



A 3-D printed vitrification device integrated with French straws

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ABSTRACT

The goal of this work was to develop prototypes of open-hardware vitrification devices for sperm cryopreservation that can be integrated with existing straw platforms. The open-hardware Vitrification Device for French Straws (VD-FS) is low-cost, customizable, 3-D printable, standardized, and allows long-term sample storage and identification. The feasibility was shown for vitrifying and storing samples with multiple configurations. The results can be improved by design alternation and evaluation of various vitrification solutions. This is the first complete open-hardware vitrification device that can be integrated with existing French-straw storage systems, providing a foundation for future community-level modifications and improvements.

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Specifications table

Hardware name	Vitrification Device for French Straws (VD-FS)
Subject area	Biological sciences
Hardware type	Biological sample handling and preparation
Closest commercial analog	Cryotec Vitrification Devices (https://us.ivfstore.com/products/cryotec-vitrification-devices?variant=33155076096077); S-Cryolock® Vitrification Device (https://us.ivfstore.com/products/s-cryolock?variant=12798333452365); Sperm VD (https://us.ivfstore.com/products/sperm-vd?variant=42371647668462)
Open source license	Creative Commons Attribution 4.0 International (CC BY 4.0)
Cost of hardware	Material cost per jig apparatus: \$0.29 Material cost per vitrification wand: \$0.01
Source file repository	https://doi.org/10.17632/b7yy72wcxx.2 https://3dprint.nih.gov/discover/3dpx-017622

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Hardware in context

Vitrification of living biological materials was introduced in the late 1900 s and has continued to advance as an emerging cryopreservation approach [1]. Compared with conventional equilibrium cooling with relatively slow rates (e.g., 1–50 °C/min) vitrification uses ultra-rapid cooling (e.g., > 5,000 °C/min) to limit or prevent formation of crystalline ice and produce an amorphous solid (i.e., ‘glass state’) [2]. To achieve relatively high cooling and warming rates, samples are usually prepared in small volumes (<5 µL) and mixed with high concentrations of cryoprotectants [3]. Vitrification has been studied and used in cryopreservation of tissues [4], somatic cells [5], gametes [2,6], and embryos [7] for animals and humans. Advantages of vitrification approaches include low cost, portability, short processing times, and potentially higher sample quality [7,8].

Despite these advantages, vitrification has not been widely adopted in applications for development of animal germplasm repositories [26], primarily due to a lack of practical, economical tools. For example, most commercially available vitrification products, such as the Cryotop® [9] and Cryolock® [10], were designed for processing of human oocytes or embryos, which can be loaded onto a thin polymer strip to achieve high cooling rates. However, smaller cells (e.g., sperm) [28] need to be loaded as droplets, which can limit the cooling rate and reduce handling efficiency. Commercial sperm vitrification products are expensive, with average costs of \$20–30 per sample [11]. In addition, the sample protection after cooling for long-term storage in these devices is provided by proprietary caps, which also contribute to the high cost. This cost is acceptable in human fertility clinics, but it is a limitation in practical applications for animal germplasm repositories addressing conservation, biomedical research, and agriculture.

Open hardware provides a promising solution to the limitation of existing vitrification tools with added potential for contributions from user communities to test, evaluate, change, improve, and standardize prototypes [12,13]. With rapidly developing consumer-level capabilities, three-dimensional (3-D) printing and computer-aided design (CAD) offer viable approaches to rapidly design and fabricate devices that can support cryogenic applications [14,15]. This provides opportunities for community-level standardization [16] and aggregate throughput [17] that would otherwise be impossible via traditional research and publications. Recently, components of 3-D printed open-hardware vitrification devices have been explored. Initial feasibility was demonstrated in simple 3-D printed loops [18] for formation of thin films of samples, and an observation pedestal [19] was developed for standardized vitrification quality assessment.

Long-term sample storage and identification are critical for practical development and management of germplasm repositories [27]. As such, a vitrification device [20] was created to fit within identification jackets from commercially available High Security sperm straws (CBS straws, IMV Technologies, L’Aigle, France), which enabled secure storage, identification printing, and sample management within existing goblets containers. The cost of CBS identification jackets is about \$0.15/each, which is relatively expensive and much-less used compared to industry-standard cryopreservation straws (‘French straws’, \$0.05/each). Created in 1963, French straws are the most used sperm cryopreservation container in global sperm banking practices. The advantages of these straws are low-cost, reliability, high-throughput, space efficiency, color variants, automated labeling, and abundant peripheral accessories. The present work aimed to develop a vitrification device incorporating French straws (VD-FS) that can be integrated with the commonly used French-straw storage system.

Hardware description

Previous studies established a standardized method for assessment of vitrification quality and introduced prototype versions of vitrification loops used to study thin-film characteristics. The work presented herein produced vitrification wands and jig apparatuses, and further explored the feasibility of vitrification by use of geometries and dimensions that can accommodate protective sleeves made from 0.5-mL French straws. French straws have a small inner diameter (about 2.4 mm), and thus present challenges to achieve vitrification because 3-D printing polymers can act as thermal insulation to reduce sample cooling rates. Specific objectives were to: (1) design various configurations of vitrification wands that could fit within standard 0.5-mL French straws; (2) design a jig apparatus to produce protective sleeves by use of French straws; (3) 3-D print vitrification wands and the jig apparatus, and (4) evaluate the feasibility of vitrification with the VD-FS prototypes. This work provided prototypes with great feasibility for community users to adopt, test, modify, optimize, and eventually standardize. As open hardware, these will provide a useful and much-needed foundation to enable community-level efforts to create more efficient devices in the future [17].

Vitrification quality is inversely related to sample volume, because higher thermal mass leads to lower cooling rates. Thus, vitrification wands (Fig. 1, Supplemental Fig. 1) were designed with multiple configurations that differed by loop length and height (different number of layers in 3-D printing slicing). The nominal height per layer was 0.2 mm when printed with 0.4-mm printer nozzles. Loop configurations included three lengths (Short:15.4 mm, intermediate:16.6 mm, and long:24.4 mm) and three heights (1 layer, 2 layers, 3 layers). The total length of the wands was identical to French straws to fit within standard cryogenic goblet storage containers.

Wands were printed with polylactic acid (PLA) thermoplastic filament, widely used for 3-D printing, and can withstand liquid nitrogen temperatures [14,21]. The ability of PLA to retain integrity after cooling and thawing allowed for a single batch (five replicates of each configuration) with nine loop configuration types to be reused throughout the duration of this study.

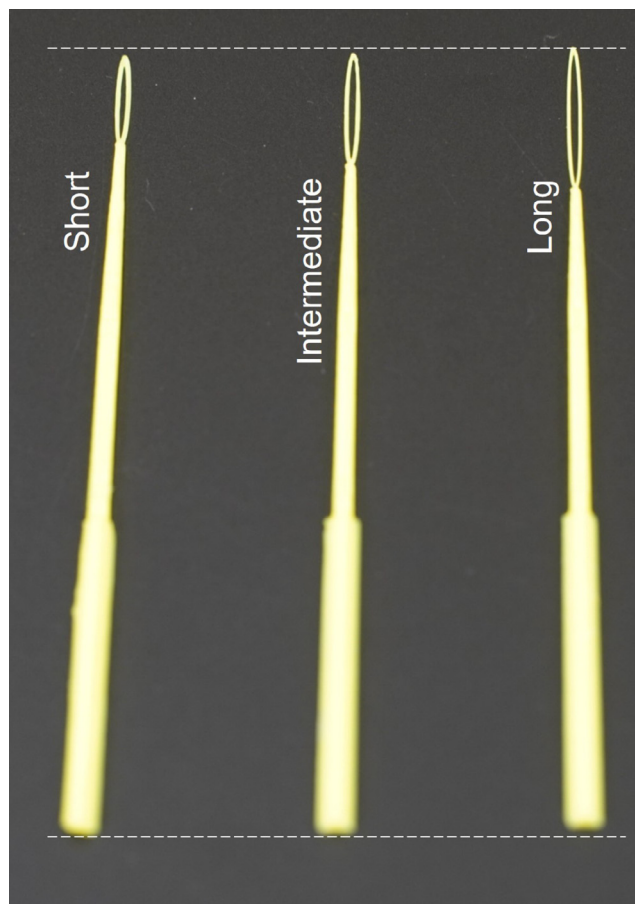


Fig. 1. Vitrification wands with different loop lengths. The total length of the wands was identical (indicated with dashed lines).

A self-contained jig apparatus (Fig. 2A) was designed to size, indent, and cut a standard 0.5-mL cryopreservation straw into two protective sleeves. This apparatus consisted (Fig. 2B) of a dock (Supplemental Fig. 2), a stylus (Supplemental Fig. 3), and a stencil (Supplemental Fig. 4). The stylus featured a tapered tip to produce a defined indentation on French straws that would slide along a groove fabricated in the vitrification wands. The stencil ensured correct positioning of the

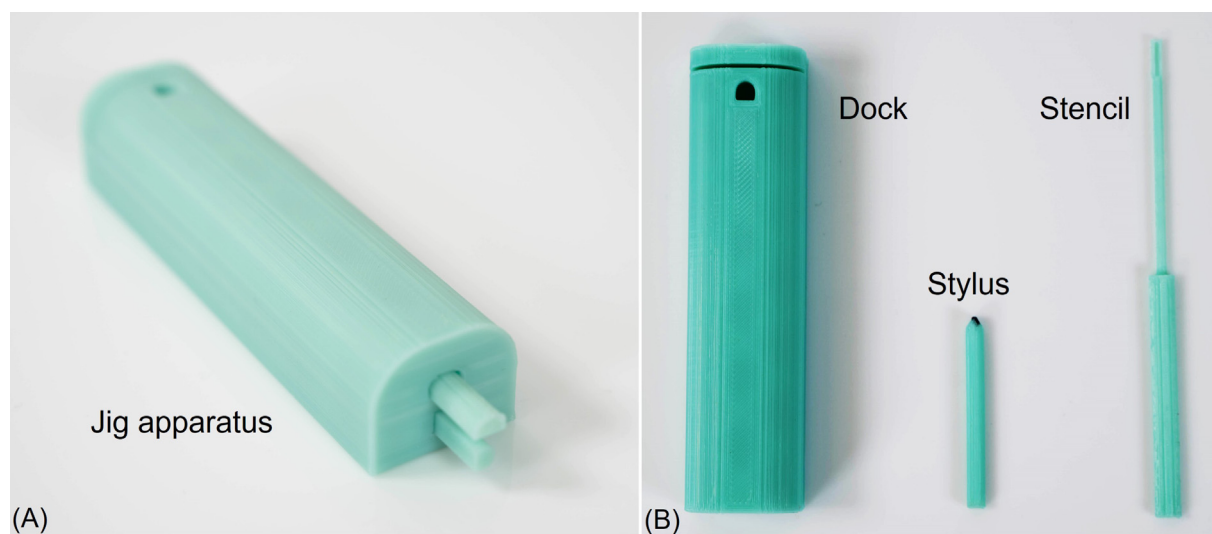


Fig. 2. A 3-D printed jig apparatus to produce protective sleeves from 0.5-mL French straws. The jig apparatus (A) comprised three components (B): dock, stylus, and stencil. These components could be self-contained.

French straws within the jig and facilitated localization of the indentation. The dock enabled positioning of a razor blade, stylus, and stencil during sleeve making. The stylus and stencil were self-contained by insertion into the dock after use.

Due to a lack of existing hardware, sperm vitrification has been performed with various improvised tools, or by adoption of devices that were originally designed for other purposes. For example, it remains necessary for vitrification devices designed for human embryos, such as the Cryotop[®], to be used in vitrification of animal sperm [22]. Medical devices specifically designed for human clinical application are costly. For example, the Sperm VD device [11] designed for vitrification with storing and labeling capabilities costs \$74 (as of 5/30/2022) for three samples (1 μ L/sample) per device. Moreover, researchers have opted for non-specific tools that were not intended for cryopreservation, such as pipette tips (originally for liquid transfer), sperm cryopreservation straws (for freezing of semen) cut at various angles, and inoculation loops (for microbiology) [23,24]. These improvised tools typically lack capabilities for standardization, labeling, identification, and protection. In addition, most of the existing tools reported are not integrated with a standardized assessment approach for vitrification quality.

Advantages of the VD-FS described herein include:

- Capability for sperm vitrification with permanent labeling, identification, and secure storage.
- Integration with existing standard French straw storage systems.
- Low cost compared to commercial products and alternative tools.
- Protective sleeves can be produced from commonly used French straws.
- Customization is available through open-hardware sharing and distribution.
- Standardization is enabled through community-level open fabrication.

Design files

The design files include 3-D drawings as editable.ipt files created in Autodesk Inventor (Autodesk, San Rafael, CA), and stereolithography (.stl) files (Table 1, Fig. 3). This work is licensed under the Creative Commons Attribution 4.0 International

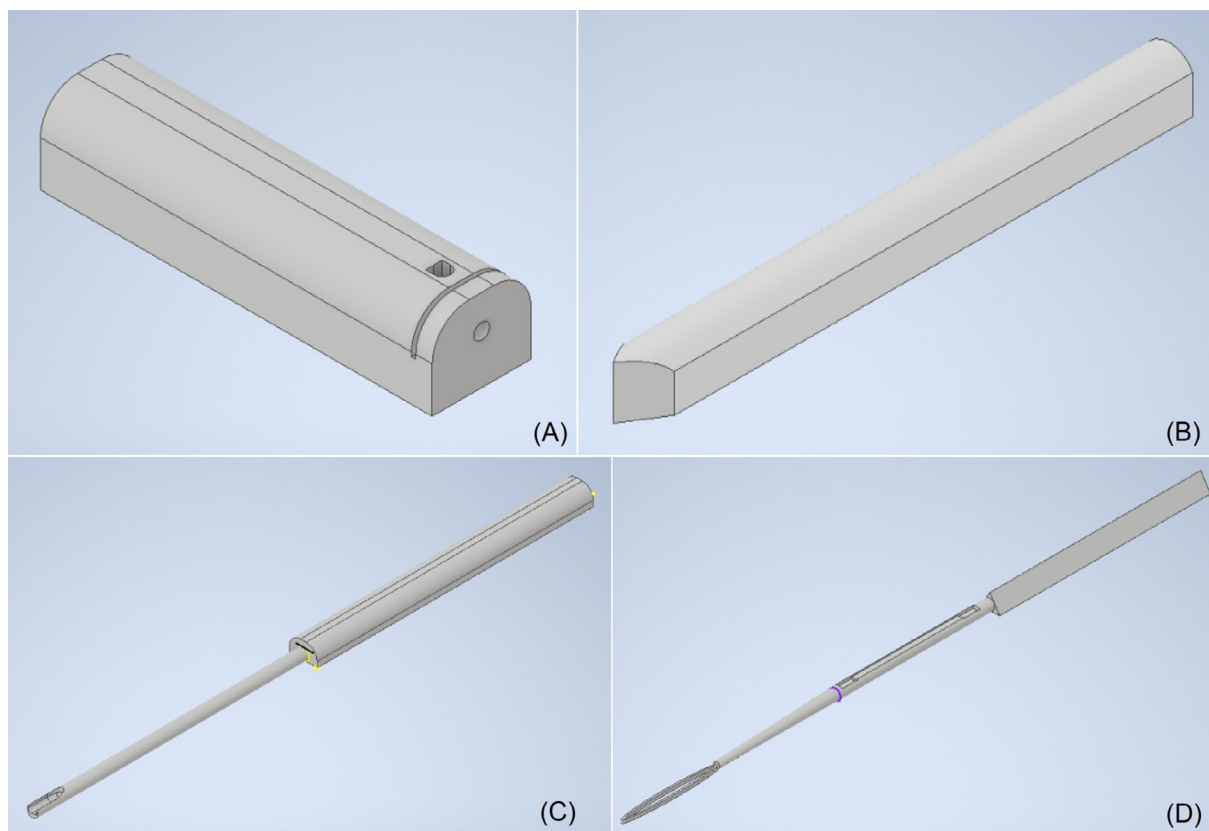


Fig. 3. Three-dimensional rendering of designs of the vitrification device for French straws (VD-FS). A jig apparatus was designed with three self-contained components including: a dock (A), stylus (B), and stencil (C). The jig apparatus produced protective sleeves from French straws to provide secure storage, labeling, and identification for samples processed with vitrification wands (D).

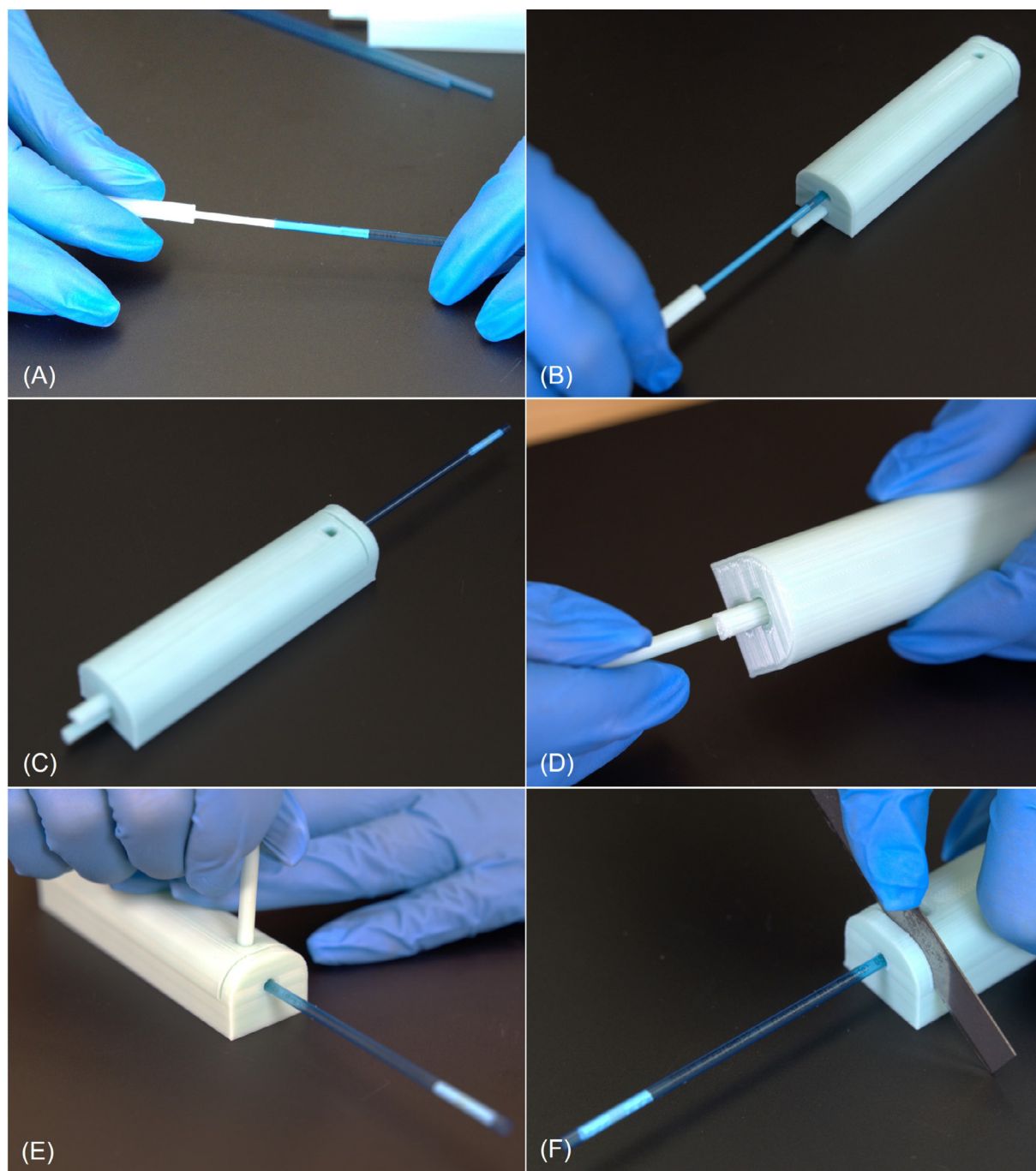


Fig. 4. Operations for creating protective sleeves by use of French straws and the jig apparatus in the vitrification device for French straws (VD-FS). A straw was loaded on the stencil (A) and inserted (B) into the dock (C). The stylus (D) was used to make an indentation (E). A single French straw can be used to create two sleeves with a razor blade (F).

(CC BY 4.0) License. To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/> or send a letter to Creative Commons, PO Box 1866, Mountain View, CA 94042, USA. All components in the files were fabricated with 1.75-mm polylactic acid (PLA) filament (ZYLtech Engineering, Houston, TX, USA) with a fused deposition modeling 3-D printer (Prusa i3, Prague, Czech Republic) and standard 0.4-mm nozzle. These components could also be printed with comparable quality by use of entry-level consumer printers such as the Ender-3 3-D printers (Creality 3D Technology, Shenzhen, China).

Table 1
File description.

File name	File type	Open source license	Location of the file
Dock	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Stylus	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Stencil	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (15.4-mm long, 1 layer)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (15.4-mm long, 2 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (15.4-mm long, 3 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (15.4-mm long, 4 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (15.4-mm long, 5 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (15.4-mm long, 6 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (16.6-mm long, 1 layer)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (16.6-mm long, 2 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (16.6-mm long, 3 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (16.6-mm long, 4 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (16.6-mm long, 5 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (16.6-mm long, 6 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (24.4-mm long, 1 layer)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (24.4-mm long, 2 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (24.4-mm long, 3 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (24.4-mm long, 4 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (24.4-mm long, 5 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2
Vitrification Wand (24.4-mm long, 6 layers)	IPT, STL	CC BY 4.0	http://dx.doi.org/10.17632/b7yy72wcxx.2

Bill of materials

The filament used in the present work (Table 2) cost about \$20 per kg, and thus the material costs for 3-D printed components were estimated as \$0.02/g. The mass of filament and printing time were provided by Simplify 3D slicing software (Simplify 3D, Cincinnati, OH, USA). A commercially available blade was used to divide single French straws into two sections. As such, the material costs were \$0.34 for a jig apparatus (including the blade) and \$0.04 per set of vitrification wand and protective sleeve.

Table 2
Bill of materials.

Component	Material	Unit	Cost (\$)/unit	Cost (\$)	Print Time
Dock	PLA	15.3 (g)	0.02	0.3	1 hr 42 min
Stylus	PLA	0.5 (g)	0.02	0.01	10 min
Stencil	PLA	0.8 (g)	0.02	0.02	6 min
Vitrification Wand	PLA	0.6 (g)	0.02	0.01	5 min
Protective sleeve	0.5-mL French straws	A single straw	0.06	0.03	–
Razor blade	Alloy Steel	1	0.01	0.01	–
Total		17.2		0.38	2 hr 3 min

Build instructions

- 1) Print vitrification wands with desired loop configurations. The slicing settings for Simplify 3D (used in the present study) and free software Ultimaker Cura (V4.6, Ultimaker, Utrecht, Netherlands) are provided in the [Table 3](#).
- 2) Print components of jig apparatus with appropriate settings ([Table 3](#)).
- 3) Label 0.5-mL French straws on each half (two labeled sleeves will be produced per straw).
- 4) Sheath a straw onto the stencil ([Fig. 4A](#)).
- 5) Insert loaded stencil into the dock through the upper opening on the end of the dock ([Fig. 4B](#)).
- 6) Push the loaded stencil into the dock as far as it will go ([Fig. 4C](#)).
- 7) Remove the stylus from the dock ([Fig. 4D](#)).
- 8) Align the stylus in the receiving slot and press to form an indentation on the straw with the tapered tip ([Fig. 4E](#)).
- 9) Place a single-edge razor blade into the perpendicular slot in the dock and slice the straw into two sections ([Fig. 4F](#)).
- 10) Repeat the steps above for the other half of the straw to create two protective sleeves ([Fig. 5A](#)).
- 11) The protective sleeve should be placed on the wand by aligning the indentation with the groove.
- 12) The sleeve should be in the 'open' position (closest to handle) prior to sample loading ([Fig. 5B](#)).
- 13) The protective sleeve can be slid along the groove into the 'closed' position to cover the loops ([Fig. 5C](#)).

Operation instructions

To perform this procedure, always wear appropriate protective gear, including insulated gloves and eye protection. Choose a Styrofoam container that is large enough to submerge a plastic storage goblet in liquid nitrogen with at least an additional inch above the goblet (to ensure submersion of vitrification wands after freezing). Approximately 4 L of liquid nitrogen was dispensed into the Styrofoam container. Loops were separated according to length, and dried and sanitized with 70% ethanol before testing. A previously published vitrification solution of 20% 1,2-propanediol, 20% 2-methoxyethanol, 20% HBSS300, and 40% methanol was prepared [20].

Each vitrification wand was dipped into the vitrification solution to form thin films on the loops, which were verified by visual observation. The vitrification wands were individually plunged in liquid nitrogen with forceps and held for approximately 10 sec ([Fig. 6A](#)). While submerged, the protective sleeves were slid ([Fig. 6B](#)) to the 'closed' position ([Fig. 6C](#)) by use of another pair of forceps. The quality of vitrification was assessed by viewing three horizontal lines on a 3-D printed pedestal previously described ([Fig. 6D](#)) [19]. Vitrification quality was assessed by visual classification into six categories ([Fig. 7](#)):

- 1) Vitrified, 2) Partially Vitrified, 3) Crystalline, 4) Shattered (S), 5) Collapsed (C), and 6) No Film (N).

Table 3

Specifications for 3-D printing of prototypes of Vitrification Device for French Straws (VD-FS).

Parameters	Specifications	Specifications
Slicing software	Simplify 3D	Ultimaker Cura
Filament manufacturer	ZYLtech Engineering	ZYLtech Engineering
Filament material	Polylactic acid	Polylactic acid
Filament diameter	1.75 mm	1.75 mm
Hotend temperature	200 °C	200 °C
Print speed	60 mm/s	60 mm/s
Nozzle diameter	0.4 mm	0.4 mm
Extrusion Multiplier	1.04	N/A
Layer height	N/A	0.2 mm
Extrusion/layer width	0.29 (Manual)	0.3 mm
Outer before inner walls	N/A	Enable
Compensate outer wall overlaps	N/A	Enable
Retraction distance	1 mm	1 mm
Retraction vertical lift	0.1 mm	0.1 mm
Retraction speed	60 mm/s	60 mm/s
Print bed temperature	60 °C	60 °C
Build surface material	Flexible magnetic surface	Flexible magnetic surface
Part cooling fan speed	100 %	100 %
First layer printing speed	20 %	20 %
Infill rate	20 %	20 %
Infill pattern	Fast honeycomb	Grid
Perimeter layer number	3	3
Top layer number	3	3
Bottom layer number	3	3
Support usage	N/A	N/A
Skirt layers	1	N/A
Skirt outlines	4	4

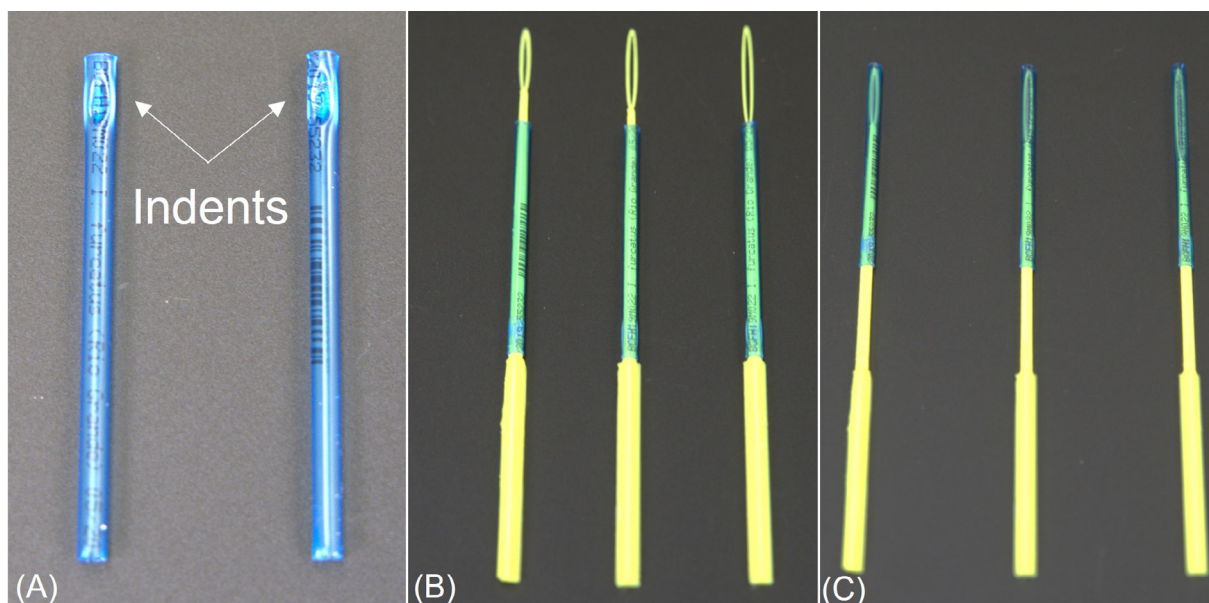


Fig. 5. Protective sleeves made from French straws by the vitrification device for French straws (VD-FS). Two sleeves with indentations (A) can be created from single 0.5-mL straw. The sleeves can be placed in an 'open' position (B) prior to use, and in a 'closed' position (C) after cooling and during cryogenic storage.

Validation and characterization

To maximize the performance of vitrification wands, prints required routine visual inspection for fabrication defects as a quality control element. The operator should perform sample loading and cooling with minimum variations of motions among wands and samples. The quality of frozen films varied among different loop configurations, because cooling rates were affected by sample volume, thermoplastic mass, and surface area. The sample volume and mass increased with the layer numbers and length of loops. Five replicates (individual wands printed in same batches) of each loop configuration were tested thrice with the procedures described above. To evaluate the feasibility of vitrification with various loop designs, wands were cooled and examined immediately without coverage of the protective sleeves. In a second test, protective sleeves were slid to the 'closed' position after cooling, transferred to a plastic goblet, stored for 14 d, and examined. Based on previous studies, only 1–3 layer numbers were tested to demonstrate feasibility and usability.

It was feasible to produce vitrification with variations in quality among different loop configurations and replicated prints (Table 4). Longer loop lengths and higher layer numbers tended to result in higher rates of film failure and lower vitrification quality. No film failure was observed in any replicates of the 1-layer short loops, whereas failures were seen in all (except for one) long loops in the three-layer configuration. At least two among three replicates showed full or partial vitrification in all (but one) short and intermediate loops. To accommodate storage within protective the sleeves, loops were designed with narrow width (1.2 mm) (Supplemental Fig. 4). This design reduced the structural strength for lateral support of loops, which could have contributed to the rate of film collapse seen in the long loops. In future designs, horizontal or axial support structures can be added and tested to reduce film failures. This was a pilot feasibility study, and thus no statistical analyses were performed. Further work can evaluate the vitrification success rate with specific cryoprotectant media in comparison to commercial vitrification devices. Throughout this work vitrification wands were reused without observable degradation to the material integrity after cooling and thawing, indicating the ability for reuse of this device.

It was feasible to (Table 5) recover vitrified samples after storage with coverage by the protective sleeves for 14 d. Wands with no films (3–6 wands for each configuration with different layer numbers) were more frequently observed, indicating that the operation of the protective sleeves after cooling and before thawing might cause sample loss. To address this, further studies can investigate the feasibility of sample plunging after the sleeves positioned at the 'closed' position. The present work evaluated the feasibility of the innovative open-hardware approach for sample vitrification and provides foundational prototypes for community users to modify, improve, optimize, and eventually standardize. The composition of cryoprotectant solutions play an important role in vitrification success [25]. In the present study, a previously reported vitrification solution for aquatic species was used for evaluation. In practical application, various vitrification solutions can be used with the VD-FS to evaluate their effects on vitrification success and perhaps improve the functionality.

Print quality was a critical factor to vitrification success. For example, sample loss could be caused by the sliding of protective sleeves after cooling, and wands printed with uneven or poorly formed grooves can perform poorly by restricting

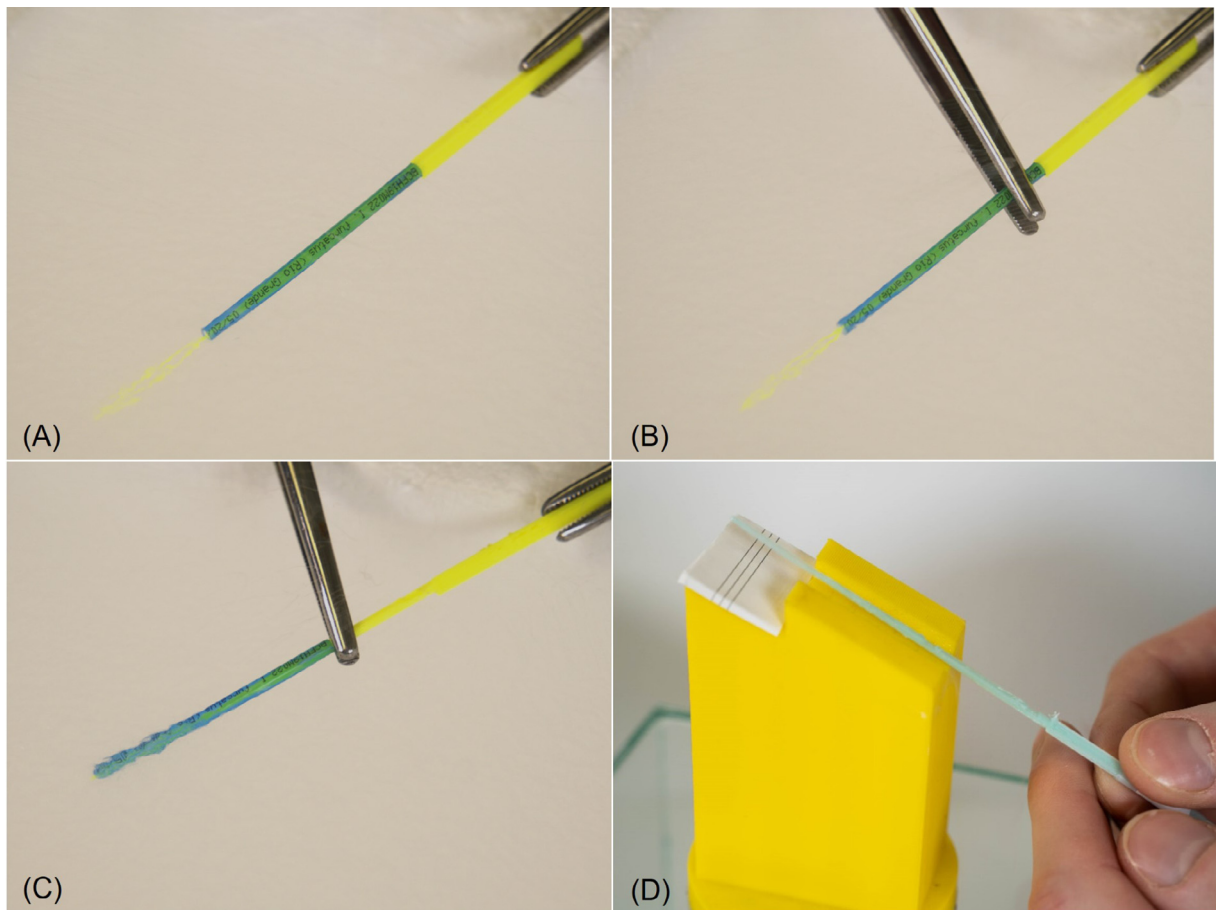


Fig. 6. Sample handling and evaluation with the vitrification devices for French straws (VD-FS). The vitrification loops loaded with sample were plunged (A) into liquid nitrogen with the protective sleeves in the 'open' position, followed by sliding of the sleeve to the 'closed' position in liquid nitrogen (B) by use of two forceps (C). For validation, vitrification quality was assessed by visual examination of the film clarity and other features by use of a 3-D printed pedestal (D).

smooth positioning. This issue was exacerbated after cooling because freezing can add difficulties for sliding. Although these defects could be addressed post-fabrication with sandpaper, there was a risk to remove the grooves by excessive sanding. As such, precision of printed parts is essential to the use of this device. In the present study, 3-D printers with 0.4-mm nozzles were used because it is the most commonly used nozzle size. Printing precision can be increased by use of smaller nozzles (e.g., 0.2 mm) but this often requires a higher skill level. In addition, printing precision can also be improved by adjustment of slicing settings and printer calibrations. Resin 3-D printing with photocurable resins and biocompatible materials has emerged in recent years [29]. Resin printers can fabricate finer details compared to fused filament fabrication, and could be evaluated for fabrication of the VD-FS.

Limitations

Several common print failures (Fig. 8) were observed and could affect usability of the VD-FS. Most failures were due to issues during filament extrusion, in combination with a lack of adequate adhesion to the build plate. Inconsistent extrusion prevented appropriate adhesion between layers (Fig. 8A). A clogged printer head could cause inconsistent extrusion, leading to sporadic clumps of material printed on the build plate (Fig. 8B). Several prints produced non-uniform loops due to stringing error, in which layers were printed with hair-like strands (Fig. 8C). Stringing errors interfered with formation of useful films with consistent surface tension. Due to these potential fabrication variations and failures, it is recommended that wands be printed, examined, tested, and readjusted before use with biological samples.

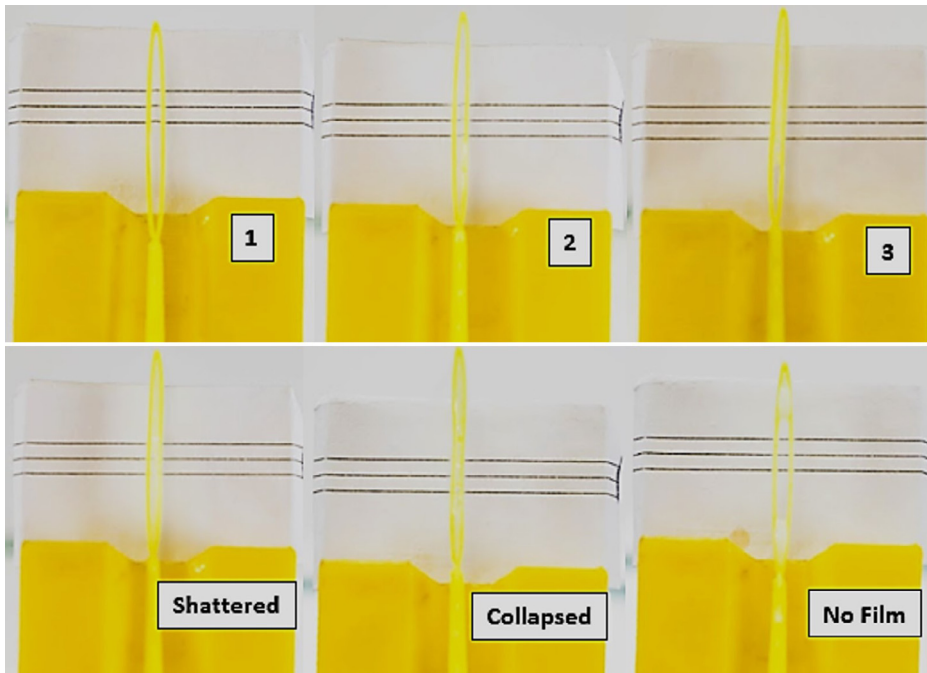


Fig. 7. Evaluation of vitrification quality after cooling. Vitrification quality was assessed by visual classification into six categories of films and loops after cooling: (1) Vitrified, (2) Partially Vitrified, (3) Crystalline, (4) Shattered, (5) Collapsed, and (6) No Film. The crystalline and shattered films can look alike, but cracks could be seen in the shattered films.

Table 4

Vitrification quality of samples examined immediately after cooling with vitrification wands of different loop lengths (S-short, I-intermediate, and L-long) and layer numbers.

Layers	Replicant	Film Failure (%) ^a			Classification		
		S	I	L	S	I	L
1	1	0	0	100	1, 2, 3	2, 1 s, 1	1c, 1c, 1c
	2	0	0	100	1, 1, 1	2, 2, 2	n, c, c
	3	0	0	67	2, 1, 3	1, 3, 2	1, 2c, 1c
	4	0	67	100	2, 1, 1	1, 2 s, 3 s	n, c, 1c
	5	0	33	33	2, 1, 1	1, 1, 2 s	2, 1e, 1c
2	1	0	0	100	1, 1, 1	1, 1, 1	c, 2c, 2c
	2	33	67	33	2, 1, 2 s	2 s, 2, 2 s	2, 3c, 1
	3	0	0	33	1, 1, 2	1, 2, 1	1 s, 2c, 2
	4	0	33	33	2, 2, 1	1, 2 s, 3	2, 2 s, 1
	5	67	0	33	1, 2 s, 3 s	2, 2, 1	1, 1, 2c
3	1	0	0	67	2, 3, 1	2, 1, 3	1, 2 s, 2 s
	2	33	33	33	3, 3 s, 2	1, 2 s, 1	3, 2 s, 2
	3	0	33	100	3, 2, 1	2, 2 s, 3	3 s, 3 s, 3 s
	4	0	33	0	2, 1, 3	1, 3 s, 1	3, 2, 3
	5	0	0	33	2, 3, 1	2, 2, 2	1, 2 s, 3

^a Rates of film failure and classifications of vitrification quality were assigned to five replicates of each configuration after three repeated trials and examination. Classifications (1-vitrified, 2-partially vitrified, 3-crystalline, s-shattered, c-collapsed, and n-no film) were assigned by transparency and attributes of frozen films. Loop configurations consisted of three different lengths (short-S, intermediate-I, and long-L) and three different layer numbers (0.2 mm nominal layer height).

Table 5

Vitrification quality of samples examined immediately after 14-d storage covered with the protective sleeves. Vitrification wands with different loop lengths (S-short, I-intermediate, and L-long) and layer numbers were evaluated.

Layers	Replicant	Film Failure (%) [*]			Classification		
		S	I	L	S	I	L
1	1	0	0	0	1	2	1c
	2	0	100	100	2	n	n
	3	100	0	100	n	2	n
	4	0	100	0	3	n	1c
	5	0	100	0	1	n	1
2	1	0	0	0	2	1	1c
	2	100	0	0	n	2	1c
	3	0	0	0	1	1	1
	4	0	0	0	2	3	2s
	5	0	0	100	3	1	n
3	1	0	0	0	1	3	2
	2	100	100	0	n	n	1
	3	0	0	0	2	2s	3
	4	0	0	100	2c	2	n
	5	100	0	0	n	2	2c

^{*} Rates of film failure and classifications of vitrification quality were assigned to five replicants of each configuration after three repeated cooling and examination. Classifications (1-vitrified, 2-partially vitrified, 3-crystalline, s-shattered, c-collapsed, and n-no film) were assigned by transparency and attributes of frozen films. Loop configurations consisted of three different lengths (short-S, intermediate-I, and long-L) and three different layer numbers (0.2-mm nominal layer height).



Fig. 8. Examples of fabrication failures that occurred in 3-D printing of the vitrification wands of the VD-FS, including inappropriate adhesion among layers (A), inconsistent adhesion to build plates (B), and stringing (C).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

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Yue Liu: Yue Liu is an Assistant Professor and Assistant Director at the Aquatic Germplasm and Genetic Resources Center (AGGRC.COM) of the School of Renewable Natural Resources in Louisiana State University Agricultural Center, Baton Rouge, Louisiana. His research focuses on development of interdisciplinary technologies to support preservation and the management of genetic resources. These technologies include open hardware, cryopreservation approaches, high-throughput processing, microfluidic systems, assisted reproduction techniques, sperm physiology, gamete biology, machine learning, and electrical-cryobiology. He has participated in dozens of projects for germplasm repository technology development for support of conservation, biomedical research, aquaculture, and fisheries management. He coordinates multiple projects funded by the National Institutes of Health (NIH). He has worked with aquatic species in various user and research communities, such as biomedical research (e.g., *zebrafish*, *Xiphophorus*, *Ambystoma*, *Xenopus*, *Aplysia*, *Hydractinia*), conservation (e.g., goodeidae), aquaculture (blue catfish, channel catfish, mussels, and oysters), and algae (e.g., microalgae).



Terrence R. Tiersch: Tiersch is a Professor and Director at the AGGRC. His career has primarily addressed technology development for the study, maintenance, and distribution of genetic resources of aquatic species. This has included years of work in the areas of reproduction and genetic improvement for aquaculture species, and germplasm repository development for aquatic species relevant to aquaculture, wild fisheries, biomedical models, and imperiled species. Over this time he has led the AGGRC and collaborated extensively throughout various aquatic species communities in the USA and abroad, and with other scientists, fish hatcheries and farmers, and state and federal agencies including, USDA, US Fish and Wildlife Service, USAID, and NOAA. Included in these collaborators are long-standing relationships with NIH biomedical stock centers such as the *Xiphophorus* Genetic Stock Center and the Zebrafish International Resource Center. The mission of AGGRC is to provide global leadership in repository development and commercialization of genetic resources of aquatic species. This work has been driven by identifying and responding to the demands of numerous and diverse user communities to move interdisciplinary innovation from research to real-world application including commercialization.