eDNA Glossary

3' end (three prime end) – Refers to the directionality of a DNA strand and is often used in the context of the amplification primer. Taq polymerase can only extend and synthesize target DNA in the 5' to 3' (upstream) direction. Therefore, amplification primers that mediate the DNA replication process are often carry a 5' and 3' notation to signify the direction of synthesis. For example, a forward primer would be denoted as 5'-ATGTCGACTGATCACC-3' and a reverse primer as 3'-TCGATCGATCTTCCAC-5'.

5" end (five prime end) – See 3' end.

A

Accuracy – Accuracy: a qualitative measure of the agreement between a measured value and a true, but generally unknown, value (see "bias", Mosher et al. 2019).

Amplicon – The short lengths of DNA obtained during the PCR process. These can be referred to as amplicons or PCR products. Amplicons for eDNA are usually 100-150 nucleotide base pairs (bp) in length, although at times are slightly smaller or larger, particularly for metabarcoding efforts where amplicons can be several hundred bp in length.

Amplification – Laboratory process where millions of copies of target DNA are produced through the process of polymerase chain reaction (PCR) to allow for visualization and measurement.

Annealing Temperature – The temperature at which primers bind to single stranded DNA template during PCR. Because the primers mediate replication of the DNA, it is essential that the annealing temperature be optimized for each primer set. Proper optimization will affect assay efficiency, sensitivity, and specificity.

Assay – An assay refers to the primers (forward and reverse) and fluorescently labeled probe that are used for species detection. Depending on the context, assay may also include the reaction chemistry (type of taq polymerase) and thermocycler conditions. Assay is often used interchangeably with the term eDNA marker.

B

Barcode genes – Refers to genes that can be used for species identifications. Different regions of DNA mutate at different rates and some change at just the right rate to be stable within a species but different between species. These are known as barcode genes. Most barcode regions are phylogenetically informative such that the higher taxonomic source of a sequence can be inferred even if the actual sequence has no exact match in a database of reference sequences. Environmental sequences can also be assigned to operational taxonomic units (OTUs), which may represent taxonomic

groups not yet recognized by systematics or not adequately represented in sequence databases (https://www.naturemetrics.co.uk/edna-glossary/).

Barcoding – Is a method of species identification based on the DNA sequence of a section of a specific gene or genes. By comparing the DNA sequence obtained from an unknown organism to that of a reference database, an individual sequence can be uniquely identified based on its similarity to sequences of known origin. This method is generally used to identify individual specimens from which a muscle, blood, hair or other tissue sample is obtained. This in in contrast to metabarcoding which allows for the simultaneous identification of many specimens from a single sample based on sequence similarity (see '*Metabarcoding*').

Bioinformatics – data processing science that takes the raw sequence data from high-throughput sequencing (often many millions of sequences) and transforms it into usable ecological data. Key steps for metabarcoding pipelines include quality filtering, trimming, merging paired ends, removal of sequencing errors such as chimeras, clustering of similar sequences into molecular taxonomic units (each of which approximately represents a species), and matching one sequence from each cluster against a reference database. The output is a species-by-sample table showing how many sequences from each sample were identified as each species (https://www.naturemetrics.co.uk/edna-glossary/).

Blank – an "empty" eDNA sample that is collected without filtering/centrifuging actual water from the sampling environment. Blanks are a type if field control used to evaluate whether there is contamination present in the samples/equipment and, by extension, if any positive detections are valid. Depending on the timing and location that the blank sample is prepared, these controls can evaluate different parts of the sample collection process. See Bottle/Field Control, Cooler Control, Pump/Equipment Control, Dry Control, Rinsite Control, and Process Control for details on specific blank collection procedures.

Bottle/Field Control – A negative field control. The control bottle is opened at the field collection site, re-sealed after 5 seconds, and then fully submerged into the field water (USFWS 2019). Bottle controls are stored with the other sample bottles both before and after being submerged. It is important to replicate the exact same storage, and transportation process used for field samples.

C

Centrifugation – Sample processing method utilizing a centrifuge whereby sample tubes (ordinarily containing a liquid) are spun at very high speeds that results in concentrating of particles suspended in the liquid to the bottom of the tube and subsequent separation of the particles from the liquid medium. For environmental DNA samples, centrifugation is an alternate concentration strategy to filtration.

Centrifuge Control – A 2-part negative field process control. The first control is created with a batch of tubes that are being centrifuged in order to collect the material at the bottom of the tube. The centrifuge process control should be labeled with the sample ID range that was included with the batch of tubes that were centrifuged at the same time.

The second centrifuge process control is created when tubes are set out to dry. Controls are prepared by placing a clean centrifuge tube in a clean rack in the drying area along with the range of samples that were placed in the hood to evaporate. The labelling for the control should contain the sample ID range that makes up the batch the control was associated with (example 1-46). Both the tube and lid should be labeled since they are separated during the evaporation process.

Community DNA – DNA extracted from a mixture of different organisms. Could be eDNA (environmental samples almost always contain DNA from a mixture of species) or organismal DNA (e.g. homogenized insect trap samples, plankton tows, larval fish samples, or gut content samples) (https://www.naturemetrics.co.uk/edna-glossary/).

Contamination – the transfer of genetic material (or derivative) from the target organism to samples or surfaces that did not naturally contain the organism's genetic material. Contamination can occur during field work (sampling) or in the laboratory if sampling and/or processing protocols are not followed (Mosher et al. 2019).

Cooler Control – A negative field control. Cooler controls remain sealed in the cooler and are not exposed to the atmosphere at the field collection site nor are they submerged in the field water supply. It is important to replicate the exact same storage, and transportation process used for field samples.

Conventional Polymerase Chain Reaction (cPCR) – See end-point PCR

Ct:(Cycle Threshold) – This is a metric used to determine a positive PCR/qPCR reaction which is measured by the accumulation of a fluorescent signal. Ct is the number of cycles required for the fluorescent signal to exceed background levels (the threshold).

D

Degradation – the process whereby DNA is broken down into smaller fragments. DNA obtained from eDNA samples is often 'degraded' and requires shorter length target amplicons for successful amplification. eDNA degrades through a variety of processes including exposure to chlorine bleach, ultraviolet light, higher temperatures, turbulence, or microbial activity.

Detection – A result that indicates a target taxon's DNA is present at the sample collected

Detection Probability – The probability of detecting an organism's DNA when present at a site, in a sample, or in a PCR replicate (also known as false negative probability; see "sensitivity" and "Type II error", Mosher et al. 2019).

Digital PCR (dPCR) – PCR assay in which a sample is partitioned into thousands of independent reactions that are each analyzed as end-point PCRs. The proportion of positive reactions can be directly related to the concentration of target in the original sample without need of a standard curve and may allow for a higher tolerance to PCR inhibitors present in the sample (see Quan et al. 2018).

Digital droplet PCR (ddPCR) – See also digital PCR. ddPCR is a method for performing digital PCR that is based on water-oil emulsion droplet technology. A sample is fractionated into 20,000 droplets, and PCR amplification of the template molecules occurs in each individual droplet. ddPCR technology uses reagents and workflows like those used for most standard probe-based qPCR assays (https://seakfhp.org/wp-content/uploads/2019/04/eDNA_terminology.pdf).

DNA extraction – The process of separating DNA from an environmental sample, organismal tissues, cellular material, or any other type of biological sample that contains DNA. Many DNA extraction methods are available, and their use is primarily determined by sample type, cost, ease of use, laboratory proficiency, efficiency, and resulting DNA quality.

Dry Control – A negative field control. Dry control filters are attached to the pump system while it is turned on, exposing the filter to the field site atmosphere and collection process, but no water is used. It is important to replicate the exact same collection, storage, and transportation process, but without filtering any water.

E

Endogenous DNA – DNA that originates from the target species local to the collection location. See also exogenous DNA.

End-point PCR (epPCR) – A PCR assay in which the presence of the target amplicon is assessed after the reaction protocol has completed. Assessment of amplification is typically made by passing resulting PCR products through an agarose gel in which they are stained and visualized. Also referred to as conventional PCR (cPCR, see also Gel Electrophoresis).

Environmental DNA (eDNA) – DNA deposited in the environment through a variety of biological processes (e.g. excretion, shedding, mucous, slime, saliva, gametes, etc.) from single-celled (i.e. bacteria) or multicellular organisms (i.e. fish, amphibians, reptiles, mollusks, etc.). eDNA can be collected in environmental samples (e.g. water, sediment, soil, air, etc.) and used to identify the organisms from which it originated. Although eDNA is most accurately referred to as a sample type, it is also often referred to as a method, laboratory application, analysis, or technique.

As defined in Taberlet et al. 2018, 1st Edition, “Environmental DNA: For Biodiversity Research and Monitoring”, eDNA is defined as: Environmental DNA is a complex mixture of genomic DNA from many different organisms found in an environmental sample (Taberlet et al. 2012a). Soil, sediment, water, or even feces are considered as environmental samples, which can also include the material resulting from filtering air or water, from sifting sediments, or from bulk samples (e.g. the whole insect content of a Malaise trap). Alternatively, environmental DNA can be defined from another perspective (i.e., the objective of the study). In this case, eDNA corresponds to DNA extracted from an environmental sample with the aim of obtaining the most comprehensive DNA-based taxonomic or functional information as possible for an ecosystem under consideration. Total eDNA contains both intracellular and extracellular

DNA (Levy-Booth et al. 2007, Pietramellara et al. 2009). Intracellular DNA originates from living cells or living multicellular organisms that are present in the environmental sample. Extracellular DNA results from cell death and subsequent destruction of cell structures, and can be degraded through physical, chemical, or biological processes. For example, DNA molecules can be cut into smaller fragments by nucleases. After its release, extracellular eDNA may be adsorbed by inorganic or organic surface-reactive particles such as clay, sand, silt, or humic substances.

eDNA assay – Laboratory procedure to detect the presence of DNA from a target species in an environmental sample.

Exogenous DNA – DNA that originates from outside of the study system and is transported into the sampling environment and/or water supply. Examples of exogenous DNA sources include but are not limited to boats (bilge water, live wells, biofilms, etc.), fishing equipment, wastewater outfalls, feces from migratory or wide-ranging species like birds, anglers and hunters transporting then disposing of carcasses.

F

False Detection – A result that indicates that a species is present when it is not.

False Non-Detection – A result that indicates that a species is not present when it is.

False Detection Probability – the probability of detecting an organism when not present at a site, in a sample, or in a PCR replicate (see “specificity” and “Type I error”, Mosher et al. 2019).

Filtration – Passing water samples through a membrane that captures eDNA-containing particles. The pore size and membrane material are important considerations in selecting a filtration strategy.

Filter Pore Size – The size of the pores in filters used to separate eDNA from collected water, often measured in µm. Filer pore size influences the sizes and quantities of retained eDNA fragments and ambient particulate matter including inhibitors. Environmental DNA detections efficiencies can be significantly affected by filter pore size choice.

Field Control – negative controls used to evaluate contamination during field sampling and transport to the lab.

Filter Control – A negative field process control. A new filter is placed in the filter cup and clean, target DNA-free water is poured through it. The filter is immediately replaced, and the field sample is processed through the same filter cup.

Flocculation – The process of adding a chemical agent to a solution to cause a target substance to be deposited as a flakes or floccules to aid in physically removel. Following eDNA sample collection,

chemicals such as lanthanum chloride can be added to water samples to cause DNA along with other particulates to form floccules that can be collected and used during subsequent analyses. Flocculation allows greater sample volumes to be collected than precipitation and is an alternate eDNA concentration strategy to filtration and centrifugation; although, it requires the transportation and storage of large amounts of liquid compared to filtration and field centrifugation.

G

g-Block – Synthetic double stranded DNA created specific to each qPCR marker. g-Blocks include both the primer and probe binding locations and serve as positive control material for amplification and quantification of a given DNA target based on serial dilutions.

Gel Electrophoresis – technique commonly used in laboratories to separate charged molecules like DNA, RNA and proteins according to their size. Charged molecules move through a gel when an electric current is passed through it.

Grab Sample/Bottle – samples taken directly by hand with an open container, by skimming or dipping at or near the surface. A dip sample is a simple extension in that the container is affixed to a handle to extend the reach of the sampling individual. The grab sample may be in the form of a filter cup that is already attached by tubing to a pump/filter apparatus, or may also be taken at a defined depth through use of a submerged sampling device (i.e. Van Dorn, Kemmerer, etc.)

H

High-throughput sequencing (HTS) – Sequencing technology that produces millions of sequences in parallel. Enables thousands of different organisms from a mixture of species to be sequenced at once, so community DNA from a sample can be sequenced. Also known as Next-Generation Sequencing (NGS) or parallel sequencing (https://www.naturemetrics.co.uk/edna-glossary).

I

Internal Positive Control (IPC) – A small amount of amplifiable DNA that is added to each environmental sample to evaluate and measure whether samples demonstrate PCR inhibition and to what degree. DNA samples that display PCR inhibition must be purified further until confidence in PCR results can be obtained.

Inhibition – Also referred to as PCR inhibition. During DNA extraction of environmental samples, various chemical compounds can co-purify along with the DNA and interfere (inhibit) the PCR amplification process. High levels of inhibition can lead to false negative results in PCR reactions. Inhibition is measured and monitored through the use of internal positive control DNA.

Inhibitor – Substances that interfere with the PCR amplification process and can cause PCR inhibition. See Inhibition.

In-situ filtration – Filtration that occurs within the sampled body of water. In-situ filtration often involves in-line filtration of water through a filter assembly attached to a pumping device.

J

K

L

Lab Control – Lab controls are both negative and positive controls. Negative lab controls assess the presence of contamination during sample processing in the laboratory, and screen for sample inhibition when necessary. Positive lab controls evaluate whether the sample DNA was successfully extracted and the PCR reaction properly prepared.

Limit of detection (LOD) – the smallest quantity of a target DNA that can be identified using a particular technique, with some level of confidence (e.g., 95%). (Mosher et al. 2019 and https://www.sciencebase.gov/catalog/item/57e92b77e4b09082500c90ea).

Limit of quantification (LOQ) – the smallest quantity of a target DNA that can be accurately quantified using a particular technique, with some level of precision (e.g., 35% Coefficient of Variation). The LOQ is typically higher than the LOD, because at very low concentrations of target DNA, the PCR may not sufficiently approximate the expected exponential growth of the DNA target upon which quantification is based (Mosher et al. 2019 and https://www.sciencebase.gov/catalog/item/57e92b77e4b09082500c90ea).

Long-term preservation - preservation methods intended to preserve eDNA samples for >24 hours (often indefinitely). Examples of commonly employed long-term eDNA preservation techniques include freezing, desiccation, and the addition of buffer solutions. The expected shelf life and viability of stored samples can vary between storage methods.

M

Marker – A DNA sequence that is diagnostic of a taxonomic group or a specific species. Presence of the target DNA is detected by using specific primers, or primer/probe combinations, in PCR, or by sequencing. The term marker is generally used to reference the primer/probe combinations required to amplify a given DNA target. The term marker and assay are often used interchangeably.

Marker validation – Controlled experimental process by which primer/probe combinations are evaluated for use on eDNA samples. Throughout the process, a marker's sensitivity, specificity, and

optimal reaction conditions must be established. Validation includes sequence comparisons to existing sequence data for both target and non-target species, controlled laboratory testing, and testing of field samples.

Metabarcoding – Rapid biodiversity assessment that combines DNA barcoding and high throughput sequencing techniques. This combination allows identification of multiple species from community DNA. PCR is carried out with taxon-specific primers (e.g. all bivalves in a sample, all fish in a sample, all salmonids in a sample, etc.), followed by high-throughput sequencing and bioinformatics processing. The process can identify hundreds of species in each sample, and 100+ different samples can be processed in parallel to reduce sequencing cost.

Metagenetic sequencing – See metabarcoding. Metagenetic sequencing is often used interchangeably with metabarcoding. Some make the subtle distinction that metabarcoding targets a single barcode gene while metagenetic sequencing targets multiple barcode genes.

Metagenomic sequencing – Sequencing of random genomic fragments from complex environmental mixtures. Metagenomics can provide a less-biased assessment of community composition because taxonomically informative sequencing is not restricted to one or a few genetic barcodes but is much less efficient than metagenetics for this purpose because most randomly captured fragments will be uninformative. Metagenomics is more traditionally used to identify functional classes of genes present in a complex microbial sample, in order to predict metabolic capabilities of the community as a whole.

Minibarcodes – The traditional DNA barcode is a 650bp portion of the COI (mitochondrial cytochrome oxidase subunit I) gene and was suitable for Sanger sequencing. Newer high throughput sequencing technologies are restricted to shorter read lengths and required a redesign of the traditional 650bp barcode. These shorter (100-450bp) barcodes are referred to a minibarcodes. Shorter barcodes are also advantageous for eDNA work as they are more reliably amplified from degraded or fragmented DNA.

MIQE standard – A minimum information standard for quantitative PCR experiments adopted by some journals and often used as a standard in peer review. eDNA experiments have QC requirements that extensively overlap with the MIQE standard. For more info see, The MIQE Guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55(4): 611-622. doi: 10.1373/clinchem.2008.112797.

Mitochondrial DNA (mtDNA) – DNA derived from the mitochondria of a cell and ordinarily is the general target for most eDNA assays. Mitochondria are organelles located in the cytoplasm of a cell and produce energy. Because mitochondria play such an important role for the cell, ordinarily a single cell contains many dozens to many hundreds of mitochondria, each containing a mitochondrial genome. By default, mitochondrial DNA increases the probability of detection of an eDNA target due to the increased number of copies residing in each cell.

N

Negative control – Samples lacking target DNA. Negative controls are incorporated in both field and laboratory steps of sample processing to monitor for false positive detections resulting from contamination.

Next-generation sequencing – Catch-all term referencing modern high-throughput sequencing technologies and their applications. Common platforms for surveying environmental sequences are Illumina, Roche 454, and Ion Torrent, in various configurations. The specifications of these platforms differ in the lengths of fragments than can be sequenced, the number of independent fragments that can be sequenced per run, the duration and cost of the sequencing run, the error distribution of the data, and the number of independent samples that can be combined in a sequencing run. The key innovation shared by all classes of next-generation sequencing technology is that thousands to millions of independent reactions can be recorded in parallel.

Nuclease-free – Certified not to have nuclease (DNA-degrading) activity above a designated threshold. It is important that eDNA storage solutions be based on nuclease-free reagents.

O

Operational taxonomic unit (OTU) – A sequence that is classified as distinct at some threshold from other environmental sequences or known reference sequences and is postulated to derive from a phylogenetically distinct group of organisms. Environmental DNA sequences generated at a barcode locus are often clustered into distinct group (OTUs) that are assigned provisional taxonomic positions and then analyzed under the assumption that the clustering process approximates the genetic distinctiveness of species or higher-level taxa. OTUs are useful for estimating abundance and diversity metrics that are like what would be obtained if each species in a community sample were fully characterized at the barcode locus, which is rarely the case in practice.

Organismal DNA - DNA sampled directly from the target organism through whole organism collection, swabbing, blood sampling, fin clips etc. This practice generally results in highly concentrated and high-quality DNA sourced from a single individual. The species identity and collection location are definitively known. Overall, there are fewer uncertainties than for eDNA.

P

PCR assay – See **Assay**

Polymerase chain reaction (PCR) – An enzymatic reaction used to make copies of a specific DNA segment through a series of heating and cooling steps. This exponential amplification process is mediated by primers and generates thousands to millions of copies of a particular DNA target. Variants of a PCR assay include end-point PCR (or “conventional” PCR), in which the presence or absence of the

intended target is inferred only after the reaction completes; quantitative PCR, in which the amount of input target DNA is inferred by monitoring the change in DNA concentration as the reaction proceeds; and digital-droplet PCR, which also quantifies the amount of input target DNA by creating many random independent reactions from the input solution and determining the proportion of which produce an amplification product.

Pooled samples/replicates – Samples or replicates that are combined prior to analysis. An example of pooled sample is a group of grab samples collected along a transect at the same site and then filtered through a single filter.

Positive control – Samples to which a known target DNA has been added. Positive controls should be incorporated in both field and laboratory steps of sample processing to identify methodological issues contributing to false negatives. These issues might include degradation during storage, PCR inhibition, or sequencing bias. Internal positive controls are added directly to samples to be analyzed, whereas external positive controls are added to a DNA-free solution. Internal positive controls are typically used to provide evidence of inhibition during a qPCR reaction, whereas external positive controls are used as evidence of reagent quality, DNA extraction efficiency, and proper thermal-cycler function.

Precipitation – The process of adding a chemical agent to a solution to cause a target substance to be deposited as a solid that can be physically removed. Following eDNA sample collection, chemicals such as ethanol-sodium acetate can be added to water samples to cause DNA to form a precipitate that can be collected and used during laboratory analysis. Precipitation is an alternate eDNA concentration strategy to filtration and centrifugation; although, its application is typically limited to small sample volumes.

Preservation – The steps taken to stabilize the eDNA in a sample by countering the main forces of degradation that threaten eDNA integrity. Environmental DNA preservation methods often take the form of short-term or long-term preservation methods.

Primary Equipment – Field equipment that comes in direct contact with the eDNA sample (e.g. sample collection bottles, filters, filter funnels, filter cups, forceps, filter storage tubes, preservation additive, etc.).

Primers – Short sections of synthesized DNA that bind to either end of the DNA segment to be amplified by PCR. Can be designed to be specific to a particular species (so that only that species' DNA will be amplified from a community DNA sample), or to be very general so that a wide range of species' DNA will be amplified. Good design of primers is one of the critical factors in DNA-based monitoring.

Primer bias – An artifact of metabarcoding studies when generic or universal primers preferentially amplify one or a group of species over others. This preferential amplification can lead to false negative detections and or cause read count data to be skewed towards the species that amplify with a higher degree of efficiency.

Probe – An oligonucleotide that complements the intended target and is added along with primers to a qPCR. The probe matches a sequence in between the two primers and does not contribute to amplification of the target sequence. The probe produces a fluorescent signal that is measured by a qPCR platform. Several types of probes are successfully used in eDNA work. See Probe-based qPCR.

Probe based qPCR – Probe-based qPCR functions by recognition of a specific sequence on the desired PCR product. Unlike SYBR® Green qPCR methods, that use an intercalating dye to bind all double-stranded DNA, probe-based qPCR uses fluorescent-labeled target-specific probes. This technique yields increased specificity and sensitivity since only specific DNA molecules will be labeled. Other fluorescent dyes can also be combined with probe-based qPCR to label and quantify various sequences.

Process Control: – A negative field control. Process controls are collected when the sample concentration process (filtering or centrifugation) is not performed at the field sample collection site (such as back a vehicle or in an equipment trailer). The two main kinds of process controls are Filter Controls and Centrifuge Controls.

Pump/Equipment Control – A negative field control. Pump controls are collected at the same locations using the same procedures and equipment as field samples, but with "clean" water (such as deionized, distilled, or well water that is known to be free of target species DNA) poured onto and pumped through the filter. It is important to replicate the exact same sample collection, storage, and transportation process for water sampled in the field.

Q

qPCR assay – See **Assay**

Quantitative PCR (qPCR) – A PCR reaction incorporating a colored dye that fluoresces during amplification, allowing quantification of a given target in the reaction. Often used with species-specific primers where detection of amplification is used to infer presence of the target species' DNA in the sample. If the target DNA is not present in the sample, no fluorescence will be detected. The high specificity of the qPCR method makes it ideal for situations where a single target is required. See also real-time PCR.

R

Real-time PCR – A PCR reaction incorporating a colored dye that fluoresces during amplification, allowing a machine to track the progress of the reaction in "real-time." This term is often used interchangeably with quantitative PCR (qPCR).

Reference Databases – A collection of DNA sequences (usually from barcode genes) that have been obtained from species of known identity. Sequences from unidentified organisms – obtained either by

Sanger sequencing or high-throughput sequencing – are compared against a reference database to make species identifications. Databases can be curated (e.g. the Barcode of Life Database – BOLD – www.boldsystems.org (Ratnasingham and Hebert, 2007) or uncurated (e.g. GenBank – www.ncbi.nlm.nih.gov). In curated databases, identifications are scrutinized and verified; in uncurated databases they are not. GenBank is therefore far more extensive than BOLD but contains many more errors.

Replication – DNA replication is the process by which copies of DNA are made during cell division. This process is artificially achieved in the laboratory through the PCR process.

Rinsate Control – A negative field control. Studies that reuse sampling equipment in the field need to decontaminate gear between sample collections (often with bleach or DNA Away solution). Rinsate controls evaluate if field decontamination was successful. A rinsate control is collected using the decontaminated gear and clean (deionized, distilled, or target DNA-free water) water, followed immediately by collection of the actual sample using the same equipment but with water from the field.

S

Sanger Sequencing – Traditional DNA sequencing. Each reaction produces a single sequence, so it only works on amplified DNA of a single species. As such, Sanger sequencing is not appropriate for use on community DNA or most eDNA sample types.

Short-term preservation – preservation methods often taken immediately after sample collection that are intended to reduce DNA degradation rates for <24 hours following collection. Cooler storage is a commonly employed short-term preservation method when collecting water samples.

Shotgun sequencing – a strategy in which all DNA sequences in a sample are sequenced in an approximately random fashion without any PCR-based selection of genetic loci. Not commonly used for eDNA at present because techniques for identifying the source organisms are not well developed or validated for this use.

Secondary Equipment – Field equipment that is used in every sample collection (coolers, bottle racks, pumps, pump tubing, “grabber arms”, etc.) but that does not contact the sample directly.

Sensitivity – The minimum number of target copies in a PCR reaction that can be detected. Typically, sensitivity is expressed as the limit of detection (LOD), which is the concentration that can be detected with reasonable certainty (95% probability is commonly used) with a given analytical procedure.

Specificity – The ability of PCR primers and/or probe to detect the appropriate target sequence rather than alternative, nonspecific targets also present in a sample. In most instances a PCR assay is referred to as specific if it amplifies only the species for which it was designed.

Standard curve – Used to calibrate qPCR assays, serially-diluted standard solutions with known concentrations of the target template are processed in conjunction with the samples to be quantified.

The standard curve estimates reaction efficiency as well as a conversion factor for calculating target copy number from the primary measured variable for each sample.

SYBR Green – A fluorescent dye that binds with all double-stranded DNA in a sample or PCR reaction. SYBR Green is used in qPCR assays to measure DNA accumulation during the amplification process. Unlike probe-based qPCR assays, SYBR Green does not convey added specificity to the assay because it will bind to any double-stranded DNA in the reaction, potentially producing non-specific fluorescence. As a result, SYBR green a less appropriate choice for species-specific detection from eDNA.

T

Taq Polymerase – an enzyme critical for DNA replication through PCR amplification. Taq polymerase binds to single stranded DNA and adds complimentary nucleotides to the DNA strand to which it is bound, creating a copy. Taq polymerase also functions in DNA proof reading, where it finds mismatched nucleotides and replaces them with complementary nucleotides.

Tertiary Equipment – Field equipment that is used to access the collection site or transport equipment to and from the collection site but is not a part of the collection system (boats, backpacks, drybags, PFDs, waders and boots, etc.).

Thermocycling – a series of temperature cycles used in PCR reactions that allows for Taq Polymerase to activate, primers to anneal, and DNA replication to occur, resulting in target DNA replication. See Polymerase Chain Reaction.

Threshold cycle (Ct) – A unit of measure referring to the intersection between an amplification curve and a threshold line. It is a relative measure of the concentration of target in the PCR reaction. Often used interchangeably with quantification cycle (Cq).

Type I Error – the probability of detecting an organism when it is not present in a sample (see "specificity" and "false positive probability", Mosher et al. 2019).

Type II Error – the probability of failing to detect the target organism when it is present in a sample (see "sensitivity" and "detection probability", Mosher et al. 2019).

U

V

W

X

Y

Z

Acronyms

cPCR - conventional end-point polymerase chain reaction
Ct – threshold cycle
Cq – quantification cycle
dPCR – digital polymerase chain reaction
ddPCR – digital droplet polymerase chain reaction
epPCR – end-point polymerase chain reaction
DNA – deoxyribonucleic acid
eDNA – environmental deoxyribonucleic acid
mtDNA – mitochondrial deoxyribonucleic acid
GenBank – Familiar name of a suite of biotechnology databases maintained by NCBI
HTS – high throughput sequencing
LOD – limit of detection
LOQ – limit of quantification
MIQE – minimum information for publication of quantitative real-time PCR experiments
NCBI – national center for biotechnology information
NGS – next-generation sequencing
OTU – operational taxonomic unit
PCR – polymerase chain reaction
qPCR – quantitative real-time polymerase chain reaction