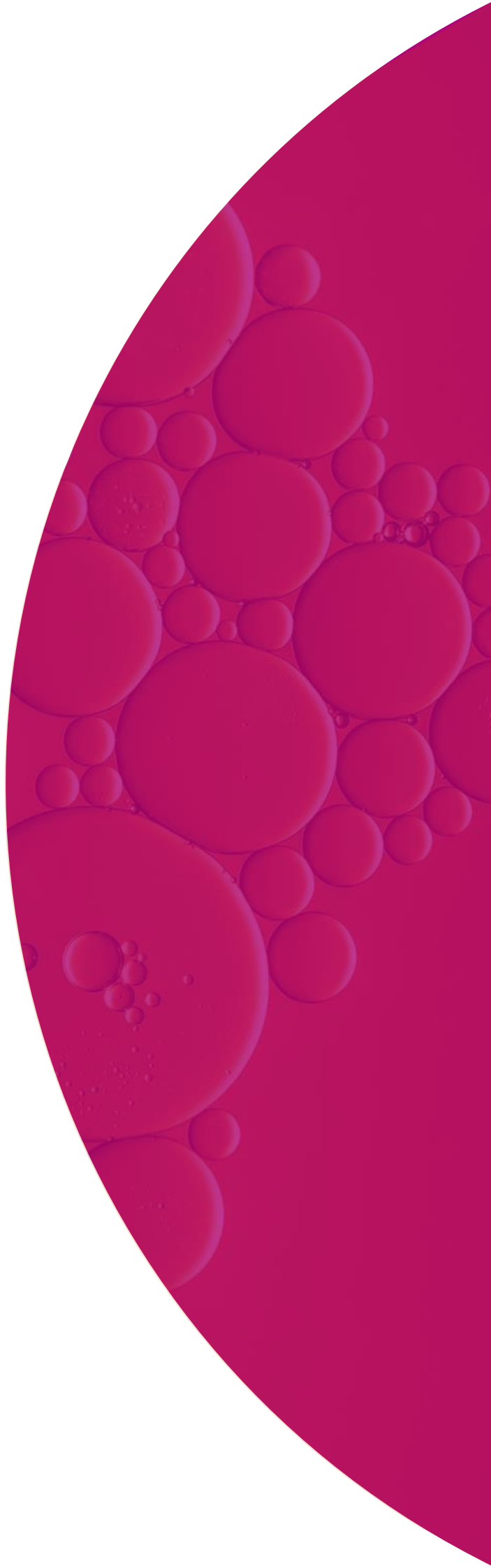


Selecting fish tissue type for sequencing

Procedure & checklist



Kits required

The major kits used in this Procedure & checklist are listed below. Additional equipment and materials required by each kit are provided in the kit specific protocols.

Equipment	Model
Nanobind® tissue kit	PacBio® (102-302-100)
SRE kit	PacBio (102-208-300)

Fish tissue type selection

This Procedure and checklist describes our recommended methods for sequencing fish tissue. Nile Tilapia is used as an example.

Extraction and sequencing performance can vary dramatically across fish tissue types.

Note: We highly recommend users begin new projects by extracting DNA from a variety of tissue types and focusing on the tissue types that generate the highest quality DNA.

The optimal tissue can vary across fish species. However, fish heart tissue generates consistent performance across various fish species and is the recommended tissue type for optimal sequencing performance.

Procedures & checklists describing the extraction of various fish tissue types are available including:

Procedure & checklist – Extracting HMW DNA from fish skeletal muscle using Nanobind kits

Procedure & checklist – Extracting HMW DNA from tilapia testis using Nanobind kits

Procedure & checklist – Extracting HMW DNA from nucleated blood using Nanobind kits

Study Synopsis

Note: Fish heart generates consistently high-quality DNA across a wide variety of fish species and superior sequencing results. This is the preferred sample type for fish.

- Fish testis generates high amounts of DNA and very good sequencing results.
- Fish kidney and fin clips are also good samples for sequencing.
- Fish spleen generates very high amounts of DNA. Quality and sequencing performance can vary.
- Fish liver is very sensitive to degradation. Liver tissue must be frozen or processed immediately after dissection or DNA sample quality will be reduced.
- Fish muscle generates low amounts of DNA and has high variability across fish species. Other sample types are recommended to be tested first.
- Fish blood is also a good sample with very high DNA content and good sequencing performance.

Tissue dissection

A live Nile Tilapia was sacrificed and dissected into individual tissue specimens by type. These included muscle, kidney, liver, brain, fin, spleen, testes, adipose, gill, etc.

Each tissue was then frozen and stored at -80°C.



DNA extraction yield and purity

Extraction notes

- Performed duplicate extractions on kidney, liver, fin, brain, testes, spleen, heart, and muscle samples.
- Followed standard TissueRuptor Protocols for kidney, fin, and heart samples.
- For liver, brain, testes, and spleen samples, we followed example protocols as provided in Procedure & checklists.
- Performed QC on extracted DNA by 1) Triplicate Nanodrop from top, middle and bottom of tube, 2) Qubit DNA BR Assay, 3) Qubit RNA BR Assay, and 4) Bio-Rad CHEF gel.

Extraction results

- Spleen and testes samples result in very high extraction yields. Consistently among the highest of all tissue types.
- Kidney also resulted in high yield.
- Heart and fin samples resulted in good yields.
- Liver and brain resulted in low but serviceable yields.
- Muscle samples failed extraction and resulted in very low yield.
- UV purity was consistently good across all tissue types

Note: Pink samples were selected for sequencing

Sample Name	Sample Input (mg)	dsDNA (μg)	%RNA	A260/A280	A260/A230
809 Kidney A	22	16.1	10.5	2.22	1.86
809 Kidney B	23	28.1	9.4	2.24	1.85
809 -80 Liver A	24	4.4	14.7	1.94	1.85
809 -80 Liver B	25	1.9	0.0	1.54	1.87
809 Fin A	23	8.0	13.0	2.12	1.81
809 Fin B	27	11.6	13.1	2.13	1.82
809 Brain A	20	2.5	14.5	1.83	1.90
809 Brain B	32	4.5	13.9	2.07	1.87
809 Testes A	25	41.9	9.3	2.31	1.86
809 Testes B	28	40.7	9.8	2.35	1.86
809 Spleen A	22	54.6	8.6	2.31	1.86
809 Spleen B	25	48.6	9.2	2.33	1.87
809 Heart A	23	7.6	12.9	2.01	1.86
809 Heart B	25	13.1	11.5	2.18	1.86

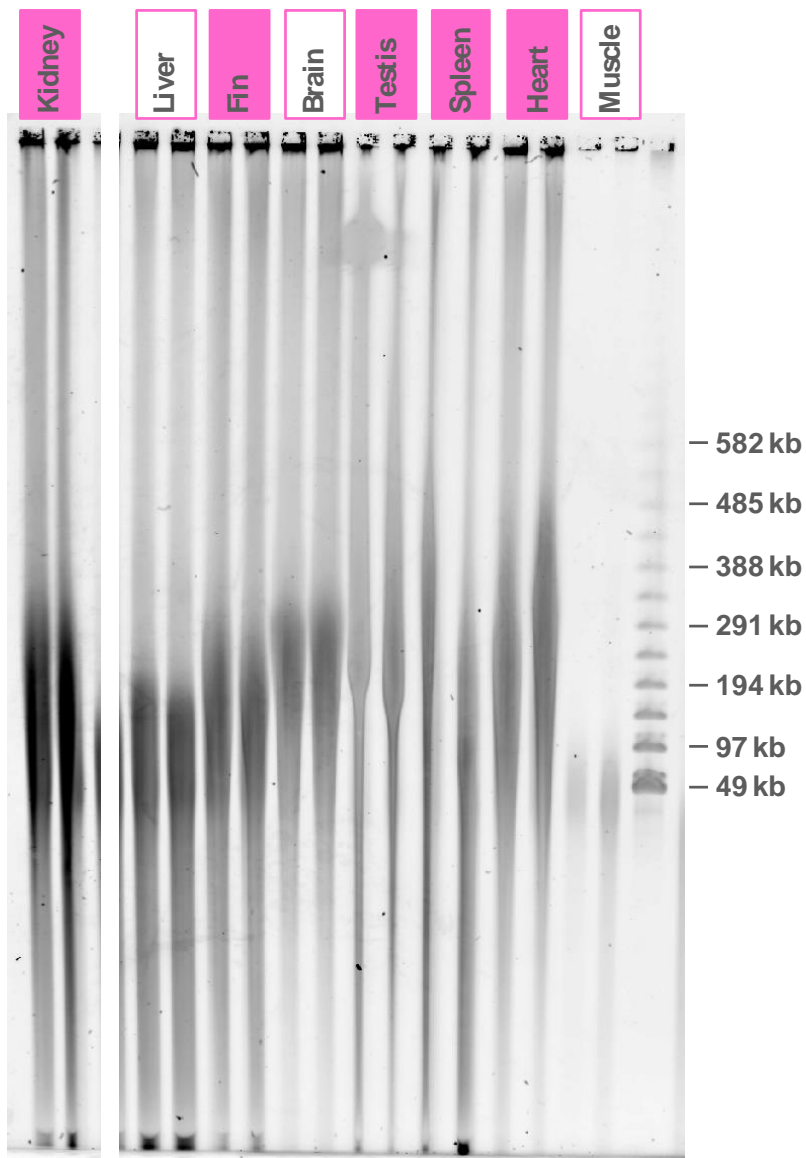
DNA size

CHEF gel notes

- DNA size was analyzed by Bio-Rad CHEF gel
- 200 ng of DNA was loaded into each lane, run using our standard 22 hr protocol, stained by SYBR Gold for 1 hr, and imaged on a GE Typhoon.

Size results

- Liver samples appeared more degraded than others. Time that the liver sample spent before being frozen dramatically affected DNA size.
- Heart sample appeared best and is consistent with experiments in other fish species.



Sequencing

Shearing

- The spleen, fin, kidney, testes, and heart samples were 5X needle sheared (1X = 1 up + 1 down stroke) using a 26G blunt end needle. Needle shearing was performed at the elution concentration.

Size selection

- The samples were then diluted to either 75 ng/μL or 150 ng/μL.
- Size selection was performed using the short read eliminator (102-208-300) according to the standard protocol.
- Measured recovery efficiency varied from 23–96%.
- Spleen sample had low recovery indicative of short DNA size.

Sample	Qubit DNA starting concentration (ng/μL)	Starting DNA sample volume (μL)	Qubit DNA final concentration (ng/μL)	Final DNA sample volume (μL)	Recovery efficiency
Spleen	136	60	30.8	60	23%
Fin	74.2	60	55.8	55	69%
Kidney	70	60	48.7	55	64%
Testes	53.3	60	55.6	55	96%
Heart	89.8	60	63.2	55	65%

Sequencing results

- Sequenced 5 tissue samples with third generation sequencing prior to HiFi (data not shown).
- Heart generated highest N50 but still maintained good throughput. Heart tissue generates consistent sequencing data across many fish species and is our recommended tissue type for fish samples.
- Spleen generated shortest read lengths but highest throughput.
- Testes generated very good read lengths and very good throughput.
- Fin and kidney generated good read lengths and very good throughput.

Revision history (description)	Version	Date
Initial release	01	July 2022
Minor updates throughout	02	December 2022

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