013</td>
<td>AD</td>
<td>44</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>159,547</td>
<td>146,251</td>
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<td>28</td>
<td>28</td>
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<td>50</td>
<td>50</td>
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<td>153</td>
<td>162</td>
<td>162</td>
<td>818,653</td>
<td>767,896</td>
<td>0.938</td>
</tr>
<tr>
<td>PAH</td>
<td>Egg-Box</td>
<td>2013</td>
<td>ADint</td>
<td>137</td>
<td>143</td>
<td>144</td>
<td>138</td>
<td>144</td>
<td>7,904,032</td>
<td>6,992,098</td>
<td>0.918</td>
</tr>
</tbody>
</table>

**Average: 91.8%**
6. Demonstrate the application and versatility of this technology through the summary of multiple back end projects that use completed PBT baselines to assign parentage to samples of unknown origin.
Chinook salmon
Estimating stock proportions of hatchery Chinook salmon at Lower Granite Dam

- Why use PBT to estimate hatchery proportions when these estimates are already generated using PIT tags?
Estimating stock proportions with PBT

- PIT tags may underestimate numbers at Lower Granite Dam when compared to window counts
  - PIT tag shedding
  - Mortality
  - Tag failure
- Can be adjusted, if underestimate is uniform across stocks
Estimating stock proportions with PBT

- PIT tags can underestimate hatchery adult returns
- Results shown below are for PIT estimates not corrected using PIT arrays at hatchery traps (not all hatcheries have these)
- Underestimation not consistent across hatchery stocks or return year

<table>
<thead>
<tr>
<th>Stock and Cohort</th>
<th>Number of PITs</th>
<th>PBT Estimate</th>
<th>PIT Tag Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>McCall (BY08)</td>
<td>36,000</td>
<td>5,000</td>
<td>4,000</td>
</tr>
<tr>
<td>McCall (BY09)</td>
<td>13,000</td>
<td>4,000</td>
<td>3,000</td>
</tr>
<tr>
<td>Sawtooth (BY08)</td>
<td>13,000</td>
<td>3,000</td>
<td>2,000</td>
</tr>
<tr>
<td>Sawtooth (BY09)</td>
<td>13,000</td>
<td>2,000</td>
<td>1,000</td>
</tr>
</tbody>
</table>

**PBT Samples**
Lower Granite = 1,025
Estimating Stock Composition of the 2012 Lower Salmon River Fishery

- Harvest estimated weekly from a roving creel
- All fish observed in the catch were scanned for CWT and tissue sampled for PBT
- Stock composition methods – described in Bernard and Clark, 1996; CJFAS
  - Tag recoveries expanded by tag and sample rates
2012 Sport Harvest-Lower Salmon R Adult Chinook

- CI's were significantly smaller using PBT
- CWT estimates tend to underestimate individual stock contribution
- Two stocks were undetected by CWT

<table>
<thead>
<tr>
<th>Stock</th>
<th>CWT</th>
<th>PBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid River</td>
<td>10</td>
<td>155</td>
</tr>
<tr>
<td>Sawtooth</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>McCall</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>Pahsimeroi</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Imnaha</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16</strong></td>
<td><strong>209</strong></td>
</tr>
</tbody>
</table>

Total Est. Harvest: 3,126
Combined PBT: 2,826
Combined CWT: 2,162

![Graph showing PBT and CWT estimates for different stocks](chart.png)
Steelhead
Objective: Estimate the proportion of Snake River hatchery smolts in a subsample of GSI assigned smolts recovered from Caspian terns and double-crested cormorants.

Results:
- Of 57 samples (all GSI assigned to reporting groups above Bonneville dam), ~64% assigned to Snake River hatcheries.
- Highest predation on smolts from Dworshak NFH.

Data courtesy of David Kuligows
Northwest Fish Science Center
Bonneville Dam

**Objective:**
- Estimate stock proportion of hatchery fish returning over Dam.
- Test and improve GSI estimation
- Estimate % of hatchery adults called “wild”

**Results:**
- ~80% of hatchery adults assigned to Snake River
- 16.1% of the steelhead phenotypically identified as "wild" were identified as unclipped hatchery fish using PBT

N = 1000
C - G Columbia and Snake River Fisheries (Sport and Tribal)

Objective: Estimate Snake River hatchery proportions within mixed stock fisheries

Alan’s Results....
Deschutes River Basin

**Objective:**
Determine the origin of stray hatchery steelhead collected in Bakeoven Creek and Buck Hollow Creek

**Results:**
- ~80% of the hatchery fish that strayed into Buck Hollow Creek were from Snake River hatcheries.
- Highest number of strays observed were from the Sawtooth hatchery

**Advantages:**
- Non-lethal sampling required
- Allows subsequent evaluation of relative reproductive success of straying hatchery versus wild steelhead

*Data courtesy of Matt Smith, Abernathy Fish Technology Center*
I Lower Granite Dam (Adults)

**Objective:**
Estimate escapement of individual hatchery stocks returning over Lower Granite Dam

**Results:**
- 11.6% of the steelhead phenotypically identified as "wild" were identified as unclipped hatchery fish using PBT

**Advantages:**
- More precise and less biased than PIT tag estimates
- Integrated with GSI program for wild adults

74,276 total hatchery steelhead over LGR Dam (SY2013)
Back at the spawning hatchery (e.g. Oxbow Hatchery)

Since all broodstock are sampled and genotyped each year, PBT also allows precise estimates of effective population size of broodstocks, inter-hatchery stray rates, and demographic characteristics of individual broodstocks and release groups.
Lower Granite Dam (Kelts)

Objective:
Estimate stock composition of hatchery kelts outmigrating downstream Lower Granite Dam

Results:
- Most Snake River hatcheries are producing kelts
- Highest percentage in SY2013 from the Pahsimeroi hatchery
- Significant differences in outmigration timing observed among stocks
Future:
Multi-regional, multi-agency effort underway to sample all steelhead and Chinook salmon hatcheries in the Columbia River basin

Current discussions on whether PBT sampling should be expanded to include PSC indicator Chinook salmon hatcheries in CA, southeast AK, and British Columbia
Next-Generation DNA Sequencing

The cost of obtaining DNA data has dropped faster than the costs of processing data on computers. From 2007 until 2012 the cost of sequencing has dropped from ~$1,000 per megabase to less than $0.1 per 1000 megabase.
• Next-Generation DNA Genotyping by Sequencing

We can use this same technology to improve our existing programs and do it at a reduced cost!

• This technology can be used to screen much larger panels of genetic markers
  ✓ Same 96 SNP genetic marker sets that we run now
  ✓ An additional ~200-400 recently developed SNPs
  ✓ Reduce consumable costs from $15/sample to $5/sample

• Empirically demonstrated (Campbell et al 2014 and references within)
• Next-Generation DNA Genotyping by Sequencing

✓ In addition, the same equipment can be used for the discovery and identification of novel Single Nucleotide Polymorphism (SNP) genetic markers.
Next-Generation DNA Genotyping by Sequencing

The ability to screen larger numbers of SNPs is likely to improve Genetic Stock Identification and the allow better quantification of hatchery straying and introgression into wild populations. This is due both to the increase in power from running more genetic markers, but also from the ability to screen genetic markers that are under selection.
• GBS neutral versus non-neutral genetic variation

✓ Up until recently, most of the genetic markers used for the study and management of steelhead and salmon populations were neutral (variation at these genes does not affect fitness and are not under selection).

✓ However, the use of only neutral markers has received scrutiny and increasingly, researchers and managers are interested in information that can be obtained from adaptive functional genes (i.e., genes that directly influence fitness).
• **GBS neutral versus non-neutral genetic variation**

Some examples:

✓ The identification of SNPs exhibiting diversification selection can provide increased accuracy and precision for genetic stock identification (Ackerman et al 2011).

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**Transactions of the American Fisheries Society**

Publication details, including instructions for authors and subscription information: [http://www.tandfonline.com/loi/utaf20](http://www.tandfonline.com/loi/utaf20)

**Single-Nucleotide Polymorphisms (SNPs) under Diversifying Selection Provide Increased Accuracy and Precision in Mixed-Stock Analyses of Sockeye Salmon from the Copper River, Alaska**

Michael W. Ackerman a c, Christopher Habicht b & Lisa W. Seeb a

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c Idaho Department of Fish and Game and Pacific States Marine Fish Commission, Eagle Fish Genetics Laboratory, 1800 Trout Road, Eagle, Idaho, 83616, USA
GBS neutral versus non-neutral genetic variation

Some examples:

- The identification of SNPs under domestication selection can provide methods for estimating actual rates of gene flow between hatchery and wild populations (Karlsson et al 2014), as opposed to simply detecting hatchery strays.
GBS neutral versus non-neutral genetic variation

Some examples:

✓ The identification of adaptive genes linked to disease resistance offers the potential to utilize marker assisted selection for fish managers to increase resistance and reduce outbreaks (Campbell et al 2014).

“identified 12 SNP markers that were highly associated with resistance to CWD and 19 markers associated with resistance to IHNV”
• IDFG recently received funding from PCSRF to purchase DNA sequencer (NextSeq 500) to perform GTseq for GSI and PBT projects and to identify new Single Nucleotide Polymorphism (SNP) genetic markers in salmon and steelhead.
Ultimate goal: Identify parents

- Where and when it was released
- Where and under what conditions it reared
- Stock and age
- Diet
- Disease
- Rearing density
- Temperature

This information could be available for all hatchery broodstock every year!

http://www.flickr.com/photos/natekay/4319654380/sizes/o/
Questions???

Idaho Department of Fish and Game Eagle Fish Genetics Lab