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Reflection on Molecular Approaches Influencing State-of-the-Art Bioremediation Design: Culturing to Microbial Community Fingerprinting to Omics

Lauren M. Czaplicki¹ and Claudia K. Gunsch, Ph.D.^{2,+,*}

¹Ph.D. Candidate, Department of Civil & Environmental Engineering, Duke University, Durham, NC 27708-0287 USA

²Associate Professor, Department of Civil & Environmental Engineering, Duke University, Durham, NC 27708-0287 USA

Abstract

Bioremediation is generally viewed as a cost effective and sustainable technology because it relies on microbes to transform pollutants into benign compounds. Advances in molecular biological analyses allow unprecedented microbial detection and are increasingly incorporated into bioremediation. Throughout history, state-of-the-art techniques have informed bioremediation strategies. However, the insights those techniques provided were not as in depth as those provided by recently developed omics tools. Advances in next generation sequencing (NGS) have now placed metagenomics and metatranscriptomics within reach of environmental engineers. As NGS costs decrease, metagenomics and metatranscriptomics have become increasingly feasible options to rapidly scan sites for specific degradative functions and identify microorganisms important in pollutant degradation. These omic techniques are capable of revolutionizing biological treatment in environmental engineering by allowing highly sensitive characterization of previously uncultured microorganisms. Omics enables the discovery of novel microorganisms for use in bioaugmentation and supports systematic optimization of biostimulation strategies. This review describes the omics journey from roots in biology and medicine to its current status in environmental engineering including potential future directions in commercial application.

Introduction

In situ bioremediation is a commonly applied treatment strategy for cleaning up contaminated sites that relies on stimulating indigenous microbes (biostimulation) and/or adding exogenous microbes (bioaugmentation) to transform pollutants into innocuous compounds. This approach has often been favored over other treatment methods because it results in little overall site disturbance (US EPA 2013). Monitored natural attenuation, the most passive of *in situ* bioremediation strategies, has long been an industry favorite. Many studies in bioremediation of polluted soil and water have relied on lab-based research involving culturable bacteria with niche degradative ability. While this strategy has worked

*Corresponding author: Phone: (919)660-5208, Fax: (919)660-5219, ckgunsch@duke.edu.

⁺A.M. A.S.C.E.

in many instances, it is estimated that only 0.001–3 percent of bacteria are culturable (Amann et al. 1995) and thus culture-based approaches may ignore a large portion of the overall microbial community.

Microorganisms are difficult to culture largely because of inherent bias in selecting enrichment culture conditions and because they often exist in communities in which synergistic relationships develop between key microbes, making it tricky if not impossible to isolate particular strains. For example, recent genomic data has shown syntrophy in dechlorinating communities. The genome of *Dehalococcoides mccartyi*, a bacterium important in chlorinated solvent remediation, lacks key enzymes causing it to produce a compound this organism cannot tolerate (carbon monoxide) when dechlorinating solvents (Zhuang et al. 2014). Thus, *Dehalococcoides mccartyi* exhibits syntrophic association with carbon monoxide-metabolizing organisms in dechlorinating communities in the environment. Culture-based studies are poorly suited for revealing synergistic relationships such as these as well as transkingdom interactions (e.g., bacteria-fungi, archaea-bacteria). For instance, several fungal genera have been shown to serve as “highways”, translocating bacteria in the subsurface (Knudsen et al. 2013; Warmink et al. 2011; Wick et al. 2006). Other filamentous eukaryotes have been seen to do the reverse, translocating the pollutant to bacterial degraders (Schamfuß et al. 2013) and it is quite likely that similar synergistic relationships could lead to enhanced bioremediation elsewhere (Jiang et al. 2015; Lade et al. 2012; Wang et al. 2012). Molecular tools may be better suited than culture-based tools to detect transkingdom interactions. Furthermore, molecular tools have proven beneficial to the fields of wastewater treatment, bioremediation, and in particular for monitored natural attenuation (ESTCP 2011), and will likely provide more insights in the future. New insights from molecular tools regarding the pollution-associated microbial community can lead to bioaugmentation and biostimulation strategies that take advantage of synergistic relationships.

Similarly, these insights can also lead to bioremediation strategies that draw on abilities of underutilized kingdoms. While the majority of bioremediation studies have focused on bacteria, there is increasing evidence that archaea and eukaryotes may also play central roles in key environmental processes. This is particularly critical in hostile environments (e.g., low pH, at temperature extremes, or low nutrient conditions) as well as when pollutant bioavailability may be limiting (Leitao 2011; Dhakar et al. 2014; Gunde-Cimerman et al. 2009; Leitao et al. 2011; Qasemian et al. 2012). For example, halophilic archaea have been shown to degrade various pollutants in saline environments that would challenge most bacterial degraders (Erdogmus et al. 2013). Also, fungal nonspecific enzymes are able to transform hydrophobic contaminants unavailable to bacteria residing in soil pore-water and groundwater aquifers (Cameron et al. 2000; Eggen 1999; Gramss et al. 1999; Yu et al. 2005). Furthermore, various wood-rotting, soil-inhabiting, and sediment fungi have been shown to break down different classes of contaminants often encountered in the field ranging from pesticides to polychlorinated biphenyls (Cameron et al. 2000; Gramss et al. 1999; Potin et al. 2004; Rafin et al. 2013; Ravelet et al. 2000; Vogel et al. 1987; Wang et al. 2014; Ye et al. 2011; Zafra et al. 2015; Zhao et al. 2013). Thus, an expanded knowledge of the membership and dynamics of pollution-associated microbiomes would likely include novel useful microbes beyond the typical bacterial candidates (e.g. *Dehalococcoides sp.* for

chlorinated solvents). Drawing on the capacity of more microbes may enable more pollutants to be degraded and lead to more sustainable environmental remediation strategies.

The molecular tools that help in understanding pollution-associated microbiomes, though different from each other, all share a focus on elucidating a part of the coding function of the cell (Figure 1). Figure 1 is a simplistic illustration of a cross-section of a generic bacterium with relevant genetic machinery and describes how deoxyribonucleic acid (DNA) is transcribed into ribonucleic acid (RNA) before it is translated into proteins. As the field of molecular biotechnology has evolved, it has generated commonly used terminology to describe specific concepts. Table 1 is a list of the common terms used in molecular biotechnology for environmental applications. These terms are relevant to both past and recent molecular advances.

Recent molecular advances have enabled metagenomics and metatranscriptomics to provide new insights that improve the field of bioremediation by allowing concurrent analysis of millions of microorganisms in their natural and polluted habitats, preempting the need to culture these organisms before delving into their pollutant-degradation capacity. Figure 2 shows the number of studies using each technique discussed herein over the past several decades. DNA-based approaches such as metagenomics and amplicon sequencing provide useful information for studying the potential function of microbial communities. For example, Suenaga et al. used metagenomic (i.e., DNA-based) analysis to reveal how aromatic pollutant degradation genes were organized in the microbial community and that they were more widespread than previously thought (Suenaga et al. 2009). Metatranscriptomic (i.e., RNA-based) approaches are used to detect activity of those microbial communities. Luo and coauthors, for instance, used metatranscriptomic analysis to detect carboxylation activity in a mixed community during benzene degradation (Luo et al. 2014). Omics methods of genomics, metagenomics, transcriptomics, and metatranscriptomics provide state-of-the-art molecular sensing of the environment and have become the basis for transformative advances in bioremediation. With omics-based methods, the field has been able to discover novel microbes with key degradative capacities in the environment and examine complex microbial interactions in the subsurface in a sensitive and rapid manner. For instance, studies can be more targeted by relying on field-based rather than culture-based observations. Furthermore, omics-based methods have provided the basis for innovative *in situ* bioremediation monitoring strategies.

The Omics Evolution

Growing from Biology: The Early Years

It may be no surprise that the omics evolution began in biology and has been largely driven by a desire to describe the microbial source of disease. Figure 3 gives a historical perspective on omics evolution by marking important milestones throughout time. Early acknowledgements of microbial activity and disease date back to the 1870s when Hansen observed the “Leprosy Bacillus” (a.k.a., *Mycobacterium leprae*) and linked that microorganism to a specific disease (Harboe 1973). Later, in 1876, Koch validated the germ theory of disease and demonstrated that *Bacillus anthracis* was linked to anthrax (Koch 1876). This discovery paved the way for other studies on disease agents using either liquids

or the diseased tissues themselves to examine pathogens (Laveran 1880; Muller 1933; Pasteur and Académie des 1861). The first environmentally relevant discovery came when nitrification was shown to be a biological process occurring in soil (Schloesing and Muntz 1877). However, most researchers steered away from the complexity of the soil environment in preference of conditions which could be more easily reproduced in the laboratory, such as those required to demonstrate fermentation and disease development (Harboe 1973; Lister 1878; Pasteur and Académie des 1861). Pure culture work remained difficult until Fanny Hesse suggested the addition of agar in the early 1880s. The agar solidified culture media and withstood high incubation temperatures. Solid media enabled mixed communities to be plated and individual colonies separated based on phenotypic differences (Hitchens and Leikind 1939). From here, studies shifted toward enrichment cultures where particular mixtures of nutrients were used to select specific microorganisms based on metabolic preferences (Beijerinck 1888). Although enrichment cultures have been critical in the characterization of numerous microbial functions, enrichment culturing still falls short in identifying some microorganisms as well as describing complex microbial interactions in the natural environment. From an environmental remediation standpoint, culturing has been critical in terms of finding useful organisms, but does not reflect how well those same organisms will perform after being introduced to a contaminated site such as in bioaugmentation. This gap has led to the perception of bioremediation as somewhat of a “black box” technology wherein remedy success is unpredictable (Bouchez et al. 2000; Mancera-Lopez 2008; Stephenson and Stephenson 1992; Stevens 1989).

Creating the Building Blocks: The Molecular Era

Once Avery, MacLeod and McCarty identified that the molecules conferring microbial virulence of bacteria were DNA (Avery et al. 1944), molecular approaches began to be considered as the best strategy to answer broader questions about microbial prevalence in the natural environment. Although many past molecular advances have contributed to the current state-of-the-art omics methods used in probing pollution-associated microbiomes, arguably the three most critical innovations were cloning, Sanger sequencing, and polymerase chain reaction (PCR).

The development of genetic cloning in 1972 enabled the transformation of the unculturable into the culturable. As Figure 4 shows, genetic cloning uses culturable organisms (often *Escherichia coli*) to transcribe DNA from unculturable organisms into RNA, which is then translated into proteins and enzymes. These proteins and enzymes can be characterized and their functions traced back to specific genes from unculturable sources. Furthermore, genetic cloning enabled clone “libraries” to be prepared from a sample of environmental DNA or RNA. Inserting environmental DNA into culturable organisms produces clone libraries within a timespan of a few days. A characteristic gene region, often the small-subunit ribosomal RNA, could be amplified using PCR, cloned and sequenced via Sanger sequencing. The resulting DNA sequence served as a genetic fingerprint for each microbe because it generally changes at the same rate as species or genera diverge from each other in evolutionary time. Therefore, comparing DNA sequences can tell how closely related two microbes are to each other.

Sanger sequencing, as Figure 5 illustrates, is a DNA sequencing method based on incorporating chain-terminating dideoxynucleotides (ddNTPs) during DNA replication (Sanger et al. 1977). When added to a sequencing reaction, these inhibitors are incorporated into the growing DNA chain, resulting in the termination of the chain. The earlier an inhibitor is incorporated, the shorter the resulting fragment will be. This results in DNA fragments of varying length, one for each basepair (bp) in the original DNA strand. Sanger sequencing is suitable for any length of DNA up to ~800 bp. The chain-terminating inhibitors in current Sanger sequencing methods have different signatures allowing for differentiation of each nucleotide, so four separate reactions (inset c of figure 5) are no longer necessary.

It was not until PCR was developed that the field could, with great specificity, target what sequences to clone into culturable organisms. The development of PCR technology by Kary Mullis in 1985 played a critical role in advancing the molecular field (Mullis et al. 1987). PCR is a fundamental tool used to exponentially amplify targeted genetic regions enabling a single precursor DNA template to generate millions of copies of DNA (Figure 6). The invention of PCR was critical as it enabled the production of large quantities of targeted DNA regions, which in turn facilitated manipulation and large-scale studies of genetic regions previously inaccessible to researchers. PCR, cloning, and Sanger sequencing enabled development of methods discussed later that informed bioremediation, including next generation sequencing (NGS) used to generate metagenomic and metatranscriptomic data.

The environmental sequencing enabled by innovations in the early years of the molecular era led to better microbe classification in monitoring remediation. In one notable study, Dojka and coauthors used cloning to monitor microbial communities associated with bioremediation of an aquifer contaminated with hydrocarbons (Dojka et al. 1998). They used cloning to examine communities in samples from three redox zones: methanogenic, methanogenic-sulfate reducing, and iron/sulfate reducing. DNA from the zones was then amplified via PCR using universal, bacteria, and archaea primers. The resulting amplicons were cloned and sequenced, revealing 104 (94 bacterial and 10 archaeal) taxa. A close relative of *Syntrophus* spp. was identified which produces energy from anaerobic organic acid oxidation resulting in acetate and hydrogen. Another organism, identified as a relative of *Methanosaeta* spp., was known to generate energy through acetoclastic methanogenesis. Through cloning, Dojka and coauthors suggested that the hydrocarbons were degrading by a pathway ending in acetoclastic methanogenesis as a result of the syntrophy detected between *Syntrophus* spp. relative and *Methanosaeta* spp. relative. Cloning, PCR, and Sanger sequencing also contributed to determining both the identity and role of oil-metabolizing marine bacteria from the Thames estuary (McKew et al. 2007), degradation and dynamics of bacteria responding to biostimulation to remedy oil spills on beaches (Roling et al. 2002), and identified which uranium and nitrate removing microbes responded to glucose and ethanol biostimulation strategies in a co-contaminated aquifer (North et al. 2004). Studies like these lead to actual improvements on bioremediation designs (McKew et al. 2007; Sheppard et al. 2014; Vishnivetskaya et al. 2010). For example, McKew et al. used results of their earlier study to design different intervention strategies such as bioaugmentation using enrichments of an indigenous bacterium and biostimulation with key nutrients (McKew et al.

2007). Through this study, they were able to conclude that bioaugmentation with *Alcanivorax borkumensis* led to sustained enhanced degradation in polluted sediments (McKew et al. 2007).

Recognizing Microbial Diversity: The Community Era

The use of other molecular era innovations have also changed research in the field of bioremediation. Two main strategies were employed to characterize community diversity and function. The first main strategy is shared by denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment polymorphism (T-RFLP). This strategy examines microbial diversity by exploiting differences in a highly conserved gene such as the rRNA gene and then separating fragments with different sequences using a variety of methods. This approach has been shown to be powerful for focusing on community-level interactions in bacteria and archaea (16S rRNA) as well as fungi (18S rRNA). The second strategy consists of targeting a known gene using quantitative PCR (qPCR) to quantify organisms within a community who possess the target gene. A similar approach known as reverse transcription quantitative PCR (RT-qPCR) can be applied to quantify the transcription of a target gene in RNA studies. A more in depth description of the most significant molecular techniques is provided below with corresponding examples of applications in bioremediation.

Denaturing Gradient Gel Electrophoresis—Denaturing gradient gel electrophoresis (DGGE) was an early mainstream molecular method used to assess microbial community dynamics (Muyzer et al. 1993). For DGGE microbial surveys, gene targets are first amplified using a combination of one forward PCR primer and one reverse PCR primer with a GC clamp (Muyzer et al. 1993; Myers et al. 1985). The resulting PCR amplicons are then run on a linear gradient gel under denaturing conditions. Typical gradient conditions range from 0 to 100% denaturing solution, often a mixture of formamide and urea (Muyzer et al. 1993). Denaturing conditions are achieved by adding this denaturant solution to the polyacrylamide gel and running the gel at a high temperature, generally ~60°C. DNA fragments differing by even single basepairs (bp) have different separation (denaturing) points along a gradient of denaturing agent primarily due to the fact that different bases have different affinities for each other (Fischer and Lerman 1983). In principal, each band on a DGGE gel corresponds to a distinct microorganism in a given community. Thus, the result of DGGE is a microbial community fingerprint, unique to each community. The primary advantage of DGGE is that it does not rely on a prior knowledge of microbial identity. DGGE microbial fingerprints can be obtained from various treatments and compared. While largely qualitative, some semi-quantitative conclusions could be reached by comparing the intensity of each band, a measure that is somewhat correlated to the starting quantity of a specific amplicon in a given treatment. Still, because PCR multiplies abundant taxa more than rarer taxa (PCR bias), these measurements are not very accurate. Originally, comparisons were performed visually, but soon thereafter, more accurate comparisons were possible using image analysis software. While no information is available about the identity of each microorganism in the fingerprint, researchers could identify particular strains by cutting prominent bands out of the gel and sequencing them.

The strengths of DGGE have led to many insights into microbial community response to bioremediation strategies. According to Web of Science, DGGE has been used in 393 bioremediation studies and 187 studies in biological wastewater treatment as of the writing of this article. In a particularly impactful study, (Macnaughton et al. 1999) examined bacterial population changes in response to different bioremediation strategies addressing a coastline experimental oil spill. Macnaughton et al. (1999) used DGGE to compare community shifts in response to four different treatments: #1 was an unoiled control; #2 involved adding oil but not additional amendments; #3 was adding both oil and nutrients; and #4 involved adding nutrients and an indigenous inoculum capable of degrading hydrocarbons. The 16S rRNA amplicons resulting from those treatments were separated using DGGE. The pattern observed in the control suggested that the baseline population structure was simple and dynamic. The pattern observed in the treatments differed greatly from the control (the unoiled plot). The DGGE patterns for the nutrient amended treatments were different from the ones without nutrients, showing a definite shift in the bacterial community structure and diversity resulting from nutrient addition. MacNaughton et al. (1999) also sequenced prominent bands from the DGGE gel to find that the α -proteobacteria and *Flexibacter-Cytophaga-bacteroides* phyla dominated the oil-treated sands. Furthermore, since α -proteobacteria were not detected in the unoiled control, it was found that members of α -proteobacteria were enriched by oil contamination, and thus are likely involved in biodegradation. Other bioremediation applications benefited from similar DGGE experimental designs. A few benefits include a better understanding community dynamics during coupled chemical oxidation and bioremediation for improved biphasic diesel soil remediation (Sutton et al. 2014), a better understanding of relationships between different polycyclic aromatic hydrocarbons (PAH) and novel microbes for PAH soil remediation (Andreoni et al. 2004; Leys et al. 2004), and a better understanding of the identity of a key player in immobilizing radioactive and toxic metals in impacted groundwater remediation (Snoeyenbos-West et al. 2000). These and other studies monitoring microbial response to bioremediation were able to contribute important information about prominent members of pollutant-impacted microbial communities that translated into improved bioremediation strategies (Colombo et al. 2011; van Herwijnen et al. 2006). For example, Colombo et al. used the study by Andreoni et al. to design their bioremediation strategy using *Sphingobium chlorophenolicum* bioaugmentation to remedy agricultural soils co-contaminated by metals and PAH mixtures (Colombo et al. 2011). As impactful as these studies were in improving bioremediation, they were still subject to the limitations of DGGE including inherent PCR bias, qualitative community structure analysis, and limited sequencing ability.

Terminal Restriction Fragment Length Polymorphism (T-RFLP)—T-RFLP has been another method commonly used in monitoring bioremediation. T-RFLP was also generally applied to the highly conserved ribosomal RNA (rRNA) genes (Liu et al. 1997; Mills et al. 2003). The first step in T-RFLP analysis involves performing PCR amplification using a normal forward primer and a fluorescently-modified reverse primer. Following amplification, amplicons are digested using multiple restriction enzymes that each target a specific DNA location. The enzyme digestion step generates distinct, fluorescently-labelled DNA fragments of varying lengths because each amplicon has a different genetic sequence. Then, fragments are separated using capillary gel electrophoresis and fluorescence is

detected, producing an electropherogram. In the electropherogram, each peak corresponds to a distinct microorganism within the community. Since the size of the peak can be correlated to a specific amount of a given microorganism within the community, T-RFLP is more quantitative than DGGE, although still not a fully quantitative method. A number of software packages have been developed to compare electropherograms between treatments and perform statistical analyses to quantify similarities. While no strain identification can be obtained from a particular terminal restriction fragment (T-RF), researchers have been able to associate T-RFs to microorganism sequences using ribotype databases. These ribotype databases have been constructed by compiling a collection of microorganism sequences, their T-RFs generated using various combinations of restriction enzymes, and their identity so that a T-RF can be quickly matched and identified.

The semi-quantitative and rapid ribotyping ability of T-RFLP has led to multiple insights gained by bioremediation. For example, Grant and coauthors gained insights into microbial shifts spurred by biostimulation of polycyclic aromatic hydrocarbon (PAH) degrading organisms (Grant et al. 2007). Soil samples were collected from a former timber treatment yard and lab-scale microcosms were constructed. The experimental design invoked T-RFLP to compare three treatments to PAH-contaminated soils: # 1 consisted of sterile soil (abiotic control); # 2 consisted of the original soil without aeration; and # 3 consisted of the original soil with aeration. DNA was extracted, purified and concentrated from soils subjected to the treatments and then T-RFLP was performed. Percentage abundance was calculated and ribotypes identified using the Ribosomal Database Project's ribotype database. Comparisons between the observed diversity and that usually found in typical soils revealed diversity to be higher in the polluted soils. Furthermore, increases in certain ribotypes were able to be inferred through the semi-quantitative metric, percentage abundance, over the period of degradation. *Bacilli*, pseudomonad, aeromonad, *Vibrio* and *Clostridia* ribotypes were the main fragments of interest, but there was a slight decrease in *Clostridia* spp. over the degradation period. Comparison with the database also facilitated classification of several ribotypes greatly increasing in the treatments over time, though these increases were similar in both Treatments #2 and #3. Although Grant et al. was able to classify many ribotypes, the one fragment that differed between Treatments 2 and 3 (unaerated and aerated) remained elusive due to insufficient coverage in the ribotype database of the Ribosomal Database Project. This study and others with similar experimental design added a semi-quantitative facet to build on DGGE's basic information about bioremediation responses. T-RFLP also introduced the practice of using databases to classify results. Using T-RFLP experimental design similar to the above, diverse important bioremediation insights were developed. These include a rapid semi-quantitative understanding of microbial communities capable of degrading alkanes in cold anaerobic climates (Ding et al. 2015), identifying microbes involved in uranium reduction in drinking water biostimulation strategy (Williams et al. 2011), and finding favorable conditions for ammonia removal by *Nitrosomonas* species in a drinking water biofilter (Feng et al. 2012). Knowing which microbial communities can remove pollutants under different conditions informs bioaugmentation strategies (Wang et al. 2013; Zhuang et al. 2012). For example, Zhuang and coauthors built on the Williams et al. study to minimize the cost of biostimulation chemicals while maintaining uranium concentrations below environmental safety standards (Zhuang et al. 2012). T-RFLP

represents an improvement on DGGE in assessing with the possibility of characterizing the entire community at once given a sufficient ribotype database. However, in the event that database ribotypes do not cover the diversity present, there is no sequence information gained, while in DGGE one may obtain sequences for prominent bands.

Quantitative PCR (qPCR)—qPCR is a powerful tool for quantifying the amount of a gene in a sample. Various approaches have been developed and the chemistry varies depending on the platform used, however the general concept remains constant. Briefly, qPCR measures fluorescence associated with an amplicon's signal as it undergoes amplification. The chemistries differ in how they assign the fluorescent signal to the amplicons. The most common chemistries used for qPCR consist of SYBR® Green and TaqMan. SYBR® Green chemistry uses a dye, SYBR® green, which only fluoresces when it inserts itself in double stranded DNA. TaqMan chemistry relies on a fluorescent probe that has been designed to attach to a specific sequence present in the target gene. The probe fluoresces as the target gene is amplified. As each cycle of PCR replicates the number of target genes exponentially, the fluorescence increases exponentially. Since increase in fluorescence is directly proportional to the amount of double stranded DNA, absolute quantification is possible if a few standards of known target gene concentration are measured at the same time as the samples. In absolute quantification qPCR, the data output is a concentration of target genes. There is also relative quantification qPCR, in which two or more samples are measured at the same time to quantify differences in a specific gene across different samples. Relative quantification qPCR results in data output in terms of fold-difference between samples (ex: one sample expresses the target gene twice or three times as much as the other). While qPCR is specific to DNA, it can also be extended to RNA targets by incorporating a reverse transcription (RT) step. It should be noted that qPCR and RT-qPCR accuracy depends on appropriate primers that correctly detect the organisms or functions of interest since primer mismatches may lead to inaccuracies (Ledeker and De Long 2013) and primer choice is often limited by the lack of robust databases. Despite that, RT-qPCR is powerful as it now provides insights into microbial activity through the measurement of particular mRNA transcripts, the precursor molecules to proteins.

The truly quantitative nature of RT-qPCR and qPCR have given many insights into microbial behavior in bioremediation scenarios. Arguably, the most notable insight might be the detection of bacteria involved in mediating degradation of chlorinated solvents. One study used qPCR to target both conserved genes and specific reductive dehalogenase (RDase) genes simultaneously in order to monitor a contaminated site for different members of *Dehalococcoides* (Ritalahti et al. 2006). The study tested three treatments in an attempt to identify *Dehalococcoides* genes *in situ* using known dehalogenating sequences. The experimental design used Taqman chemistry and absolute qPCR to quantify gene copy number in three model scenarios of increasing complexity: dechlorinating enrichment cultures, chloroethane-dechlorinating microcosms, and samples from a contaminated site. In the enrichment cultures, qPCR data suggested that the sum of the three RDase genes was equal to the total *Dehalococcoides* spp. cell number. However, this was not supported by qPCR in the samples from the contaminated site and the microcosm. With qPCR, the authors were able to quantitatively conclude that there were novel degradation genes and the field

has been underestimating the number of genes involved in dechlorinating communities. RT-qPCR and qPCR have also shed light on other environmental microbial processes in bioremediation. Using similar qPCR and RT-qPCR experimental designs, pyrite was found to stimulate denitrification communities in a nitrate-contaminated aquifer (Torrento et al. 2011), oxidized uranium was found to support growth of uranium immobilizing communities in contaminated groundwater aquifers (Sanford et al. 2007), and bioremediation of volatile organic pollutants was enhanced using phenylacetate (Gunsch et al. 2006). qPCR improves on the semi-quantitative quality of T-RFLP because it is directly quantitative, but it requires specific *a priori* knowledge of the sequences in order to quantify them, as T-RFLP does not. Also, qPCR approaches do not allow higher level assessment of community structure or diversity as DGGE and T-RFLP do. The molecular era shift towards non-culture based methods has enabled researchers to more accurately decipher microbial interactions as well as to focus on microorganisms carrying out key reactions that may be minor proportions of the overall community.

Sequencing Microbial Diversity: The Early Omics Era

While community-level analyses provided valuable insight, they could not examine all of the whole genomes that harbor the community's capabilities. Whole-genome shotgun sequencing was the early omics era innovation that combined cloning and Sanger sequencing for large-scale environmental sequencing applications. In whole-genome shotgun sequencing, DNA is fragmented into three different sizes (approximately 1000, 8000, and 40,000 bp) and cloned into host organisms. Once the host cells are grown, DNA is extracted and then sequenced using Sanger sequencing (Figure 5). Then, the DNA sequences are pieced together into the genome. Venter and coauthors used whole-genome shotgun sequencing to examine diversity and assemble genomes of organisms living in the Sargasso Sea (Venter et al. 2004). This whole-genome sequencing effort served as a proof of concept for environmental genomics, paving the way for the future of environmental omics.

Once again, however, it was the pursuit of human health-related knowledge that motivated the innovation needed to make environmental inquiry feasible: sequencing the human genome. The first human genome was completed in 2003 using whole-genome shotgun sequencing at a cost of \$2.7 billion dollars over 15 years (Bernard et al. 2010; Lander et al. 2001). The Human Genome Project and the parallel efforts in yeast and worms laid out the strategy to deal with large-scale genomic sequencing. Researchers realized that although it was possible to perform thousands of sequencing reactions via Sanger sequencing, it would be much faster and ultimately cheaper if there were a higher throughput option. This drive for technological improvement also benefited other fields including that of environmental remediation by expanding analytical capabilities and thus technological feasibility assessments.

The Human Genome Project escalated the need to improve sequencing technologies and created the need for inexpensive and simpler methods (Lander et al. 2001). The promise of genomic knowledge coupled with medical applications spurred the sequencing industry to offer increasingly affordable platforms until the ability to sequence an entire genome for \$1000 (the thousand-dollar genome) was close to a reality (Wolinsky 2007). Early

improvements on Sanger sequencing introduced capillary replacements that sorted DNA fragments more efficiently than agarose gels used in the past. Shortly thereafter, pyrosequencing employed an innovative sequencing technology to add a high-throughput dimension to sequencing.

Advancing Omics: The High Throughput Sequencing Era

Omics tools have enabled biological insights into microbial capacity in unprecedented detail. Experimental designs often center around three strategies: amplicon surveys, metagenomics, and metatranscriptomics. Amplicon-based surveys of microbial communities provide general information regarding taxa in a community. The targeted gene regions can be any section of DNA that is conserved enough to design PCR primers. Common DNA targets range from ribosomal RNA regions which provide phylogenetic information, to genes that are involved in carrying out certain functions (functional genes). It is important to note that certain gene regions change at the genus evolutionary rate (e.g., fungal 18S rRNA), while others change at the species evolutionary rate (e.g., fungal intertransgenic spacer) and this rate can even change within the taxa of interest. Thus, the level of detail provided by amplicon-based techniques depends on how well the gene is conserved and genes should be carefully selected to match the scope of the specific study. Further information can loosely be inferred with respect to relative abundance. However, inferences should be carefully drawn between relative abundance of specific taxa and functional importance of that taxa as PCR bias can artificially inflate the numbers of the abundant taxa. Furthermore, some taxa may have more copies of the target gene and may appear proportionally more prevalent than in reality (Gonzalez et al. 2012). However, creative methods of tagging the genomic DNA before PCR have been shown to overcome some of the PCR bias challenges (Lundberg et al. 2013). Large searchable online databases such as SILVA, Greengenes and the Ribosomal Database Project have been constructed to parallel the ribotype databases of the past. These are gradually becoming more robust as researchers contribute more and more sequences to them (Wang et al. 2007; Yilmaz et al. 2013). Still, there is a strong need for improved databases for bacteria, archaea, and fungi to increase the power of such analyses, as the ability to accurately characterize a microbial community and its functional attributes is directly correlated to the quality and quantity of data available in these databases.

Metagenomics methods, in contrast, give more detail regarding a specific degradative ability of a microbial community because they sequence entire genomes, not only one section of the genome as in amplicon-based methods. Also, in contrast to amplicon-based methods, metagenomics tends to be much more costly. This approach involves extracting genomic DNA and shearing the DNA into fragments. The challenge in this approach lies in the data analysis wherein all the short DNA sequences must be pieced together to reassemble the genomes. However, while computationally challenging, successful implementation of this approach may provide a more accurate reflection of population dynamics at a given site. A more detailed overview of metagenomics analysis is given in Bragg and Tyson (Bragg and Tyson 2014). These data can provide much better identification at the species and, possibly, even at the strain level compared to the amplicon-based approach due to increased sequence information for each microbe.

Metatranscriptomic approaches can be used to inform which of the enzyme systems are expressed and determine how fungi, bacteria, and/or archaea can be further stimulated. Similar to metagenomic approaches, metatranscriptomic approaches are also more expensive than amplicon-based methods. RNA-level analyses provide an indirect measure of microbial activity and for this reason may be a better target than DNA for estimating the potential degradation capacity of a particular microbial community. In principal, the number of transcripts in a sample reflect which genes are expressed and, by extension, provide some indication as to which enzymes may be active under a certain set of treatment conditions. In a similar fashion to the metagenomic analysis described previously, metatranscriptomic analysis is carried out for all of the organisms present in a given sample, providing a snapshot of a given community's activity profile. By definition, metatranscriptomic analyses are not limited to pure cultures or well-known genes, which is a tremendous advantage over previous approaches that required *a priori* genetic knowledge making it challenging to work with uncharacterized and unsequenced microorganisms (Gunsch et al. 2007). In metatranscriptomic approaches, total RNA is extracted, reverse transcribed into complementary DNA (cDNA) which is sequenced, and finally mapped back onto genomes. Then, a combination of in depth bioinformatic and biostatistical analyses identify metabolic pathways which are up- and down-regulated providing key insights into degradative pathways as well as potential inhibitory interactions between microbial competitors. For more detail on the various approaches to prepare samples for metatranscriptomic analysis, see the comparison by (Alberti et al. 2014; He et al. 2010). Thus, by integrating metagenomics and metatranscriptomics, it is likely that bioremediation strategies could be implemented more efficiently by ensuring that the indigenous microbial system is stimulated to its full potential. It should be noted that the biggest limitation is currently computational, resulting mostly from the scarcity of relevant genomic data. However, as the relevant databases grow, so will the strength and influence of omics techniques in informing bioremediation strategies.

As described above, metatranscriptomic approaches have the ability to reveal which genes are up-regulated under specific treatment conditions and may even be able to reveal novel degradative genes. Thus, this approach is powerful for helping in the identification of key environmental parameters and microbial interactions controlling the successful expression of key degradative functions. One current limitation to metatranscriptomic analyses is that the relative abundance of genes cannot yet be measured in a sensitive enough fashion especially as the transcript number of the genes of interest are small relative to housekeeping genes. However, the information gathered concerning the differentially expressed genes can then be carried over to RT-qPCR analysis for a more accurate measurement.

Pyrosequencing was the first sequencing technique to bring sequencing beyond cloning into the high-throughput realm. Pyrosequencing preparation involves first attaching specific sequences of DNA to the target DNA strands. During sequencing, these modified strands will bind to complementary DNA sequences on special capture beads. Once each bead has a single strand of DNA, emulsion PCR is performed, the bead captures the resulting copy, and the cycle is repeated until many copies of the same DNA sequence entirely cover the bead. Finally, the DNA capture beads are placed into wells where DNA polymerases add nucleotides one-by-one and release light. The pyrosequencing detector then records

whichever base was present when the light was released (signaling the base addition to the complementary strand of DNA to the one attached to the capture bead). Pyrosequencing was a significant milestone technique because it made knowing both the sequence of organisms as well as an estimate of their number a feasible undertaking.

Pyrosequencing quickly found use in environmental remediation applications. For example, pyrosequencing was used to monitor bacterial response to ethanol injection in order to stimulate uranium reduction in a groundwater plume (Cardenas et al. 2010). Cardenas and coauthors found remediation to be achieved using ethanol injection in a system of hydraulically controlled areas. The experimental design involved 16S rRNA amplicon pyrosequencing organisms in three different hydraulically controlled areas. The pyrosequencing effort resulted in 97,610 sequences for 17 samples from the different conditions. These results revealed communities dominated by a few taxa, the top 10 most abundant taxa represented at least 40% of the sequences in each sample. The study delved further into the sequences to classify the main taxa adapting to the three different conditions. *Castellaniella* and *Rhodanobacter* dominated the community in areas of low pH, heavy metals, and bioactivity. In areas of high sulfate, iron (III), and uranium (VI) reducing bacteria, *Desulfovibrio* spp., *Anaeromyxobacter* spp. and *Desulfosporosinus* spp., indicated where uranium was actively reduced. The abundance of those bacteria was found to correlate to the hydraulic connectivity to the injection site and thus the authors were able to conclude that bacteria were directly responding to electron donor addition. Amplicon surveys using pyrosequencing studies greatly contributed to other pollutant-microbe interactions also. Some areas pyrosequencing enlightened include environmental responses to emerging contaminants (Chariton et al. 2014), resilience of the impacted soil microbiome against pollutants (Kato et al. 2015), and fungal diversity of lignocellulose degrading genes in soils for future bioremediation application (Barbi et al. 2014).

However, large-scale pyrosequencing projects were still too expensive for widespread use. This motivated the next generation of sequencing technologies to become even more high-throughput. NGS accomplished the \$1000 genome. At present, it is possible to sequence a few samples in a run shared with others using advanced chemistry kits so that the cost per sample is less than \$100. Advances in next generation sequencing have made comprehensive biological environmental inquiry feasible in various fields, including that of bioremediation. There are several platforms that operate using NGS technology. Though there is a range of platforms available to suit various applications with their non-Sanger-based high-throughput sequencing, this section will focus on the most common platforms and a more in depth description of other methods can be found elsewhere (Glenn 2011; Myllykangas et al. 2012; Shendure and Ji 2008). It is important to keep in mind that one of the main criticisms of NGS is the generation of short DNA reads. Early reagents were designed to suit single genome study and produced short DNA reads, 50–100 bp long, that would get pieced together into a genome. Short DNA reads do not suit amplicon studies because they are rarely long enough to cover the part of the gene unique to each genus or species. Thus, microbial identification using short DNA reads is difficult. Now, there are improved sets of reagents that can be used to extend this read length to beyond 300 bp, producing sufficient sequence length to discern genus, if not species, in bacteria, fungi, and archaea.

Currently, there are two common NGS platforms used in environmental sequencing: Illumina and Ion Torrent (Salipante et al. 2014). These are considered second generation sequencing platforms. Because it is generally easier to identify members of the microbial community using longer sequences, the Illumina MiSeq platform is most commonly used as longer reads can be generated while still being cost-effective; however shorter gene regions have been successfully studied using other technologies including the Ion Torrent platform (Geml et al. 2014; Lindahl et al. 2013). PacBio and Oxford Nanopore platforms are also worth mentioning, though their technologies, considered to be third generation, have not been extensively used in environmental microbial studies (Glenn 2011). As the incorrect base identification (error rate) for Pacific Biosciences (PacBio) and Oxford Nanopore improve, they will likely be used more widely. These four technologies operate based on different principles. The following is a short overview of each technology, though the most recent platform details and illustrative animations can be found at the respective manufacturer's website. Illumina uses sequencing-by-synthesis wherein bases are recognized as they bind to the elongating DNA strand. Illumina's sequencing-by-synthesis recognizes these bases by their unique light signals (Illumina 2016). The Illumina detector is highly sensitive and can discern many sequence signals at once, resulting in extremely high-throughput technology. Ion Torrent also uses sequencing-by-synthesis, but detects protons released when nucleotides get incorporated into DNA instead of detecting light (Rothberg et al. 2011). Light detection is a part of PacBio's basic principle, but in a different manner than in Illumina technology. In contrast, Oxford Nanopore does not rely on DNA synthesis or light detection as part of their sequencing reaction. Oxford Nanopore measures electrical current changes in an electrically resistive membrane caused by DNA or RNA chains passing one at a time through it (Eisenstein 2012; Mikheyev and Tin 2014). The hole in the membrane is on the order of nanometers wide so that only one strand of DNA or RNA can fit at a time. However, the membrane is thick enough to envelop a portion of the DNA or RNA strand. Each combination of bases in the strand disturbs the electrical current in a different way. Therefore, sequence information is inferred by pulling the DNA or RNA strand through the membrane so that the membrane can sense the bases on the entire strand. A table detailing a few common instruments based on these platforms along with the 2014 update to the price comparison first published in 2011 is presented in Table 2 (Glenn 2011). Error rate is important to consider in addition to cost and read length when choosing an NGS platform, as the platforms range in accuracy a fair amount (<1% error with Illumina's platforms to ~14% on PacBio's RS). It is worth noting that the prices associated with these sequencing technologies have decreased significantly since the first versions of these instruments were made available (Pettersson et al. 2009). As a result, environmental inquiry has increasingly become mainstream and has necessitated the creation of entirely new fields such as computational biology and bioinformatics, which focus on the analysis of the large datasets generated.

Bioinformatics and Biostatistics

While there are challenges associated with performing the various analyses described in this manuscript, the biggest challenges moving forward are primarily concerned with the biostatistical and bioinformatics side of the analysis as the various NGS platforms generate a large amount of sequence data and associated quality scores. Various tools have been

developed to analyze data and are generally divided according to the starting sequence type (i.e., amplicon, metatranscriptome or metagenome). The structure for these protocols are generally similar and allow for essentially the same treatments for each data type. First, quality filtering is performed, followed by sequence identification, and, finally visual representations of the system are developed. Quantitative Insights into Molecular Ecology (QIIME) is one common tool used to treat sequences generated by the amplicon approach (Bittinger et al. 2010). QIIME is a customizable software package that removes low quality sequences, groups the remaining sequences into operational taxonomic units (OTUs) that parallel either genera or species depending on the gene region and similarity threshold, and assigns taxonomy. Once taxonomy is assigned to the OTUs, the relative abundance of taxa in various samples can be visualized in various forms such as barcharts, phylogenetic relatedness trees, or nonmetric multidimensional scaling plots using another QIIME script, R, or any desired statistics software (Dixon 2003). Currently, QIIME operates most smoothly with 16S rRNA bacterial surveys. For fungi, it is slightly more cumbersome at the taxonomy step when the output needs to be reformatted and taxonomy can then be assigned using appropriate databases such as SILVA, UNITE or those available in RDP Classifier (Abarenkov et al. 2010; Wang et al. 2007; Yilmaz et al. 2013). MOTHUR is an alternate to the QIIME protocol which is commonly used for amplicon-based surveys (Schloss et al. 2009). MG-RAST and the Galaxy framework are the preferred toolbox for metagenomic analyses (Blankenberg et al. 2001; Glass et al. 2010). As for metatranscriptomic analyses, there are few toolboxes available, however the Galaxy framework appears to be most straight-forward as it requires minimal programming familiarity, is very reproducible, and highly transparent (Blankenberg et al. 2001).

One example of how NGS informs bioremediation remedies was when metagenomic and amplicon-based approaches were integrated in a study performed on microbial mats treating wastewater from hydraulic fracturing (Akyon et al. 2015). This study coupled physical observations of biodegradation with amplicon surveys and metagenomics to describe the microbial community and degradation capacity of different microbial mats. The experimental design examined microbial mats in both synthetic produced water and actual produced water from hydraulic fracturing. Metagenomic sequencing used Illumina's Nextera XT DNA sample preparation kit. Sample preparation can be labor intensive and slight variations in protocols can cause different results. Thus, standard kits such as the Nextera XT kit improve the reliability, consistency, and overall practicality of omic techniques. Sequencing was performed using Illumina MiSeq with the V2 reagent chemistry. QIIME was used to treat amplicon data and Greengenes supported taxonomy classification. Metagenomic data was processed using CLC Genomics Workbench and MG-RAST to annotate genomes. Subsystems pathways and the KEGG Orthology functional database assigned function to the genes. Akyon et al. found that microbial mats were capable of removing 1.45 mg of chemical oxygen demand per gram each day at a total dissolved solids concentration of 91,351 mg/L. Metagenomic and amplicon-based community analysis found a highly adaptive microbial community that changed depending on the specific waste water treated and possessed functions capable of removing guar gum (which contributes to chemical oxygen demand) under salinities relevant to hydraulic fracturing produced waters. This suggested, with great confidence, that microbial mats are capable of treating hydraulic

fracturing produced waters. Similar environmental studies used metagenomic and amplicon-based approaches to find a level of ethylenediaminetetraacetic acid (EDTA) that enabled bioaugmented bacteria to sustain effective hydrocarbon biodegradation in the microbial community (Al Kharusi et al. 2016) and to track how bioaugmentation affects the *in situ* community to enhance degradation (Festa et al. 2016).

Concluding Remarks

Over the last several decades, investigations relating to the biological treatment of pollutants have been improved using new molecular tools. These improvements are continuing with the development of inexpensive metagenomic and metatranscriptomic protocols which promise to provide researchers with even more relevant information. Metagenomic analyses enable rapid scanning of the pollution-associated microbiome for microbes that possess metabolic capacity to degrade toxins, discovering new microbes for use in bioremediation. Identifying key degraders and microbial synergies in highly polluted waters and soils can lead to optimized assemblies of microbial communities capable of addressing new problem sites. Metatranscriptomic analyses could complement metagenomics analyses by pointing to the best conditions to stimulate degradative gene expression, creating better bioremediation approaches. However, the power of omics to improve bioremediation approaches requires the continuous population of sequence databases and more user-friendly protocols to enable quick, efficient predictions of bioremediation outcomes. Chemical analyses of proteins (proteomics) and metabolites (metabolomics) are beginning to be used to complement genomic and transcriptomic studies. For example, Dore et al. predicted the proteins and extracellular enzymes secreted by a fungus from analyzing the genome and transcriptome and then used liquid chromatography combined with mass spectrometry to detect what was actually secreted by the fungus under environmental conditions (Dore et al. 2015). In another study, Konopka and Wilkins used meta-transcriptomics and meta-proteomics to predict and verify *Geobacter spp.* response to a carbon biostimulation amendment (Konopka and Wilkins 2012). The potential impact of omics on environmental remediation calls for training environmental engineers in interdisciplinary skills that equip them to use omic data to inform new remedies to address other emerging problems in a sustainable way through bioremediation.

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Figure 1.

Coding function of the cell illustrated by a simplified cross section of a generic bacterium (a). Most bacteria contain at least two important features to aid in the genetic process—the chromosome and the ribosome. The chromosome consists of DNA and is where transcription takes place (b). DNA consists of adenine (A), thymine (T), cytosine (C) and guanine (G) bases. These bases form bonds with the bases on the opposite strand: A always pairs with T and C always pairs with G. When the microorganism needs to carry out a function, RNA polymerase transcribes the section needed into a single-stranded RNA transcript (and substitutes uracil (U) for T). The RNA transcript then directs the ribosome to assemble amino acids into proteins (c).

Figure 2.

Molecular tools used to inform studies in bioremediation and wastewater treatment throughout the past few decades according to advanced searches of the Web of Science database. Query topics for each decade and technology were as follows: TS=(Cloning AND bioremediation) OR TS=(cloning AND wastewater AND treatment); TS=(DGGE AND bioremediation) OR TS=(DGGE AND wastewater AND treatment); TS=((quantitative PCR) AND bioremediation) OR TS=((quantitative PCR) AND wastewater AND treatment) OR TS=((reverse transcript* quantitative PCR) AND bioremediation) OR TS=((reverse transcript* quantitative PCR) AND wastewater AND treatment); TS=((ion torrent) AND bioremediation) OR TS=((Ion torrent) AND wastewater AND treatment) OR TS=((Illumina) AND bioremediation) OR TS=((Illumina) AND wastewater AND treatment) OR TS=((nanopore) AND bioremediation) OR TS=((nanopore) AND wastewater AND treatment) OR TS=((pacific biosciences) AND bioremediation) OR TS=((pacific biosciences) AND wastewater AND treatment).

Figure 3.
Chronology of Key Discoveries in the Field of Microbiology and Molecular Biology
Relevant to Bioremediation.

Figure 4.

Genetic cloning overview. Genetic cloning consists of three steps. First, the unculturable DNA fragment is attached to a well-studied host organism's circular DNA (a). Then, the altered DNA is inserted into the host organism (b). Finally, the host organism multiplies, copying the unculturable fragment (c).

Figure 5.

Sanger Sequencing. Sanger sequencing is a common method used to sequence DNA, one strand at a time. First, the strands must be separated (a). Then, a primer marks the place where DNA polymerase begins adding nucleotides (b). Four sequencing reactions happen, one for each base A, T, C, and G. In the G reaction (c), DNA polymerase incorporates dATP, dCTP, dGTP, and dTTP until it adds a chain-inhibiting ddGTP to complement the C base, stopping the process. Here, a ddGTP is incorporated to complement the 7th and 8th base, inferring that the 7th and 8th bases in the sequence are Cs. (d) Incorporating these chain-inhibiting bases creates fragments of different sizes from each of the four different reactions. These different fragments are then sorted by length and their complementary bases inferred (the G reaction fragments tell the location of the Cs, the As give T locations, etc.).

Figure 6.
Polymerase Chain Reaction

Table 1

Table of Commonly used terms

Term	Definition
Amplicon	A DNA or RNA segment that results from artificial amplification, often via polymerase chain reaction.
Bioinformatics	The use and development of methods and software tools to analyze and interpret biological data.
Biostatistics	The application of statistics to biological topics.
Conserved gene	A span of DNA sequence that is shared among members at a taxonomic level and varies little between members within that level.
Copy number	The number of times a specific DNA sequence appears within an organism's genome.
ddNTP	the collective abbreviation for the four dideoxynucleotides ddATP (dideoxyadenosine triphosphate), ddCTP (dideoxycytosine triphosphate), ddGTP(dideoxyguanine triphosphate), ddTTP (dideoxythymine triphosphate)
Emulsion PCR	Polymerase chain reaction performed within small droplets of one liquid in a second liquid in which it is not miscible or soluble.
GC clamp	A short sequence of guanine and cytosine bases
G/C content	The percentage of guanine and cytosine bases
Genome	The sum of all of the DNA in an organism.
Housekeeping gene	A sequence of DNA that codes for a basic cellular function and is therefore expressed at all times.
Hybridization	The process of allowing complementary single-stranded DNA or RNA strands to form a single double stranded molecule.
Metagenome	The sum of all of the genes in every organism present in a given environment
Metatranscriptome	The sum of all of the transcripts expressed by organisms under a set of conditions.
Operational taxonomic unit (OTU)	A group of DNA sequences that resulted from amplicon-based NGS. These parallel species or genera, depending on variation in the target gene region amplified.
Up or Down Regulated Gene	A differential expression in gene copy numbers relative to baseline expression obtained by comparing gene copy number resulting from a given treatment with that resulting from a control.
Transcriptome	The sum of all of the messenger RNA (transcripts) transcribed by an organism.

Table 2

NGS Platform Comparison

Instrument	Sequencing Principle	Maximum Read Length	Cost per Gigabase, USD	Error Rate,% ^a
Illumina MiSeq v3	Synthesis fluorescence	600 bp	109	~0.1
Illumina HiSeq 2500- rapid run	Synthesis fluorescence	300 bp	46	~0.1
Illumina NextSeq 500	Synthesis fluorescence	300 bp	40	~0.1
Ion Torrent PGM 318 chip	pH fluctuations	400 bp	460	~1
Ion Torrent Proton I	pH fluctuations	175 bp	82	~1
Pacific Bioscience RS II	Synthesis fluorescence	3000	1111	14 ^b
Oxford Nanopore GridION 8000 (forecast)	Nanopore	10000	10	4

^aError rates are based on 2012 manufacturer supplied data and are not entirely comparable as the technologies are prone to different kinds of error rates (ex. Illumina tends towards substituting erroneous bases while Ion Torrent tends towards inserting extra bases and deleting bases) (Glen 2011)

^bError rate for RS chemistry, RS II data unavailable (Quail et al. 2012).