

Research Citation Guide

# Find qPCR Solutions for Your Research Questions



# Quantitative PCR and qRT-PCR

Quantitative PCR (qPCR) and quantitative real-time PCR (qRT-PCR) technology combines DNA, cDNA, or RNA amplification with real-time monitoring of the amplified product in order to calculate the initial quantity of the specific target of interest. We offer a comprehensive approach to qPCR by simplifying the challenges you face from sample preparation through data analysis. Researchers have used our qPCR systems to successfully customize, optimize, and validate their assays – and this research citation guide will show you how they did it.

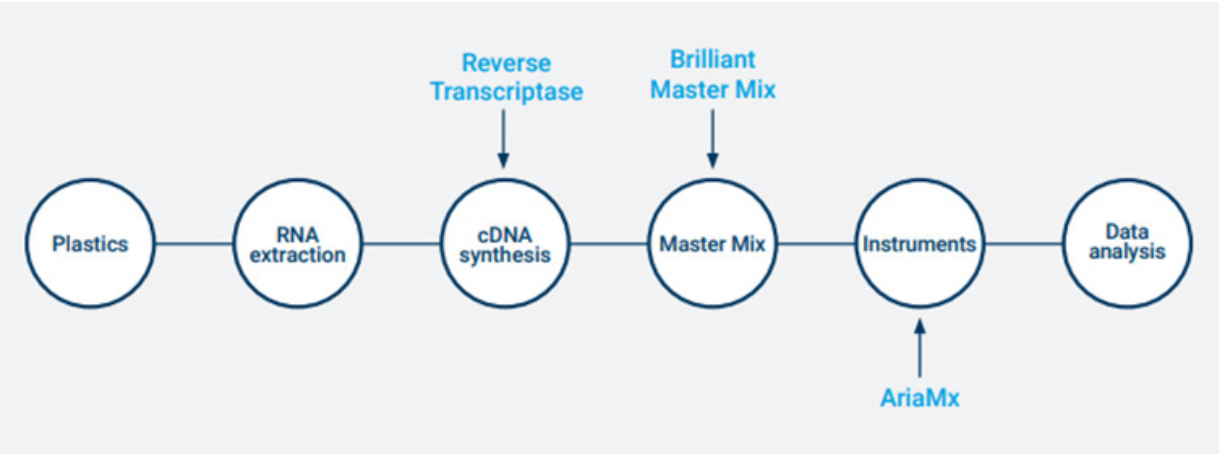


Figure 1. Agilent solutions for qPCR and RT-qPCR workflows.

# Excellent qPCR tools for every application

Real-Time PCR is employed in multiple areas of research such as next-generation sequencing (NGS), forensics, and food, drug, and agricultural research. Agilent qPCR instruments, software, and reagents have been developed with a multitude of your applications in mind, including:

- NGS library quantification
- Cell culture technologies
- Highly specific miRNA detection

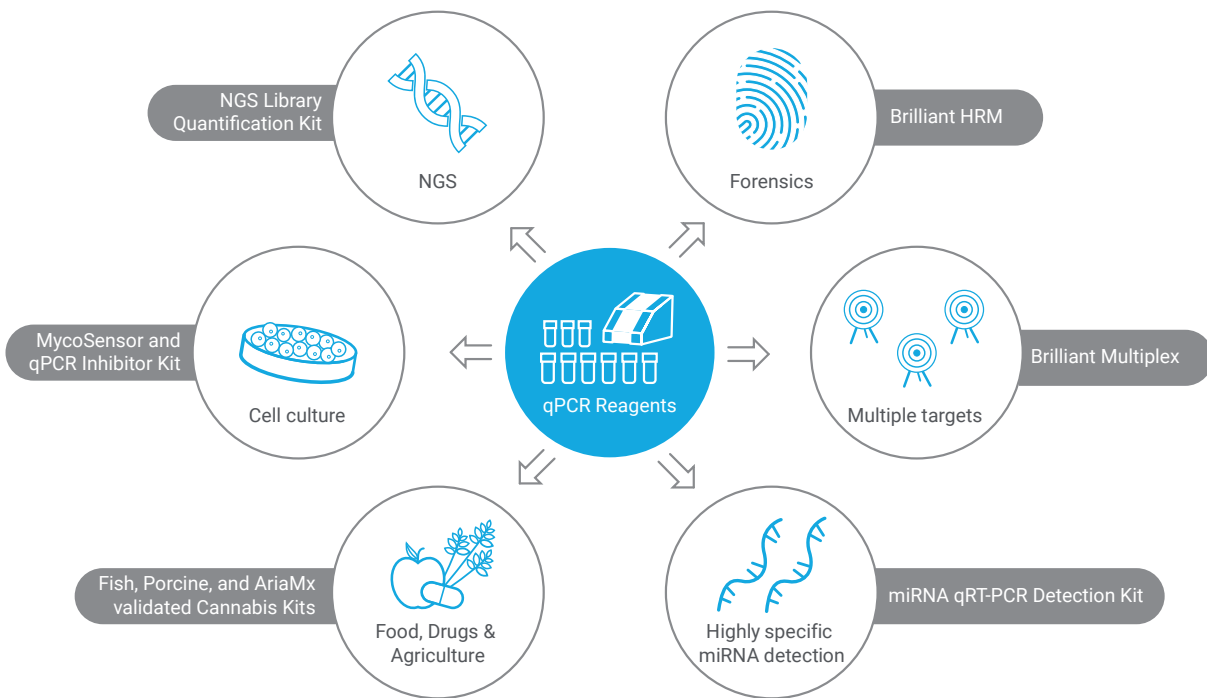


Figure 2. Agilent qPCR and qRT-PCR solutions can be used for various applications.

# Table of Contents

<b>Agriculture</b>	First Molecular Detection of <i>Toxoplasma gondii</i> in Vegetable Samples in China Using Qualitative, Quantitative Real-Time PCR and Multilocus Genotyping	6
	Establishment of a Novel Probe-Based RT-qPCR Approach for Detection and Quantification of Tight Junctions Reveals Age-Related Changes in the Gut Barriers of Broiler Chickens	7
<b>Environmental Science and Pollution Research</b>	Abdominal Contact of Fluvalinate Induces Olfactory Deficit in <i>Apis mellifera</i>	8
	Temperature Effects During a Sublethal Chronic Metal Mixture Exposure on Common Carp ( <i>Cyprinus carpio</i> )	9
	Gestational and Lactational Exposure to Triclosan Causes Impaired Fertility of F1 Male Offspring and Developmental Defects in F2 Generation	10
<b>Fish and Insect Pathology</b>	Local Immune Depression in Baltic Cod ( <i>Gadus morhua</i> ) Liver Infected with <i>Contracaecum osculatum</i>	11
	Rapid identification and genotyping of the honeybee pathogen <i>Paenibacillus larvae</i> by combining culturing and multiplex quantitative PCR	12
<b>Microbiome Research</b>	Comparison of Several Real-Time PCR Kits Versus a Culture-Dependent Algorithm to Identify Enteropathogens in Stool Samples	13
	Slight Disruption in Intestinal Environment by Dextran Sodium Sulfate Reduces Egg Yolk Size Through Dysfunction of Ovarian Follicle Growth	14
<b>Plant Pathology</b>	<i>De Novo</i> Assembly of <i>Amorpha fruticosa</i> L. Transcriptome in Response to Drought Stress Provides Insight into the Tolerance Mechanisms	15
	Mechanism Underlying Potato Spindle Tuber Viroid Affecting Tomato ( <i>Solanum lycopersicum</i> ): Loss of Control Over Reactive Oxygen Species Production	16
<b>Population Ecology</b>	Comprehensive Health Assessment of Green Turtles <i>Chelonia mydas</i> Nesting in Southeastern Florida, USA	17

<b>Stem Cell Research</b>	Discovery of a Chemical Compound that Suppresses Expression of BEX2, a Dormant Cancer Stem Cell-Related Protein	18
	Enhancement of Anti-Inflammatory and Immunomodulatory Effects of Adipose-Derived Human Mesenchymal Stem Cells by Making Uniform Spheroid on the New Nano-Patterned Plates	19
<b>Vaccine and Immunity Research</b>	Immunity to TBEV Related Flaviviruses with Reduced Pathogenicity Protects Mice from Disease but Not from TBEV Entry into the CNS	20
	Effects of Avian Infectious Bronchitis with Newcastle Disease and Marek's Disease Vaccinations on the Expression of Toll-Like Receptors and Avian $\beta$ -Defensins in the Kidneys of Broiler Chicks	21
	Age-Related Dysfunction of p53-Regulated Phagocytic Activity in Macrophages	22
<b>Virology</b>	Real-Time PCR Assay Development for the Control of Vaccine Against Hemorrhagic Fever with Renal Syndrome Discriminating the Eight Genotypes of the Porcine Circovirus Type 2 with TaqMan-Based Real-Time PCR	23
	Discriminating the Eight Genotypes of the Porcine Circovirus Type 2 with TaqMan-Based Real-Time PCR	24
	Real-Life Evaluation of a Rapid Extraction-Free SARS-CoV-2 RT-PCR Assay (COVID-19 PCR Fast-L) for the Diagnosis of COVID-19	25
	Rapid SARS-CoV-2 variant monitoring using PCR confirmed by whole genome sequencing in a high-volume diagnostic laboratory	26
<b>Cancer Research and Therapies</b>	Propionate of a microbiota metabolite induces cell apoptosis and cell cycle arrest in lung cancer	27
<b>Gene expression</b>	Identification of putative miRNA biomarkers in early rheumatoid arthritis by genome-wide microarray profiling: A pilot study	28

# First Molecular Detection of *Toxoplasma gondii* in Vegetable Samples in China Using Qualitative, Quantitative Real-Time PCR and Multilocus Genotyping

Scientific Reports, 2019.

doi: <https://doi.org/10.1038/s41598-019-54073-6>

## Authors

Anna Lass, Liqing Ma,  
Ioannis Kontogeorgos, Xueyong Zhang,  
Xiuping Li, and Panagiotis Karanis

## Abstract

*Toxoplasma gondii* infection is becoming an increasing problem in China but there is no data concerning contamination of vegetables intended for consumption with this parasite. The aim of the present study was to investigate fresh vegetables originated from open markets located in the Xining City, the Qinghai-Tibet Plateau (QTP), P.R. China for their contamination with *T. gondii*. A total of 279 fresh vegetable samples were collected and analyzed using real-time PCR assay targeting B1 gene and multilocus genotyping. *T. gondii* DNA was found in 10 (3.6%) samples tested; eight of them represented *T. gondii* type I and remaining two *T. gondii* type II. The approximate level of contamination of positive vegetable samples, estimated based on quantitative real-time PCR (qPCR), ranged between less than one and 27000 *T. gondii* oocysts per sample, with majority not exceeding several oocysts per sample. The results of the study confirmed that *T. gondii* is present in vegetables offered in open markets in the Qinghai province, P.R. China; eating them unwashed and raw may therefore pose a threat to consumers. This is the first investigation describing *T. gondii* detection in fresh vegetables intended for consumption collected from the territory of P.R. China using sensitive molecular tools.

This is an open access article distributed under the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Anna Lass, Liqing Ma, Ioannis Kontogeorgos, Xueyong Zhang, Xiuping Li, Panagiotis Karanis. First Molecular Detection of *Toxoplasma gondii* in Vegetable Samples in China Using Qualitative, Quantitative Real-Time PCR and Multilocus Genotyping. *Sci. Rep.* 2019, 9, 17581. doi: <https://doi.org/10.1038/s41598-019-54073-6>

# Establishment of a Novel Probe-Based RT-qPCR Approach for Detection and Quantification of Tight Junctions Reveals Age-Related Changes in the Gut Barriers of Broiler Chickens

PLoS ONE, 2021

doi: <https://doi.org/10.1371/journal.pone.0248165>

## Authors

J. Sophia von Buchholz, Ivana Bilic, Jörg R. Aschenbach, Michael Hess, Taniya Mitra, and Wageha A. Awad

## Abstract

Tight junctions (TJs) play a dominant role in gut barrier formation, therefore, resolving the structures of TJs in any animal species is crucial but of major importance in fast growing broilers. They are regulated in molecular composition, ultrastructure and function by intracellular proteins and the cytoskeleton. TJ proteins are classified according to their function into barrier-forming, scaffolding and pore-forming types with deductible consequences for permeability. In spite of their importance for gut health and its integrity limited studies have investigated the TJs in chickens, including the comprehensive evaluation of TJs molecular composition and function in the chicken gut. In the actual study sequence-specific probes to target different TJ genes (*claudin* 1, 3, 5, 7, 10, 19, *zonula occludens 1* (*ZO1*), *occludin* (*OCLN*) and *tricellulin* (*MD2*)) were designed and probe-based RT-qPCRs were newly developed. *Claudin* (*CLDN*) 1, 5, *ZO1* and *CLDN* 3, 7, *MD2* were engulfed in multiplex RT-qPCRs, minimizing the number of separate reactions and enabling robust testing of many samples. All RT-qPCRs were standardized for chicken jejunum and caecum samples, which enabled specific detection and quantification of the gene expression. Furthermore, the newly established protocols were used to investigate the age developmental changes in the TJs of broiler chickens from 1–35 days of age in the same organ samples. Results revealed a significant increase in mRNA expression between 14 and 21 days of age of all tested TJs in jejunum. However, in caecum, mRNA expression of some TJs decreased after 1 day of age whereas some TJs mRNA remained constant till 35 days of age. Taken together, determining the segment-specific changes in the expression of TJ- proteins by RT-qPCR provides a deeper understanding of the molecular mechanisms underpinning pathophysiological changes in the gut of broiler chickens with various etiologies.

2021 von Buchholz et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

J. Sophia von Buchholz, Ivana Bilic, Jörg R. Aschenbach, Michael Hess, Taniya Mitra, Wageha A. Awad. Establishment of a Novel Probe-Based RT-qPCR Approach for Detection and Quantification of Tight Junctions Reveals Age-Related Changes in the Gut Barriers of Broiler Chickens. *PLoS ONE* 2021, 16(3), e0248165. doi: <https://doi.org/10.1371/journal.pone.0248165>

# Abdominal Contact of Fluvalinate Induces Olfactory Deficit in *Apis mellifera*

*Pesticide Biochemistry and Physiology*, 2020.

doi: <https://doi.org/10.1016/j.pestbp.2020.02.005>

## Authors

SooHo Lim, Ural Yunusbaev,  
Rustem Ilyasov, Hyun Sook Lee,  
and Hyung Wook Kwon

## Abstract

$\tau$ -Fluvalinate (fluvalinate) is a highly selective pyrethroid insecticide compound used for controlling ectoparasitic mites that cause major damages in honey bee colonies. Although honey bees have resistance and low toxicity to this xenobiotic chemical, little is known about the effects of this chemical on sensory modulation and behaviors in honey bees. Here we addressed the effect on olfactory cognition at the behavioral, molecular, and neurophysiological levels. First, we found that topical application of fluvalinate to honeybee abdomen elicited somewhat severe toxicity to honey bees. Furthermore, honeybees treated with sublethal doses of fluvalinate showed a significant decrease in olfactory responses. At the molecular level, there was no change in gene expression levels of odorant receptor co-receptor (Orco), which is important for electrical conductivity induced by odorant binding in insects. Rather, small neuropeptide F (sNPF) signaling pathway was involved in olfactory fluctuation after treatment of fluvalinate. This indicates that olfactory deficits by abdominal contact of fluvalinate may stem from various internal molecular pathways in honey bees.



# Temperature Effects During a Sublethal Chronic Metal Mixture Exposure on Common Carp (*Cyprinus carpio*)

Frontiers in Physiology, 2021

doi: <https://doi.org/10.3389/fphys.2021.651584>

## Authors

Giovanni Castaldo, Marion Pillet, Leen Ameryckx, Lieven Bervoets, Raewyn M. Town, Ronny Blust, and Gudrun De Boeck

## Abstract

The aquatic environment is the final sink of various pollutants including metals, which can pose a threat for aquatic organisms. Waterborne metal mixture toxicity might be influenced by environmental parameters such as the temperature. In the present study, common carp were exposed for 27 days to a ternary metal mixture of Cu, Zn, and Cd at two different temperatures, 10 and 20°C. The exposure concentrations represent 10% of the 96 h-LC50 (concentration lethal for the 50% of the population in 96 h) for each metal (nominal metal concentrations of Cu: 0.08 µM; Cd: 0.02 µM and Zn: 3 µM). Metal bioaccumulation and toxicity as well as changes in the gene expression of enzymes responsible for ionoregulation and induction of defensive responses were investigated. Furthermore the hepatosomatic index and condition factor were measured as crude indication of overall health and energy reserves. The obtained results showed a rapid Cu and Cd increase in the gills at both temperatures. Cadmium accumulation was higher at 20°C compared to 10°C, whereas Cu and Zn accumulation was not, suggesting that at 20°C, fish had more efficient depuration processes for Cu and Zn. Electrolyte (Ca, Mg, Na, and K) levels were analyzed in different tissues (gills, liver, brain, muscle) and in the remaining carcasses. However, no major electrolyte losses were observed. The toxic effect of the trace metal ion mixture on major ion uptake mechanisms may have been compensated by ion uptake from the food. Finally, the metal exposure triggered the upregulation of the metallothionein gene in the gills as defensive response for the organism. These results, show the ability of common carp to cope with these metal levels, at least under the condition used in this experiment.

This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/) (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Giovanni Castaldo, Marion Pillet, Leen Ameryckx, Lieven Bervoets, Raewyn M. Town, Ronny Blust, Gudrun De Boeck. Temperature Effects During a Sublethal Chronic Metal Mixture Exposure on Common Carp (*Cyprinus carpio*). *Front. Physiol.* 2021, 12, 651584. doi: [10.3389/fphys.2021.651584](https://doi.org/10.3389/fphys.2021.651584)

# Gastational and Lactational Exposure to Triclosan Causes Impaired Fertility of F1 Male Offspring and Developmental Defects in F2 Generation

*Environmental Pollution*, 2020.

doi: <https://doi.org/10.1016/j.envpol.2019.113617>

## Authors

Priyanka, Ayushi Trivedi, Priyanka Maske, Chandrashekhar Mote, and Vikas Dighe

## Abstract

Triclosan (5-chloro-2-(2, 4-dichlorophenoxy) phenol, TCS), is a broad-spectrum antimicrobial agent and extensively used in household and daily daycare products. Recently, several reports have demonstrated the endocrine disruptive action of TCS to alter the testicular steroidogenesis. However, the gestational and lactational effects of TCS exposure on F1 offspring has not been studied. Present study aimed to investigate the effect of gestational and lactational exposure to TCS on F1 male progeny and its effect on fertility. Pregnant dams (F0) were administered with different doses of TCS (0.1, 4, 40 and 150 mg/kg b. wt./day) and Diethylstilbestrol (1 µg/kg b. wt./day), as a positive control daily by subcutaneous injection during Gestation Day 6 to Postnatal Day 21. Delayed testicular descent was observed at 150 mg/kg b. wt./day dose group. Dose-dependent decrease in testosterone level, sperm count and motility was observed. Significantly decreased expression of steroid hormone receptors (AR, ER $\alpha$  and ER $\beta$ ), StAR and aromatase were observed in F1 male rats; indicating its prolonged effect on spermatogenesis and steroidogenesis in adulthood and poor development in F2 fetuses. Further, gestational and lactational exposure to TCS has negative impact on the fertility of F1 male rats. The F1 male rats were found sub-fertile with increased (%) pre- and post-implantation loss (at 40 and 150 mg/kg b.wt./day dose) with a simultaneous decrease in litter size. The significant decrease in mean fetal weight and crown-rump length (CRL) of F2 fetuses were observed at 0.1, 4, 40 and 150 dose groups indicating impaired development of F2 fetuses caused by TCS exposure.

Present study emphasizes for the first time that TCS exposure during the vulnerable developmental time point (gestation and lactation) adversely affects reproductive functions and fertility of F1 male rats, which were transmitted to F2 generations leading to reduced CRLs and weights of F2 fetuses.

Reprinted with permission from Elsevier B.V., Copyright (2021). All rights reserved.

Priyanka, Ayushi Trivedi, Priyanka Maske, Chandrashekhar Mote, Vikas Dighe. Gestational and Lactational Exposure to Triclosan Causes Impaired Fertility of F1 Male Offspring and Developmental Defects in F2 Generation. *Environ. Pollut.* 2020, 257, 113617. doi: <https://doi.org/10.1016/j.envpol.2019.113617>

# Local Immune Depression in Baltic Cod (*Gadus morhua*) Liver Infected with *Contracaecum osculatum*

*Journal of Helminthology*, 2020

doi: [10.1017/S0022149X19001111](https://doi.org/10.1017/S0022149X19001111)

## Authors

H. Marnis, P.W. Kania, K. Syahputra, S. Zuo, and K. Buchmann

## Abstract

Third-stage larvae of the anisakid nematode *Contracaecum osculatum* infecting cod (*Gadus morhua*) liver elicit a host immune response involving both innate and adaptive factors, but the reactions differ between liver and spleen. Inflammatory reactions occur in both liver and spleen, but a series of immune effector genes are downregulated in liver infected with nematodes whereas these genes in spleen from the same fish are upregulated. A series of novel primer and probe sets targeting cod immune responses were developed and applied in a real-time quantitative polymerase chain reaction set-up to measure the expression of immune-relevant genes in liver and spleen of infected and uninfected cod. In infected liver, 12 of 23 genes were regulated. Genes encoding cytokines associated with inflammatory reactions (IL-1 $\beta$ , IL-6, IL-8) were significantly upregulated, whereas genes encoding effector molecules, assisting the elimination of pathogens, C-reactive protein (CRP)-PII, hepcidin, lysozyme G1, lysozyme G2, C3 and IgDm, were significantly downregulated. The number of downregulated genes increased with the parasite burden. In spleen, 14 of 23 immune genes showed significant regulation and nine of these were upregulated, including genes encoding CRPI, CRPII, C3, hepcidin and transferrin. The general gene expression level was higher in spleen compared to liver, and although inflammation was induced in nematode-infected liver, the effector molecule genes were depressed, which suggests a worm-induced immune suppression locally in the liver.

# Rapid identification and genotyping of the honeybee pathogen *Paenibacillus larvae* by combining culturing and multiplex quantitative PCR

*Open Veterinary Journal*, 2020.

doi: [10.4314/ovj.v10i1.9](https://doi.org/10.4314/ovj.v10i1.9)

## Authors

Beims H, Janke M, von der Ohe W, Steinert M

## Abstract

American Foulbrood (AFB) is a devastating disease of honey bee (*Apis mellifera*) larvae caused by the spore-forming, Gram-positive bacterium *Paenibacillus larvae*. In most countries, the law requires mandatory reporting of AFB to the veterinary authority.

To speed up detection and genotyping of *P. larvae* spores, we compared different culturing protocols on Columbia sheep blood agar and developed a new multiplex quantitative polymerase chain reaction to distinguish between the two relevant *P. larvae* genotypes enterobacterial repetitive intergenic consensus (ERIC) I and ERIC II.

As confirmed by *P. larvae* reference strains and field isolates, the new identification and genotyping protocol halves the time of current workflows, lessens labor-intension, allows a higher throughput of samples for monitoring, and permits a faster intervention to prevent the spread of AFB.

# Comparison of Several Real-Time PCR Kits Versus a Culture-Dependent Algorithm to Identify Enteropathogens in Stool Samples

Scientific Reports, 2020

doi: <https://doi.org/10.1038/s41598-020-61202-z>

## Authors

Silvia Valledor, Inés Valledor,  
María Concepción Gil-Rodríguez,  
Cristina Seral, and Javier Castillo

## Abstract

This study aims to validate the current diagnostic method for the clinical detection of gastroenteritis. We analyzed 400 stool samples to detect three of the most common enteropathogens: *Salmonella spp.*, *Campylobacter spp.*, and *Yersinia enterocolitica*. All specimens were tested with a routine clinical diagnosis algorithm and with five real-time PCR assays. A total of 98 specimens (24.5%) were positive for enteropathogens. We found 24 samples positive for *Salmonella enterica*, 71 positive for *Campylobacter spp.*, and 4 positive for *Yersinia enterocolitica*. All evaluated methods exhibited a good performance in identifying *Salmonella* and *Yersinia enterocolitica*, being the highest positive percent agreement (PPA) value of 95.8% and 100%, respectively. The clinical algorithm showed the highest PPA value identifying *Salmonella*, due to the enrichment in selenite broth. However, the evaluated methods showed notable differences in the identification of *Campylobacter* species, obtaining a wide range of PPA values: 59.2%–100%. The clinical algorithm showed the lowest PPA value since it was only able to detect *Campylobacter jejuni* and *Campylobacter coli* species. This study revealed the importance of implementing the real-time PCR technique in a clinical algorithm: it improved the accuracy of the diagnosis and provided results in a shorter time compared to routine clinical methods.

This article is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

Silvia Valledor, Inés Valledor, María Concepción Gil-Rodríguez, Cristina Seral, Javier Castillo. Comparison of Several Real-Time PCR Kits Versus a Culture-Dependent Algorithm to Identify Enteropathogens in Stool Samples. *Sci. Rep.* 2020, 10, 4301. doi: <https://doi.org/10.1038/s41598-020-61202-z>

# Slight Disruption in Intestinal Environment by Dextran Sodium Sulfate Reduces Egg Yolk Size Through Dysfunction of Ovarian Follicle Growth

*Frontiers in Physiology*, 2021.

doi: [10.3389/fphys.2020.607369](https://doi.org/10.3389/fphys.2020.607369)

## Authors

Takahiro Nii, Takashi Bungo, Naoki Isobe, and Yukinori Yoshimura

## Abstract

Intestinal environments such as microbiota, mucosal barrier function, and cytokine production affect egg production in laying hens. Dextran sodium sulfate (DSS) is an agent that disrupts the intestinal environment. Previously, we reported that the oral administration of dextran sodium sulfate (DSS: 0.9 g/kg BW) for 5 days caused severe intestinal inflammation in laying hens. However, the DSS concentration in the previous study was much higher to induce a milder disruption of the intestinal environment without heavy symptoms. Thus, the goal of this study was to determine the effects of a lower dose of DSS on the intestinal environment and egg production in laying hens. White Leghorn laying hens (330-day old) were orally administered with or without 0.225 g DSS/kg BW for 28 days (DSS and control group: n = 7 and 8, respectively). Weekly we collected all laid eggs and blood plasma samples. Intestinal tissues, liver, ovarian follicles, and the anterior pituitary gland were collected 1 day after the final treatment. Lower concentrations of orally administered DSS caused (1) a decrease in the ratio of villus height/crypt depth, *occludin* gene expressions in large intestine and cecal microbiota diversity, (2) a decrease in egg yolk weight, (3) an increase in VLDL in blood plasma, (4) and enhanced the egg yolk precursor accumulation in the gene expression pattern in the follicular granulosa layer, (5) an increase in *FSH* and *IL-1 $\beta$*  gene expression in the pituitary gland, and (6) an increase in concentration of plasma lipopolysaccharide binding protein. These results suggested that the administration of the lower concentration of DSS caused a slight disruption in the intestinal environment. This disruption included poor intestinal morphology and decreased cecal microbiome diversity. The change in the intestinal environment decreases egg yolk size without decreasing the VLDL supply from the liver. The decrease in egg yolk size is likely to be caused by the dysfunction of egg-yolk precursor uptake in ovarian follicles. In conclusion, the oral administration of a lower dose of DSS is a useful method to cause slight disruptions of intestinal environment, and the intestinal condition decreases egg yolk size through dysfunction of ovarian follicle.

2021 Nii, Bungo, Isobe and Yoshimura. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Takahiro Nii, Takashi Bungo, Naoki Isobe, Yukinori Yoshimura. Slight Disruption in Intestinal Environment by Dextran Sodium Sulfate Reduces Egg Yolk Size Through Dysfunction of Ovarian Follicle Growth. *Front. Physiol.* 2021, 11, 1812. doi: [10.3389/fphys.2020.607369](https://doi.org/10.3389/fphys.2020.607369)

# De Novo Assembly of *Amorpha fruticosa* L. Transcriptome in Response to Drought Stress Provides Insight into the Tolerance Mechanisms

Peer J Plant Biology, 2021

doi: <https://doi.org/10.7717/peerj.11044>

## Authors

Xinzhu Sun, Songmiao Hu, Xin Wang,  
He Liu, Yun wei Zhou, and Qingjie Guan

## Abstract

*Amorpha fruticosa* L. is a deciduous shrub that is native to North America and has been introduced to China as an ornamental plant. In order to clarify the drought resistance characteristics of *Amorpha fruticosa* L. and excavate the related genes involved in drought resistance regulation pathway, the mechanism of drought resistance stress of *Amorpha fruticosa* L. was revealed by the changes of transcriptome of *Amorpha fruticosa* L. under drought stress. Through the changes of the transcriptome of *Amorpha fruticosa* L. under drought stress, the mechanism of anti-stress of *Amorpha fruticosa* L. could be revealed.

This is an open access article distributed under the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Xinzhu Sun, Songmiao Hu, Xin Wang, He Liu, Yun wei Zhou, Qingjie Guan. De Novo Assembly of *Amorpha fruticosa* L. Transcriptome in Response to Drought Stress Provides Insight into the Tolerance Mechanisms. *PeerJ*. 2021, 9, e11044. doi: <https://doi.org/10.7717/peerj.11044>

# Mechanism Underlying Potato Spindle Tuber Viroid Affecting Tomato (*Solanum lycopersicum*): Loss of Control over reactive Oxygen Species Production

*Journal of General Plant Pathology*, 2021.

doi: <https://doi.org/10.1007/s10327-021-01000-1>

## Authors

Misato Fujibayashi, Takahiro Suzuki,  
and Teruo Sano

## Abstract

Severe infection of the tomato cultivar 'Rutgers' with potato spindle tuber viroid (PSTVd) variants resulted in systemic stunting, leaf malformation, and vein necrosis; accompanied by a rapid increase in reactive oxygen species (ROS) and abnormally high expression of the stress-responsive microRNAs, miR398 and miR398a-3p. The severity of the symptoms, PSTVd accumulation, the high expression of miR398 and miR398a-3p, and the high level of ROS production were positively correlated with the pathogenesis of the PSTVd variants. In contrast, the cytosolic and chloroplast-localized Cu/Zn-superoxide dismutase genes *SISOD4* and *SISOD3*, respectively that encode the ROS scavenging enzymes were down-regulated and negatively correlated with the severity of the symptoms. Conversely, in the PSTVd-infected, PSTVd-tolerant (almost asymptomatic) tomato cultivar 'Moneymaker' in which PSTVd accumulation was suppressed, the ROS production was as low as in the control, miR398 and miR398a-3p were down-regulated, and almost all the SOD genes were up-regulated. The results indicated that in the PSTVd-sensitive tomato cultivar 'Rutgers', infection with the severe PSTVd variant caused suppression of the cytosolic and chloroplast-localized Cu/Zn-SOD genes via PSTVd-induced overexpression of miR398 and miR398a-3, respectively. This condition significantly reduced or eliminated the normal ROS scavenging function and an excess of harmful ROS in cells and tissues seem to have caused severe pathological symptoms accompanied by necrosis.

Reprinted with permission from the publisher (2021). Springer Nature Switzerland AG. Part of Springer Nature.

Misato Fujibayashi, Takahiro Suzuki, Teruo Sano. Mechanism Underlying Potato Spindle Tuber Viroid Affecting Tomato (*Solanum lycopersicum*): Loss of Control Over Reactive Oxygen Species Production. *J. Gen. Plant. Pathol.* 2021, 87, 226-235. doi: <https://doi.org/10.1007/s10327-021-01000-1>



# Comprehensive Health Assessment of Green Turtles *Chelonia mydas* Nesting in Southeastern Florida, USA

*Endangered Species Research*, 2020

doi: <https://doi.org/10.3354/esr01036>

## Authors

Annie Page-Karjian, Ryan Chabot,  
Nicole I. Stacy, Ashley S. Morgan,  
Roldán A. Valverde, Sydney Stewart,  
Christina M. Coppentrath,  
Charles A. Manire, Lawrence H. Herbst,  
Christopher R. Gregory, Branson W. Ritchie,  
and Justin R. Perrault

## Abstract

Important indicators of population health needed for large-scale sea turtle population recovery efforts include demographics, disease and mortality trends, condition indices, and baseline blood data. With this comprehensive health assessment of adult female green sea turtles *Chelonia mydas* nesting on Juno Beach, Florida, USA, we (1) established comprehensive baseline health indices; (2) identified individuals with evidence of infection by chelonid alphaherpesviruses 5 and 6 (ChHV5, ChHV6), which are implicated in fibropapillomatosis and respiratory and skin disease, respectively; and (3) compared measured health indices between turtles that did versus those that did not test positive for ChHV5 and/or ChHV6. All 60 turtles included in the study were in good body condition with no external fibropapillomatosis tumors. Hematological and biochemical reference intervals were established. Via quantitative PCR (qPCR), 5/60 turtles (8%) tested positive for ChHV5, and all turtles were negative for ChHV6. Of 41 turtles tested for antibodies to ChHV5 and ChHV6, 29% and 15% tested positive, respectively, and 10% tested positive for antibodies to both viruses. Notably, there were no statistically significant differences between health variables for nesting turtles that tested positive for ChHV5 DNA versus those that tested negative; and also no differences between turtles that tested positive for ChHV5 or ChHV6 antibodies and those that did not. This suggests that these viruses are enzootically stable in Florida's adult green turtles. This study provides a health profile of nesting green turtles in southeastern Florida applicable to temporal and spatial investigations of this and other populations.

This is an open access article distributed under the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Annie Page-Karjian, Ryan Chabot, Nicole I. Stacy, Ashley S. Morgan, Roldán A. Valverde, Sydney Stewart, Christina M. Coppentrath, Charles A. Manire, Lawrence H. Herbst, Christopher R. Gregory, Branson W. Ritchie, Justin R. Perrault. Comprehensive Health Assessment of Green Turtles *Chelonia mydas* Nesting in Southeastern Florida, USA. *Endanger. Species Res.* 2020, 42, 21-35. doi: <https://doi.org/10.3354/esr01036>

# Discovery of a Chemical Compound that Suppresses Expression of BEX2, a Dormant Cancer Stem Cell-Related Protein

*Biochemical and Biophysical Research Communications*, **2021**.

doi: <https://doi.org/10.1016/j.bbrc.2020.11.022>

## Authors

Satoshi Saijoh, Mao Nakamura-Shima, Rie Shibuya-Takahashi, Ryo Ito, Akira Sugawara, Tomoko Yamazaki, Takayuki Imai, Yukinori Asada, Kazuto Matsuura, Wataru Iwai, Yuta Wakui, Makoto Abue, Sadafumi Kawamura, Yu Katayose, Haruna Fujimori, Mai Mochizuki, Jun Yasuda, Kazunori Yamaguchi, Kazuo Sugamura, Kennichi Satoh, Yukio Katori, and Keiichi Tamai

## Abstract

Cancer stem cells (CSCs) are believed to cause cancer metastasis and recurrence. *BEX2* (brain expressed X-linked gene 2) is a CSC-related gene that is expressed in dormant CSCs in cholangiocarcinoma and induces resistance against chemotherapy. The aim of the present study was to identify small compounds that have activity to inhibit *BEX2* expression and result in the attenuation of CSC-related phenotypes. We screened 9600 small chemical compounds in high-throughput screening using cholangiocarcinoma cell line HuCCT1 expressing *BEX2* protein fused with NanoLuc, and identified a compound, BMPP (1, 3-Benzenediol, [4-(4-methoxyphenyl)-1H-pyrazol-3-yl]). BMPP was found to exert decreasing effects on *BEX2* protein expression and  $G_0$  phase population of the tumor cells, and increasing effects on ATP levels and chemotherapeutic sensitivity of the cells. These findings indicate that BMPP is a valuable chemical compound for reducing dormant CSC-related phenotypes. Thus, the identification of BMPP as a potential CSC suppressor provides scope for the development of novel therapeutic modalities for the treatment of cancers with *BEX2* overexpressing CSCs.

Reprinted with permission from Elsevier B.V., Copyright (2020). All rights reserved.

Satoshi Saijoh, Mao Nakamura-Shima, Rie Shibuya-Takahashi, Ryo Ito, Akira Sugawara, Tomoko Yamazaki, Takayuki Imai, Yukinori Asada, Kazuto Matsuura, Wataru Iwai, Yuta Wakui, Makoto Abue, Sadafumi Kawamura, Yu Katayose, Haruna Fujimori, Mai Mochizuki, Jun Yasuda, Kazunori Yamaguchi, Kazuo Sugamura, Kennichi Satoh, Yukio Katori, Keiichi Tamai. Discovery of a Chemical Compound that Suppresses Expression of *BEX2*, a Dormant Cancer Stem Cell-Related Protein. *Biochem. Biophys. Res. Co.* **2021**, 537, 132-139. doi: <https://doi.org/10.1016/j.bbrc.2020.11.022>

# Enhancement of Anti-Inflammatory and Immunomodulatory Effects of Adipose-Derived Human Mesenchymal Stem Cells by Making Uniform Spheroid on the New Nano-Patterned Plates

*Biochemical and Biophysical Research Communications*, 2021

doi: <https://doi.org/10.1016/j.bbrc.2021.03.026>

## Authors

Sangho Lee, Hyo-Sop Kim, Byoung-Hoon Min, Byoung Geun Kim, Shin Ae Kim, Hyeyoung Nam, Minsuk Lee, Minsun Kim, Hye Yeon Hwang, Alex Inkeun Leesong, Margaret Minsun Leesong, Jae-Ho Kim, and Jun-Seop Shin

## Abstract

Human mesenchymal stem cells (MSCs) are known to have anti-inflammatory and immunomodulatory functions; thus, several MSC products have been applied as cell therapy in clinical trials worldwide. Recent studies have demonstrated that MSC spheroids have superior anti-inflammatory and immunomodulatory functions to a single cell suspension. Current methods to prepare MSC spheroids include hanging drop, concave microwell aggregation, spinner flask, and gravity circulation. However, all these methods have limitations such as low scalability, easy cell clumping, low viability, and irregular size distribution. Here, we present a nano-patterned culture plasticware named PAMcell 3D plate to overcome these limitations. Nano-sized silica particles (700 nm) coated with RGD peptide were arrayed into fusiform onto the PLGA film. This uniform array enabled the seeded MSCs to grow only on the silica particles, forming uniform-sized semi-spheroids within 48 h. These MSC spheroids have been shown to have enhanced stemness, anti-inflammatory, and immunomodulatory functions, as revealed by the increased expression of stem cell markers (Oct4, Sox2, and Nanog), anti-inflammatory (IL-10, TSG6, and IDO), and immunomodulatory molecules (HGF, VEGF, CXCR4) both at mRNA and protein expression levels. Furthermore, these MSC spheroids demonstrated an increased palliative effect on glycemic control in a multiple low-dose streptozotocin-induced diabetes model compared with the same number of MSC single cell suspensions. Taken together, this study presents a new method to produce uniform-sized MSC spheroids with enhanced anti-inflammatory and immunomodulatory functions in vitro and in vivo.

Reprinted with permission from Elsevier B.V., Copyright (2021). All rights reserved.

Sangho Lee, Hyo-Sop Kim, Byoung-Hoon Min, Byoung Geun Kim, Shin Ae Kim, Hyeyoung Nam, Minsuk Lee, Minsun Kim, Hye Yeon Hwang, Alex Inkeun Leesong, Margaret Minsun Leesong, Jae-Ho Kim, Jun-Seop Shin. Enhancement of Anti-Inflammatory and Immunomodulatory Effects of Adipose-Derived Human Mesenchymal Stem Cells by Making Uniform Spheroid on the New Nano-Patterned Plates. *Biochem. Bioph. Res. Co.* 2021, 552, 164-169. doi: <https://doi.org/10.1016/j.bbrc.2021.03.026>

# Immunity to TBEV Related Flaviviruses with Reduced Pathogenicity Protects Mice from Disease but Not from TBEV Entry into the CNS

*Vaccines*, **2021**.

doi: <https://doi.org/10.3390/vaccines903019622>

## Authors

Monique Petry, Martin Palus,  
Eva Leitzen, Johanna Garcia Mitterreiter,  
Bei Huang, Andrea Kröger, Georges MGM  
Verjans, Wolfgang Baumgärtner,  
Guus F. Rimmelzwaan, Daniel Růžek, Albert  
Osterhaus, and Chittappen Kandiyil Prajeeth

## Abstract

Tick-borne encephalitis virus (TBEV) is a leading cause of vector-borne viral encephalitis with expanding endemic regions across Europe. In this study we tested in mice the efficacy of preinfection with a closely related low-virulent flavivirus, Langat virus (LGTV strain TP21), or a naturally avirulent TBEV strain (TBEV-280) in providing protection against lethal infection with the highly virulent TBEV strain (referred to as TBEV-Hypr). We show that prior infection with TP21 or TBEV-280 is efficient in protecting mice from lethal TBEV-Hypr challenge. Histopathological analysis of brains from nonimmunized mice revealed neuronal TBEV infection and necrosis. Neuroinflammation, gliosis, and neuronal necrosis was however also observed in some of the TP21 and TBEV-280 preinfected mice although at reduced frequency as compared to the nonimmunized TBEV-Hypr infected mice. qPCR detected the presence of viral RNA in the CNS of both TP21 and TBEV-280 immunized mice after TBEV-Hypr challenge, but significantly reduced compared to mock-immunized mice. Our results indicate that although TBEV-Hypr infection is effectively controlled in the periphery upon immunization with low-virulent LGTV or naturally avirulent TBEV 280, it may still enter the CNS of these animals. These findings contribute to our understanding of causes for vaccine failure in individuals vaccinated with TBE vaccines.

This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Monique Petry, Martin Palus, Eva Leitzen, Johanna Garcia Mitterreiter, Bei Huang, Andrea Kröger, Georges MGM Verjans, Wolfgang Baumgärtner, Guus F. Rimmelzwaan, Daniel Růžek, Albert Osterhaus, Chittappen Kandiyil Prajeeth. Immunity to TBEV Related Flaviviruses with Reduced Pathogenicity Protects Mice from Disease but Not from TBEV Entry into the CNS. *Vaccines* **2021**, 9(3), 196. doi: <https://doi.org/10.3390/vaccines9030196>

# Effects of Avian Infectious Bronchitis with Newcastle Disease and Marek's Disease Vaccinations on the Expression of Toll-Like Receptors and Avian $\beta$ -Defensins in the Kidneys of Broiler Chicks

*Poultry Science*, 2020

doi: <https://doi.org/10.1016/j.psj.2020.08.071>

## Authors

Masahiro Shimizu, Takahiro Nii,  
Naoki Isobe, and Yukinori Yoshimura

## Abstract

The aim of this study was to determine the effect of vaccinations for avian infectious bronchitis with Newcastle disease (IB/ND) and Marek's disease (MD) on the expression of toll-like receptors (TLR) that recognize viral RNA and microbial DNA, and AvBD in chick kidneys. Day-old chicks were vaccinated with MD or IB/ND vaccines or received no treatment (control group). The gene expression of TLR and AvBD in the kidneys of 3-day-old chicks and 10-day-old chicks was examined using real-time PCR. The localization of AvBD2 and AvBD4 was examined by immunohistochemistry at day three only. At 3 days of age, the expression of TLR7 and TLR21 was significantly higher in the IB/ND group (but not in the MD group) than in the control group. Conversely, at 10 days of age there was no significant difference in the expression of the three TLR between groups. In the 3-day-old chicks the expression levels of AvBD4, 5, 6, and 7 were higher in the MD group than in the control group. Furthermore, at this age, the expression levels of other AvBD were not significantly different between the control and vaccination (MD and IB/ND) groups. At 10 days of age, no AvBD expression was affected by MD and IB/ND vaccinations. Immunohistochemistry results localized AvBD2 in the leukocytes in the interstitial tissue and AvBD4 in the surface of microvillus epithelial cells of renal tubules, and in the epithelial cells of the collecting ducts and ureter. The localization of AvBD2 and AvBD4 was identified in all chicks. We suggest that the expression of innate immune molecules (including TLR and AvBD) in kidneys could be modulated by MD and IB/ND vaccination when performed at the day-old stage. Although the effects of both vaccinations may subside within 10 days, the enhanced expression of those innate immune molecules may support the innate immunodefense function in the kidneys of young chicks.

Reprinted with permission from Elsevier B.V., Copyright (2020). All rights reserved.

Masahiro Shimizu, Takahiro Nii, Naoki Isobe, Yukinori Yoshimura. Effects of Avian Infectious Bronchitis with Newcastle Disease and Marek's Disease Vaccinations on the Expression of Toll-Like Receptors and Avian  $\beta$ -Defensins in the Kidneys of Broiler Chicks. *Poultry Sci.* 2020, 99(12), 7092-7100. doi: <https://doi.org/10.1016/j.psj.2020.08.071>.

# Age-Related Dysfunction of p53-Regulated Phagocytic Activity in Macrophages

*Biochemical and Biophysical Research Communications*, 2020.

doi: <https://doi.org/10.1016/j.bbrc.2020.05.121>

## Authors

Yohko Yamaguchi, Kohei Kaida,  
Yusuke Suenaga, Akihito Ishigami,  
Yoshiro Kobayashi, and Kisaburo Nagata

## Abstract

Aging promotes polarization of M2-like macrophages to M1-like macrophages and reduces their phagocytic ability. However, the molecular mechanisms underlying these aging-related changes remain poorly understood. Here, we demonstrate that p53 regulates phagocytic activity in macrophages from young mice but not in those from old ones. Macrophages from both old and young mice expressed functional p53 to induce target genes including p21 and Mdm2. In macrophages from young mice, chemically induced p53 decreased phagocytic activity and c-Myc levels, with the latter change reducing M2-related genes. However, in macrophages from old mice, phagocytic activity and c-Myc expression were independent of p53 activity. Furthermore, c-Myc suppression did not affect M2-related genes in old-mouse macrophages. These results demonstrate that dysregulation of p53 function is a molecular mechanism underlying reduced phagocytic activity in aged-mouse macrophages.

Reprinted with permission from Elsevier B.V., Copyright (2020). All rights reserved.

Yohko Yamaguchi, Kohei Kaida, Yusuke Suenaga, Akihito Ishigami, Yoshiro Kobayashi, Kisaburo Nagata. Age-Related Dysfunction of p53-Regulated Phagocytic Activity in Macrophages. *Biochem. Bioph. Res. Co.* 2020, 529(2), 462-466. doi: <https://doi.org/10.1016/j.bbrc.2020.05.121>

# Real-Time PCR Assay Development for the Control of Vaccine Against Hemorrhagic Fever with Renal Syndrome

*Problems of Virology*, 2021

doi: <https://doi.org/10.36233/0507-4088-30>

## Authors

Maria Sergeevna Egorova,  
Svetlana Sergeevna Kurashova,  
Aidar Airatovich Ishmukhametov,  
Maria Vladimirovna Balovneva,  
Andrey Andreevich Deviatkin,  
Marina Viktorovna Safonova,  
Sergey Viktorovich Ozherelkov,  
Yusuf Khadzhibekovich Khapchaev,  
Alexandra Sergeevna Balkina,  
Alla Vladimirovna Belyakova,  
Tamara Kazbekovna Dzagurova, and  
Evgeny Aleksandrovich Tkachenko

## Abstract

Hemorrhagic fever with renal syndrome (HFRS) holds a leading place among natural focal human diseases in Russian Federation. There is no etiotropic therapy for the disease now. The vaccine prophylaxis is the most effective method to control this infection. The main criteria for inactivated vaccines evaluation are its immunogenicity and specific activity.

The study purposes were to develop a sensitive and specific real-time PCR method for viral RNA quantification in the inactivated vaccine and to study the correlation between the viral RNA amount and vaccine immunogenicity.

This article is licensed under a [Creative Commons Attribution 4.0](https://creativecommons.org/licenses/by/4.0/) International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

Maria Sergeevna Egorova, Svetlana Sergeevna Kurashova, Aidar Airatovich Ishmukhametov, Maria Vladimirovna Balovneva, Andrey Andreevich Deviatkin, Marina Viktorovna Safonova, Sergey Viktorovich Ozherelkov, Yusuf Khadzhibekovich Khapchaev, Alexandra Sergeevna Balkina, Alla Vladimirovna Belyakova, Tamara Kazbekovna Dzagurova, Evgeny Aleksandrovich Tkachenko. Real-Time PCR Assay Development for the Control of Vaccine Against Hemorrhagic Fever with Renal Syndrome. *Probl. Virol.* 2021, 66(1), 65-73. doi: <https://doi.org/10.36233/0507-4088-30>

# Discriminating the Eight Genotypes of the Porcine Circovirus Type 2 with TaqMan-Based Real-Time PCR

*Virology Journal*, 2021.

doi: <https://doi.org/10.1186/s12985-021-01541-z>

## Authors

Ellen Kathrin Link, Matthias Eddicks, Liangliang Nan, Mathias Ritzman, Gerd Sutter, and Robert Fux

## Abstract

The porcine circovirus type 2 (PCV2) is divided into eight genotypes including the previously described genotypes PCV2a to PCV2f and the two new genotypes PCV2g and PCV2h. PCV2 genotyping has become an important task in molecular epidemiology and to advance research on the prophylaxis and pathogenesis of PCV2 associated diseases. Standard genotyping of PCV2 is based on the sequencing of the viral genome or at least of the open reading frame 2. Although, the circovirus genome is small, classical sequencing is time consuming, expensive, less sensitive and less compatible with mass testing compared with modern real-time PCR assays. Here we report about a new PCV2 genotyping method using qPCR.

This article is licensed under a [Creative Commons Attribution 4.0](https://creativecommons.org/licenses/by/4.0/) International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

Ellen Kathrin Link, Matthias Eddicks, Liangliang Nan, Mathias Ritzman, Gerd Sutter, Robert Fux. Discriminating the Eight Genotypes of the Porcine Circovirus Type 2 with TaqMan-Based Real-Time PCR. *Viol. J.* 2021, 18(70). doi: <https://doi.org/10.1186/s12985-021-01541-z>



# Real-Life Evaluation of a Rapid Extraction-Free SARS-CoV-2 RT-PCR Assay (COVID-19 PCR Fast-L) for the Diagnosis of COVID-19

*Journal of Medical Virology*, 2021

doi: <https://doi.org/10.1002/jmv.27039>

## Authors

Ignacio Torres, Jamal Qualai, Eliseo Albert, Felipe Bueno, Dixie Huntley, Sandrine Poujois, María Teresa Gil, and David Navarro

## Abstract

Timely and rapid diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection by reverse transcription-polymerase chain reaction (RT-PCR) is paramount to the control of the coronavirus disease 2019 (COVID-19) pandemic. Attempts have been made to shorten molecular testing turnaround by skipping nucleic acid extraction, thus performing RT-PCR directly on heat-treated respiratory specimens. A number of either "in-house"-developed or commercial (i.e., Cepheid Xpert Xpress SARS-CoV-2 assay) RT-PCR or loop-mediated amplification protocols have been developed and found to display clinical sensitivities ranging from 75% to 98% when compared to RT-PCR preceded by viral RNA extraction.<sup>1-9</sup> Here, we conducted a real-life evaluation of the performance of a commercially-available free-extraction RT-PCR, the Ascires COVID-19 PCR Fast-L (Sistemas Genómicos), which is multiplexed to amplify two conserved sequences within ORF-1ab/1a (FAM and CY5 channels), one of which lies within the RdRP gene (FAM channel) and returns qualitative results in less than 1 h. In this assay, nasopharyngeal (NP) specimens are transferred to 1 ml of transport/extraction buffer containing proteinase K provided by the manufacturer, placed in a dry bath at 60°C for 5 min, then at 98°C for 2 min, and finally on ice for cooling. Target amplification is carried out using the AriaMx Real-Time PCR System (Agilent), and results are analyzed and interpreted automatically by The AriaMx Software version 1.5. Thermal cycling conditions are shown in the footnote of Table 1. The assay includes an internal heterologous DNA control (HEX probe). According to the manufacturer, the limit of detection (LOD) of the assay is approximately 4000 copies/ml (95% confidence interval [CI]).

# Rapid SARS-CoV-2 Variant Monitoring Using PCR Confirmed by Whole Genome Sequencing in a High-Volume Diagnostic Laboratory

*Journal of Clinical Virology*, 2021.

doi: <https://doi.org/10.1016/j.jcv.2021.104906>

## Authors

Andreas Lind, Regine Barlinn,  
Elisabeth Toverud Landaas, Lise Lima  
Andresen, Kirsti Jakobsen, Cathrine Fladeby,  
Mariann Nilsen, Pål Marius Bjørnstad,  
Arvind Y.M. Sundaram, Teodora Ribarska,  
Fredrik Müller, Gregor D. Gilfillan,  
and Mona Holberg-Petersen

## Abstract

The emerging SARS-CoV-2 variants of concern (VoC), B.1.1.7, B.1.351 and P.1, with increased transmission and/or immune evasion, emphasize the need for broad and rapid variant monitoring. Our high-volume laboratory introduced a PCR variant assay (Variant PCR) in January 2021 based on the protocol by Vogels et al.

To assess whether Variant PCR could be used for rapid B.1.1.7, B.1.351 and P.1 screening, all positive SARS-CoV-2 airway samples were prospectively tested in parallel using both the Variant PCR and whole genome sequencing (WGS).

In total 1,642 SARS-CoV-2 positive samples from individual patients were tested within a time span of 4 weeks. For all samples with valid results from both Variant PCR and WGS, no VoC was missed by Variant PCR (totalling 399 VoC detected). Conversely, all of the samples identified as “*other lineages*” (i.e., “*non-VoC lineages*”) by the Variant PCR, were confirmed by WGS.

The Variant PCR based on the protocol by Vogels et al., is an effective method for rapid screening for VoC, applicable for most diagnostic laboratories within a pandemic setting. WGS is still required to confirm the identity of certain variants and for continuous surveillance of emerging VoC.

Reprinted with permission from Elsevier B.V., Copyright (2021). All rights reserved.

Andreas Lind, Regine Barlinn, Elisabeth Toverud Landaas, Lise Lima Andresen, Kirsti Jakobsen, Cathrine Fladeby, Mariann Nilsen, Pål Marius Bjørnstad, Arvind Y.M. Sundaram, Teodora Ribarska, Fredrik Müller, Gregor D. Gilfillan, Mona Holberg-Petersen. Rapid SARS-CoV-2 variant monitoring using PCR confirmed by whole genome sequencing in a high-volume diagnostic laboratory. *Journal of Clinical Virology*, Volume 141, 2021, 104906, ISSN 1386-6532, <https://doi.org/10.1016/j.jcv.2021.104906>.

# Propionate of a Microbiota Metabolite Induces Cell Apoptosis and Cell Cycle Arrest in Lung Cancer

*Molecular Medicine Reports*, 2019

doi: <https://doi.org/10.3892/mmr.2019.10431>

## Authors

Kim, K., Kwon, O., Ryu, T. Y., Jung, C., Kim, J., Min, J., Kim, D., Son, M., and Cho, H.

## Abstract

Short-chain fatty acids (SCFAs; butyrate, propionate and acetate) are metabolites derived from the gut microbiota via dietary fiber fermentation. In colon cancer, treatment with SCFAs, mainly butyrate and propionate, suppresses cell proliferation, migration and invasion. Furthermore, although sodium butyrate is known to induce cell apoptosis in lung cancer, the anticancer effects of sodium propionate (SP) on lung cancer are not well understood. In the present study, SP treatment induced cell cycle arrest, especially in the G2/M phase, and cell apoptosis in the H1299 and H1703 lung cancer cell lines. As determined by reverse transcription-quantitative PCR and western blotting, Survivin and p21 expression levels were significantly affected by SP treatment, suggesting that SP treatment suppressed cell proliferation in these lung cancer cell lines. Thus, it was proposed that the SP-mediated regulation of Survivin and p21 in lung cancer may be applicable to lung cancer therapy.

Kim et al. This is an open access article distributed under the terms of Creative Commons Attribution License.

Kim, K., Kwon, O., Ryu, T. Y., Jung, C., Kim, J., Min, J., Kim, D., Son, M., Cho, H. Propionate of a microbiota metabolite induces cell apoptosis and cell cycle arrest in lung cancer. *Molecular Medicine Reports*, 2019. 20, 1569-1574. <https://doi.org/10.3892/mmr.2019.10431>

# Identification of Putative miRNA Biomarkers in Early Rheumatoid Arthritis by Genome-Wide Microarray Profiling: A Pilot Study

*Gene*, 2019.

doi: <https://doi.org/10.1016/j.gene.2019.144081>

## Authors

M.F. Romo-García, Y. Bastian,  
M. Zapata-Zuñiga, N. Macías-Segura,  
J.D. Castillo-Ortiz, E.E. Lara-Ramírez,  
J.C. Fernández-Ruiz, A.J. Berlanga-Taylor,  
R. González-Amaro, C. Ramos-Remus,  
J.A. Enciso-Moreno,  
and J.E. Castañeda-Delgado

## Abstract

Despite the existing research, the etiology of rheumatoid arthritis (RA), an autoimmune disease remains poorly understood with early and accurate diagnosis difficult to achieve. MicroRNAs (miRNAs) play an important role in biological processes as modulators of transcription and translation. Previous studies have demonstrated a downregulation of several genes in early RA stages and in addition, miRNAs may serve as early biomarkers of subclinical changes in early RA.

When comparing the four groups (ANOVA  $P < 0.01$ , fold change  $> 4$ ), we found 253 differentially expressed miRNAs. Of these, 97 miRNAs were identified as overexpressed in early rheumatoid arthritis. The validation of miRNA microarray expression was performed in a set by RT-qPCR and showed strong agreement with microarray expression data. The putative targets of overexpressed microRNAs in early RA were significantly enriched in apoptosis, tolerance loss and Wnt pathways. Moreover, ROC analysis showed values of AUC 0.76 and  $P < 0.05$  for miR 361-5p, identifying this miRNA as a potential biomarker of disease.

We identified specific microRNAs associated with early rheumatoid arthritis and proposed them as early biomarkers of disease. Our results provide novel insight into immune disease physiopathology and describe unreported microRNAs in RA with potential for clinical use.

Reprinted with permission from Elsevier B. V., Copyright (2019) All rights reserved.

M.F. Romo-García, Y. Bastian, M. Zapata-Zuñiga, N. Macías-Segura, J.D. Castillo-Ortiz, E.E. Lara-Ramírez, J.C. Fernández-Ruiz, A.J. Berlanga-Taylor, R. González-Amaro, C. Ramos-Remus, J.A. Enciso-Moreno, J.E. Castañeda-Delgado. Identification of putative miRNA biomarkers in early rheumatoid arthritis by genome-wide microarray profiling: A pilot study. *Gene* 720, 144081 (2019). <https://doi.org/10.1016/j.gene.2019.144081>.

**AriaMx is For Research Use Only. Not for use in diagnostic procedures.**  
**AriaDx is For In Vitro Diagnostic Use.**  
PR7000-3486

This information is subject to change without notice.

© Agilent Technologies, Inc. 2022  
Published in the USA, September 20, 2022  
5994-3856EN

