Cytotechnology **37:** 143–151, 2001.

Characterization of rainbow trout cell lines using microsatellite DNA profiling

G.M.L. Perry[1], G.J. McDonald[1], M.M. Ferguson[1], R.C. Ganassin[2] and N.C. Bols[3,*]
[1]Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada; [2]Department of Biology, Malaspina University College, Nanaimo, BC V9R 5S5, Canada; [3]Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1 (N.C.B.), Canada; [*]Author for correspondence (e-mail: ncbols@sciborg.uwaterloo.ca)

Received 28 February 2001; accepted in revised form 4 June 2001

Key words: Authentication, DNA profiling, Fish cell lines, Microsatellite, Rainbow trout

Abstract

Ten microsatellite loci (*Omy27DU*, *Omy325(A3)UoG*, *OmyFGT5TUF*, *OmyFGT14TUF*, *OmyFGT15TUF*, *OmyFGT23TUF*, *Omy77DU*, *Ssa20.19NUIG*, *Ots1BML*, and *One18ASC*) were amplified using the polymerase chain reaction to create genetic profiles for nine cell lines (RTG-2, RTH-149, RTL-W1, RTgill-W1, RTS-11, RTS-34st, RTP-2, RTP-91E and RTP-91F) from rainbow trout (*Oncorhynchus mykiss*) and one cell line (CHSE-214) from Chinook salmon (*O. tschawytscha*). A cell line (PHL) from a non-salmonid, the Pacific herring (*Clupea harengus pallasi*), was included as a control. The ten loci clearly revealed the uniqueness of each cell line, except for two cell lines (RTP-91E and RTP-91F) from the same fish. RTP-91E and RTP-91F were identical at all loci except *Ssa20.19NUIG*. The most useful locus for demonstrating uniqueness was *Ots1BML*. The information was used to demonstrate that an uncharacterized rainbow trout cell line (Clone 1A) was in fact CHSE-214, illustrating the usefulness of multiplexed microsatellites for the creation of genetic profiles for salmonid cell lines and for the testing of cell line cross-contamination.

Abbreviations: CCLC – cross cell line contamination, SSR – simple sequence repeat, bp – base pairs, PBC – peripheral blood cells, PCR – polymerase chain reaction

Introduction

Although not used as long and as intensively as mammalian cell lines, cell lines from fish are proving to have many important research uses in a wide range of disciplines. Historically, fish cell lines were developed in the 1960s to isolate and study viruses causing economically important fish diseases (Wolf 1988). More recently, they have been applied in biomedical research (Hightower and Renfro 1988), biotechnology (Bols 1991; Li et al. 1998) and toxicology (Babich and Borenfreund 1991; Bols et al. 1997; Segner 1998). Basic research into aspects of fish biology, including immunology, physiology, endocrinology, genetics and development, have also incorporated the use of fish cell lines (Bols and Lee 1993; Ganassin et al. 2000).

A problem that has arisen with the use of animal cell lines is cross cell line contamination (CCLC) (Markovic and Markovic 1998; MacLeod et al. 1999). This concern has been examined intensively in mammalian cell lines, particularly human ones, because misidentification of tumor cell lines has invalidated millions of dollars in cancer research (Nelson-Rees et al. 1980). Several different techniques have been used to genetically discriminate mammalian cell lines (Kaplan and Hukku 1998) of which DNA profiling is the most recent and powerful (King et al. 1994; Dirks et al. 1999; Matsuo et al. 1999). Microsatellites (simple sequence repeats; SSR) consist of short tan-

dem repeats of two to five base pairs (bp) flanked by unique DNA, usually less than 300 nt in total length (Park and Moran 1994). Complementary primers designed for the unique flanking sequences allow the specific amplification of the SSR through the polymerase chain reaction (PCR) (Newton and Graham 1997). SSR are generally considered to be extremely useful molecular markers for a variety of reasons including codominant expression, relatively low mutation rate and high polymorphism (Park and Moran 1994). Furthermore, microsatellite variation is easily scored since allele size intervals correspond to the number of repeat units (O'Reilly and Wright 1995). Several SSR loci can be amplified simultaneously (multiplex PCR) by labeling them with different fluorescent dye molecules (O'Reilly et al. 1996; Wenburg et al. 1996; Fishback et al. 1999). Thus when fragments from different loci overlap in size range they can be distinguished without confusion. Although little work has been done on authenticating fish cell lines using DNA markers, DNA profiling is a promising approach with numerous aquacultural and evolutionary applications (Ferguson and Danzmann 1998).

In this paper we have evaluated the utility of using SSR to distinguish among rainbow trout (*Oncorhynchus mykiss*) cell lines. The unequivocal uniqueness of ten salmonid cell lines, including nine from rainbow trout and one from chinook salmon (*O. tschawytscha*), was established through the analysis of ten microsatellite loci. Two of the rainbow trout cell lines, derived from the same individual, were genetically divergent at one locus due to a two bp shift in one allele. In the course of this work we also uncovered an example of CCLC while examining a putative rainbow trout cell line.

Table 1. Cell lines surveyed, tissue/species source and citation source.

Cell line	Tissue, Species	Reference
RTG-2	gonad, *O. mykiss*	Wolf and Quimby (1962)
RTH-149	hepatoma, *O. mykiss*	Fryer et al. (1981)
RTS11	spleen, *O. mykiss*	Ganassin and Bols (1998)
RTgill	gill, *O. mykiss*	Bols et al. (1994)
RTL-WI	liver, *O. mykiss*	Lee et al. (1993)
RTS34st	spleen, *O. mykiss*	Ganassin and Bols (1999)
RTP-2	pituitary, *O. mykiss*	Bols et al. (1995)
RTP-91E	pituitary, *O. mykiss*[1]	Tom et al. (2001)
RTP-91F	pituitary, *O.mykiss*[1]	Tom et al. (2001)
Clone 1A	blood, *O. mykiss* (putative)	unpublished
CHSE-214	embryo, *O. tshawytscha*	Lannan et al. (1984)
PHL	larvae, *C. harengus*	Ganassin et al. (1999)

[1]both cell lines derived from the same individual fish, same organ.

Materials and methods

The cell lines and their origins are listed in Table 1. They were routinely maintained as described previously (Bols et al. 1994). Except for RTG-2, RTH 149 and CHSE-214, which were obtained from the American Type Culture Collection (ATCC) (Manassas, VA), the cell lines were developed in the laboratory of Dr. Bols. One of these lines, RTgill-W1 (CRL-2523), is now available from the ATCC. All of the cell lines have been described previously except Clone 1A. Clone 1A was developed from primary cultures of rainbow trout peripheral blood cells (PBC). PBC were pelleted by low speed centrifugation. The buffy coat was removed, washed in the basal medium, Leibovitz's L-15, and the cells plated in L-15 into 25 cm flasks (Canadian Life Technologies, Burlington, ON). Some cells attached over a period of 1 to 2 days, and at this point the medium was removed and replaced with L-15 with 10% fetal bovine serum (FBS). After approximately three weeks several colonies appeared. Cells from one of the colonies were selectively removed by scraping, and replated into a 12.5-cm flask. These cells were grown up and cryopreserved.

Approximately 2×10^6 cells of the various cell lines were supplied on ice either pre-pelleted by centrifugation or suspended in sterile filtered Liebovitz's L-15 medium (Canadian Life Technologies, Burlington, ON) and pelleted on arrival (14,000 rpm) at the Department of Zoology, University of Guelph (Guelph, ON). Most often the cells were from cultures that had been grown routinely in the laboratory for several months. However, in some cases, frozen vials of cell lines were supplied as well. Cyrovials of RTG-2, RTH-149, and CHSE-214 were obtained from ATCC and analyzed without first culturing the cells. As well, vials of RTL-W1 that had been cryopreserved in 1989 at early passages were analyzed without first culturing the cells. A cell line (PHL) from a non-salmonid, the Pacific herring (*Clupea harengus pallasi*), was provided to illustrate the specificity of the loci included in the analysis.

DNA was extracted using a modified protocol based on Bardakci and Skibinski (1994). Pellets were

washed 2–3 times in STE extraction buffer (0.1 M NaCl, 0.05 M Tris-HCl, 0.01 M Na^2EDTA, pH 8.0) to remove all traces of medium. Resuspended pellets were digested for 14–18 h in 500 μl STE, 15 μl 20% SDS (solutions from Fisher Scientific) and 30 μl proteinase K (10 mg ml^{-1}; Roche Molecular Biochemicals) at 37 °C. DNA suspensions were subjected to a standard phenol-chloroform:isoamyl alcohol (24:1; Roche Molecular Biochemicals) extraction with a single phenol step. DNA pellets were resuspended in autoclaved double-deionized water and DNA concentration was determined using a GeneQuant RNA/DNA Calculator (Pharmacia LKB Biochrom Ltd.) with 320nm absorbance correction recommended by Ferrero et al. (1998).

All PCR amplifications were performed on a PTC-100HB-96V (tm) programmable thermocycler (MJ Research, Inc.) using disposable 0.2-ml microplates (Diamed Lab Supplies, Inc.). PCR cocktail preparation and electrophoretic gel separation were performed using fluorescently labeled primers (PCR reagents from Roche Molecular Biochemicals, labeled primers from Gibco BRL). Both the octaplex (the simultaneous amplification of eight SSR loci) and hexaplex (six loci) PCR systems of Fishback et al. (1999) were used to genotype the cell lines submitted for analysis in order to establish their identity. However, two loci were discarded from the hexaplex system due to anticipated age-related primer degradation or excessive length overlap with amplified fragments from another locus (Table 2). The remaining SSR loci used (Table 2) were all from salmonid species, and included seven derived from rainbow trout (*O. mykiss; Omy*), one from Atlantic salmon (*Salmo salar; Ssa*), one from sockeye (*O. nerka; One*) and one from chinook salmon (*O. tshawytscha; Ots*). Genotypes were determined using the method of Fishback et al. (1999): PCR products were separated by size on a Perkin-Elmer/Applied Biosystems Inc (PE/ABI) Prism™ 377 DNA sequencer using a 4.5% denaturing polyacrylamide gel (polyacrylamide from Fisher Scientific). Fragment size was determined using the software program GENESCAN™ 3.1 (PE/ABI) by comparison of fragment sizes with Genescan© −350 Tamra internal size standard (PE/ABI), which was run in each lane with PCR product. Allele peaks were visualized and genotypes collected using GENOTYPER™ 1.1r8 (PE/ABI) software (see Figure 1 for sample electropherogram) and allele identities were recorded as length in bp. Cell line genotype at each locus surveyed was determined and a common 'genetic fingerprint' for each line was determined, from which lines with identical genotypes were identified.

Results

The twelve cell lines and the ten SSR loci in this study are listed respectively in Tables 1 and 2. Nine cell lines were from rainbow trout (RT) and have been described previously. Two (RTP-91E and RTP91F) of these nine RT lines were from the same fish. An uncharacterized cell line, Clone 1A, was thought to have arisen from rainbow trout as well. The remaining two cell lines were from Chinook salmon embryo (CHSE-214) and Pacific herring larvae (PHL). Effectiveness of multiplex amplification was generally quite high (Figure 1).

All ten salmonid SSR loci were successfully amplified from the RT cell lines (Table 3). The multilocus genotype of the putative RT cell line, Clone 1A, was identical to that of CHSE-214 (Table 3; see also Figure 1). Four alleles at the locus *OmyFGT23TUF* amplified, indicating duplicated expression (i.e. a non-diploidised locus) at this locus for Clone 1A and CHSE-214. At four loci (*OmyFGT5TUF*, *OmyFGT14TUF*, *Omy77DU* and *Ots1BML*), the alleles in CHSE-214 were not found in any of the RT cell lines except the putative RT cell line, Clone 1A. The SSR loci did not amplify in PHL, with the exception of a single fragment (~69 bp) amplified with *Ssa20.19NUIG*; this was distinct from any of the alleles detected in the salmonid cell lines and was not consistently observed.

Except for the two pituitary cell lines from the same rainbow trout, the RT cell lines were clearly distinguished from one another by comparing SSR genotypes (Table 4). The minimum number of loci at which genotypic differences occurred between any pair of cell lines was five, which was the case for RTP-91E versus RTP-2. The maximum number of differences was ten, which occurred for several cell line pairs. RTP-91E and RTP-91F, developed from the same individual fish, were identical at all loci except one (*Ssa20.19NUIG*). Some loci were more useful than others in demonstrating the uniqueness of the cell lines. Each of the rainbow trout cell lines from a different fish was unique at *Ots1BML* (Table 3). *Omy77DU* also provided effective discrimination among the cell lines, with genotypes being different in each of the lines except two (Table 3).

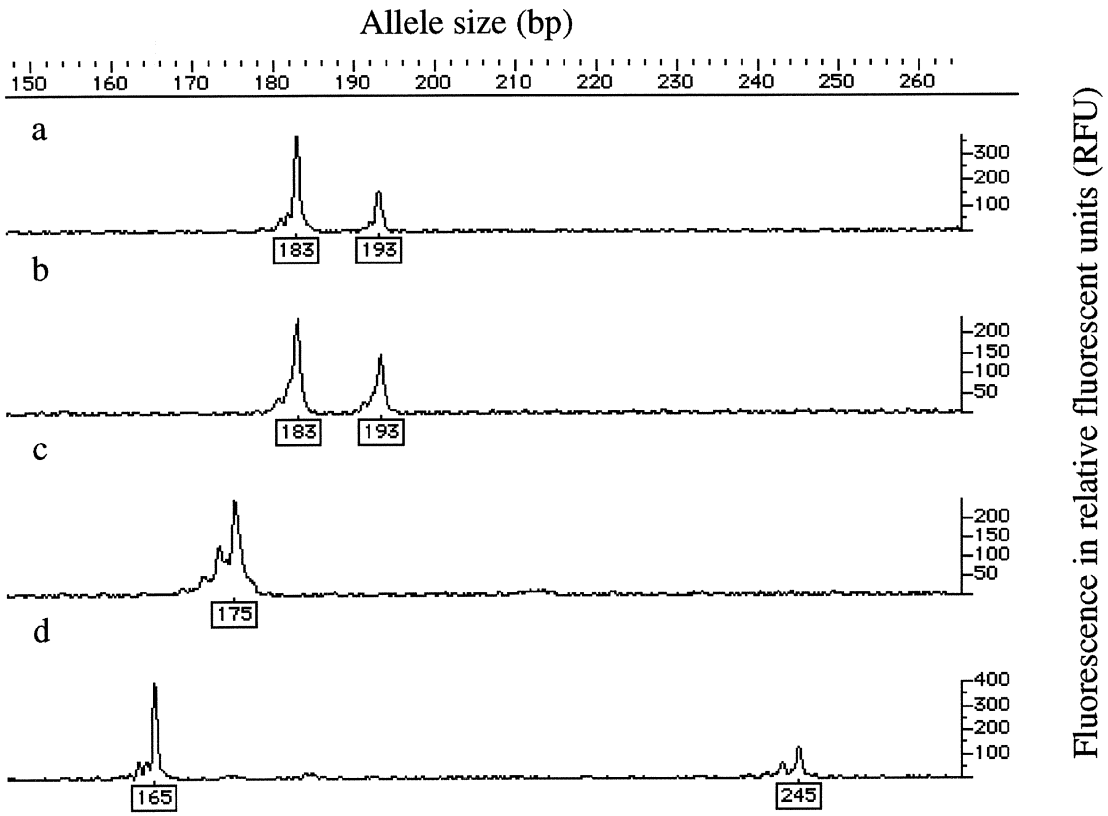

Figure 1. Electropherogram output from GENOTYPER™ 1.1r8 (PE/ABI) at the locus *Ots1BML* for four cell lines: a) Clone 1A, b) CHSE-214, c) RTH-149 and d) RTL-WI. Note identical genotype for Clone 1A and CHSE-214. Allele size in base pairs(bp) indicated on upper X-axis. Allelic size determined from first, strongest peak; subsequent peaks represent copying errors by *Taq* enzyme during PCR (see Newton and Graham (1997)). Fluorescent ouput for each locus measured in relative fluorescent units (RFU).

Clone 1A and CHSE-214 were identical at all loci (Table 4). The probability of this occurring by chance is extremely low. Genetic variability in actual salmonid populations is highly source population- and/or subpopulation-specific (see O'Reilly and Wright (1995), O'Reilly et al. (1996), Ferguson and Danzmann (1998)). In order to avoid bias from literature allele frequencies, we calculated the probability of identical genotypes occurring by chance (genetic identity) for two lines under a ''worst-case'' scenario of equal allelic frequency ($p = q = 0.5$). The probability of genetic identity due to random chance was therefore $(0.5)(0.5)^n$, where n is the number of heterozygous loci genotyped, or $(0.5)(0.5)^5 = 9.77 \times 10^{-4}$ assuming that the genetic signature observed for these two lines at *OmyFGT23TUF* was the result of a pair of duplicated loci. Homozygous loci were not included since it was considered possible that these SSR might be fixed in chinook salmon. However, the probability of both homeologous loci being identical in two lines presumptively from different species (rainbow trout and chinook salmon) is extremely low, and the calculated value thus represents an upper bound on the likelihood of genetic identity. Therefore, we conclude that Clone 1A arose from cell line cross-contamination and was actually CHSE-214. CHSE-214 was being grown in the laboratory during the period when the primary culture of rainbow trout peripheral blood cells (PBC) that led to Clone 1A was prepared. The routine laboratory practice was to use separate bottles of medium and solutions for each cell line, but a common bottle of L-15 might accidentally have been used to wash PBC cells. Prior contamination of this bottle with CHSE-214 would have led to their transfer to the primary culture.

An additional experiment was undertaken to evaluate this possibility. Some putative Clone-1A cells were suspended in L-15 without FBS, at a density of 100 cells ml^{-1}, and placed into a 100 ml glass bottle, which was put into a refrigerator at 5 °C. At seven

Table 2. Microsatellite loci used to characterize the cell lines. Primers were labeled with fluorescent dyes for the two multiplex sets as described in Fishback et al. (1999). Core repeat sequences are from the isolating clone used in each source reference; na: sequence not available.

SSR Locus	Forward primer	Core sequence	Reverse primer	Source
Omy325(A3)UoG[1,3]	GAACTTTGACTCCTCATTGTGAG	$(GT)_{22}$	Hex-CGGAGTCCGTATCCTTCCC	O'Connell et al. (1997)
Omy27DU[1]	TTTATGTCATGTCAGCCAGTG	na	Hex-TTTATGGCTGGCAACTAATGT	Heath et al. (in press)
One18ASC[1]	ATGGCTGCATCTAATGGAGAGTAA	$(CA)_{16}$	Hex-AAACCACACACACTGTACGCCAA	Scribner et al. (1996)
Ssa20.19NUIG[1,3]	TCAACCTGGTCTGCTTCGAC	$(CA)_{n}$	Fam-CTAGTTTCCCCAGCACAGCC	Sanchez et al. (1996)
OmyFGT23TUF[1]	(T. Sakamoto, pers. comm.)	$(GT)_{18}$	(T. Sakamoto, pers. comm.)	Sakamoto (1996)
OmyFGT14TUF[1]	(T. Sakamoto, pers. comm.)	$(CA)_{10}$	(T. Sakamoto, pers. comm.)	Sakamoto (1996)
OmyFGT5TUF[2]	TCCAGCCAGACACACACG	$(CA)_{26}$	Tet-TCCTTTTCTTCCCTTTCTTTCC	Sakamoto et al. (1994)
Omy77DU[2]	CGTTCTCTACTGAGTCAT	GGGGTCA $(GA)_5$ACAGGG$(GA)_{25}$	Tet-GTCTTTAAGGCTTCACTGCA	Morris et al. (1996)
OmyFGT15TUF[2]	(T. Sakamoto, pers. comm.)	$(GT)_8$	(T. Sakamoto, pers. comm.)	Sakamoto (1996)
Ots1BML[2]	Fam - GGAAAGAGCAGATGTTGTT	$(TG)_3N_8(TG)_3N_4(TG)_3N_2(TG)_7N_{16}(TG)_9$	TGAAGCAGCAGATAAAGCA	Banks et al. (1999)

[1]Group 1 fluorescent multiplex set, Fishback et al. (1999). *Omy325(A3)UoG* is modified from the original SSR sequence as reported by O'Connell et al. 1997 (see Fishback et al. 1999).
[2] Group 2 fluorescent multiplex set, Fishback et al. (1999). Two of the loci were dropped from the original hexaplex set. *Omy207UoG* failed to amplify (likely due to primer age) and *Omy301UoG* had excessive overlaps with *Ots1* (McDonald, unpub.)
[3] Primers labeled radioactively (γ^{33}P). *Omy325UoG* and *Ssa20.19NUIG* were originally amplified with both γ^{33}P labeled primer and with the Group 1 fluorescent multiplex to test for scoring differences between radioactive and fluorescent methodologies.

Table 3. Microsatellite genotypes of rainbow trout and chinook salmon cell lines.

Line	*Omy325 UoG*[1,3]	*Omy27 DU*[1]	*One18 ASC*[1]	*OmyFGT23 TUF*[1]	*OmyFGT14 TUF*[1]	*Ssa20.19 NUIG*[1,3]	*OmyFGT5 TUF*[2]	*Omy77 DU*[2]	*Ots1 BML*[2]	*OmyFGT15 TUF*[2]
RTG-2	122/122	105/107	174/180	103/103	208/208	79/83	174/184	100/106	163/167	161/161
RTH-149	134/134	107/107	174/178	97/113	204/204	75/81	212/212	120/122	175/175	159/159
RTL-W1	130/132	105/107	184/184	103/115	208/208	83/83	174/184	100/100	165/245	163/163
RTgill-W1	122/122	105/107	174/180	115/117	204/204	83/83	174/174	126/126	161/169	161/161
RTS-11	122/148	107/107	174/174	103/115	206/208	83/87	184/184	100/130	167/241	161/167
RTS-34st	122/138	105/107	174/174	115/117	204/208	79/83	174/178	100/116	165/167	161/161
RTP-2	132/138	107/107	174/174	103/103	204/204	83/83	174/174	96/96	165/165	161/161
RTP-91E	130/132	105/105	174/174	103/121	204/204	83/83	174/174	100/116	169/171	161/161
RTP-91F	130/132	105/105	174/174	103/121	204/204	83/85	174/174	100/116	169/171	161/161
CHSE-214	148/148	99/105	178/178	—[1]	234/234	83/85	208/208	136/136	185/193	161/161
Clone 1A	148/148	99/105	178/178	—[1]	234/234	83/85	208/208	136/136	185/193	161/161

[1]Clone 1A and CHSE-214 both appear as 149/135/121/111; locus may be duplicated.

Table 4. Comparison of genotypic identity between 11 salmonid cell lines at 10 microsatellite (SSR) loci. Values indicate number of SSR loci that exhibited identical genotypes between pairs of cell lines.

Cell line	RTG-2	RTH-149	RTL-W1	RTgill-W1	RTS-11	RTS-34st	RTP-2	RTP-91E	RTP-91F	CHSE-214	Clone 1A
RTG-2	10	—	—	—	—	—	—	—	—	—	—
RTH-149	0	10	—	—	—	—	—	—	—	—	—
RTL-W1	3	0	10	—	—	—	—	—	—	—	—
RTgill-W1	4	1	2	10	—	—	—	—	—	—	—
RTS-11	0	1	1	0	10	—	—	—	—	—	—
RTS-34st	3	0	1	3	1	10	—	—	—	—	—
RTP-2	2	2	1	4	2	2	10	—	—	—	—
RTP-91E	1	1	2	4	1	3	5	10	—	—	—
RTP-91F	1	1	1	3	1	3	4	9	10	—	—
CHSE-214	1	0	0	1	0	1	1	1	2	10	—
Clone 1A	1	0	0	1	0	1	1	1	2	10	10

day intervals for up to 4 weeks, the bottle was shaken and three ml aliquots of cell suspension removed and placed into 12.5-cm^2 culture flasks. FBS was added to give a final concentration of 10%, and the flasks incubated at 18 °C. They were examined by phase contrast microscopy on the following day for attached cells, and periodically for up to 3 weeks for the appearance of cell colonies. Even after 4 weeks of storage at 5 °C in serum-free medium, some Clone 1A cells were still capable of forming colonies.

The SSR profile for most cell lines appeared stable with cell culturing. For RTL-W1, cryopreserved samples from ten years earlier at 10 to 20 passages gave the same SSR profile as working cultures at 60 to 70 passages. Cells from working cultures of RTG-2 and RTH-149 also gave the same results as cryopreserved cells from ATCC. However, one polymorphism at *Ssa20.19NUIG* was noted between the two lines RTP-91E and RTP-19F (Tables 3 and 4), both of which originate from the same pituitary tissue in the same individual rainbow trout (Tom et al. 2001). Despite this genotypic difference, it seems highly likely that the genetic origin of these two lines was the same, since they were identical at all other loci. Using the method above, the likelihood of false genetic identity for the heterozygous loci at which RTP-91E and RTP-91F were identical is $(0.5)(0.5)^4 = 0.00391$; this estimate is excessively conservative given that both cell lines clearly seem to be rainbow trout lines and that homozygous loci would be the result of recombination, not fixation. In that event, the probability of false genetic identity decreases dramatically to $(0.5)(0.5)^9 = 3.81 \times 10^{-6}$. The use of equal allelic frequency values is reasonably conservative given observed variability in aquacultural (Fishback et al. 1999; McDonald 2001) and wild salmonid populations (Morris et al. 1996; Wenburg et al. 1996).

Discussion

SSR loci were superior to any of the previously

described methods for distinguishing between teleost cell lines. Techniques for authenticating fish cell lines have included cytotoxic-antibody assays and isozymes (Greene et al. 1966; Wester et al. 1975; Lidgerding et al. 1984; Lannan et al. 1984). None of these methods were able to distinguish cell lines from the same species. For example, isozyme profiles were successful in distinguishing cell lines from different teleost families but could not distinguish between all cell lines from different cyprinids (Lidgerding et al. 1984) or between cell lines from the same salmon species (Lannan et al. 1984). Analysis using SSR loci clearly illustrated the uniqueness of eight rainbow trout cell lines.

Instability of SSR locus genotypes over time could potentially limit their usefulness as identification markers of cell lines. However, the experience with mammalian cell lines is that DNA fingerprints are stable over long periods of routine passage (Kaplan and Hukku 1998). Several observations suggest that this might be the case with the rainbow trout cell lines as well. The genotypes of a working RTL-W1 culture and of cryopreserved RTL-W1 were identical at all 10 loci. Likewise, the genotype for a cryovial of RTG-2 cells obtained directly from ATCC was the same as for RTG-2 that had been grown in the laboratory for several years. A possible example of instability was the case of RTP-91E and RTP-91F, which were obtained from the same fish, but differed in one locus (*Ssa20.19NUIG*). At this locus RTP-91F but not RTP-91E was heterozygous. This may have been due to a mutational event in one of the original lines followed by an increase in frequency to fixation or near fixation. Selection of small volumes of culture for cell passage might have promoted the likelihood of such an event. The development of hemizygosity for several loci has been reported for sublines of the human cell line, HeLa (Yan et al. 1996). Our results suggest that SSR stability is high for salmonid cell lines, but emphasises the need for multilocus analysis to account for occasional mutation due to the considerable amount of single-cell replication followed by cell passage, which might act as a founder event for genetic variants at low frequency in the culture population. Although RTG-2 and CHSE-214 were derived from several pooled embryos (Wolf and Quimby 1962; Lannan et al. 1984), there was a very low incidence of multiallelic loci in these two lines (with an exception for *OmyFGT23TUF*, which may be unreduced from the ancestral duplicated state). This suggests that these lines are now each composed of cells originating from a single individual, although cells from other individuals might be present at levels undetectable by SSR PCR (i.e. 5–10%; see Markovic and Markovic (1998)). Differences in cell multiplication between individuals coupled with extensive culturing and cell passage may have driven certain lines to fixation by selection and the disproportionate founding of new cultures from certain cell lines.

As well as illustrating the power of DNA profiling, the example of CCLC uncovered here illustrates the need for vigilance in maintaining good laboratory procedures, which have been detailed for fish cell lines by Lannan (1994). A retrospective view of how the confusion over Clone 1A arose points to contamination of the initial primary culture with CHSE-214 through the use of a common bottle of the basal medium, L-15. Two routine laboratory practices should have ensured that this would not have happened. Separate bottles of media and buffers should have been used exclusively for each cell line, and pipettes that had been used to pipette cells should never have been dipped back into a bottle of medium or buffer. Not following these procedures appears to be particularly critical for CHSE-214, and possibly for other fish cell lines. This is because CHSE-214 survived in a bottle of unsupplemented L-15 at 5 °C for several weeks.

The subject of CCLC likely could become more important in the future as more fish cell lines become available and are carried by single laboratories, but DNA profiling with SSR should help identify problems. As of 1994, the existence of 159 known cell lines derived from 34 families of bony fish were reported, with an indeterminate number of additional unreported lines also in existence (Fryer and Lannan 1994). The largest number of fish cell lines (36) were derived from salmonids (Osteichthyes: Salmonidae). Rainbow trout account for roughly 40% of salmonid cultures (Fryer and Lannan 1994), the highest taxonomic representation overall. The predominance of salmonid cell lines in general (and rainbow trout lines in particular) indicates the usefulness of our system. The ten SSR loci used in this study demonstrated the uniqueness of different rainbow trout cell lines. Rapid generation of multilocus genetic information was also made possible by the use of multiplexed PCR reactions. However, as a routine procedure, probably fewer loci could be used as some loci (*One18ASC*, *Omy27DU*) showed few differences. A particularly powerful locus was *Omy77DU*, which revealed different genotypes in each of the eight cell lines. With

the rapid pace of development of SSR and other DNA markers for other fish taxa (O'Connell et al. 1997; Roy et al. 2000), probes should become available to conduct DNA profiling on cell lines from a wide range of fish species.

Acknowledgements

The research was supported by grants from the Natural Science and Engineering Research Council of Canada (NSERC) to MMF and NCB.

References

Babich H. and Borenfreund E. 1991. Cytotoxicity and genotoxicity assays with cultured fish cells: a review. Toxic. In Vitro. 5: 91–100.

Banks M.A., Blouin M.S., Baldwin B.A., Rashbrook V.K., Fitzgerald H.A., Blankenship S.M. et al. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tschawytscha*). J. Hered. 90: 281–288.

Bardakci F. and Skibinski D.O.F. 1994. Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity 73: 117–123.

Bols N.C. 1991. Biotechnology and aquaculture: The role of cell cultures. Biotech. Adv. 9: 31–49.

Bols N.C. and Lee L.E.J. 1993. Cell lines: availability, propagation and isolation. In: Hochachka P.W. and Mommsen T.P. (eds), Biochemistry and Molecular Biology of Fishes Vol. 3. Elsevier, Amsterdam, pp. 145–159.

Bols N.C., Barlian A., Chirino-Trejo S.J., Caldwell S.J., Goegan P. and Lee L.E.J. 1994. Development of a cell line from primary cultures of rainbow trout, *Oncorhynchus mykiss*, gills. J. Fish Dis. 17: 601–611.

Bols N.C., Yang B.Y., Lee L.E. and Chen T.T. 1995. Development of a rainbow trout pituitary cell line that expresses growth hormone, prolactin, and somatolactin. Mol. Mar. Biol. Biotechnol. 4: 154–163.

Bols N.C., Whyte J., Clemons J., Tom D., van den Heuvel M. and Dixon D.G. 1997. Use of liver cell lines to develop toxic equivalency factors and to derive toxic equivalent concentrations in environmental samples. In: Zelikoff J.T. (ed.), Ecotoxicology: Responses, Biomarkers and Risk Assessment. SOS Publications, Fair Haven, pp. 329–350.

Dirks W., MacLeod R.A., Jager K., Milch H. and Drexler H.G. 1999. First searchable database for DNA profiles of human cell lines: sequential use of fingerprint techniques for authentication. Cell. Mol. Biol. 45: 841–853.

Ferrero M., Castaño A., Gonzalez A., Sanz F. and Becerril C. 1998. Characterization of RTG-2 fish cell line by random amplified polymorphic DNA. Ecotoxicol. Environ. Saf. 40: 56–64.

Ferguson M.M. and Danzmann R.G. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? Can. J. Fish Aquat. Sci. 55: 1553–1563.

Fishback A.G., Danzmann R.G., Sakamoto T. and Ferguson M.M. 1999. Optimization of semi-automated microsatellite multiplex Polymerase Chain Reaction systems for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 172: 247–254.

Fryer J.L. and Lannan C.N. 1994. Three decades of fish cell culture: a current listing of cell lines derived from fishes. J. Tissue Cult. Meth. 16: 87–94.

Fryer J.L., McCain B.B. and Leong J.C. 1981. A cell line derived from rainbow trout (*Salmo gairdneri*) hepatoma. Fish Pathol. 15: 193–200.

Ganassin R.C. and Bols N.C. 1998. Development of a monocyte/macrophage-like cell line, RTS11, from rainbow trout spleen. Fish & Shellfish Immunol. 8: 457–476.

Ganassin R.C. and Bols N.C. 1999. A stromal cell line from rainbow trout spleen, RTS34ST, that supports the growth of rainbow trout macrophages and produces conditioned medium with mitogenic effects on leukocytes. In Vitro Cell. Dev. Biol. 35: 80–86.

Ganassin R.C., Sanders S.M., Kennedy C.J., Joyce E.M. and Bols N.C. 1999. Development and characterization of a cell line from Pacific herring,*Clupea harengus* pallasi, sensitive to both naphthalene cytotoxicity and infection by viral hemorrhagic septicemia virus. Cell. Biol. Toxicol. 15: 99–309.

Ganassin R.C., Schirmer K. and Bols N.C. 2000. Cell and tissue culture. In: Ostrander K.G. (ed.), The Laboratory Fish. Academic Press, New York, pp. 631–651.

Greene A.E., Goldner H. and Coriell L.L. 1966. The species identification of poikilothermic tissue culture cells by the cytotoxic antibody test. Growth 30: 305–313.

Heath D.D., Pollard S. and Herbinger C.M. Genetic differentiation and isolation by distance in steelhead trout (*Oncorhynchus mykiss*) populations in British Columbia. Heredity (in press).

Hightower L.E. and Renfro J.L. 1988. Recent applications of fish cell culture to biomedical research. J. Exp. Zool. 248: 290–302.

Kaplan J. and Hukku B. 1998. Cell line characterization and authentication. Methods Cell. Biol. 57: 203–216.

King B., Lichtenstein A., Berenson J. and Kacinski B.M. 1994. Polymerase chain reaction-based microsatellite typing assay used for tumor cell line identification. Amer. J. Path. 144: 486–491.

Lannan C.N. 1994. Fish cell culture: a protocol for quality control. J. Tissue Cult. Meth. 16: 95–98.

Lannan C.N., Winton J.R. and Fryer J.L. 1984. Fish cell lines: Establishment and characterization of nine cell lines from salmonids. In Vitro 20: 671–676.

Lee L.E.J., Clemons J.H., Bechtel D.G., Caldwell S.J., Han K.B., Pasitschniak-Arts M. et al. 1993. Development and characterization of a rainbow trout liver cell line expressing cytochrome P450-dependent monooxygenase activity. Cell. Biol. Toxicol. 9: 279–294.

Li F., An H., Seymour T.A., Bradford C.S., Morrissey M.T., Bailey G.S. et al. 1998. Molecular cloning, sequence analysis and expression distribution of rainbow trout (*Oncorhynchus mykiss*) cystatin C. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 121: 135–143.

Lidgerding B.C., Phelps S.R. and Schill W.B. 1984. Fish cell lines: characterization by isozyme analysis. In Vitro 20: 167–171.

MacLeod R.A., Dirks W.G., Matsuo Y., Kaufmann M., Milch H. and Drexler H.G. 1999. Widespread intraspecies cross-contamination of human tumor cell lines arising at source. Int. J. Cancer 83: 555–563.

Markovic O. and Markovic N. 1998. Cell cross-contamination in

cell cultures: the silent and neglected danger. In Vitro Cell. Dev. Biol. 34: 1–8.

Matsuo Y., Nishizaki C. and Drexler H.G. 1999. Efficient DNA fingerprinting method for the identification of cross-culture contamination of cell lines. Hum. Cell 12: 149–154.

McDonald G.J. 2001. Relatedness determination and detection of spawning time QTL in rainbow trout (*Oncorhynchus mykiss*), M.Sc., University of Guelph, Department of Zoology, Guelph, ON, Canada.

Morris D.B., Richard K.R. and Wright J.M. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Can. J. Fish Aquat. Sci. 53: 120–126.

Nelson-Rees W.A., Flandermeyer R.R. and Daniels D. 1980. T-1 cells are HeLa and not from normal human kidney origin. Science 209: 719–720.

Newton C.R. and Graham A. 1997. PCR: second edition. Springer-Verlag, New York.

O'Connell M., Danzmann R.G., Cornuet J.M., Wright J.M. and Ferguson M.M. 1997. Differentiation of rainbow trout populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. Can. J. Fish Aquat. Sci. 54: 1391–1399.

O'Reilly P.T. and Wright J.M. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish Biol. 47: 29–55.

O'Reilly P.T., Hamilton L.C., McConnell S.K. and Wright J.M. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotideand tetranucleotide microsatellites. Can. J. Fish Aquat. Sci. 53: 2292–2298.

Park L.K. and Moran P. 1994. Developments in molecular genetic techniques in fisheries. Rev. Fish Biol. Fisheries 4: 272–299.

Roy N.K., Maceda L. and Wirgin I. 2000. Isolation of microsatellites in striped bass *Morone saxatilis* (Teleostei) and their preliminary use in population identification. Mol. Ecol. 9: 827–829.

Sakamoto T. 1996. Study for the introduction of positional cloning method using DNA markers in fisheries science, PhD, Tokyo University of Fisheries.

Sakamoto T., Okamoto N. and Ikeda Y. 1994. Dinucleotide repeat polymorphism of rainbow trout, *FGT5*. J. Anim. Sci. 72: 27–68.

Sanchez J.A., Clabby C., Ramos D., Blanco G., Flavin F., Vazquez E. et al. 1996. Protein and microsatellite single locus variability in *Salmo salar* L (Atlantic salmon). Heredity 77: 423–432.

Scribner K.T., Gust J.R. and Fields R.L. 1996. Isolation and characterization of novel microsatellite loci: Cross-species amplification and population genetics applications. Can. J. Fish Aquat. Sci. 53: 841–883.

Segner H. 1998. Fish cell lines as a tool in aquatic toxicology. In: Braunbeck T., Hinton D.E. and Streit B. (eds), Ecotoxicology. Birkhäuser, Basel, pp. 1–38.

Tom D.J., Lee L.E.J., Lew J. and Bols N.C. 2001. Induction of 7-ethoxyresorufin-o-deethylase activity by planar chlorinated hydrocarbons and polycyclic aromatic hydrocarbons in cell lines from the rainbow trout pituitary. Comp. Biochem. Physiol A. Mol. Integr. Physiol. 128: 185–198.

Wenburg J.K., Olsen J.B. and Bentzen P. 1996. Multiplexed systems of microsatellites for genetic analysis in coastal cutthroat trout (*Oncorhynchus clarki clarki*) and steelhead (*Oncorhynchus mykiss*). Mol. Mar. Biol. Biotechnol. 5: 273–283.

Wester W., Ellender R.D. and Brundage B. 1975. Characterization of lactic dehydrogenase isoenzymes from 6 fish cell lines. J. Miss. Acad Sci. 20: 47–47.

Wolf K. 1988. Fish Viruses and Fish Viral Diseases. Comstock Publishing Associates, Ithaca.

Wolf K. and Quimby M.C. 1962. Established eurythermic line of fish cells *in vitro*. Science 135: 1065–1066.

Yan Y., Ottenbreit M., Hukku B., Mally M., Chou S. and Kaplan J. 1996. DNA fingerpirnting of human cell lines using PCR amplification of fragment length polymorphisms. In Vitro Cell Dev. Biol. 32: 656–662.