3-2020

Detection and Quantification of Bacterial Species Important to Mental and Physical Health

David G. Leonard

DETECTION AND QUANTIFICATION OF BACTERIAL SPECIES IMPORTANT TO MENTAL AND PHYSICAL HEALTH

THESIS

David G. Leonard, Captain, USAF

AFIT-ENV-MS-20-M-224

DEPARTMENT OF THE AIR FORCE
AIR UNIVERSITY

AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

AFIT-ENV-MS-20-M-224

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THESIS

Presented to the Faculty

Department of Systems Engineering and Management

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Engineering Management

David G. Leonard, BS

Captain, USAF

March 2020

AFIT-ENV-MS-20-M-224

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Committee Membership:

Lt Col. A. J. Hoisington, PhD
Chair

Dr. Camilla A. Mauzy
Member

Maj J. D. Delorit, PhD
Member

Abstract

The human gut microbiome contains an abundance of microorganisms which could influence mental health as well as physical health. These microorganisms produce chemicals which affect the brain and the body in various ways. Probiotic bacteria and yeasts have been studied to determine effects they have on mice, rats, and humans to illustrate the importance these microorganisms on health. Studies have shown that adding beneficial microorganisms to the human diet can have positive effects on mental and physical health, to include lessening symptoms of depression and anxiety, lessen gastro-intestinal inflammation, displacing pathogens, and improving immunomodulatory response. A quantitative way to identify these microorganisms would be beneficial for future research and future use. Utilizing quantitative polymerase chain reaction, qPCR, to identify and quantify these probiotic microorganisms, and the data required to create assays and standard curves, it is possible to estimate the quantity of DNA of the associated bacteria from a sample. Methods, procedures, and materials were created or compiled for the purpose of growing the species, extracting the DNA, and amplifying the DNA via qPCR. These methods, procedures, materials, and the data and the standard curves created from qPCR were all compiled into a reference guide helpful in identifying and quantifying the bacteria important to human health in future endeavors.

Acknowledgments

To my wife, thank you for the support, love, and the continued encouragement to achieve higher education. I am grateful for my boys who kept our home lighthearted during times of stretching and stress. I would like to express my sincerest appreciation to my faculty advisor Lt Col. Andrew Hoisington and my committee, for their guidance, support, and mentorship throughout the course of this effort. I would, also, like to thank my sponsor, Air Force Research Laboratory, for seeing a need for this research.

David G. Leonard

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DETECTION AND QUANTIFICATION OF BACTERIAL SPECIES IMPORTANT TO MENTAL AND PHYSICAL HEALTH

Chapter 1: Introduction

Background

Recently, a multitude of research has been conducted to understand the gut-brain axis, specifically the connection of the microbiome and its related compounds to physical performance, cognitive ability, and mental health. Through diet or supplement, numerous studies suggest these microorganisms produce a variety of chemicals that affect systemic inflammation, neuroinflammation, cognitive function, and emotional behavior [1]. Beyond the chemicals that affect mental and physical health, some microorganisms can effect serotonin production [2] or release dopamine [3]. Humans have existed and developed alongside these microorganisms to where a symbiosis exists [4]. A category of foods that has the potential to contain large quantities of these probiotic microorganisms is fermented food. Although humans have created fermented foods and beverages for thousands of years, people have changed their preference of preserving from fermentation to chemically preserving foods [5]. The change in preserving left many people without these foods and microorganisms in their typical diet. Due to the resurgence of home preserving and more traditional ways of preparing food, it is necessary to identify the microorganisms to which people may be exposed. Members within the various branches of the Department of Defense are exposed to multitudes of stressors, pathogens, and environments, all of which effect mental and physical readiness.

Problem Statement

Despite advancement in research regarding the gut microbiome and the connection to mental and physical health, methods utilizing existing microbial tools to provide a comprehensive and accurate bacterial species level quantification of the potentially positive bacteria are missing. The purpose of this research was to develop the methods and protocol for qPCR assays of multiple gut bacteria with species in the genera of *Lactobacillus* and *Bifidobacterium*.

Research Objectives

The end product of this thesis was to provide a reference guide to assist future research into these bacteria. In order to create this product, the following research objectives were accomplished.

1) Identify microorganisms associated to be beneficial to human mental and physical health.
2) Develop, test, and verify methods by which to grow, quantify cells, and extract DNA of the beneficial microorganisms as well as primers and methods to create qPCR assays.
3) Compile a reference guide containing all methods, materials, melt curves, amplification curves, and standard curves for each of the microorganisms chosen.

Preview

Chapter 2, "Review of Microorganisms, Fermented Foods, and Health Benefits," is a comprehensive review of current academic literature in regards to fermented foods and beverages, their associated microorganisms, and the possible health benefits conferred. This article investigates the biological processes by which the microorganisms responsible for fermentation may convey benefits to individuals. Furthermore, various fermented foods are investigated to determine what effects they may have on health. Finally, Chapter 2 summarizes the microorganisms and associated effects on mental and physical health. The target journal for this paper is *Journal of Food Science and Technology*.

Chapter 3, "Quantitative PCR Assays to Identify and Quantify *Lactobacillus* and *Bifidobacterium* Species Which Affect Mental and Physical Health," provides details on the methods, materials, and procedure used to create the qPCR assays. Based on research in Chapter 2 and procurement time, nine bacteria with species in the genera of *Lactobacillus* and *Bifidobacterium* were grown and amplified. All nine species were grown, DNA was extracted, and qPCR was performed in triplicate for each step. Upon completion of qPCR, standard curves and the corresponding equations were created. Finally, amplification curve plots, melt curve plots, and standard curve plots were compiled with all methods and materials to create a reference guide in the form of data sheets for future research. The target journal for this paper is *Journal of DoD Research and Engineering*.

Chapter 4, "qPCR Data Sheets for Immunomodulatory Bacteria," displays the end product from all the research in Chapter 2 and all the experimentation in Chapter 3. The

methods, materials, and procedures to recreate the various plots and data have been compiled and organized for future research or future application. Although the results are listed in Chapter 4, the analysis of the results is in Chapter 3. The target journal for this paper is *Journal of DoD Research and Engineering*.

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Chapter 2: Literature Review of Microorganisms, Fermented Foods, and Health Benefits

Chapter Overview:

The purpose of this chapter is to provide a comprehensive review of current academic literature regarding fermented foods and beverages, their associated microorganisms, and their possible health benefits. This article investigates the biological processes by which the microorganisms responsible for fermentation may convey benefits to individuals, specifically the chemicals, butyrate, GABA, serotonin, and dopamine. Furthermore, various fermented foods and beverages of the western diet are investigated to determine what effects they may have on health. Finally, studies on microorganisms and their associated effects on mental and physical health are investigated and summarized. This chapter provides the basis of what microorganisms are selected for qPCR in Chapter 3.

Publication Intention:

Title: Health Benefits of Fermented Foods conferred by their Microorganisms Responsible

Publication: *Journal of Food Science and Technology*

Review: Health Benefits of Fermented Foods conferred by their Microorganisms

David G. Leonard[1], Justin D. Delorit[1], Camilla A. Mauzy[2], Andrew J. Hoisington[1,3,4]

[1]Department of Systems Engineering and Management, Air Force Institute of Technology, Wright-Patterson AFB, OH 45433, USA

[2]711 HPW/RHDJ, Air Force Research Laboratory, Wright Patterson Air Force Base, Dayton, OH, USA

[3]Military and Veteran Microbiome: Consortium for Research and Education (MVM-CoRE), Denver, CO 80220, USA

[4]Veterans Health Administration, Rocky Mountain Mental Illness Research Education and Clinical Center (MIRECC), Denver Veterans Affairs Medical Center (VAMC), Denver, CO 80220, USA

Abstract:

Humans have been fermenting to create multitudes of other food and drink products for thousands of years. Fermented foods, via the microorganisms used to create them, provide benefits to the human microbiome. The microorganisms in the human gut influence mental health as well as physical health by production of chemicals which may affect the brain via transmission through multiple pathways including the vagus nerve. Transmission of chemicals by these methods could be the way microorganisms improve mood and lessen symptoms of depression and anxiety. A need has arisen in recent years to study the microorganisms responsible for fermented foods in the Western diet. Microorganisms have been studied in vitro, in vivo, and in humans to determine their effects on mental and physical health. A benefit of the fermentation process is the cultivation of these microorganisms, increasing the exposure of benefits to humans. Studies have shown that adding beneficial microorganisms to the human diet, like those found in fermented foods, can have positive effects on mental and physical health, to include lessening symptoms of depression and anxiety, lessen gastro-intestinal inflammation, displacing harmful microorganisms, and improving immunomodulatory response.

Introduction:

For thousands of years, humans have fermented foods and beverages to preserve, alter the flavor, and provide health benefits. From pickled vegetables and fruits, yogurt and cheese, to wine and beer, humans have experimented with fermenting a multitude of foods and beverages. Louis Pasteur was one of the first scientists to recognize the connection between microorganisms and their role in the fermentation process [1]. Fermentation is a biological process by which microorganisms, namely bacteria and yeast, consume sugars and carbohydrates to produce byproducts, such as alcohol, lactic acid, and carbon dioxide [2].

Fermentation is vital to the production of cheese, yogurt, beer, wine, select bread, and certain sausages [3]. There is widespread consumption of these foods and a growing recognition of the potential health benefits of microorganisms on human health [4]. For example, milk fermented into cheese or yogurt decreases symptoms associated with lactose intolerance [4]. The foods and beverages created through fermentation have increased health benefits due in part to the microorganisms used in the process [5]. Probiotics are associated with fermented foods but are not all encompassing. Through the consumption of fermented foods, microorganisms transit into the human digestive tract and produce a variety of chemicals that affect the gut and brain. These chemicals affect mood, decrease inflammation, and act as an antidepressant [1]. The purpose of this paper is to review fermented food and beverage health benefits, investigate the associated microorganisms, and summarize potential biological processes by which microorganisms may confer benefits to individuals.

Biological Processes by which Microorganisms Confer Benefits

The human gut microbiome is influenced by foods people consume [1]. Microbiota and the effect on the gut-brain axis is a more extensive topic than the purpose of this paper, but a simplified explanation is needed to understand the method in which these microorganisms "communicate" with the brain. Brain function and gut function influence each other through chemical signals [6]. This influence can be mental depression and intestinal inflammation [6] or finishing a satisfying meal and a release of dopamine [7]. The vagus nerve is the conduit in which the chemical signals transfer back and forth between internal organs and the brain [1]. Chemicals produced by microorganisms in the gut can be transported to the brain through penetration of the gut epithelial wall, absorption by enteric nerves, and then conducted via the vagus nerve. These chemicals can also be absorbed into the blood stream during natural digestive processes for transportation to the brain [6]. The gut microorganisms considered beneficial produce vitamins, organic acids, which may reduce more harmful bacteria, and reinforce the epithelial barrier of the intestinal lining [3]. The epithelial barrier along the intestinal wall prevents antigens from passing into the blood stream, preventing pathogens from infecting the brain and the rest of the body. These microorganisms can also produce chemicals that effect mood, mental health, and physical health. Butyrate, gamma-Aminobutyric acid (GABA), dopamine, and serotonin are some of the chemicals that are secreted by gut microorganisms that affect mental and physical health [1]. Butyrate is a chemical that can penetrate the blood brain barrier, induce a positive mood, act as an antidepressant, as well as decrease inflammation [1]. Some species of *Bifidobacterium* have the ability to produce butyrate. Butyrate affects the gut by

increased cell proliferation and differentiation, improved epithelial barrier function, stimulation of mucin synthesis, and is an anti-inflammatory agent [8]. Positive changes to insulin sensitivity, cholesterol sensitivity and regulation of fluid and electrolyte uptake are affected by butyrate [8]. Butyrate may also be important in the prevention and treatment of diet induced obesity and colon cancers [8].

Gamma-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the central nervous system. Species of *Lactobacillus* and *Bifidobacterium* in the human gut have the ability to produce GABA. This tranquilizing neurotransmitter has been studied as a way to treat inflammatory bowel disease (IBS) [1] and depression [56]. The use of GABA in the treatment of depression has been successful in patients who, previously, treatments have not worked [9]. Changes in GABA and GABA receptors have also been observed to effect mood disorders, anxiety disorders, ability to overcome fear, and changes in spatial working memory [9].

Serotonin and dopamine are neurotransmitters whose level fluctuations are associated with changes in the intensity of the symptoms associated with anxiety and depression [10], [11]. Serotonin is used by the body to regulate the nervous system, the gastro-intestinal system, the cardiovascular system, the respiratory system, and mood [10]. The production of serotonin requires tryptophan, a chemical that *Bifidobacteria infantis* produces [12]. Dopamine is used to induce reward effects for certain actions, like the contentment felt after consuming a good meal [7], and is produced by some species of *Bacillus* that live in the gut [13]

Health benefits associated with fermented foods:

In Western society, there has been both a resurgence of interest and a rising abundance of health benefit claims [14]. Yet, the pace of foundational academic research has lagged behind the commercial industry. Researchers have identified the need for in-vitro, animal, and human studies on potential beneficial health impacts [14]-[16]. While there is not a consensus on the benefits, multiple fermented foods have been preliminarily studied to include yogurt and milk, kimchi and sauerkraut, wine, beer, kombucha, and pickled vegetables and fruits.

Select microorganisms can utilize lactose as a substrate in yogurt and fermented milk, resulting in the proliferation of bacterial cells. Bacteria intentionally added for fermentation are often *Lactobacillus bulgaricus* and *Streptococcus thermophiles,* but other species of *Lactobacillus* and *Bifidobacterium* are used as well [15]. To ensure microbial consistency, the milk is sterilized before adding the desired microorganisms that convert the liquid into the intended product. There are multiple claims on the probiotic impacts of yogurt and milk. For example, a randomized, double blind study of 64 men with type 2 diabetes mellitus, observed consumption of probiotic yogurt resulted in both antidiabetic and antioxidant outcomes compared to control [15]. In another probiotic milk drink study of 132 participants funded by Yakult, consumption of the drink improved the mood of subjects that were initially poor [16].

Kimchi and sauerkraut are produced through the lactic fermentation of cabbage. Traditionally in Korea, naturally occurring lactic acid bacteria such as *Lactobacillus plantarum, Lactobacillus brevis, Pediococcus cerevisiae, Streptococcus faecalis,* and *Leuconostoc mesenteroides* ferment Napa cabbage into Kimchi [17]. Traditional German

sauerkraut is also produced utilizing naturally occurring lactic acid bacteria, *L. mesenteroides, Leuconostoc fallax, L. plantarum,* and *L. brevis* to ferment white cabbage. Although it is common to use naturally occurring bacteria, industrial processes have noted improved quality by the addition of *Lactococcus lactis, Pediococcus dextrinicus, Lactobacillus sakei, L. plantarum, Lactobacillus casei,* and *Lactobacillus curvatus* [18]. The process is similar to create kimchi and sauerkraut, only with variations on spices and accompanying ingredients. In one study, 22 participants demonstrated a significant correlation between a diet including fermented kimchi and decreased body fat, improved blood pressure, decreased fasting insulin levels, decreased fasting glucose levels, and decreased total cholesterol levels over a diet containing non-fermented kimchi [17]. Fermented kimchi was also shown to have an anti-obesity effect in mice fed a high fat diet. Lower levels of serum insulin, serum leptin, and epididymal fat were observed compared to the mice that consumed only a high fat diet [19]. Sauerkraut demonstrated anti-inflammatory effects, through the increased production of nitric oxide production inhibitors, as well as antioxidant effects in murine macrophages compared to non-fermented cabbage [20].

Wine is a produced from the fermentation of fruit juice, most popularly grapes. The microorganism used in the production of wine is the yeast, *Saccharomyces cerevisiae.* Researchers attempted to determine the link between red wine consumption and lipopolysaccharide serum concentrations, which is associated with liver disease. The study concluded that while consumption of red wine does not directly reduce post meal lipopolysaccharide serum concentrations, it does increase the abundance of *Bifidobacterium* and *Prevotella*, which may in-turn lower lipopolysaccharide serum

concentrations [21]. Studies in moderate consumption of wine have also shown that is beneficial to the cardiovascular system [22]. Benefits to cardiovascular health were shown in a study on concentrations of inflammatory markers four hours after the consumption of wine. In addition, a significant correlation was demonstrated between wine consumption and the reduction of factors associated with the development of atherosclerosis such as blood pressure, plasma glucose, and LDL-cholesterol, [23]. In a longitudinal study on the connection between alcohol consumption and depression, 13,619 university graduate students were administered biennial surveys about personal alcohol consumption and depression. Results showed a U-shaped correlation between alcohol consumption, primarily wine, and depression. Out of the four categories of alcohol consumption (none, minimal, moderate, heavy), moderate consumption displayed the lowest risk of depression [24].

Beer is produced from the fermentation of cereal grains and water. The yeasts used are *Saccharomyces pastorianus* and *S. cerevisiae*, depending on the type of beer brewed. A study on moderate beer consumption showed a protective effect on the cardiovascular system through the bolstering of the atheroprotective profile of HDL, which lessened cholesterol build up [25]. The association between change in HDL levels and alcohol consumption was also demonstrated in a longitudinal study of 71,379 members of the Kailuan community in Tangshan City, China. That study illustrated moderate beer consumption was associated with lowering total cholesterol over light, heavy, or no consumption [26].

Kombucha is an effervescent sweet tea beverage that has been brewed in China for over 2000 years [27]. The fermentation process uses a symbiotic culture of bacteria

and yeast to convert the sugars and tea into B-vitamins, gluconic acid, acetic acid, fructose, and trace amounts of alcohol. Acetic acid bacteria, *Acetobacter* and *Gluconacetobacter,* are the majority of the bacteria active during the fermentation process, but *Lactobacillus* and other microorganisms can be found as well [27]. Multiple reviews denote health benefits such as anti-carcinogenic and anti-diabetic effects, treatments for gastric ulcers, high cholesterol, and liver detoxification for kombucha [27]-[29]. Studies demonstrated that kombucha conferred anti-microbial properties, in-vitro [30], and prevented weight loss in diabetic rats [31] and mice [32].

Pickled vegetables and fruit are created by soaking in a brine, a vinegar, or by fermentation for the purpose lowering the pH to limit bacterial growth to select species. When pickling by fermentation, the desired bacteria outcompete other present microorganisms due to resistance to low pH, high salt concentrations, and high alcohol concentrations [33]. The primary bacterial genera associated in the pickling of vegetables and fruit is *Lactobacillus*, which are naturally occurring on vegetables and fruit. During the fermentation pickling process, nutritional quality and digestibility are enhanced, while toxins and anti-nutritional compounds are lowered [33]. A study, utilizing mass spectrometry, was performed to determine the quantity of bioactive peptides in raw, acidified, and fermented pickled cucumbers. They discovered the quantity of peptides is greater in fermented cucumbers than in either raw or acidified cucumbers [34].

Microorganisms Associated with Fermentation

Due to similar processes of fermentation, the foods and beverages noted previously contain many of the same microorganisms. For example, *Bifidobacterium, Lactobacillus, S. thermophilus,* and *S. cerevisiae* are present in fermented foods and may have positive effects on human physical and mental health [1]. Studies on the consumption of foods containing these microorganisms, reduced gastro-intestinal inflammation [35]-[37], decreased symptoms of depression and anxiety [38]-[42], increased cognitive function [43], improved immunomodulatory response [44]-[45], and boosted overall mood [12], [13], [46]-[48]. A summary of some of the microorganisms associated with fermentation and their benefits are illustrated in Table 1.

Table 1. Associated Health Benefits of Select Microorganisms used in Fermentation

Microorganisms	Benefits	Reference
Bifidobacterium bifidum	reduction in functional gastrointestinal disorders, reduced psychological stress	[35]
Bifidobacterium breve	prevent the growth of *E. coli* and *Candida albicans,* alleviates symptoms of diarrhea, lower anxiety levels in mice that were bred to be anxious, improve cognitive function, lower depression	[1], [38], [43]
Bifidobacteria infantis	increases serotonin production	[12]
Bifidobacterium lactis	improve symptoms of irritable bowel syndrome and improve mood when used with other psychobiotics	[1], [46]
Bifidobacterium longum	anti-inflammatory, lower cholesterol, antioxidant, reduction anxiety, cortisol levels, depression, improvement of cognition and coping skills, antidepressant effect	[1], [39], [40]
Lactobacillus acidophilus	reduction in stress and fatigue effects, potential in stabilizing and fortifying the gastrointestinal system against disease and infection	[36], [37]
Lactobacillus brevis	anti-inflammatory, alleviate symptoms of IBS	[1], [49]
Lactobacillus casei	some effect on combating some of the issues associated with chronic fatigue syndrome	[41]
Lactobacillus helveticus	lower blood pressure in those with hypertension, prevent anxiety and cognitive impairment, prevent inflammation and anxiety from a high fat diet, and fight pathogens, remove allergens, and enhance absorption of nutrients	[50]-[53]
Lactobacillus lactis	improves mood	[47]
Lactobacillus paracasei	contracted common cold less, exhibited cold symptoms for shorter amount of time, effects on positive mood	[48]
Lactobacillus planetarium	bolsters immune activity and improved stress management	[44], [45]
Lactobacillus reuteri	anti-inflammatory, insulin modulation	[54], [55]
Lactobacillus rhamnosus GG	fewer symptoms of depression and anxiety	[42]
Saccharomyces cerevisiae	anti-stress, anti-fatigue	[56]
Streptococcus thermophilus	treats diarrhea symptoms, increases healthy gut flora	[37]
Weisselia koreensis OK1-6	counteracts effects of high fat diet	[19]

The *Bifidobacterium* genera of bacteria are found in fermented food, either through natural occurrence or being added to aid the fermentation process. Studies have shown that it has positive effects on mental and physical health. *Bifidobacterium bifidum*, commonly detected in yogurts and fermented milk products, has been investigated as a potential probiotic in promotion of health [1]. In an open label study of 37 patients with functional gastrointestinal disorders, participants were administered fermented milk containing *B. bifidum* for four weeks. Upon conclusion of the study, the researchers correlated consumption of *B. bifidum* to a reduction of functional gastrointestinal disorders and reduced psychological stress [35]. Another species of *Bifidobacterium*, *Bifidobacterium breve*, can also be found in fermented milk drinks [1]. *B. breve* demonstrated that it prevents the growth of *Escherichia coli* and *Candida albicans,* which reduced symptoms associated with diarrhea [1]. *B. breve* also lowered the anxiety and depression symptoms in people diagnosed with schizophrenia [38] and in an Alzheimer's disease mouse model, increased cognitive function [43]. *Bifidobacterium lactis*, can also be found in fermented milk drinks and yogurts. *B. lactis* can decrease bloating symptoms associated with irritable bowel syndrome [46]. *Bifidobacterium longum*, can be found in yogurts and fermented milk drinks. *B. longum* in the human gut may lower cholesterol, have antioxidant properties, reduce anxiety, and reduce depression [1]. Consumption of *B. longum* was demonstrated beneficial in the management of stress by score changes of three questionnaires taken after a social stressor inducing game [39]. *B. longum* was administered to swimmers in a double-blind study and the results gathered from blood serum and saliva showed a decrease in anti-inflammatory cytokine IL-1ra, improved cognition, and self-reported faster physical recovery [40].

The *Lactobacillus* genera of lactic acid bacteria can also be found in fermented food. *Lactobacillus acidophilus* is in fermented milk drinks, kimchi, sauerkraut, and yogurt. A study examined chronic fatigue syndrome via a forced swim test observed rats who consumed *L. acidophilus* had a reduction in stress and fatigue effects [36]. *L. acidophilus* aids in the protection of the gastrointestinal system against disease and infection through production of lactic acid and other organic acids [37]. *L. brevis*, can be found in bread, pickles, kimchi, sauerkraut and fermented milk drinks [1]. In a randomized, double-blind study to determine the interaction between *L. brevis* and symptoms of irritable bowel syndrome, individuals who took *L. brevis* had higher serum anti-inflammatory cytokines and decreased abdominal pain compared to the placebo group [49]. Another *Lactobacillus* bacterium, *L. casei* is found in yogurt and cheeses. A randomized, double-blind study investigating the effects of *L. casei* strain *Shirota* on chronic fatigue syndrome (CFS) was conducted on 39 patients. CFS symptoms include persistent fatigue, cognitive dysfunction, headaches, anxiety, and depression [41]. The study showed that *L. casei Shirota* affected some of the issues associated with CFS through changes in Beck Depression Inventory and Beck Anxiety Inventory scores associated with a decrease in anxiety over the course of the study [41]. *Lactobacillus helveticus*, formerly known as *Lactobacillus delbrueckii* (*bulgaricus*), can be found in cheeses and fermented milk drinks [1]. *L. helveticus* prevented anxiety and cognitive impairment in rats [50], [51], prevented inflammation and anxiety that stem from a high fat diet [52], inhibited the growth of pathogens, and enhanced absorption of nutrients in mice [53]. *L. lactis*, can be found in cheeses, and fermented milk drinks [1] and is known to improve mood when given in combination with other types of healthy gut bacteria

[47]. *Lactobacillus paracasei*, can be found in fermented milk drinks, wine, some sausages, and some cheeses. In a randomized, double-blind study involving the susceptibility to the common cold, it was noted that subjects who took a supplement containing *L. paracasei*, were diagnosed with the common cold less often, where during the 12 week intervention, 61.4% of the placebo group reported occurrence of a cold, 53% of those taking the *L. paracasei* supplement reported occurrence of a cold. Additionally, the participants administered *L. paracasei* and did get a cold, had reduced severity of symptoms compared to individuals who did not take the supplement [48]. Researchers also noted a positive mood lasting longer for the *L. paracasei* participants in comparison to the placebo group [48]. *L. plantarum*, can be found in some sausages, sauerkraut, pickles, and kimchi. *L. plantarum* was found to be beneficial to natural killer cells whose function is to destroy virally-infected cells assist and with other immune responses [45]. In a double-blind study involving 171 subjects with natural killer cell counts less than 50%, it was found that the subjects who consumed the yogurt with *L. plantarum* HOKKAIDO showed an increase in natural killer cell activity and showed lower stress markers [44]. The yogurt containing *L. plantarum* HOKKAIDO may help with immune activity and with decrease the biological markers associated with stress. Another *Lactobacillus* microorganism, *Lactobacillus reuteri*, has shown promise in effecting insulin and c-peptide secretions, important to people who have issues with sugar regulation [54]. *L. reuteri* also has anti-inflammatory properties demonstrated in vivo and in vitro [55]. *L. rhamnosus*, can be found in fermented milk drinks as well as fermented oatmeal. In a double-bind study of postpartum depression in 423 women, it was found that the women who took the supplement containing *L. rhamnosus* during and after

pregnancy exhibited fewer symptoms of depression and anxiety than those taking the placebo [42]. The combined views of these studies indicate that *Lactobacillus* has promise as a probiotic to benefit human physical and mental health.

There are other bacteria and yeasts commonly used to ferment food and drink to which health effects are not as well studied. For example, *S. cerevisiae* is yeast that can be found in wine, beer, bread, or in supplement form. A study on the anti-stress and anti-fatigue effects of *S. cerevisiae* was performed on rats who consumed fermented rice bran. The study showed a correlation between the consumption of the fermented rice bran and anti-stress by the change in weight of the spleen, thyroid, thymus, and adrenal gland over the control. It also showed anti-fatigue effects over the control with extended performance times during the swim test [56]. *S. thermophilus*, is bacteria that can be found in yogurts, cheeses, and fermented milk drinks. *S. thermophilus* has shown beneficial in treating diarrhea and has a secondary benefit of increasing other healthy microorganisms, such as *Bifidobacterium* [37].

Beneficial microorganisms may work more efficiently as a community. When *Bifidobacterium* and *Lactobacillus* species were administered orally in a multispecies probiotic, a study demonstrated improved mood, as illustrated by changes in MRI measures [57]. The same study also noted positive changes in behavioral scores on self-reported assessments to include LEIDS-r questionnaire [57], Positive and Negative Affect Schedule, Symptoms Checklist 90, Allgemine Depressionsskala [57]. Similar results were observed in another study which measured changes in mood via LEIDS-r questionnaire [58].

Conclusion

Studies performed utilizing in-vitro models, animal subjects, or human subjects demonstrated the benefits theses microorganisms can convey. Fermented foods, the microorganisms they contain, and the chemicals produced by those microorganisms are beneficial to those who consume them. Fermented food, as the delivery method of providing probiotic microorganisms to the human gut microbiome, has shown to have positive effects on test subjects, but similar effects were shown with the administration of probiotic supplements. The benefit to utilizing food as the delivery method would be the abundance of and readily availability of fermented foods and beverages in the Western diet. Additional studies into fermented foods should provide more insight to further examine and expand how microorganisms affect mental and physical health. The need to identify and quantify the microorganisms associated with fermented foods exists. The benefits of adding these probiotic microorganisms to treatments can only be justified once enough clinical trials exist to demonstrate effectiveness.

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Chapter 3: Quantitative PCR Assays to Identify and Quantify *Lactobacillus* and *Bifidobacterium* Species Which Affect Mental and Physical Health

Chapter Overview:

This chapter provides details on the methods, materials, and procedure used to create the qPCR assays. Details are illustrated for the growth, cell count, cell extraction, and DNA extraction of nine species of bacteria in the genera of *Lactobacillus* and *Bifidobacterium*. The quantitative amplification of the DNA is illustrated as well as the methods required to replicate the qPCR. Upon completion of qPCR, standard curves and the corresponding equations were created. Amplification curve plots, melt curve plots, and standard curve plots were compiled with all methods and materials to create a reference guide for future research detailed in Chapter 4. Although the results are listed in Chapter 4, the analysis of the results is in Chapter 3. Finally, a discussion about the DNA quality and the efficiency of the standard curves provides insight into the repeatability and usability of the qPCR assays.

Publication Intention:

Title: Quantitative PCR Assays to Identify and Quantify *Lactobacillus* and *Bifidobacterium* Species Which Affect Mental and Physical Health

Publication: *Journal of DoD Research and Engineering*

Quantitative PCR Assays to Identify and Quantify Lactobacillus and Bifidobacterium Species Which Affect Mental and Physical Health

David G. Leonard[1], Zachary C. Goris[1], Justin D. Delorit[1], Camilla A. Mauzy[2], Andrew J. Hoisington[1,3,4]

[1]Department of Systems Engineering and Management, Air Force Institute of Technology, Wright-Patterson AFB, OH 45433, USA

[2]711 HPW/RHDJ, Air Force Research Laboratory, Wright Patterson Air Force Base, Dayton, OH, USA

[3]Military and Veteran Microbiome: Consortium for Research and Education (MVM-CoRE), Denver, CO 80220, USA

[4]Veterans Health Administration, Rocky Mountain Mental Illness Research Education and Clinical Center (MIRECC), Denver Veterans Affairs Medical Center (VAMC), Denver, CO 80220, USA

Abstract

Microorganisms in the gut may effect mental and physical health. The use of fermented foods or probiotic bacteria in the treatment of these issues is the next step in holistic health management. It is necessary to have a quantitative way to identify these microorganisms to determine what exists in samples. Utilizing qPCR to identify and quantify them provides the assays and standard curves necessary to determine the presence and abundance of DNA from the associated bacteria in a sample. In order to have these assays and standard curves, the methods, procedures, and materials must be created or compiled. Each species was grown, the cells counted, and DNA extracted, qualified, and quantified. The DNA was amplified using primers determined through research into previous quantification and identification studies. Standard curves were plotted from the data created using QuantStudio 6 Flex software. These equations, plots and the data created will be helpful in identifying and quantifying the bacteria potentially important to human health in future research and application.

Introduction

It is necessary to have a valid method of identifying and quantifying the gut microorganisms which hold the potential to convey positive effects to mental and physical health. A connection exists between the presence of these microorganisms in an individual's gut and their mental and physical state [1]. The importance of taking a holistic approach to human health has been the subject of multiple reviews [2]-[4]. Studies on the possible benefits conferred by these microorganisms include, reduced gastro-intestinal inflammation [1],[5]-[9], decreased symptoms of depression and anxiety [10]-[12], increased cognitive function [11], [12], improved immunomodulatory response [13]-[16], and boosted overall mood [15]. Connecting the quantity of these probiotic microorganisms to the possible health benefits requires a way to identify and quantify them in a sample from the gut microbiome.

Identification and quantification of the microorganisms in the gut microbiome is possible through the use of quantitative polymerase chain reaction (qPCR). After the review of multiple probiotic microorganisms that are found in the human gut, the species listed in Table 1 were selected. These microorganisms were selected for the following reasons: found in common foods, positive effects on mental and physical health [1], availability and lead time for procurement, and being considered generally recognized as safe (GRAS) through the Food and Drug Administration (FDA) [17]. Although, *Lactobacillus brevis* is not listed on the FDA GRAS list, it is considered a probiotic not subject to FDA guidelines [17]. Each of these microorganisms can be found in commons foods such as yogurt, cheeses, fermented sausages, and fermented vegetables [1].

Table 1. Associated Health Benefits of Select Microorganisms

Microorganisms	Benefits	References
Bifidobacterium breve	prevent the growth of *E. coli* and *Candida albicans,* alleviates symptoms associated with diarrhea, lower anxiety levels in mice that were bred to be anxious, improve cognitive function, lower depression	[1], [6], [11]
Lactobacillus acidophilus	reduction in stress and fatigue effects, potential in stabilizing and fortifying the gastrointestinal system against disease and infection	[7], [8]
Lactobacillus brevis	anti-inflammatory, alleviate symptoms of IBS	[1], [9]
Lactobacillus casei	some effect on combating some of the issues associated with chronic fatigue syndrome	[18]
Lactobacillus delbrueckii sub. *bulgaricus*	increase production of immature T cells enhancing immune response	[14]
Lactobacillus helveticus	lower blood pressure in those with hypertension, prevent anxiety and cognitive impairment in animal models, prevent inflammation and anxiety that stem from a high fat diet, and fight pathogens, remove allergens, and enhance absorption of nutrients in mice	[19], [12], [10], [15]
Lactobacillus paracasei	contracted common cold less, exhibited cold symptoms for shorter amount of time, effects on positive mood	[16]
Lactobacillus planetarium	enhanced immune response and with dealing with stress	[17], [20]
Lactobacillus rhamnosus GG	fewer symptoms of depression and anxiety	[13]

Polymerase chain reaction (PCR) is the process by which DNA is duplicated; the DNA undergoes multiple cycles of heating, until the strands separate; cooling, until primers bond to the strands; and extending the primers with a DNA polymerase [21]. Small concentrations of DNA can be amplified into larger quantities through the use of PCR. Quantitative polymerase chain reaction (qPCR) adds fluorescent probes to PCR testing, which can detect amplifications of the target DNA in the reaction vessel in real time [22]. The cycle threshold method and the standard curve method are ways to analyze the data created through the qPCR process [23]. The cycle threshold method measures the change in fluorescent signal of the reporter dye to the number of amplification cycles

undergone [24]. The standard curve method is an absolute quantification method that requires the amplification of serial dilutions of the same DNA [24]. The cycle threshold is plotted logarithmically against the quantity of DNA. This method provides an estimate DNA quantity based on the cycle threshold determined in future qPCR tests. Analyzing the melt curve at the end of the qPCR process allows the ability to verify the sample amplified is the targeted sample [25]. The melt curve can also serve as a quality check to verify if contaminants were present within a sample, since each DNA has its own temperature of dissociation. Multiple peaks in the melt curve indicate multiple strains of DNA. The use of qPCR can provide data to analyze the DNA according to the cycle threshold method, the standard curve method, and the production of melt curves. The data and plots created will be compiled into a technical reference guide that will assist in future research and application.

This paper details the methods required to culture the bacterial species from a freeze-dried state, how the plating was performed, and how the species where extracted, creating a known bacterial dilution. Furthermore, the methods and techniques required to extract and quantify the DNA utilizing qPCR are detailed. Finally, the process by which standard curves were created and the quality control method is identified. The purpose of this paper is to provide methods, materials, and procedures to create assays and standard curves for nine species of probiotic bacteria.

Method and Materials

Culture Growth

Lactobacillus species were received freezes-dried and revived by combining with a broth. The broth, sterilized in an autoclave at 121ºC for 25 minutes, consisted of 5.5 g BD Difco™ Lactobacilli MRS Broth (Becton, Dickinson and Company, DIFCO 288130, Franklin Lakes, New Jersey) and 100 ml deionized water. Inoculation occurred by transferring 1 ml room-temperature broth to the vial containing the *Lactobacillus* species freeze-dried pellet. The aliquot of broth and *Lactobacillus* species was next transferred to a test tube with 4 ml of broth. A broth/agar mixture was used as a growth medium for each of the *Lactobacillus* cultures. The broth/agar mixture was created by mixing 27.5 g broth (BD 288130), 500 ml deionized water, and 7.5 g agar. The broth/agar mixture was sterilized in an autoclave at 121ºC for 40 minutes. Once cooled to room temperature, the broth/agar mixture and the inoculated broth were ready for plating.

As with the *Lactobacillus* species, a broth was required to inoculate the *Bifidobacterium* cultures from a freeze-dried state. The broth, sterilized in an autoclave at 121ºC for 25 minutes, was created by mixing 3.0 g Tryptic Soy Broth (Becton, Dickinson and Company, DIFCO 211825, Franklin Lakes, New Jersey) and 95 ml deionized water. The sterilized mixture was cooled to ~47ºC before gently mixing in 5 ml room temperature sheep's blood (defibrinated), (ThermoFisher Scientific, R54012, Waltham, Massachusetts). Inoculation occurred by transferring 1 ml of the room-temperature broth/sheep's blood mixture to the vial containing the *Bifidobacterium* species freeze-dried pellet. Next the aliquot of broth/sheep's blood and *Bifidobacterium* species was transferred to a test tube with 4 ml of broth/sheep's blood. A broth/sheep's blood/agar

mixture was used as a growth medium for each of the *Bifidobacterium* cultures. The broth/agar mixture was created by mixing 15.0 g broth (BD 211825), 7.5 g agar, and 475 ml deionized water, then sterilized in an autoclave at 121ºC for 40 minutes. The broth/agar mixture was then cooled to ~47ºC before 25 mL of room temperature sheep blood (defibrinated) was gently mixed in. Once cooled to room temperature, the broth/sheep's blood/agar mixture and the inoculated broth were ready for plating.

Plating

The method for plating each species is the same; the only change is in the growth media and species being cultured. 100 mm petri dish plate, (Fisherbrand, FB0875712, Waltham, Massachusetts), were used to grow each bacteria. The broth/agar mixture was heated to 55 ºC and 30 ml was poured onto three plates per species. Each plate was poured in such a manner as to ensure no bubbles were created. The plates were then placed into a 37 ºC incubator for three hours to dry. Once drying was complete, 0.1 ml of the inoculated broth was pipetted near an edge of the plate. A disposable sterile inoculating loop, (Globe Scientific, 2875-25, Mahwah, New Jersey), was used to inoculate the plates by dipping the loop into the broth and then spreading it onto the plate.

Once inoculated, the plates, up to 12, were placed in an AnaeroPack System Jar (Mitsubishi Gas Chemical Company Inc, R685025, Tokyo, Japan) with an AnaeroPouch (Mitsubishi Gas Chemical Company Inc, R681001, Tokyo, Japan), and a RT Anaero-Indicator (Mitsubishi Gas Chemical Company Inc, R684002, Tokyo, Japan) to provide an anaerobic environment for the cultures to grow. The AnaeroPack System was sealed and placed in an incubator at 37 ºC for at least 48 hours or until sufficient growth was noted.

Cell Extraction

The number of colonies on each plate was counted and segmented into groups of ~200. A known dilution for each plate was created using 1 ml of Phosphate Buffered Saline (PBS) 10X solution (Fisher Bioreagents, BP399-1, Waltham, Massachusetts) and ~200 colonies. This sample was vortexed for 10 seconds or until thoroughly mixed. Cell counts were accomplished with three 6 µl measurements per dilution in a 4-Chip Hemocytometer (Bulldog Bio, DHC-N420, Portsmouth New Hampshire), and magnified 40X with a Zeiss Axioskop 50 (Carl Zeiss Microscopy, Jena, Germany). The counts were made in an "X" pattern on the hemocytometer with the mean taken per chip. This provided a range of cell counts to determine the number of cells in each 1 ml dilution. The cell counts per species in cells per microliter are illustrated in Table 3.

DNA Extraction

The method from bacterial growth to DNA sample is illustrated in Figure 1. The DNA was extracted from each PBS and bacteria dilution using a DNeasy® PowerSoil® Pro Kit (QIAGEN, 47014, Hilden, Germany). Three 250 µl samples were taken from the dilution and processed in accordance with the DNeasy® PowerSoil® Pro Kit instructions [26], with the exception being during the first vortex step. In place of vortexing for 10 minutes, the sample in the PowerBead tube was placed into a MP Fast Prep -24™5G Sample Preparation System (M.P. Biomedicals LLC, Santa Ana, California) and lysed at 6.0 m/s for 30 seconds. These steps produced 100 µl of extracted DNA. With DNA was extracted, 2 µl tests of each DNA sample were measured in triplicate in a NanoDrop™ One (ThermoFisher Scientific, Madison, Wisconsin) to determine the concentration (ng/µl) and the quality (A260/A280). The samples were stored at -86 ºC until quantitative analysis was performed

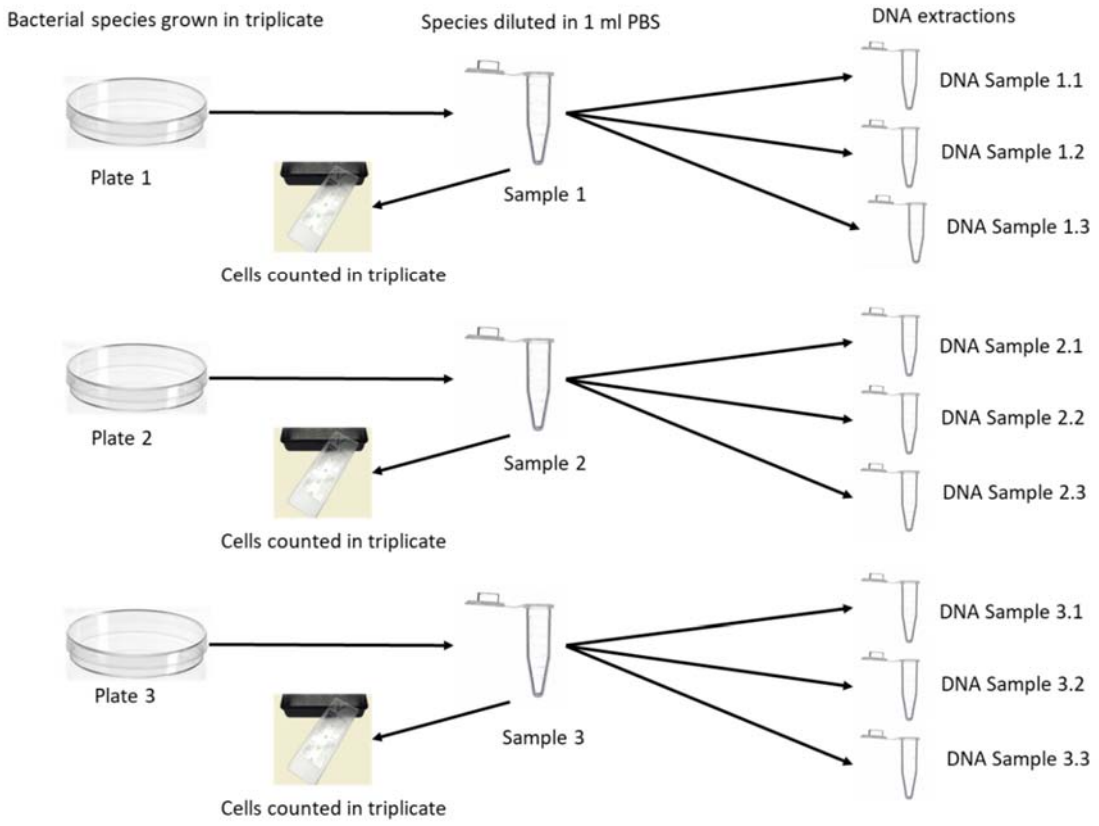


Figure 1: From Growth to Dilution with Cell Count to DNA Extraction

Quantitative Amplification

Quantitative analysis of the extracted DNA samples was performed by qPCR. The extracted DNA was serially diluted by five orders of magnitude, ranging from 1:1 to 1:10,000. Into each well of a 384-well plate, aliquots of 5 µl Powerup SYBR Green Master Mix (Applied Biosystems, A25742, Foster City, California), 1 µl of 100 nanomole Forward Primer, 1 µl of 100 nanomole Reverse Primer, 2 µl UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen, 10977015, Waltham, Massachusetts), and 1 µl DNA at dilution was added. For each DNA sample, 15 wells of DNA at dilution and 3 wells of negative control were tested. The forward and reverse primers are shown

in Table 2. The forward primers, reverse primers, and the qPCR cycling method for *B. breve* [27], *L. casei* [28], *L. rhamnosus* GG [29], *L. helveticus* [30], *L. brevis* [31], *L. acidophilus*, *L. plantarum*, *L. delbrueckii* sub. *bulgaricus*, and *L. paracasei* [32] were identified from previous studies. Although the researched qPCR methods performed, *L. delbrueckii* sub. *bulgaricus* and *L. brevis* required additional cycles to be added for complete data. Each amplification was repeated in triplicate, including the negative control, which used DNA/RNA free ultra-purified water instead of sample DNA. The 384-well plate was sealed and placed into a PlateFuge MicroCentrifuge (Southwest Science, C2000, Trenton, New Jersey) for a quick spin to ensure no air bubbles were present in the wells. The 384-well plate was placed into a QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific, Waltham, Massachusetts) and cycled according to the methods found through research (Table 2).

Table 2: List of select bacteria species, their primers, and qPCR cycle method

Species	Forward Primer	Reverse Primer	qPCR cycling method	Reference
B. breve	CCG GAT GCT CCA TCA CAC	ACA AAG TGC CTT GCT CCC T	40 cycles: Denaturation: 94 °C - 20 sec Annealing: 55°C - 20 sec Extension: 72°C - 50 sec	[27]
L. acidophilus	GTT AAG GCT GTT GAT GTA ACA AC	CTT CCC AGA TAA TTC AAC TAT CGC TTA	35 cycles: Denaturation: 95 °C - 20 sec Annealing/Extension: 55°C - 2 min	[32]
L. brevis	GCA AGC CTA TCG CGC AAA	CCG TCA ATT CCT TTG AGT TT	40 cycles: Denaturation: 94 °C - 1 min Annealing: 55°C - 2 min Extension: 74°C - 2 min	[31] Number of cycles extended
L. casei	CAG ACT GAA AGT CTG ACG G	GCG ATG CGA ATT TCT TTT TC	30 cycles: Denaturation: 93 °C - 30 sec Annealing: 57°C - 30 sec Extension: 72°C - 30 sec Final extension: 72°C - 2 min	[28]
L. delbrueckii sub. *bulgaricus*	CAC TTG TAC GTT GAA AAC TGA ATA TCT TAA	CGA ACT CTC TCG GTC GCT TT CCG	50 cycles: Denaturation: 95 °C - 20 sec Annealing/Extension: 55°C - 2 min	[32] number of cycles extended
L. helveticus	TGC TAA GGG TAT TCC TGC AAC	GCG TTA GTG TTT GCT GAG TCA TA	35 cycles: Denaturation: 94 °C - 45 sec Annealing: 58°C - 45 sec Extension: 72°C - 1 min Final extension: 72°C - 7 min	[30]
L. paracasei	ACA TCA GTG TAT TGC TTG TCA GTG AAT AC	CCT GCG GGT ACT GAG ATG TTT C	35 cycles: Denaturation: 95 °C - 20 sec Annealing/Extension: 55°C - 2 min	[32]
L. plantarum	TGG ATC ACC TCC TTT CTA AGG AAT	TGT TCT CGG TTT CAT TAT GAA AAA ATA	40 cycles: Denaturation: 95 °C - 1 min Annealing: 58°C - 30 sec Extension: 72°C - 1 min Final extension: 72°C - 5 min	[32]
L. rhamnosus GG	ATC AAC AGG CTC AGT GA	CAT GTT GTG CGC TTG GAA AA	40 cycles: Denaturation: 95 °C - 15 sec Annealing/extension: 60°C - 1 min	[29]

Results

Utilizing the data collected from cell counting and the NanoDrop One, three samples out of the nine DNA extractions created per species were selected to analyze via qPCR based on low variation amongst DNA quality and DNA quantity, higher DNA quantity, and DNA quality being close to 1.8. The remaining samples were stored at -86 ºC to keep for future use. The DNA samples selected from each species, the DNA quantity, the DNA quality, and cell counts from the known dilution are in Table 3.

Standard curves were created, for each species, using the data generated through use of the QuantStudio 6 Flex System from each qPCR run. The quantity of DNA was plotted against the cycle threshold (Ct) along a logarithmic scale to create the standard curve. An equation for the best fit line as well as the coefficient of determination (R^2) was determined for each sample. These plots and equations are displayed in figures 4a-4i.

Table 3: DNA quality, concentration, and cell quantification per species of bacteria

		DNA quantity (ng/µl)		DNA quality (A260/A280)		Cell Count (cells/ml)
Species	sample	mean	stan. dev.	mean	stan. dev.	
B. breve	2.1	19.63	0.666	1.87	0.006	8.74×10^9
B. breve	3.1	25.5	0.557	1.76	0.038	8.74×10^9
B. breve	3.3	20.73	0.231	1.77	0.015	8.04×10^9
L. acidophilus	1.2	12.77	0.513	1.67	0.04	5.47×10^9
L. acidophilus	2.2	8.57	0.551	1.81	0.155	5.73×10^9
L. acidophilus	3.3	18.13	0.379	1.79	0.021	1.05×10^{10}
L. brevis	2.2	21.43	0.808	1.79	0.04	2.43×10^9
L. brevis	2.3	19.87	0.058	1.79	0.026	2.43×10^9
L. brevis	3.2	19.83	0.351	1.8	0.06	1.69×10^9
L. casei	1.1	12.57	0.321	1.82	0.067	3.24×10^9
L. casei	2.1	9.7	0.3	2	0.023	3.24×10^9
L. casei	2.2	10.2	0.265	1.82	0.036	3.24×10^9
L. delbrueckii sub. *bulgaricus*	1.1	10.67	0.416	1.79	0.106	8.93×10^9
L. delbrueckii sub. *bulgaricus*	2.2	13.73	1.26	1.81	0.006	9.08×10^9
L. delbrueckii sub. *bulgaricus*	3.2	12.67	3.04	1.78	0.047	9.10×10^9
L. helveticus	2.1	77.87	0.751	1.81	0.015	3.38×10^9
L. helveticus	2.2	78.47	0.473	1.83	0.006	3.38×10^9
L. helveticus	2.3	75.07	0.404	1.84	0.006	3.38×10^9
L. paracasei	1.3	40.83	0.379	1.8	0.015	1.36×10^{10}
L. paracasei	3.1	32.73	0.306	1.81	0.025	1.08×10^{10}
L. paracasei	3.2	32.77	1.01	1.81	0.023	1.08×10^{10}
L. plantarum	1.2	22.53	0.513	1.85	0.07	8.69×10^9
L. plantarum	2.2	24.4	0.608	1.78	0.012	9.79×10^9
L. plantarum	2.3	25.83	1.56	1.81	0.057	9.79×10^9
L. rhamnosus GG	1.1	35.87	6.62	1.78	0.068	1.73×10^9
L. rhamnosus GG	1.3	40.1	3.58	1.82	0.031	1.73×10^9
L. rhamnosus GG	2.2	34.07	6.03	1.77	0.052	1.83×10^9

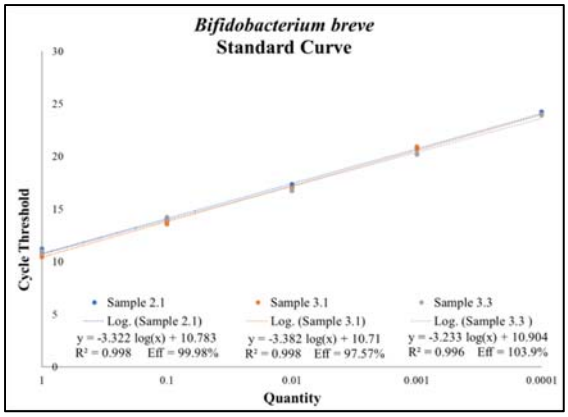


Fig. 4a

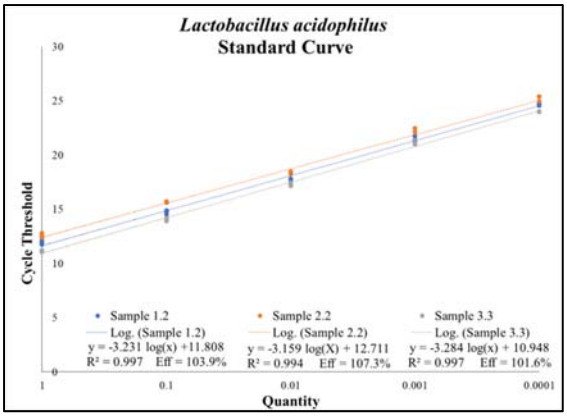


Fig. 4b

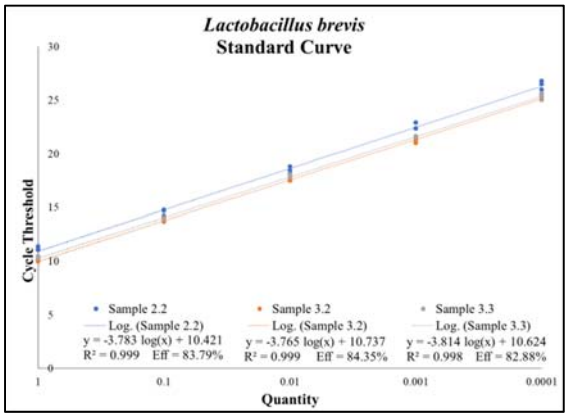


Fig. 4c

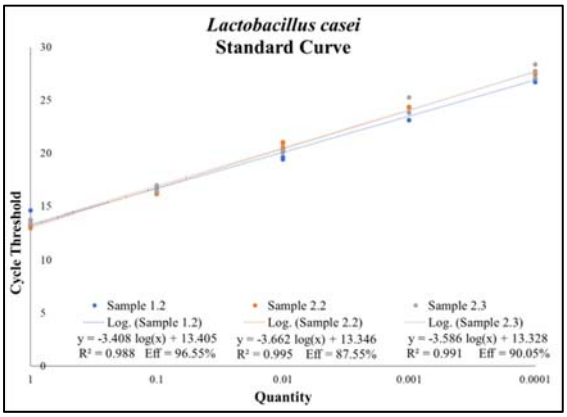
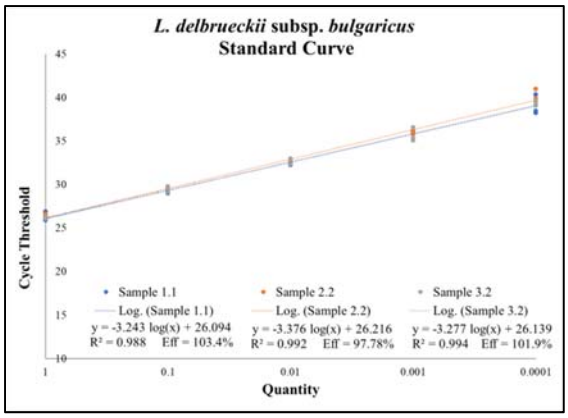


Fig. 4d



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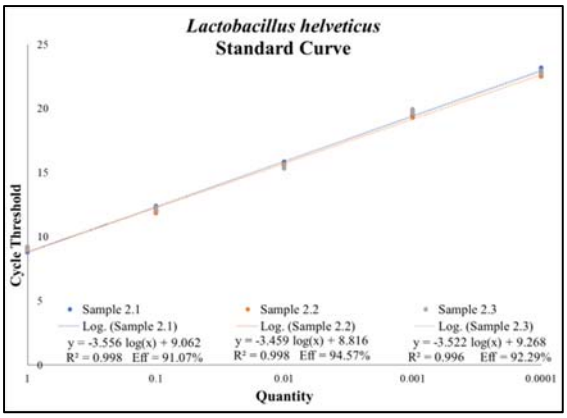

Fig. 4f

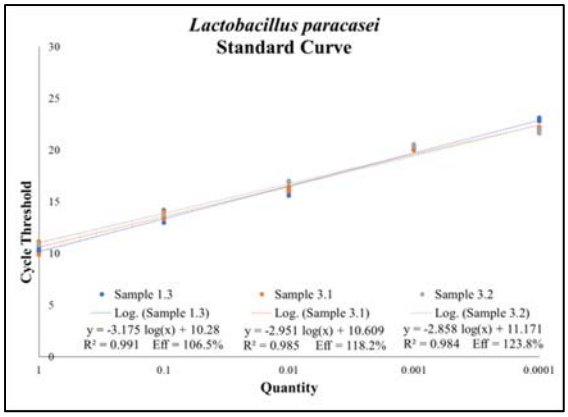

Fig. 4g

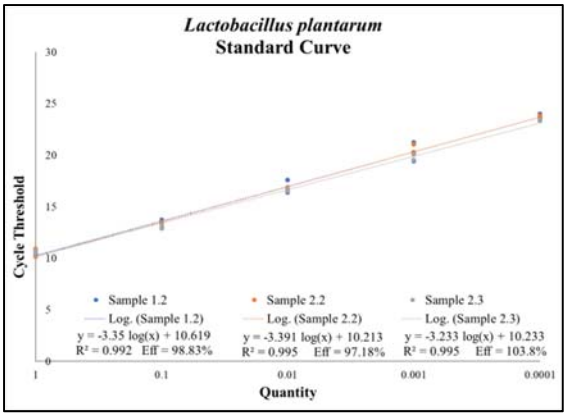

Fig. 4h

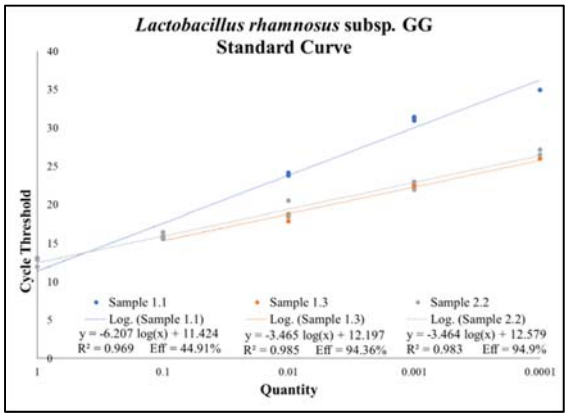

Fig. 4i

Figure 4a-i: Standard Curves by species with equations

Discussion

The DNA extraction samples used to create standard curves were selected by the DNA quality and DNA quantity. The quality of the DNA extraction is measured as the A260/A280 number. The concentration of nucleic acid in the DNA extraction is proportional to the A260 number [33]. The DNA samples were selected for quality close to 1.8 [33]. Samples with lower standard deviations in quality were selected over samples with higher standard deviations in quality. The DNA quantity was also a factor in deciding which of the DNA extraction to use. DNA extractions with DNA quantity

higher than 20 ng/µl were preferred [33]. Some of the DNA extractions from the DNA tested did not have a quantity over 20 ng/µl, so the extraction chosen for qPCR was based off the A260/A280 number and standard deviations.

The standard curves created will help in the absolute quantification of future samples of the tested bacteria. Absolute quantification relies on previously created standard curves for the known DNA. The standard curves created from the select bacteria species displayed (Figure 4a-i) have equations expressed as Equation 1, with y representing the cycle threshold, x representing the quantity, m representing the slop of the line, and b representing the y-intercept.

$$y = m\log(x) + b \qquad (1)$$

$$x = 10^{\left(\frac{y-b}{m}\right)} \qquad (2)$$

Solving for quantity from the Ct, Equation 2, it is possible to take another sample of bacterial DNA, quantified by qPCR, and determine the quantity of DNA in the sample from these standard curves. Values for equation 2 to determine each standard curve by species is listed in Table 4. The reliability of standard curves is determined by the R^2 value, and the efficiency. The R^2 value represents how much of the data is captured within the equation for the line. The efficiency of the standard curve is how many cycles each 10 fold dilution is apart with 100% efficiency being 3.3 cycles [34]. Preferred values for efficiency range from 90% to 100% [34]. The equations and values for R^2, efficiency, and error are list in Table 4.

Table 4: Variables for the equation: $quantity = 10^{(\frac{Ct-b}{m})}$, with R^2, efficiency, and error values to determine standard curves by species

Species	Sample #	m	b	R^2	eff %	error
B. breve	2.1	-3.322	10.783	0.998	99.98	0.042
B. breve	3.1	-3.382	10.710	0.998	97.57	0.047
B. breve	3.3	-3.233	10.904	0.996	103.9	0.054
L. acidophilus	1.2	-3.231	11.808	0.997	103.9	0.051
L. acidophilus	2.2	-3.159	12.711	0.994	107.3	0.071
L. acidophilus	3.3	-3.284	10.948	0.997	101.6	0.049
L. brevis	2.2	-3.783	10.421	0.999	83.79	0.040
L. brevis	2.3	-3.765	10.737	0.999	84.35	0.030
L. brevis	3.2	-3.814	10.624	0.998	82.88	0.046
L. casei	1.2	-3.408	13.405	0.988	96.55	0.109
L. casei	2.2	-3.662	13.346	0.995	87.55	0.077
L. casei	2.3	-3.586	13.328	0.991	90.05	0.100
L. delbrueckii subsp. *bulgaricus*	1.1	-3.243	26.094	0.988	103.4	0.100
L. delbrueckii subsp. *bulgaricus*	2.2	-3.376	26.216	0.992	97.78	0.085
L. delbrueckii subsp. *bulgaricus*	3.2	-3.277	26.139	0.994	101.9	0.073
L. helveticus	2.1	-3.556	9.062	0.998	91.07	0.040
L. helveticus	2.2	-3.459	8.816	0.998	94.57	0.044
L. helveticus	2.3	-3.522	9.268	0.996	92.29	0.059
L. paracasei	1.3	-3.175	10.280	0.991	106.5	0.088
L. paracasei	3.1	-2.951	10.609	0.985	118.2	0.103
L. paracasei	3.2	-2.858	11.171	0.984	123.8	0.102
L. plantarum	1.2	-3.35	10.619	0.992	98.83	0.087
L. plantarum	2.2	-3.391	10.213	0.995	97.18	0.068
L. plantarum	2.3	-3.233	10.233	0.995	103.8	0.063
L. rhamnosus GG	1.1	-6.207	11.424	0.969	44.91	0.349
L. rhamnosus GG	1.3	-3.465	12.197	0.985	94.36	0.163
L. rhamnosus GG	2.2	-3.464	12.579	0.983	94.90	0.138

The efficiencies of the qPCR results from the DNA extractions are within the preferred range with the exception of all samples of *L. brevis* and one sample of *L. rhamnosus* GG. This could be due to the sample containing PCR inhibitors or incorrect primer design [34]. The data created from all samples of *L. brevis* could be used as reference, but should be reaccomplished to for better efficiency values. Sample 1.1 of *L. rhamnosus* GG should be discarded, but was included for consistency in data displayed.

Conclusion

The assays created can be used in the holistic treatment of mental and physical health afflictions. Specifically, following the methods above, samples can be extracted to determine cell counts of specific microorganism from a subject to determine the abundance of each microorganism and to assist in multiple areas of study into these microorganisms. Some of these areas of research include the addition of probiotics alongside pharmaceutical prescriptions [2], continued research into the gut-brain axis [35]-[37], or furthering studies into the probiotic properties of fermented foods [38], [39].

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Chapter 4: qPCR Data Sheets for Immunomodulatory Bacteria

Chapter Overview:

This chapter provides the product created from research in Chapter 2 and the results from chapter 3. Details are illustrated for the all data required to replicate the qPCR assays of the nine species of bacteria in the genera of *Lactobacillus* and *Bifidobacterium*. The quantitative amplification of the DNA is illustrated as well as the methods required to replicate the qPCR. Amplification curve plots, melt curve plots, and standard curve plots, with equations, were compiled as well as all methods and materials. The end product of all the research and experimentation are theses data sheets. Future research and application of gut microorganisms could benefit from the sheets in this chapter.

Publication Intention:

Title: qPCR Data Sheets for Immunomodulatory Bacteria

Publication: *Journal of DoD Research and Engineering*

Bacterial Species: Bifidobacterium breve (ATCC15700)

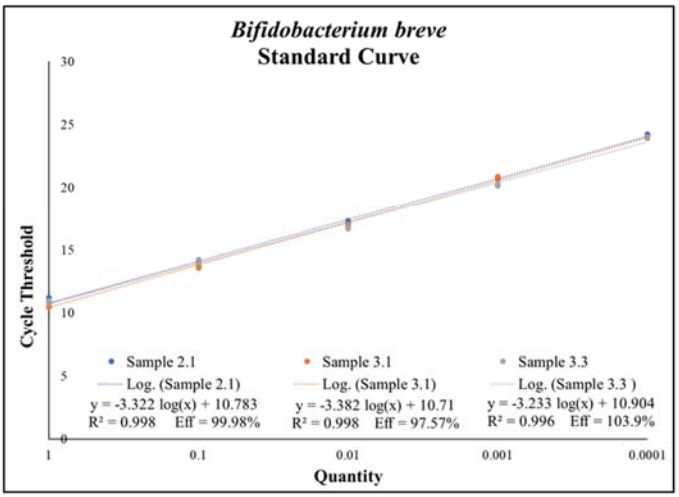
Cell Concentration: 8.74 x 10^9 cells/ml
DNA Concentration: 19.63 ng/µl

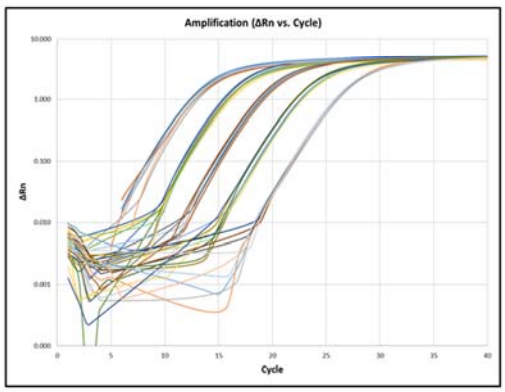
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.87

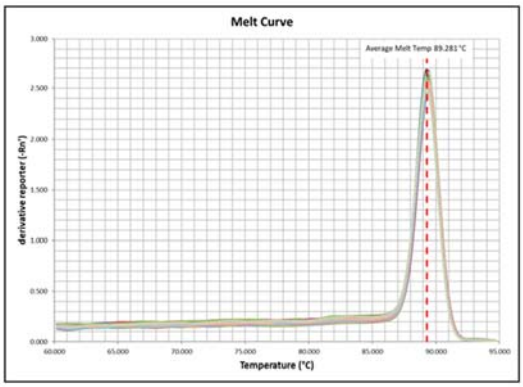
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl -100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
CCG GAT GCT CCA TCA CAC
Reverse Primer:
ACA AAG TGC CTT GCT CCC T

qPCR Method:
Hold 50 °C - 2 min
Hold 95 °C - 10 min
40 cycles:
Denaturation: 94 °C - 20 sec
Annealing: 55°C - 20 sec
Extension: 72°C - 50 sec
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Bacterial Species: Lactobacillus acidophilus (ATCC4356)

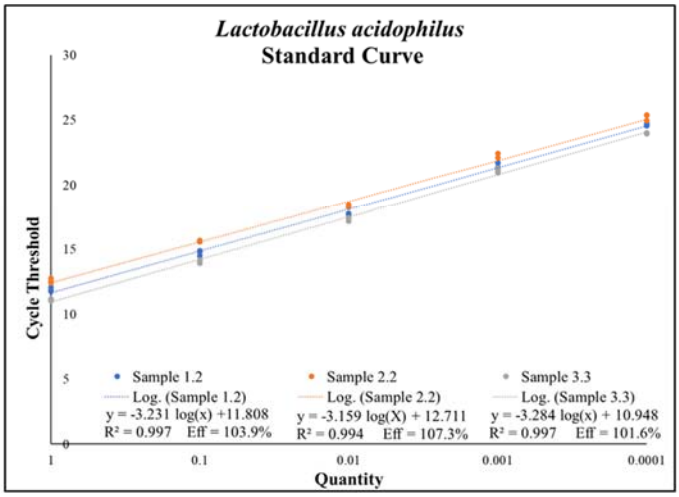
Cell Concentration: 5.73 x 10^9 cells/ml
DNA Concentration: 8.57 ng/µl

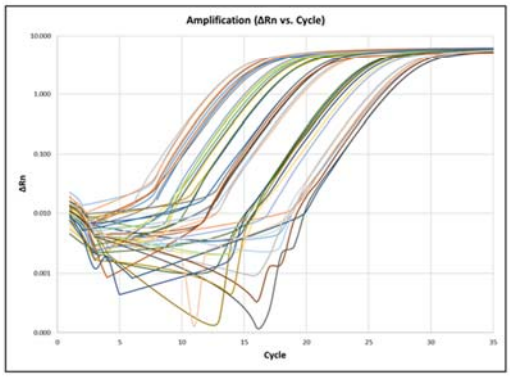
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.81

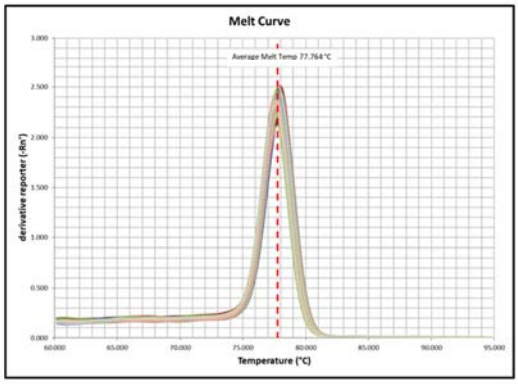
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl - 100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
GTT AAG GCT GTT GAT GTA ACA AC
Reverse Primer:
CTT CCC AGA TAA TTC
AAC TAT CGC TTA

qPCR Method:
Hold 50 °C - 2 min
Hold 95 °C - 10 min
35 cycles:
Denaturation: 95 °C - 20 sec
Annealing/Extension:
55°C - 2 min
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Bacterial Species: Lactobacillus brevis (ATCC367)

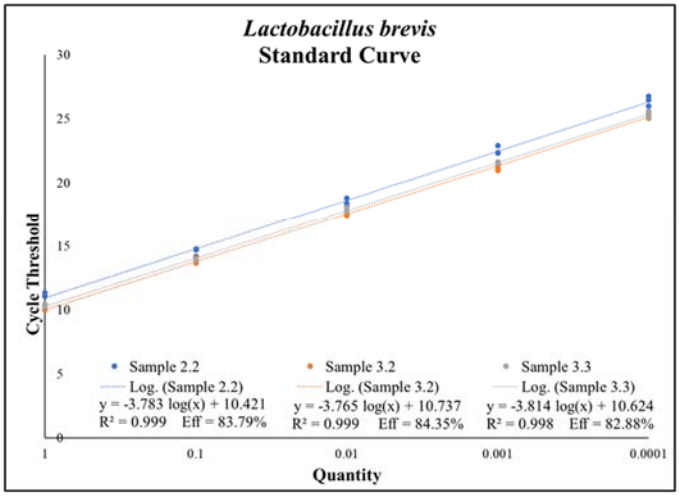
Cell Concentration: 2.43 x 10^9 cells/ml
DNA Concentration: 21.43 ng/µl

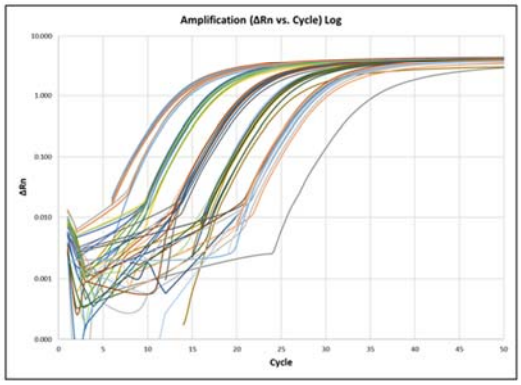
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.79

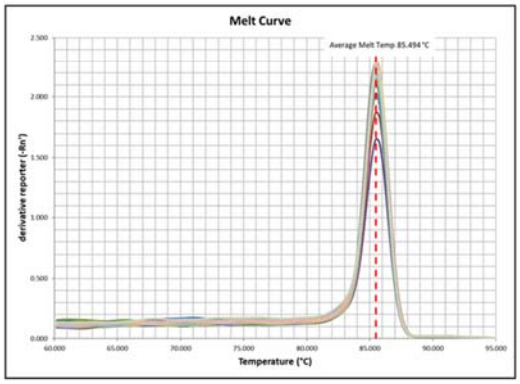
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl -100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
GCA AGC CTA TCG CGC AAA
Reverse Primer:
CCG TCA ATT CCT TTG AGT TT

qPCR Method:
Hold 50 °C - 2 min
Hold 95 °C - 10 min
40 cycles:
Denaturation: 94 °C - 1 min
Annealing: 55°C - 2 min
Extension: 74°C - 2 min
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Bacterial Species: Lactobacillus casei (ATCC393 NFP)

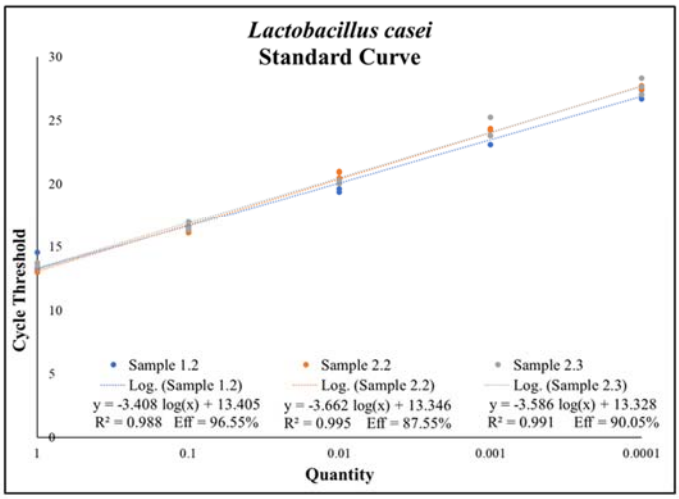
Cell Concentration: 2.24 x 10^9 cells/ml
DNA Concentration: 10.2 ng/µl

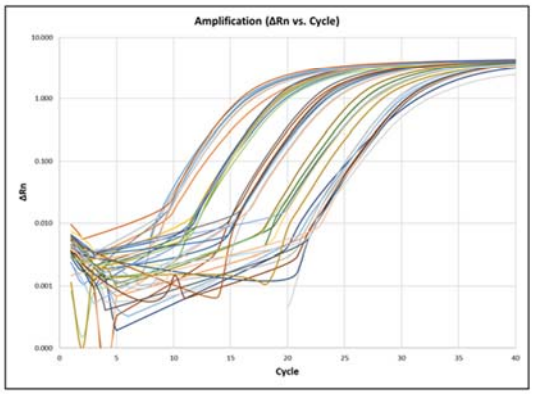
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.82

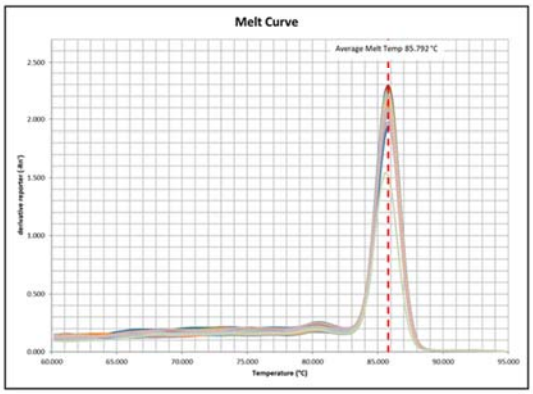
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl -100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
CAG ACT GAA AGT CTG ACG G
Reverse Primer:
GCG ATG CGA ATT TCT TTT TC

qPCR Method:
Hold 50°C - 2 min
Hold 95°C - 10 min
30 cycles:
Denaturation: 93°C - 30 sec
Annealing: 57°C - 30 sec
Extension: 72°C - 30 sec
Final Extension: 72°C - 2 min
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Bacterial Species: Lactobacillus delbrueckii subsp. bulgaricus (ATCC11842)

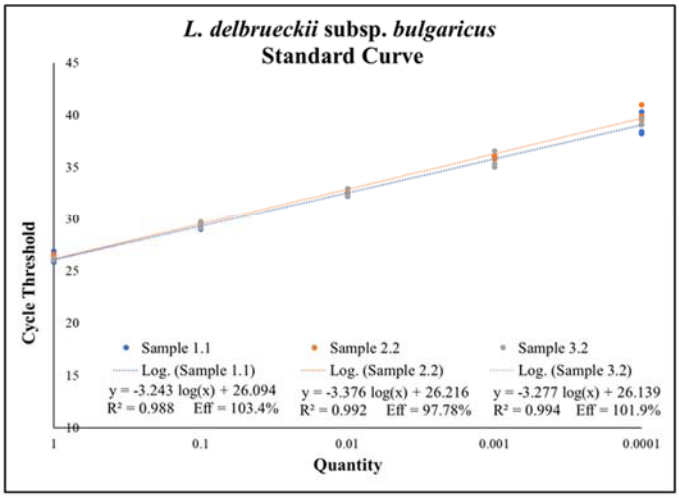
Cell Concentration: 9.08 x 10^9 cells/ml
DNA Concentration: 13.73 ng/µl

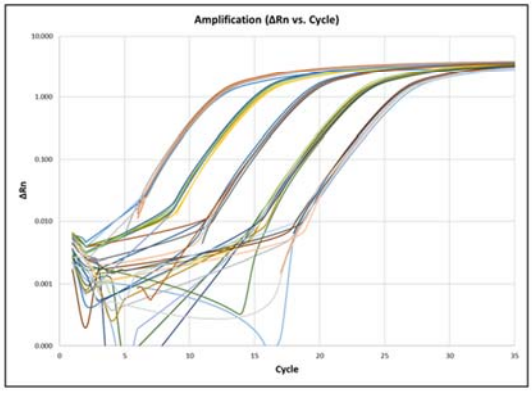
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.81

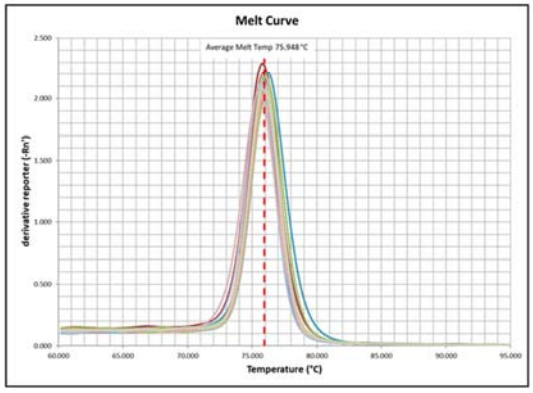
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl -100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
CAC TTG TAC GTT GAA
AAC TGA ATA TCT TAA
Reverse Primer:
CGA ACT CTC TCG GTC GCT TT CCG

qPCR Method:
Hold 50 °C - 2 min
Hold 95 °C - 10 min
50 cycles:
Denaturation: 95 °C - 20 sec
Annealing/Extension:
55°C - 2 min
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Bacterial Species: Lactobacillus helveticus (ATCC15009)

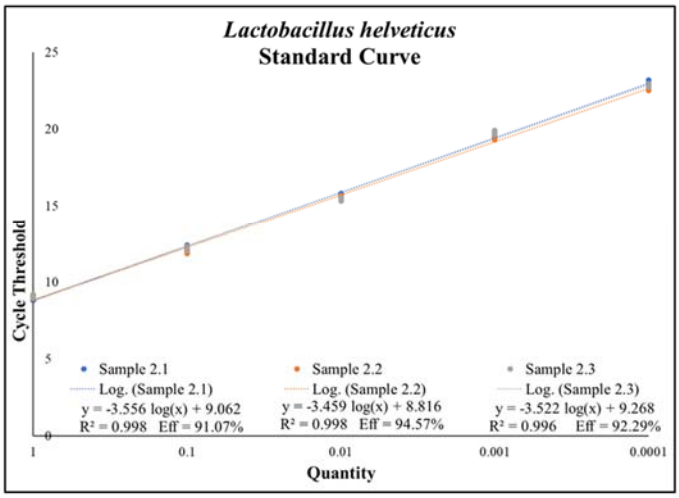
Cell Concentration: 3.38 x 10^9 cells/ml
DNA Concentration: 78.47 ng/µl

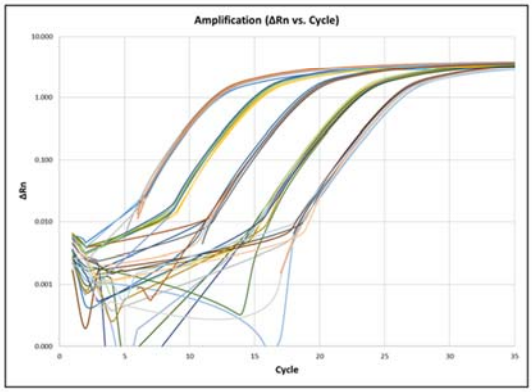
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.83

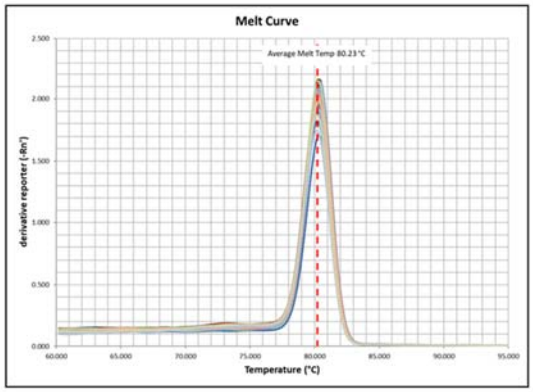
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl -100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
TGC TAA GGG TAT TCC TGC AAC
Reverse Primer:
GCG TTA GTG TTT GCT GAG TCA TA

qPCR Method:
Hold 50 °C - 2 min
Hold 95 °C - 10 min
40 cycles:
Denaturation: 94 °C - 20 sec
Annealing: 55°C - 20 sec
Extension: 72°C - 50 sec
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Bacterial Species: Lactobacillus paracasei (ATCC25302)

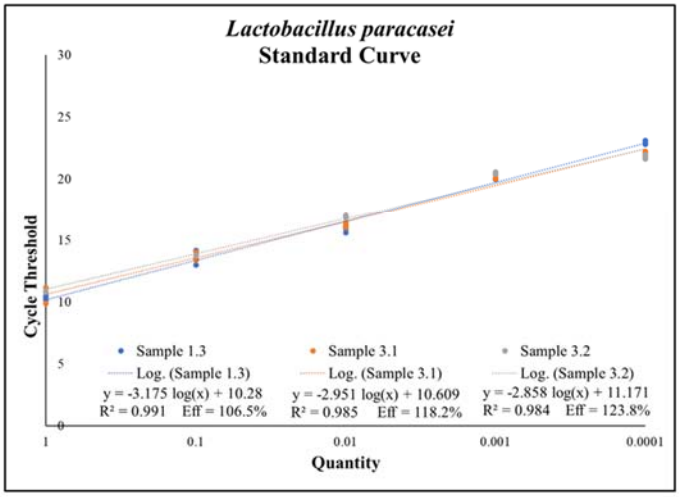
Cell Concentration: 1.36 x 10^{10} cells/ml
DNA Concentration: 40.83 ng/µl

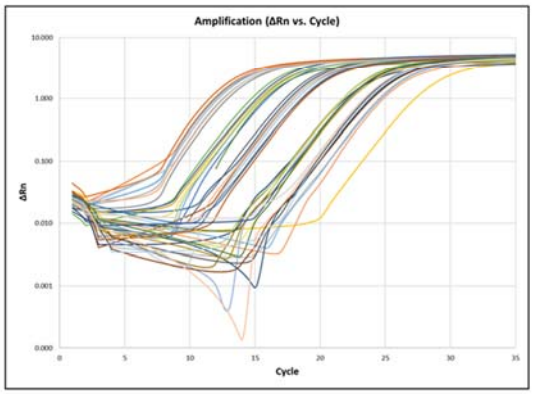
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.8

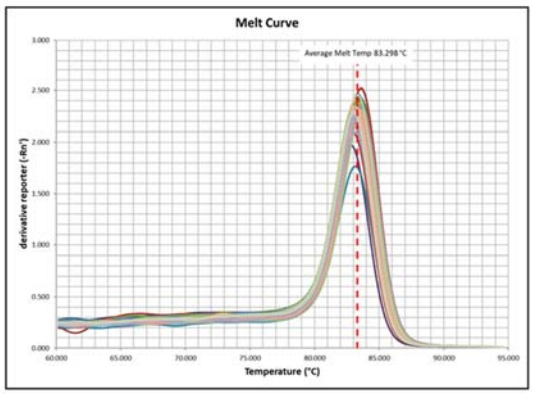
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl -100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
ACA TCA GTG TAT TGC
TTG TCA GTG AAT AC
Reverse Primer:
CCT GCG GGT ACT GAG ATG TTT C

qPCR Method:
Hold 50 °C - 2 min
Hold 95 °C - 10 min
35 cycles:
Denaturation: 95 °C - 20 sec
Annealing/Extension:
55°C - 2 min
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Bacterial Species: Lactobacillus plantarum (ATCC8014)

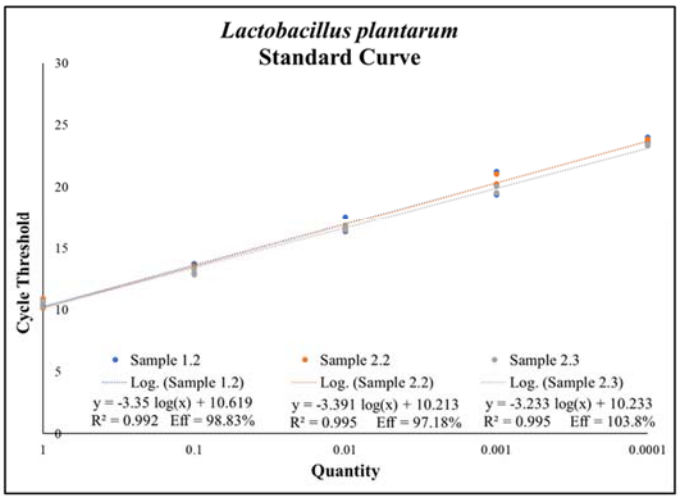
Cell Concentration: 9.79 x 10^9 cells/ml
DNA Concentration: 24.4 ng/µl

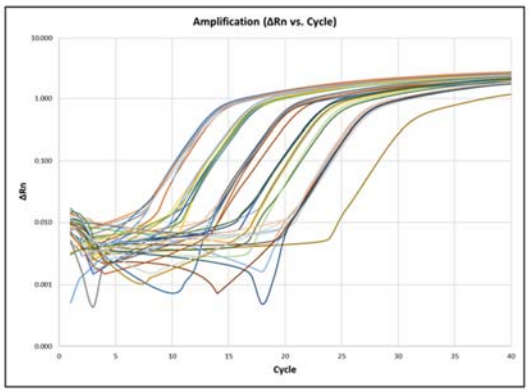
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.78

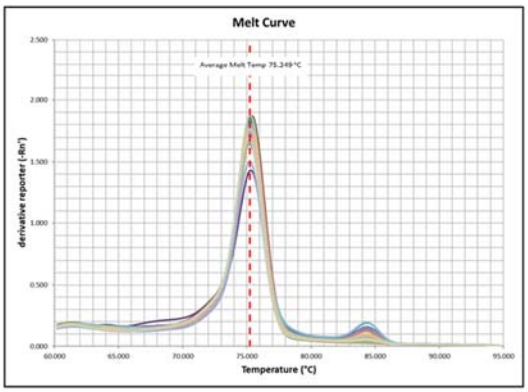
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl -100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
TGG ATC ACC TCC TTT CTA AGG AAT
Reverse Primer:
TGT TCT CGG TTT CAT
TAT GAA AAA ATA

qPCR Method:
Hold 50 °C - 2 min
Hold 95 °C - 10 min
40 cycles:
Denaturation: 95 °C - 1 min
Annealing: 58°C - 30 sec
Extension: 72°C - 1 min
Final Extension: 72°C - 5 min
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Bacterial Species: Lactobacillus rhamnosus subsp. GG (ATCC53103 NFP)

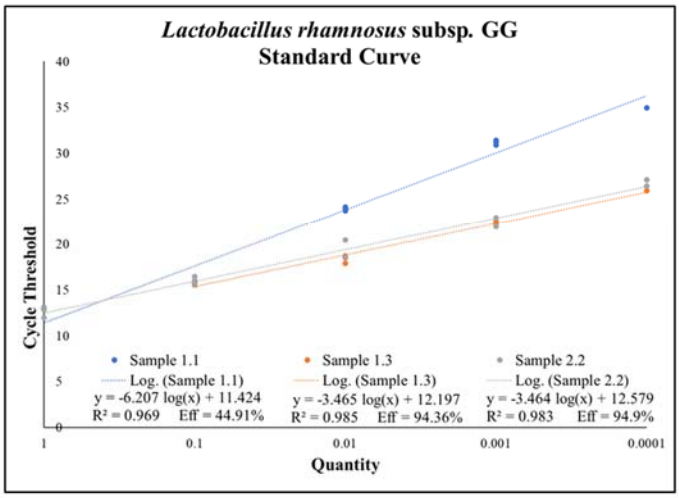
Cell Concentration: 1.73 x 10^9 cells/ml
DNA Concentration: 35.7 ng/µl

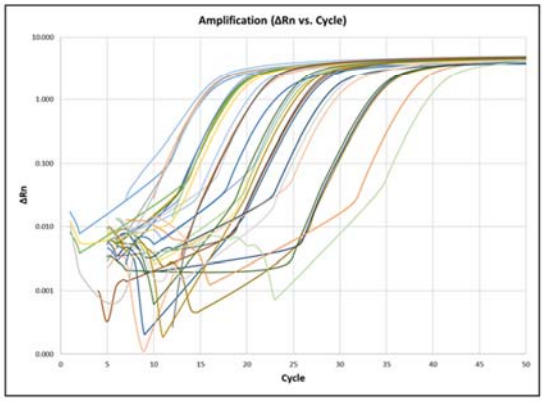
Extraction: DNeasy® PowerSoil® Pro
260/280 ratio: 1.78

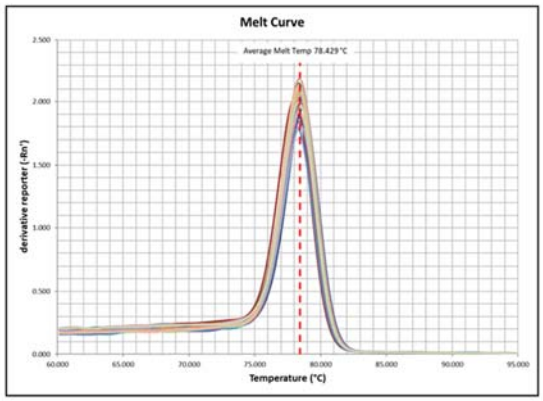
qPCR Mix:
5 µl - Powerup SYBR Green Master Mix
1 µl -100 nM Forward Primer
1 µl - 100 nM Reverse Primer
2 µl - DNA/RNA free water
1 µl - DNA at dilution

Forward Primer:
ATC AAC AGG CTC AGT GA
Reverse Primer:
CAT GTT GTG CGC TTG GAA AA

qPCR Method:
Hold 50°C - 2 min
Hold 95°C - 10 min
40 cycles:
Denaturation: 95°C - 20 sec
Annealing/Extension:
60°C - 1 min
Melt Curve







Results are shown from Quantstudio 6. Any questions on this protocol can be directed to Lt Col Andrew Hoisington (Andrew.Hoisington@afit.edu)

Chapter 5: Conclusions and Recommendations

Conclusions of Research

There is a significant connection between the gut microbiome and effects on mental and physical health. Future research into the gut brain axis will require an established method to identify and quantify the microorganisms which occupy the human gut. The objectives of this thesis were to:

1) Identify microorganisms associated to be beneficial to human mental and physical health.
2) Develop methods by which to grow, quantify cells, and extract DNA of the beneficial microorganisms as well as primers and methods to create qPCR assays.
3) Compile a reference guide containing all methods, materials, melt curves, amplification curves, and standard curves for each of the microorganisms chosen.

In the review of multiple microorganisms, many were identified as beneficial to mental and physical health (Chapter 2, Table 1). These microorganisms exist on food naturally or can be added to control the fermentation process. Evidence from multiple studies demonstrate the possible health benefits humans could garner from these microorganisms. Although some of the studies confine their research to in-vitro and animal studies, there is a benefit to studying effects on human. Symptoms associated with depression, anxiety, and systemic inflammations are some of the overlapping conditions which were found to be treated by microorganisms. The microorganisms selected for

qPCR are only a small portion in the total community of beneficial microorganisms discovered in research. The selected microorganisms complete the first objective. Chapter 3 ties the selected microorganisms to objective two. The methods and materials outlined in Chapter 3 led to the successful creation of nine qPCR assays. Using information found within other studies into qPCR assays, the methods to produce successful and reliable amplifications and standard curves were compiled and verified. The final objective was met with the creation of a reference guide, Chapter 4, which can be used for future research and studies utilizing these nine bacteria species.

Significance of Research

The benefits that could be garnered from this thesis is the addition of probiotic bacteria to the treatment of depression, anxiety, and systemic inflammation. Health professionals could test a patient's gut microbiome to determine if there are specific bacterial species missing. Prescribing a probiotic of missing bacterial species could alleviate the symptoms which accompany the condition for which they are being treated. Additional areas of research that could benefit from the create standard curves include the addition of probiotics alongside pharmaceutical prescriptions [1], continued research into the gut-brain axis [2]-[4], or furthering studies into the probiotic properties of fermented foods [5], [6].

Recommendations for Future Research

The selected bacterial species are only a small percentage of the microorganisms which could affect mental and physical health. Only nine species of the seventeen identified in Chapter 2 were used to create information in the reference guide. The

additional eight plus multitudes of others that have shown some connection to benefit health could be added to the reference guide, with continued additions until all known microorganisms associated with human health are covered. Additional qPCR assays, methods, materials, procedures, and the data collected will be required to create a complete guide for future research and utilization. A complete reference guide would provide additional tools for health professionals, food scientists, and Department of Defense researchers to assist in finding new ways to apply the benefits these microorganisms can confer.

Bibliography

[1] P. C. Calder, S. R. Carding, G. Christopher, D. Kuh, S. C. Langley-Evans, and H. McNulty, "A holistic approach to healthy ageing: how can people live longer, healthier lives?," *Journal of Human Nutrition and Dietetics*, vol. 31, no. 4, pp. 439–450, Aug. 2018, doi: 10.1111/jhn.12566.

[2] J. F. Cryan et al., "The Microbiota-Gut-Brain Axis," *Physiological Reviews*, vol. 99, no. 4, pp. 1877–2013, Oct. 2019, doi: 10.1152/physrev.00018.2018.

[3] A. Evrensel and M. E. Ceylan, "The Gut-Brain Axis: The Missing Link in Depression," *Clinical Psychopharmacology and Neuroscience*, vol. 13, no. 3, pp. 239–244, Dec. 2015, doi: 10.9758/cpn.2015.13.3.239.

[4] S. M. O'Mahony, G. Clarke, Y. E. Borre, T. G. Dinan, and J. F. Cryan, "Serotonin, tryptophan metabolism and the brain-gut-microbiome axis," *Behavioural Brain Research*, vol. 277, pp. 32–48, Jan. 2015, doi: 10.1016/j.bbr.2014.07.027.

[5] E. M. Selhub, A. C. Logan, and A. C. Bested, "Fermented foods, microbiota, and mental health: ancient practice meets nutritional psychiatry," *Journal of Physiological Anthropology*, vol. 33, no. 1, p. 2, Dec. 2014, doi: 10.1186/1880-6805-33-2.

[6] C. Stanton, R. P. Ross, G. F. Fitzgerald, and D. V. Sinderen, "Fermented functional foods based on probiotics and their biogenic metabolites," *Current Opinion in Biotechnology*, vol. 16, no. 2, pp. 198–203, Apr. 2005, doi: 10.1016/j.copbio.2005.02.008.

Appendix A

Experiment Results Report

2020-01-07 112415

Experiment Summary

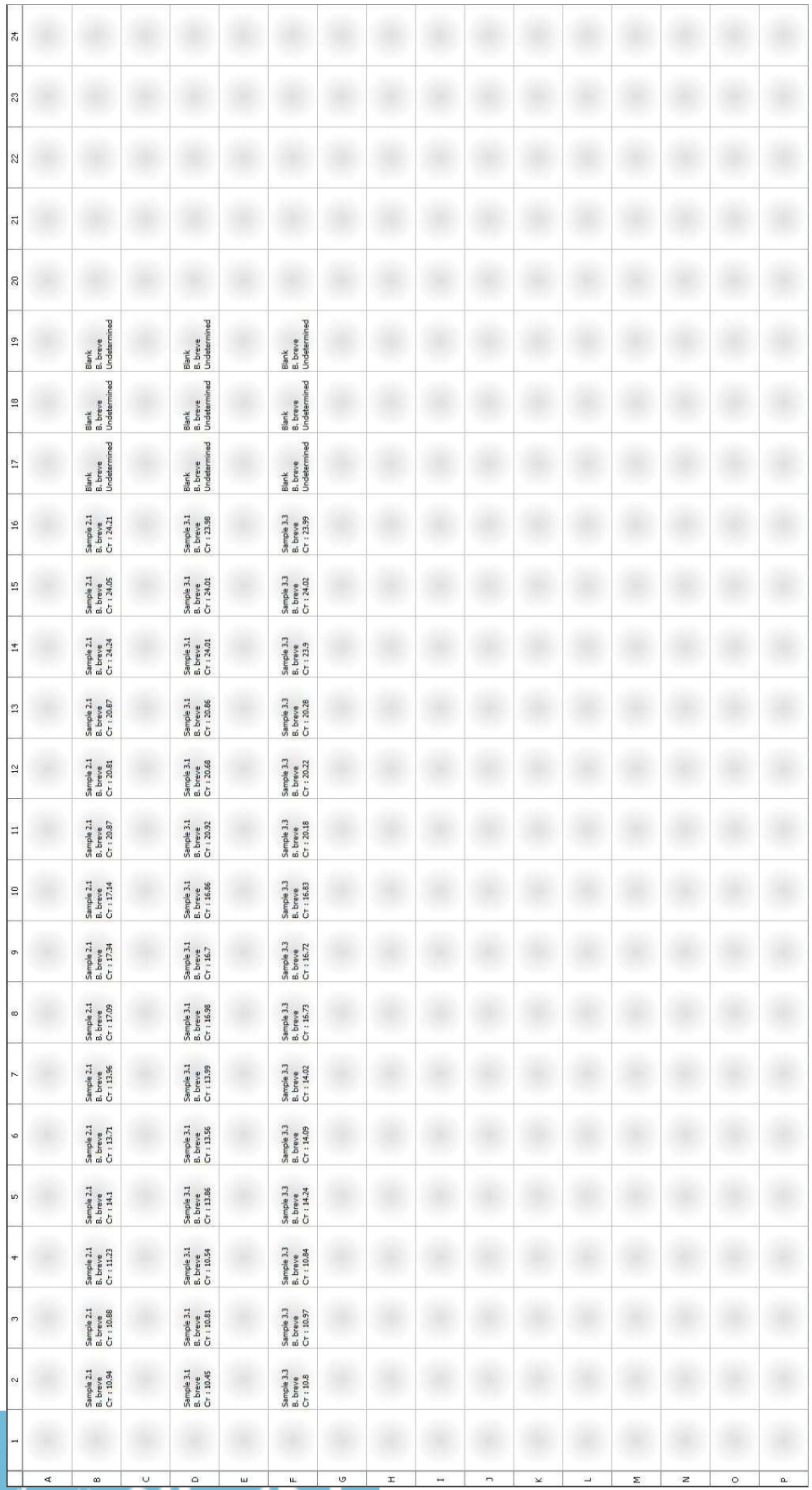
Experiment Name: 2020-01-07 112415
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_B_breve_20200107_standard curve.eds
Run Started: 01-07-2020 17:09:59 PST
Run Finished: 01-07-2020 19:11:28 PST
Run Duration: 121 minutes 29 seconds
Date Modified: 01-07-2020 14:09:51 PST
Date Created: 01-07-2020 11:24:15 PST
User:
Number of Wells Used: 54
Number of Wells with Results: 54
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 3
Comments:
Quantification Cycle Setting: CT

Reagent Information

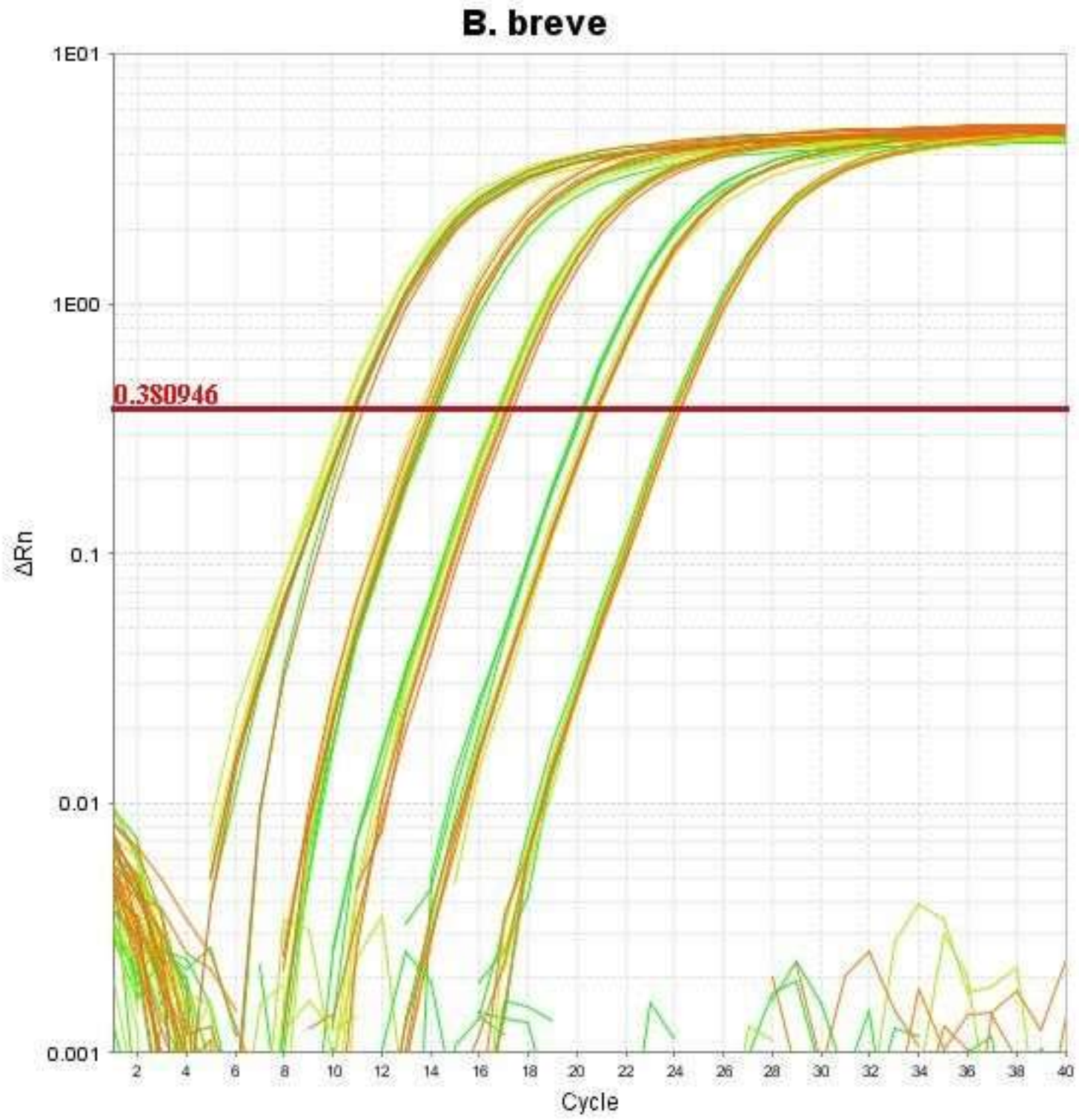
Results Summary

Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
Blank	B. breve				
Sample 2.1	B. breve				
Sample 3.1	B. breve				
Sample 3.3	B. breve				

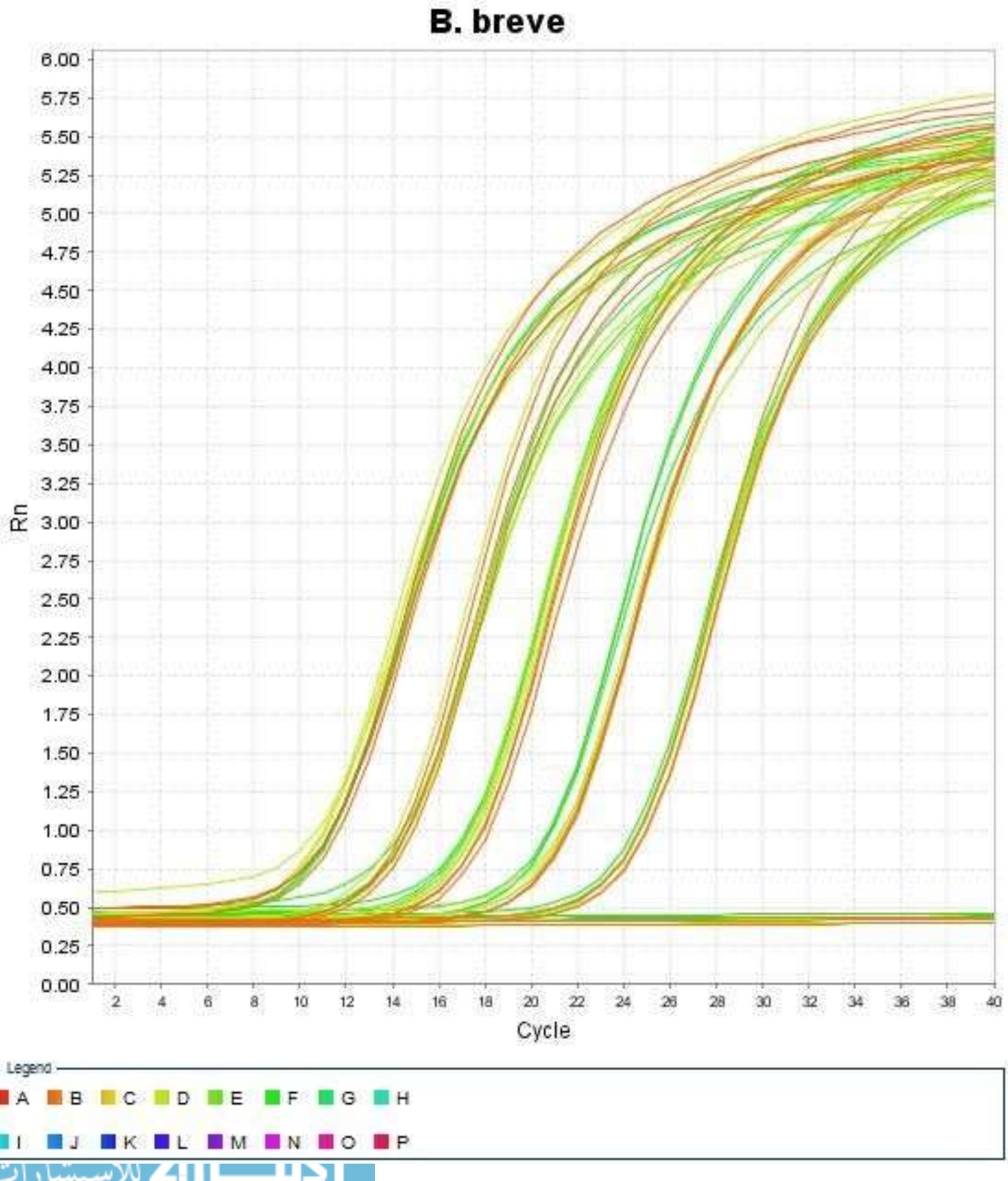
Plate Layout



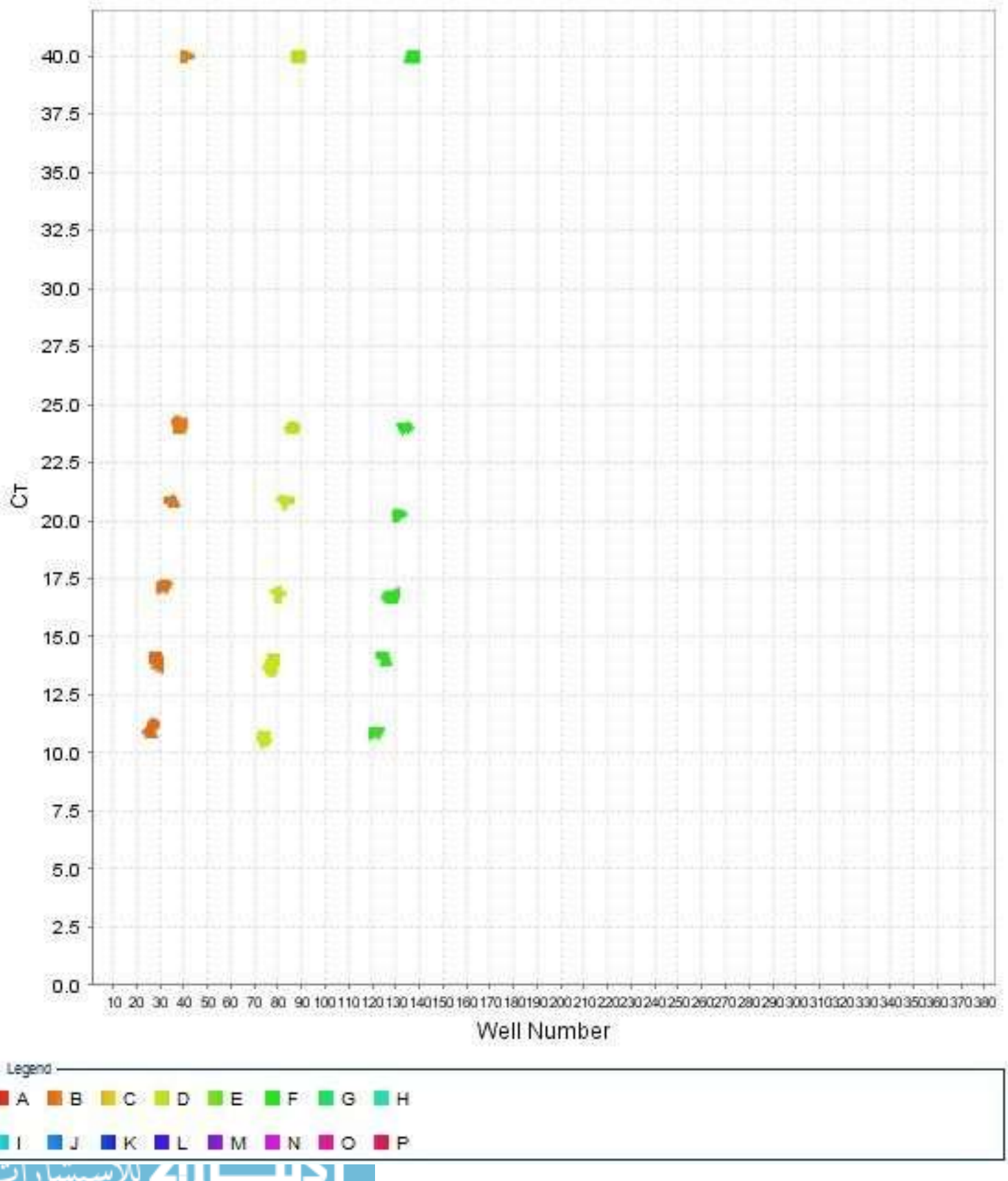
Amplification Plot (ΔRn vs. Cycle)



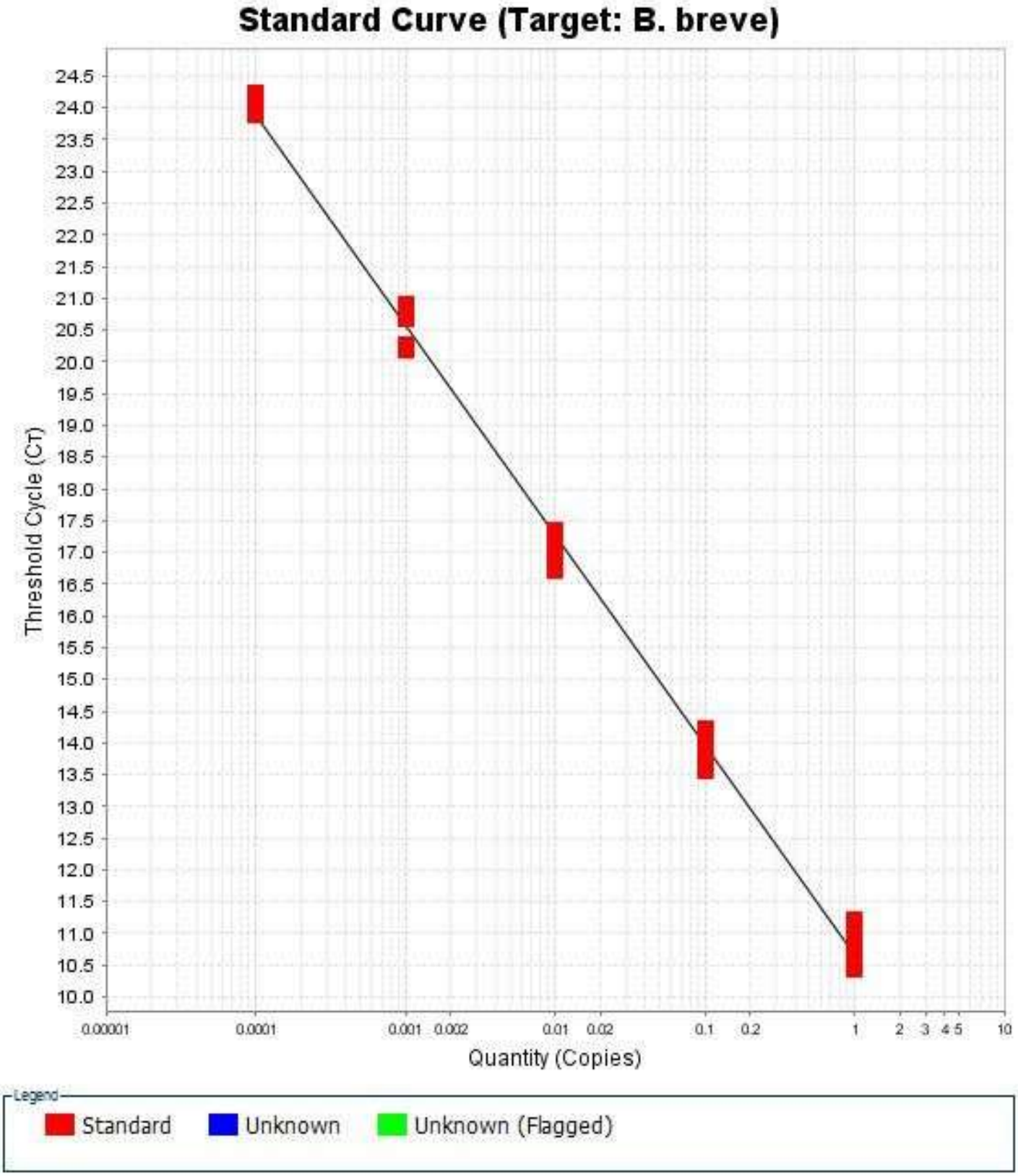
Amplification Plot (Rn vs. Cycle)



Amplification Plot (CT vs. Well)

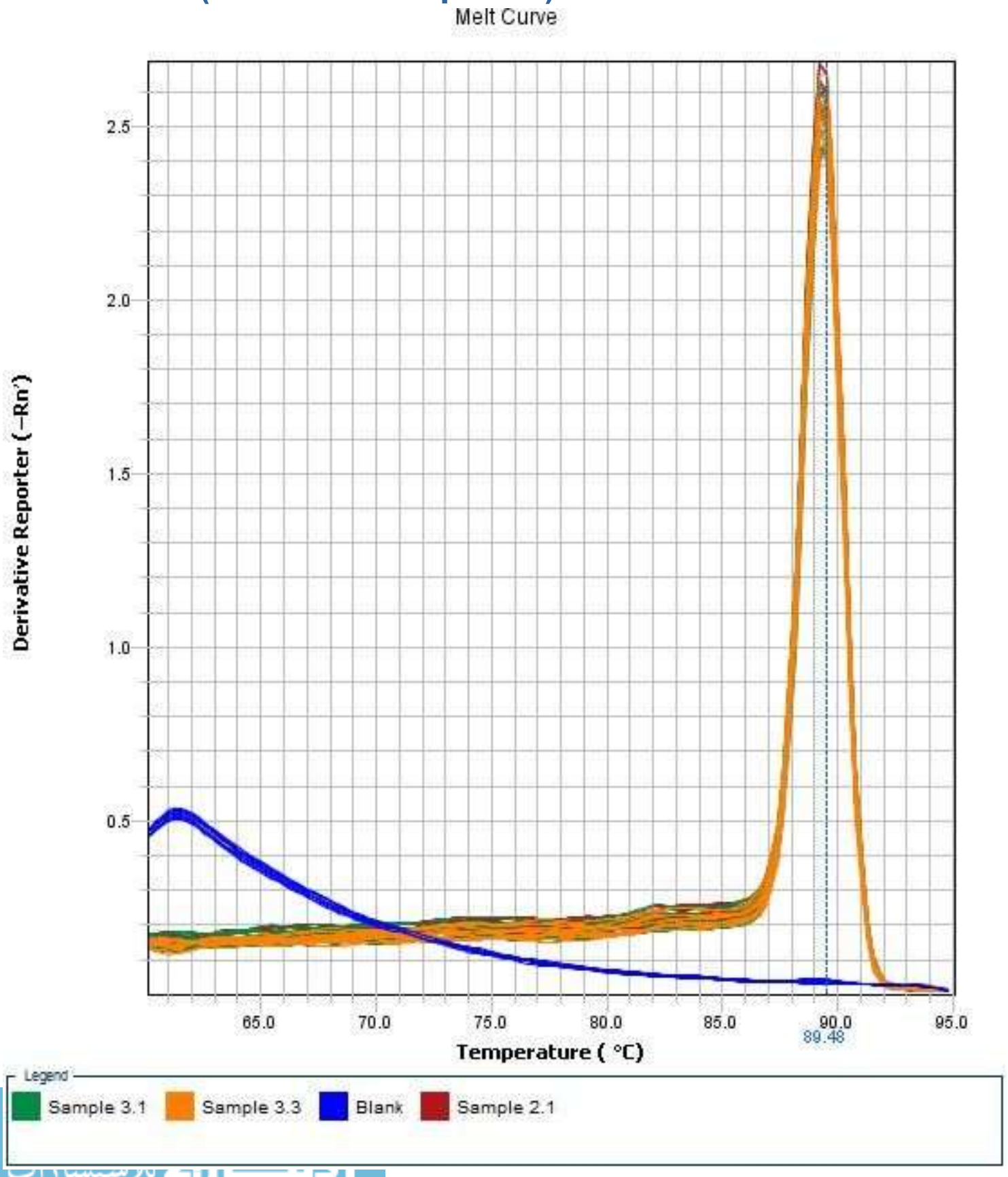


Standard Curves

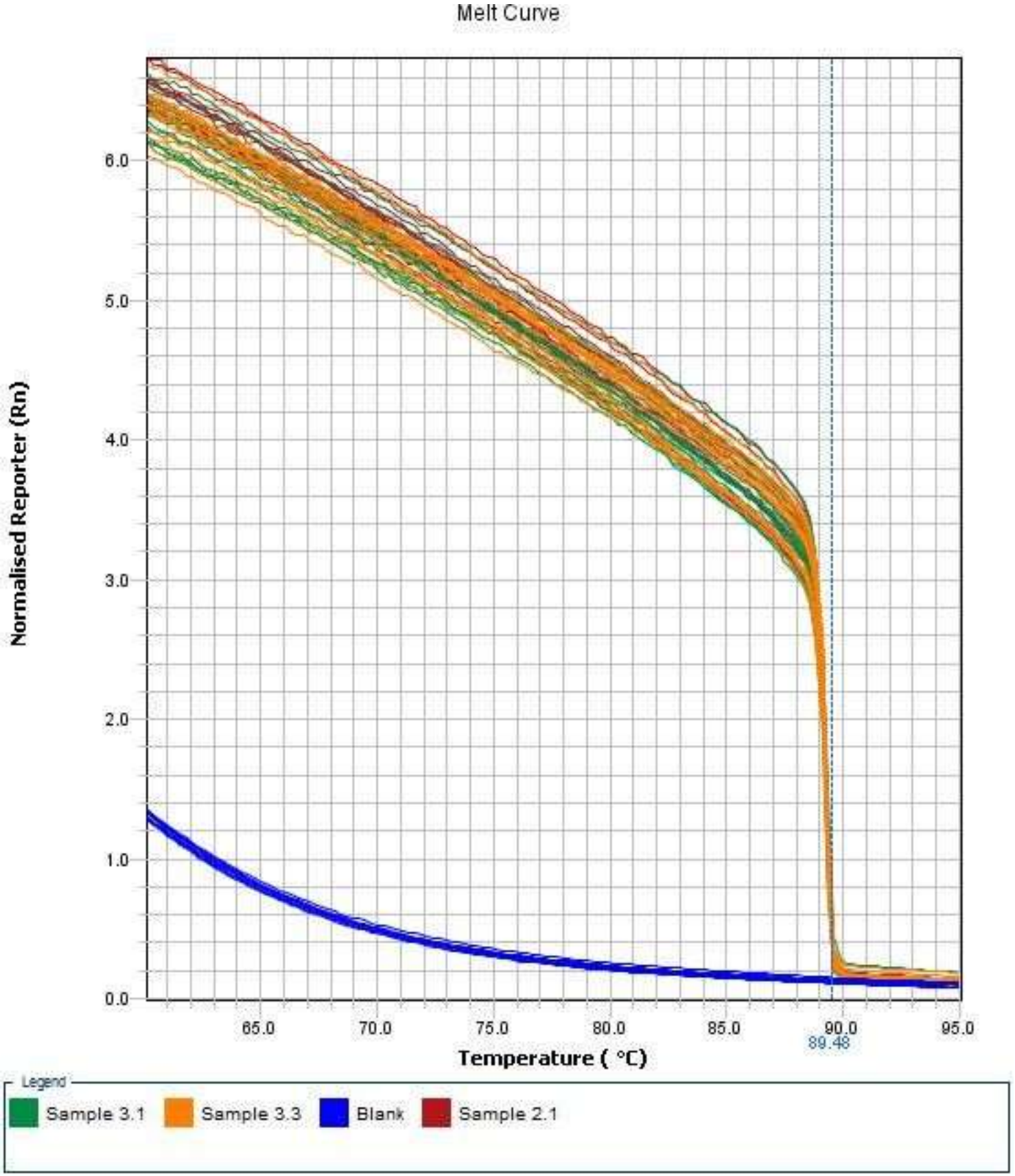


slope:-3.3116 Y-Intercept:10.654 R²:0.996 Eff%:100.433

Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	CT	CT Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	Sample 2.1	B. breve	S	1.000	10.937	11.017	0.185		89.344		
B3	Sample 2.1	B. breve	S	1.000	10.884	11.017	0.185		89.344		
B4	Sample 2.1	B. breve	S	1.000	11.229	11.017	0.185		89.344		
B5	Sample 2.1	B. breve	S	0.100	14.098	13.922	0.194		89.344		
B6	Sample 2.1	B. breve	S	0.100	13.714	13.922	0.194		89.344		
B7	Sample 2.1	B. breve	S	0.100	13.955	13.922	0.194		89.344		
B8	Sample 2.1	B. breve	S	0.010	17.089	17.187	0.131		89.344		
B9	Sample 2.1	B. breve	S	0.010	17.336	17.187	0.131		89.344		
B10	Sample 2.1	B. breve	S	0.010	17.137	17.187	0.131		89.344		
B11	Sample 2.1	B. breve	S	0.001	20.873	20.851	0.039		89.344		
B12	Sample 2.1	B. breve	S	0.001	20.806	20.851	0.039		89.344		
B13	Sample 2.1	B. breve	S	0.001	20.874	20.851	0.039		89.344		
B14	Sample 2.1	B. breve	S	0.000	24.236	24.165	0.099		89.344		
B15	Sample 2.1	B. breve	S	0.000	24.051	24.165	0.099		89.344		
B16	Sample 2.1	B. breve	S	0.000	24.208	24.165	0.099		89.344		
B17	Blank	B. breve	N		UND.				61.386		
B18	Blank	B. breve	N		UND.				61.386		
B19	Blank	B. breve	N		UND.				61.386		
D2	Sample 3.1	B. breve	S	1.000	10.450	10.601	0.188		89.344		
D3	Sample 3.1	B. breve	S	1.000	10.812	10.601	0.188		89.476		
D4	Sample 3.1	B. breve	S	1.000	10.541	10.601	0.188		89.344		
D5	Sample 3.1	B. breve	S	0.100	13.863	13.804	0.219		89.344		
D6	Sample 3.1	B. breve	S	0.100	13.561	13.804	0.219		89.344		
D7	Sample 3.1	B. breve	S	0.100	13.986	13.804	0.219		89.344		
D8	Sample 3.1	B. breve	S	0.010	16.975	16.846	0.136		89.344		
D9	Sample 3.1	B. breve	S	0.010	16.704	16.846	0.136		89.344		
D10	Sample 3.1	B. breve	S	0.010	16.859	16.846	0.136		89.344		
D11	Sample 3.1	B. breve	S	0.001	20.917	20.820	0.121		89.344		
D12	Sample 3.1	B. breve	S	0.001	20.685	20.820	0.121		89.344		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	CT	CT Mean		Qty Mean	Qty SD	Tm1	Tm2	Tm3
D13	Sample 3.1	B. breve	S	0.001	20.859	20.820	0.121			89.344		
D14	Sample 3.1	B. breve	S	0.000	24.011	24.002	0.017			89.344		
D15	Sample 3.1	B. breve	S	0.000	24.012	24.002	0.017			89.344		
D16	Sample 3.1	B. breve	S	0.000	23.982	24.002	0.017			89.344		
D17	Blank	B. breve	N		UND.					61.518	89.212	
D18	Blank	B. breve	N		UND.					61.386		
D19	Blank	B. breve	N		UND.					61.386		
F2	Sample 3.3	B. breve	S	1.000	10.801	10.872	0.091			89.344		
F3	Sample 3.3	B. breve	S	1.000	10.974	10.872	0.091			89.344		
F4	Sample 3.3	B. breve	S	1.000	10.839	10.872	0.091			89.344		
F5	Sample 3.3	B. breve	S	0.100	14.239	14.117	0.111			89.344		
F6	Sample 3.3	B. breve	S	0.100	14.089	14.117	0.111			89.344		
F7	Sample 3.3	B. breve	S	0.100	14.023	14.117	0.111			89.344		
F8	Sample 3.3	B. breve	S	0.010	16.735	16.761	0.056			89.344		
F9	Sample 3.3	B. breve	S	0.010	16.723	16.761	0.056			89.344		
F10	Sample 3.3	B. breve	S	0.010	16.826	16.761	0.056			89.344		
F11	Sample 3.3	B. breve	S	0.001	20.178	20.225	0.052			89.344		
F12	Sample 3.3	B. breve	S	0.001	20.216	20.225	0.052			89.344		
F13	Sample 3.3	B. breve	S	0.001	20.281	20.225	0.052			89.344		
F14	Sample 3.3	B. breve	S	0.000	23.900	23.970	0.062			89.344		
F15	Sample 3.3	B. breve	S	0.000	24.020	23.970	0.062			89.344		
F16	Sample 3.3	B. breve	S	0.000	23.990	23.970	0.062			89.212		
F17	Blank	B. breve	N		UND.					61.386		
F18	Blank	B. breve	N		UND.					61.386		
F19	Blank	B. breve	N		UND.					61.386		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	54	Targets Used	1
Well Setup	54	Flagged Wells	0	Samples Used	4

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	0	
HIGHSD	High standard deviation in replicate group	0	
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	0	
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp**	m1,x1 m4,x4
Cover Temperature	105.0	**Filter Set**	
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	40	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	600	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	94.0	20	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	55.0	20	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	72.0	50	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	15	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_	60.0	60	0.0	0

PER_SECOND

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_PER_SECOND	95.0	15	0.0	0

Experiment Results Report

2020-01-03 123815

Experiment Summary

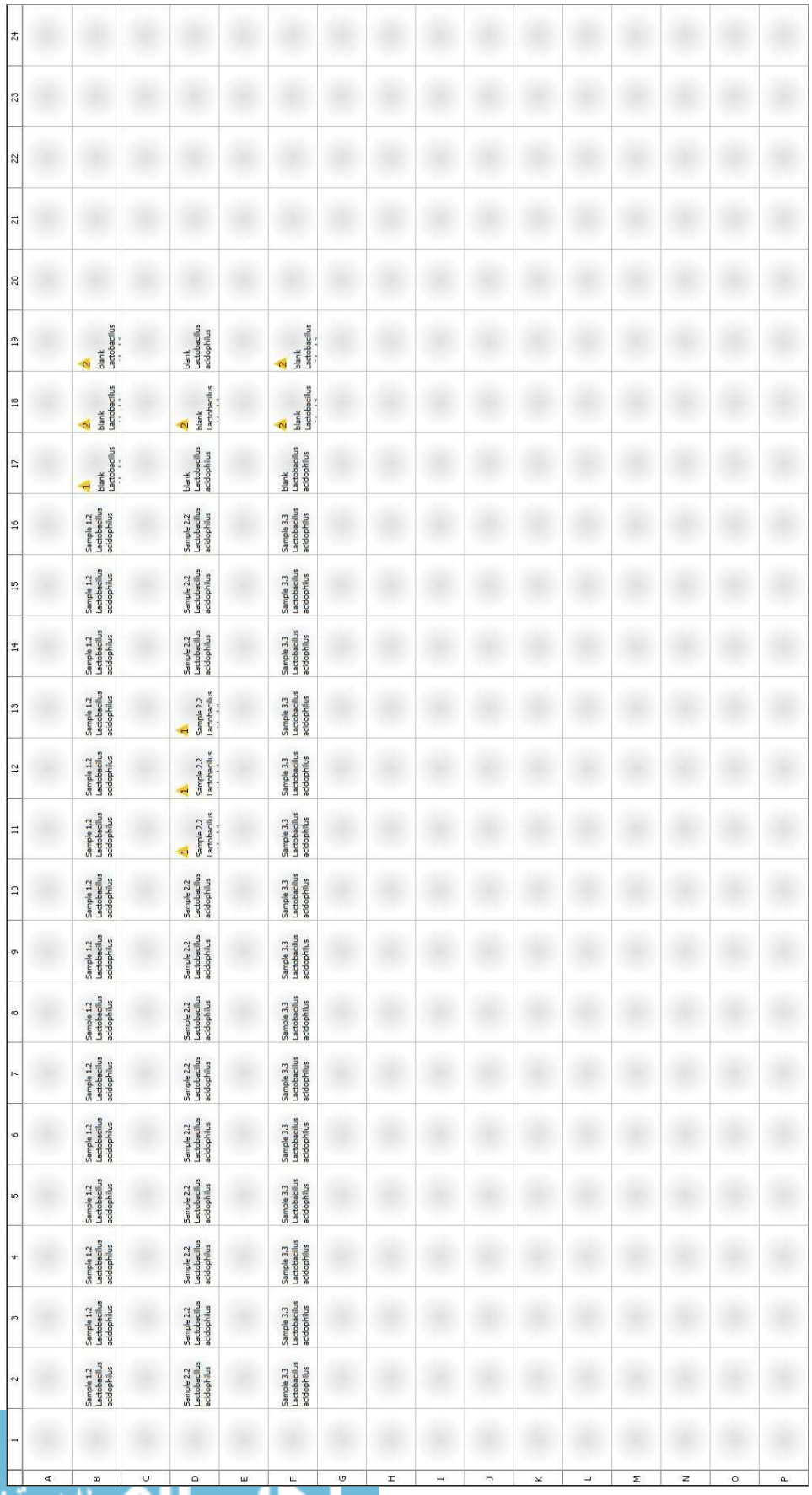
Experiment Name: 2020-01-03 123815
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_L_acidophilus_20200103_standardcurve1.eds
Run Started: 01-03-2020 17:51:40 PST
Run Finished: 01-03-2020 20:13:06 PST
Run Duration: 141 minutes 26 seconds
Date Modified: 01-03-2020 15:35:25 PST
Date Created: 01-03-2020 12:38:15 PST
User:
Number of Wells Used: 54
Number of Wells with Results: 54
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 2
Comments: L. acidophilus
Quantification Cycle Setting: CT

Reagent Information

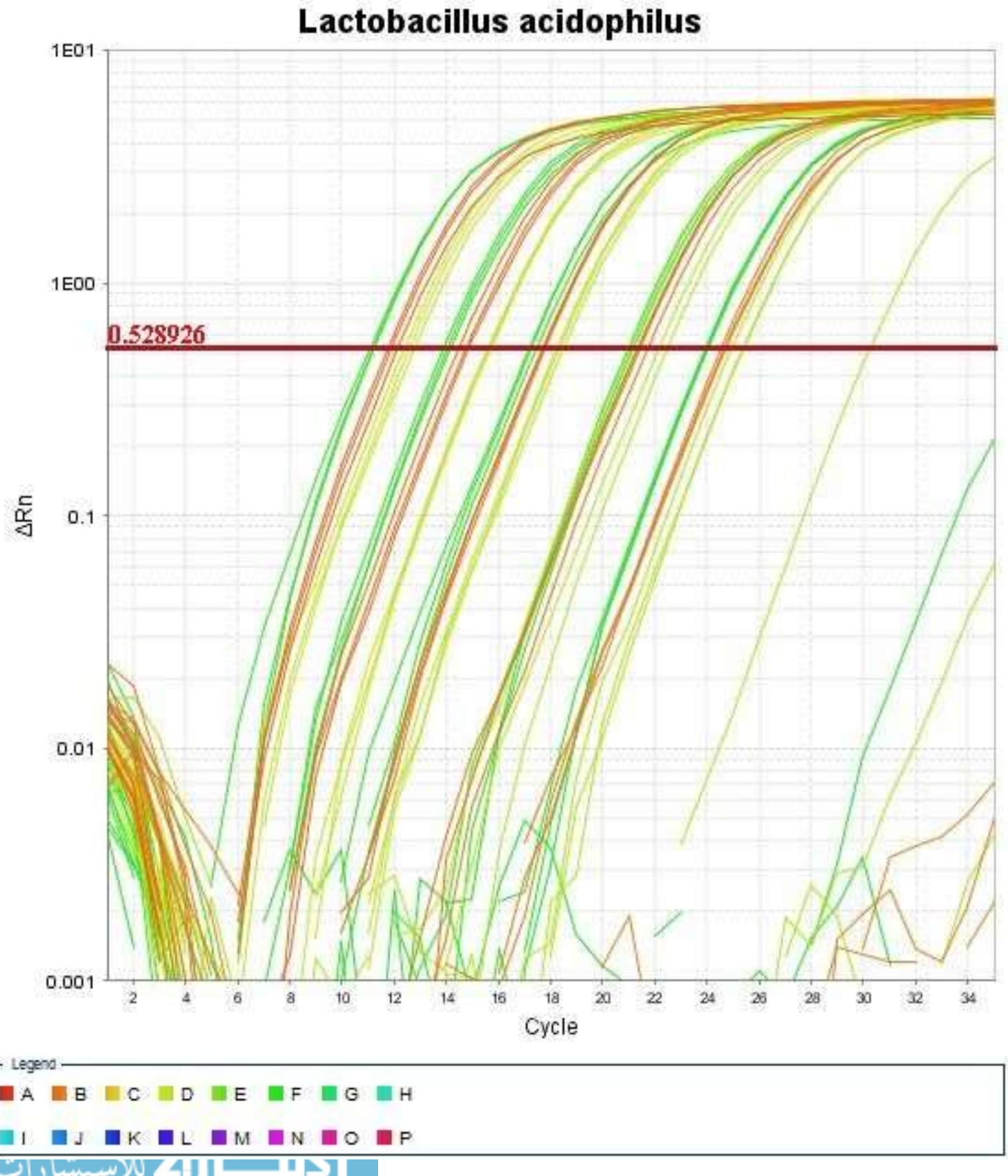
Results Summary

Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
Sample 1.2	Lactobacillus acidophilus				
Sample 2.2	Lactobacillus acidophilus				
Sample 3.3	Lactobacillus acidophilus				
blank	Lactobacillus acidophilus	0.000		30.249	

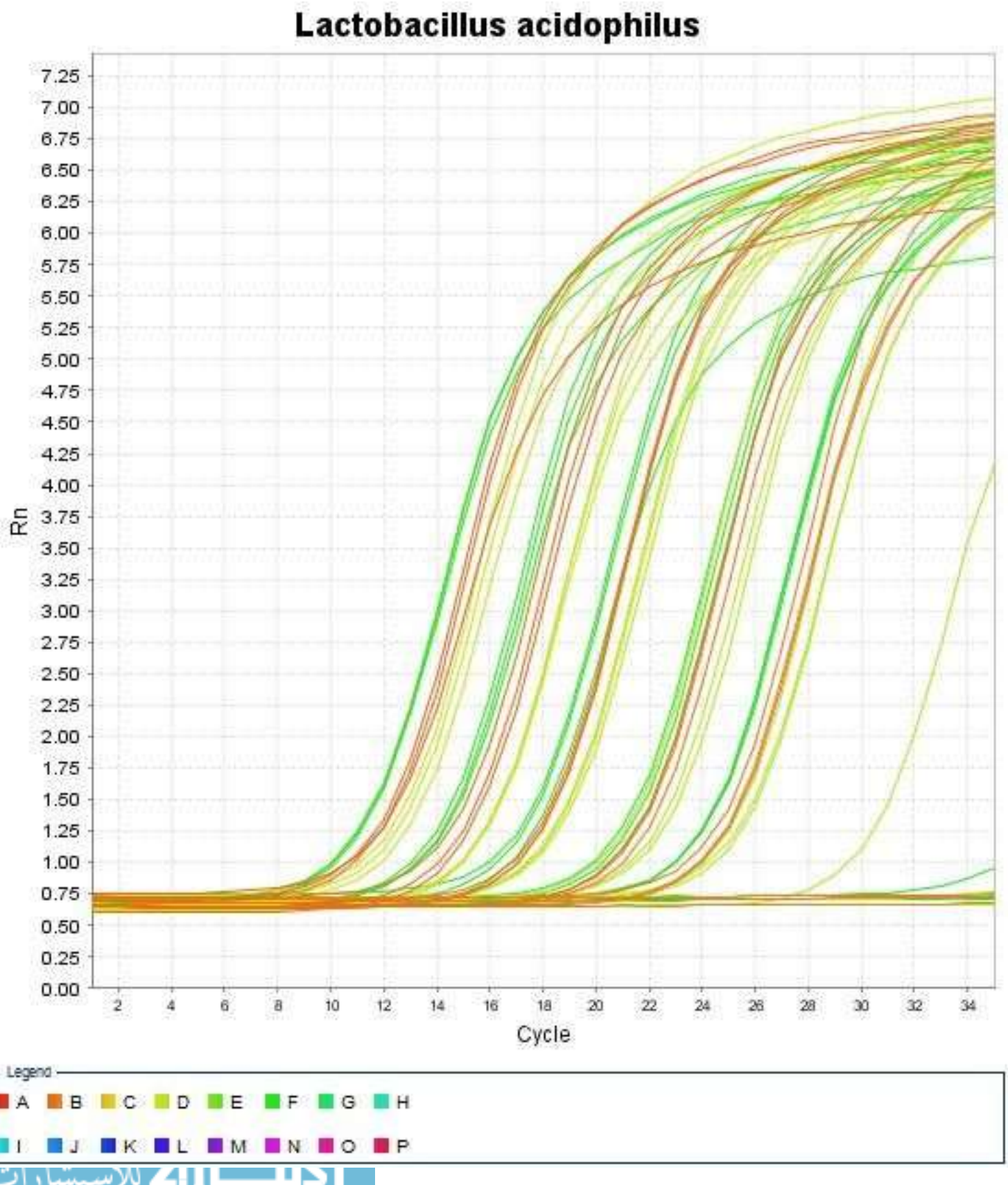
Plate Layout



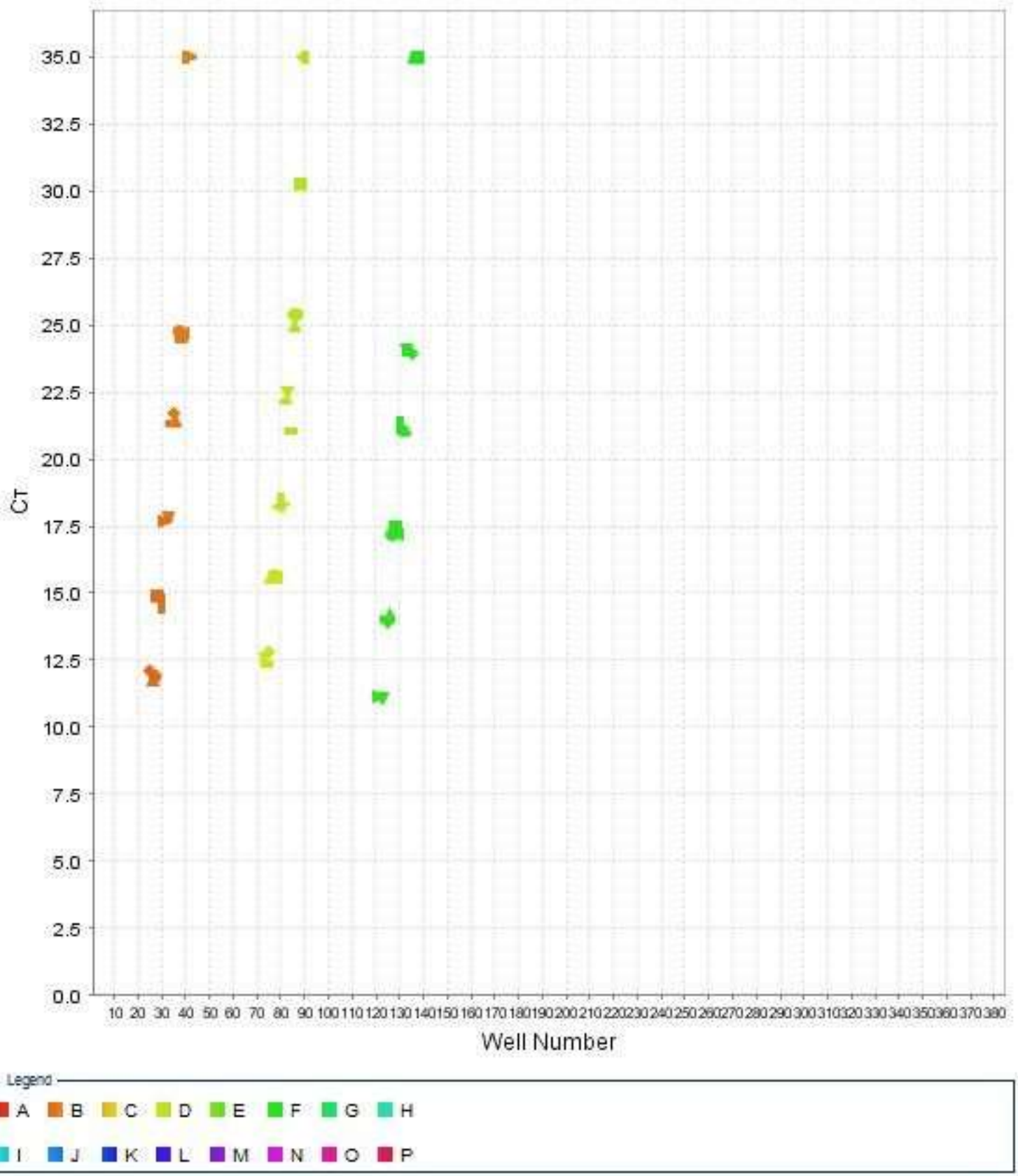
Amplification Plot (ΔRn vs. Cycle)



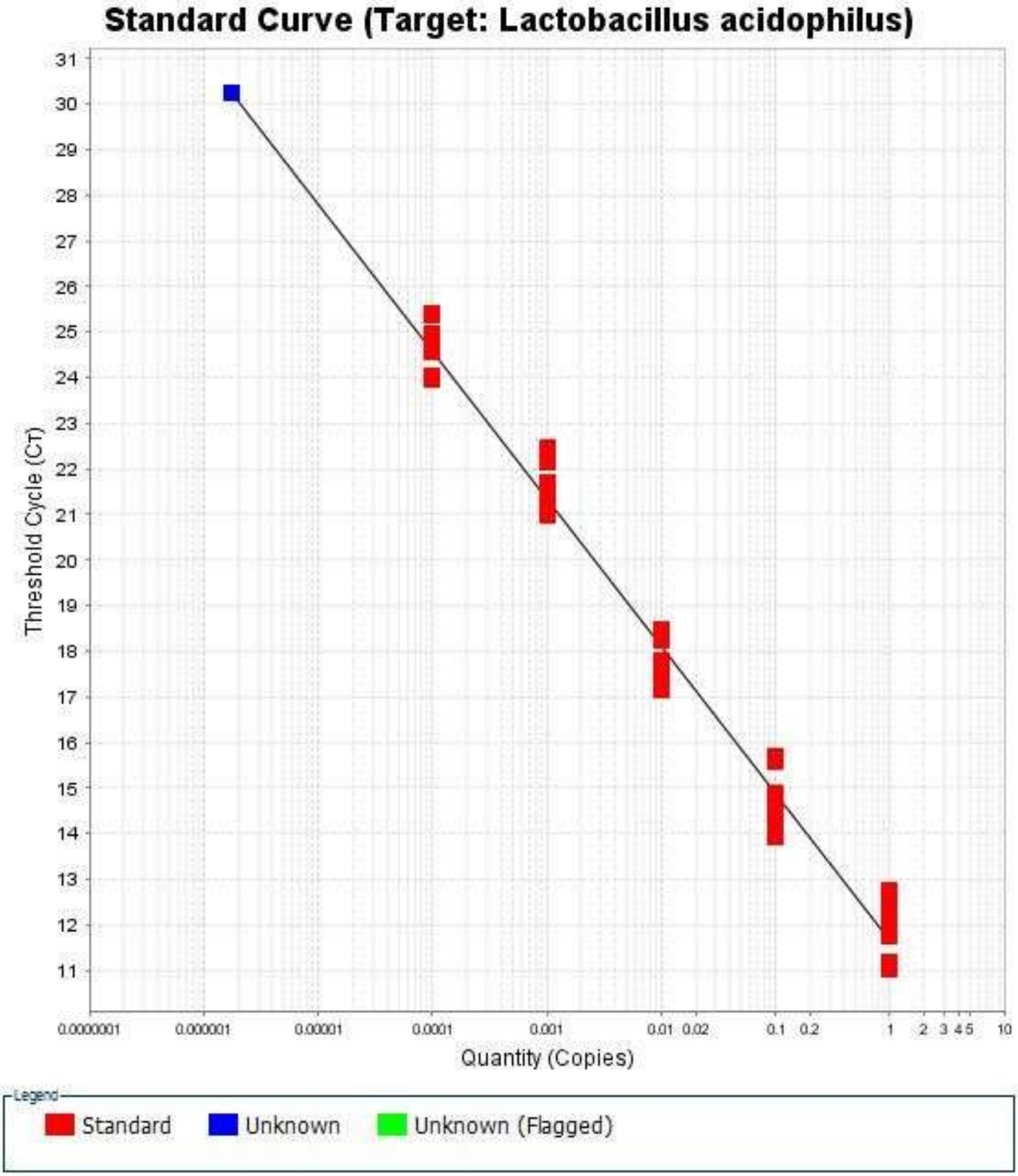
Amplification Plot (Rn vs. Cycle)



Amplification Plot (CT vs. Well)

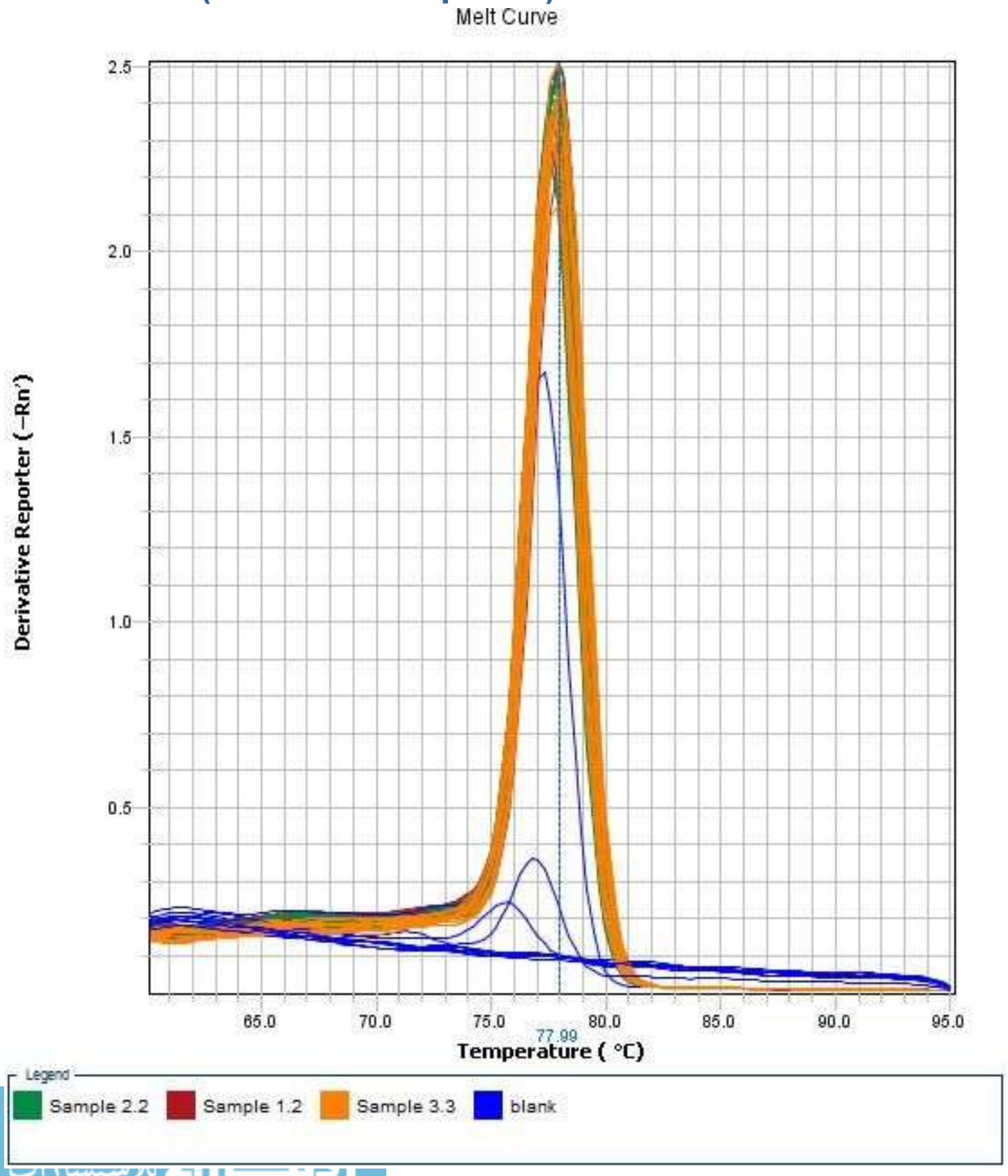


Standard Curves

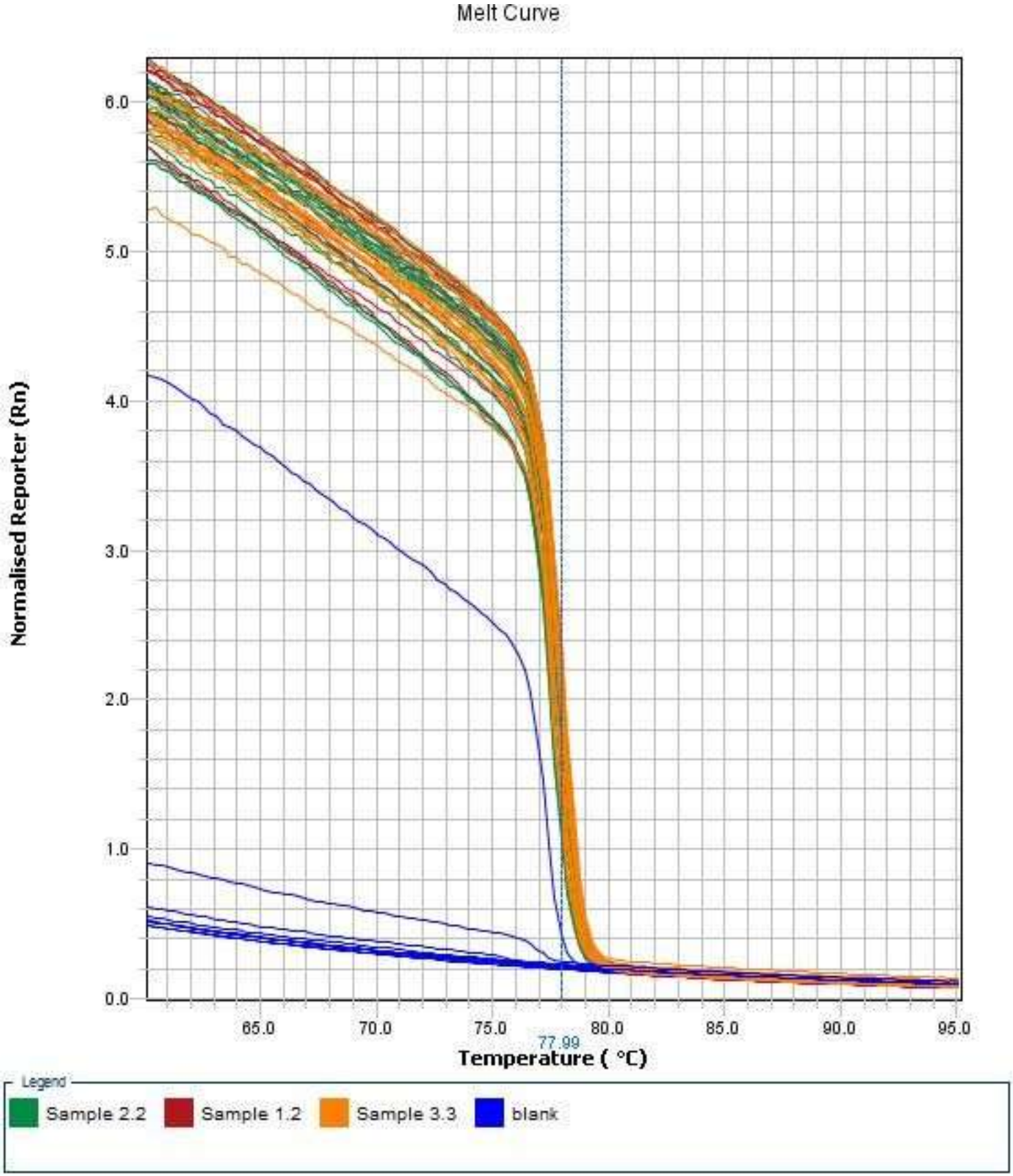


slope:-3.2232 Y-Intercept:11.6732 R²:0.984 Eff%:104.292

Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	Sample 1.2	Lactobacillus acidophilus	S	1.000	12.096	11.906	0.176		77.989		
B3	Sample 1.2	Lactobacillus acidophilus	S	1.000	11.749	11.906	0.176		77.989		
B4	Sample 1.2	Lactobacillus acidophilus	S	1.000	11.873	11.906	0.176		77.989		
B5	Sample 1.2	Lactobacillus acidophilus	S	0.100	14.895	14.727	0.201		77.858		
B6	Sample 1.2	Lactobacillus acidophilus	S	0.100	14.783	14.727	0.201		77.858		
B7	Sample 1.2	Lactobacillus acidophilus	S	0.100	14.504	14.727	0.201		77.858		
B8	Sample 1.2	Lactobacillus acidophilus	S	0.010	17.695	17.739	0.050		77.726		
B9	Sample 1.2	Lactobacillus acidophilus	S	0.010	17.729	17.739	0.050		77.726		
B10	Sample 1.2	Lactobacillus acidophilus	S	0.010	17.793	17.739	0.050		77.726		
B11	Sample 1.2	Lactobacillus acidophilus	S	0.001	21.313	21.483	0.214		77.726		
B12	Sample 1.2	Lactobacillus acidophilus	S	0.001	21.724	21.483	0.214		77.726		
B13	Sample 1.2	Lactobacillus acidophilus	S	0.001	21.413	21.483	0.214		77.726		
B14	Sample 1.2	Lactobacillus acidophilus	S	0.000	24.753	24.668	0.098		77.594		
B15	Sample 1.2	Lactobacillus acidophilus	S	0.000	24.561	24.668	0.098		77.594		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Cт SD	Qty Mean	Qty SD	Tm1	Tm2	Tm3
B16	Sample 1.2	Lactobacillus acidophilus	S	0.000	24.691	24.668	0.098			77.594		
B17	blank	Lactobacillus acidophilus	U		UND.	30.249		0.000		61.648		
B18	blank	Lactobacillus acidophilus	U		UND.	30.249		0.000		61.516		
B19	blank	Lactobacillus acidophilus	U		UND.	30.249		0.000		61.516		
D2	Sample 2.2	Lactobacillus acidophilus	S	1.000	12.564	12.577	0.195			77.989		
D3	Sample 2.2	Lactobacillus acidophilus	S	1.000	12.388	12.577	0.195			77.989		
D4	Sample 2.2	Lactobacillus acidophilus	S	1.000	12.778	12.577	0.195			77.858		
D5	Sample 2.2	Lactobacillus acidophilus	S	0.100	15.623	15.636	0.050			77.858		
D6	Sample 2.2	Lactobacillus acidophilus	S	0.100	15.692	15.636	0.050			77.858		
D7	Sample 2.2	Lactobacillus acidophilus	S	0.100	15.594	15.636	0.050			77.858		
D8	Sample 2.2	Lactobacillus acidophilus	S	0.010	18.249	18.355	0.127			77.726		
D9	Sample 2.2	Lactobacillus acidophilus	S	0.010	18.495	18.355	0.127			77.726		
D10	Sample 2.2	Lactobacillus acidophilus	S	0.010	18.321	18.355	0.127			77.726		
D11	Sample 2.2	Lactobacillus acidophilus	S	0.001	22.132	21.878	0.744			77.594		
D12	Sample 2.2	Lactobacillus acidophilus	S	0.001	22.463	21.878	0.744			77.594		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Cт SD	Qty Mean	Qty SD	Tm1	Tm2	Tm3
D13	Sample 2.2	Lactobacillus acidophilus	S	0.001	21.041	21.878	0.744			77.726		
D14	Sample 2.2	Lactobacillus acidophilus	S	0.000	25.375	25.245	0.248			77.594		
D15	Sample 2.2	Lactobacillus acidophilus	S	0.000	24.958	25.245	0.248			77.462		
D16	Sample 2.2	Lactobacillus acidophilus	S	0.000	25.400	25.245	0.248			77.462		
D17	blank	Lactobacillus acidophilus	U	0.000	30.249	30.249		0.000		77.199		
D18	blank	Lactobacillus acidophilus	U		UND.	30.249		0.000		61.516		
D19	blank	Lactobacillus acidophilus	U		UND.	30.249		0.000		75.617		
F2	Sample 3.3	Lactobacillus acidophilus	S	1.000	11.151	11.126	0.071			77.989		
F3	Sample 3.3	Lactobacillus acidophilus	S	1.000	11.180	11.126	0.071			77.989		
F4	Sample 3.3	Lactobacillus acidophilus	S	1.000	11.045	11.126	0.071			77.989		
F5	Sample 3.3	Lactobacillus acidophilus	S	0.100	14.044	14.041	0.129			77.858		
F6	Sample 3.3	Lactobacillus acidophilus	S	0.100	13.910	14.041	0.129			77.858		
F7	Sample 3.3	Lactobacillus acidophilus	S	0.100	14.168	14.041	0.129			77.858		
F8	Sample 3.3	Lactobacillus acidophilus	S	0.010	17.162	17.275	0.147			77.858		
F9	Sample 3.3	Lactobacillus acidophilus	S	0.010	17.441	17.275	0.147			77.726		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Cт SD	Qty Mean	Qty SD	Tm1	Tm2	Tm3
F10	Sample 3.3	Lactobacillus acidophilus	S	0.010	17.221	17.275	0.147			77.858		
F11	Sample 3.3	Lactobacillus acidophilus	S	0.001	21.328	21.148	0.177			77.726		
F12	Sample 3.3	Lactobacillus acidophilus	S	0.001	21.142	21.148	0.177			77.726		
F13	Sample 3.3	Lactobacillus acidophilus	S	0.001	20.973	21.148	0.177			77.726		
F14	Sample 3.3	Lactobacillus acidophilus	S	0.000	24.034	23.992	0.037			77.594		
F15	Sample 3.3	Lactobacillus acidophilus	S	0.000	23.980	23.992	0.037			77.594		
F16	Sample 3.3	Lactobacillus acidophilus	S	0.000	23.962	23.992	0.037			77.594		
F17	blank	Lactobacillus acidophilus	U		UND.	30.249		0.000		76.803		
F18	blank	Lactobacillus acidophilus	U		UND.	30.249		0.000		61.384		
F19	blank	Lactobacillus acidophilus	U		UND.	30.249		0.000		61.648		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	54	Targets Used	1
Well Setup	54	Flagged Wells	9	Samples Used	4

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	6	B17, B18, B19, D18, F18, F19
HIGHSD	High standard deviation in replicate group	3	D11, D12, D13
NOAMP	No amplification	5	B18, B19, D18, F18, F19
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	0	
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp Filter Set**	m1,x1 m4,x4
Cover Temperature	105.0		
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	35	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	600	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	20	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	55.0	120	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	15	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	60.0	60	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_	95.0	15	0.0	0

PER_SECOND

Experiment Results Report

2020-01-10 143244

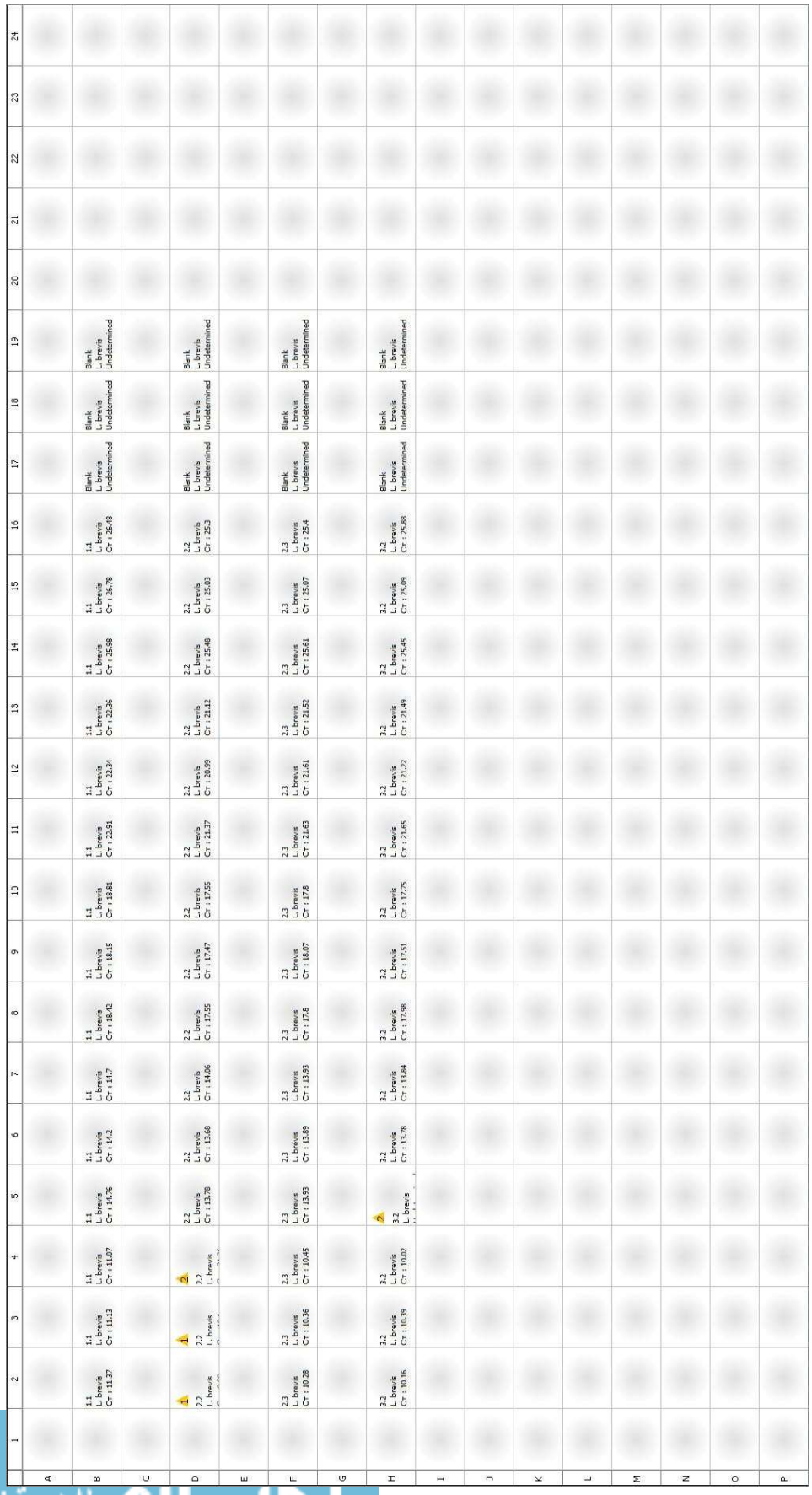
Experiment Summary

Experiment Name: 2020-01-10 143244
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_LBrevis_01102020_StandardCurve.eds
Run Started: 01-10-2020 21:03:18 PST
Run Finished: 01-11-2020 02:22:51 PST
Run Duration: 319 minutes 32 seconds
Date Modified: 01-10-2020 21:21:06 PST
Date Created: 01-10-2020 14:32:44 PST
User:
Number of Wells Used: 72
Number of Wells with Results: 72
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 3
Comments:
Quantification Cycle Setting: CT

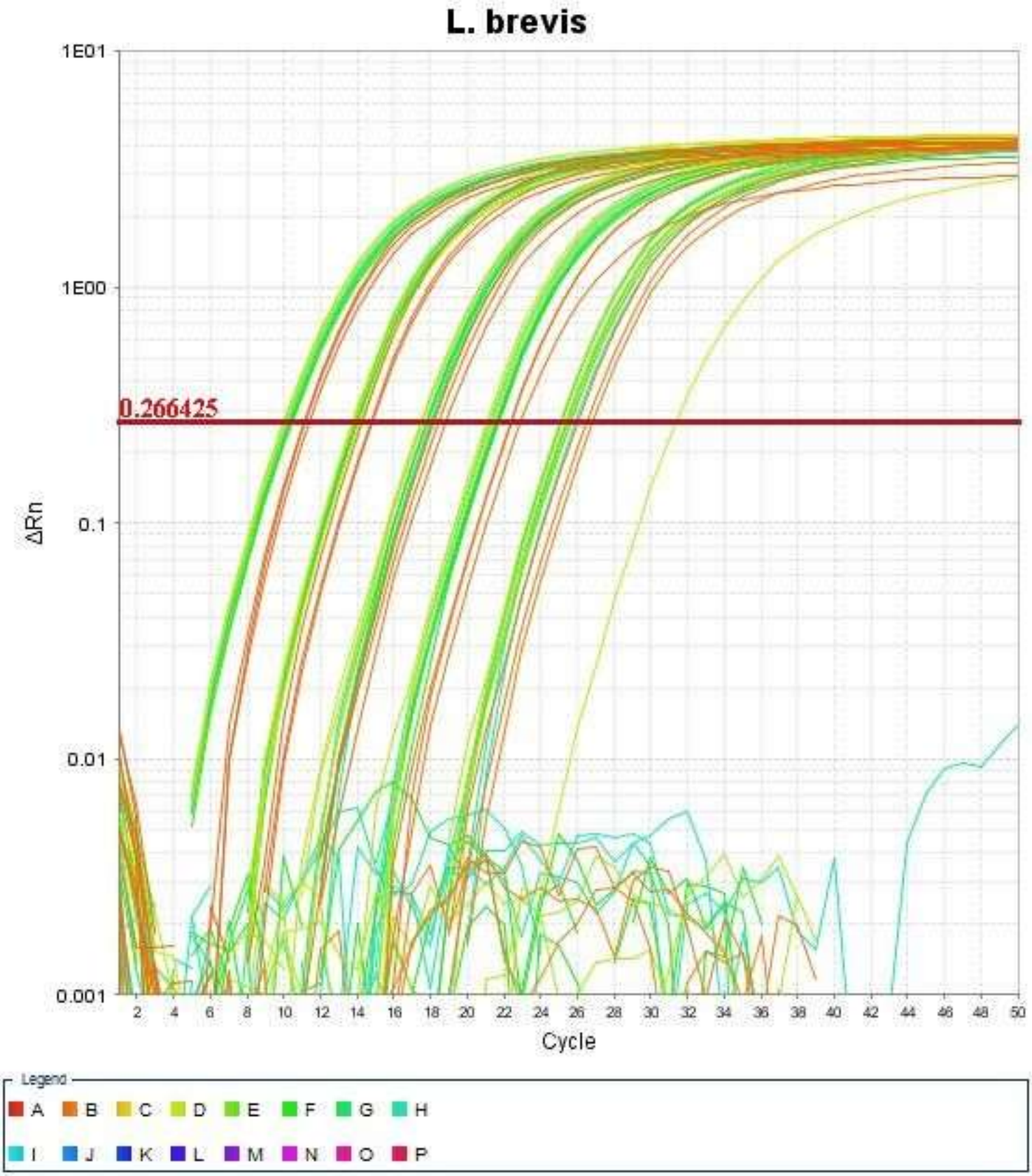
Reagent Information

Results Summary

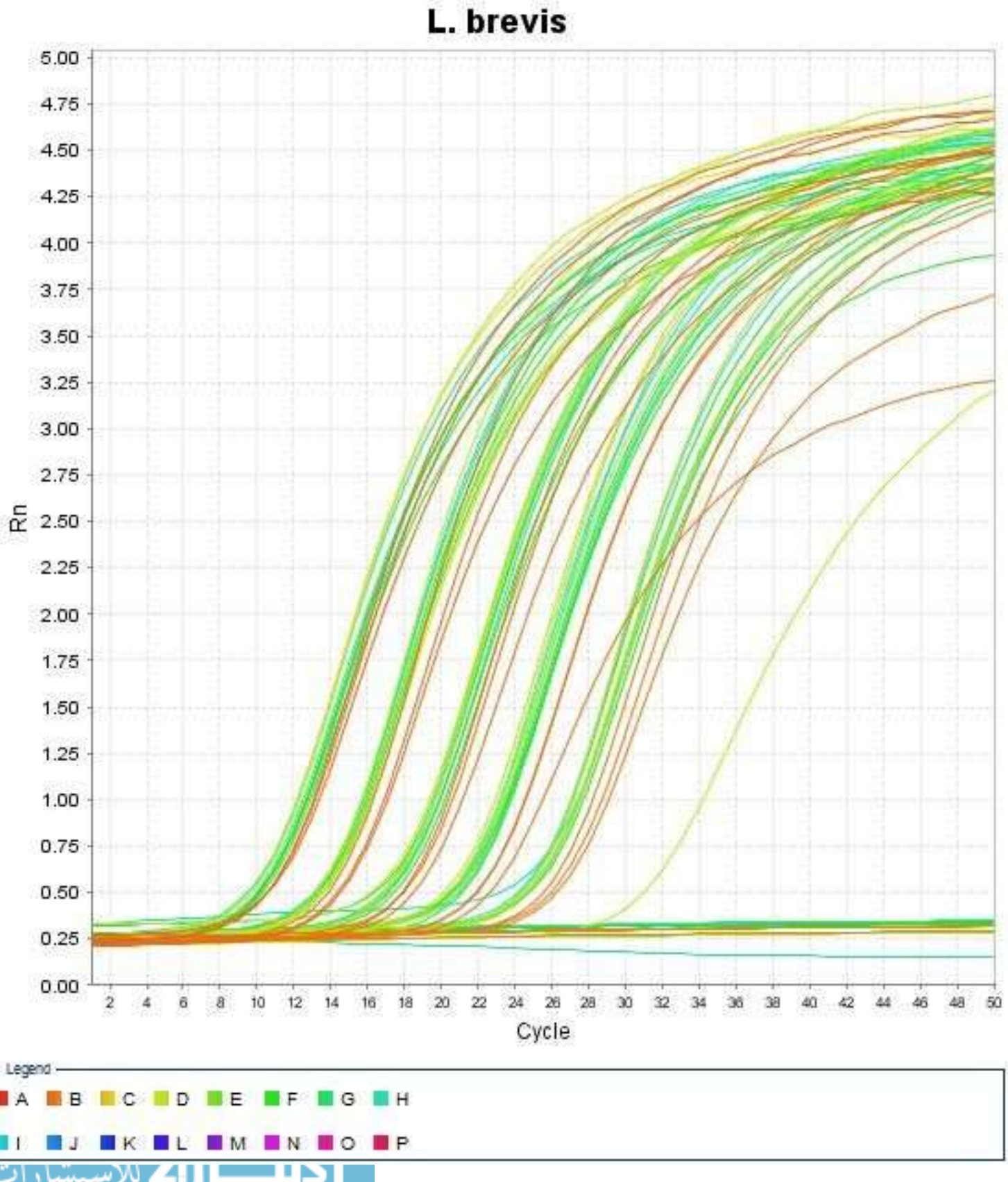
Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
1.1	L. brevis				
2.2	L. brevis				
2.3	L. brevis				
3.2	L. brevis				
Blank	L. brevis				



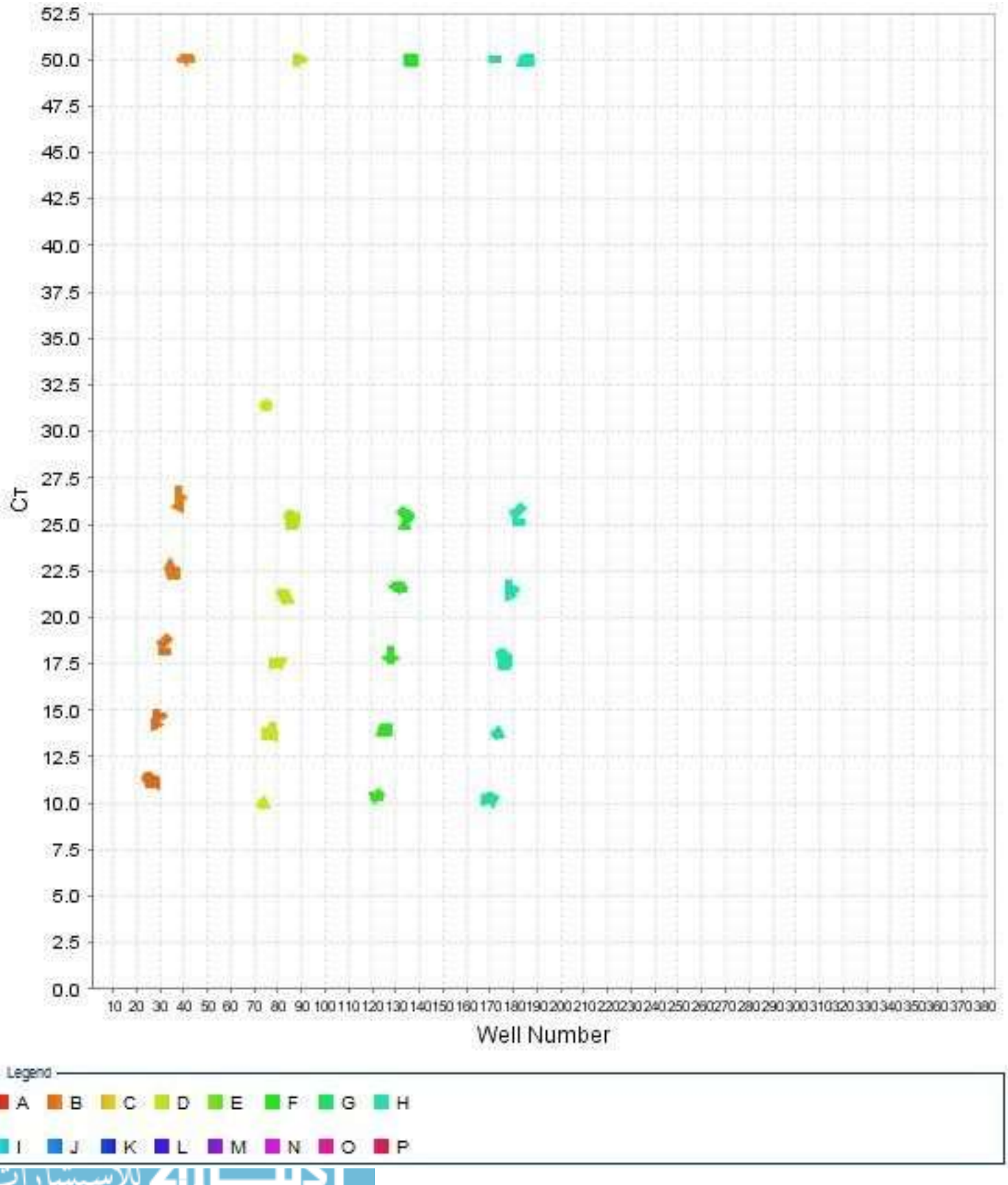
Amplification Plot (ΔRn vs. Cycle)



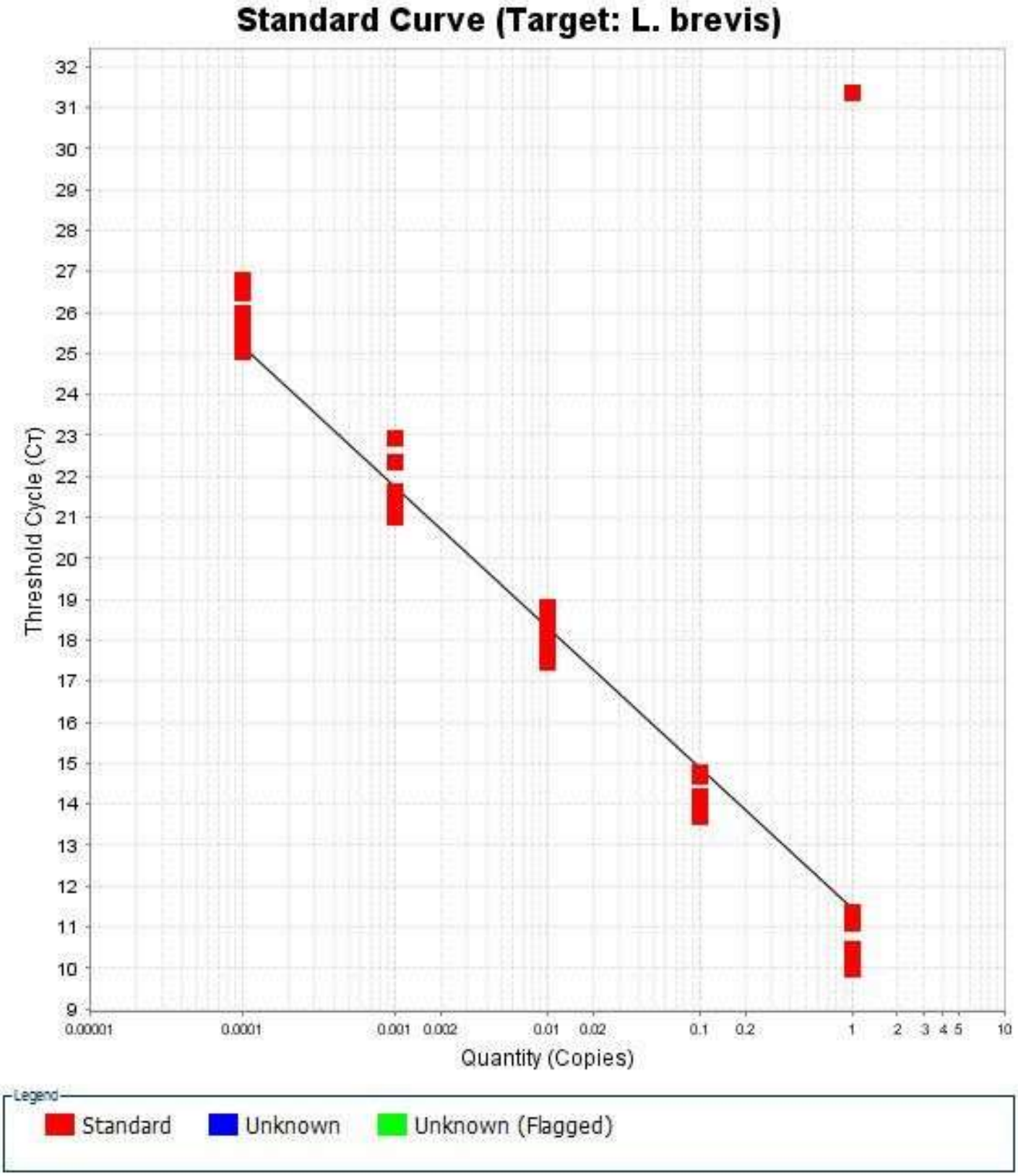
Amplification Plot (Rn vs. Cycle)



Amplification Plot (CT vs. Well)

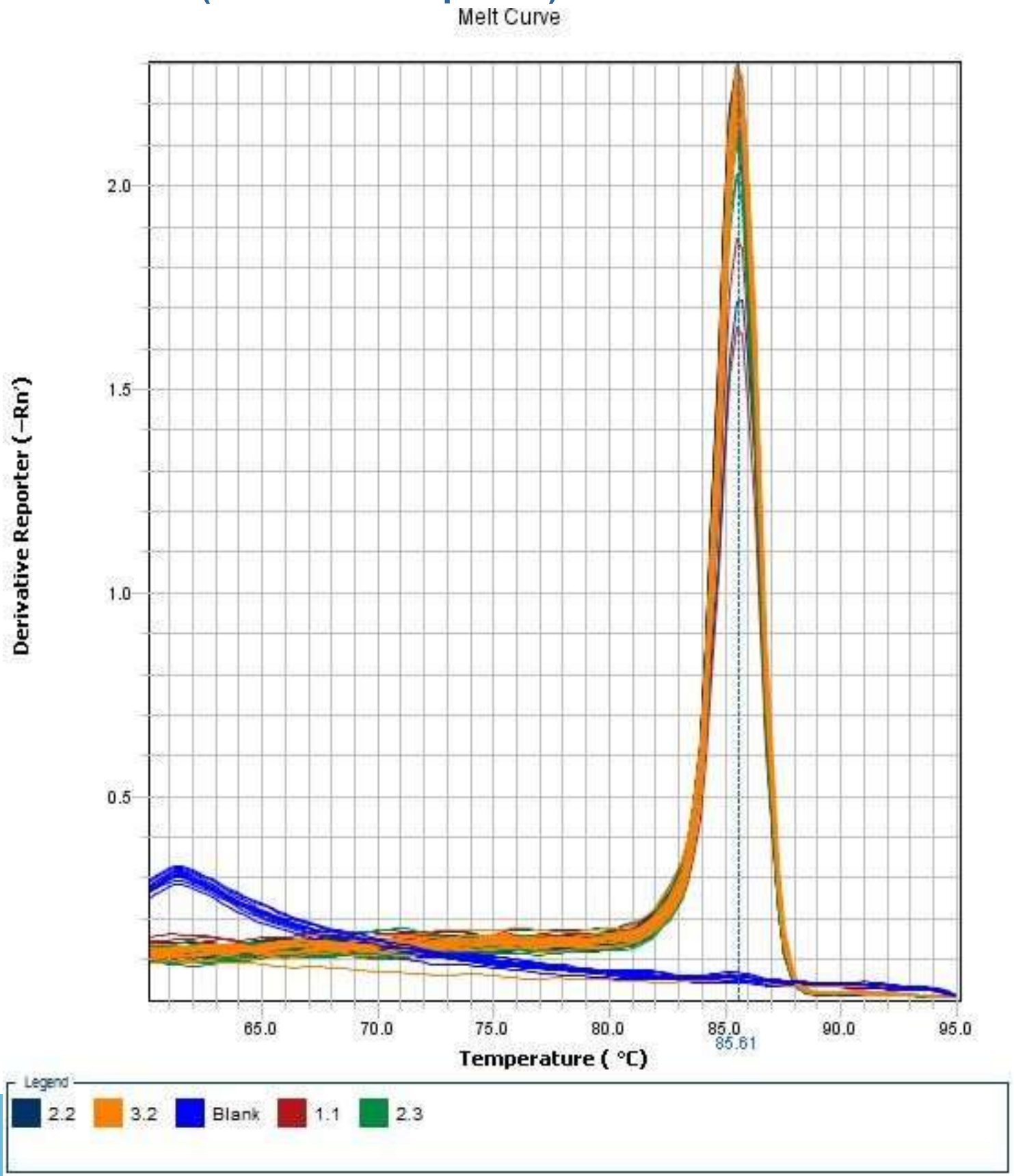


Standard Curves

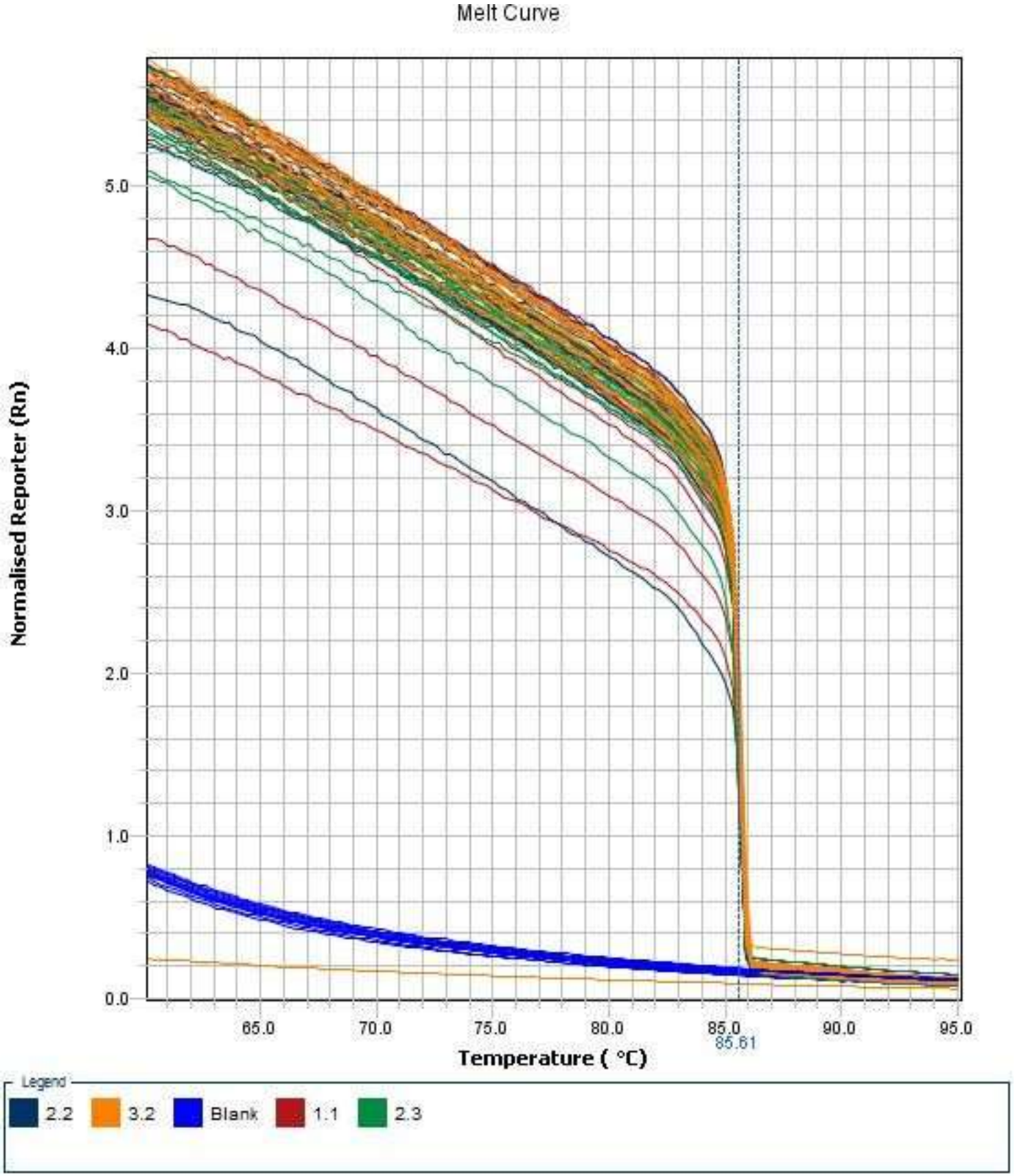


slope:-3.4378 Y-Intercept:11.4365 R²:0.765 Eff%:95.382

Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	1.1	L. brevis	S	1.000	11.375	11.191	0.161		85.615		
B3	1.1	L. brevis	S	1.000	11.127	11.191	0.161		85.615		
B4	1.1	L. brevis	S	1.000	11.073	11.191	0.161		85.483		
B5	1.1	L. brevis	S	0.100	14.762	14.552	0.308		85.483		
B6	1.1	L. brevis	S	0.100	14.199	14.552	0.308		85.483		
B7	1.1	L. brevis	S	0.100	14.696	14.552	0.308		85.483		
B8	1.1	L. brevis	S	0.010	18.421	18.457	0.332		85.483		
B9	1.1	L. brevis	S	0.010	18.145	18.457	0.332		85.483		
B10	1.1	L. brevis	S	0.010	18.805	18.457	0.332		85.615		
B11	1.1	L. brevis	S	0.001	22.914	22.540	0.324		85.615		
B12	1.1	L. brevis	S	0.001	22.343	22.540	0.324		85.615		
B13	1.1	L. brevis	S	0.001	22.364	22.540	0.324		85.615		
B14	1.1	L. brevis	S	0.000	25.985	26.415	0.402		85.615		
B15	1.1	L. brevis	S	0.000	26.780	26.415	0.402		85.615		
B16	1.1	L. brevis	S	0.000	26.480	26.415	0.402		85.615		
B17	Blank	L. brevis	N		UND.				61.385	84.825	
B18	Blank	L. brevis	N		UND.				61.254	89.960	
B19	Blank	L. brevis	N		UND.				61.385		
D2	2.2	L. brevis	S	1.000	9.993	17.149	12.306		85.483		
D3	2.2	L. brevis	S	1.000	10.096	17.149	12.306		85.483		
D4	2.2	L. brevis	S	1.000	31.359	17.149	12.306		85.615		
D5	2.2	L. brevis	S	0.100	13.782	13.841	0.200		85.483		
D6	2.2	L. brevis	S	0.100	13.677	13.841	0.200		85.483		
D7	2.2	L. brevis	S	0.100	14.064	13.841	0.200		85.483		
D8	2.2	L. brevis	S	0.010	17.550	17.521	0.048		85.483		
D9	2.2	L. brevis	S	0.010	17.466	17.521	0.048		85.483		
D10	2.2	L. brevis	S	0.010	17.547	17.521	0.048		85.483		
D11	2.2	L. brevis	S	0.001	21.367	21.156	0.194		85.483		
D12	2.2	L. brevis	S	0.001	20.985	21.156	0.194		85.483		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	Ct	Ct Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
D13	2.2	L. brevis	S	0.001	21.115	21.156	0.194		85.483		
D14	2.2	L. brevis	S	0.000	25.480	25.272	0.225		85.483		
D15	2.2	L. brevis	S	0.000	25.034	25.272	0.225		85.483		
D16	2.2	L. brevis	S	0.000	25.301	25.272	0.225		85.483		
D17	Blank	L. brevis	N		UND.				61.385		
D18	Blank	L. brevis	N		UND.				61.385		
D19	Blank	L. brevis	N		UND.				61.254		
F2	2.3	L. brevis	S	1.000	10.281	10.364	0.087		85.615		
F3	2.3	L. brevis	S	1.000	10.358	10.364	0.087		85.615		
F4	2.3	L. brevis	S	1.000	10.454	10.364	0.087		85.615		
F5	2.3	L. brevis	S	0.100	13.935	13.918	0.027		85.483		
F6	2.3	L. brevis	S	0.100	13.887	13.918	0.027		85.483		
F7	2.3	L. brevis	S	0.100	13.932	13.918	0.027		85.483		
F8	2.3	L. brevis	S	0.010	17.803	17.891	0.157		85.483		
F9	2.3	L. brevis	S	0.010	18.072	17.891	0.157		85.483		
F10	2.3	L. brevis	S	0.010	17.798	17.891	0.157		85.615		
F11	2.3	L. brevis	S	0.001	21.631	21.586	0.061		85.615		
F12	2.3	L. brevis	S	0.001	21.610	21.586	0.061		85.615		
F13	2.3	L. brevis	S	0.001	21.517	21.586	0.061		85.483		
F14	2.3	L. brevis	S	0.000	25.606	25.360	0.271		85.483		
F15	2.3	L. brevis	S	0.000	25.069	25.360	0.271		85.483		
F16	2.3	L. brevis	S	0.000	25.404	25.360	0.271		85.483		
F17	Blank	L. brevis	N		UND.				61.385	85.220	
F18	Blank	L. brevis	N		UND.				61.385		
F19	Blank	L. brevis	N		UND.				61.517	84.956	
H2	3.2	L. brevis	S	1.000	10.162	10.191	0.188		85.615		
H3	3.2	L. brevis	S	1.000	10.391	10.191	0.188		85.615		
H4	3.2	L. brevis	S	1.000	10.019	10.191	0.188		85.615		
H5	3.2	L. brevis	S	0.100	UND.	13.805	0.043		62.044		
H6	3.2	L. brevis	S	0.100	13.775	13.805	0.043		85.483		
H7	3.2	L. brevis	S	0.100	13.835	13.805	0.043		85.483		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	CT	CT Mean		Qty Mean	Qty SD	Tm1	Tm2	Tm3
H8	3.2	L. brevis	S	0.010	17.983	17.748	0.238			85.615		
H9	3.2	L. brevis	S	0.010	17.508	17.748	0.238			85.615		
H10	3.2	L. brevis	S	0.010	17.754	17.748	0.238			85.615		
H11	3.2	L. brevis	S	0.001	21.651	21.457	0.216			85.615		
H12	3.2	L. brevis	S	0.001	21.225	21.457	0.216			85.615		
H13	3.2	L. brevis	S	0.001	21.494	21.457	0.216			85.615		
H14	3.2	L. brevis	S	0.000	25.447	25.473	0.392			85.615		
H15	3.2	L. brevis	S	0.000	25.095	25.473	0.392			85.483		
H16	3.2	L. brevis	S	0.000	25.877	25.473	0.392			85.615		
H17	Blank	L. brevis	N		UND.					61.385	85.220	
H18	Blank	L. brevis	N		UND.					61.385	91.145	
H19	Blank	L. brevis	N		UND.					61.517		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	72	Targets Used	1
Well Setup	72	Flagged Wells	4	Samples Used	5

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	1	H5
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	1	H5
HIGHSD	High standard deviation in replicate group	3	D2, D3, D4
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	1	D4
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp**	m1,x1 m4,x4
Cover Temperature	105.0	**Filter Set**	
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	50	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	600	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	94.0	60	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	55.0	120	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	74.0	120	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	15	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_	60.0	60	0.0	0

PER_SECOND

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_PER_SECOND	95.0	15	0.0	0

Experiment Results Report

2020-01-13 103751

Experiment Summary

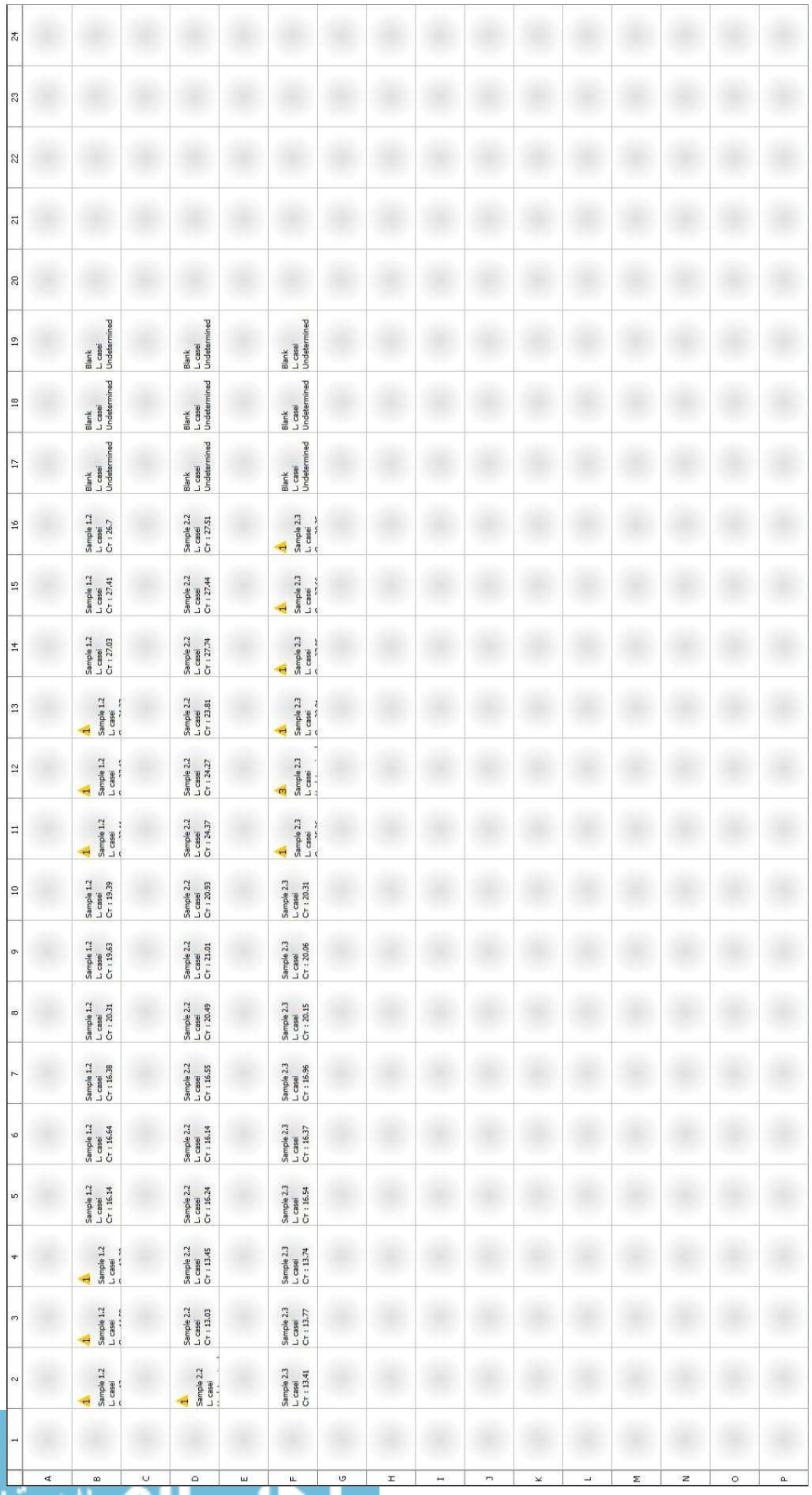
Experiment Name: 2020-01-13 103751
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_L_casei_20200113_standardcurve2.eds
Run Started: 01-13-2020 20:42:55 PST
Run Finished: 01-13-2020 22:44:01 PST
Run Duration: 121 minutes 6 seconds
Date Modified: 01-13-2020 17:42:07 PST
Date Created: 01-13-2020 10:37:50 PST
User:
Number of Wells Used: 54
Number of Wells with Results: 54
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 3
Comments:
Quantification Cycle Setting: CT

Reagent Information

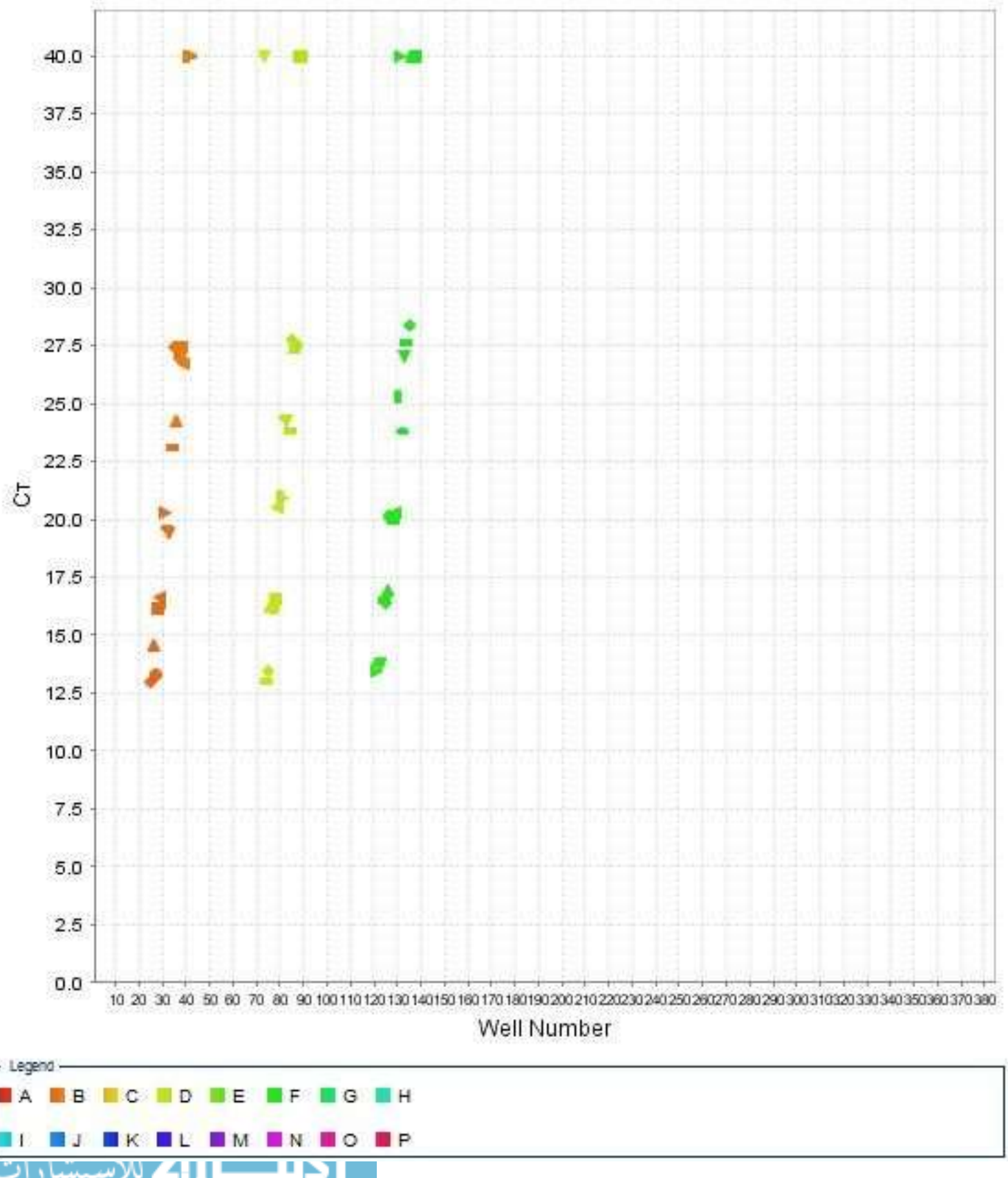
Results Summary

Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
Blank	L. casei				
Sample 1.2	L. casei				
Sample 2.2	L. casei				
Sample 2.3	L. casei				

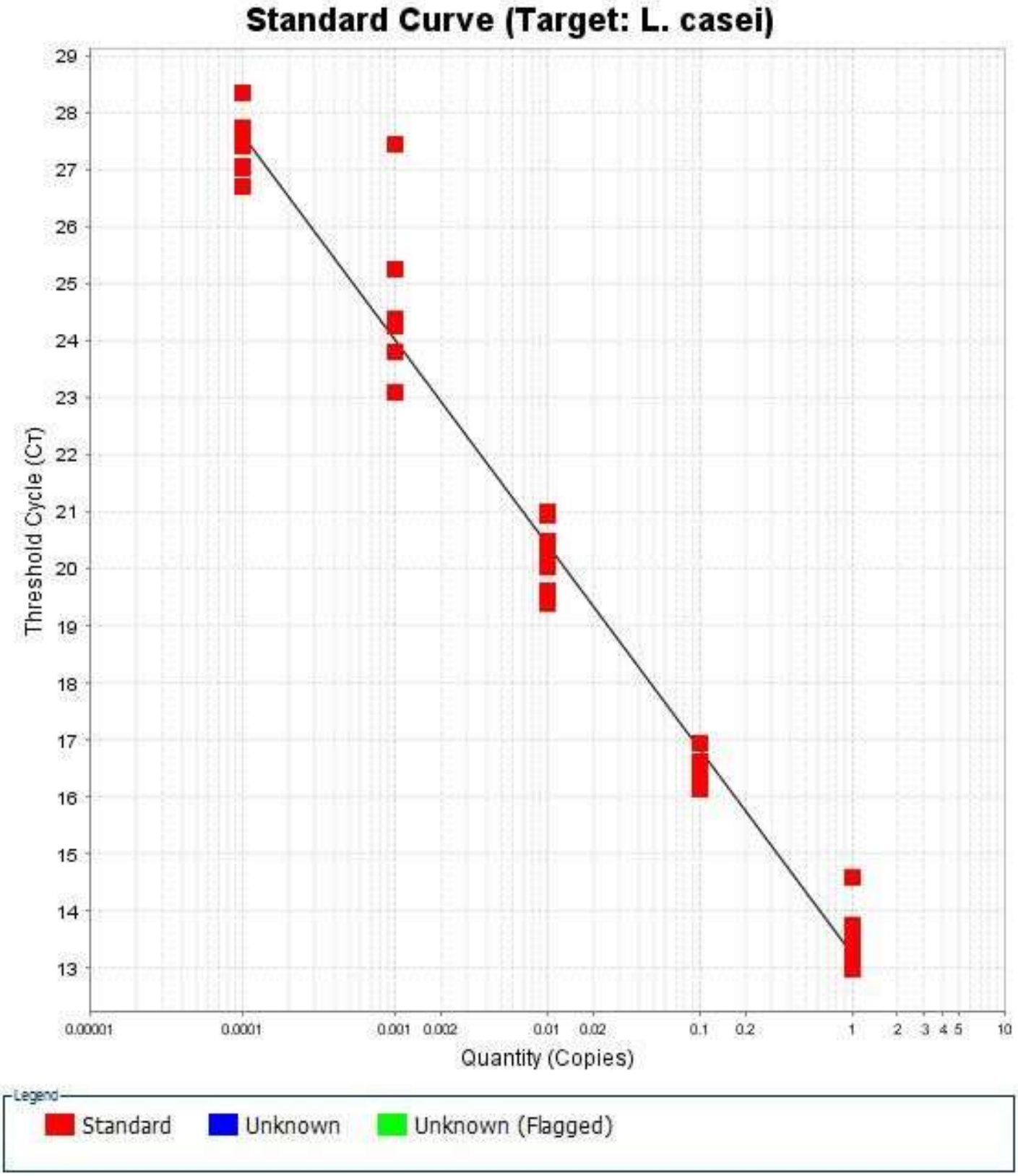
Plate Layout



Amplification Plot (CT vs. Well)

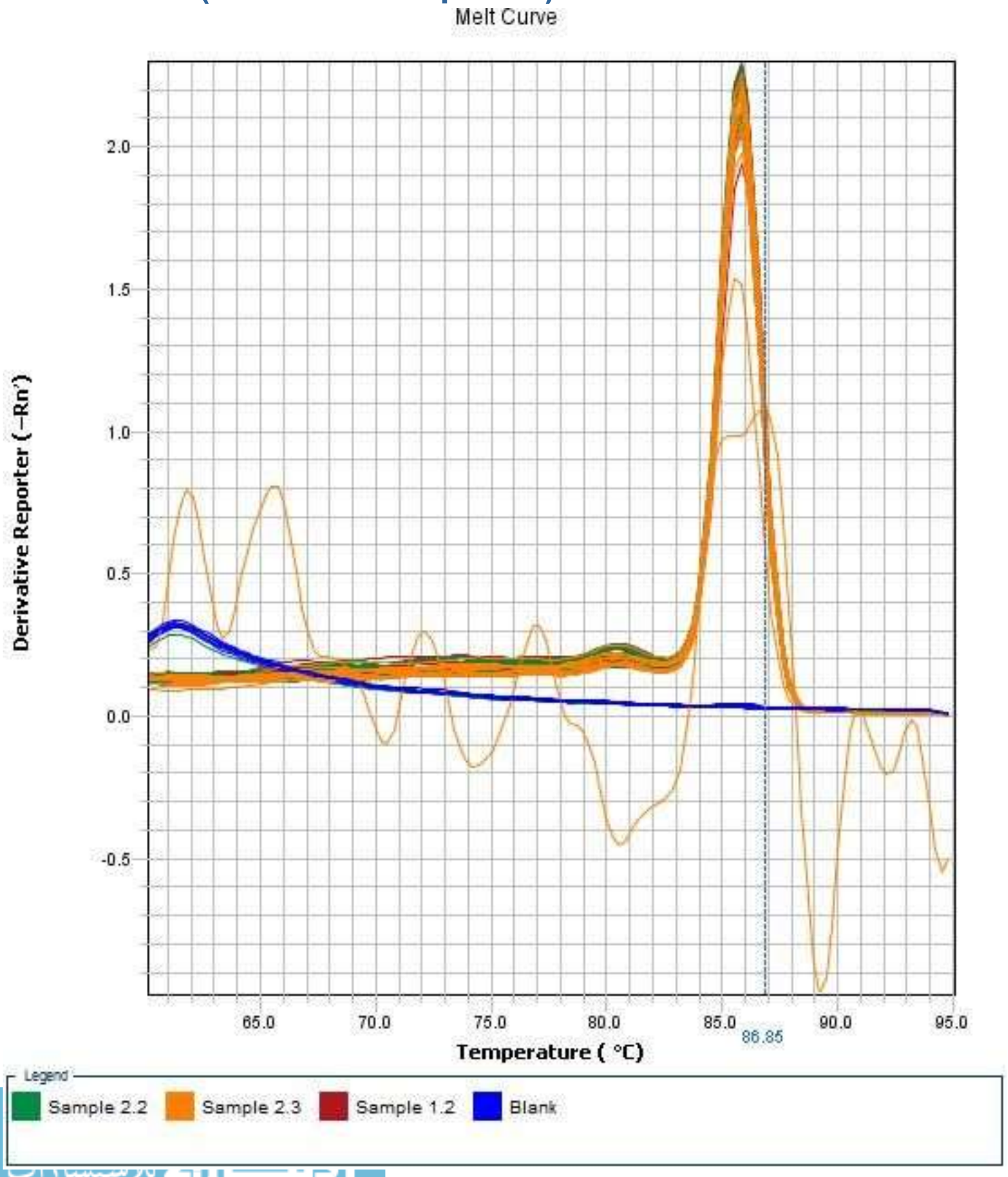


Standard Curves

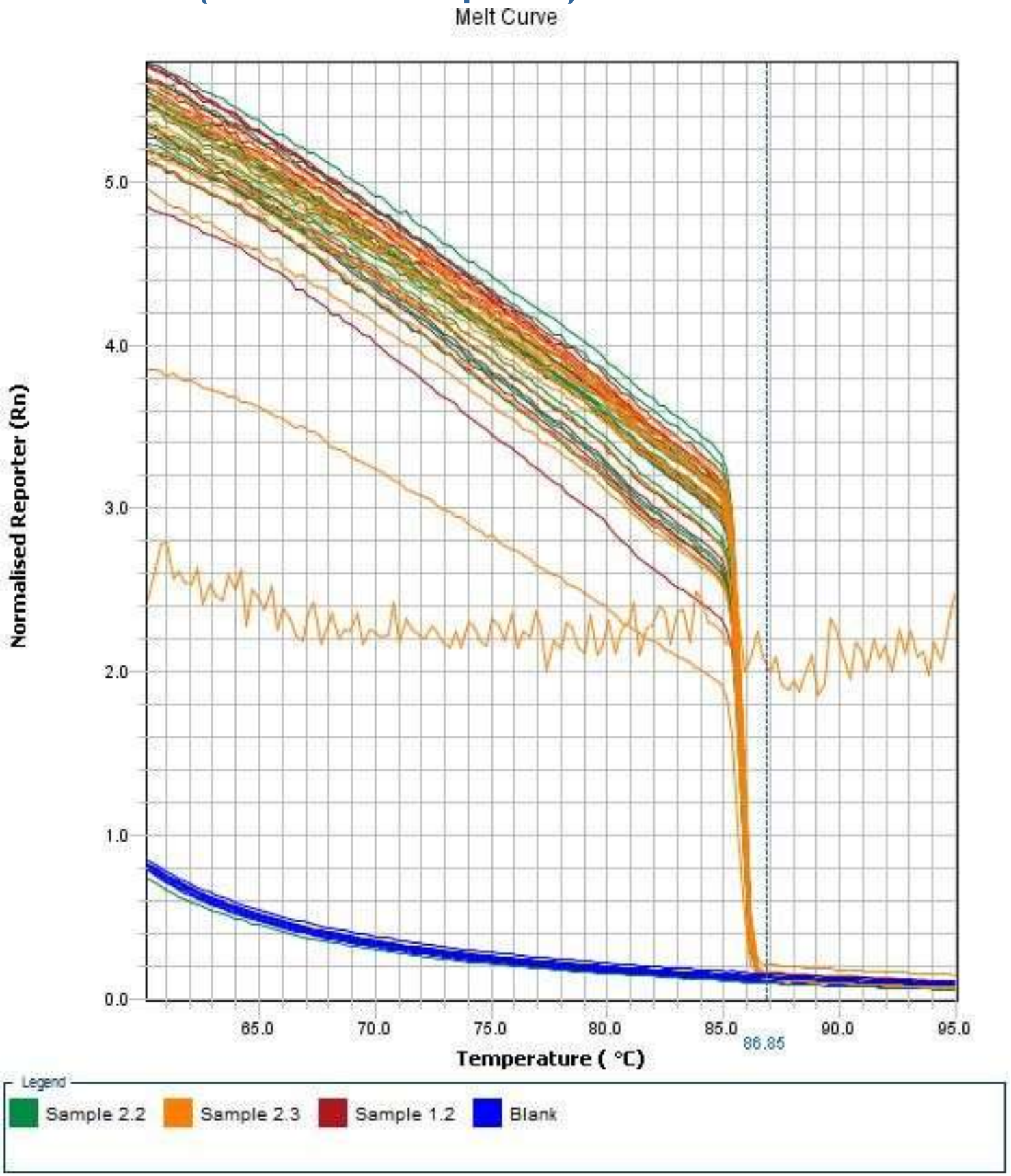


slope:-3.5908 Y-Intercept:13.2401 R^2:0.979 Eff%:89.884

Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	Sample 1.2	L. casei	S	1.000	12.999	13.624	0.848		85.798		
B3	Sample 1.2	L. casei	S	1.000	14.589	13.624	0.848		85.798		
B4	Sample 1.2	L. casei	S	1.000	13.283	13.624	0.848		85.798		
B5	Sample 1.2	L. casei	S	0.100	16.139	16.387	0.249		85.798		
B6	Sample 1.2	L. casei	S	0.100	16.638	16.387	0.249		85.798		
B7	Sample 1.2	L. casei	S	0.100	16.383	16.387	0.249		85.798		
B8	Sample 1.2	L. casei	S	0.010	20.313	19.777	0.480		85.798		
B9	Sample 1.2	L. casei	S	0.010	19.630	19.777	0.480		85.798		
B10	Sample 1.2	L. casei	S	0.010	19.388	19.777	0.480		85.798		
B11	Sample 1.2	L. casei	S	0.001	23.109	24.936	2.239		85.798		
B12	Sample 1.2	L. casei	S	0.001	27.434	24.936	2.239		85.798		
B13	Sample 1.2	L. casei	S	0.001	24.266	24.936	2.239		85.798		
B14	Sample 1.2	L. casei	S	0.000	27.027	27.048	0.357		85.798		
B15	Sample 1.2	L. casei	S	0.000	27.414	27.048	0.357		85.798		
B16	Sample 1.2	L. casei	S	0.000	26.701	27.048	0.357		85.666		
B17	Blank	L. casei	N		UND.				61.411	85.139	
B18	Blank	L. casei	N		UND.				61.411		
B19	Blank	L. casei	N		UND.				61.279		
D2	Sample 2.2	L. casei	S	1.000	UND.	13.241	0.294		61.411	85.402	
D3	Sample 2.2	L. casei	S	1.000	13.033	13.241	0.294		85.798		
D4	Sample 2.2	L. casei	S	1.000	13.449	13.241	0.294		85.798		
D5	Sample 2.2	L. casei	S	0.100	16.239	16.308	0.212		85.666		
D6	Sample 2.2	L. casei	S	0.100	16.140	16.308	0.212		85.666		
D7	Sample 2.2	L. casei	S	0.100	16.547	16.308	0.212		85.798		
D8	Sample 2.2	L. casei	S	0.010	20.493	20.812	0.279		85.798		
D9	Sample 2.2	L. casei	S	0.010	21.011	20.812	0.279		85.798		
D10	Sample 2.2	L. casei	S	0.010	20.932	20.812	0.279		85.798		
D11	Sample 2.2	L. casei	S	0.001	24.375	24.152	0.301		85.798		
D12	Sample 2.2	L. casei	S	0.001	24.272	24.152	0.301		85.798		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
D13	Sample 2.2	L. casei	S	0.001	23.809	24.152	0.301		85.798		
D14	Sample 2.2	L. casei	S	0.000	27.742	27.565	0.157		85.798		
D15	Sample 2.2	L. casei	S	0.000	27.444	27.565	0.157		85.666		
D16	Sample 2.2	L. casei	S	0.000	27.509	27.565	0.157		85.666		
D17	Blank	L. casei	N		UND.				61.411	85.271	
D18	Blank	L. casei	N		UND.				61.411		
D19	Blank	L. casei	N		UND.				61.411		
F2	Sample 2.3	L. casei	S	1.000	13.412	13.639	0.197		85.798		
F3	Sample 2.3	L. casei	S	1.000	13.768	13.639	0.197		85.798		
F4	Sample 2.3	L. casei	S	1.000	13.736	13.639	0.197		85.798		
F5	Sample 2.3	L. casei	S	0.100	16.536	16.622	0.303		85.798		
F6	Sample 2.3	L. casei	S	0.100	16.371	16.622	0.303		85.798		
F7	Sample 2.3	L. casei	S	0.100	16.959	16.622	0.303		85.798		
F8	Sample 2.3	L. casei	S	0.010	20.146	20.169	0.127		85.798		
F9	Sample 2.3	L. casei	S	0.010	20.055	20.169	0.127		85.798		
F10	Sample 2.3	L. casei	S	0.010	20.306	20.169	0.127		85.798		
F11	Sample 2.3	L. casei	S	0.001	25.262	24.535	1.029		85.798		
F12	Sample 2.3	L. casei	S	0.001	UND.	24.535	1.029		86.852		
F13	Sample 2.3	L. casei	S	0.001	23.807	24.535	1.029		85.798		
F14	Sample 2.3	L. casei	S	0.000	27.052	27.687	0.651		85.666		
F15	Sample 2.3	L. casei	S	0.000	27.655	27.687	0.651		85.666		
F16	Sample 2.3	L. casei	S	0.000	28.353	27.687	0.651		85.666		
F17	Blank	L. casei	N		UND.				61.279	85.534	
F18	Blank	L. casei	N		UND.				61.279		
F19	Blank	L. casei	N		UND.				61.411		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	54	Targets Used	1
Well Setup	54	Flagged Wells	13	Samples Used	4

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	2	D2, F12
HIGHSD	High standard deviation in replicate group	11	B2, B3, B4, B11, B12, B13, F11, F13, F14, F15, F16
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	1	F12
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	0	
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	1	F12
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp**	m1,x1 m4,x4
Cover Temperature	105.0	**Filter Set**	
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	40	1	false
Hold Stage	1	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	600	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	93.0	30	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	57.0	30	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	72.0	30	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	72.0	120	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_	95.0	15	0.0	0

	PER_SECOND

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	60.0	60	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_PER_SECOND	95.0	15	0.0	0

Experiment Results Report

2020-01-10 100938

Experiment Summary

Experiment Name: 2020-01-10 100938
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_L_delbruki_B_bifidum_20200110_standardcurve.eds
Run Started: 01-10-2020 16:34:47 PST
Run Finished: 01-10-2020 20:16:00 PST
Run Duration: 221 minutes 12 seconds
Date Modified: 01-10-2020 15:14:17 PST
Date Created: 01-10-2020 10:09:38 PST
User:
Number of Wells Used: 108
Number of Wells with Results: 54
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 2
Comments:
Quantification Cycle Setting: CT

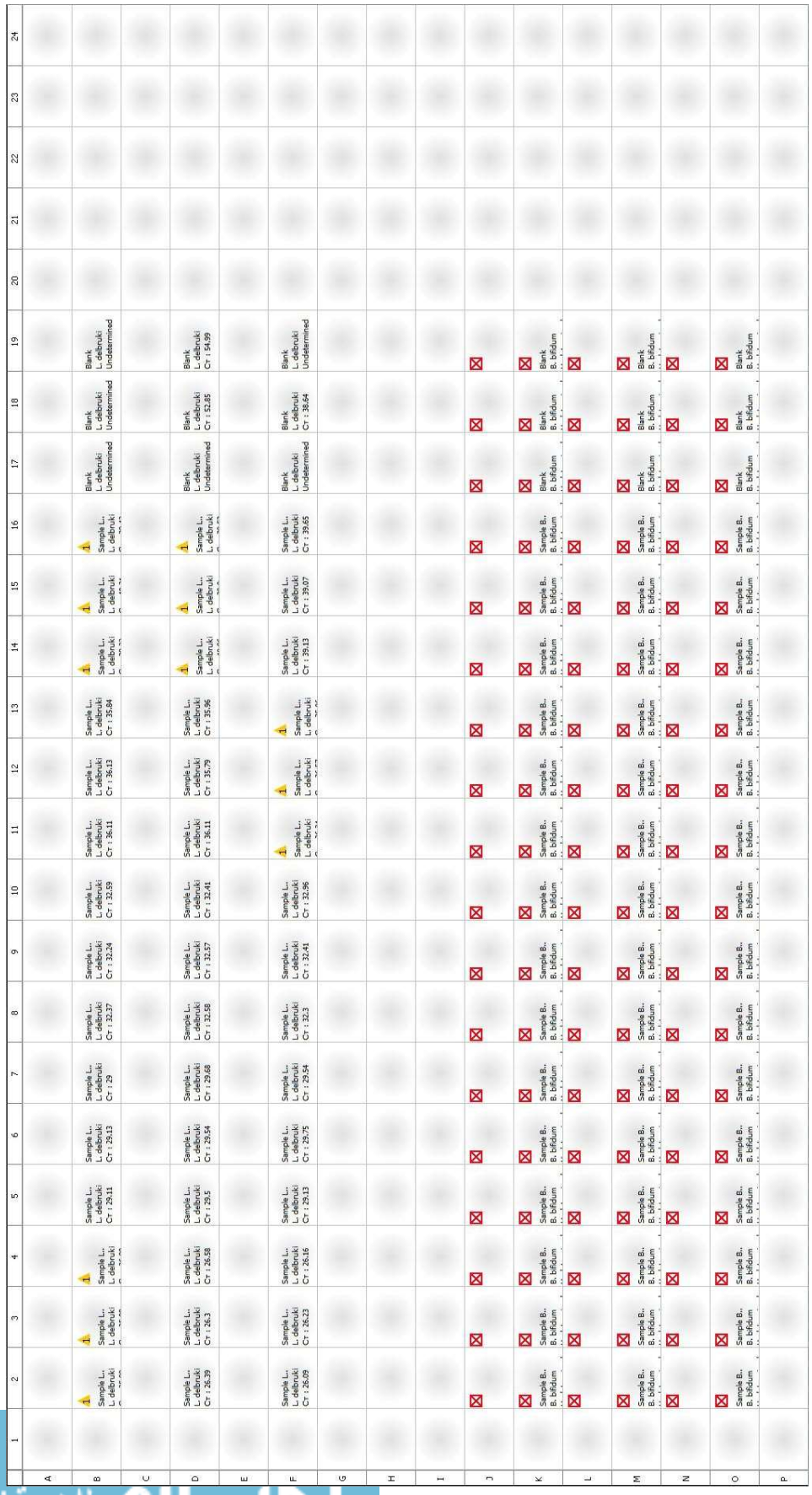
DATA FOR B. BIFIDUM NOT USED FROM THIS FILE

Reagent Information

Results Summary

Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
Blank	B. bifidum				
Sample B1.1	B. bifidum				
Sample B2.3	B. bifidum				
Sample B3.2	B. bifidum				
Blank	L. delbruki				
Sample L1.1	L. delbruki				
Sample L2.2	L. delbruki				
Sample L3.2	L. delbruki				

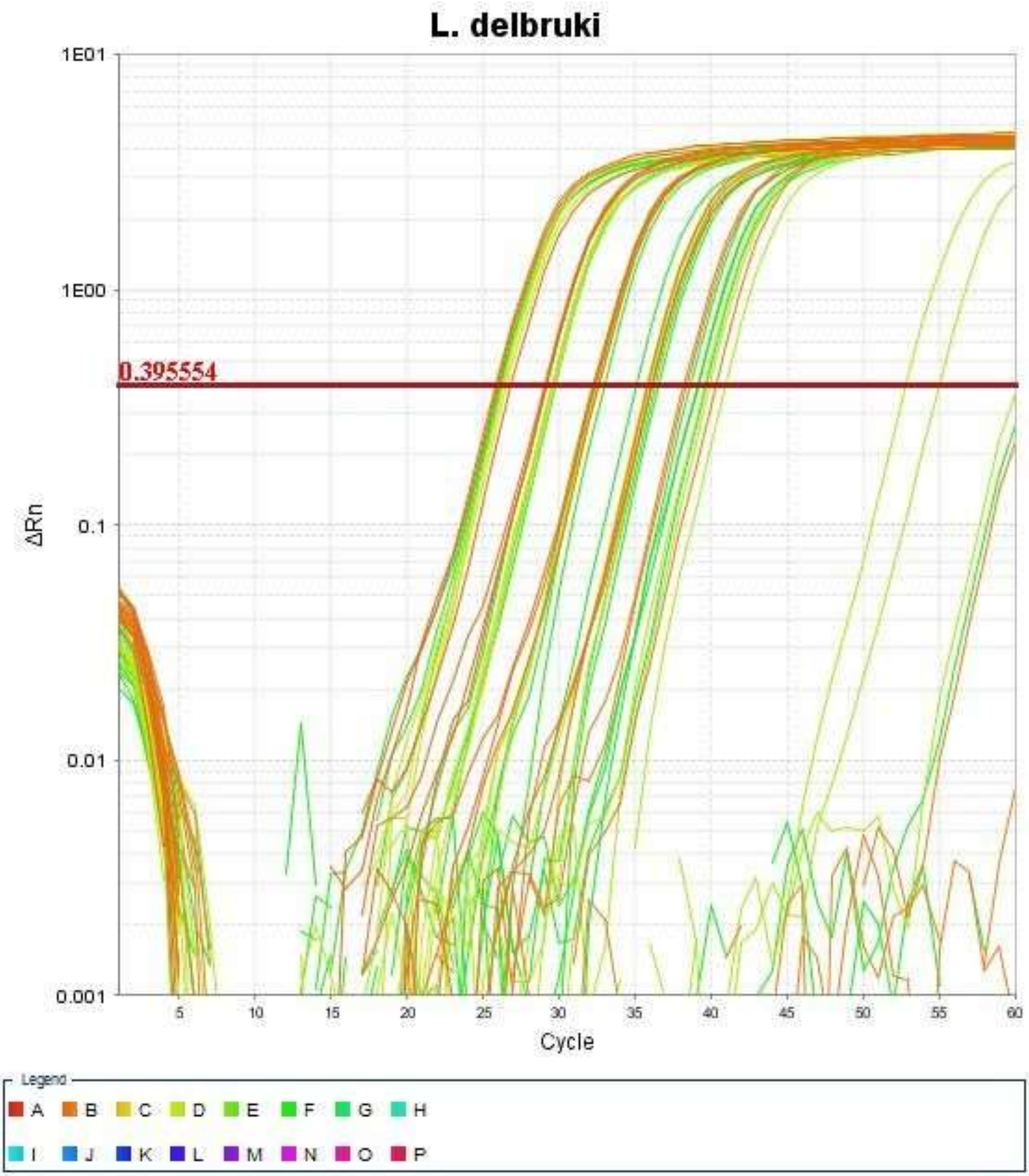
DATA FOR B. BIFIDUM NOT USED FROM THIS FILE



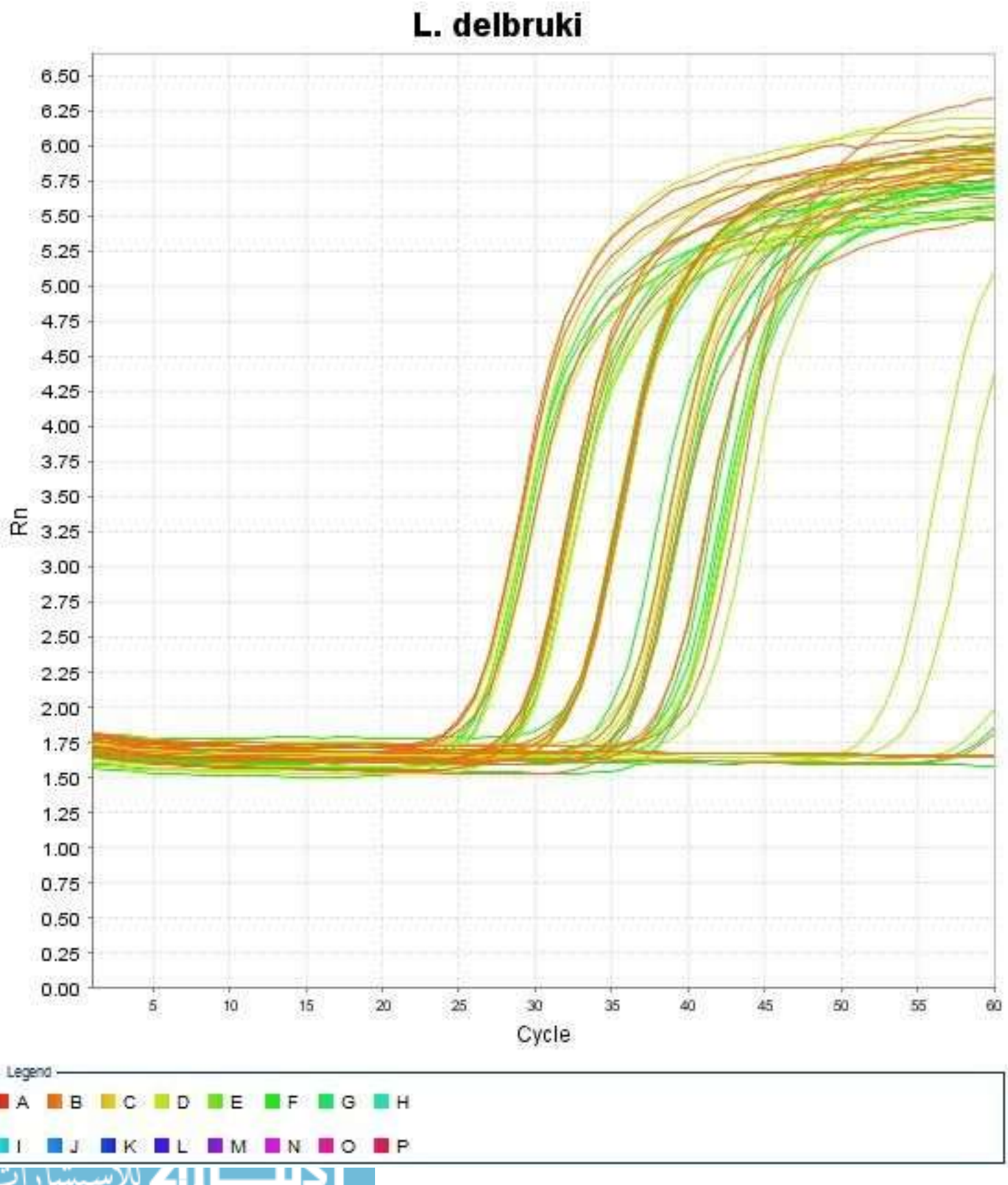
DATA FOR B. BIFIDUM NOT USED FROM THIS FILE

Plate Layout

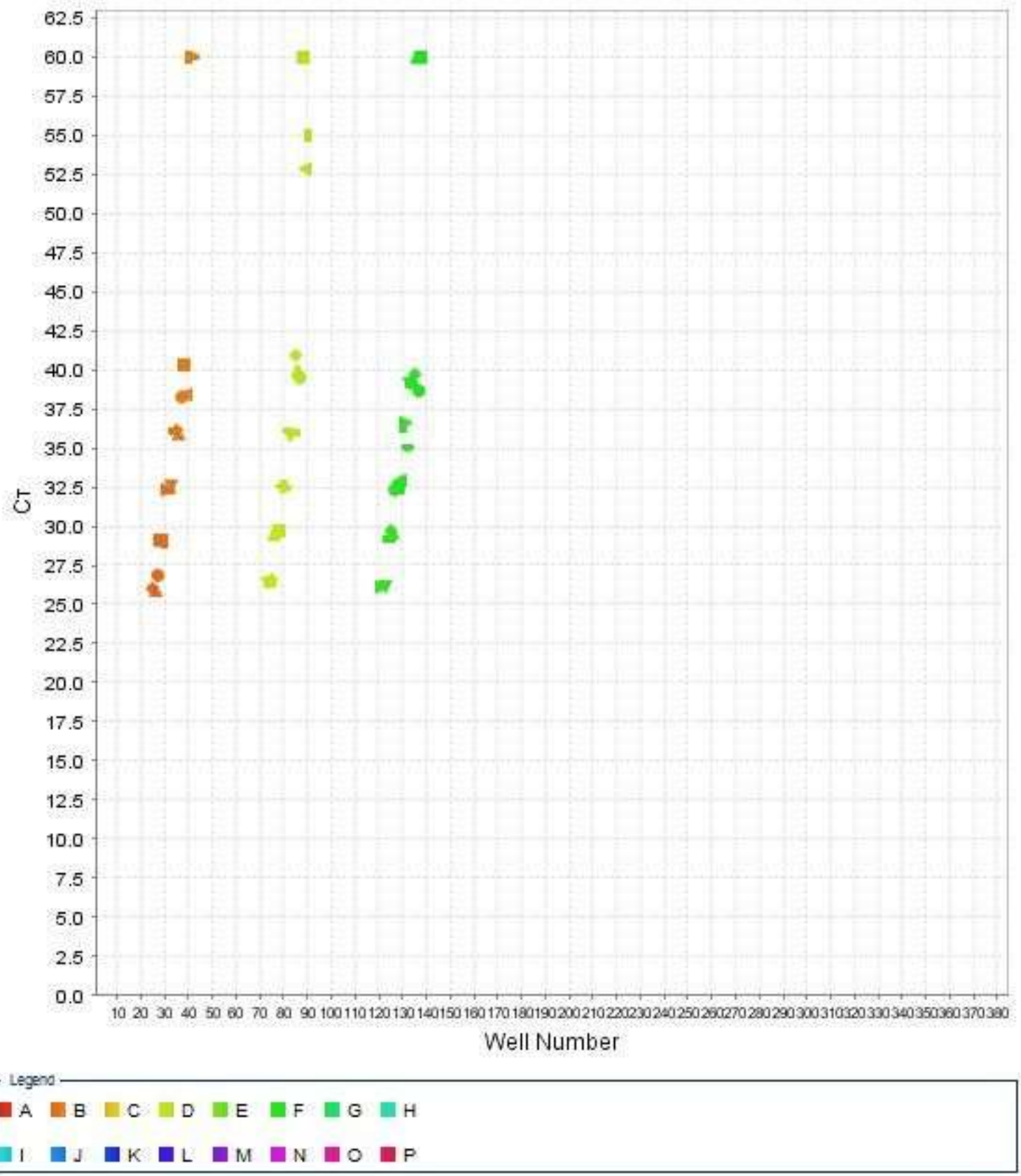
Amplification Plot (ΔRn vs. Cycle)



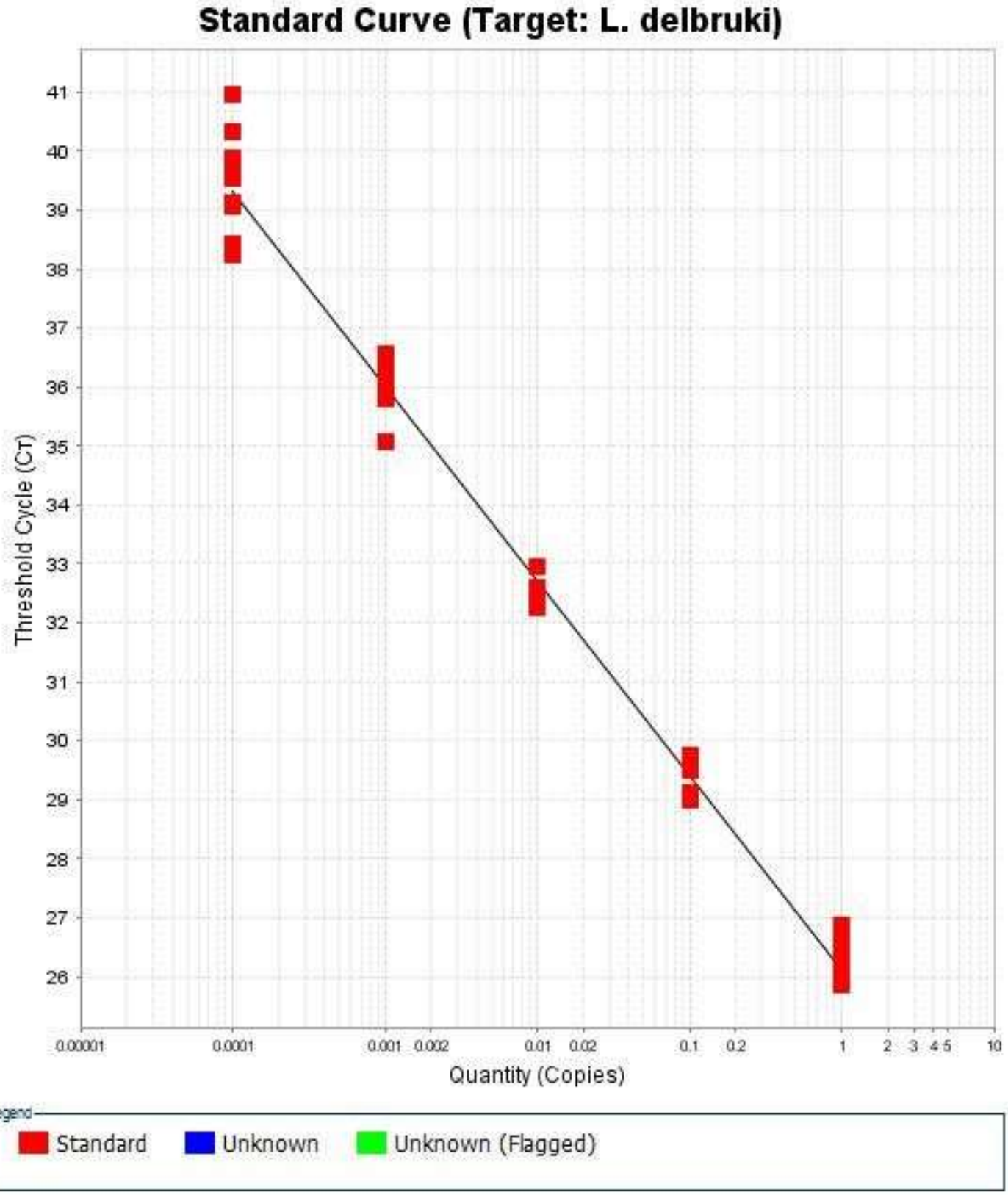
Amplification Plot (Rn vs. Cycle)



Amplification Plot (CT vs. Well)



Standard Curves



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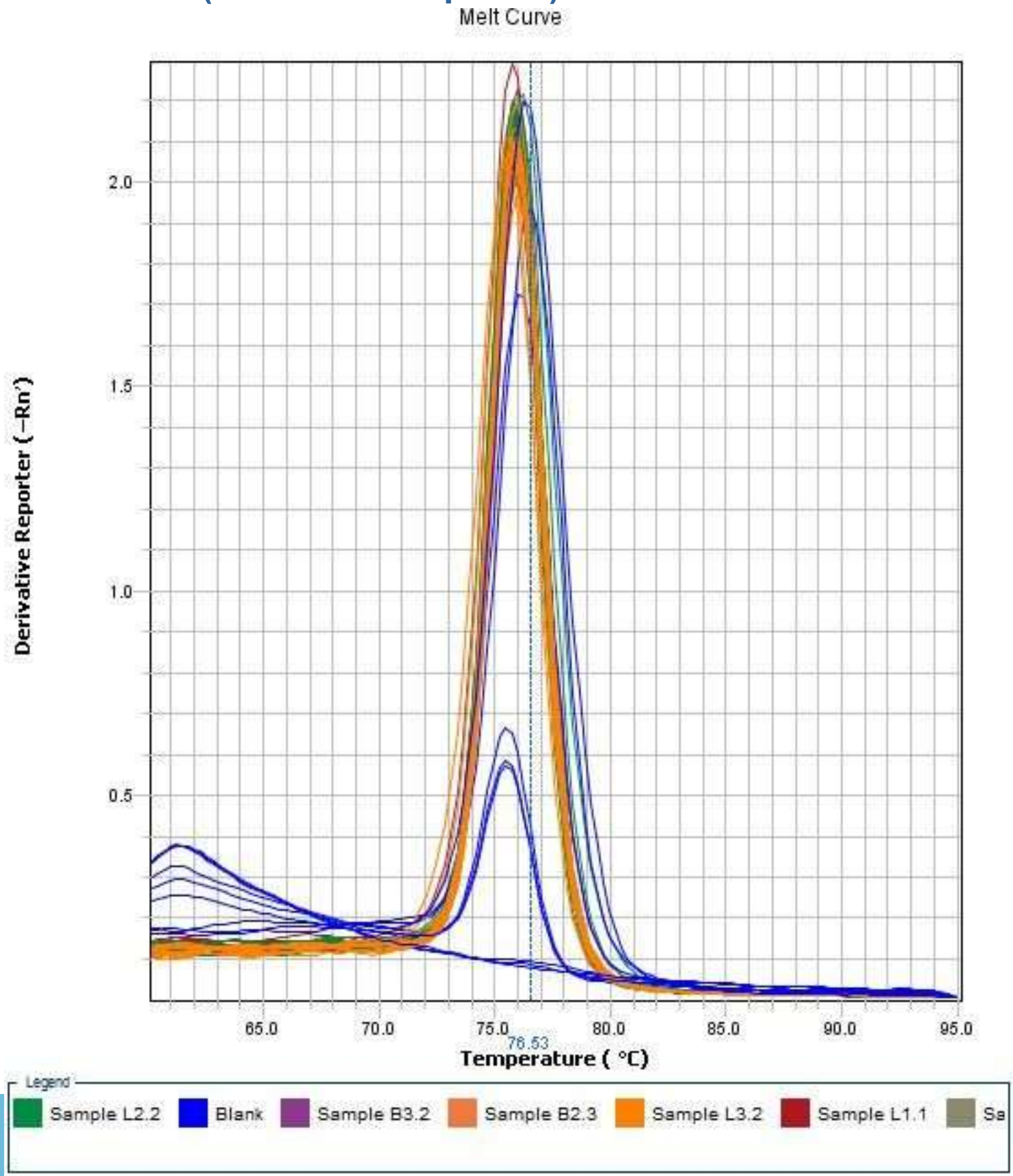
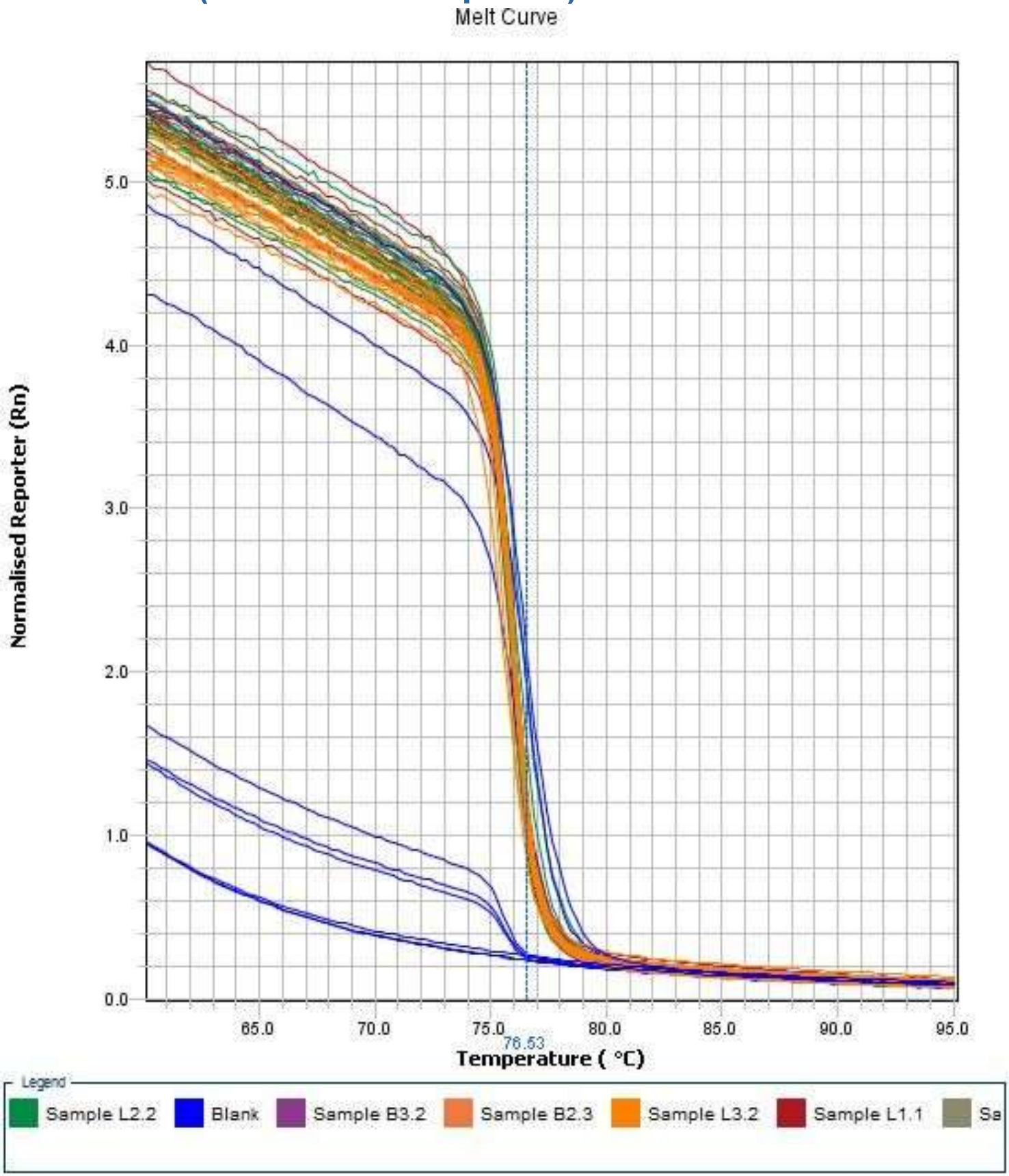
Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	Cт	Cт Mean		Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	Sample L1.1	L. delbruki	S	1.000	25.994	26.249	0.548			76.007		
B3	Sample L1.1	L. delbruki	S	1.000	25.875	26.249	0.548			76.007		
B4	Sample L1.1	L. delbruki	S	1.000	26.878	26.249	0.548			75.875		
B5	Sample L1.1	L. delbruki	S	0.100	29.114	29.082	0.074			76.007		
B6	Sample L1.1	L. delbruki	S	0.100	29.135	29.082	0.074			76.007		
B7	Sample L1.1	L. delbruki	S	0.100	28.997	29.082	0.074			75.875		
B8	Sample L1.1	L. delbruki	S	0.010	32.372	32.402	0.177			75.875		
B9	Sample L1.1	L. delbruki	S	0.010	32.242	32.402	0.177			75.875		
B10	Sample L1.1	L. delbruki	S	0.010	32.591	32.402	0.177			76.007		
B11	Sample L1.1	L. delbruki	S	0.001	36.110	36.029	0.163			75.875		
B12	Sample L1.1	L. delbruki	S	0.001	36.135	36.029	0.163			75.875		
B13	Sample L1.1	L. delbruki	S	0.001	35.841	36.029	0.163			75.875		
B14	Sample L1.1	L. delbruki	S	0.000	38.225	38.985	1.154			75.875		
B15	Sample L1.1	L. delbruki	S	0.000	40.313	38.985	1.154			75.743		
B16	Sample L1.1	L. delbruki	S	0.000	38.417	38.985	1.154			75.875		
B17	Blank	L. delbruki	N		UND.					61.517		
B18	Blank	L. delbruki	N		UND.					75.480		
B19	Blank	L. delbruki	N		UND.					61.385		
D2	Sample L2.2	L. delbruki	S	1.000	26.391	26.423	0.141			76.007		
D3	Sample L2.2	L. delbruki	S	1.000	26.300	26.423	0.141			76.007		
D4	Sample L2.2	L. delbruki	S	1.000	26.577	26.423	0.141			76.007		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	CT	CT Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
D5	Sample L2.2	L. delbruki	S	0.100	29.496	29.571	0.093		76.007		
D6	Sample L2.2	L. delbruki	S	0.100	29.543	29.571	0.093		76.138		
D7	Sample L2.2	L. delbruki	S	0.100	29.675	29.571	0.093		75.875		
D8	Sample L2.2	L. delbruki	S	0.010	32.577	32.520	0.094		75.875		
D9	Sample L2.2	L. delbruki	S	0.010	32.572	32.520	0.094		75.875		
D10	Sample L2.2	L. delbruki	S	0.010	32.412	32.520	0.094		75.875		
D11	Sample L2.2	L. delbruki	S	0.001	36.106	35.955	0.156		75.875		
D12	Sample L2.2	L. delbruki	S	0.001	35.793	35.955	0.156		75.875		
D13	Sample L2.2	L. delbruki	S	0.001	35.965	35.955	0.156		75.875		
D14	Sample L2.2	L. delbruki	S	0.000	40.961	40.129	0.744		75.743		
D15	Sample L2.2	L. delbruki	S	0.000	39.896	40.129	0.744		75.875		
D16	Sample L2.2	L. delbruki	S	0.000	39.529	40.129	0.744		76.007		
D17	Blank	L. delbruki	N		UND.				75.480		
D18	Blank	L. delbruki	N		52.850				76.533		
D19	Blank	L. delbruki	N		54.993				76.138		
F2	Sample L3.2	L. delbruki	S	1.000	26.088	26.160	0.072		76.007		
F3	Sample L3.2	L. delbruki	S	1.000	26.232	26.160	0.072		76.007		
F4	Sample L3.2	L. delbruki	S	1.000	26.160	26.160	0.072		76.007		
F5	Sample L3.2	L. delbruki	S	0.100	29.131	29.474	0.314		76.007		
F6	Sample L3.2	L. delbruki	S	0.100	29.749	29.474	0.314		76.007		
F7	Sample L3.2	L. delbruki	S	0.100	29.542	29.474	0.314		76.007		
F8	Sample L3.2	L. delbruki	S	0.010	32.304	32.555	0.350		75.875		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
F9	Sample L3.2	L. delbruki	S	0.010	32.406	32.555	0.350		75.875		
F10	Sample L3.2	L. delbruki	S	0.010	32.955	32.555	0.350		75.875		
F11	Sample L3.2	L. delbruki	S	0.001	36.341	35.990	0.813		75.875		
F12	Sample L3.2	L. delbruki	S	0.001	36.569	35.990	0.813		75.875		
F13	Sample L3.2	L. delbruki	S	0.001	35.061	35.990	0.813		75.875		
F14	Sample L3.2	L. delbruki	S	0.000	39.132	39.285	0.320		75.611		
F15	Sample L3.2	L. delbruki	S	0.000	39.071	39.285	0.320		75.480		
F16	Sample L3.2	L. delbruki	S	0.000	39.653	39.285	0.320		75.875		
F17	Blank	L. delbruki	N		UND.				61.385		
F18	Blank	L. delbruki	N		38.644				76.402		
F19	Blank	L. delbruki	N		UND.				75.480		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	54	Targets Used	2
Well Setup	108	Flagged Wells	12	Samples Used	7

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	0	
HIGHSD	High standard deviation in replicate group	12	B2, B3, B4, B14, B15, B16, D14, D15, D16, F11, F12, F13
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	0	
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp**	m1,x1 m4,x4
Cover Temperature	105.0	**Filter Set**	
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	60	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	600	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	20	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	55.0	120	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	15	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	60.0	60	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_	95.0	15	0.0	0

PER_SECOND

Experiment Results Report

2020-01-03 151049

Experiment Summary

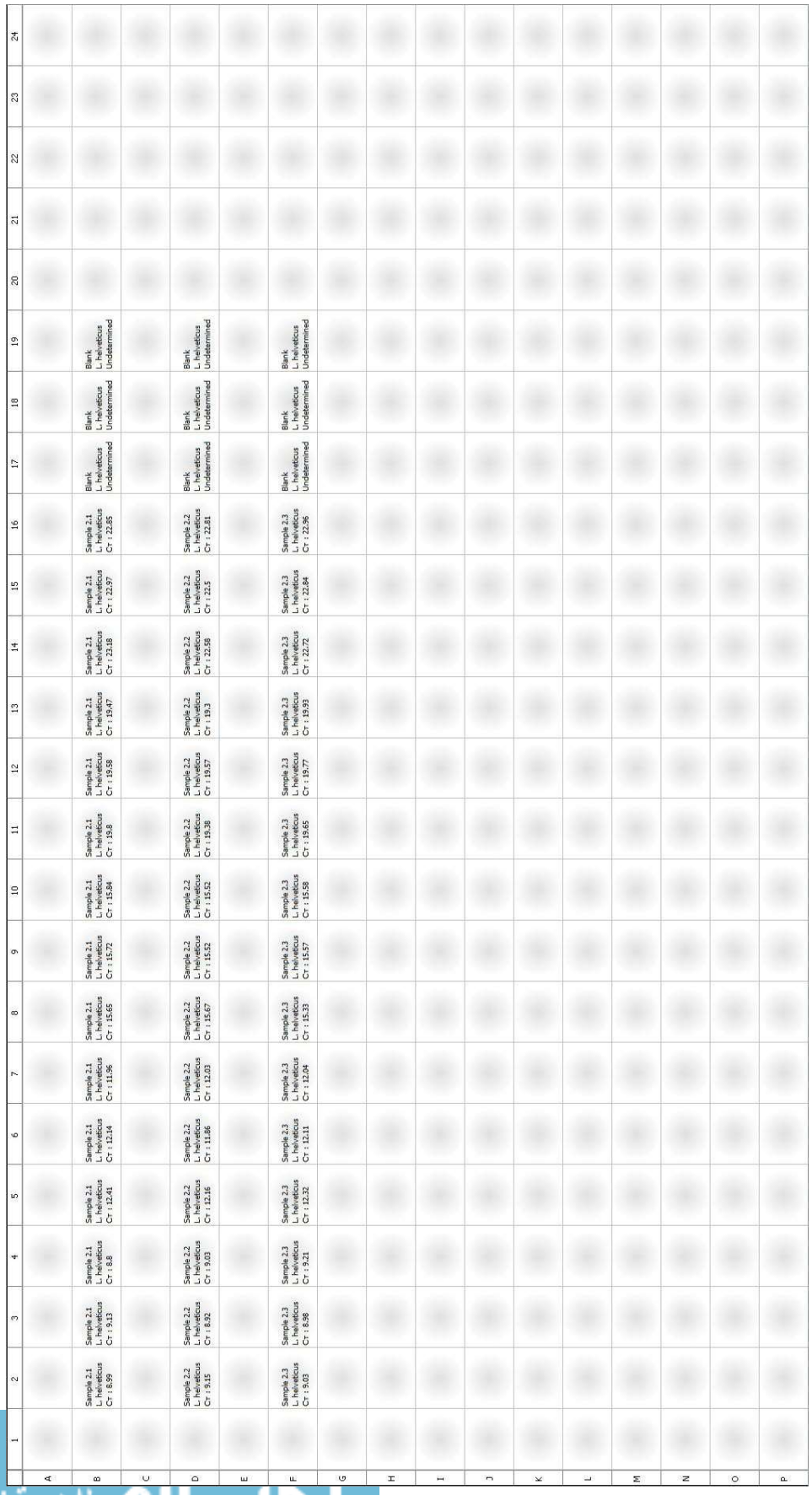
Experiment Name: 2020-01-03 151049
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_L_helveticus_20200103_standardcurve.eds
Run Started: 01-03-2020 20:21:31 PST
Run Finished: 01-03-2020 22:51:05 PST
Run Duration: 149 minutes 33 seconds
Date Modified: 01-03-2020 17:49:39 PST
Date Created: 01-03-2020 15:10:49 PST
User:
Number of Wells Used: 54
Number of Wells with Results: 54
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 3
Comments:
Quantification Cycle Setting: CT

Reagent Information

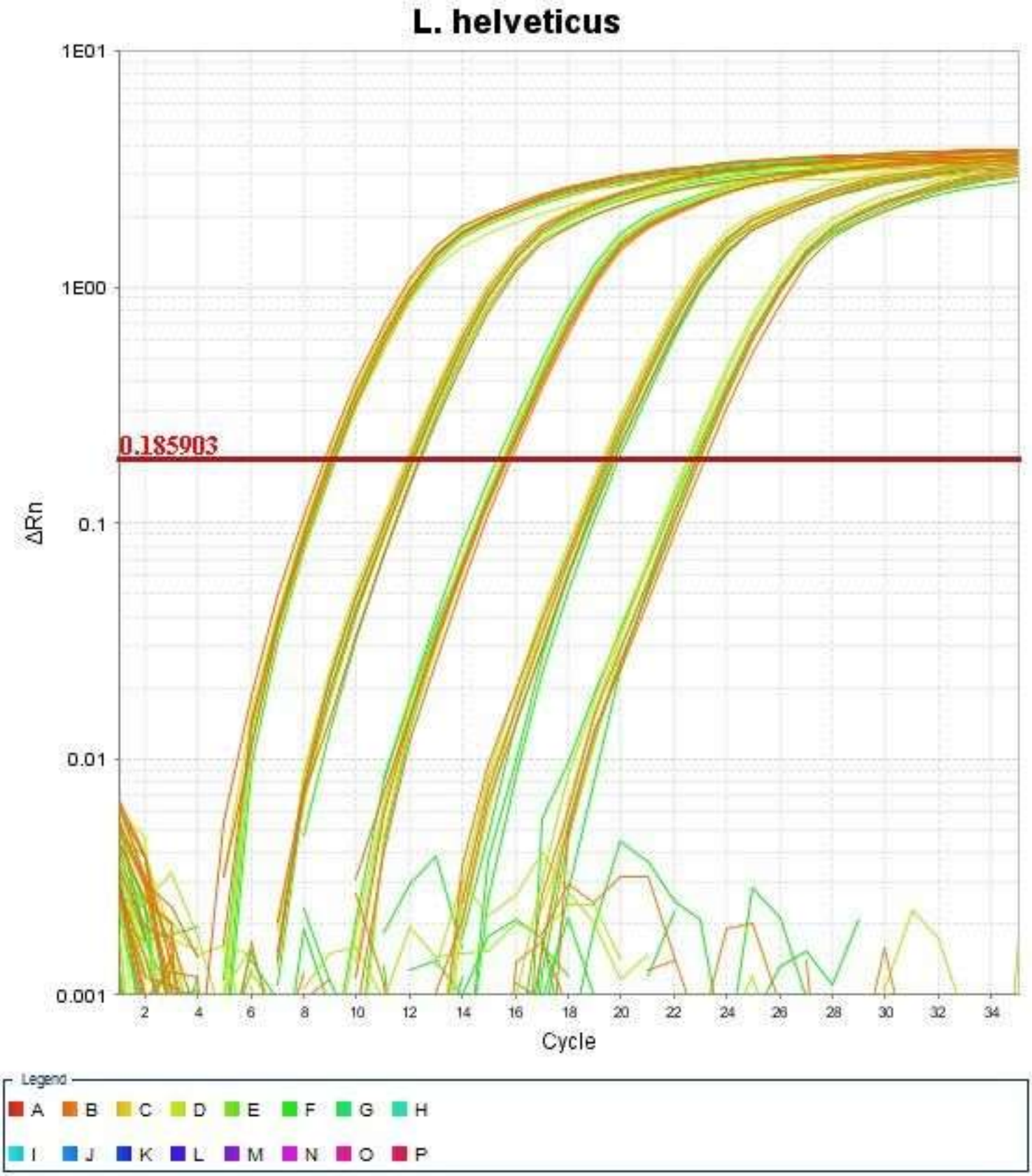
Results Summary

Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
Blank	L. helveticus				
Sample 2.1	L. helveticus				
Sample 2.2	L. helveticus				
Sample 2.3	L. helveticus				

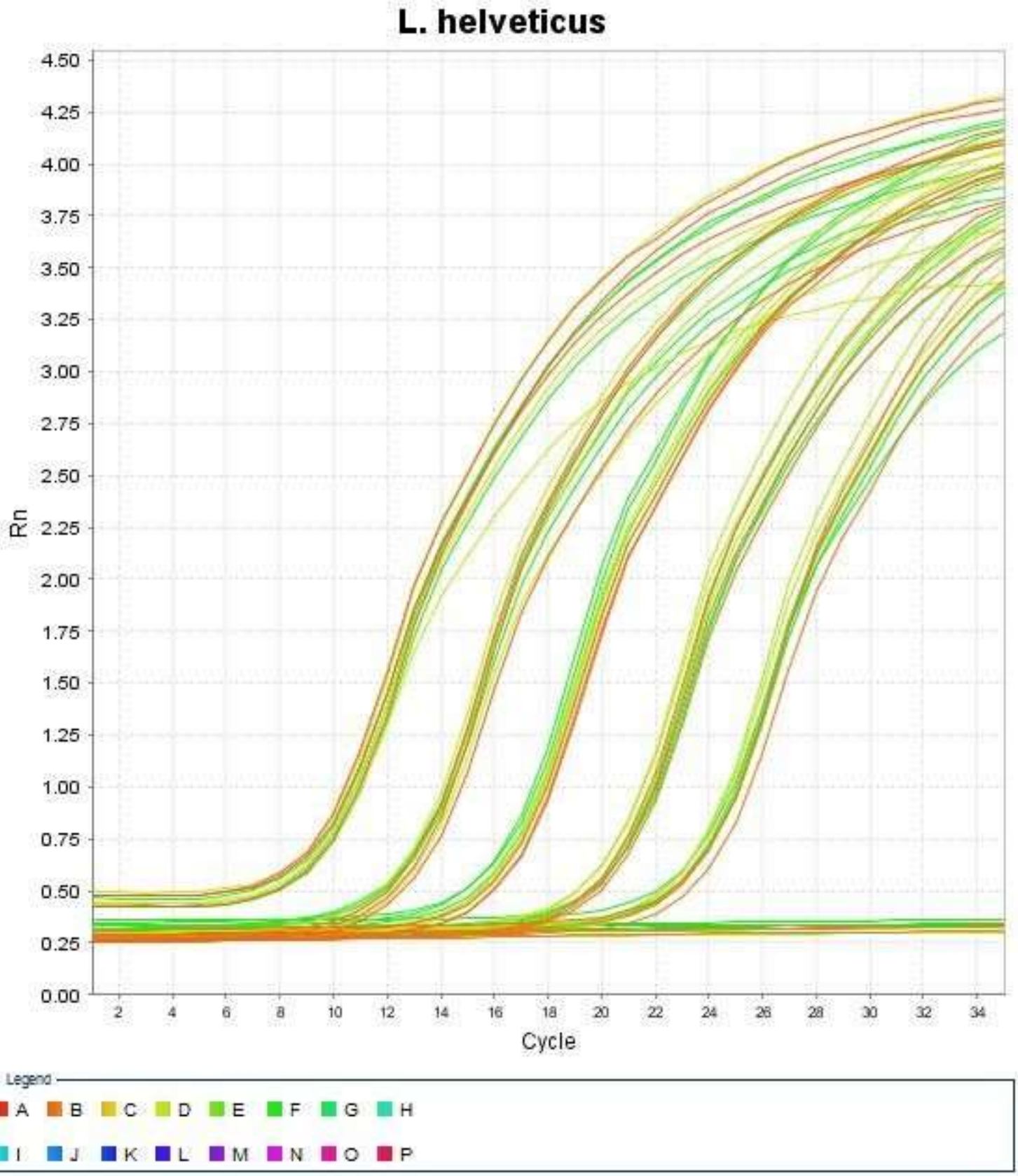
Plate Layout



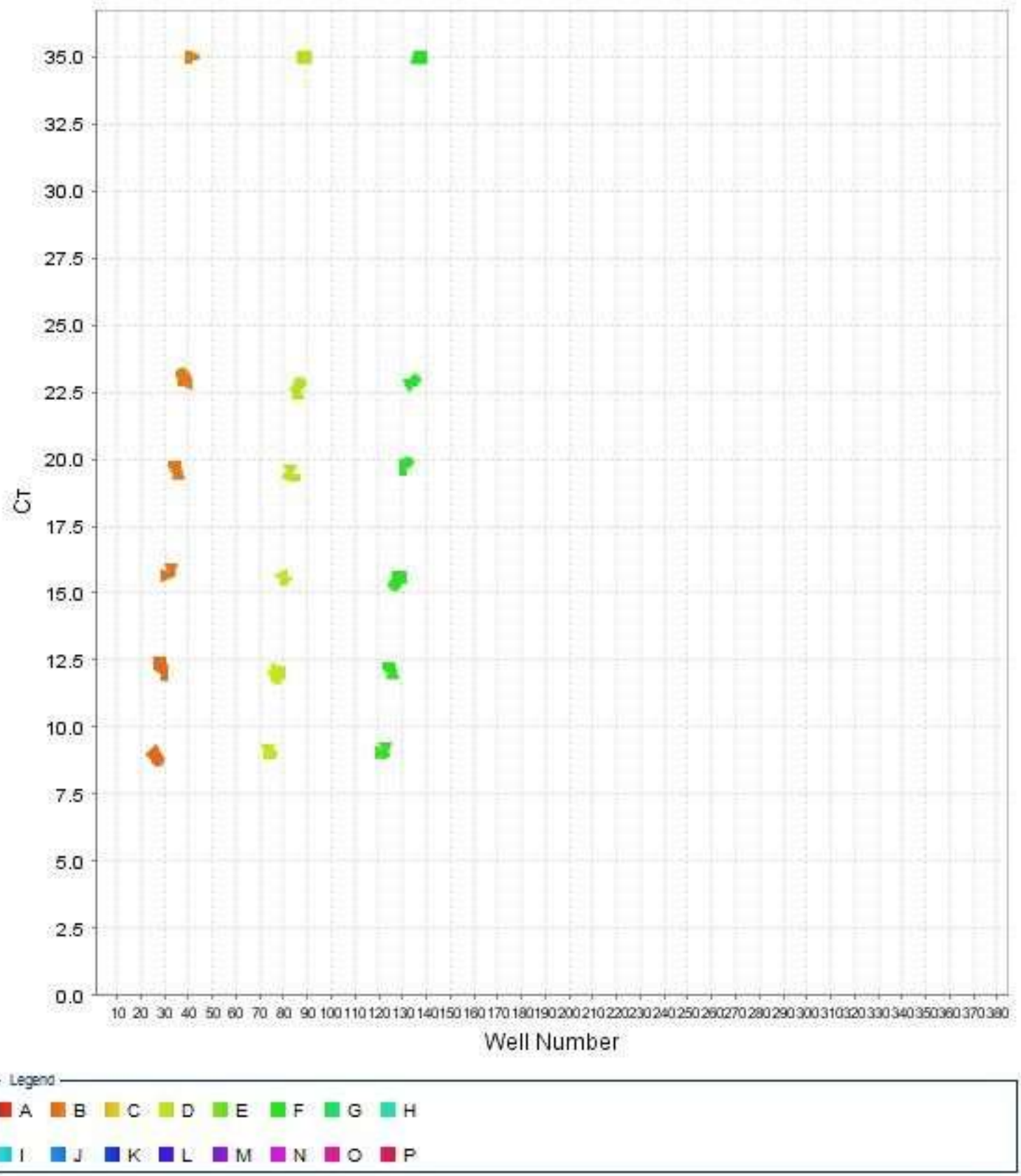
Amplification Plot (ΔRn vs. Cycle)



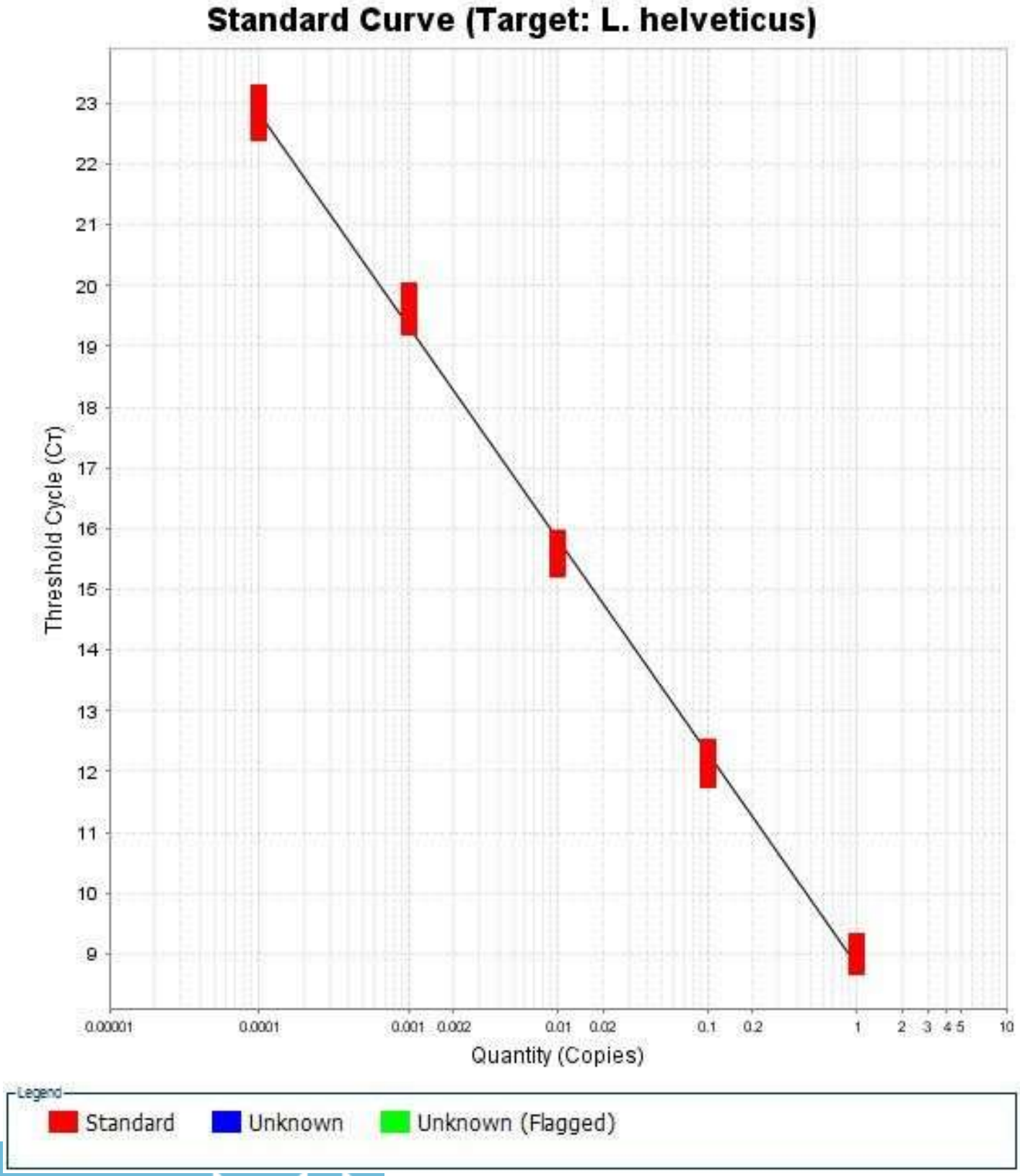
Amplification Plot (Rn vs. Cycle)



Amplification Plot (CT vs. Well)

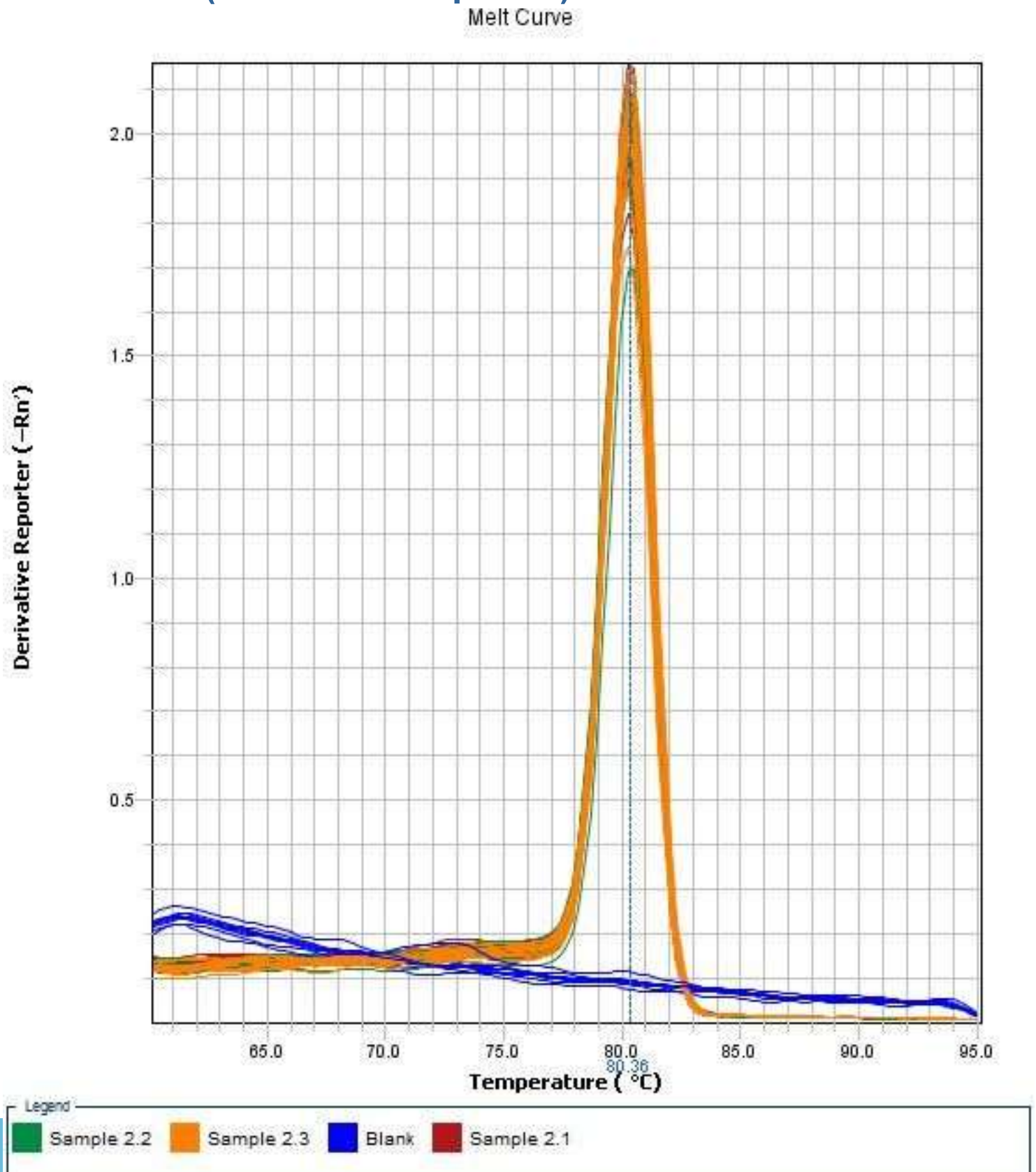


Standard Curves

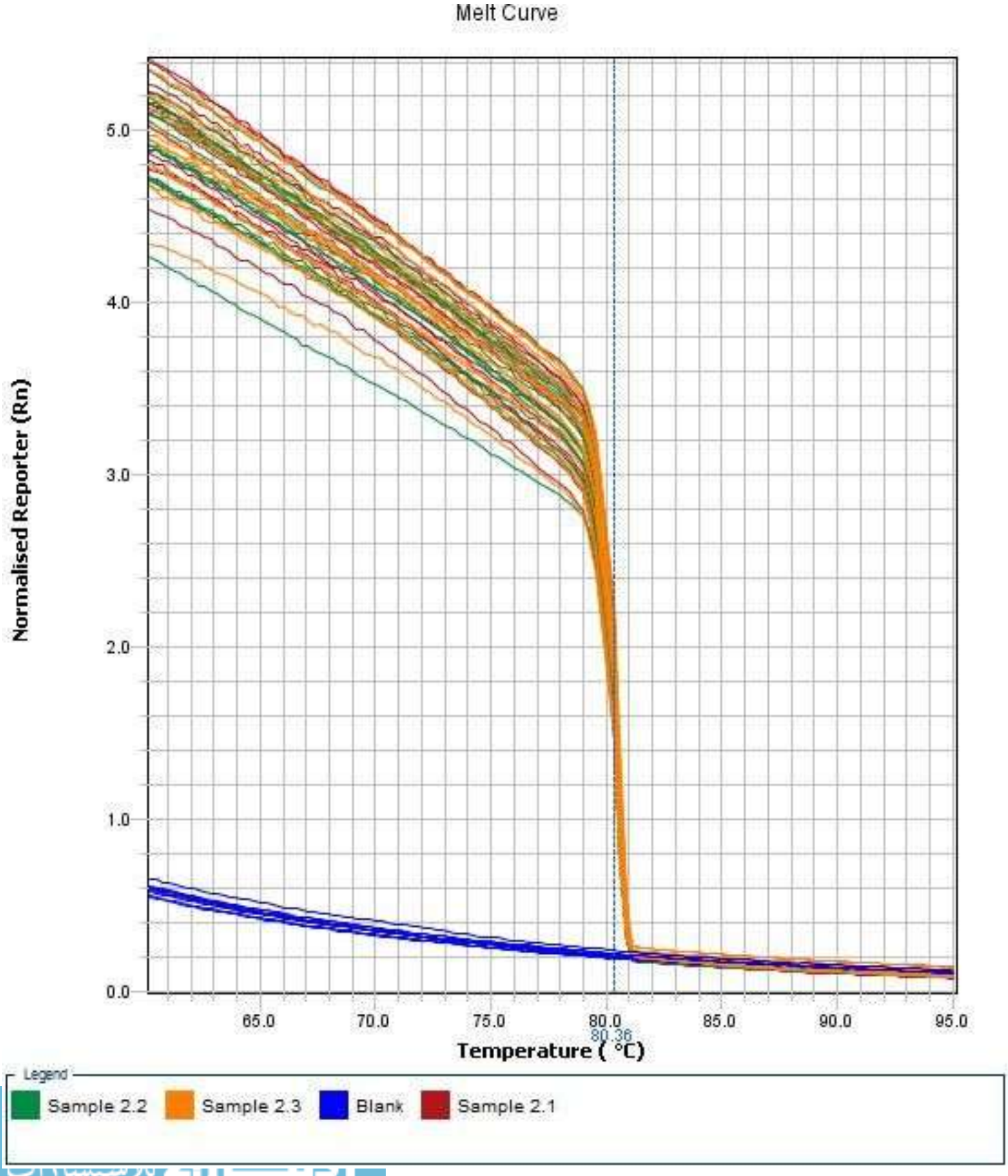


slope:-3.5078 Y-Intercept:8.8185 R^2:0.997 Eff%:92.788

Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	CT	CT Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	Sample 2.1	L. helveticus	S	1.000	8.994	8.974	0.166		80.361		
B3	Sample 2.1	L. helveticus	S	1.000	9.130	8.974	0.166		80.361		
B4	Sample 2.1	L. helveticus	S	1.000	8.799	8.974	0.166		80.361		
B5	Sample 2.1	L. helveticus	S	0.100	12.406	12.171	0.222		80.361		
B6	Sample 2.1	L. helveticus	S	0.100	12.142	12.171	0.222		80.361		
B7	Sample 2.1	L. helveticus	S	0.100	11.964	12.171	0.222		80.230		
B8	Sample 2.1	L. helveticus	S	0.010	15.655	15.736	0.093		80.230		
B9	Sample 2.1	L. helveticus	S	0.010	15.716	15.736	0.093		80.230		
B10	Sample 2.1	L. helveticus	S	0.010	15.837	15.736	0.093		80.361		
B11	Sample 2.1	L. helveticus	S	0.001	19.797	19.614	0.167		80.230		
B12	Sample 2.1	L. helveticus	S	0.001	19.575	19.614	0.167		80.230		
B13	Sample 2.1	L. helveticus	S	0.001	19.470	19.614	0.167		80.230		
B14	Sample 2.1	L. helveticus	S	0.000	23.177	22.998	0.168		80.230		
B15	Sample 2.1	L. helveticus	S	0.000	22.971	22.998	0.168		80.230		
B16	Sample 2.1	L. helveticus	S	0.000	22.845	22.998	0.168		80.230		
B17	Blank	L. helveticus	N		UND.				61.278		
B18	Blank	L. helveticus	N		UND.				61.409		
B19	Blank	L. helveticus	N		UND.				61.409		
D2	Sample 2.2	L. helveticus	S	1.000	9.151	9.034	0.114		80.361		
D3	Sample 2.2	L. helveticus	S	1.000	8.924	9.034	0.114		80.361		
D4	Sample 2.2	L. helveticus	S	1.000	9.027	9.034	0.114		80.361		
D5	Sample 2.2	L. helveticus	S	0.100	12.162	12.018	0.150		80.361		
D6	Sample 2.2	L. helveticus	S	0.100	11.862	12.018	0.150		80.230		
D7	Sample 2.2	L. helveticus	S	0.100	12.031	12.018	0.150		80.361		
D8	Sample 2.2	L. helveticus	S	0.010	15.674	15.571	0.089		80.230		
D9	Sample 2.2	L. helveticus	S	0.010	15.524	15.571	0.089		80.230		
D10	Sample 2.2	L. helveticus	S	0.010	15.515	15.571	0.089		80.230		
D11	Sample 2.2	L. helveticus	S	0.001	19.377	19.416	0.136		80.230		
D12	Sample 2.2	L. helveticus	S	0.001	19.568	19.416	0.136		80.230		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	CT	CT Mean		Qty Mean	Qty SD	Tm1	Tm2	Tm3
D13	Sample 2.2	L. helveticus	S	0.001	19.304	19.416	0.136			80.230		
D14	Sample 2.2	L. helveticus	S	0.000	22.578	22.631	0.161			80.230		
D15	Sample 2.2	L. helveticus	S	0.000	22.504	22.631	0.161			80.230		
D16	Sample 2.2	L. helveticus	S	0.000	22.812	22.631	0.161			80.230		
D17	Blank	L. helveticus	N		UND.					61.409		
D18	Blank	L. helveticus	N		UND.					61.278		
D19	Blank	L. helveticus	N		UND.					61.146		
F2	Sample 2.3	L. helveticus	S	1.000	9.033	9.076	0.120			80.361		
F3	Sample 2.3	L. helveticus	S	1.000	8.983	9.076	0.120			80.361		
F4	Sample 2.3	L. helveticus	S	1.000	9.211	9.076	0.120			80.361		
F5	Sample 2.3	L. helveticus	S	0.100	12.319	12.157	0.145			80.361		
F6	Sample 2.3	L. helveticus	S	0.100	12.114	12.157	0.145			80.361		
F7	Sample 2.3	L. helveticus	S	0.100	12.038	12.157	0.145			80.361		
F8	Sample 2.3	L. helveticus	S	0.010	15.334	15.496	0.140			80.230		
F9	Sample 2.3	L. helveticus	S	0.010	15.568	15.496	0.140			80.230		
F10	Sample 2.3	L. helveticus	S	0.010	15.585	15.496	0.140			80.361		
F11	Sample 2.3	L. helveticus	S	0.001	19.646	19.782	0.143			80.230		
F12	Sample 2.3	L. helveticus	S	0.001	19.770	19.782	0.143			80.230		
F13	Sample 2.3	L. helveticus	S	0.001	19.930	19.782	0.143			80.230		
F14	Sample 2.3	L. helveticus	S	0.000	22.715	22.839	0.121			80.230		
F15	Sample 2.3	L. helveticus	S	0.000	22.844	22.839	0.121			80.230		
F16	Sample 2.3	L. helveticus	S	0.000	22.956	22.839	0.121			80.230		
F17	Blank	L. helveticus	N		UND.					61.146		
F18	Blank	L. helveticus	N		UND.					61.541		
F19	Blank	L. helveticus	N		UND.					61.541		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	54	Targets Used	1
Well Setup	54	Flagged Wells	0	Samples Used	4

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	0	
HIGHSD	High standard deviation in replicate group	0	
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	0	
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp**	m1,x1 m4,x4
Cover Temperature	105.0	**Filter Set**	
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	35	1	false
Hold Stage	1	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	600	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	94.0	45	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	58.0	45	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	72.0	60	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	72.0	420	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_	95.0	15	0.0	0

	PER_SECOND

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	60.0	60	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_PER_SECOND	95.0	15	0.0	0

Experiment Results Report

2020-01-06 165629

Experiment Summary

Experiment Name: 2020-01-06 165629
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_L_delbruki_L_paracasei_standardcurve_20200106.eds
Run Started: 01-06-2020 22:08:39 PST
Run Finished: 01-07-2020 00:30:04 PST
Run Duration: 141 minutes 25 seconds
Date Modified: 01-06-2020 19:28:30 PST
Date Created: 01-06-2020 16:56:29 PST
User:
Number of Wells Used: 108
Number of Wells with Results: 54
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 2
Comments:
Quantification Cycle Setting: CT

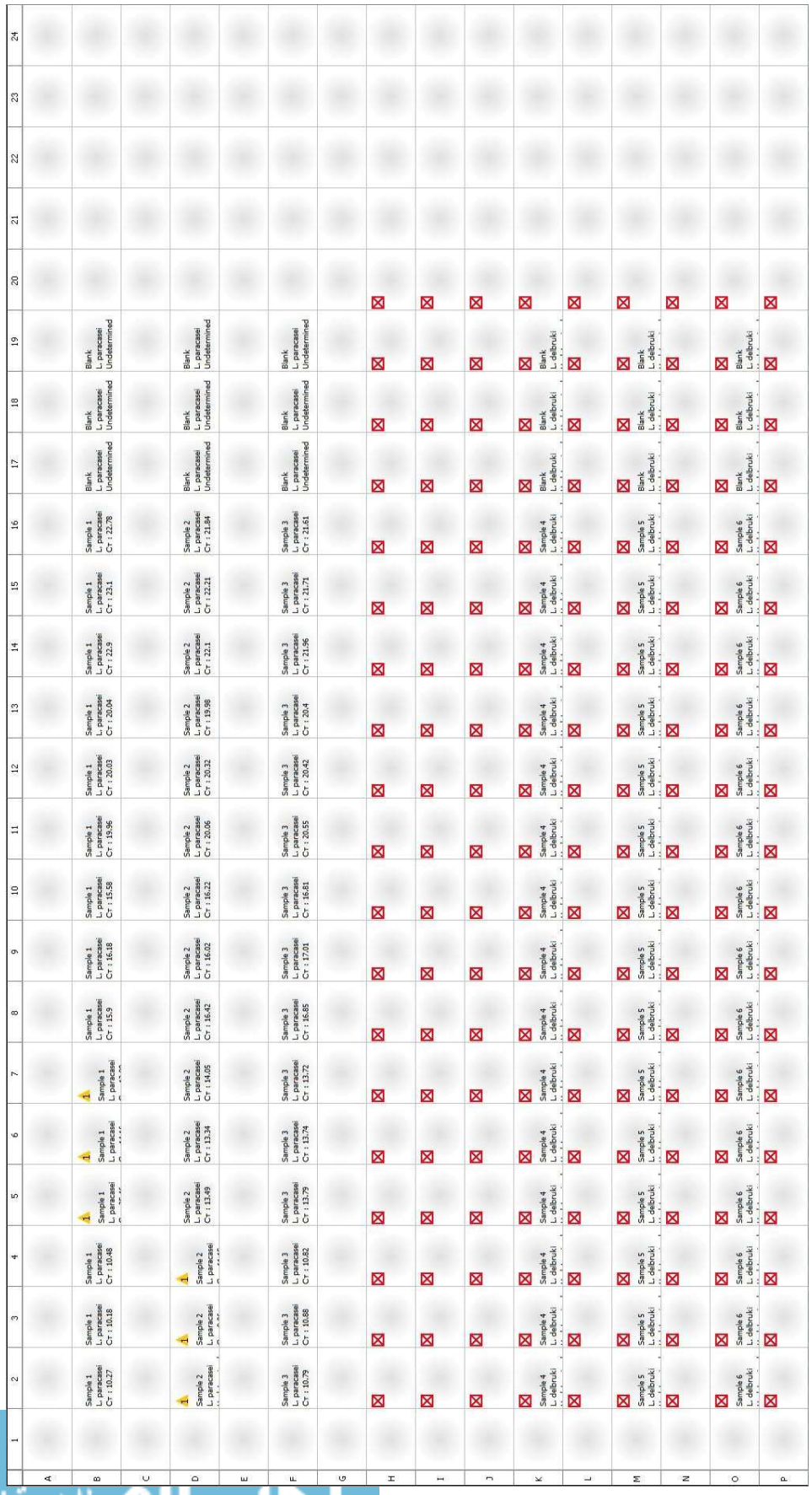
DATA FOR L. DELBRUECKII NOT USED FROM THIS FILE

Reagent Information

Results Summary

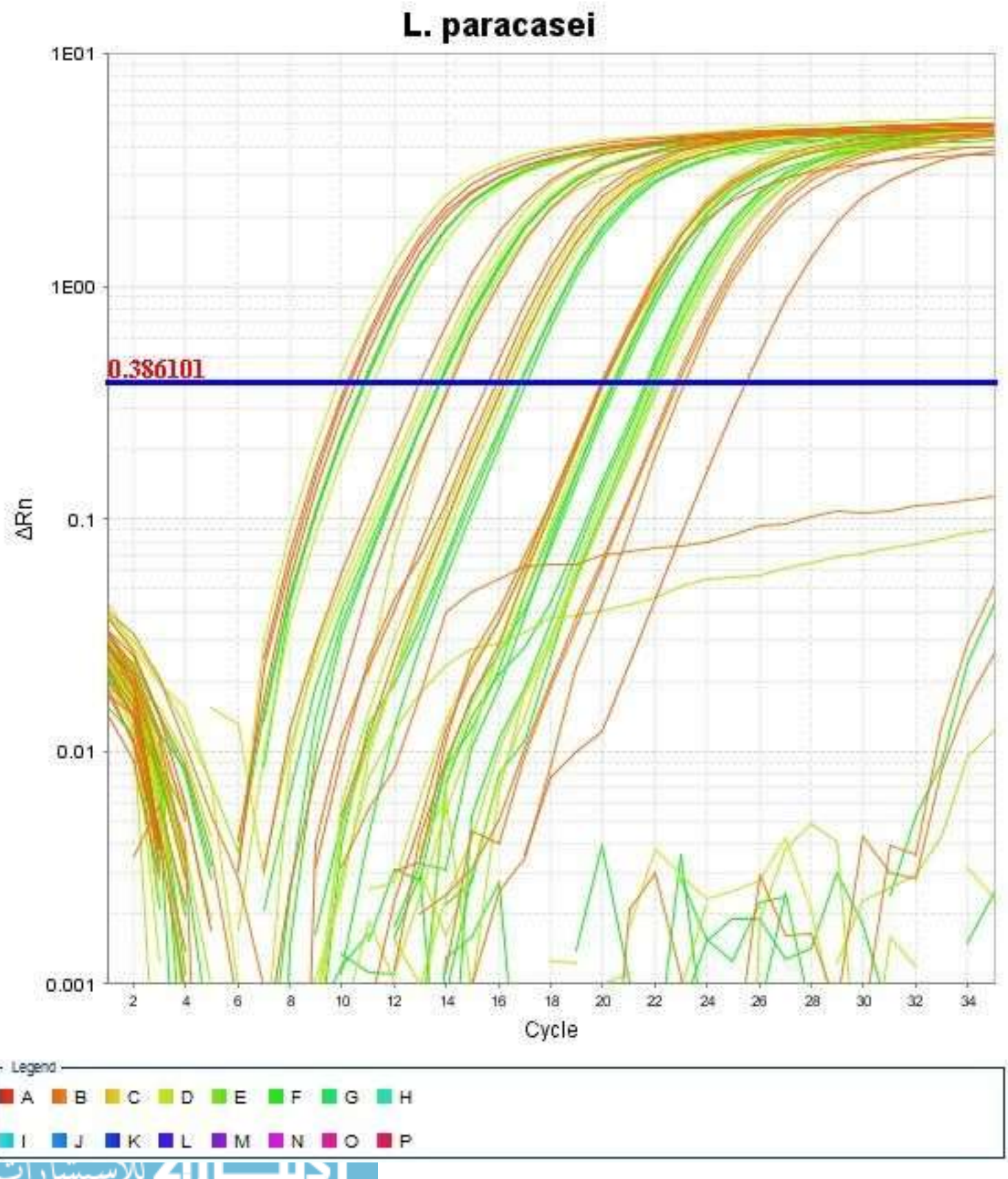
Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
Blank	L. delbruki				
Sample 4	L. delbruki				
Sample 5	L. delbruki				
Sample 6	L. delbruki				
Blank	L. paracasei				
Sample 1	L. paracasei				
Sample 2	L. paracasei				
Sample 3	L. paracasei				

DATA FOR L. DELBRUECKII NOT USED FROM THIS FILE

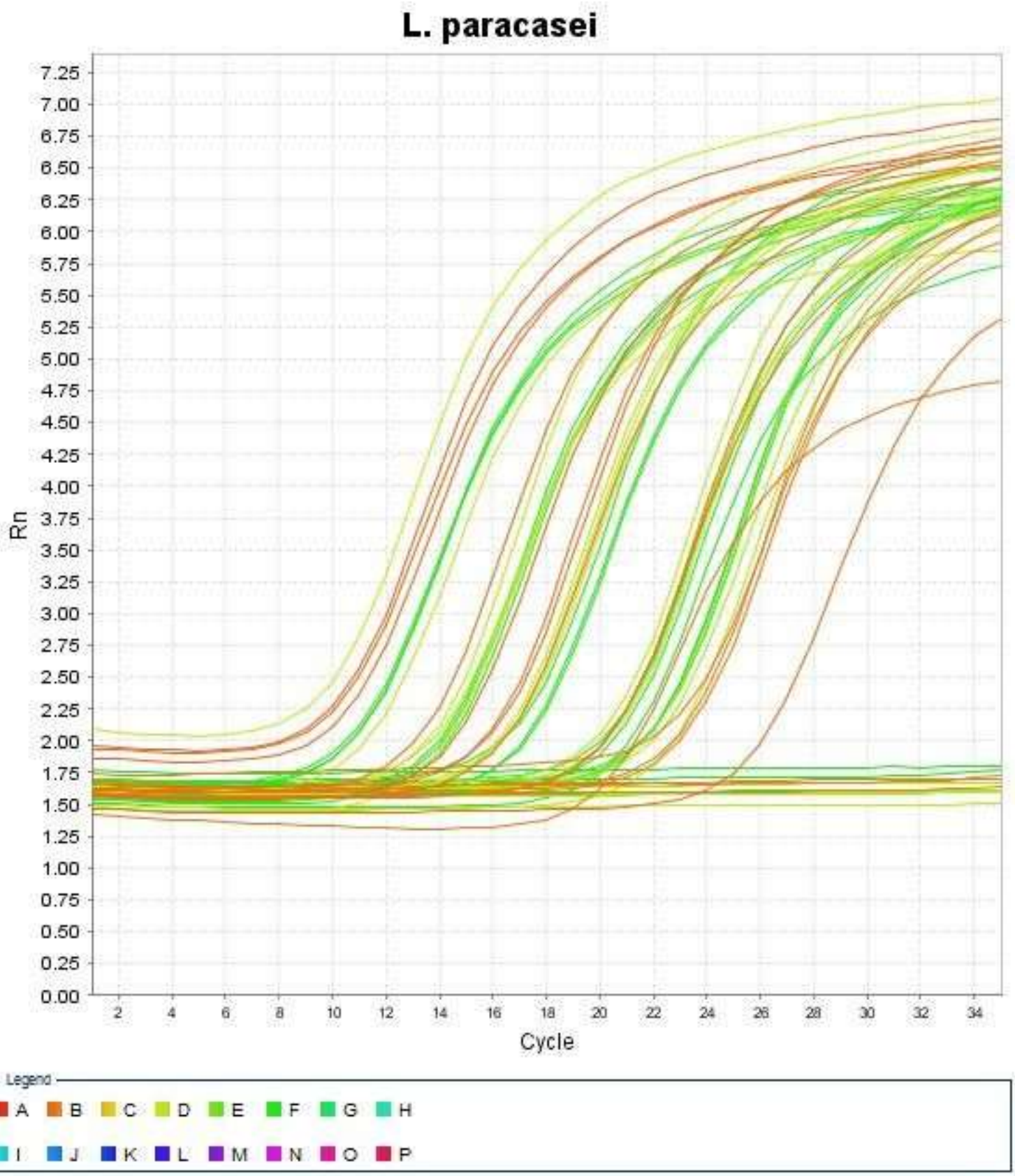


DATA FOR L. DELBRUECKII NOT USED FROM THIS FILE

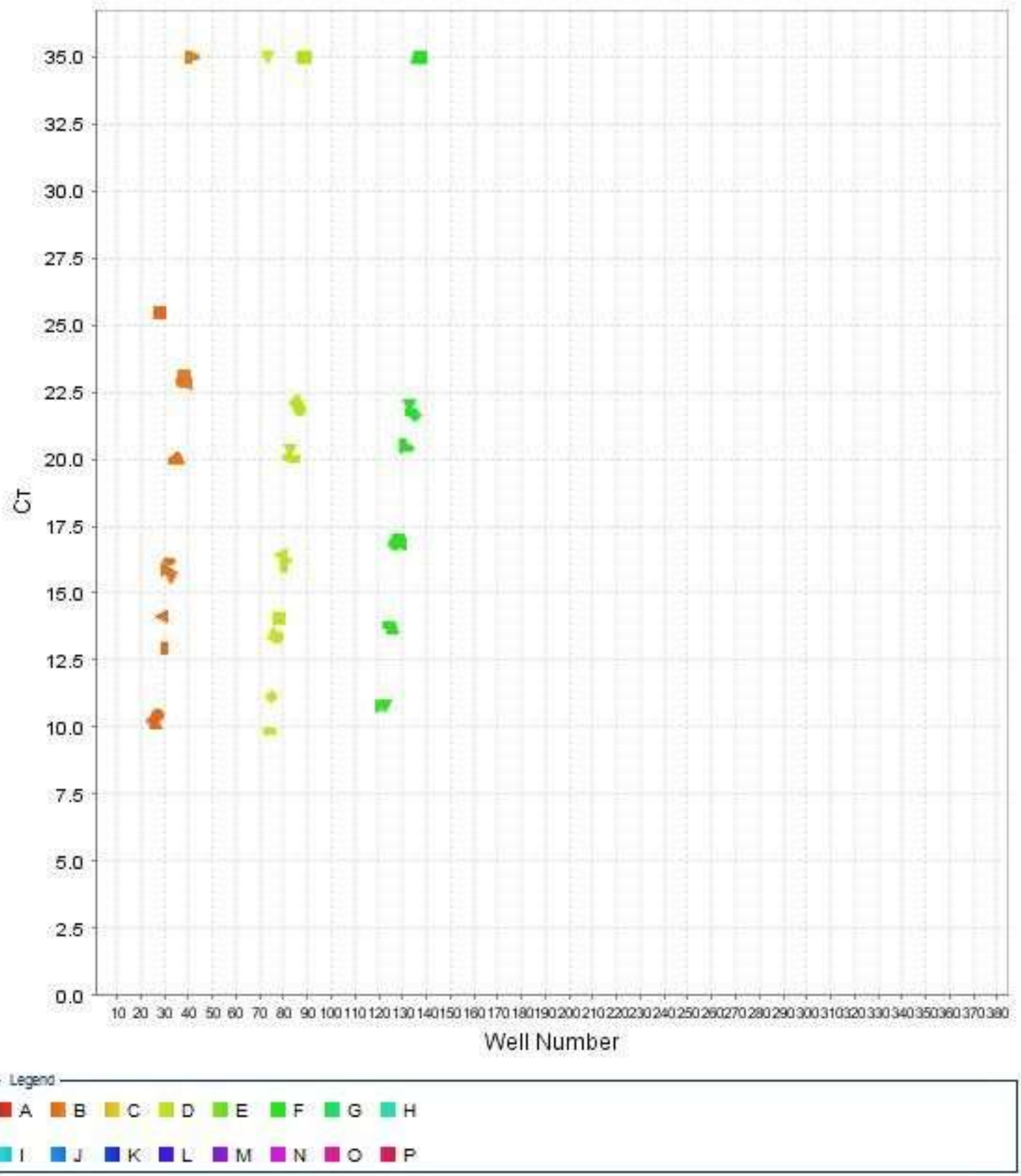
Amplification Plot (ΔRn vs. Cycle)



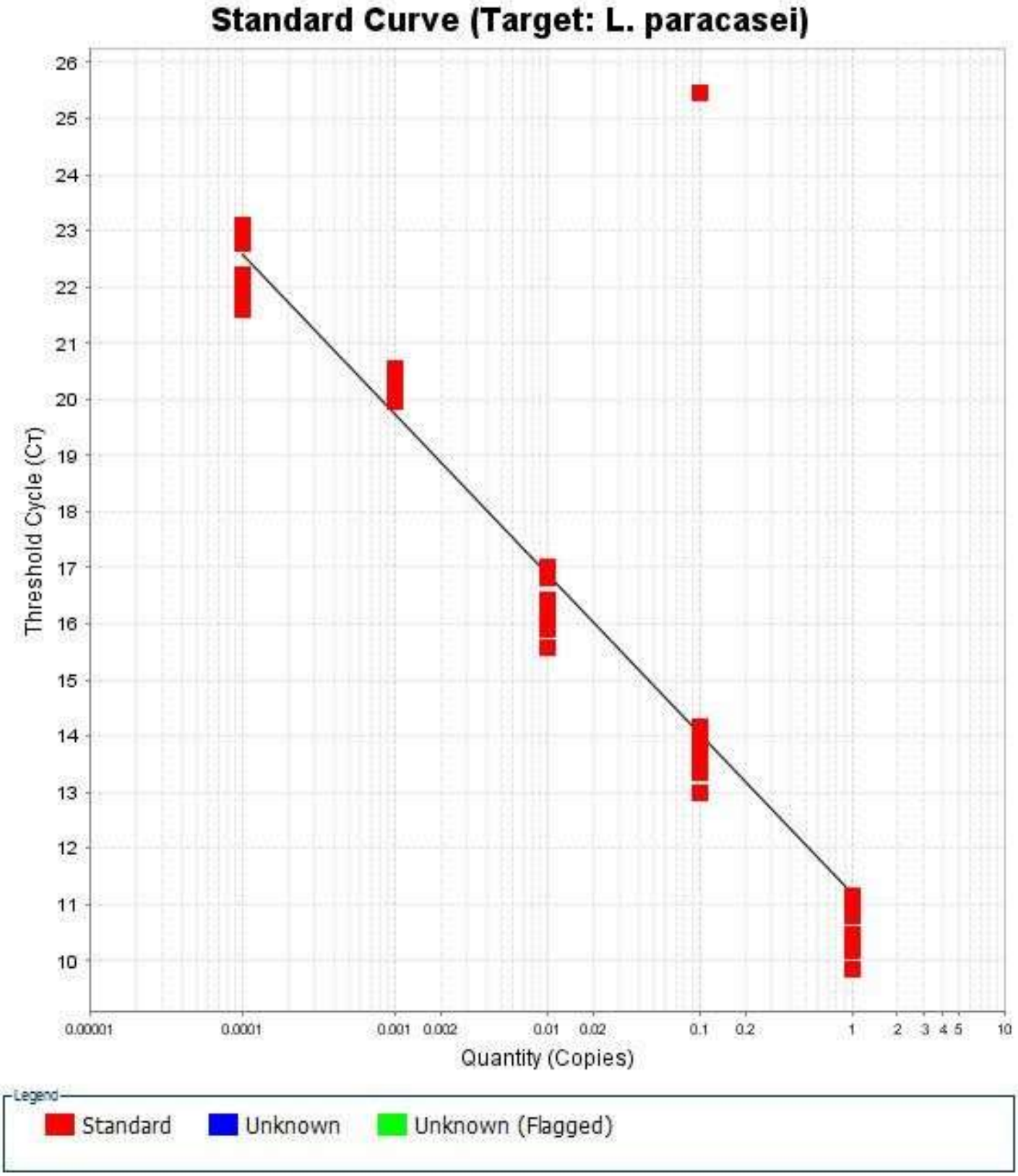
Amplification Plot (Rn vs. Cycle)



Amplification Plot (CT vs. Well)

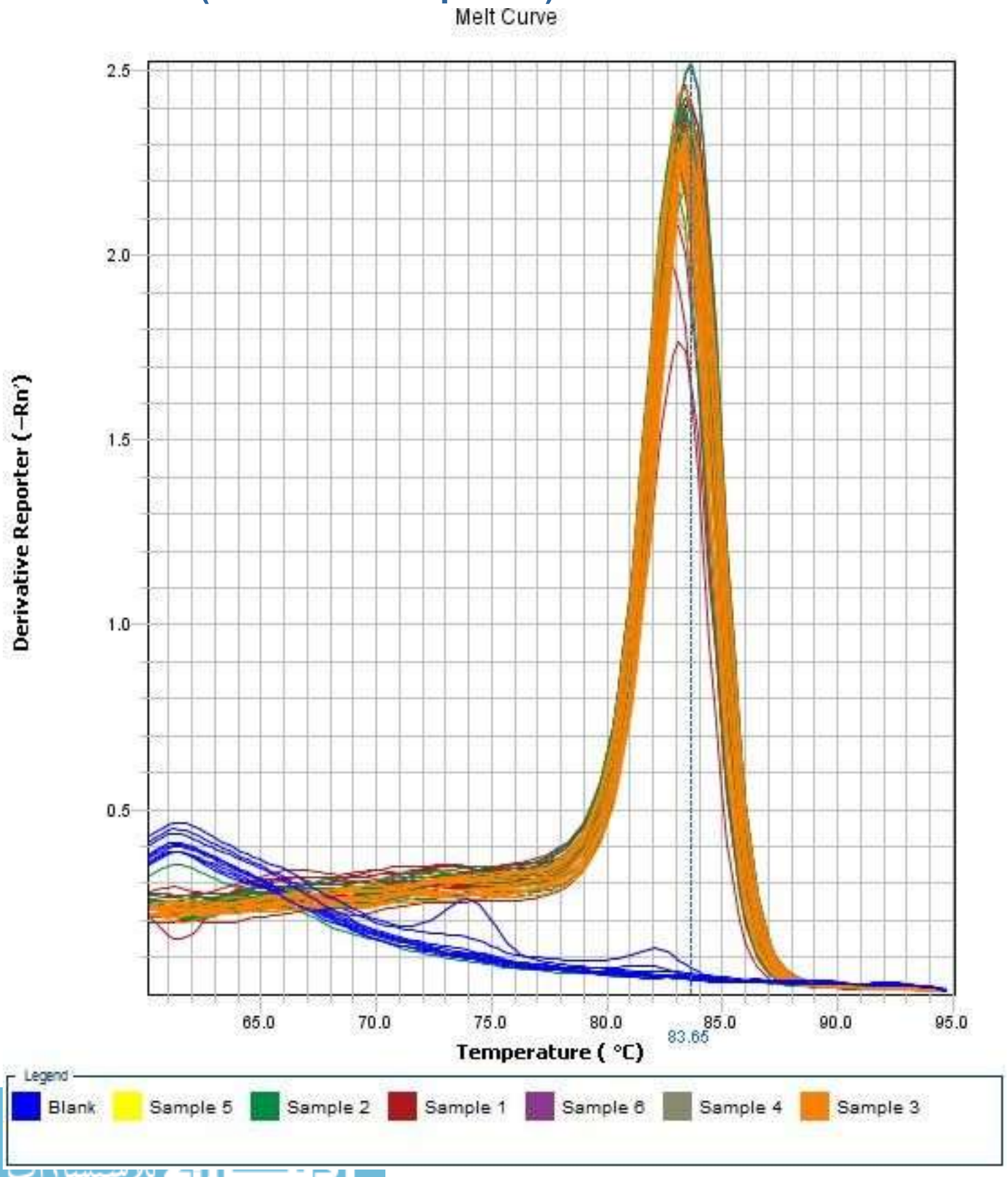


Standard Curves

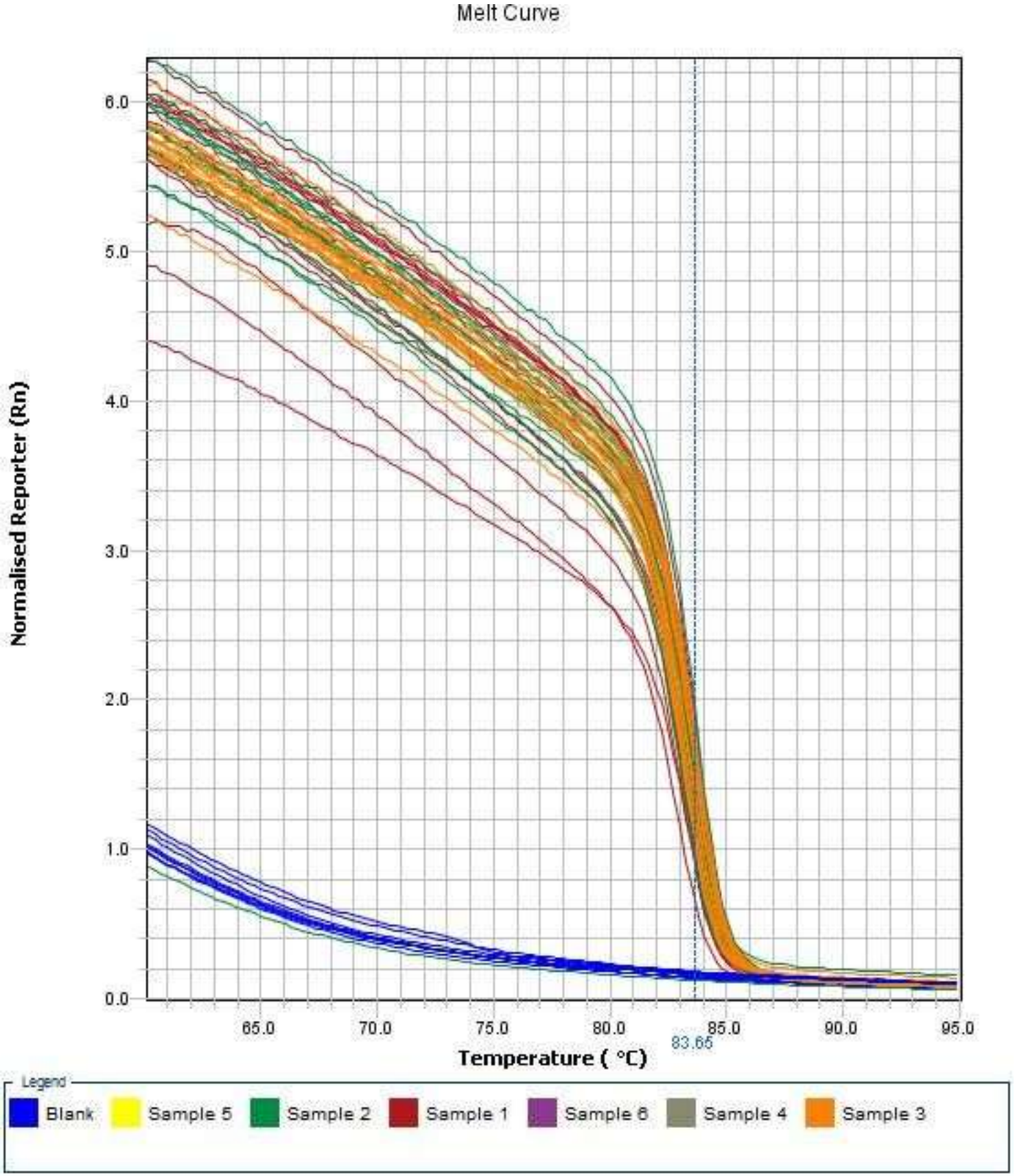


slope:-2.8472 Y-Intercept:11.1791 R²:0.825 Eff%:124.503

Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	Cт	Cт Mean		Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	Sample 1	L. paracasei	S	1.000	10.271	10.309	0.156			83.651		
B3	Sample 1	L. paracasei	S	1.000	10.176	10.309	0.156			83.651		
B4	Sample 1	L. paracasei	S	1.000	10.480	10.309	0.156			83.520		
B5	Sample 1	L. paracasei	S	0.100	25.461	17.532	6.892			82.861		
B6	Sample 1	L. paracasei	S	0.100	14.158	17.532	6.892			83.388		
B7	Sample 1	L. paracasei	S	0.100	12.978	17.532	6.892			83.520		
B8	Sample 1	L. paracasei	S	0.010	15.899	15.887	0.301			83.388		
B9	Sample 1	L. paracasei	S	0.010	16.181	15.887	0.301			83.388		
B10	Sample 1	L. paracasei	S	0.010	15.580	15.887	0.301			83.388		
B11	Sample 1	L. paracasei	S	0.001	19.959	20.008	0.043			83.124		
B12	Sample 1	L. paracasei	S	0.001	20.029	20.008	0.043			83.124		
B13	Sample 1	L. paracasei	S	0.001	20.036	20.008	0.043			83.256		
B14	Sample 1	L. paracasei	S	0.000	22.903	22.930	0.160			82.993		
B15	Sample 1	L. paracasei	S	0.000	23.102	22.930	0.160			82.993		
B16	Sample 1	L. paracasei	S	0.000	22.784	22.930	0.160			82.993		
B17	Blank	L. paracasei	N		UND.					61.383		
B18	Blank	L. paracasei	N		UND.					61.252	81.675	
B19	Blank	L. paracasei	N		UND.					82.070	61.383	
D2	Sample 2	L. paracasei	S	1.000	UND.	10.504	0.910			61.383		
D3	Sample 2	L. paracasei	S	1.000	9.861	10.504	0.910			83.651		
D4	Sample 2	L. paracasei	S	1.000	11.147	10.504	0.910			83.520		
D5	Sample 2	L. paracasei	S	0.100	13.487	13.627	0.377			83.388		
D6	Sample 2	L. paracasei	S	0.100	13.340	13.627	0.377			83.388		
D7	Sample 2	L. paracasei	S	0.100	14.054	13.627	0.377			83.388		
D8	Sample 2	L. paracasei	S	0.010	16.419	16.222	0.198			83.256		
D9	Sample 2	L. paracasei	S	0.010	16.024	16.222	0.198			83.388		
D10	Sample 2	L. paracasei	S	0.010	16.225	16.222	0.198			83.388		
D11	Sample 2	L. paracasei	S	0.001	20.061	20.120	0.175			83.124		
D12	Sample 2	L. paracasei	S	0.001	20.317	20.120	0.175			83.124		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	CT	CT Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
D13	Sample 2	L. paracasei	S	0.001	19.983	20.120	0.175		83.124		
D14	Sample 2	L. paracasei	S	0.000	22.096	22.050	0.191		82.993		
D15	Sample 2	L. paracasei	S	0.000	22.213	22.050	0.191		82.993		
D16	Sample 2	L. paracasei	S	0.000	21.840	22.050	0.191		82.993		
D17	Blank	L. paracasei	N		UND.				61.383		
D18	Blank	L. paracasei	N		UND.				61.252		
D19	Blank	L. paracasei	N		UND.				61.252		
F2	Sample 3	L. paracasei	S	1.000	10.788	10.829	0.049		83.651		
F3	Sample 3	L. paracasei	S	1.000	10.884	10.829	0.049		83.651		
F4	Sample 3	L. paracasei	S	1.000	10.817	10.829	0.049		83.651		
F5	Sample 3	L. paracasei	S	0.100	13.794	13.752	0.038		83.520		
F6	Sample 3	L. paracasei	S	0.100	13.744	13.752	0.038		83.388		
F7	Sample 3	L. paracasei	S	0.100	13.720	13.752	0.038		83.388		
F8	Sample 3	L. paracasei	S	0.010	16.853	16.890	0.106		83.256		
F9	Sample 3	L. paracasei	S	0.010	17.010	16.890	0.106		83.256		
F10	Sample 3	L. paracasei	S	0.010	16.808	16.890	0.106		83.388		
F11	Sample 3	L. paracasei	S	0.001	20.554	20.459	0.083		83.124		
F12	Sample 3	L. paracasei	S	0.001	20.421	20.459	0.083		83.124		
F13	Sample 3	L. paracasei	S	0.001	20.401	20.459	0.083		83.124		
F14	Sample 3	L. paracasei	S	0.000	21.959	21.759	0.181		82.993		
F15	Sample 3	L. paracasei	S	0.000	21.710	21.759	0.181		82.993		
F16	Sample 3	L. paracasei	S	0.000	21.607	21.759	0.181		82.993		
F17	Blank	L. paracasei	N		UND.				73.901	61.383	
F18	Blank	L. paracasei	N		UND.				61.383		
F19	Blank	L. paracasei	N		UND.				61.252	89.317	

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	54	Targets Used	2
Well Setup	108	Flagged Wells	6	Samples Used	7

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	1	D2
HIGHSD	High standard deviation in replicate group	5	B5, B6, B7, D3, D4
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	0	
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp**	m1,x1 m4,x4
Cover Temperature	105.0	**Filter Set**	
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	35	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	600	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	20	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	55.0	120	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	15	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	60.0	60	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_	95.0	15	0.0	0

PER_SECOND

Experiment Results Report

2020-01-08 095816

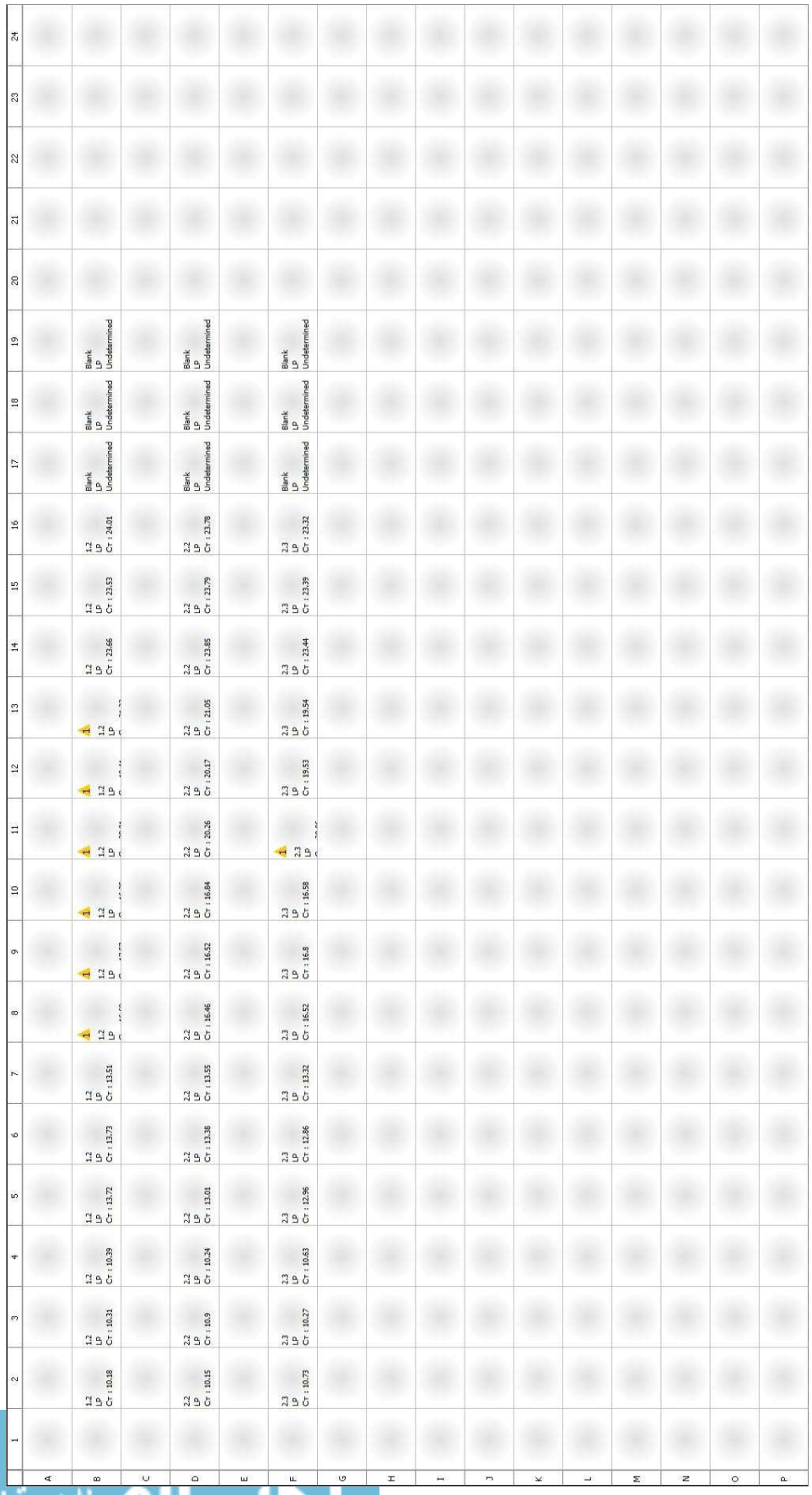
Experiment Summary

Experiment Name: 2020-01-08 095816
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_L_plantarum_20200108_standardcurve.eds
Run Started: 01-08-2020 15:31:56 PST
Run Finished: 01-08-2020 18:09:53 PST
Run Duration: 157 minutes 56 seconds
Date Modified: 01-08-2020 13:08:14 PST
Date Created: 01-08-2020 09:58:16 PST
User:
Number of Wells Used: 54
Number of Wells with Results: 54
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 3
Comments:
Quantification Cycle Setting: CT

Reagent Information

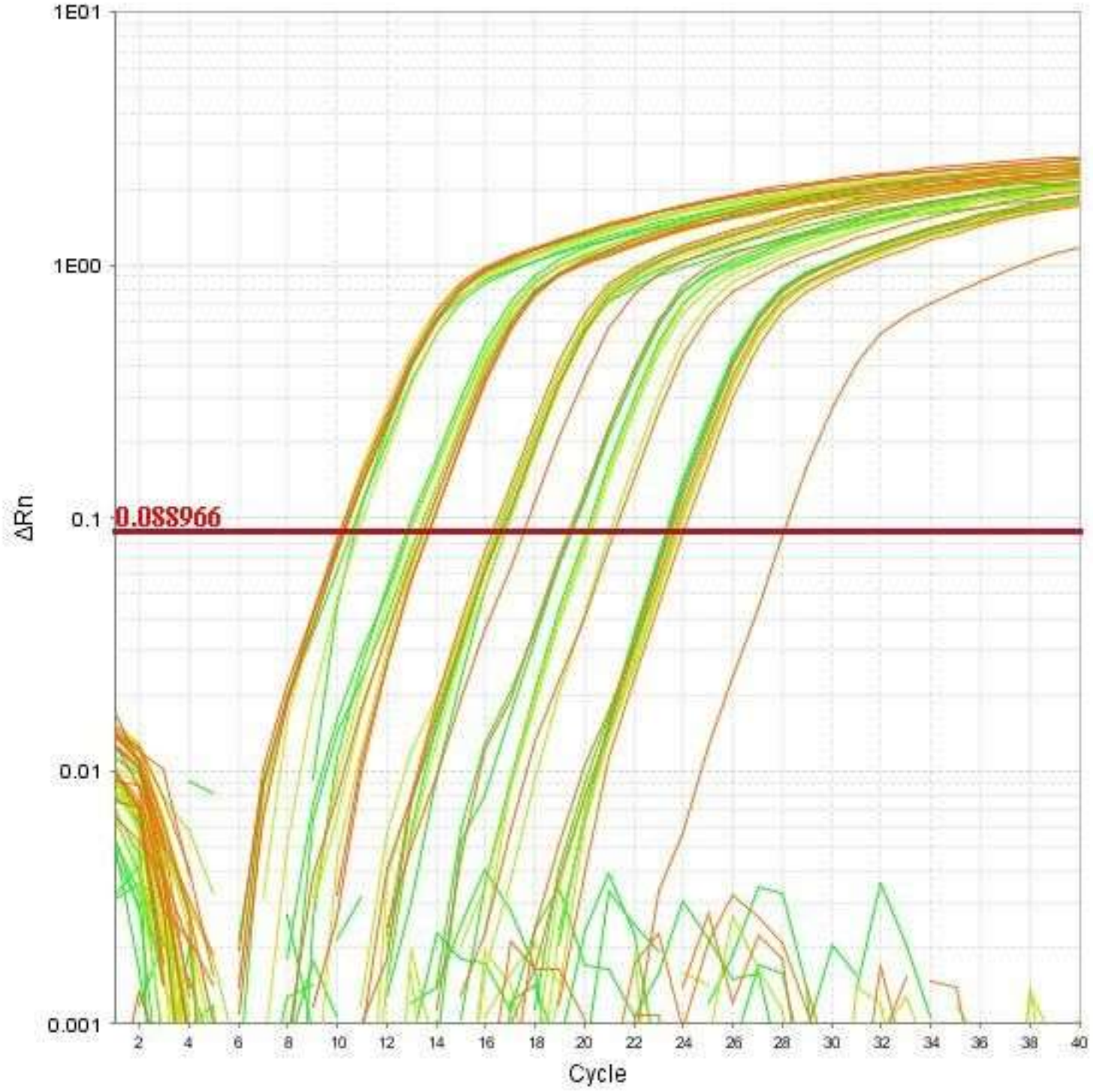
Results Summary

Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
1.2	L. Plantarum				
2.2	L. Plantarum				
2.3	L. Plantarum				
Blank	L. Plantarum				



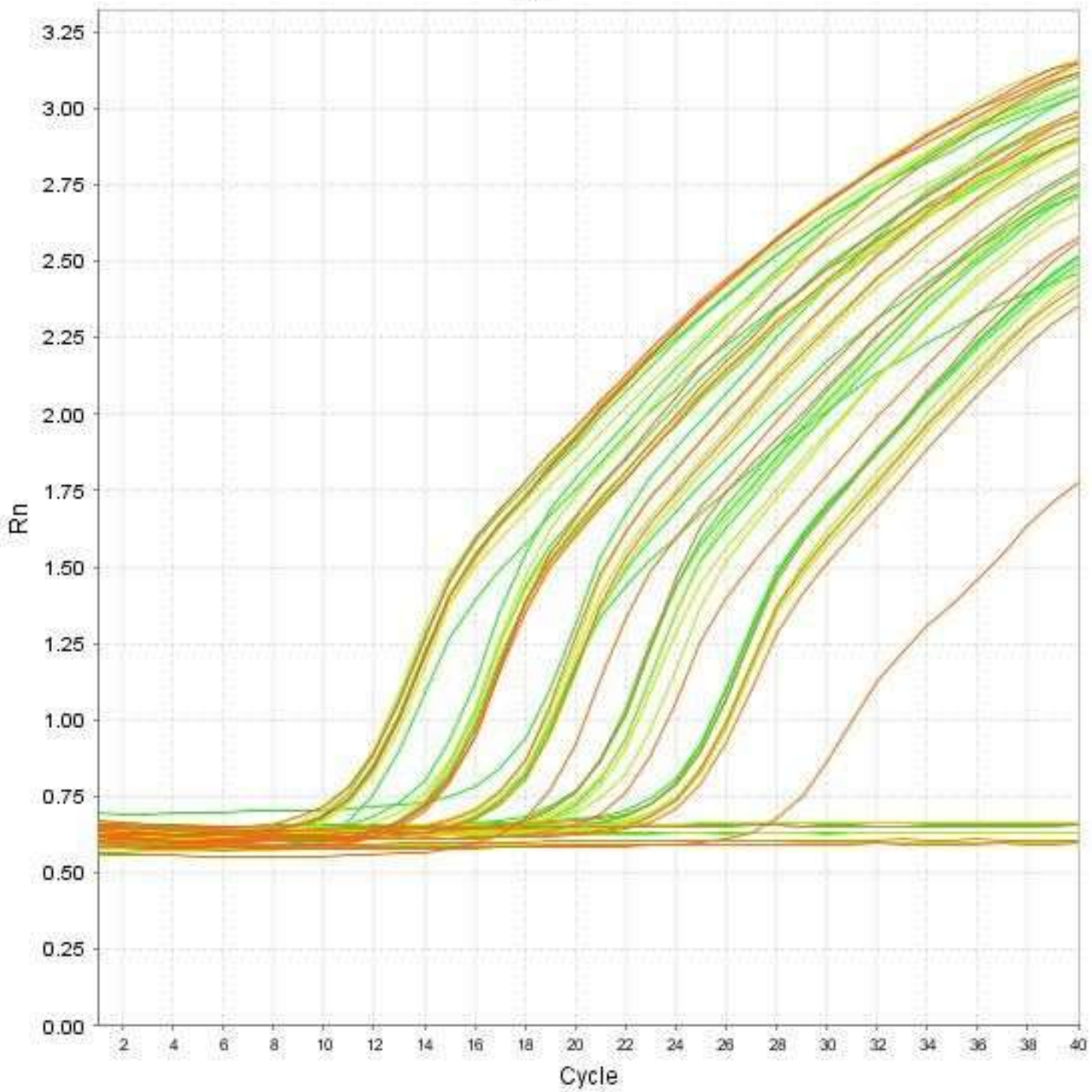
Amplification Plot (ΔRn vs. Cycle)

L. Plantarum

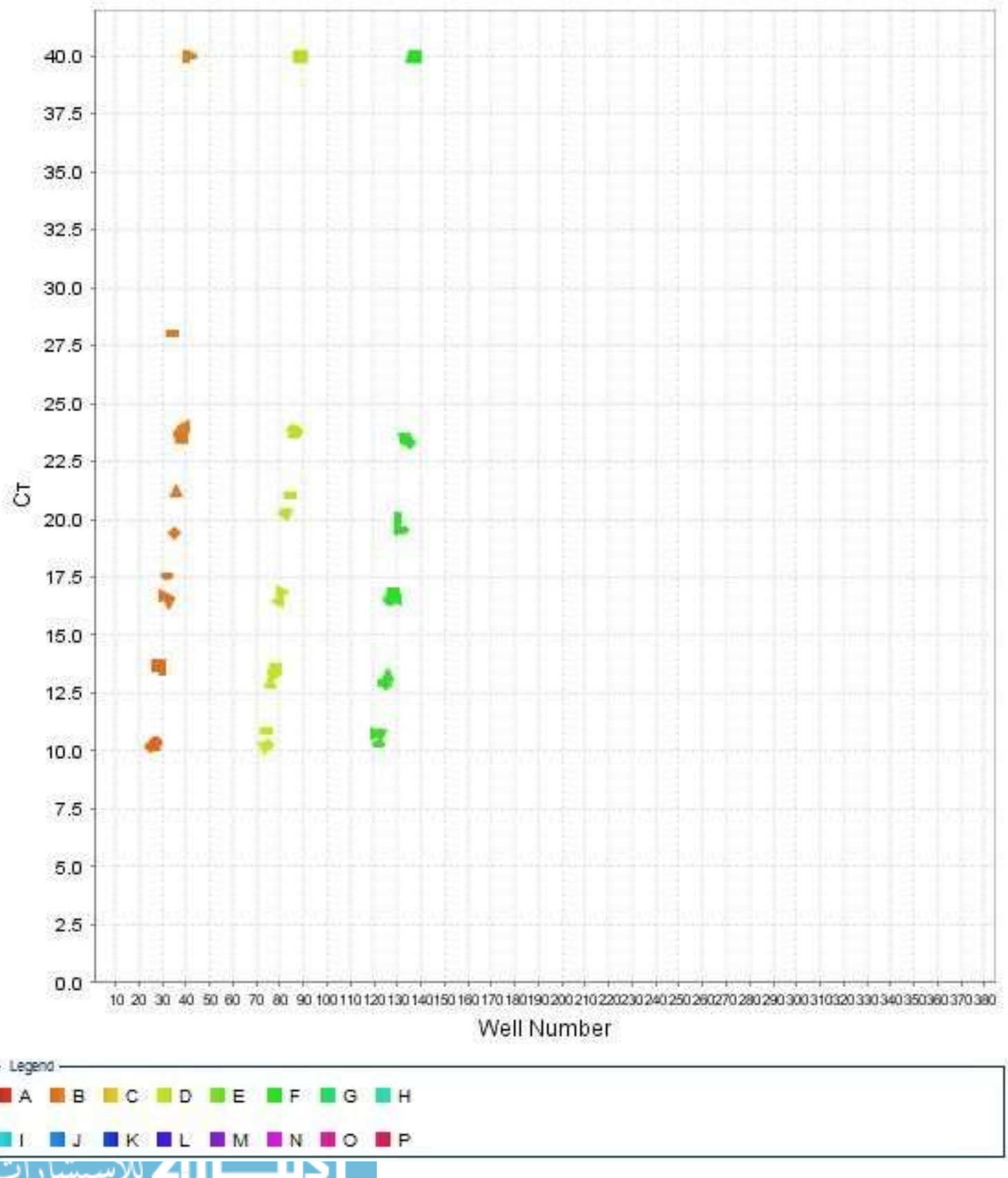


Amplification Plot (Rn vs. Cycle)

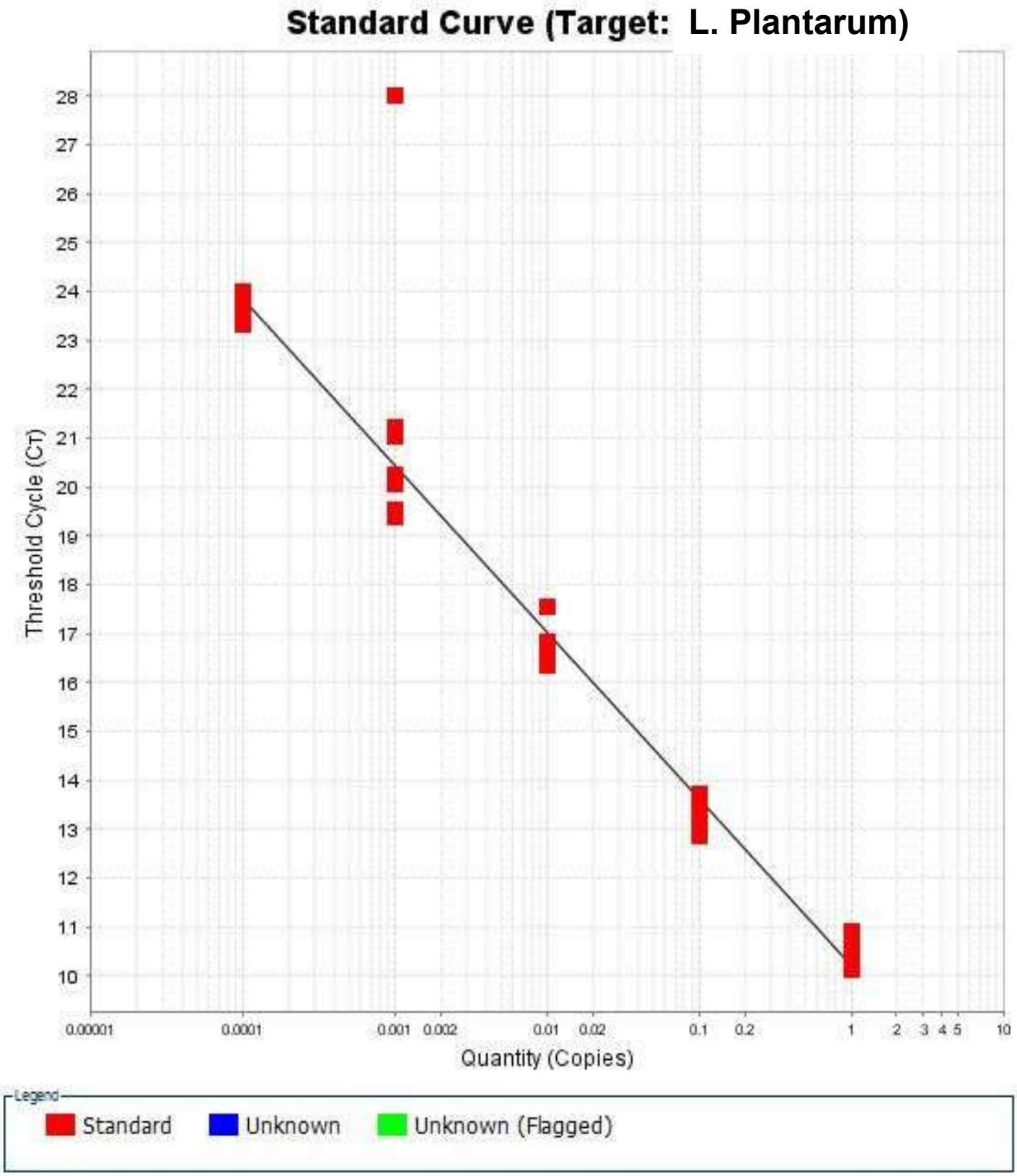
L. Plantarum



Amplification Plot (CT vs. Well)

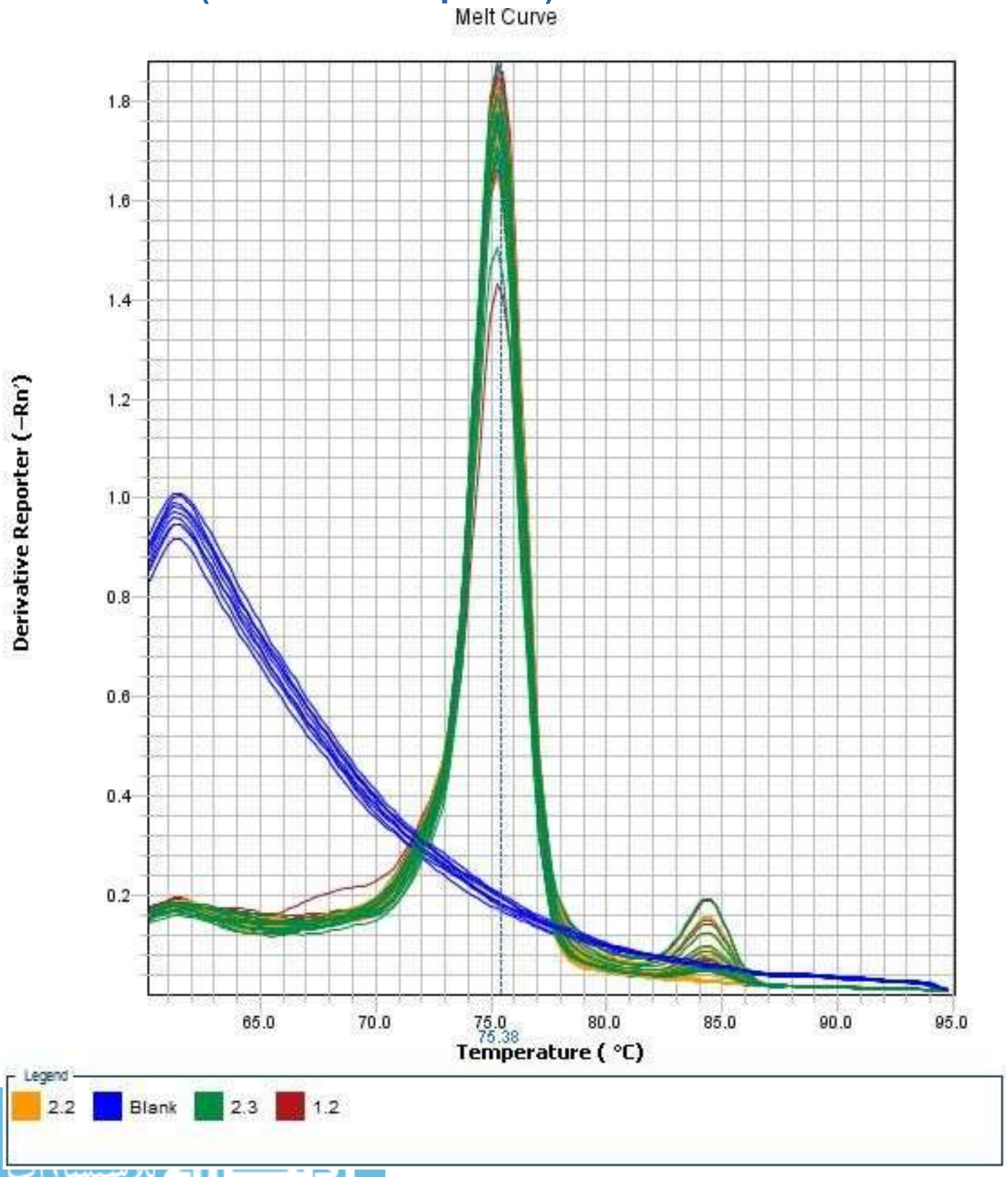


Standard Curves

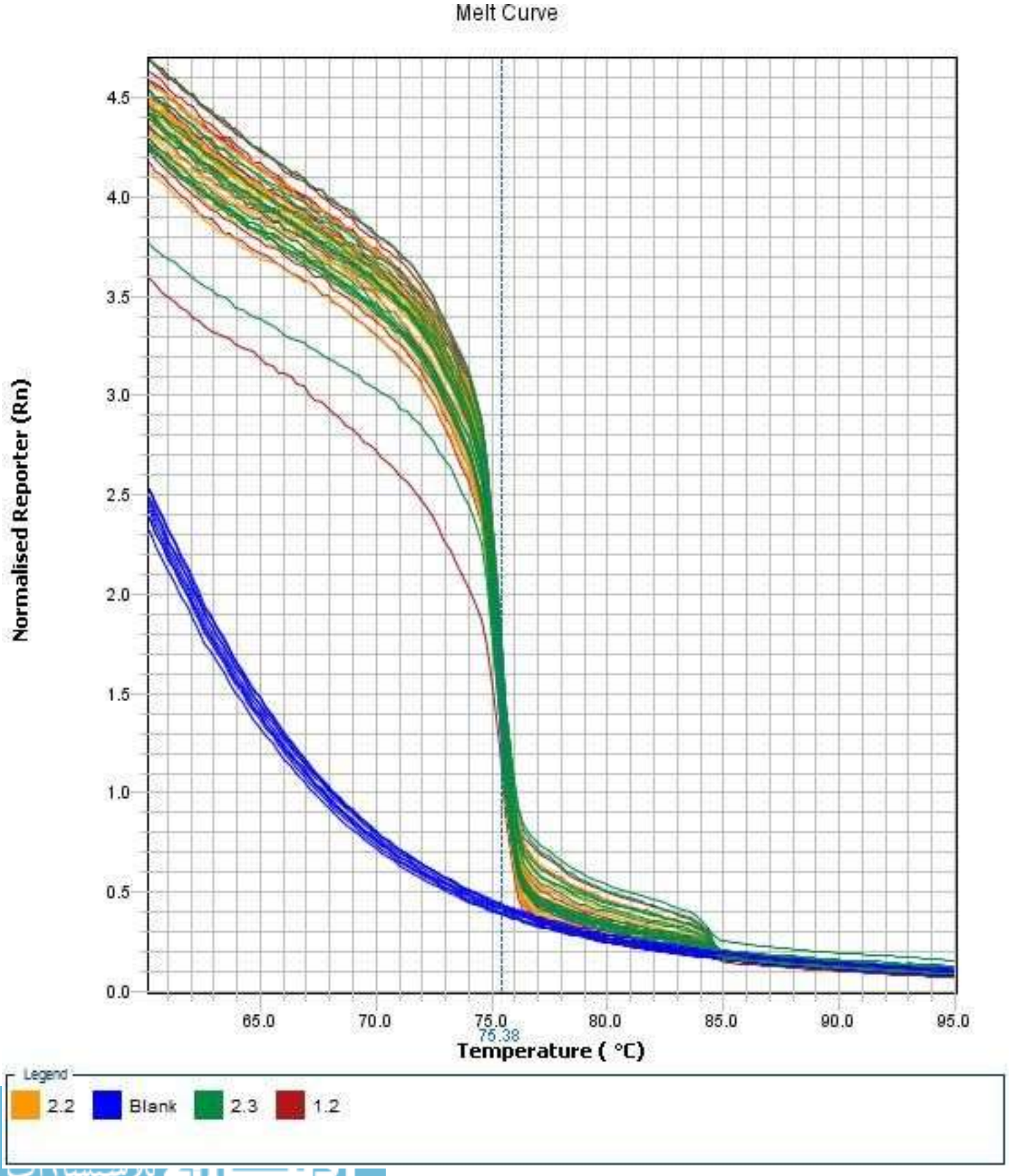


slope:-3.4124 Y-Intercept:10.2015 R^2:0.94 Eff%:96.358

Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	Cт	Cт Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	1.2	LP	S	1.000	10.177	10.291	0.107		75.381		
B3	1.2	LP	S	1.000	10.307	10.291	0.107		75.381		
B4	1.2	LP	S	1.000	10.389	10.291	0.107		75.381		
B5	1.2	LP	S	0.100	13.724	13.653	0.125		75.381		
B6	1.2	LP	S	0.100	13.726	13.653	0.125		75.249		
B7	1.2	LP	S	0.100	13.508	13.653	0.125		75.381		
B8	1.2	LP	S	0.010	16.690	16.872	0.628		75.249		
B9	1.2	LP	S	0.010	17.571	16.872	0.628		75.249		
B10	1.2	LP	S	0.010	16.354	16.872	0.628		75.249		
B11	1.2	LP	S	0.001	28.014	22.884	4.536		75.249		
B12	1.2	LP	S	0.001	19.405	22.884	4.536		75.249		
B13	1.2	LP	S	0.001	21.233	22.884	4.536		75.381		
B14	1.2	LP	S	0.000	23.656	23.731	0.248		75.249		
B15	1.2	LP	S	0.000	23.529	23.731	0.248		75.249		
B16	1.2	LP	S	0.000	24.008	23.731	0.248		75.249		
B17	Blank	LP	N		UND.				61.411		
B18	Blank	LP	N		UND.				61.411		
B19	Blank	LP	N		UND.				61.411		
D2	2.2	LP	S	1.000	10.147	10.431	0.410		75.381		
D3	2.2	LP	S	1.000	10.900	10.431	0.410		75.381		
D4	2.2	LP	S	1.000	10.244	10.431	0.410		75.249		
D5	2.2	LP	S	0.100	13.012	13.314	0.276		75.249		
D6	2.2	LP	S	0.100	13.379	13.314	0.276		75.249		
D7	2.2	LP	S	0.100	13.552	13.314	0.276		75.249		
D8	2.2	LP	S	0.010	16.465	16.609	0.203		75.249		
D9	2.2	LP	S	0.010	16.521	16.609	0.203		75.249		
D10	2.2	LP	S	0.010	16.842	16.609	0.203		75.249		
D11	2.2	LP	S	0.001	20.260	20.494	0.487		75.249		
D12	2.2	LP	S	0.001	20.168	20.494	0.487		75.249		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	CT	CT Mean	Qty Mean	Qty SD	Tm1	Tm2	Tm3
D13	2.2	LP	S	0.001	21.053	20.494	0.487		75.249		
D14	2.2	LP	S	0.000	23.849	23.804	0.039		75.249		
D15	2.2	LP	S	0.000	23.787	23.804	0.039		75.249		
D16	2.2	LP	S	0.000	23.776	23.804	0.039		75.249		
D17	Blank	LP	N		UND.				61.411		
D18	Blank	LP	N		UND.				61.411		
D19	Blank	LP	N		UND.				61.411		
F2	2.3	LP	S	1.000	10.733	10.546	0.241		75.249		
F3	2.3	LP	S	1.000	10.274	10.546	0.241		75.381		
F4	2.3	LP	S	1.000	10.630	10.546	0.241		75.381		
F5	2.3	LP	S	0.100	12.963	13.047	0.245		75.249		
F6	2.3	LP	S	0.100	12.856	13.047	0.245		75.249		
F7	2.3	LP	S	0.100	13.323	13.047	0.245		75.249		
F8	2.3	LP	S	0.010	16.516	16.630	0.149		75.249		
F9	2.3	LP	S	0.010	16.798	16.630	0.149		75.249		
F10	2.3	LP	S	0.010	16.576	16.630	0.149		75.249		
F11	2.3	LP	S	0.001	20.062	19.708	0.306		75.249		
F12	2.3	LP	S	0.001	19.528	19.708	0.306		75.249		
F13	2.3	LP	S	0.001	19.535	19.708	0.306		75.249		
F14	2.3	LP	S	0.000	23.437	23.383	0.059		75.249		
F15	2.3	LP	S	0.000	23.391	23.383	0.059		75.117		
F16	2.3	LP	S	0.000	23.321	23.383	0.059		75.117		
F17	Blank	LP	N		UND.				61.411		
F18	Blank	LP	N		UND.				61.411		
F19	Blank	LP	N		UND.				61.411		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	54	Targets Used	1
Well Setup	54	Flagged Wells	7	Samples Used	4

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	0	
HIGHSD	High standard deviation in replicate group	6	B8, B9, B10, B11, B12, B13
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	1	F11
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp Filter Set**	m1,x1 m4,x4
Cover Temperature	105.0		
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	40	1	false
Hold Stage	1	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	120	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	60	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	58.0	30	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	72.0	60	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	72.0	300	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_	95.0	15	0.0	0

	PER_SECOND

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	60.0	60	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_PER_SECOND	95.0	15	0.0	0

Experiment Results Report

2019-12-06 113816

Experiment Summary

Experiment Name: 2019-12-06 113816
Experiment Type: Standard Curve
BarCode:
File Name: qPCR_LGG_20191206_standardcurve1.eds
Run Started: 12-06-2019 19:25:19 PST
Run Finished: 12-06-2019 21:26:44 PST
Run Duration: 121 minutes 25 seconds
Date Modified: 12-06-2019 16:18:10 PST
Date Created: 12-06-2019 11:38:16 PST
User:
Number of Wells Used: 54
Number of Wells with Results: 54
Instrument Name: 278862532
Instrument Type: QuantStudio™ 6 Flex System
Instrument Serial Number: 278862532
Model/Block Type: QuantStudio™ 6 Flex System / 384-Well Block
Stage/Step for analysis : Stage 2, Step 2
Comments:
Quantification Cycle Setting: CT

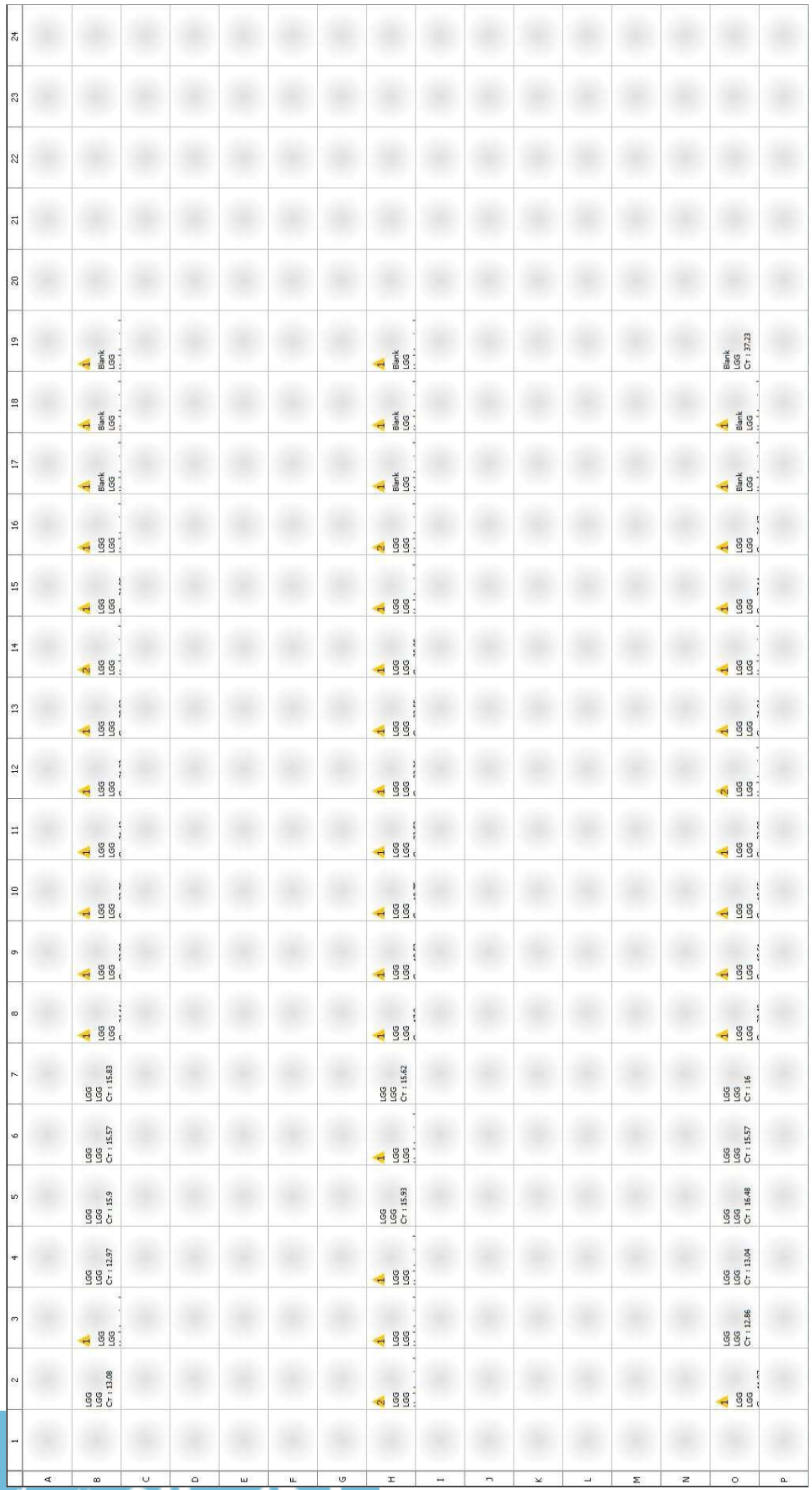
L. rhamnosus GG

Reagent Information

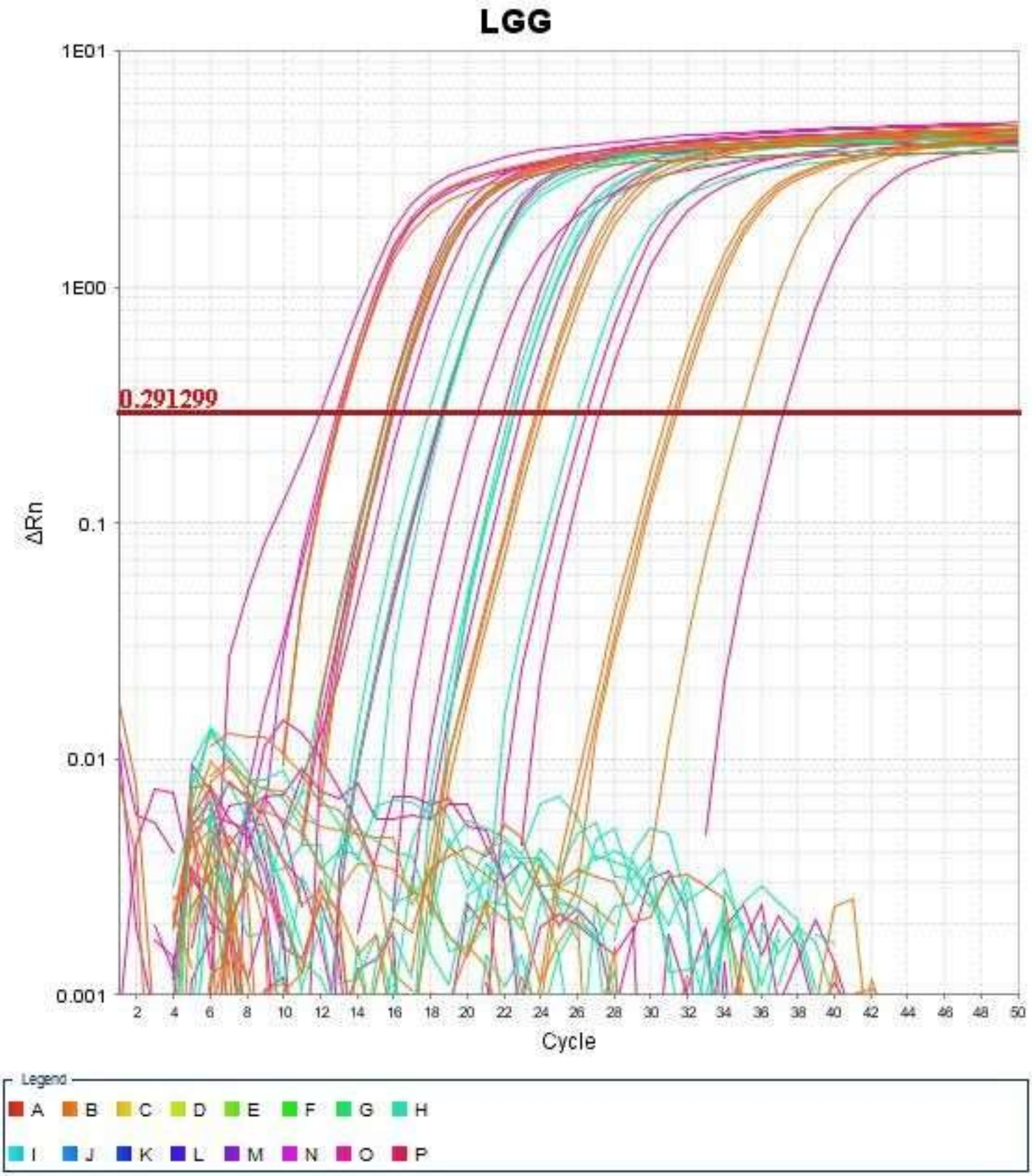
Results Summary

Sample	Target	Quantity (Mean)	Quantity (Std Dev)	CT (Mean)	CT (Std Dev)
Blank	LGG	0.000		37.231	
LGG	LGG				

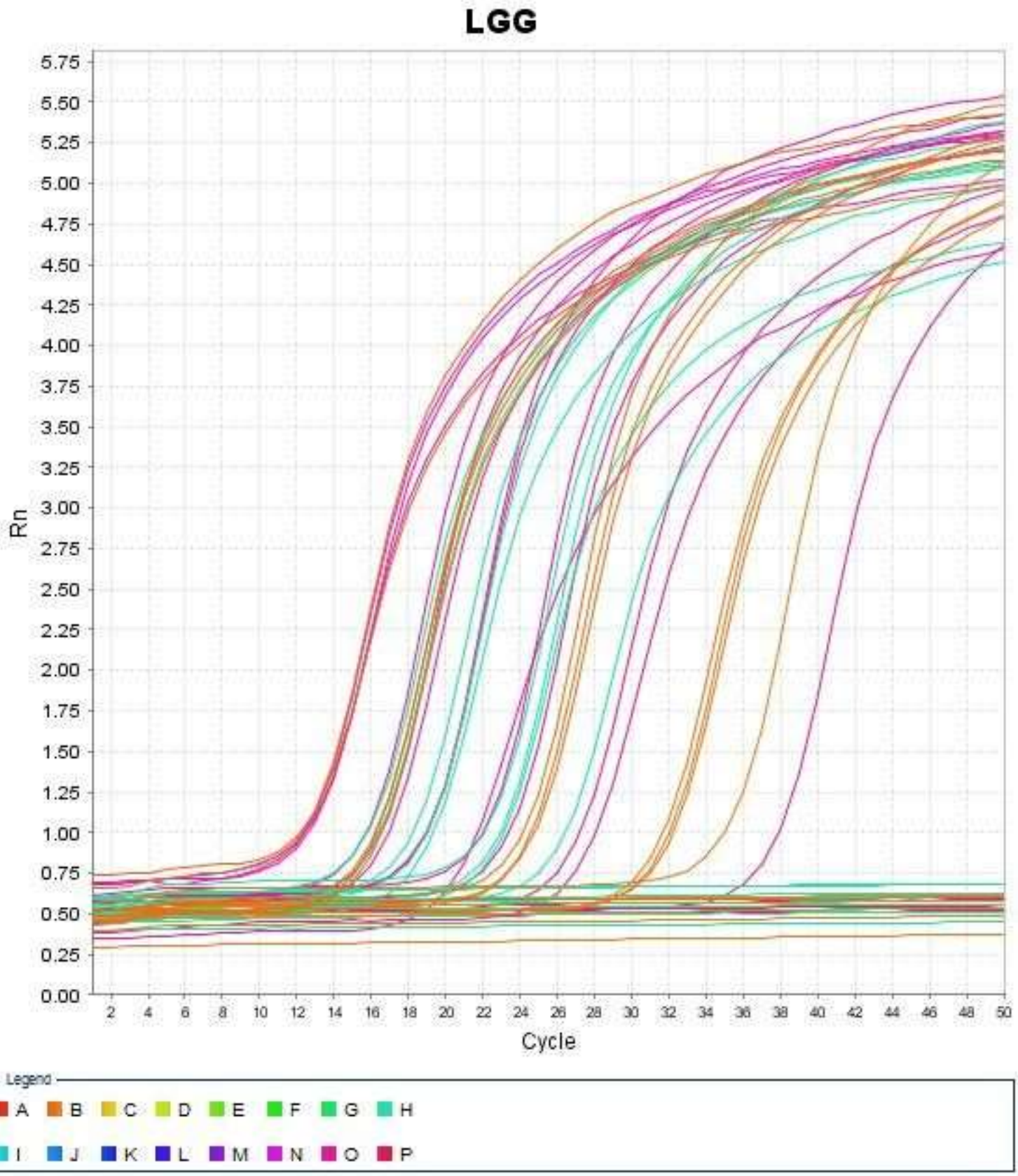
Plate Layout



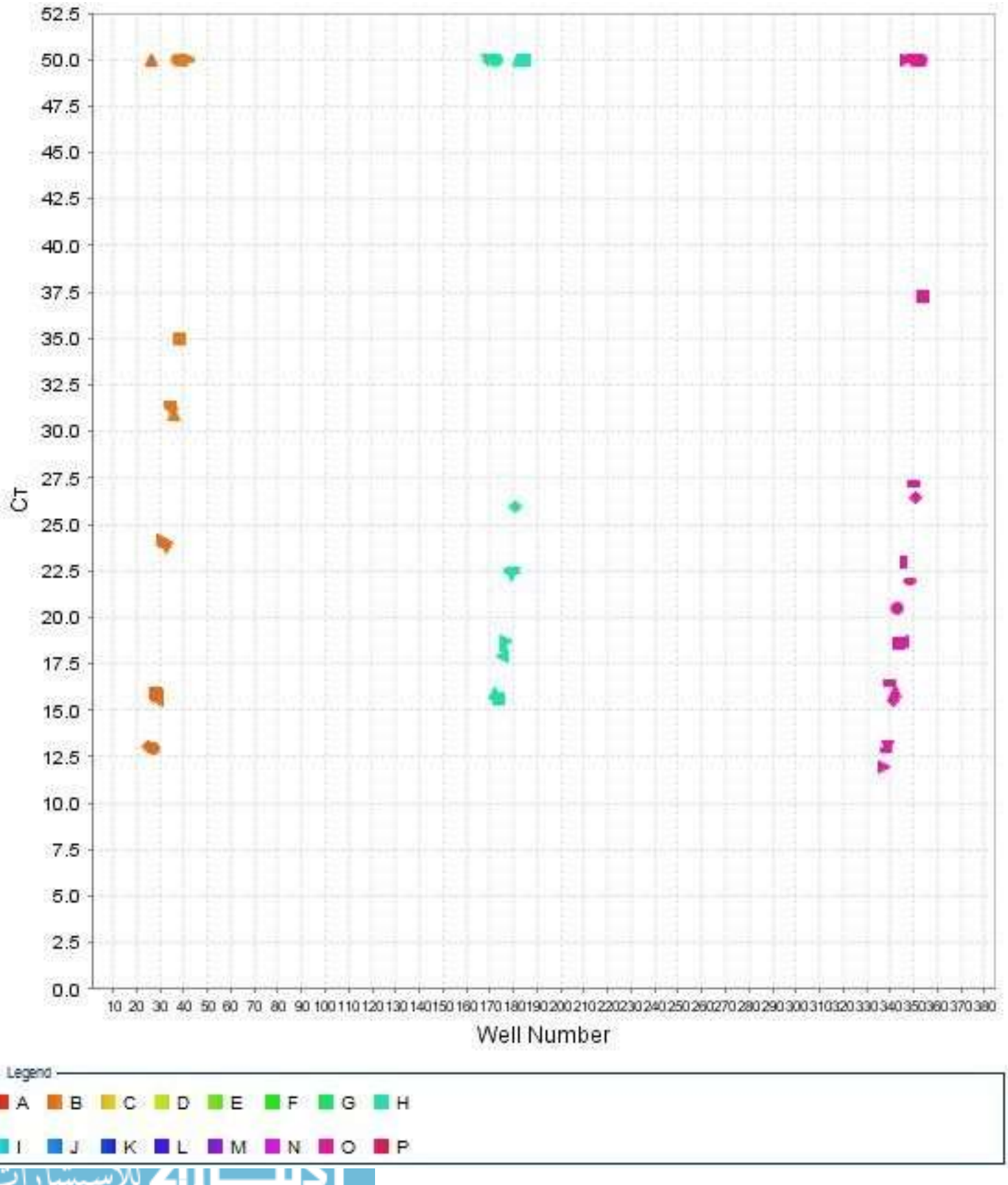
Amplification Plot (ΔRn vs. Cycle)



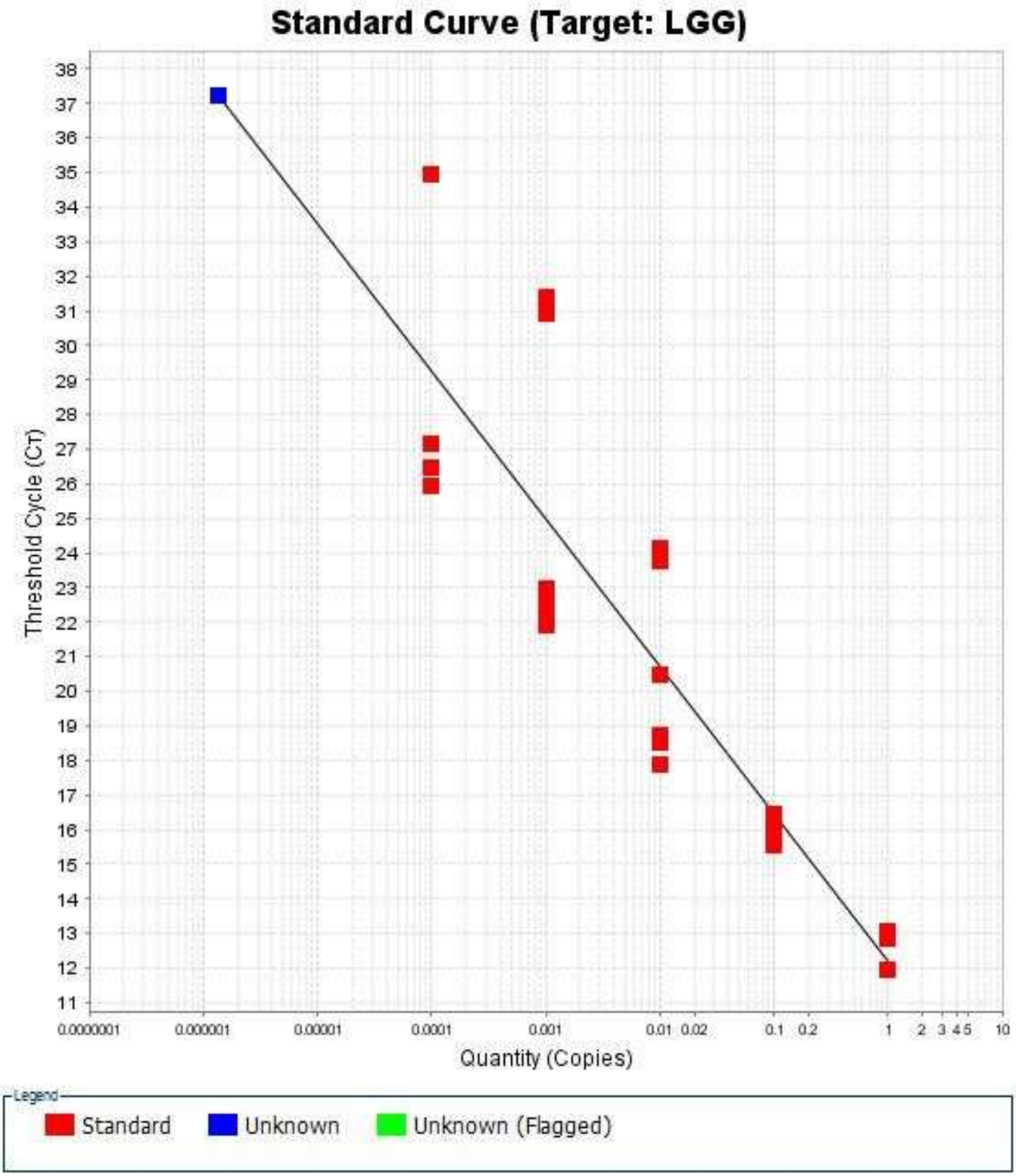
Amplification Plot (Rn vs. Cycle)



Amplification Plot (CT vs. Well)

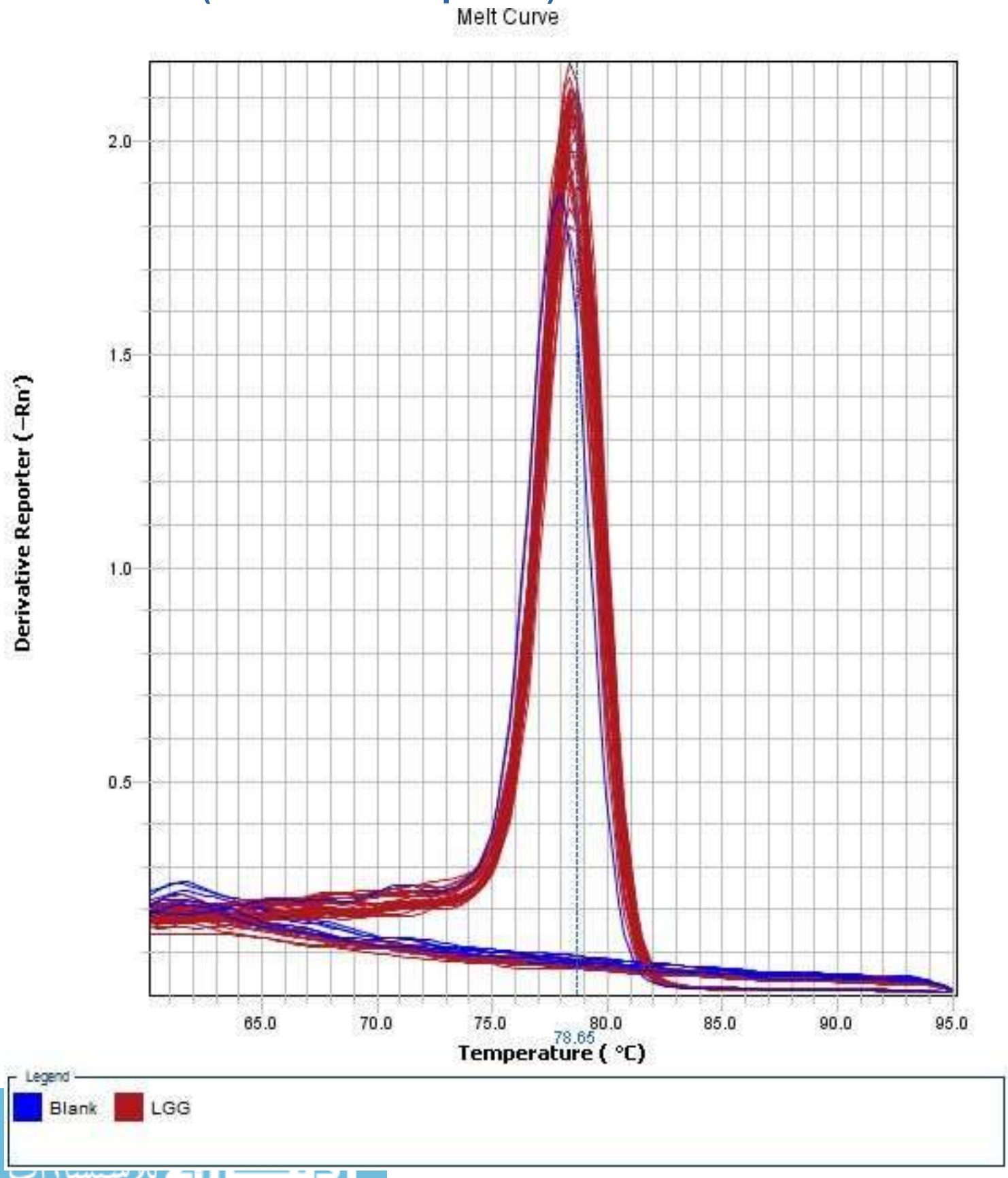


Standard Curves

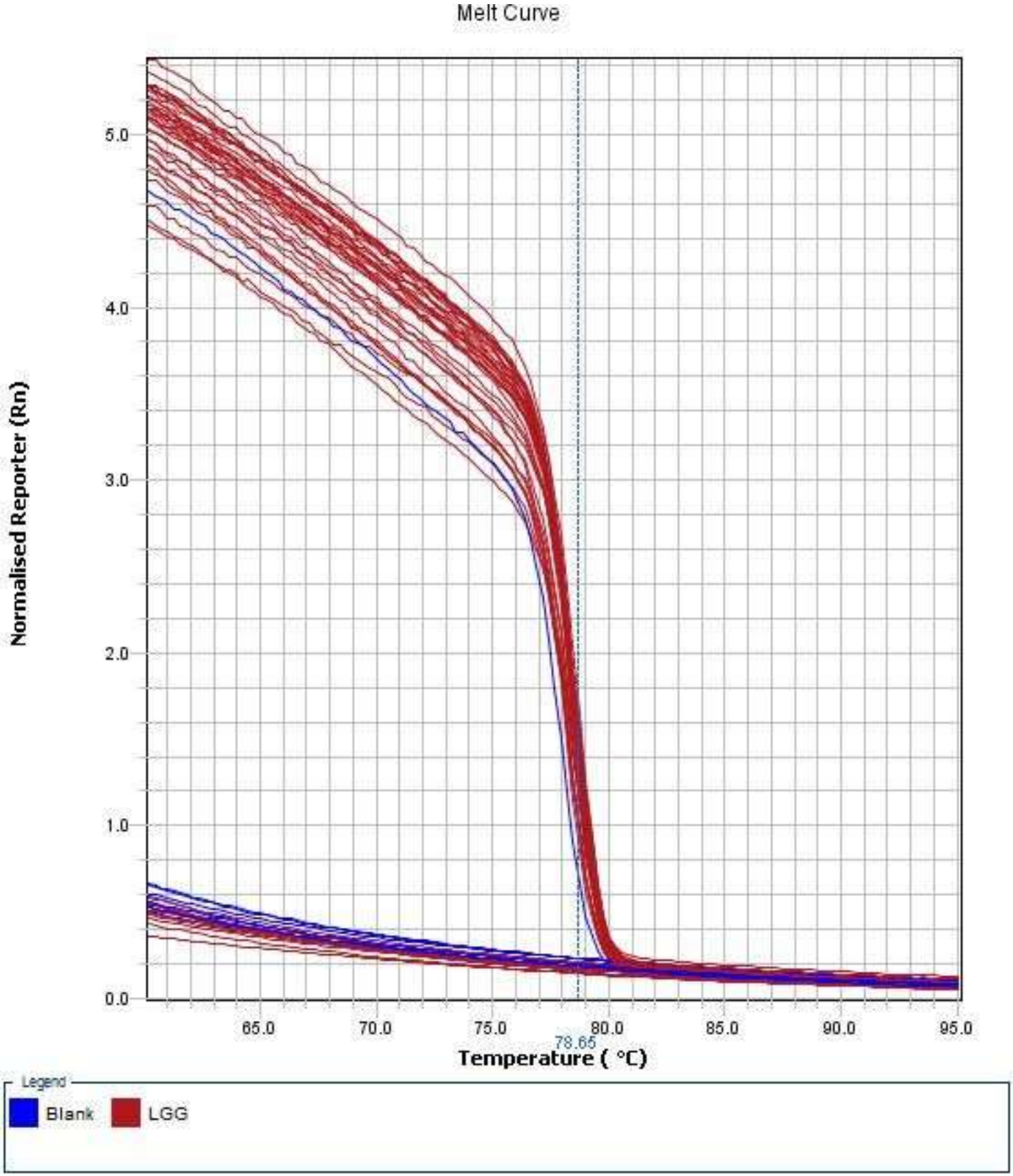


slope:-4.26 Y-Intercept:12.1956 R²:0.78 Eff%:71.689

Melt Curve (Derivative Reporter)



Melt Curve (Normalized Reporter)



Results Table

Well	Sample	Target	Task	Qty	CT	CT Mean	CT SD	Qty Mean	Qty SD	Tm1	Tm2	Tm3
B2	LGG	LGG	S	1.000	13.084	12.785	0.462			78.646		
B3	LGG	LGG	S	1.000	UND.	12.785	0.462			61.385	77.987	
B4	LGG	LGG	S	1.000	12.967	12.785	0.462			78.646		
B5	LGG	LGG	S	0.100	15.903	15.861	0.301			78.514		
B6	LGG	LGG	S	0.100	15.568	15.861	0.301			78.514		
B7	LGG	LGG	S	0.100	15.833	15.861	0.301			78.514		
B8	LGG	LGG	S	0.010	24.138	20.522	2.646			78.382		
B9	LGG	LGG	S	0.010	23.882	20.522	2.646			78.382		
B10	LGG	LGG	S	0.010	23.756	20.522	2.646			78.382		
B11	LGG	LGG	S	0.001	31.415	25.725	4.535			78.250		
B12	LGG	LGG	S	0.001	31.229	25.725	4.535			78.250		
B13	LGG	LGG	S	0.001	30.923	25.725	4.535			78.250		
B14	LGG	LGG	S	0.000	UND.	28.627	4.240			77.987	69.422	93.271
B15	LGG	LGG	S	0.000	34.946	28.627	4.240			77.987		
B16	LGG	LGG	S	0.000	UND.	28.627	4.240			61.253		
B17	Blank	LGG	U		UND.	37.231		0.000		61.385		
B18	Blank	LGG	U		UND.	37.231		0.000		61.253		
B19	Blank	LGG	U		UND.	37.231		0.000		61.253	89.713	
H2	LGG	LGG	S	1.000	UND.	12.785	0.462			61.253		
H3	LGG	LGG	S	1.000	UND.	12.785	0.462			61.517		
H4	LGG	LGG	S	1.000	UND.	12.785	0.462			61.385		
H5	LGG	LGG	S	0.100	15.926	15.861	0.301			78.646		
H6	LGG	LGG	S	0.100	UND.	15.861	0.301			61.253		
H7	LGG	LGG	S	0.100	15.617	15.861	0.301			78.514		
H8	LGG	LGG	S	0.010	17.899	20.522	2.646			78.514		
H9	LGG	LGG	S	0.010	18.516	20.522	2.646			78.514		
H10	LGG	LGG	S	0.010	18.745	20.522	2.646			78.514		
H11	LGG	LGG	S	0.001	22.519	25.725	4.535			78.382		
H12	LGG	LGG	S	0.001	22.259	25.725	4.535			78.250		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

Well	Sample	Target	Task	Qty	CT	CT Mean	CT SD	Qty Mean	Qty SD	Tm1	Tm2	Tm3
H13	LGG	LGG	S	0.001	22.546	25.725	4.535			78.382		
H14	LGG	LGG	S	0.000	25.954	28.627	4.240			78.250		
H15	LGG	LGG	S	0.000	UND.	28.627	4.240			61.517		
H16	LGG	LGG	S	0.000	UND.	28.627	4.240			61.385		
H17	Blank	LGG	U		UND.	37.231		0.000		61.649		
H18	Blank	LGG	U		UND.	37.231		0.000		61.385		
H19	Blank	LGG	U		UND.	37.231		0.000		61.517		
O2	LGG	LGG	S	1.000	11.972	12.785	0.462			78.514		
O3	LGG	LGG	S	1.000	12.857	12.785	0.462			78.514		
O4	LGG	LGG	S	1.000	13.042	12.785	0.462			78.514		
O5	LGG	LGG	S	0.100	16.476	15.861	0.301			78.514		
O6	LGG	LGG	S	0.100	15.566	15.861	0.301			78.514		
O7	LGG	LGG	S	0.100	15.997	15.861	0.301			78.514		
O8	LGG	LGG	S	0.010	20.491	20.522	2.646			78.514		
O9	LGG	LGG	S	0.010	18.614	20.522	2.646			78.382		
O10	LGG	LGG	S	0.010	18.652	20.522	2.646			78.382		
O11	LGG	LGG	S	0.001	22.977	25.725	4.535			78.382		
O12	LGG	LGG	S	0.001	UND.	25.725	4.535			61.517	93.271	87.869
O13	LGG	LGG	S	0.001	21.936	25.725	4.535			78.250		
O14	LGG	LGG	S	0.000	UND.	28.627	4.240			61.385		
O15	LGG	LGG	S	0.000	27.143	28.627	4.240			78.382		
O16	LGG	LGG	S	0.000	26.466	28.627	4.240			78.382		
O17	Blank	LGG	U		UND.	37.231		0.000		61.780	90.636	
O18	Blank	LGG	U		UND.	37.231		0.000		61.253		
O19	Blank	LGG	U	0.000	37.231	37.231		0.000		77.855		

Task Legends: S = Standard, N = NTC, U = Unknown, UND. = Undetermined

QC Summary

Total Wells	384	Processed Wells	54	Targets Used	1
Well Setup	54	Flagged Wells	41	Samples Used	2

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CTFAIL	C_T algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on C_T range	0	
EXPFAIL	Exponential algorithm failed	19	B3, B14, B16, B17, B18, B19, H2, H3, H4, H6, H15, H16, H17, H18, H19, O12, O14, O17, O18
HIGHSD	High standard deviation in replicate group	21	B8, B9, B10, B11, B12, B13, B15, H8, H9, H10, H11, H12, H13, H14, O8, O9, O10, O11, O13, O15, O16
NOAMP	No amplification	4	B14, H2, H16, O12
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	1	O2
PRFDROP	Passive reference signal changes near C_T	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

TC Protocol

Thermal Protocol Details

Block Type	384-Well Block	**Run Mode**	Standard
Sample Volume	10.0	**Melt Curve Ramp**	m1,x1 m4,x4
Cover Temperature	105.0	**Filter Set**	
PCR Filter Set	m1,x1 m2,x2 m3,x3 m4,x4 m5,x5		

Stage	No. of Repetitions	Starting Cycle	Auto Delta Enabled
Hold Stage	1	1	false
Cycling Stage	50	1	false
Melt Curve Stage	1	1	false

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	50.0	120	0.0	0

Step Hold Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	120	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	15	0.0	0

Step Cycling Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Enabled	1.6	DEGREES_PER_SECOND	60.0	60	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	95.0	15	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	1.6	DEGREES_PER_SECOND	60.0	60	0.0	0

Step Melt Curve Stage

Collection Flag	Ramp Rate	Ramp Rate Unit	Temperature	Hold Time	Auto Delta Temperature	Auto Delta Hold Time
Disabled	0.05	DEGREES_	95.0	15	0.0	0

PER_SECOND

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
06-03-2020	Master's Thesis	September 2018 – March 2020

TITLE AND SUBTITLE

Detection and quantification of bacterial species important to mental and physical health

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Leonard, David G., Captain, USAF

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(S)

Air Force Institute of Technology
Graduate School of Engineering and Management (AFIT/ENV)
2950 Hobson Way, Building 640
WPAFB OH 45433-8865

8. PERFORMING ORGANIZATION REPORT NUMBER

AFIT-ENV-MS-20-M-224

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Research Laboratory
2760 Q Street, Bldg. 837, WPAFB, OH 45433
Phone:312-674-9535 Email:camilla.mauzy@us.af.mil
ATTN: Dr. Camilla Mauzy

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

DISTRUBTION STATEMENT A. APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The human gut microbiome contains an abundance of microorganisms which could influence mental health as well as physical health. These microorganisms produce chemicals which affect the brain and the body in various ways. Probiotic bacteria and yeasts have been studied to determine effects they have on mice, rats, and humans to illustrate the importance these microorganisms on health. Studies have shown that adding beneficial microorganisms to the human diet can have positive effects on mental and physical health, to include lessening symptoms of depression and anxiety, lessen gastro-intestinal inflammation, displacing pathogens, and improving immunomodulatory response. A quantitative way to identify these microorganisms would be beneficial for future research and future use. Utilizing quantitative polymerase chain reaction, qPCR, to identify and quantify these probiotic microorganisms, and the data required to create assays and standard curves, it is possible to estimate the quantity of DNA of the associated bacteria from a sample. Methods, procedures, and materials were created or compiled for the purpose of growing the species, extracting the DNA, and amplifying the DNA via qPCR. These methods, procedures, materials, and the data and the standard curves created from qPCR were all compiled into a reference guide helpful in identifying and quantifying the bacteria important to human health in future endeavors.

15. SUBJECT TERMS

Microbiome, mental health, physical health, lactic acid bacteria, qPCR assay, probiotic

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Lt Col Andrew J. Hoisington, AFIT/ENV
U	U	U	UU	19	19b. TELEPHONE NUMBER *(Include area code)* (937) 255-6565, ext 4826 (Andrew.hoisington@afit.edu)

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39-18