Developing a real time LAMP assay for *P. perurans* – penside molecular diagnostics

and

An *in vitro* model for studying the innate immune response to *P. perurans* in gill cells

Amanda Bayley, Susie Gunning, Irene Cano-Cejas, Richard Paley*

Aquatic Animal Disease, Centre for Environment, Fisheries and Aquaculture Science, Weymouth, UK,
richard.paley@cefas.co.uk

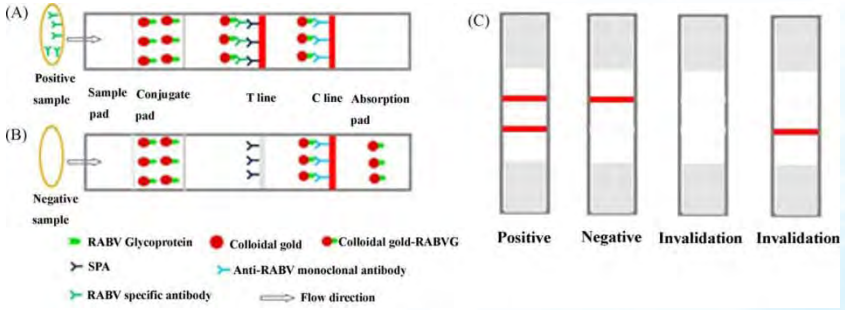
Cefas

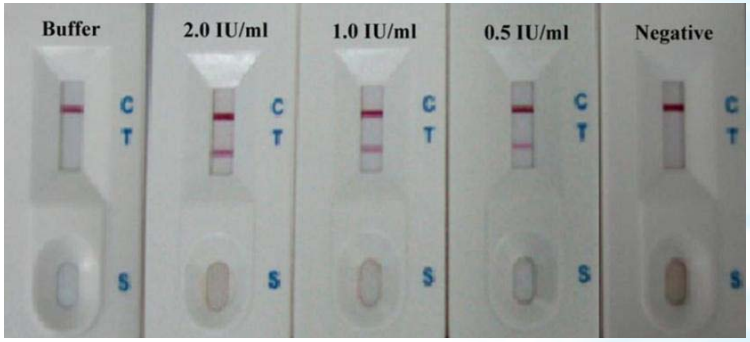
Pond-side tests

- Pen-side, Point of Care
- Rapid, easy to perform, cheap
- Provide opportunity for quicker decisions and management action
- OIE, EFSA reports promoting their investigation, development and potential use.

Pond-side tests

- Lateral flow devices
- Antibody based
- Often suitable but sometimes issues of specificity (cross reactivity) and sensitivity





Rabies

Wang *et al*., 2010

Cefas

Nucleic acid based tests – taking PCR out of the lab

Smiths, Bio-Seeq Vet Lab

Idaho Technology, RAZOR

Cepheid, GeneXpert

Enigma, Mini laboratory

Cefas

epistem

Genedrive®

Roche

cobas® Liat System

Cefas

LAMP - Loop mediated isothermal amplification

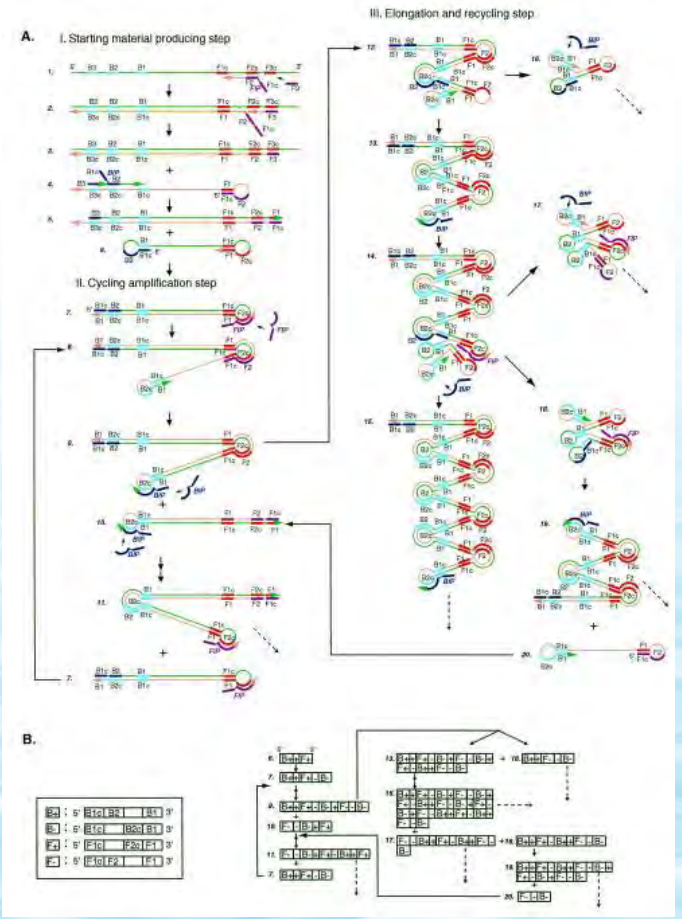
Nucleic Acids Research, 2000, Vol. 28, No. 12 e63

Loop-mediated isothermal amplification of DNA

Tsugunori Notomi[1,3,*], Hiroto Okayama[2], Harumi Masubuchi[1], Toshihiro Yonekawa[1], Keiko Watanabe[1], Nobuyuki Amino[3] and Tetsu Hase[1]

[1]Eiken Chemical Co. Ltd, 1381-3 Shimoishigami, Ohtawara, Tochigi 324-0036, Japan, [2]Department of Biochemistry and Molecular Biology, The University of Tokyo, Graduate School of Medicine, Bunkyo-ku, Tokyo 113-0033, Japan and [3]Department of Laboratory Medicine, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Received February 1, 2000; Revised April 8, 2000; Accepted April 15, 2000

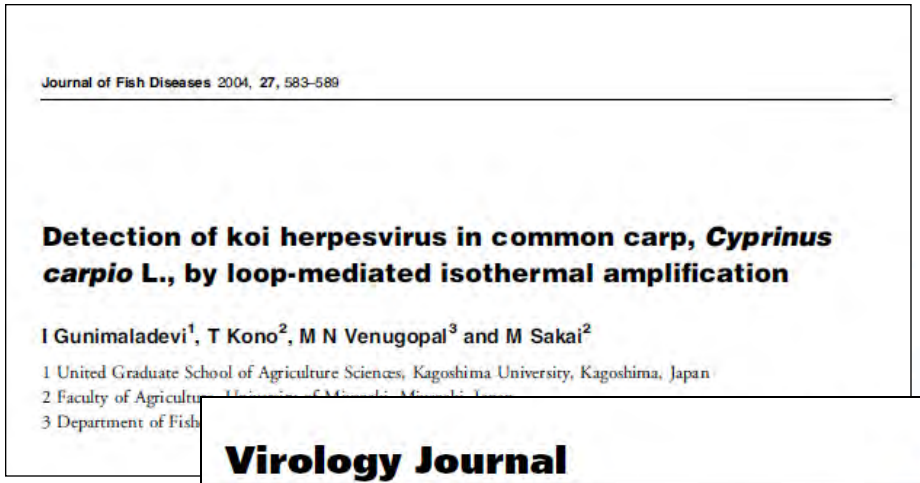


- 60-65 ˚C – *Bst* polymerase
- Rapid
- Specific
- Sensitive
- Simple equipment

Eiken Chemical Company

Cefas

LAMP assays for KHV



Journal of Fish Diseases 2004, 27, 583–589

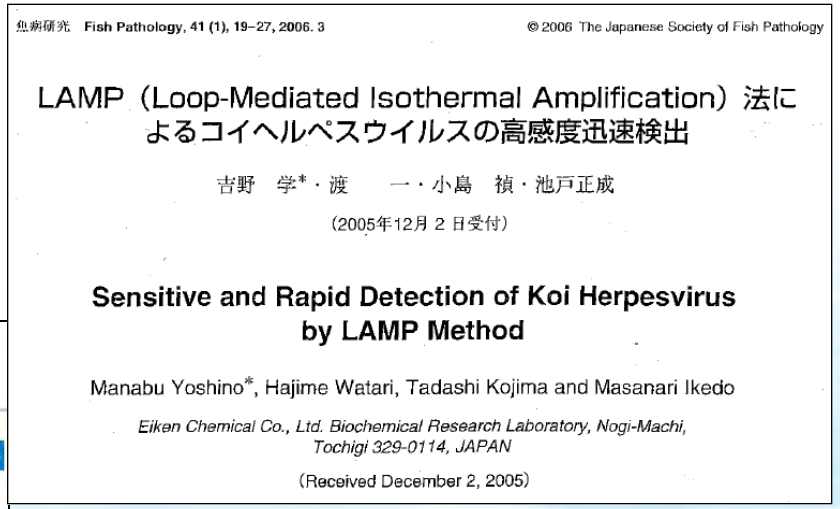
Detection of koi herpesvirus in common carp, *Cyprinus carpio* L., by loop-mediated isothermal amplification

I Gunimaladevi[1], T Kono[2], M N Venugopal[3] and M Sakai[2]

1 United Graduate School of Agriculture Sciences, Kagoshima University, Kagoshima, Japan

2 Faculty of Agricultu

3 Department of Fish



魚病研究 Fish Pathology, 41 (1), 19–27, 2006. 3 © 2006 The Japanese Society of Fish Pathology

LAMP（Loop-Mediated Isothermal Amplification）法によるコイヘルペスウイルスの高感度迅速検出

吉野 学*・渡 一・小島 禎・池戸正成

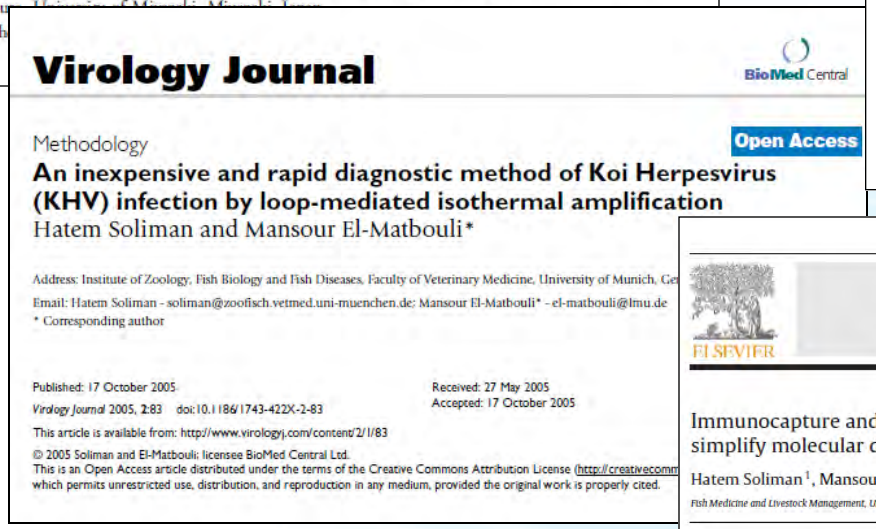
（2005年12月 2 日受付）

Sensitive and Rapid Detection of Koi Herpesvirus by LAMP Method

Manabu Yoshino*, Hajime Watari, Tadashi Kojima and Masanari Ikedo

Eiken Chemical Co., Ltd. Biochemical Research Laboratory, Nogi-Machi, Tochigi 329-0114, JAPAN

(Received December 2, 2005)



Virology Journal

BioMed Central

Methodology

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An inexpensive and rapid diagnostic method of Koi Herpesvirus (KHV) infection by loop-mediated isothermal amplification

Hatem Soliman and Mansour El-Matbouli*

Address: Institute of Zoology, Fish Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Munich, Ge

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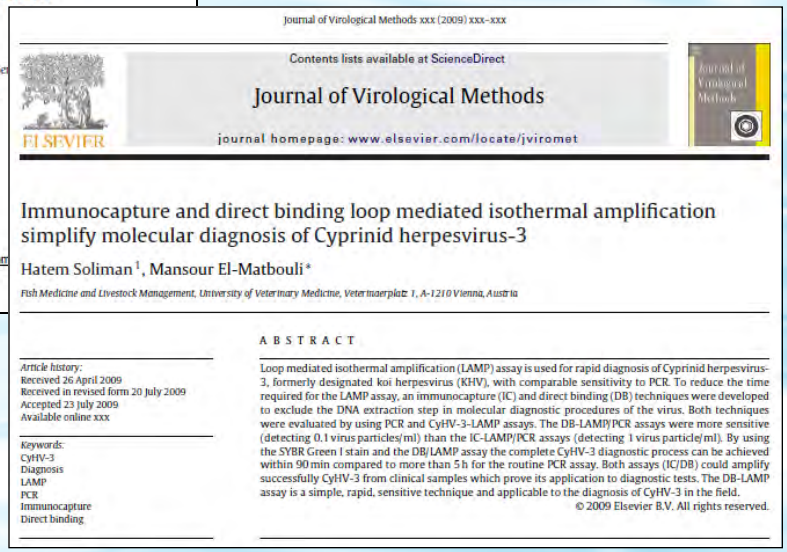

Journal of Virological Methods xxx (2009) xxx–xxx

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journal homepage: www.elsevier.com/locate/jviromet

ELSEVIER

Immunocapture and direct binding loop mediated isothermal amplification simplify molecular diagnosis of Cyprinid herpesvirus-3

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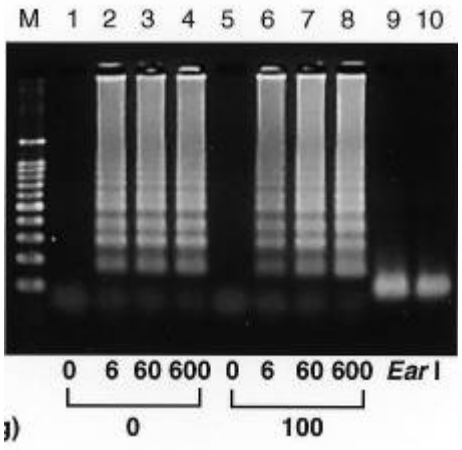
Keywords:
CyHV-3
Diagnosis
LAMP
PCR
Immunocapture
Direct binding

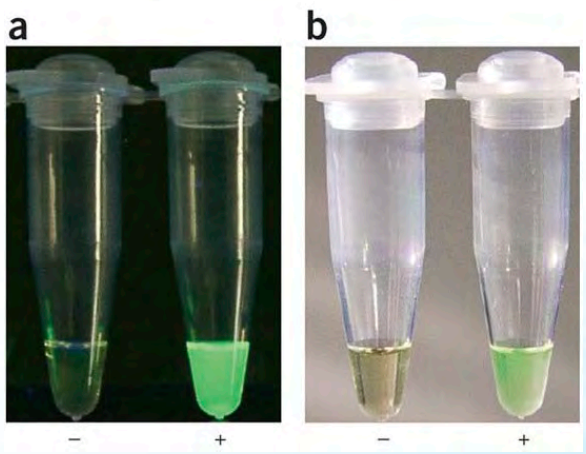
ABSTRACT

Loop mediated isothermal amplification (LAMP) assay is used for rapid diagnosis of Cyprinid herpesvirus-3, formerly designated koi herpesvirus (KHV), with comparable sensitivity to PCR. To reduce the time required for the LAMP assay, an immunocapture (IC) and direct binding (DB) techniques were developed to exclude the DNA extraction step in molecular diagnostic procedures of the virus. Both techniques were evaluated by using PCR and CyHV-3-LAMP assays. The DB-LAMP/PCR assays were more sensitive (detecting 0.1 virus particles/ml) than the IC-LAMP/PCR assays (detecting 1 virus particle/ml). By using the SYBR Green I stain and the DB/LAMP assay the complete CyHV-3 diagnostic process can be achieved within 90 min compared to more than 5 h for the routine PCR assay. Both assays (IC/DB) could amplify successfully CyHV-3 from clinical samples which prove its application to diagnostic tests. The DB-LAMP assay is a simple, rapid, sensitive technique and applicable to the diagnosis of CyHV-3 in the field.

Cefas

Visualisation

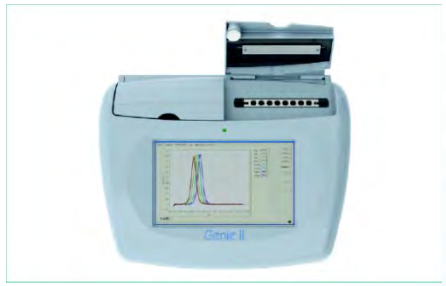




- Gel electrophoresis, end point in tube fluorescence or turbidity
- Varied sensitivities at least equal to standard PCR
- 60-90 min. run + gel running time

Cefas

Real time LAMP apparatus



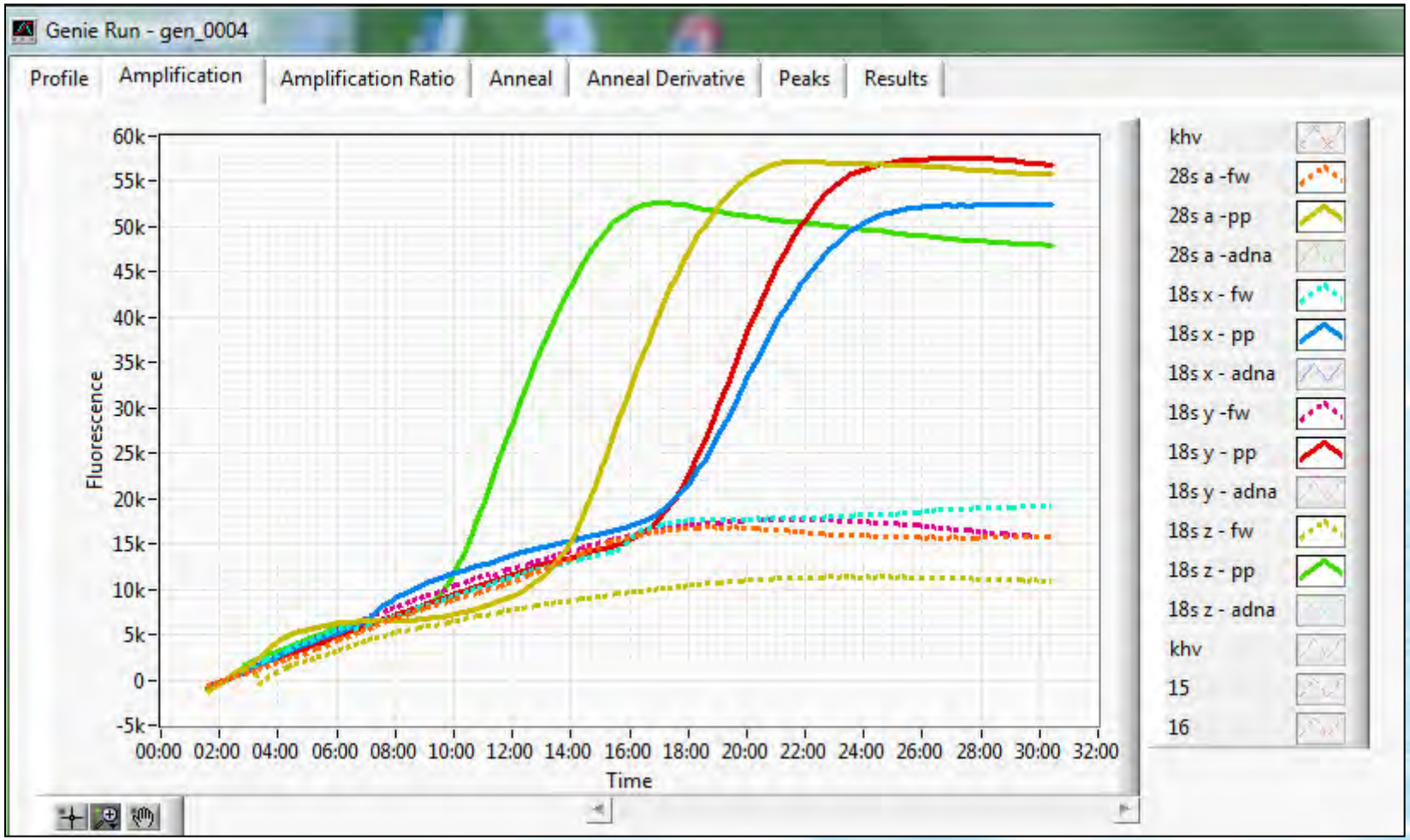
OptiGene

- Genie I, II and III
- Programmable gradient heating blocks
- Fluorimeter
- Intercalating dye fluorescence
- Real time analysis
- Small portable, field based.
- Proprietary mastermix including recombinant polymerase (Geobacillus sp)
- Closed tube (limited potential for contamination)
- Bluetooth and Wifi enabled

Cefas

Paramoeba perurans assays – 28s and 18s rRNA

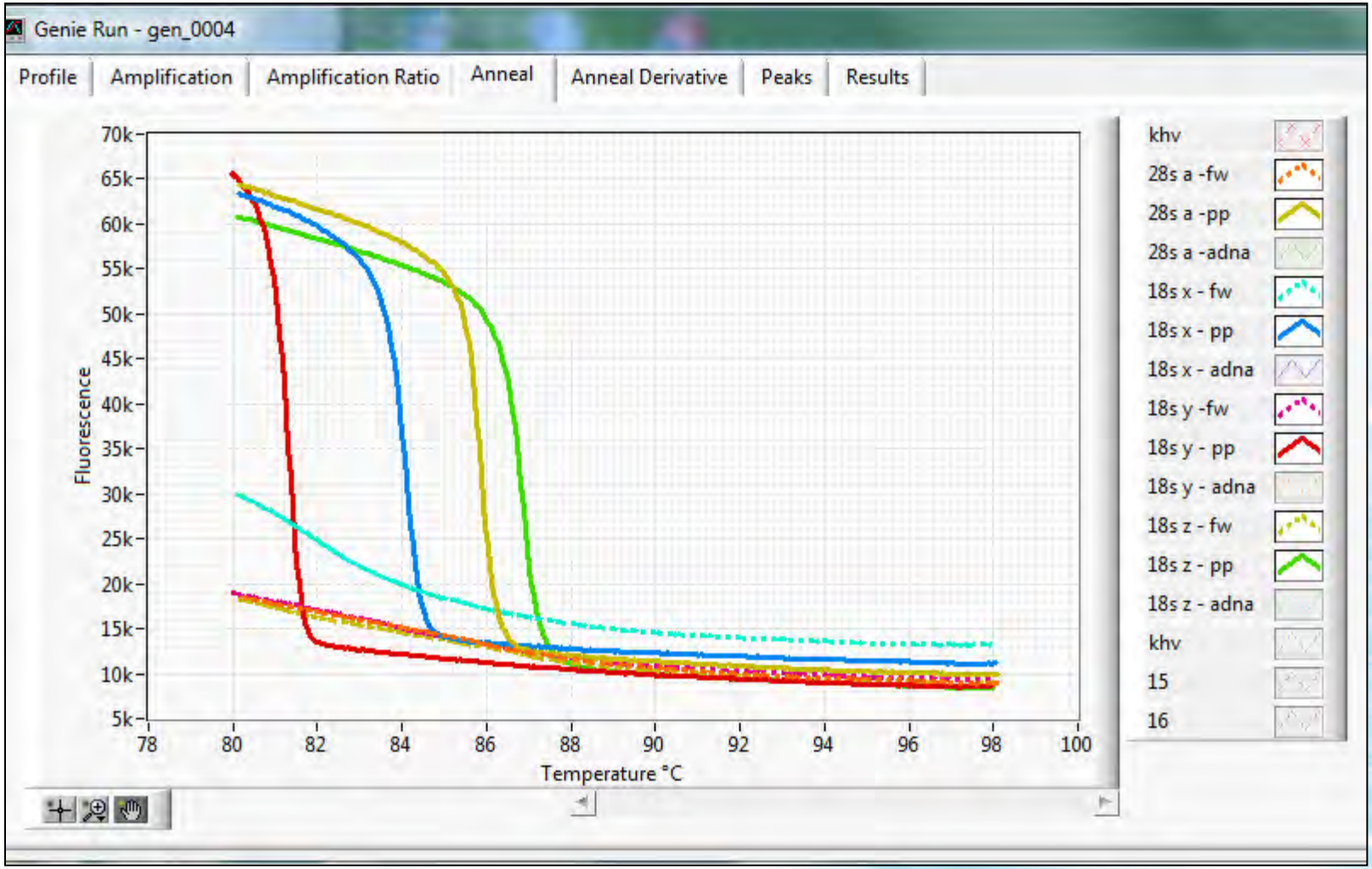
- Amplification



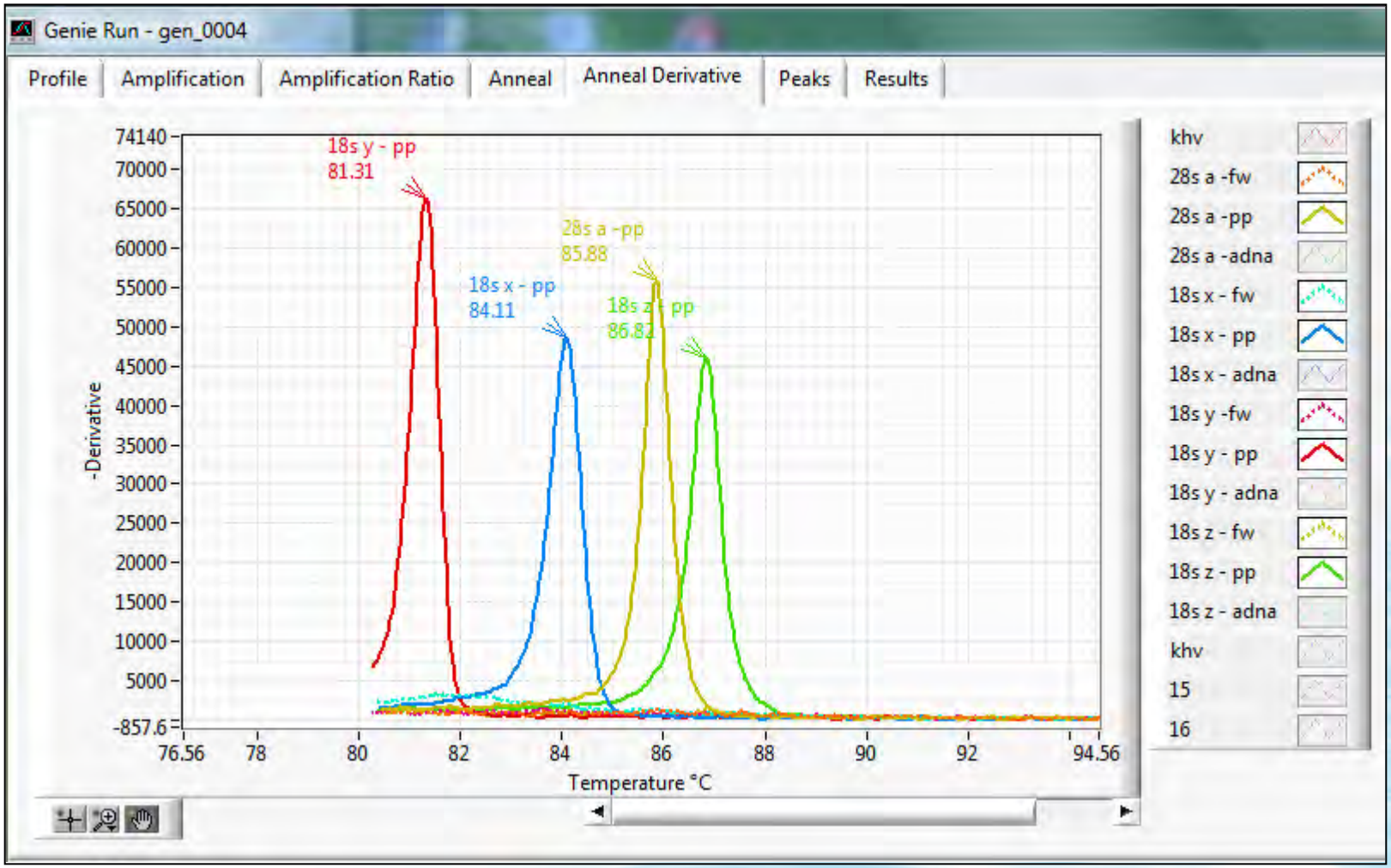
Courtesy - Susie Gunning

Cefas

- Anneal/melt temperature

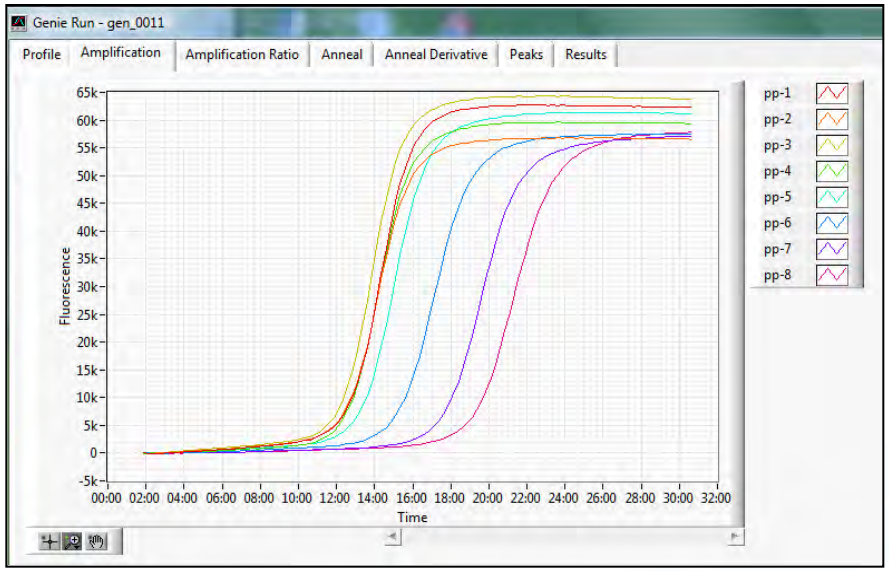


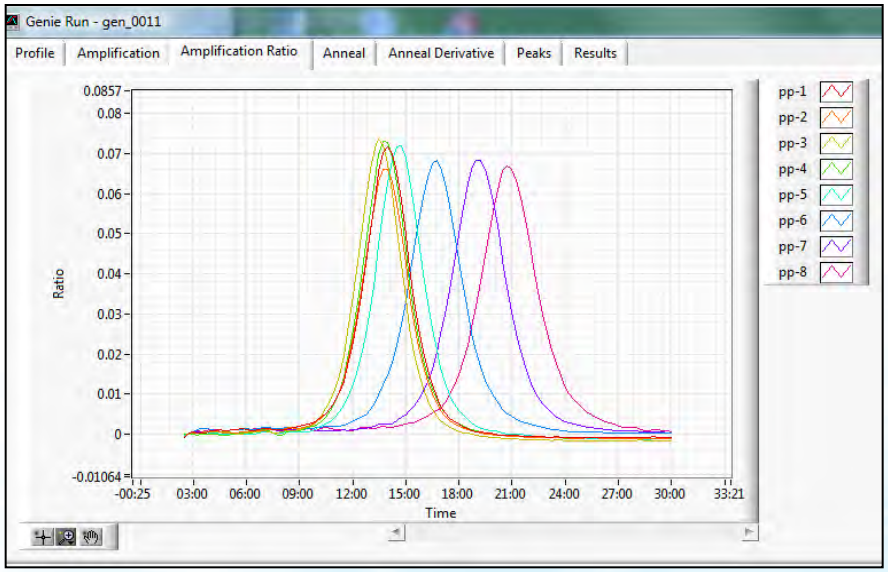
- Anneal/melt temperature

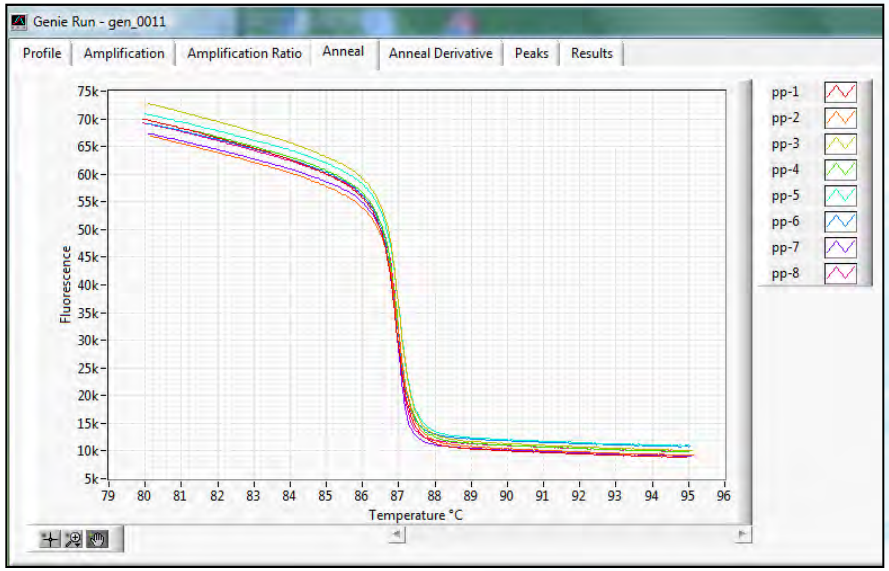


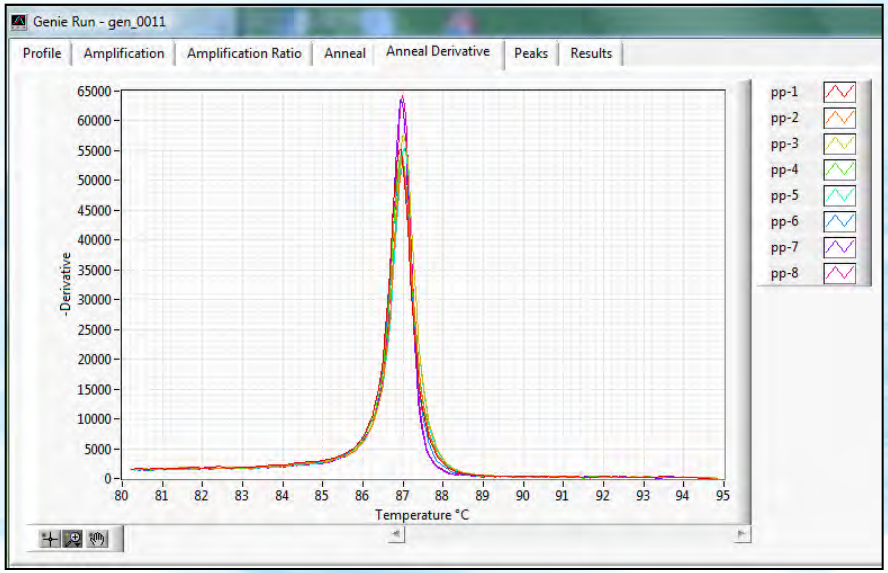
Cefas

Gradients to optimise isothermal amplification



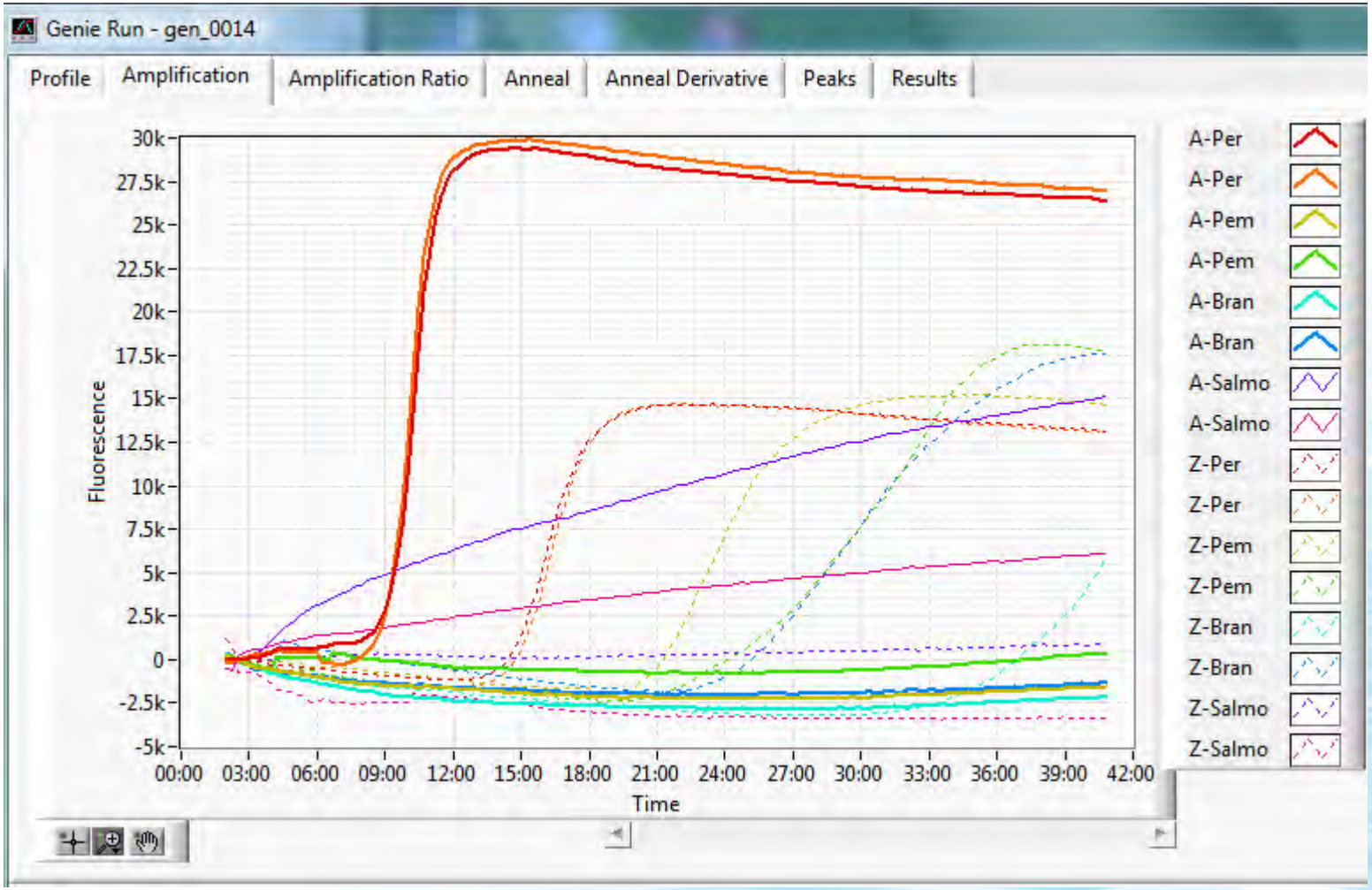






Cefas

Specificity



Cefas

Other isothermal technologies

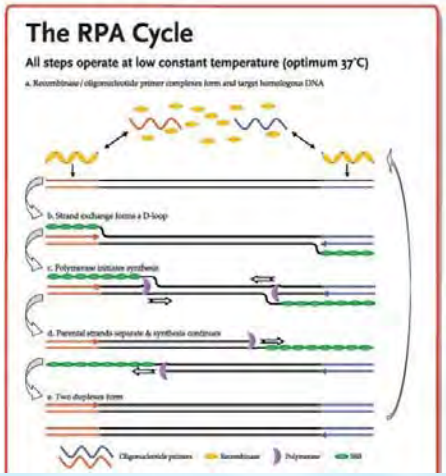
TwistDx

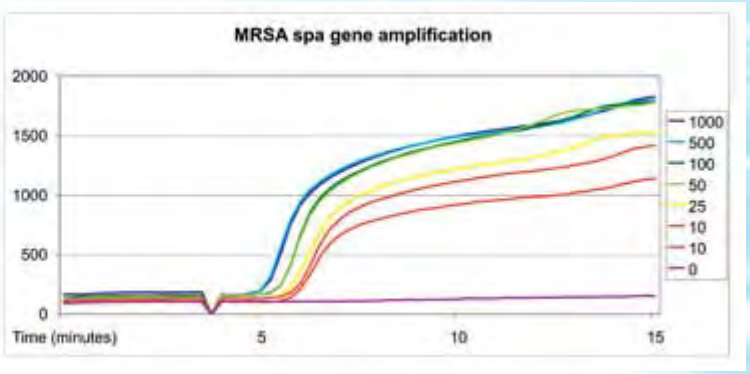
Based on recombinase + polymerase activity

37 ºC

TwistAmp™ exo

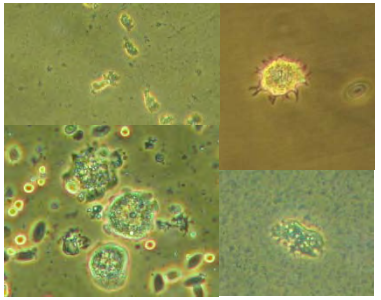
Twista™ portable real-time fluorometer

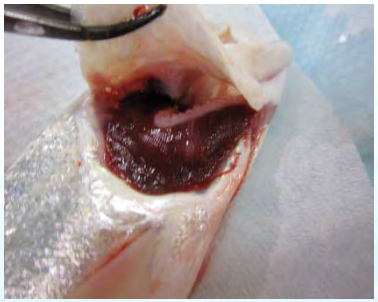


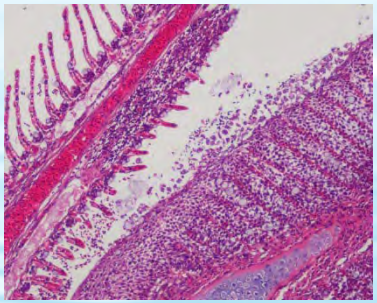


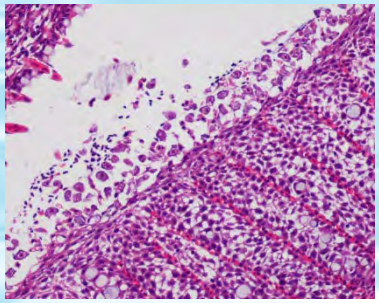
Cefas

Future work:









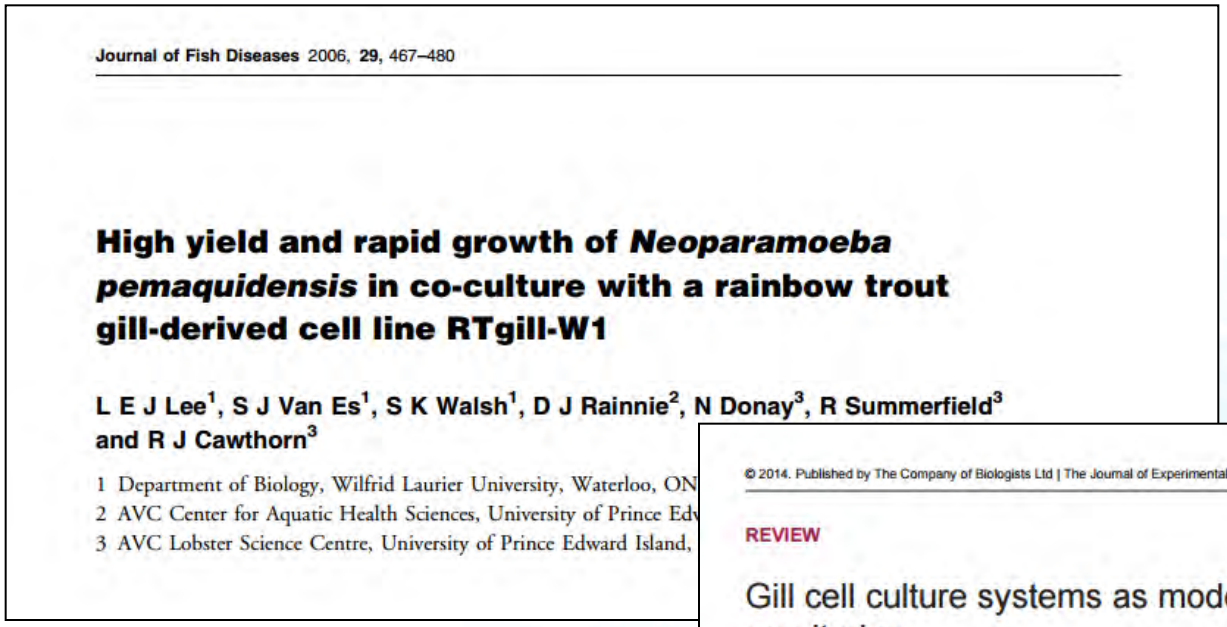
- H2020
- Finalise assay development
- Confirm analytical specificity and sensitivity
- Compare with qPCR
- Sample preparation
- Semi-quantification
- Test on challenge derived samples

- Field testing for diagnostic specificity and sensitivity and utility – industry collaboration welcomed

OptiGene

Cefas

An *in vitro* model for studying the innate immune response to *P. perurans* in gill cells

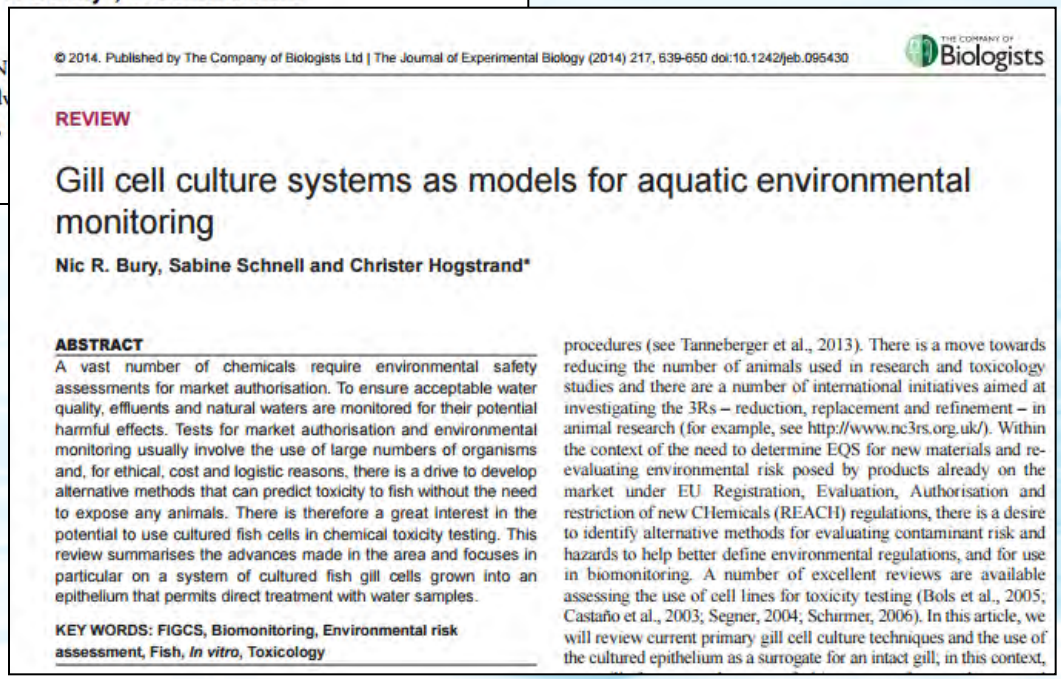


Journal of Fish Diseases 2006, 29, 467–480

High yield and rapid growth of *Neoparamoeba pemaquidensis* in co-culture with a rainbow trout gill-derived cell line RTgill-W1

L E J Lee[1], S J Van Es[1], S K Walsh[1], D J Rainnie[2], N Donay[3], R Summerfield[3] and R J Cawthorn[3]

1 Department of Biology, Wilfrid Laurier University, Waterloo, ON
2 AVC Center for Aquatic Health Sciences, University of Prince Ed
3 AVC Lobster Science Centre, University of Prince Edward Island,



© 2014. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2014) 217, 639-650 doi:10.1242/jeb.095430

REVIEW

Gill cell culture systems as models for aquatic environmental monitoring

Nic R. Bury, Sabine Schnell and Christer Hogstrand*

ABSTRACT

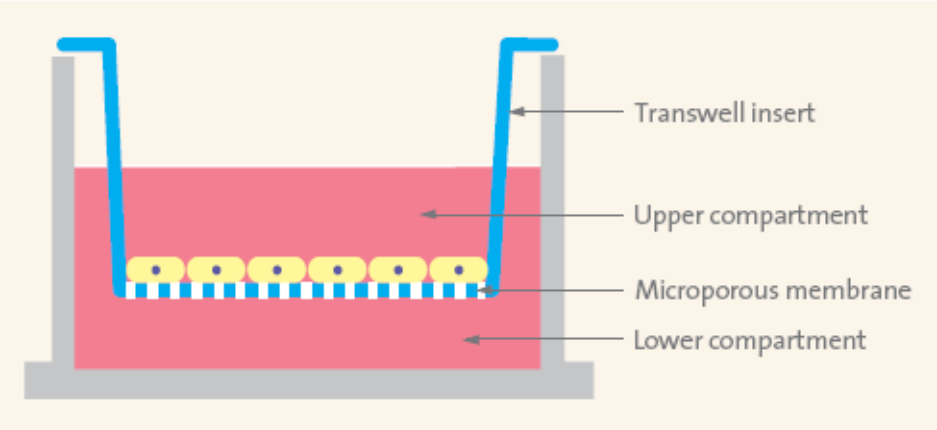
A vast number of chemicals require environmental safety assessments for market authorisation. To ensure acceptable water quality, effluents and natural waters are monitored for their potential harmful effects. Tests for market authorisation and environmental monitoring usually involve the use of large numbers of organisms and, for ethical, cost and logistic reasons, there is a drive to develop alternative methods that can predict toxicity to fish without the need to expose any animals. There is therefore a great interest in the potential to use cultured fish cells in chemical toxicity testing. This review summarises the advances made in the area and focuses in particular on a system of cultured fish gill cells grown into an epithelium that permits direct treatment with water samples.

KEY WORDS: FIGCS, Biomonitoring, Environmental risk assessment, Fish, *In vitro*, Toxicology

procedures (see Tanneberger et al., 2013). There is a move towards reducing the number of animals used in research and toxicology studies and there are a number of international initiatives aimed at investigating the 3Rs – reduction, replacement and refinement – in animal research (for example, see http://www.nc3rs.org.uk/). Within the context of the need to determine EQS for new materials and re-evaluating environmental risk posed by products already on the market under EU Registration, Evaluation, Authorisation and restriction of new CHemicals (REACH) regulations, there is a desire to identify alternative methods for evaluating contaminant risk and hazards to help better define environmental regulations, and for use in biomonitoring. A number of excellent reviews are available assessing the use of cell lines for toxicity testing (Bols et al., 2005; Castaño et al., 2003; Segner, 2004; Schirmer, 2006). In this article, we will review current primary gill cell culture techniques and the use of the cultured epithelium as a surrogate for an intact gill; in this context,

Transwell® Permeable Supports

CORNING



The Journal of Experimental Biology (2014) doi:10.1242/jeb.095430

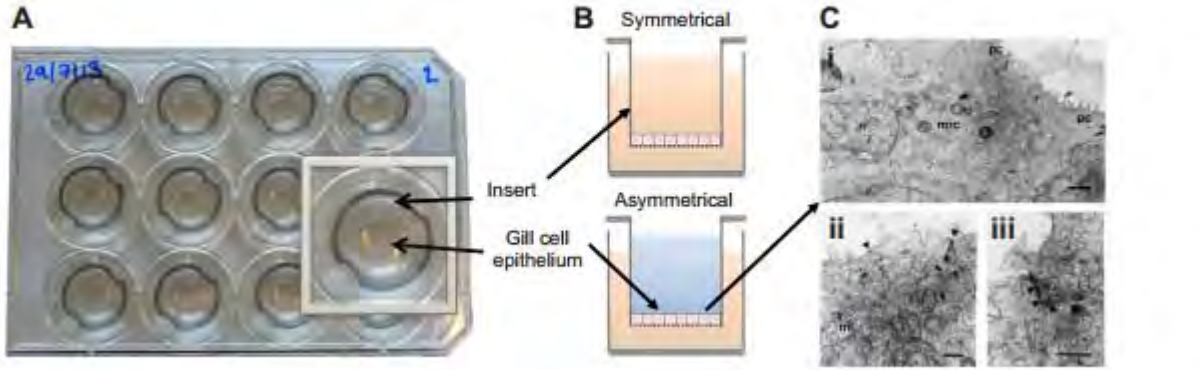


Fig. 1. The primary gill cell culture insert system. (A) Multiwell cell culture plate with inserts containing semipermeable supports used to culture gill cell epithelia.

Cefas

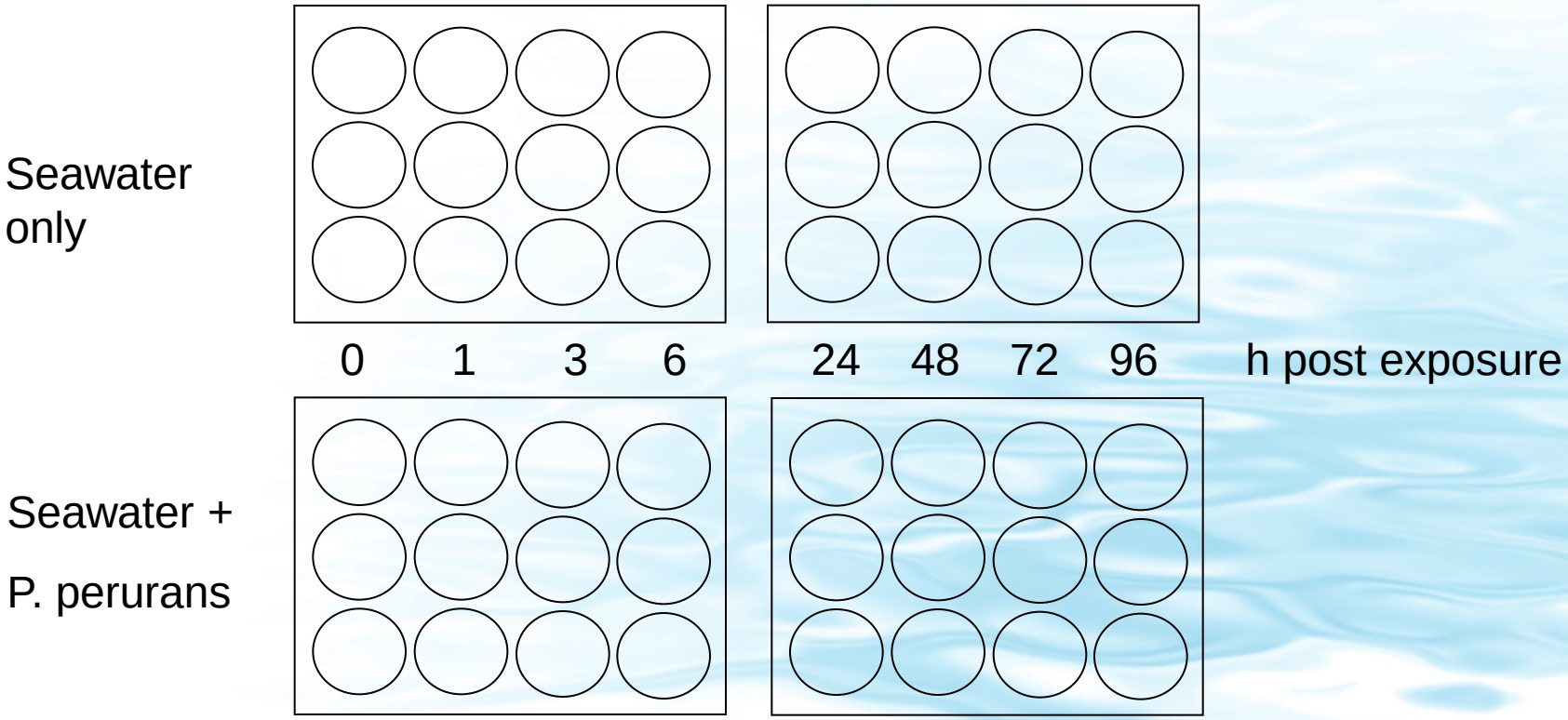
RTG-W1 cells seeded onto transwells

Allowed to establish for 48h (L15 medium top and bottom)

Media in transwell exchanged for 50% seawater/L15

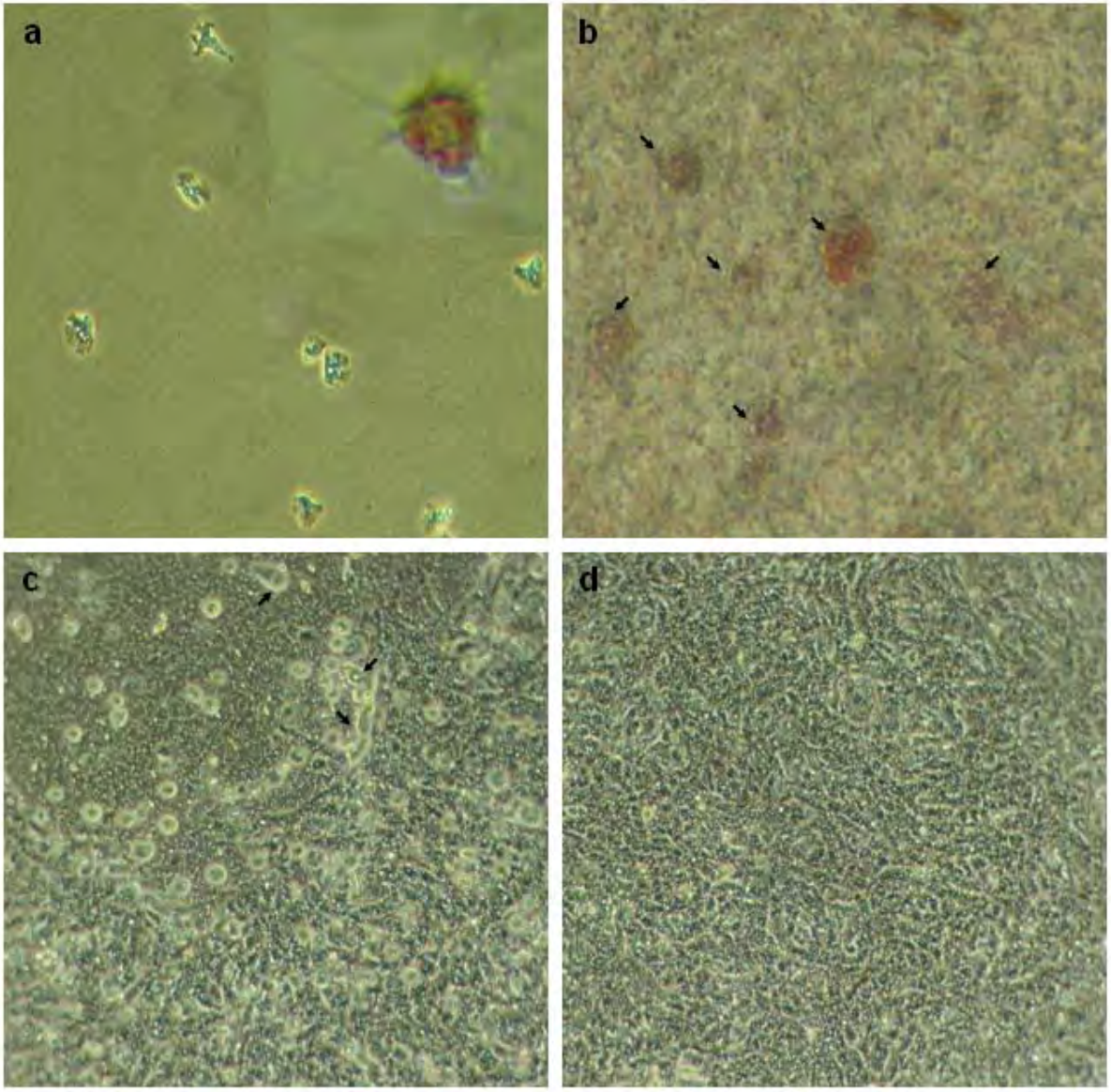
24h later media exchanged again for 100% seawater ± amoebae ($4x10^3$)

x3 wells RNA extracted at various time points



Cefas

Rainbow trout gill cell monolayer (RTgill-W1)



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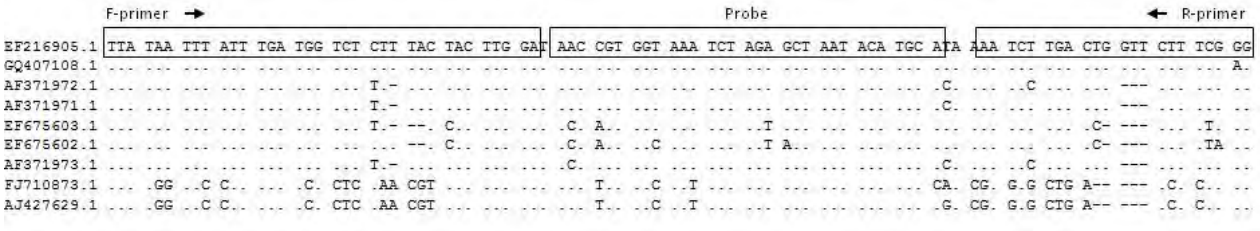
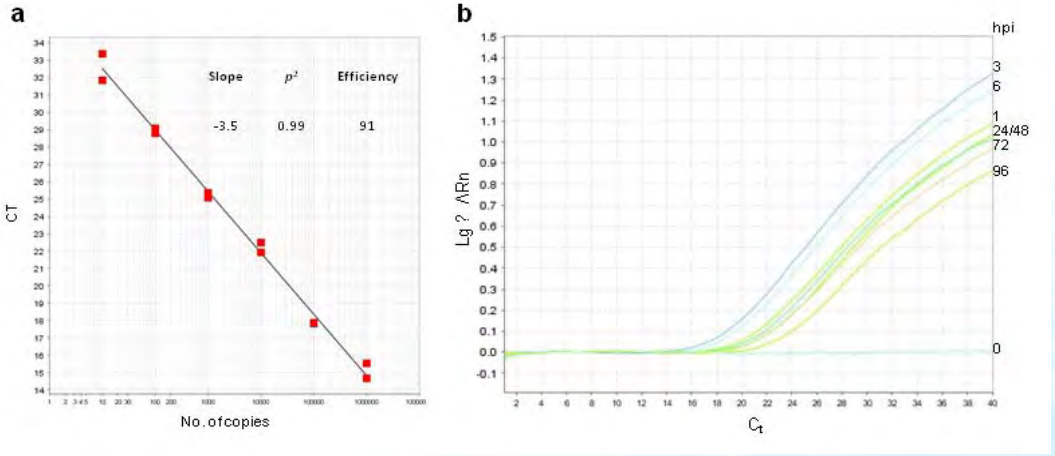




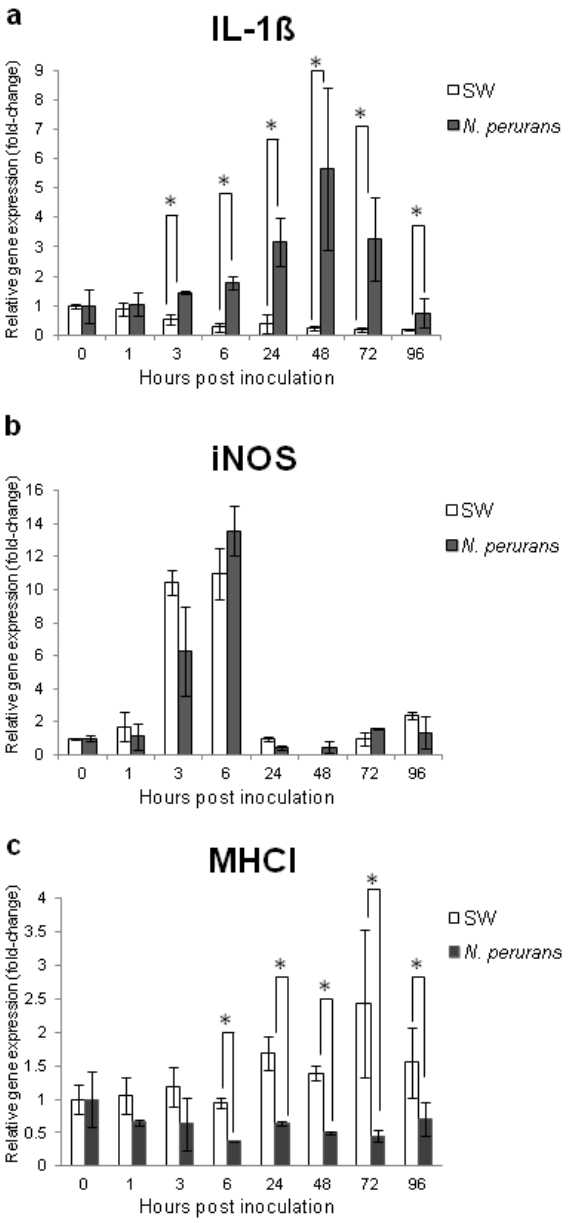
Hours pi	C_t (average ±sd)	No. of copies (average)
1	18.8 ± 2.7	$18x10^6$
3	16.7 ± 0.7	$32x10^6$
6	16.8± 0.6	$28x10^6$
24	18.1± 0.5	$9x10^6$
48	19.2 ± 0.8	$4x10^6$
72	19.9 ± 0.2	$2x10^6$
96	20.0 ± 0.1	$1x10^6$

qPCR enumeration of amoebae

Amoebae alive but not visibly replicating

Slow decline in copy number

Cefas



Immune gene expression

Interleukin-1β – pro inflamatory cytokine

Inducible nitric oxide synthase

MHC1 – antigen presenting

(Bridle 2006; Morrison 2007)

Relative to EF1α

Normalised to time zero

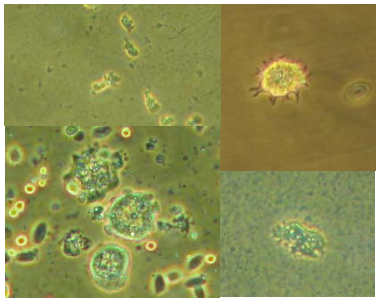
Cefas

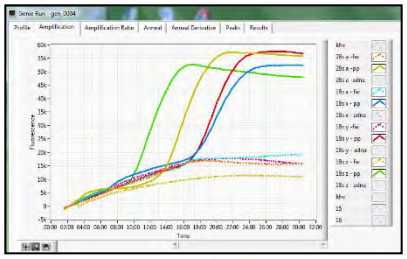
Further development and potential uses:

- Develop optimal growth conditions for the amoebae – additional nutrient (malt/yeast extract) to the seawater
- Expand host response analysis – RNA seq, microarrays
- Compare different amoeba species
- Compare *P. p* strains with different virulence
- Assess amoebicidal compounds *in vitro,* effect on epithelial layer, transepithelial transport of compounds
- Salmon cells – primary cultures – in vitro model for e.g. selective breeding - harvest and assess sib families at fry stage
- Open to collaborations!

Cefas

Acknowlegments





Ben Perry, Iain McEwan - Fish Vet Group

Barbara Nowak – Uni Tasmania

Microbiology team

Aquatic Pests and Pathogens (APP) group

Amanda Bayley

Irene Cano-Cejas

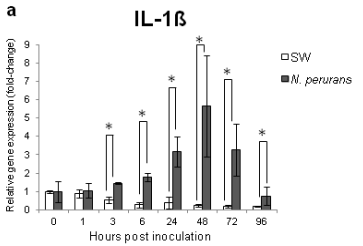
Susie Gunning

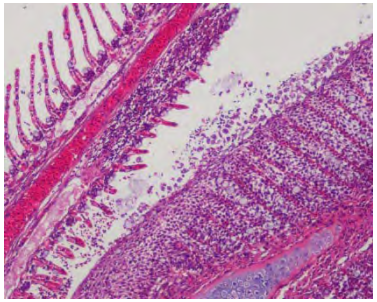
Steve Feist

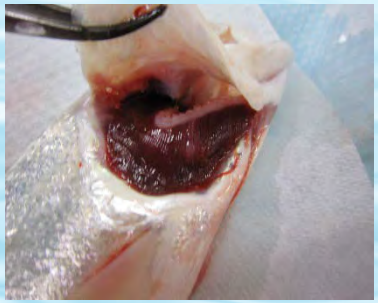
Histology lab

Aquaria facility

Cefas Seedcorn project DP325







Thanks for your attention

Cefas