

Collection and Curation

Questions

- Need for standardized protocols?
- What to collect and curate?
- How long to store?
- Baseline vs. Fishery samples?

Baseline vs. Fishery Mixture Collections

- Potential for reanalysis
 - ✓ determined by management needs
- Other uses for samples
 - ✓ population identification
 - ✓ monitoring of anthropogenic changes
 - ✓ gene expression – requires different handling protocols
- Associated data available
 - ✓ individual location, date, age, other
- Desire to update based on new markers
 - ✓ cheaper and easier to reanalyze existing samples
- Interlab standardization of scoring

Standardized Protocols

- Fishery Mixtures vs. Baseline
- Sample collection
 - ✓ sampling protocol
 - ✓ type of sample
 - ✓ method of collection
- Extraction Methods
- Curation of samples
 - ✓ method of storage
 - ✓ duration of storage

What to collect and store?

- Tissues
- DNA
- PCR Products

What to collect and store?

- Tissues
 - ✓ quantity
 - ✓ type
 - ✓ collection method
 - ✓ storage method
- DNA
- PCR Products

Tissues: type

Tissue	Advantages	Limitations
Opercle Punch	Easy to collect; high DNA yield	Delamination can cause problems for bulk collections
Scale	Easy to collect	Cross contamination can cause problems; extraction consumes scale(s); low DNA yield; no detection of previous sampling
Axillary process (located above pelvic fin)	Easy to collect; maintains integrity in bulk collections; high DNA yield; easy to sample appropriate amount.	Amount of tissue limited by size of process (generally not a problem)
Fin clip	Easy to collect; medium DNA yield; easiest type on live fish	Delamination may cause problems with bulk collections
Heart, muscle, liver	Tissues may be available in allozyme archives; heart and liver have high DNA yield, muscle medium DNA yield; may be useful for multiple techniques	Generally lethal to collect

Tissues: collection method

	Advantages	Limitations	Description
Vials, individually labeled (Alcohol, DMSO, Frozen)	Linkable to individual data; most common technique	Vials must be individually labeled, filled with preservative or frozen, and capped. Each tissue must be cut by hand.	
Vials, bar-coded (Alcohol, DMSO, Frozen)	Linkable to individual data; no individual labeling required; vials non-collection specific; used in Alaska and Canada	Must have field and lab barcode reader. Vials must be filled with preservative or frozen and capped. Each tissue must be cut by hand.	Factory-printed vials that do not repeat numbers.
Bulk (Alcohol, DMSO and Frozen)	Rapid and easy field collection, only single bottle must be labeled.	Not linkable to individual data. Each tissue must be cut by hand.	Typically multiple individuals collected into a single 125 or 250 ml bottle of alcohol.

Tissues: collection method

	Advantages	Limitations	Description
Blotter Paper (Dry)	Linkable to individual data. Potentially easy to subsample by automation. Store at room temperature, no maintenance, low archive volume. Used in Pacific Northwest and California.	Must dry to preserve. Limited tissue types (fin).	Placed on paper and then in envelopes or multiple individuals placed on single divided sheet
FTA paper (Dry) http://www.whatman.com/products/?pageID=7.31.31	Linkable to individual data. Potentially easy to subsample by automation. Tissue collection and DNA extraction in single step.	Must dry to preserve. Tissue must be individually homogenized onto paper. Limited or untested for salmon. Eliminates ability to archive tissue.	Sample placed on FTA paper and dried. Paper contains extraction reagents, DNA sticks to the paper and can be released for analysis.

Tissue: preservation and storage

	Advantages	Limitations
Alcohol	Long term preservation well documented; rapid dehydration; easy/cheap to obtain. Room temperature storage.	Requires hazmat shipping, approved storage and ventilation, alcohol levels must be monitored and maintained in vials
DMSO	Appropriate for shipping to "dry" communities.	Preservation properties less reliable than alcohol
Frozen	Long term preservation well documented; shipment on blue or wet ice does not require HAZMAT; useful for multiple techniques	Must remain frozen during storage; degradation over repeated freeze/thaw cycles; must guard against loss due to power or freezer failure
Dry	Dry, room temperature storage; easily cut for extraction; storage volume varies; may bypass extraction step	Must be dried after collection to avoid degradation; may be difficult to dry in wet environment; unknown length of preservation in different ambient conditions

What to collect and store?

- Tissues
 - ✓ type
 - ✓ quantity
 - ✓ method of collection
- DNA
 - ✓ extraction method
 - ✓ frozen
 - ✓ dried
- PCR Products

DNA: extraction methods

	Chelex	Phenol/chloroform	Silica purification columns	Magnetic Beads without robotics	Magnetic Beads with robotics
Chemical/disposable cost/sample	\$0.05	\$0.20	\$2.00	\$2.00	\$2.00
Personnel time (192 fish)	2 hr	16 hr	5 hr	5 hr	2.5 hr
Total cost/sample	\$0.36	\$2.70	\$2.78	\$2.78	\$2.39
Turnaround time (192 fish, 1 person)	3 hrs	16hr	6 hrs - overnight	5 hrs	5 hrs
Suitability for Archive	No	Yes	Yes	Yes	Yes
DNA yield	High but dirty	High	Medium	Medium to high	Medium to high
Toxicity	Low	High	Medium	Low	Low
Automatable	No	No	Yes	No	Yes
Capital cost	\$500	\$3,000	\$8,000	\$3,000	\$60,000

What to collect and store?

- Tissues
 - ✓ type
 - ✓ quantity
 - ✓ method of collection
- DNA
 - ✓ extraction method
 - ✓ frozen
 - ✓ dried
- PCR Products
 - ✓ utility?

PCR Products: storage

- Longevity
 - ✓ short term
- Usefulness
 - ✓ until data are scored
- Standardization not required
 - ✓ lab specific standards based on SOP

Storage and Curation

- Space required
 - ✓ differs with method of collection and preservation
 - ✓ increases with duration of storage
- Personnel time
 - ✓ alcohol storage increases curatorial time
- Availability
 - ✓ need to share among labs
 - ✓ quantity of tissue

Recommendations: Standardized Protocols

- Fishery Mixture Samples
 - ✓ random sample of fishery
 - ✓ at sea sampling beneficial
 - ✓ standardization less important
 - tissue type, collection method, and extraction method
- Baseline Samples
 - ✓ random sample
 - ✓ individual collection and storage
 - ✓ silica purification column extraction

Recommendations: Tissue Type

- Non-lethal sample
- Scale collection desirable for ageing
 - ✓ less desirable for DNA
- Fin or axillary process
 - ✓ more important for baseline samples

Recommendations: Storage and Curation

- Storage Medium
 - ✓ alcohol most common
- Desirability of commodity to store
 - ✓ Tissue > DNA >>> PCR Products
- Fishery Mixture Samples
 - ✓ store at a minimum until management applications are complete and agreed to by parties (genotyping completed)
- Baseline Samples
 - ✓ should be stored indefinitely

THANK YOU

Questions or Comments?