

Invitromatics, invitrome, and invitroomics: introduction of three new terms for in vitro biology and illustration of their use with the cell lines from rainbow trout

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Abstract The literature on cell lines that have been developed from rainbow trout (RT) (*Oncorhynchus mykiss*) is reviewed to illustrate three new terms: invitromatics, invitrome, and invitroomics. Invitromatics is defined as the history, development, characterization, engineering, storage, and sharing of cell lines. RT invitromatics differs from invitromatics for humans and other mammals in several ways. Nearly all the RT cell lines have developed through spontaneous immortalization. No RT cell line undergoes senescence and can be described as being finite, whereas many human cell lines undergo senescence and are finite. RT cell lines are routinely grown at 18–22°C in free gas exchange with air in basal media developed for mammalian cells together with a supplement of fetal bovine serum. An invitrome is defined as the grouping of cell lines around a theme or category. The broad theme in this article is all the cell lines that have ever been created from *O. mykiss*, or in other words, the RT invitrome. The RT invitrome consists of approximately 55 cell lines. These cell lines can also be categorized on the basis of their storage and availability. A curated invitrome constitutes all the cell lines in a repository and for RT consists of 11 cell lines. These consist of epithelial cell lines, such as RTgill-W1, and fibroblast cell lines, such as RTG-2. RTG-2 can be purchased from a scientific company and constitutes the commercial RT invitrome. Cell lines that are exchanged between researchers are termed the informally shared invitrome and for RT consists of over 35

cell lines. Among these is the monocyte/macrophage cell line, RTS11. Cell lines whose existence is in doubt are termed the zombie invitrome, and for RT, approximately 12 cell lines are zombies. Invitroomics is the application of cell lines to a scientific problem or discipline. This is illustrated with the use of the RT invitrome in virology. Of the RT invitrome, RTG-2 was the most commonly used cell line to isolate viruses. Fifteen families of viruses were studied with RT invitrome. RT cell lines were best able to support replication of viruses from the *Herpesviridae*, *Iridoviridae*, *Birnaviridae*, *Togaviridae*, and *Rhabdoviridae* families.

Keywords Cell lines · Rainbow trout · *Oncorhynchus mykiss* · New terms · Invitromatics · Invitrome · Invitroomics · Fish virology

Introduction

Three new terms are proposed here for in vitro biology: invitromatics, invitrome, and invitroomic. They are being proposed in order to more easily consolidate past discoveries and to provide direction for future research. The underpinnings for all three terms are cell lines. A cell line is a population of cells from a multicellular animal or plant that can be propagated outside the organism through the serial transfer (subcultivation) of cells from one fabricated culture vessel to another (Schaeffer 1990). As the production and maintenance of animal and plant cell lines is quite different, this article will deal with only animals, although acknowledging that the definitions could have use in plant in vitro biology as well. Rainbow trout (RT) will be used as an exemplar of how these terms might be applied in animal in vitro biology. RT is chosen because the authors have been involved in developing many cell lines from this species and because the number of RT cell

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lines is substantial enough for the terms to be illustrated but not so large as to be overwhelming, which might be the case with the human cell lines.

Animal invitromatics is being defined here as the science and history of establishing, characterizing, engineering, storing, and distributing cell lines. An analogy can be found in the discipline of informatics. “Informatics” has been described as the “study and practice of creating, storing, finding, manipulating and sharing information” (University of Washington 2016). This is similar to the one proposed here for invitromatics but “information” has been replaced with cell line. The use of the term animal invitromatics is being restricted to the science and history of the basic establishment, characterization and genetic engineering of cell lines whereas specific uses of cell lines will be described as invitroomics, which will be defined later in this Introduction. The preparation of primary cell cultures might be considered as part of invitromatics because this is a necessary step for establishing cell lines. A primary culture starts with the placement of an animal’s cells from tissues and organs directly into culture and ends when the primary culture is either used in an experiment or subcultured for the first time; at which point, it becomes a cell line (Schaeffer 1990). Primary cultures are excluded here on the basis that they are usually not stored and lack a life after they have been experimented on. The preparation of primary cell cultures certainly has a science, with methods varying with the cell type, tissue or organ being studied, but primary cell cultures are usually “one and done” and have no subsequent history. By contrast, cell lines take on a life of their own that can lead to very diverse and complex issues. An illustration of this complexity is the best-selling book *The Immortal Life of Henrietta Lacks* that provides among its many achievements a biography on the early life of the human cell line, HeLa (Skloot 2010).

The invitrome is being defined as one or more cell lines grouped around a common theme. Thus, the RT invitrome is all the cell lines that have ever been derived from *Oncorhynchus mykiss* and is a subset of the fish invitrome, which in turn is a subset of the animal invitrome. However other properties of the animals from which the cell lines were developed might be used to define an invitrome. For example, animal habitat could be used. In this case, the marine invitrome would be all the cell lines that had been developed from marine multicellular animals. Cell lines might be grouped around the organs from which they were initiated so that there would be for example a liver invitrome or brain invitrome. Histological organization might be another descriptor so that invitromes might be clustered around tissue type, such fibroblast or epithelial invitromes, or functional cell type, such as a hepatocyte invitrome or neuronal invitrome. Additionally, all the cell lines from tumors would be a cancer invitrome. Besides their origins, cell

lines might be grouped around specific functional properties, such as their ability to support replication of viruses.

Invitroomics is being defined as the use of cell lines to study the cellular and molecular biology of multicellular organisms or to manufacture useful products. Invitroomics would include employing cell lines to understand complex processes in many disciplines, including development biology, physiology, immunology, toxicology, and virology. Therefore, the word might be most easily employed as an adjective, “invitroomic.” For example, invitroomic toxicology would be the use of cell lines to study a toxicology problem; invitroomic cardiology, the use of cell lines to study the heart. Production of commercial products might be called invitroomic manufacturing. At the end of this article, the use of the word invitroomics will be illustrated with the example of RT invitroomic virology.

Rainbow Trout (RT) Invitromatics

The topics that would encompass the invitromatics for a species are briefly reviewed here for rainbow trout (RT). These involve the history, maintenance, development, characterization, storage, and distribution of RT cell lines (Fig. 1a).

History The history of RT invitromatics begins with the establishment of the rainbow trout gonadal cell line, RTG-2 (Wolf and Quimby 1962). RTG-2 was the first fish cell line and so also begins fish invitromatics. To this day, RT cell lines continue to be developed, with perhaps the most recent ones being RT-ovfl and RTmilt5 from reproductive fluids (Vo et al. 2015) and HK1532 from the head kidney (Maisey et al. 2016).

Maintenance of RT cell lines Detailed maintenance protocols have been presented elsewhere (Wolf and Quimby 1969; Wolf 1979; Wolf and Ahne 1982; Bols and Lee 1994; Dayeh et al. 2005, 2013) and are not that dissimilar from those for mammalian cells so here the emphasis is on some of the considerations that are important for culturing cells from coldwater fish, like RT.

Temperature RT cell lines are routinely grown at 18–22°C, which is often reported as room temperature. However, cells survive for at least a week in cultures held at any temperature between 0 and 28°C. This is referred to as the RT cellular endurance zone (Bols et al. 1992). The cells will proliferate from 4–5 to 24–25°C (Plumb and Wolf 1971; Mosser et al. 1986; Van Oostrom and Bols 1991), which has been defined as the proliferation zone (Bols et al. 1992). For RTG-2 cultures routinely grown at room temperature, shifting cultures to 24°C and above will initiate heat shock protein (HSP) synthesis (Mosser et al. 1986; Bols et al. 1992) and development of thermotolerance (Mosser et al. 1987; Mosser and Bols 1988).

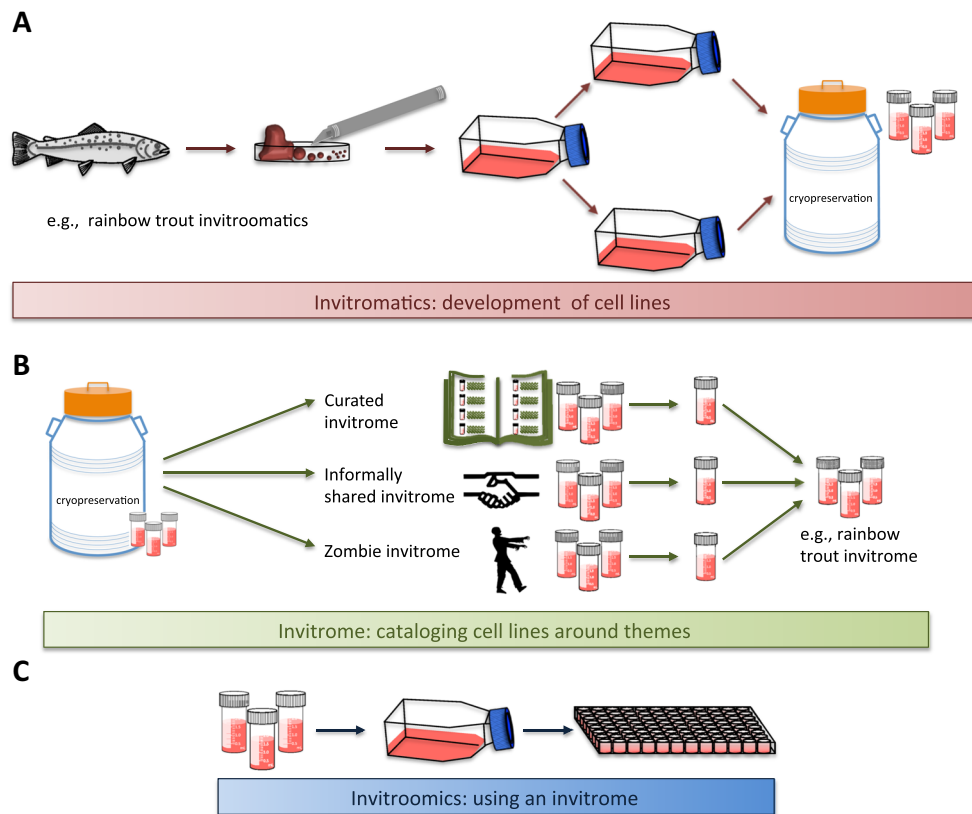


Fig. 1 A schematic that uses rainbow trout (RT) to illustrate the distinctions between three new terms for in vitro biology. Invitromatics is the preparation, storage, and distribution of cell lines (a). All the cell lines that have been cataloged around a theme constitute an invitrome. One theme is the availability of the cell lines. On this basis, this article defines several invitromes, with three of them being the curated invitrome, the informally shared invitrome, and the zombie invitrome

(b). From these invitromes, the cell lines can be grouped in many other ways, such around the species from which the cell lines were developed. This article reviews the RT invitrome. Invitroomics is the utilization of the invitrome for investigating scientific problems (c). For example, invitroomic virology and invitroomic toxicology would be the utilization of cells lines to investigate respectively viruses and toxicants.

Media The complete medium for the growth of RT cell lines consists of a basal medium with a supplement of serum (Bols and Lee 1994), although RTG-2 can survive for several wk in basal medium alone (Lee et al. 1988). A basal medium is a buffered aqueous solution of nutrients that always includes a hexose, bulk ions, amino acids, and vitamins. Basal media were developed for mammalian cells but work well for fish cells and are commercially available. Although RT cell lines can be maintained without antibiotics, most often penicillin and streptomycin are added to complete the medium. The choice of basal media depends on the availability of incubators.

Based on the buffering mechanism, two different classes of basal media have been used to develop and maintain RT cell lines. In one class, pH is maintained by sodium bicarbonate (NaHCO_3) and a CO_2 rich atmosphere, usually 5% CO_2 and 95% air. Eagle's Minimum Essential Medium (MEM), which was used to develop RTG-2 (Wolf and Quimby 1962), and modifications of MEM belong in this class. These require a CO_2 -incubator to provide a CO_2 atmosphere necessary for the

bicarbonate buffering system to work. CO_2 -incubators have to be modified to maintain temperatures below room temperature so are often not available. To get around this problem, basal media in this class have been modified in several ways to be less dependent on a CO_2 atmosphere. Without a CO_2 -incubator, culture pH can be maintained with these basal media when they have been prepared with Hank's salts and/or have organic buffers, such as N-2-hydroxyethylpiperazine-N'-2'-ethanesulfonic acid (HEPES) or tris (hydroxymethyl)-aminomethane(tris)-hydrochloride (TRIS). STE-137 from steelhead, which is sea-run RT, grew in a MEM/TRIS based medium without sodium bicarbonate and in free gas exchange with air (Kleeman et al. 1970). In MEM/HEPES, RTG-2 appeared to grow better than in MEM (Wolf and Quimby 1973a).

The second basal media class was formulated specifically for use in free gas exchange with air. Most commonly, this is Leibovitz's L-15, which maintains physiological pH through a combination of salts, high basic amino acid concentrations, and replacing glucose with galactose. L15 has been the basal medium for developing and using many RT cell lines,

including RTL-W1 and RTgill-W1 (Lee et al. 1993; Bols et al. 1994). Another basal media of this class is CO₂-Independent Medium, which unlike L-15 contains sodium bicarbonate, and has been used to isolate RT cell lines from the liver and pituitary (Chen et al. 2004, 2010).

Commonly glutamine is added to basal media before use with mammalian cells, but for RT and other fish cell lines, this was not necessary when the basal medium was L15 (Bols et al. 1994). However, when MEM was the basal medium, growth was poor without the addition of glutamine.

Usually, RT cell lines are grown in basal media with 10% fetal bovine serum (FBS) but this has varied with some cell lines. For RTS11, 30% FBS was essential for the early stages of establishment but latter for routine growth was dropped to 15% (Ganassin and Bols 1998). RTL-W1 was maintained for some years with 5% FBS (Lee et al. 1993). Although at various times salmonid sera have been commercially available, they have usually been too expensive to be routine alternative to FBS.

Adherence versus suspension With the exception of two immune cell lines, all RT cell lines are anchorage dependent: they grow adherent to the surfaces of tissue culture vessels, usually plastic. By contrast, RTS11, which is a monocyte/macrophage cell line (Ganassin and Bols 1998), and HK1532, which is a T cell line (Maisey et al. 2016), grow in suspension or loosely adherent to tissue culture plastic and likely could be grown in suspension bioreactors. Under some conditions, adherent cell lines can form aggregates. In culture vessels that were manufactured not to support cell attachment, RTS34st aggregated to form spheroids (Xing et al. 2008). When added to conventional tissue culture plastic vessels after a week in suspension, the spheroids attached and cells migrated out over the growth surface, indicating that the cells had remained viable in suspension. Some but not all RT cell lines show strict contact-dependent inhibition of growth. When the RT liver cell line, RTL-W1, was maintained for a long time without being passaged, aggregates formed on the monolayer (Malhao et al. 2013).

Subculturing For the passaging of adherent RT cell lines, cells are removed from the vessel surface with trypsin but alternatives such as TrypLE work as well (Bols and Lee 1994). A usual passaging ratio for such cell lines is 1 to 2 or 3. For difficult to detach cells, alternatives such plant proteolytic extracts (Lee et al. 1986) or non-enzymatic cell dissociation solutions have been used (Bols and Lee 1994). The time between routine subcultivations declines during cell line development and usually stabilizes at between 7 and 14 d (Lannan et al. 1984; Lee et al. 1993). The population-doubling time has been reported at between 3 and 7 d (Chen et al. 2010).

Establishing RT cell lines

Spontaneous immortalization Most RT cell lines have arisen through spontaneous immortalization. Spontaneous immortalization is the initiation of an indefinitely growing cell line through just the routine procedures of conventional cell culturing, with no addition of biological, chemical or physical agents. RT cell lines are judged to be immortal because lines, such as RTL-W1, have been subcultivated for 2- to 3-yr periods at 1 to 2 or 1 to 3 split ratios, which approximate a population doubling, over a hundred times, without any signs of cultures deterioration. Also continued passaging does not cause the cells to acquire senescence-associated β -galactosidase (SA β -Gal) (Vo et al. 2015), which for mammalian cells is a marker of replicative senescence (Dimri et al. 1995). Spontaneous immortalization occurs for cells from all RT life stages and tissue or organs, although immortalization is rare for cells of the immune system. Two very different general mechanisms can be advanced to explain why cells are so easy to immortalize from apparently normal RT tissues/organs.

One explanation is that cell lines develop from naturally immortal cells. Most tissues and organs of vertebrates have tissue-resident somatic stem cell populations (Chandel et al. 2016). Possibly small populations of immortal stem cells in RT tissues/organs have given rise to the cell lines. Alternatively, in light of the observation that telomerase activity is high in all RT organs (Klapper et al. 1998; Yoda et al. 2002), perhaps all the cells are immortal and any of them can lead to cell lines, which would then be expected to contain telomerase. Telomerase activity has been detected in at least one RT cell line, RTHDF (Ossum et al. 2004). However, the telomerase activity in all RT organs might reflect the indeterminate growth of RT and not the lack of barriers to continuous proliferation.

The second general explanation would be that most RT cells in vivo do have barriers to continuous proliferation, but the barriers are lost spontaneously in some or all of cells upon being placed in culture. One barrier could be replicative senescence, but if so, this barrier must be overcome very early upon the cells being placed in culture. When monitored during the development of RT cell lines from reproductive fluids, SA β -Gal staining was seen in primary cultures from milt but disappeared as the cells were passaged (Vo et al. 2015). For cultures from ovarian fluid, SA β -Gal staining was never seen and like many classic RT cell lines did not reappear on continued passaging of cells (Vo et al. 2015).

Although rare, the spontaneous immortalization of avian and mammalian cells offer some clues as to how proliferation barriers might be breached with RT cells. For the cells of higher vertebrates, changes in the expression and/or activity of genes involved in the p53 and Rb pathways contributed to immortalization (Christman et al. 2006). Therefore, RT cell immortalization might involve changes in the tumor

suppressor proteins, p53 and Rb, and their signaling networks. However, recently culture conditions, especially cytokines and growth factors, have thought to be critical in the immortalization of mouse liver endothelial cells (Zhao et al. 2015). Indeed, several observations with RT cells suggest a unique combination and/or concentration of polypeptide growth factors early in the cell culturing might be critical in order for the proliferation to become continuous. Exposure to the recombinant RT Ea4 peptide, which is related to pro-insulin-like growth factor 1 (IGF1), appeared necessary for cells in primary RT pituitary cultures to evolve into immortal cell lines (Chen et al. 2010). Essential to the early development of RTS11 was the use of 30% FBS, which later could be dropped to 15% (Ganassin and Bols 1998). The development of several RT cell lines has started by allowing the primary culture to become over confluent and to let them sit in this state for several weeks without changing the medium before the first sub-cultivation. During this time, autocrine and/or paracrine growth factors might have accumulated that facilitated immortalization.

Directed immortalization Attempts have been made to directly immortalize RT cells. Heart cells were transduced with polyoma middle T antigen, leading to an endothelial cell line (RTH) (Luque et al. 2014). Whether the T antigen had played a role in RTH development is hard to say, given the ease with which RT cells spontaneously immortalize. However, T antigen has been a common immortalization method for a variety of cell types from different mammalian species (Banerjee et al. 2016).

Basic characterization

Species identification Species identification of fish cell lines has improved dramatically over the last 10 years but distinguishing between lines from the same species has yet to become routine. Amplifying and sequencing a 653 bp region of the mitochondrial cytochrome c oxidase I gene has been shown to unambiguously identify several thousand species of fish, including RT (Hubert et al. 2008; Ward et al. 2009). This technique, called DNA barcoding, has been used to confirm the species identity of several RT cell lines, including RTgutGC (Kawano et al. 2011). For distinguishing between RT cell lines, several different techniques have been tried. Although having some success at identifying the species origin of a cell line, isoenzyme analysis and proteomics have largely been unsuccessful at distinguishing between different RT cell lines (Lannan et al. 1984; Lidgerding et al. 1984; Wagg and Lee 2005). The most success has been from analyzing microsatellite loci (Perry et al. 2001). Nine RT cell lines could be distinguished from one another when 10 microsatellite loci were analyzed, but this is a technique that is not readily available to every cell culture laboratory.

Mycoplasma Assessing for mycoplasma is a part of invitroomatics quality control. Mycoplasma refers to any species in the Mollicutes, a class of bacteria with approximately 200 species (Razin and Hayflick 2010). Culturists have long been concerned with mycoplasma because of the ease with which they can contaminate cultures without overtly changing the culture appearance. Over the years, many methods for detecting mycoplasma in animal cell lines have been developed. These fall into three general categories: culture tests, polymerase chain reaction (PCR), and fluorescent DNA staining (Young et al. 2010). Fluorescent staining is technically the easiest method and is done with fluorescent nucleic acid dyes, such as Hoescht H33258 or DAPI (4',6-diamidino-2-phenylindole). When contaminated cultures are stained, cell nuclei appear as large fluorescent structures; mycoplasma, as small punctate fluorescent staining over and/or around the cell surface (Chen 1977). Staining gives no information as to whether the punctate fluorescent staining over cells are inside or on the surface of cells or to the identity of the contaminating mycoplasma species.

During the course of their development, cell lines either might acquire mycoplasma accidentally from within the cell culture facility or have had mycoplasma from the start, being present in the organ from which the primary cultures were initiated. The accidental acquisition is covered later in the invitroomics section, whereas their possible presence in the starting material is considered here. Mucosa membranes of at least some vertebrates contain mycoplasma as commensals and this appears to be the case for the fish gut. Molecular-based methods have revealed multiple mycoplasma species in the intestinal microbiota of RT (Etyemez and Balcazar 2015), Atlantic salmon (Holben et al. 2002; Zarkasi et al. 2014; Llewellyn et al. 2016), and other fish (Bano et al. 2007). Yet, only two mycoplasma species have been grown from fish. *Acholeplasma laidlawii* was isolated from bass, bluegills, crappies, and sunfish (Francis-Floyd et al. 1998); *Mycoplasma mobile*, from a tench (Kirchoff et al. 1987). However, the isolation and identification of a mycoplasma species from RT has yet to be reported. We have stained several primary RT cell cultures with H33258 and have seen no signs of mycoplasma. On the other hand, H33258 staining has indicated mycoplasma in several fish cell lines and mycoplasma can be grown from fish cell cultures. H33258 staining revealed mycoplasma in cultures at certain passages for two RT cell lines, STE-137 (Lannan et al. 1984) and RTgill-W1 (Bols et al. 1994). The first report of mycoplasma in any fish cell line was with RTG-2 and the contaminant was identified through culture tests as *A. laidlawii* (Kunze et al. 1972). Since then, *A. laidlawii* has been identified by microbiological tests in 11 different fish cell lines (Emerson et al. 1979; Frerichs 1996) and *Mycoplasma arginini* in three (Frerichs 1996).

Overall, an endogenous entry route for the appearance of mycoplasma in fish cell lines is possible but more evidence is needed to support this route.

Several approaches for removing mycoplasma from mammalian cell lines have been developed and many mammalian cell lines have been cleaned up (Uphoff and Drexler 2002; Uphoff et al. 2012), but eradication has been attempted infrequently with fish cell lines. Technically, the simplest eradication methods involve antibiotics and new commercial antibiotic preparations (e.g., Plasmocin) for this purpose continue to become available (Uphoff et al. 2012). One concern about this approach has been the possibility that properties of the cell line change because of treatment with the mycoplasma antibiotics. However, for human embryonic stem cell lines, Plasmocin was found not to affect pluripotency (Romorini et al. 2013). The only literature report of an RT cell line been cleaned of mycoplasma is for RTgill-W1 (Bols et al. 1994), although some cell repositories (see Table 1) note one or two their fish cell lines have been cleaned. For RTgill-W1, success was achieved by treating cultures in succession with two commercial antibiotic preparations: mycoplasma removal agent (MRA) and BM cyclin. MRA contains a nondisclosed fluoroquinolone; BM cyclin, derivatives of pleuromutilin and tetracycline. Whether other fish cell lines have been cleaned up but have gone unreported would be interesting to know, especially the details of successful treatments.

Although cultures can be cleaned, deciding how to proceed with infected cell lines continues to be difficult. Often the advice has been to discard them, but this is not always practical when cell lines are under development. RT cell lines can take 1 to 2 yr to establish and so discovering mycoplasma late in the process makes discarding them difficult. Such cell lines probably should be cryopreserved, but only thawed when they can be handled separately from other cell lines and attempts can be made to clean them. In the future, better procedures and drugs for cleaning cells of mycoplasma might be developed, allowing contaminated cell lines to be rescued.

Karyotype Karyotyping has been done on some RT cell lines. The diploid chromosome number for RT can vary with the strain but commonly is reported as being between 58 and 64 (Thorgaard 1983; Colihuegue et al. 2001). RTG-2 had a modal chromosome number of 59 (Wolf and Quimby 1962). However, other RT cell lines have been found to be heterploid, with at least one modal number over 100 (Lee et al. 1993; Bols et al. 1994; Kawano et al. 2011). Early in the history of fish cell line development, karyotyping was done to give some assurance of species of origin, but now species identification can be done more easily and accurately through DNA barcoding. However, determining whether other RT cell lines fall into either diploid or heteroploid classes will be interesting to document because this might suggest two different spontaneous immortalization mechanisms. From work with

Table 1 Repositories with rainbow trout (RT) cell lines

Country	Name	Acronym	Address	Website
Australia	CellBank Australia		Children's Medical Research Institute Locked Bag 23 Wentworthville NSW 2145, Australia	http://www.cellbankaustralia.com/
Brazil	Banco De Celulas Do Rio De Janeiro	BCRJ	Our Lady of Grace Avenue, number 50, Building 32—PTX—Technology Park Xerem—Duque de Caxias, Rio De Janeiro, Brazil	http://bcrcj.org.br/
England	European Collection of Authenticated Cell Cultures	ECACC	Public Health England Porton Down Salisbury	http://ecacc.org.uk
Germany	Collection of Cell Lines in Veterinary Medicine	CCLV	SP4 0JG, England, UK Friedrich-Loeffler-Institut Stüdlufer 10 D-17493 Greifswald—Insel Riems Germany	https://www.fli.de/en/services/collection-of-cell-lines-in-veterinary-medicine-cclv/
Germany Italy	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna—Biobanking of Veterinary Resources	DSMZ IZSLER	Inhoffenstraße 7B 38124 Braunschweig Germany Zooprofilattico Istituto di Lombardia and Emilia Romagna Via Bianchi 7/9—25124 Brescia Italy	https://www.dsmz.de/ http://www.izslr.org/
Taiwan United States	Bioresource Collection and Research Center American Type Culture Collection	BCRC ATCC	331, Shih-Pin Rd., Hsinchu 30062, Taiwan P.O. Box 1549 Manassas, VA 20108 USA	http://www.bcrf.firdi.org.tw/ http://www.atcc.org

mammalian cells (Agapova et al. 1996), heteroploidy would suggest the involvement of p53. Indeed, inhibiting p53 in RTgill-W1 induced polyploidy (Zeng et al. 2016).

Cellular morphology The predominant cell shape in cultures of RT cell lines is usually noted as either being epithelial-like, fibroblast-like or other and is used to suggest a possible origin from either epithelial or connective tissue. In cultures of fibroblast-like lines, the cells are bipolar, usually long, and become more noticeably arranged in parallel as cultures become confluent. By contrast, in cultures of epithelial-like lines, the cells are very flat with outlines varying from irregular to cobblestone. The epithelial cultures can be dynamic with respect to shape. For example, the most common shapes in cultures of the epithelial-like RTP-2 changed as the cells grew from low density to confluency (Bols et al. 1995). Most RT cell lines are epithelial-like (Tables 2, 3, and 4). Attempts to correlate epithelial and fibroblast shapes with expression of cytoskeletal components are frustrated by the complex distribution of intermediate filament proteins in RT tissues (Schaffeld et al. 2002) and the paucity of commercial antibodies that react well with fish cytoskeletal proteins (Malhao et al. 2013). Cultures with the most diverse cellular morphologies are for immune cells. The monocyte/macrophage cell line,

RTS11, has a complex mixture of cell shapes in suspension and on the culture surface (Ganassin and Bols 1998).

In-depth characterization of RT cell lines

Increasing the characterization of RT cell lines beyond their basic features can increase their value in research. An example of such an in depth characterization has recently been published for the liver cell line, RTL-W1, for which cell lineage and stem cell properties were assessed (Malhao et al. 2013). RTL-W1 had characteristics of bile preductular epithelial cells (BPDECs), which are considered bipolar progenitor cells capable of differentiating into both hepatocytes and biliary cell lineages (Alison et al. 2001; Malhao et al. 2013). Characterization can also be done to increase the value of the cell line for a particular discipline or purpose. Examples of this will be given for toxicology, endocrinology, and virology. For toxicology, seven different RT cell lines were characterized for the expression of ATB binding cassette (ABC) transport proteins because they might be involved in cellular transport of ecotoxicants (Fischer et al. 2011). ABC transporters were constitutively expressed in all the cell lines. Aryl hydrocarbon receptor (AhR) signaling pathways were characterized in two RT cell lines to improve their utility in studying dioxin-like compounds (Pollenz and Necela

Table 2 The curated rainbow trout (RT) invitrome: RT cell lines in repositories

Cell line designation	Tissue of origin	Morphology	Repository	Repository #	Reference
D-11 ^a	Liver	Fibroblast	DSMZ	ACC 77	Ahne 1985
R1	Liver	Fibroblast	DSMZ	ACC 56	Ahne 1985
RTG-2	Mixed; testis; ovary	Fibroblast	ATCC ECACC IZSLER BCRC CCLV DSMZ CellBank Australia	CCL-55 90102529 BS CL 76 BCRC 60445 CCLV-RIE 0088 ACC 352 90102529	Wolf et al. 1960 Wolf and Quimby 1962
RTG-2/f			CCLV	CCLV-RIE 0686	Karger et al. 2010
RTG-P1 ^b	Gonadal (mixed testis/ovary)	Fibroblast	ATCC	CRL-2829	Collet et al. 2004
RTgill-W1	Gill	Epithelial	ATCC BCRJ	CRL-2523 BCRJ 0362	Bols et al. 1994
RTH-149	Liver (hepatoma)	Epithelial	ATCC	CRL-1710	Lannan et al. 1984
RTO	Ovary	Fibroblast	IZSLER	BS CL 77	Li 1974
SOB-15	Liver	Epithelial	ATCC	CRL-2301	Ostrander et al. 1995
STE-137	Embryo	Epithelial	ECACC CellBank Australia	95122020 95122020	Lannan et al. 1984
TPS	Kidney (anterior)	Epithelial/fibroblast	ECACC CellBank Australia	96112839 96112839	Diago et al. 1995

^a Subclone of the original R1 cell line and later identified as originating from a Chinook salmon (*Oncorhynchus tshawytscha*). Please see DMSZ website for species status of D11 and R1

^b Engineered cell lines. Please see text under “Engineering RT cell lines” section

Table 3 Informally shared rainbow trout (RT) invitrome: RT cell lines in current use but not in repositories

Cell line designation	Tissue of origin	Morphology	Reference
HK1532	Head kidney	Lymphoid (T cell)	Maisey et al. 2016
mRTP1B	Pituitary	Epithelial	Chen et al. 2010
mRTP1E	Pituitary	Fibroblast	Chen et al. 2010
mRTP1F	Pituitary	Pleomorphic	Chen et al. 2010
mRTP1K	Pituitary	Epithelial	Chen et al. 2010
mRTP2A	Pituitary	Epithelial	Chen et al. 2010
RTbrain	Brain	Glial-like	Vo et al. 2014
RTee	Early embryo	Epithelial	Bols et al. 2004
RTgutGC	Intestine	Epithelial	Kawano et al. 2011
RTH	Heart	Endothelial	Luque et al. 2014
RTHDF	Skin	Fibroblast	Ossum et al. 2004
RTL-W1	Liver	Epithelial	Lee et al. 1993
RT-milt5	Milt	Fibroblast	Vo et al. 2015
RTO-2	Ovary	Fibroblast	Vo et al. 2015
RT-ovf1	Ovarian fluid	Epithelial	Vo et al. 2015
RTP-2	Pituitary	Epithelial	Bols et al. 1995
RTP-91E	Pituitary	Epithelial	Tom et al. 2001
RTP-91F	Pituitary	Fibroblast	Tom et al. 2001
RTS11	Spleen	Macrophage-like	Ganassin and Bols 1998
RTS34st	Spleen	Stromal/epithelial	Ganassin and Bols 1999
RTS34st(neo)	Spleen	Stromal/epithelial	Fan et al. 2004
RTT	Tail	Fibroblast	Fernandez et al. 1993
RT-testis	Testis	Epithelial	Vo et al. 2015
TPS-2	Kidney (anterior)	Stromal/reticulo-endothelial	Fierro-Castro et al. 2012
TSS	Spleen	Stromal/reticulo-endothelial	Fierro-Castro et al. 2012
5C2	Embryo	Epithelial	Ristow et al. 1998
6B	Embryo	Epithelial	Ristow et al. 1998
11B	Embryo	Fibroblast	Ristow et al. 1998
23B2	Embryo	Fibroblast	Ristow et al. 1998
24 BC	Embryo	Epithelial	Ristow et al. 1998
25B	Embryo	Epithelial	Ristow et al. 1998
26B	Embryo	Epithelial	Ristow et al. 1998
49B2	Embryo	Epithelial	Ristow et al. 1998
58B2C	Embryo	Epithelial	Ristow et al. 1998

1998). For endocrinology, five RT pituitary cell lines were characterized for the expression of growth hormone and prolactin genes (Chen et al. 2010). For virology, the response of RT cell lines to synthetic double-stranded (ds) RNA, which is a viral mimic, were compared to evaluate their antiviral mechanisms. Double-stranded RNA has multiple effects on rainbow trout cell lines. Morphologically, homotypic aggregation and apoptosis was induced by dsRNA in the monocyte/macrophage cell line, RTS11 (DeWitte-Orr et al. 2005; 2007a). Additionally, dsRNA induced an antiviral state in RTS11 by upregulating interferon-stimulated genes (DeWitte-Orr et al. 2007b); this induction is

also dependent on dsRNA length as longer length induced stronger response (Poynter and DeWitte-Orr 2015).

Neoplastic characterization of RT cell lines

RT cell lines have been developed from tumors. RTH-149 was established from an alfatoxin-induced hepatoma (Fryer et al. 1981) and four cell lines (RTH1B1A, RTH1B1D, RTH1B2A, and RTH1B2C) were isolated from liver tumors induced with dibenzo[a,l]pyrene (DBP) (Chen et al. 2004). A RT mesothelioma and a nephroblastoma were the sources for cell lines

Table 4 Rainbow trout (RT) zombie invitrome: RT cell lines mentioned in the literature but not used in the last 20 yr

Cell line designation	Tissue of origin	Morphology	Reference
RBS	Spleen	Fibroblast	Wolf and Mann 1980
RBTE 45	Embryo	Not stated	Ristow and de Avila 1994
RF	Gonads		Wolf and Mann 1980
RTE	Embryo	Epithelial	Fernandez et al. 1993
RTE-2	Embryo	Epithelial	Fernandez et al. 1993
RTF-1	Fry	Fibroblast	Wolf and Mann 1980
RTL-4	Liver	Epithelial	Watanabe et al. 1987
RTM	Mesothelioma	Not stated	Carter et al. 1996
RTN	Nephroblastoma	Mixed	Wolf and Mann 1980
No name given in paper	Spleen	Reticuloendothelial	Moritomo et al. 1990
No name given in paper	Spleen	Epithelial and fibroblast	Flaño et al. 1998

Cell lines in repositories but not used for the last 20 yr are not considered zombie cell lines because their status is known and they can be purchased

referred to respectively as RTM (Carter et al. 1996) and RTN (Wolf and Mann 1980). Some of these showed features characteristic of mammalian neoplastic cells in vitro. The liver cell lines formed colonies in soft agar. As judged by immunocytochemistry, RTH-149 and RTM showed higher expression than RTG-2 for the oncogenes ras and c-myc (Carter et al. 1996).

Whether RT cell lines acquire neoplastic properties either through just the act of being continuously cultivated or as a result of carcinogen treatment has been investigated infrequently. In one report RTL-W1 was able to grow in agar without treatment (Malhao et al. 2013) and in another only after the RTL-W1 had been treated with aflatoxin (Bechtel and Lee 1994). In the case of untreated RTL-W1, the growth in agar was thought to reflect the stem cell properties of the cell line rather than neoplastic transformation (Malhao et al. 2013). Overall, the tumorigenicity of RT cell lines from tumors or from normal tissues is unknown because there is no appropriate in vivo tumor assay available to answer the question.

Engineering RT cell lines

Many attempts that have been made to engineer RT cell lines using either virus transduction or transfection produced mixed results. Three cases of virus transduction attempts and two cases of transfection attempts are described below.

Of the three transduction attempts, two were done using baculoviral vectors and one using adenoviral vectors. Both efforts to transduce RTG-2 with baculoviral vectors failed. Leisy et al. (2003) attempted to transduce RTG-2 with a recombinant baculovirus containing a β -galactosidase reporter gene (*Ac-CALacZ*) but β -galactosidase activity was not seen in RTG-2. Yokoo et al. (2013) tried to transduce RTG-2 with a baculovirus-*Drosophila* Mos1 (*AcB-GHmos*) recombinant system; however, transgene expression was not detected in RTG-2 even though it

was detected in CHSE-214 and EPC cells. Transduction of RT cell lines using adenoviral vector, instead of baculoviral vectors, produced more success. All four RT cell lines, RTG-2, RTgill-W1, RTS-34st, and RTS-pBK, expressed the β -galactosidase reporter gene when they were transduced with a recombinant human adenovirus, AdCA35*lacZ*, containing the *lacZ* gene controlled by the MCMV-IE promoter (Rainbow and Zacal 2008).

Two transfection attempts of RT cell lines also produced mixed outcomes. After failure to propagate striped jack nervous necrosis virus (SJNNV) on RTG-2 using standard inoculation procedure with infectious virus (Iwamoto et al. 2000), lipofectin transfection of RTG-2 with purified SJNNV RNA also resulted in lack of virus propagation (Iwamoto et al. 2001). However, one of the few successfully engineered RT cell lines is RTG-P1 (Collet et al. 2004). RTG-P1 was created by the transfection of RTG-2 with firefly luciferase gene controlled by the Mx1 gene promoter. The Mx1 gene is an IFN-inducible gene; therefore, this cell line has been used to study the innate immune response. Exposure of RTG-P1 to polyI:C resulted in increased luminescence suggesting an induction of the Mx1 gene; furthermore, exposure of RTG-P1 to viral hemorrhagic septicemia virus (VHSV) also caused increased luciferase activity in the cell line (Collet et al. 2004). In addition to VHSV, other fish viruses, infectious pancreatic necrosis virus (IPNV), infectious hematopoietic necrosis virus (IHNV), and salmonid alphavirus (SAV) also induced luciferase activity in RTG-P1 (Saint-Jean et al. 2010; Park et al. 2011; Collet et al. 2013).

Storing RT cell lines

Cell lines must be stored in some manner for them to be useful as experimental material for invitroomics, and like the cell lines of other vertebrates, RT cell lines can be stored at very low temperatures with cryoprotectants. Most commonly, the cryoprotectant is 10% dimethylsulfoxide (DMSO) in

complete growth medium, but other cryoprotectants, such as 10% glycerol, have been used successfully. The procedural steps for freezing and thawing are the same as used for mammalian cells, although thawing at temperatures above room temperature must be watched closely so as not to heat kill them (Bols and Lee 1994). Storage is usually done in liquid nitrogen at -196°C . In our labs, the cryovials are placed overnight in the atmosphere above the liquid nitrogen in the dewar and then lowered into the liquid nitrogen. The presumption is that in liquid nitrogen, the cells can be stored indefinitely, and we have successfully thawed RT cell lines after storage for over 30 yr in liquid nitrogen. RT cell lines in DMSO can also be stored at -80°C , and we have stored them successfully under these conditions 1 to 2 yr but have not tried longer.

On at least one occasion, attempts to cryopreserve a newly developed RT cell line were unsuccessful. Early on in their development, the RT monocyte/macrophage RTS11 failed to be cryopreserved in 10% DMSO and storage in liquid nitrogen (Ganassin and Bols 1998). Different methods of cooling cryovials were tried prior to them being placed in liquid nitrogen but without success. Yet overtime, as RTS11 continued to be grown, the cells began to cryopreserve successfully, by the same standard cryopreservation methods that had been used for other cell lines. Possibly the cell density was higher, up to 10 million cells per 1.5 mL cryovial, contributed to the later success.

Overall cryopreservation of RT cell lines seems to be routine, but procedures likely could still be improved. Different cryoprotectants could be tried, with the natural cryoprotectant trehalose being one possibility (Stokich et al. 2014). A programmable cooler could be used to freeze cells at different controlled rates to identify the optimal freezing rate. Cells could also be stored in cryogenic freezers at -150°C , which would remove the necessity to constantly keep dewars filled with liquid nitrogen.

If the equipment for cryopreservation is not available, RT cell lines can be kept alive at $4-5^{\circ}\text{C}$, without having to change the medium. The cells are first grown in L15/FBS at 18 to 21°C in non-vented flasks. After the cultures have reached confluency, the complete volume of the flask is filled with L15/FBS and flask caps are tightened. The flasks are then placed in a laboratory fridge at $4-5^{\circ}\text{C}$ and can be left unattended for 1–2 yr. When proliferating cells are needed, the flasks are returned to $18-21^{\circ}\text{C}$. After a day, the cultures can be subcultivated to being routine growth again.

Distribution of RT cell lines

Repositories, scientific companies, and scientific colleagues make up the distribution network for RT cell lines. The relative merits of each are discussed in the Invitrome section. The repositories with RT cell lines are listed in Table 1. Cells can be shipped frozen in cryovials and/or alive in flasks. Cryovials can

be sent in liquid nitrogen dry shippers or on dry ice. Liquid nitrogen dry shippers are perhaps the most convenient method for both the shipping and receiving labs but are expensive. Dry ice is hazardous material for airlines so cryovials being sent this way requires more regulatory hurdles for the shipper and additionally the receiving lab should likely thaw the cryovials immediately on getting them in order to get the best cell recovery. RT cell lines in culture flasks have survived shipment by several commercial couriers. One key to success is to use small flasks (12.5 or 25 cm^2) and to fill the whole flask volume with medium so the cells are always covered with medium, even if the package is upside down. Another key to sending RT cell lines in flasks is to ship during periods of cool weather.

Invitrome: Catalog of Cell Lines

The invitrome is being defined as the grouping of cell lines around a theme (Fig. 1b). For this article, the overarching theme is a single species, *O. mykiss*. Yet cell lines can be cataloged in many other ways in order to help researchers to use and think about them. One way is based on cell line storage and availability and this is discussed after first presenting the RT invitrome.

Rainbow trout (RT) invitrome All the cell lines reported from *O. mykiss* constitute the RT invitrome and, currently, consist of approximately 55 cell lines. This number is derived from using scientific indexing services such as the Web of Science and the “cellosaurus” search engine (<http://web.expasy.org/cgi-bin/cellosaurus/search>). These cell lines can be grouped in numerous additional ways, and below is a way that should help potential users of RT cell lines.

Invitromes based on the storage and availability of cell lines Invitromes can be defined by the storage and availability of the cell lines. When this was considered for RT cell lines, five invitromes could be distinguished. A similar framework likely can be applied to cell lines from any animal species, from insects to birds.

Curated invitromes Curated invitromes refer to the cell line collections of agencies that devote resources and personnel to cell line cataloging and quality testing and are committed to the long-term storage and distribution of cell lines. Usually, these agencies are non-profit national and international repositories. The costs of cell line accession, storage, and shipping are recovered by charging a fee for each cell line. Repositories are the best sources for cell lines because any problems of misidentification and mycoplasma usually have been sorted out before they are distributed. Repositories also have standard operating procedures and schedules for shipping and for dealing with cell importation regulations. The movement of

cell lines between countries varies with countries (Geraghty et al. 2014).

Repositories with at least one RT cell line are given in Table 1. RT cell lines that are available from repositories are presented in Table 2 and constitute the RT curated invitrome. Choosing a repository is perhaps most often driven by geography but there might be additional factors, such as cost or quality. Other institutions might have curated invitromes, although they are less likely to contain fish cell lines. These include large academic institutions, government laboratories, and pharmaceutical/biotech companies. Like repositories, these agencies can strictly restrict the movement of cells, material, and people into their facilities to help assure the quality of their cell lines. Unlike repositories, their cell lines might not be routinely available for distribution.

Commercial invitromes Cell lines are available from commercial sources. The cell lines available in this way are usually a small selection of those in repositories. The RT commercial invitrome contains at least one cell line, RTG-2, which is available from Sigma (9010259, St Louis, MO). By contrast, many mammalian cell lines are commercially available and clients can order customized cell lines from some companies, such as SB Drug Discovery (Glasgow, Scotland).

Informally shared invitromes Many cell lines are cryopreserved but are not in repositories but instead are passed informally between scientists. Excluded from this group are cell lines in repositories and commercially available but other limits are arbitrary and might depend on the species. For RT, the cell lines that have been described or used in the last 20 years are listed in Table 3 and constitute the informally shared invitrome. In this invitrome are some recently developed RT cell lines for which there has not been time to see whether others want to use them and whether they are shareable. Despite this, many RT cell lines clearly fit the definition for an informally shared invitrome. Two examples are RTL-W1 from the liver and RTS11 from the spleen monocyte/macrophage lineage (Lee et al. 1993; Ganassin and Bols 1998). The only cost for these is shipping. However, obtaining cell lines from this invitrome is less regularized. Small source laboratories do not always have the resources to immediately ship cells and the receiving laboratory might have to be more directly involved in negotiating any regulations about importing cell lines into a country. The quality of this invitrome is not as assured as with cell lines from formal repositories. However, the quality is likely adequate for many purposes, and regardless of the cell line source, quality is an ongoing concern during the routine maintenance of any cell line, as discussed later under invitroomics. A final worry about cell lines not in repositories is that they might be lost when laboratories shut down. On the other hand, if cryopreserved in several laboratories, hopefully, a cell line will remain

cryopreserved in at least one and be available for future use. However, future scientists might not necessarily know where to find the cells and the cell line could become part of the zombie invitrome.

Zombie invitromes This refers to all the cell lines that have been described in the literature either in detail or only in passing but have not been placed in repositories and have not been reported as being used much further. As the loss of a cell line is rarely noted in publications, the reader is left to wonder about their status. They might be lost, but alternatively some might still be cryopreserved in liquid nitrogen somewhere, waiting to be thawed. This potential for re-emergence into scientific endeavors suggests the moniker, zombie cell line. Even if they remain dead, they have provided some information about what cell lines are possible. For RT, Table 4 lists the cell lines that have been noted in the literature but have not been mentioned for the last 20 yr and constitute the RT zombie invitrome. Two interesting members are RTM and RTN (Wolf and Mann 1980; Carter et al. 1996) because they are two of only three cell lines from RT tumors. Finally, the zombie invitrome might be the fate of many cell lines that are not deposited in repositories because future scientific interests are hard to predict. Applications for a cell line in common use now might disappear and consequently so might the use of the cell line. Thus, the incentive to keep the cells cryopreserved would disappear and so might the cell line. On the hand, new applications might be found for the cell line much later, and so if the cells have been kept cryopreserved, the cell line could return to the flask of the living.

Cryptic invitromes Some cell lines might be developed and cryopreserved but never used in the literature. For example, they might be started for projects that subsequently change direction. As long as their cryopreservation is maintained, they could be used but would be known only to the developers and hidden from the larger scientific community. How common this situation is hard to judge but we have cryopreserved new cell lines from fish that have yet to be formally characterized.

Invitroomics: Use of Invitromes

Invitroomics is the application of cell lines to a scientific problem and is done in many disciplines (Fig. 1c). For example, the RT invitrome is important in aquaculture (Bols 1991), toxicology (Bols et al. 2005) and virology (Wolf and Mann 1980). However, several issues can confound the use of an invitrome and distract from the science. These are briefly illustrated here with the RT invitrome along with several suggestions on how to deal with them, followed by short summary of how the RT invitrome has been used in virology.

Cross contamination Occasionally, a cell line that is understood to have a particular identity is found subsequently to have a different one. This is cross contamination and was recognized as a problem for mammalian cell lines in the 1960s but continues even to this day (Lucey et al. 2009; Di Girolamo et al. 2016). Although less well documented, cross contamination also has been found with fish cell lines (Winton et al. 2010). The causes of cross contamination can vary and usually are hard to prove retrospectively. One recommendation for reducing the problem is to confirm the cell line identity as often as is possible, although this can be hard if the cell lines are from the same species. However, analyzing microsatellite loci allowed RT cell lines to be distinguished (Perry et al. 2001). Another recommendation is to maintain “good laboratory practice.” “Good practice” for fish cell culturing was presented years ago (Wolf and Quimby 1973b) and specific steps have been listed recently for this issue with mammalian cells (Di Girolamo et al. 2016). For all animal cell culturing, an important habit is to have a separate bottle of medium for each cell line, and after a volume of medium has been drawn out with a pipette, the pipette must never be returned to the medium bottle. This is to avoid introducing cells into the stock bottle of medium, which later may be used to culture another cell line. This contamination route might be more likely with cell lines from coldwater fish, such as RT and other salmonids, than with mammalian cell lines. When CHSE-214 cells were deliberately added to a bottle of medium and the bottle put into a fridge, some cells survived and could be cultured from the bottle even after several weeks of storage at 4–5°C (Perry et al. 2001).

Mycoplasma contamination Mycoplasma are thought to be inadvertently introduced into animal cell cultures in several ways (Drexler and Uphoff 2002), and considering these might help in reducing contamination when working with fish cell lines. One contamination route is media components (Low 1974). This would likely be same for piscine and mammalian cell cultures because media come from the same commercial sources. The researcher themselves can be an important source of mycoplasma contamination (Drexler and Uphoff 2002). However, this might be less likely for cell lines from coldwater fish, such as rainbow trout, because presumably human mycoplasma would adapt poorly to the low growth temperatures for these cell lines. For human and rodent cells, mycoplasma-infected cell lines are themselves the most important source (McGarrity 1976). The handling of cell cultures creates aerosols, and when cultures are contaminated, the aerosol contains and spreads mycoplasma throughout the tissue culture facility (O’Connell et al. 1964; McGarrity 1976). This is probably how mycoplasma spread into fish cell cultures but two further issues likely exacerbate the problem. As was discussed earlier, only repositories and a few other agencies have the resources to assure regularly that cell lines are

free of mycoplasma but relatively few fish cell lines are in repositories. The fish cell culture facilities are often in academic institutions where rigorously restricting the movement of cells, people and material in and out of cell culture facilities is rarely possible.

Starting experiments with cells from a cell line that had been shown to be free of mycoplasma would appear to offer the best assurance that contamination has not compromised the results. Yet contamination might take place at any time, including during the course of the experiment. The mycoplasma in fish cell cultures appear to be very slow growing and confirming their presence might take place wk after some of the cells have been used in an experiment. For example, when RTgill-W1 was being developed and before the cell line had been cleaned, mycoplasma could be consistently detected only in flask cultures that had been allowed to sit undisturbed for 2 to 4 wk without the media having been changed (Bols et al. 1994). If a 1-wk-old flask of these cells had been used to set up an experiment that lasted 3 d, the number of mycoplasma would likely have been vanishingly small and had no effect on the experiment. Rather than throw away the results from such experiments because of possible mycoplasma contamination, we would recommend presenting evidence of the mycoplasma level immediately at the end of an experiment. For this, the most practical test would be H33258 staining because nearly every lab would be able to do this, as the only equipment needed would be a fluorescent microscope. In each experiment, some wells or culture flasks would be set aside, with cells growing on slides or coverslips for staining. Even whole flasks could be stained if an inverted fluorescent microscope were available (Bols and Lee 1994). One set of these would be stained immediately at the end of the experiment and another 1–3 wk later, depending how long the cells could be maintained without media changes. These would be scored for the numbers of cells with multiple small punctate fluorescent staining on or closely around them.

Three general outcomes are possible from the H33258 test. If no punctate fluorescent staining were to be seen at either time point, the cells would be reported as having been mycoplasma negative during the experiments. As all mycoplasma have DNA and most adhere to cells (Drexler and Uphoff 2002), false negatives would be unlikely. Secondly, if no punctate fluorescent staining were seen at the end of the experiment but appeared at the later time point, the cells would be reported as having been negative for the experiment but possibly having been present in the stock culture. Thirdly, if they were present at the end of the experiment and their numbers had increased at the later time point, the cells would be concluded to have had mycoplasma both in the stock culture and during the course of the experiment. We would recommend that such results be published but together with some measure of the level of the contamination, such as the percentage of mycoplasma positive cells as determined with H33258.

False positives might be caused by structures such as micronuclei but a fluorescent picture of the stained culture might help the reader to assess this. Also repeating the experiment in the presence of antibiotics that target mycoplasma might confirm whether the experiment had indeed been influenced by mycoplasma. Overall transparency on this issue will help the scientific community build up a more detailed picture of how mycoplasma might influence fish cells and help work to ways to eliminate them and to improve the quality of invitromes and the power of invitroomics.

Stability Whether characteristics of a cell line are lost or gained with prolonged passaging has long been a worry but has been difficult to document with RT cell lines because of the time that would have to be committed to make a convincing case. During the early development of an RT cell line, the time for a culture to become confluent after a sub-cultivation shortens as the cycle of growth to confluency followed by splitting 1 to 2 or 1 to 3 is repeated. Eventually this time stabilizes after approximately 5 to 20 passages, depending on the cell line. As primary cultures would be expected to start with mainly diploid cells, the karyotype clearly changes during the development of heteroploid RT cell lines but whether the karyotype has stabilized has rarely been investigated. However, for RTH-149, the modal chromosome numbers were different at passage 20 versus passage 141 (Fryer et al. 1981; Lannan et al. 1984). The loss of a specific property has been noted with a pituitary cell line. Expression of growth hormone and prolactin genes failed to be maintained upon the prolonged culturing of RTP-2 (Bols et al. 1995; Chen et al. 2010). Therefore, reporting the passage number range at which RT cell lines are used in experiments is recommended so the stability of a particular property can be established as the cells are used over the years in different labs.

Use of the RT invitrome in virology

Virology was the original application for animal cells lines and was the impetus for RT cell line development as well. Cell lines can be a diagnostic tool for detecting viruses because many viruses on many cell lines cause cytopathic effects (CPE), indicating their presence and allowing them to be isolated and quantified. Some cell lines support the propagation of viruses. This allows the study of the single cell reproductive phase of the virus and agents that might interfere with it, including the antiviral mechanisms of the host cells. Additionally some cell lines produce sufficient virus to allow elucidation of viral structure and generation of diagnostic antibodies and vaccines. In the original description of RTG-2, the cells were shown to develop CPE in response to infectious pancreatic necrosis virus (IPNV) (Wolf and Quimby 1962). Subsequently, many viruses have been studied with the RT invitrome. These are listed in Table 5. In total, viruses from 15 families were studied with RT

cell lines; six of these families are double-stranded DNA viruses and two are double-stranded RNA viruses. Three of the families are positive sense ssRNA viruses and three are negative sense ssRNA viruses. In addition, one retrovirus, walleye dermal sarcoma virus was studied with RTgill-W1. RTG-2 was the most used out of all the RT cell lines, likely because it was the first fish cell line developed and the only RT cell line to be recommended by the OIE World Organisation for Animal Health for the isolation of fish viruses (OIE 2016).

Some of the concerns associated with the use of an invitrome have been realized in fish virology. Cross contamination was observed with EPC, which is one of the most widely used cell lines for virus work but is found to be fathead minnow rather than carp (Winton et al. 2010). One of the few documented effects of mycoplasma on an RT cell line activity was on virus propagation (Emerson et al. 1979). By itself, *A. laidlawii* caused cellular granularity and destruction when added to RTG-2 cultures at high levels but not low ones. At low levels, *A. laidlawii* appeared to enhance IPNV and IHNV infection. This was seen as a 2- to 100-fold increase in IPNV and IHNV titres. Possibly, *A. laidlawii* did not increase virus propagation but instead sensitized the RTG-2 to cellular destruction by the viruses so that viral CPE occurred at lower viral numbers than in control cultures. Whether viral susceptibility remains stable or changes as RT cell lines are passaged would be interesting to know as this could improve their use in virology. For the Chinook salmon embryo cell line, CHSE-214, susceptibility varied with different CHSE-214 lineages but did not necessarily correlate with the number of subcultivations (McAllister 1997). Despite these concerns, the RT invitrome has been successfully and widely used in virology, and below, this literature is briefly summarized under the headings for the Baltimore classification of viruses.

Double-stranded DNA viruses (dsDNA) The RT invitrome has been mostly used to study *Iridoviridae* and *Herpesviridae* families within the dsDNA viruses. Viruses of the *Iridoviridae* family have mostly been reported to replicate poorly in RT cell lines (Table 5; Ariel et al. 2009; Pham et al. 2015). Of all the RT cell lines examined, RTG-2 appears to be the most capable of supporting these viruses (Table 5). Yet this capacity pales in comparison to cells lines of other species such as fathead minnow EPC, Chinook salmon CHSE-214, and Bluegill fry BF-2 (Ariel et al. 2009; Pham et al. 2015). Interestingly, primary RT monocyte and macrophage cells and the monocyte/macrophage RTS11 cell line appear to be specifically targeted by frog virus 3 (FV3) and grouper iridovirus (GIV) to undergo apoptosis very early during the infection cycle (Pham et al. 2012, 2015).

For the *Herpesviridae* family, RTG-2 and RTF-1 support the propagation of Salmonid herpesvirus type 1 and 2 (Wolf et al. 1978; Kimura et al. 1983). RTG-2, RTgill-W1, and RTL-W1 allow the replication of cutthroat trout virus (CTV) as judged by increases in genome copy number when measured by RT-qPCR. Yet, no CPE was seen with CTV on the three cell

Table 5 Viruses studied with the rainbow trout (RT) invitrome

Family name (genome) and name of virus	Virus abbreviation	Cell lines	Virus yield ^a	Selected references
Adenoviridae (dsDNA)				
Recombinant human adenovirus	AdCA35lacZ	RTG-2	No data	Rainbow and Zacal 2008
		RTgill-W1	No data	Rainbow and Zacal 2008
		RTS-pBK	No data	Rainbow and Zacal 2008
		RTS-34st	No data	Rainbow and Zacal 2008
Snake adenovirus type 1	SnAdV-1	RTG-2	No data	Juhasz and Ahne 1993
Alloherpesviridae (dsDNA)				
<i>Anguilla</i> herpesvirus 1	AngHV-1	RTG-2	None	Davidse et al. 1999
Koi Herpesvirus (Cyprinid herpesvirus 3)	KHV (CyHV-3)	RTG-2	No data	Oh et al. 2001
Cyprinid herpes virus 2	CyHV-2	RTG-2	Yes (unspecified)	Daněk et al. 2012
Channel catfish virus (Ictalurid herpesvirus 1)	CCV	RTG-2	None	Wolf and Darlington 1971
		RTE	None	Fernandez et al. 1993
		RTE-2	None	Fernandez et al. 1993
		RTT	None	Fernandez et al. 1993
Gadid herpesvirus 1	GaHV-1	RTG-2	No data	Marcos-Lopez et al. 2012
Baculoviridae (dsDNA)				
Autographa californica multinucleocapsid nucleopolyhedrovirus (AcMNPV)	Ac-CAlacZ	RTG-2	None	Leisy et al. 2003
Herpesviridae (dsDNA)				
Cutthroat trout virus	CTV	RTG-2	~10 ⁸ GEq/mL	von Nordheim et al. 2016
		RTgill-W1	~10 ¹⁰ GEq/mL	von Nordheim et al. 2016
		RTL-W1	~10 ⁹ GEq/mL	von Nordheim et al. 2016
Percid herpesvirus 1 (<i>Herpesvirus vitreum</i>)	PeHV-1	RTG-2	None	Kelly et al. 1983
Salmonid herpesvirus type 1	SalHV-1	RTG-2	~10 ⁵ PFU/mL	Wolf et al. 1978
		RTF-1	~10 ⁵ PFU/mL	Wolf et al. 1978
		RTE	~10 ⁵ TCID ₅₀ /mL	Fernandez et al. 1993
		RTE-2	~10 ⁵ TCID ₅₀ /mL	Fernandez et al. 1993
Salmonid herpesvirus type 2 (<i>Oncorhynchus masou virus</i>)	SalHV-2 (OMV)	RTG-2	~10 ⁶ TCID ₅₀ /mL	Kimura et al. 1983
		RTE	~10 ⁴ TCID ₅₀ /mL	Fernandez et al. 1993
		RTE-2	~10 ¹ TCID ₅₀ /mL	Fernandez et al. 1993
Steelhead herpesvirus	SHV	RTG-2	No data	Hedrick et al. 1986
		RTE	~10 ² TCID ₅₀ /mL	Fernandez et al. 1993
		RTT	~10 ² TCID ₅₀ /mL	Fernandez et al. 1993
White sturgeon herpesvirus 2 (Acipenserid herpesvirus 2)	WSHV-2	RTG-2	No data	Watson et al. 1995
Iridoviridae (dsDNA)				
Bohle iridovirus	BIV	RTG-2	~10 ⁸ TCID ₅₀ /mL	Speare and Smith 1992
Doctor fish virus	DFV	RTG-2	Yes (unspecified)	Ariel et al. 2009
European catfish virus	ECV	RTG-2	Yes (unspecified)	Ariel et al. 2009
European sheatfish virus	ESV	RTG-2	Yes (unspecified)	Ariel et al. 2009
Epizootic hematopoietic necrosis virus	EHNV	RTG-2	~10 ⁵ TCID ₅₀ /mL	Langdon et al. 1986
Frog virus 3	FV3	RTG-2	~10 ⁷ TCID ₅₀ /mL	Pham et al. 2015
		RTHDF	None	Pham et al. 2015
		RTgill-W1	None	Pham et al. 2015
		RTgut-GC	None	Pham et al. 2015

Table 5 (continued)

Family name (genome) and name of virus	Virus abbreviation	Cell lines	Virus yield ^a	Selected references
		RTL-W1	~10 ⁵ TCID ₅₀ /mL	Pham et al. 2015
		RTS11	None	Pham et al. 2015
		RTO	No data	Wolf and Mann 1980
Grouper iridovirus	GIV	RTS11	None	Pham et al. 2012
Guppy virus 6	GF6	RTG-2	Yes (unspecified)	Ariel et al. 2009
Lymphocystis disease virus	LCDV	RTG-2	No data	Wolf et al. 1966
Pike-perch iridovirus	PPIV	RTG-2	No data	Tapiovaara et al. 1998
Rana esculenta virus	REV	RTG-2	Yes (unspecified)	Ariel et al. 2009
Red sea bream iridovirus	RSIV	RTG-2	~10 ⁴ TCID ₅₀ /mL	Nakajima and Sorimachi 1994
Short-finned eel virus	SERV	RTG-2	~10 ⁴ TCID ₅₀ /mL	Ariel et al. 2009
Soft-shelled turtle iridovirus	STIV	RTG-2	No data	Chen et al. 1999
Tadpole edema virus	TEV	RTG-2	~10 ² TCID ₅₀ /mL	Wolf et al. 1968
Tiger frog virus	TFV	RTG-2	None	Weng et al. 2002
<i>Nimaviridae</i> (dsDNA)				
White spot syndrome virus	WSSV (WSBV)	RTG-2	No data	Heo et al. 2000
<i>Birnaviridae</i> (dsRNA)				
Infectious pancreatic necrosis virus	IPNV	RTG-2	~10 ⁸ TCID ₅₀ /mL	Wolf and Quimby 1962
		RBS	No data	Wolf and Mann 1980
		RTF-1	No data	Wolf and Mann 1980
		RTH	None	Luque et al. 2014
		RTH-149	~10 ⁹ TCID ₅₀ /mL	Lannan et al. 1984
		STE-137	~10 ⁹ TCID ₅₀ /mL	Lannan et al. 1984
		RTE	~10 ⁵ TCID ₅₀ /mL	Fernandez et al. 1993
		RTE-2	~10 ⁷ TCID ₅₀ /mL	Fernandez et al. 1993
		RTT	~10 ⁶ TCID ₅₀ /mL	Fernandez et al. 1993
Marine birnavirus	MABV	RTG-2	No data	Jung et al. 2008
<i>Reoviridae</i> (dsRNA)				
Blue gill virus	BGV	RTG-2	~10 ² TCID ₅₀ /mL	Hoffman et al. 1969
Bluetongue virus	BTV	RTG-2	No data	Wechsler and McHolland 1988
Chum salmon reovirus	CSV	RTG-2	~10 ² TCID ₅₀ /mL	DeWitte-Orr and Bols 2007
		RTS11	~10 ¹ TCID ₅₀ /mL	DeWitte-Orr and Bols 2007
		RTE	None	Fernandez et al. 1993
		RTE-2	~10 ⁴ TCID ₅₀ /mL	Fernandez et al. 1993
		RTT	None	Fernandez et al. 1993
Golden shiner virus	GSV	RTG-2	No data	Plumb et al. 1979
<i>Nodaviridae</i> (ssRNA(+))				
Dicentrarchus labrax encephalitis virus	DIEV	RTG-2	~10 ⁸ FFU/mL	Delsert et al. 1997
Striped Jack nervous necrosis virus	SJNNV	RTG-2	No data	Fukuda et al. 1996
<i>Picornaviridae</i> (ssRNA(+))				
Fathead minnow picornavirus	FHMPV	RTG-2	No data	Phelps et al. 2014
<i>Togaviridae</i> (ssRNA(+))				
Eastern equine encephalomyelitis virus	EEEV	RTG-2	~10 ⁶ PFU/mL	Officer 1964
Salmonid alpha virus	SAV	RTG-2	~10 ⁴ TCID ₅₀ /mL	Graham et al. 2008
Sleeping disease virus	SDV	RTG-2	~10 ⁷ PFU/mL	Villoing et al. 2000
Salmon pancreas disease virus	SPDV	RTG-2	~10 ⁵ PFU/mL	Lopez-Doriga et al. 2001

Table 5 (continued)

Family name (genome) and name of virus	Virus abbreviation	Cell lines	Virus yield ^a	Selected references
Venezuelan equine encephalomyelitis virus	VEEV	RTG-2	~10 ⁵ PFU/mL	Officer 1964
Western equine encephalomyelitis virus	WEEV	RTG-2	No data	Wolf and Mann 1980
<i>Orthomyxoviridae</i> (ssRNA(-))				
European eel virus	EV-2	RTG-2	None	Nagabayashi and Wolf 1979
Infectious salmon anemia virus	ISAV	RTG-2	None	Falk et al. 1997
		RTgill-W1	~10 ³ TCID ₅₀ /mL (by IIF)	Falk et al. 1997
<i>Paramyxoviridae</i> (ssRNA(-))				
Fer-de-Lance virus	FDLV	RTG-2	No data	Clark et al. 1979
<i>Rhabdoviridae</i> (ssRNA(-))				
Eel virus American	EVA	RTG-2	No data	Somamoto et al. 2000
		RTE	No data	Fernandez et al. 1993
		RTE-2	No data	Fernandez et al. 1993
		RTT	No data	Fernandez et al. 1993
Eel virus European	EVE	RTG-2	~10 ⁸ TCID ₅₀ /mL	Okamoto et al. 1985
Eel virus European X	EVEX	RTG-2	No data	Galiniier et al. 2012
		RTE	No data	Fernandez et al. 1993
		RTE-2	No data	Fernandez et al. 1993
		RTT	No data	Fernandez et al. 1993
Epizootic ulcerative syndrome viruses	EUS Viruses	RTG-2	None	Lio-Po et al. 2000; Lilley and Frerichs 1994
<i>Hirame rhabdovirus</i>	HIRRV	RTG-2	~10 ⁶ TCID ₅₀ /mL	Kimura et al. 1986
		RTE	No data	Fernandez et al. 1993
		RTE-2	~10 ⁸ TCID ₅₀ /mL	Fernandez et al. 1993
		RTT	No data	Fernandez et al. 1993
Infectious hematopoietic necrosis virus	IHNV	RTG-2	~10 ⁶ TCID ₅₀ /mL	Clark and Soriano 1974
		RBS	No data	Wolf and Mann 1980
		RTF-1	No data	Wolf and Mann 1980
		RTH-149	~10 ⁷ TCID ₅₀ /mL	Lannan et al. 1984
		STE-137	~10 ⁷ TCID ₅₀ /mL	Lannan et al. 1984
		RTS	~10 ⁷ PFU/flask	Ristow and de Avila 1994
		RTBE45	~10 ⁸ PFU/flask	Ristow and de Avila 1994
		RTE	~10 ⁵ TCID ₅₀ /mL	Fernandez et al. 1993
		RTE-2	~10 ⁷ TCID ₅₀ /mL	Fernandez et al. 1993
		RTT	~10 ⁶ TCID ₅₀ /mL	Fernandez et al. 1993
Pike fry rhabdovirus	PFR	RTG-2	~10 ⁵ PFU/mL	Ahne 1979
		RTE	No data	Fernandez et al. 1993
		RTE-2	No data	Fernandez et al. 1993
		RTT	No data	Fernandez et al. 1993
Scophthalmus maximus rhabdovirus	SMRV	RTG-2	No data	Zhang et al. 2007
Spring viraemia of carp virus	SVCV	RTG-2	~10 ⁴ PFU/mL	Ahne 1979
		RTE	No data	Fernandez et al. 1993
		RTE-2	No data	Fernandez et al. 1993
		RTT	No data	Fernandez et al. 1993
Ulcerative disease rhabdovirus	UDRV	RTG-2	None	Frerichs et al. 1989
Viral hemorrhagic septicemia virus	VHSV	RTG-2	~10 ⁶ TCID ₅₀ /mL	Campbell and Wolf 1969
		RTF-1	No data	Wolf and Mann 1980

Table 5 (continued)

Family name (genome) and name of virus	Virus abbreviation	Cell lines	Virus yield ^a	Selected references
		RTH	Yes (unspecified)	Luque et al. 2014
		RTH-149	~10 ⁶ TCID ₅₀ /mL	Lannan et al. 1984
		STE-137	~10 ⁵ TCID ₅₀ /mL	Lannan et al. 1984
		RTgill-W1	~10 ⁸ TCID ₅₀ /mL	Pham et al. 2013
		RTS11	None	Tafalla et al. 2008
Retroviridae (ssRNA(RT))				
Walleye dermal sarcoma virus	WDSV	RTgill-W1	None	Rovnak et al. 2007
Unclassified (Likely RNA)				
Erythrocytic inclusion body syndrome virus	EIBS Virus	RTG-2	None	Leek 1987

dsDNA double-stranded DNA, dsRNA double-stranded RNA, ssRNA(+) positive sense single-stranded RNA, ssRNA(-) negative sense single-stranded RNA, ssRNA-RT RNA viruses that replicate through a DNA intermediate

^a All titer values were reported in the unit provided by the original article but rounded down to the nearest log for consistent presentation within the *table*. When a range is given, the top end of the range was taken and the presented value was rounded down to the nearest log

GEq—genome equivalent as determined by real time PCR

IFF—indirect immunofluorescence staining

PFU—plaque-forming unit

TCID₅₀—tissue culture infectious dose 50

None—cells were exposed to virus but CPE or virus replication were not observed

No data—cell morphology and the ability of viruses to induce CPE or replicate in the cell lines were not reported by the authors

lines (von Nordheim et al. 2016). The responses of RTG-2, RTgill-W1, and RTL-W1 to CTV contrast with the responses of RTS11 to FV3 and GIV. In the first case, virus was produced but no CPE were observed; whereas in the second case, CPE were observed but no viruses produced. These observations offer many potential directions for future studies and illustrate the importance of having a reasonably large invitrome for studying the interactions between viruses and the cells of a potential host.

Double-stranded RNA viruses (dsRNA) Only two families of dsRNA viruses, *Birnaviridae* and *Reoviridae*, have been studied with the RT invitrome. Viruses in the *Birnaviridae* family are very pathogenic to rainbow trout, causing severe pancreatic necrotic diseases (Crane and Hyatt 2011), whereas those in the *Reoviridae* family are less pathogenic and typically have no disease association (Winton 1981). The main virus studied in the *Birnaviridae* family is IPNV. IPNV replicates to titres of at least 10⁸ TCID₅₀/mL in RTG-2, RTH-149, and STE-137 (Wolf and Quimby 1962; Lannan et al. 1984). In contrast, viruses in the *Reoviridae* family have a very low capacity to replicate in RT cell lines, with many, such as Blue gill virus, Bluetongue virus, and Chum salmon virus, replicating to low titres (Hoffman et al. 1969; Wechsler and McHolland 1988; DeWitte-Orr and Bols 2007), in the range of 10² TCID₅₀/mL in RTG-2 (Table 5). The limited ability of reoviruses and the robust ability of birnaviruses to replicate

in RT cell line may be associated with their respectively low and high pathogenicity to RT.

Single-stranded RNA viruses, positive sense (ssRNA(+))

For positive sense ssRNA viruses, the RT invitrome has been used most often to study viruses in the *Togaviridae* family. Fish togaviruses cause pancreas disease and sleeping disease (Crane and Hyatt 2011). Salmonid alpha virus (SAV), sleeping disease virus (SDV), and salmon pancreas disease virus (SPDV) are all capable of replicating in RTG-2, producing virus yields ranging from 10⁴ TCID₅₀/mL to 10⁷ PFU/mL (Table 5). Interestingly, even mammalian togaviruses, Venezuelan equine encephalomyelitis virus (VEEV), and Eastern equine encephalomyelitis virus (EEEV) were capable of replicating to ~10⁵ and ~10⁶ PFU/mL in RTG-2 when incubated at 22°C. The other two families of positive sense ssRNA viruses studied with RTG-2 are *Nodaviridae* and *Picornaviridae* (Fukuda et al. 1996; Delsert et al. 1997; Phelps et al. 2014). However, RTG-2 produced only *Dicentrarchus labrax* encephalitis virus (DIEV), as measured by immunofluorescence (Delsert et al. 1997). Similar to CTV of the dsDNA family, DIEV appears to replicate without killing the RT cells.

Single-stranded RNA viruses, negative sense (ssRNA(-))

The RT invitrome is used most often to study the *Rhabdoviridae* family of the ssRNA(-) category. Some viruses in this family cause the most economically important viral diseases of RT. Viral hemorrhagic septicemia is caused by

VHSV; infectious hematopoietic necrosis, by IHNV. Most fish rhabdoviruses are capable of replicating in RT cell lines, with VHSV and IHNV having been demonstrated to replicate to at least 10^5 TCID₅₀/mL in three to four different RT cell lines (Table 5). Although replicating to high titer in RT fibroblast and epithelial cell lines, VHSV does not appear to be able to replicate and produce titer in monocyte/macrophage cell line RTS11. Virus entry into RTS11 is detected through the expression of the N gene; however, inhibition of replication appears to occur before the cells can translate viral mRNA as no virus proteins were detected (Tafalla et al. 2008; Pham et al. 2013). Two other virus families in the ssRNA(-) category studied with the RT invitrome are the *Orthomyxoviridae* and the *Paramyxoviridae*. European eel virus (EV-2) and Infectious salmon anemia virus (ISAV) of the *Orthomyxoviridae* family, and Fer-de-Lance virus (FDLV) of the *Paramyxoviridae* were unable to replicate in RTG-2 (Table 5). ISAV replication was detected by immunofluorescence in RTgill-W1 at a low titer of 10^3 TCID₅₀/mL but no CPE was seen (Falk et al. 1997).

RNA viruses that replicate through a DNA intermediate (ssRNA(RT)) Walleye dermal sarcoma virus (WDSV), of the retroviridae family, appears to be the only fish retrovirus to be studied with the RT invitrome. RTgill-W1 became infected with WDSV but was unable to support propagation of the virus (Rovnak et al. 2007).

Summary

In summary, the literature on RT cell lines has been reviewed to illustrate three new terms: invitromatics, invitrome and invitroomics. Succinctly, these terms refer respectively to the development, the catalog, and the use of animal cell lines. The review should aid those considering the use of RT cell lines for investigating a wide range of scientific problems but especially virology ones. Hopefully, the new terms will be broadly helpful and can be applied by those using other animal cell lines. Certainly, they could be applied relatively easily to the species for which only a few cell lines are available.

Although collecting the information for all the cell lines from some species, such as *Mus musculus*, *Cricetulus griseus*, and *Homo sapiens*, might be arduous because of the exceptionally large number of cell lines, the term “invitrome” can still be conveniently used to describe subsets of them. Among the cell lines in the murine invitrome would be all the murine cell lines that had been genetically modified through cell fusion, transfection, and gene editing or obtained from transgenic mice (Obinata 2007) and all the cell lines that had been derived from tumors. All the cell lines from a particular tumor type might constitute a specific invitrome, such as the murine mammary tumor invitrome. The Chinese hamster (*C. griseus*) invitrome would be large because many variant Chinese

hamster ovary (CHO) cell lines have been developed for biotechnology purposes (Xu et al. 2012). These might be called the CHO invitrome. The human invitrome would also be large and include embryonic stem cell lines and all the cell lines that have been derived from patients with various diseases or inherited disorders. These cell lines might be organized around the nature of the patient. For example, the Lesch-Nyhan invitrome would be all the cell lines from patients with an inherited deficiency in hypoxanthine-guanine phosphoribosyltransferase activities. Some repositories, such as the Coriell Institute for Medical Research, offer multiple cell lines from the same individual. Therefore, an individual patient or animal might be considered to have an invitrome.

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