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IN THE DISTRICT COURT OF THE FOURTH JUDICIAL DISTRICT OF THE

STATE OF IDAHO, IN AND FOR THE COUNTY OF ADA

STATE OF IDAHO, Plaintiff, V. BRYAN C. KOHBERGER, Defendant.	Case No. CR01-24-31665 MOTION TO AMEND WITNESS LIST AND SUPPLEMENT EXPERT DISCLOSURES

COMES NOW the State of Idaho, by and through the Latah County Prosecuting Attorney, and respectfully moves the Court for permission to amend the State's witness list and to supplement previously filed expert disclosures by adding Dr. Christian Westring as a potential expert witness.

The State makes this Motion to add the above-referenced witness to testify about an issue that the State believed had been resolved—namely, that Defendant was found *not* to be a contributor to a DNA mixture found under one victim's fingernail. The State proposed a stipulation to this effect, but Defendant informed the State that he will not so stipulate because he

prefers to present testimony about this fact. Thus, the State will likely need to call Dr. Christian Westring, to testify about the DNA mixture so that the jury can understand the State's position and not be misled into believing that there is factual dispute where none exists.

On February 24, 2025, Defendant filed a "Motion in Limine #5 Re: Inconclusive Data." This motion centered around a DNA mixture found under victim Madison Mogen's fingernail (Item 13.1) and the Idaho State Police Forensic Laboratory's (ISP Lab) conclusion that it was "inconclusive" whether Defendant was a contributor to this mixture. As Dr. Westring explained in his affidavit (see below), that conclusion is a result of limitations imposed on ISP Lab staff by certain internal policies; but the State did not intend to argue or imply that Defendant was a contributor to the DNA mixture, and it has maintained this position since the issue was first raised.

Thus, the State retained Dr. Westring to review the DNA testing and the computational analysis of the data. In response to the Defendant's Motion, on April 7, 2025, the State filed an affidavit for Dr. Westring (attached as Exhibit S-1). In summary, Dr. Westring opined: (1) that there is no published standard that dictates how to determine analytical thresholds; (2) ISP Lab concluded that the analysis was inconclusive with regards to Bryan Kohberger in accordance with their published standard operating procedures; and (3) based, on Dr. Westring's independent analysis, Bryan Kohberger was excluded as a potential contributor for Item 13.1. This motion was heard by the court on April 9, 2025. The Court issued an Order on April 18, 2025, ruling that the Defendant's motion was "DENIED as moot." (*See* page 6)

On May 9, 2025, the State sent the following proposed stipulation of facts to the defense for their consideration:

> A DNA sample taken from underneath a fingernail of Madison Mogen contained a mixture of DNA from three individual contributors. Madison Mogen and Kaylee

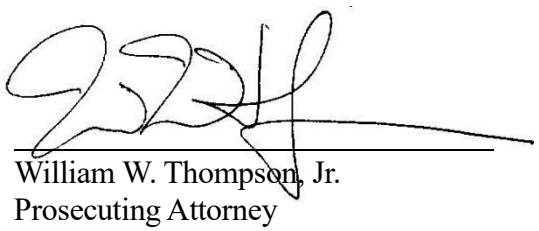
> Goncalves were identified as two of the three contributors to the mixture. Defendant, Bryan C. Kohberger, was excluded as a contributor to the mixture.

The proposed stipulation is attached as Exhibit S-2.

A month later, on Sunday, June 8, 2025, the State emailed defense counsel regarding the proposed stipulation since the State had not received a response to the above proposal. On June 9, 2025, defense counsel informed the State that they would not agree to the stipulation so that Defendant could present testimony on the issue.

Based on the above, it appears it may now become necessary for the State to call Dr. Westring as an expert witness in the State's case-in-chief to ensure that the jury understands the State's position that Defendant is not a contributor to the DNA mixture and also understands why the ISP lab's reported results were described as "inconclusive." If the State cannot call Dr. Westring, the jury would be left with the misperception that the State sought to suggest that Defendant could be a contributor to the DNA mixture, which has never been its intent and would raise a variety of concerns under Idaho Rule of Evidence 403. Consequently, the State respectfully seeks the Court's permission to amend the State's witness list to add Dr. Westring and to file an expert disclosure to incorporate Dr. Westring already disclosed analysis and opinions.

RESPECTFULLY SUBMITTED this 12th day of June 2025.



William W. Thompson, Jr.
Prosecuting Attorney

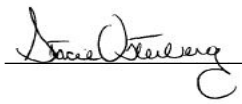
CERTIFICATE OF DELIVERY

I hereby certify that true and correct copies of the MOTION TO AMEND WITNESS LIST AND SUPPLEMENT EXPERT DISCLOSURES were served on the following in the manner indicated below:

Anne Taylor
Attorney at Law
PO Box 2347
Coeur D Alene, ID 83816

☐ Mailed
☒ E-filed & Served / E-mailed
☐ Faxed
☐ Hand Delivered

Dated this 12th day of June 2025.



Gustav & Sons, LLC
Forensic Science Services

AFFIDAVIT

April 5th, 2025

William W. Thompson, Jr.
Latah County Prosecuting Attorney
522 S. Adams St., Ste. 211
Moscow, Idaho 83843

RE: Case Name: *Idaho v. Bryan C. Kohberger*
Gustav & Sons Case Number: GS2025-001

Background and Professional Qualifications

1) I hold a doctoral degree in Biology from the University of Denver. I have more than 20 years of professional experience as a forensic biologist. I have worked on hundreds, if not thousands, of cases involving complex issues pertaining to serological testing and forensic DNA analysis.

2) I have and have been qualified as an expert witness molecular biology and forensic biology in state (Pennsylvania, New Jersey, New York, North Carolina, Connecticut), and local (Superior Court of the District of Columbia) courts.

3) I am the owner and principal investigator at Gustav & Sons, LLC, a forensic science company providing consultative services to crime laboratories, law enforcement agencies, and legal officials/practitioners.

4) I am the Laboratory Director for Niagara County Sheriff's Office Forensic Laboratory – an accredited forensic science laboratory in the state of New York providing testing services in forensic biology, drug chemistry, toxicology, firearms and tool marks.

5) I served as the Director for the Center for Crime and Forensics at Purdue University Northwest, with the rank of Professor of Practice in the College of Engineering and Sciences. I taught university courses at the undergraduate and post-graduate level in forensic science, including serology and the analysis of autosomal Short Tandem Repeat (STR) DNA and lineage specific (Y-STR and mitochondrial) DNA analyses.

6) I served as Laboratory Director at NMS Labs, an accredited forensic science laboratory system providing testing services in forensic biology, drug chemistry, blood alcohol content, fingerprints, firearms and tool marks, and crime scene analysis.

7) I have also served as the Quality Manager, where I was responsible for maintaining the Quality Assurance program, including implementing the FBI Quality Assurance Standards for DNA Testing Laboratories ("QAS"), the recommendations of the Scientific Working Group on DNA Analysis Methods ("SWGDAM"), and the recommendations of the International Society for Forensic Genetics ("ISFG"), and facilitating the ANAB accreditation program.

8) I have been qualified as a certified DNA assessor where I was responsible for all aspects of quality assurance and accreditation compliance, including adherence to recognized standards from ISO 17025, ASCLD/LAB *Supplemental Requirements*, and the FBI Quality Assurance Standards for DNA Testing Laboratories (QAS).

9) I am a member of the Biological Data Interpretation and Reporting Subcommittee for the Organization of Scientific Area Committees (OSAC). OSAC is a joint initiative of NIST and the Department of Justice to support the development and promulgation of forensic science standards and guidelines for the United States. My specific focus is the development of standards and guidelines related to forensic laboratory DNA interpretation. In this capacity, I currently serve as the Chair for the Thresholds Task Group, and I'm also a member of the leadership team for the Terminology Task Group.

10) I have served as the Serology and Trace DNA Technical Leader in the Section for Forensic Genetics at the University of Copenhagen (Denmark). Through the Department of Forensic Genetics, I have conducted training programs in DNA analysis and forensic biology for law enforcement and the legal community.

11) In connection with my professional positions in forensic science, I have conducted training programs in forensic biology, evidence handling for crime laboratories, law enforcement agencies, and legal professionals.

12) I am a member in good standing of the International Society for Forensic Genetics, the American Academy of Forensic Sciences and the American Society of Crime Laboratory Directors, and I contribute to the field of forensic biology through publication in professional peer-reviewed literature; my publications are set forth in my curriculum vitae, attached hereto as Addendum A.

13) I am the Principal Investigator in the forensic science research program at the Niagara County Sheriff's Office Forensic Laboratory. I also have productive research collaborations with a broad range of labs and/or organizations, including UC Davis, the BATFE, NFSTC, University of Denver, Center for Forensic Science, Research, and Education, among others. My research programs in forensic science are focused on some of the complex issues faced by working crime laboratories, including various aspects of forensic DNA analysis. This includes DNA recovery techniques, DNA inhibition from firearms and explosives, and the application of mathematically derived thresholds for DNA STR interpretation. Previous research activities have been funded

by the National Institute of Justice Forensic DNA Research and Development program, the Department of Defense, the National Institutes of Health and the National Science Foundation.

14) Additional experience and qualifications are provided in the attached CV.

15) As part of my professional position, I have served as an independent DNA expert in forensic biology on matters pertaining to serology/DNA testing, review of DNA discovery materials, and providing expert opinion and testimony at trial, pretrial hearings, and depositions. I am very familiar with the types of discovery materials that are produced by case-working laboratories in the course of laboratory testing.

16) As a DNA expert, I am familiar with how to bridge best-practice recommendations from professional organizations, such as the Scientific Working Group on DNA Analysis Methods (SWGDAM), and the recommendations from the International Society for Forensic Genetics (ISFG), with forensic laboratory testing procedures.

17) I was retained by the Latah County Prosecutors Office (William W. Thompson, Jr., Prosecuting Attorney) in the matter of *Idaho v. Bryan C. Kohberger* to review the DNA testing conducted by the Idaho State Police Forensic Services (hereafter, ISP Lab) and the computational analysis of the ISP data conducted by Cybergenetics (Pittsburgh, Pennsylvania).

18) I have reviewed the ISP laboratory report authored by analyst Jade Miller (dated February 6, 2023) in regards to the results of DNA testing of Item 13.1 (sample from body of person A, Madison Mogen). I have also been provided with, and have carefully reviewed, the discovery materials provided by Cybergenetics including the associated case file (pdf file) for TrueAllele containing the files generated during the computational analysis. Lastly, I have also reviewed the standard operating procedures and associated validation reports produced by the ISP Lab.

19) As part of my review, I have taken into consideration the applicable recommendations from SWGDAM; OSAC; the Office of the US Attorney General; the National Commission on Forensic Science and the President's Council of Advisors on Science and Technology. These documents provide recommendations for forensic casework analyses that are intended to promote rigorous, scientifically sound, and reliable forensic DNA testing and data interpretation.

Fundamentals of Human DNA Testing for Forensic Applications

20) By way of technical background, there are various validated approaches to the analysis of human biological material (serology and/or DNA) for forensic applications. This includes commercial chemistries that are used to analyze samples for the presence of biological fluids/cells (*e.g.* blood, semen, saliva, etc.), as applicable. In addition, other commercial chemistries are used to analyze human (male and female) autosomal/nuclear chromosomes (*i.e.*, chromosomes other than the X or Y chromosomes), and male-specific chemistries that are used to selectively analyze the human male Y chromosome. Lastly, some samples may also be tested for mitochondrial DNA (mtDNA) sequencing, when appropriate.

21) Following DNA extraction, using the current generation of commercial DNA quantitation kits (*e.g.*, ABI Quantifiler™ Trio DNA Quantification Kit, Promega's PowerQuant™ System, Plexor® HY System, or similar) it is possible to quantify the total yield of human and Y-chromosome (male specific) DNA prior to downstream DNA analysis. DNA quantitation is routinely employed by forensic testing laboratories to determine the quantity of human (and male) DNA and is required by CODIS participating laboratories. It is possible to reliably and reproducibility detect human/male DNA from only a one human cell [1] [2] [3].

22) In general, autosomal and Y-chromosome analyses employ comparisons of the genotypes present at Short Tandem Repeat (STR) loci to that of known DNA profile (with the exception of DNA base searches).

23) Following amplification (all DNA STR analyses), specialized genetic analysis software (*e.g.*, GeneMapper® IDX, GeneMarker®, OSIRIS, Sequencher®, etc.) is used to analyze the detected signal that meets laboratory-predefined quality criteria for interpretability and/or reporting.

24) Using the Promega PowerPlex® Fusion System in combination with the Applied Biosystems 3130 or 3500xL Genetic Analyzer detection instrument, it is possible to reliably and reproducibility detect complete DNA profiles from approximately 100 picograms (.1ng) of DNA (approximately 17 human cells) and partial DNA profiles from 50 picograms of DNA (9 or fewer human cells), or less. This level of sensitivity makes it possible to detect and characterize human DNA from a wide variety of evidence, including touched objects[4].

25) No DNA profile is considered or known to be absolutely unique to any individual human. The use of the term "match" in the context of DNA testing, therefore, means that two profiles (*e.g.*, a reference profile and an evidentiary profile) cannot be distinguished from each other at the points of comparison for which genetic data are available. Different DNA typing technologies have different capabilities with respect to the power of discrimination between different humans. Autosomal STR testing has the highest power of discrimination such that the chance that an unrelated person selected at random from the general population will "match" the full DNA profile from an item of evidence by coincidence is less than one in 1 in 7 trillion. Male Y-STR DNA profiles and mtDNA profiles (both called haplotypes) are shared by all paternally related males and all maternally related persons (males and females), respectively. As a result, the power of discrimination associated with YSTR and mtDNA profiles is significantly less such that the chance that an unrelated person selected at random from the general population will "match" the

[1] Applied Biosystems (2017) Quantifiler™ HP and Trio DNA Quantitation Kits USER GUIDE, Publication Number 4485354 (https://tools.thermofisher.com/content/sfs/manuals/4485354.pdf)

[2] Promega PowerQuant® System TECHNICAL MANUAL, Revised 1/20, TMD047 (https://www.promega.com/-/media/files/resources/protocols/technical-manuals/101/powerquant-system-technical-manual.pdf)

[3] Promega Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems, Revised 09/17 TM293 (https://www.promega.com/-/media/files/resources/protocols/technical-manuals/0/plexor-hy-system-for-the-applied-biosystems-7500-and-7500-fast-real-time-pcr-systems-protocol.pdf?la=en)

[4] PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual (revised 7/20) (https://www.promega.com/-/media/files/resources/protocols/technical-manuals/tmd/powerplex-fusion-system-protocol.pdf?rev=55bf6471c78e44549d5e49c15ef5bb6e&sc_lang=en)

full Y-STR or mtDNA haplotype from an item of evidence by coincidence is typically measured as being one in only several hundred to several thousand. As the quality of a DNA profile decreases, so too does the power of discrimination and the change of an adventitious "match". Hence, partial and/or degraded profiles should be viewed with caution.

26) Partial/Incomplete DNA profiles/haplotypes occur when genetic data are obtained for only a portion of the points of comparison that were targeted. There are several reasons why only a partial profile/haplotype might be obtained from an item of evidence. The amount of DNA recovered for testing may be "limiting" (*i.e.*, trace DNA) relative to the sensitivity of the DNA profiling kit employed; the DNA may be partially degraded (*i.e.*, chemically/environmentally broken down) and/or the user-defined interpretation criteria (*e.g.*, basis used to identify DNA alleles) may result in an artificial loss or failure to evaluate data at some points of comparison. The interpretation of partial profiles, therefore, must be approached with heightened caution.

27) The interpretation of DNA profile/haplotype data typically involves comparisons between known/reference profiles and questioned/evidentiary profiles. The purpose of comparing known and questioned profiles is to determine whether a profile of interest may be excluded as a potential contributor. In some cases, it may be the opinion of the analyst that the comparison is inconclusive as of complex mixtures, limited data sets, degradation, and other common factors.

28) Comparisons that result in an opinion of "can be excluded" or "inconclusive" may be reported without the need to calculate a statistical weight for the opinion, however, Likelihood Ratio calculations may be reported in these instances (and may be reported as the reciprocal = 1/LR). In contrast to this, a comparison that results in an opinion of "cannot be excluded" (or other linguistic equivalent) generally requires that the significance/weight associated with a "match" be calculated using appropriate statistical tools. Without an estimate/indication of the weight of a "match", the comparison is not helpful or meaningful.

29) With respect to DNA profiles/haplotypes, the statistical measure of the weight of an opinion of "cannot be excluded" is typically based on the statistically estimated rarity of a coincidental match to the DNA profile of interest. This depends on a number of factors including, but not limited to, the completeness of the profile/haplotype.

30) No DNA testing technology is considered to be absolute in its ability to detect human DNA. In other words, it is well understood in the field of molecular biology and forensic genetics that all DNA testing chemistries, including DNA quantitation and STR amplification, have defined limits of sensitivity/detection as is usually described in the user manual. A negative result (where the controls work appropriately and as expected) can either be interpreted as "no DNA detected", or, "less than a detectable quantity of DNA is present for the given technology". Based on these results, it is not possible to distinguish between these two alternatives.

Fundamentals of Human DNA Profiles and their Interpretation

31) There are various technologies used for human identification, including the Promega PowerPlex® Fusion System utilized by the ISP Lab in this case. DNA testing results are derived through the application of a commercial DNA amplification kit followed by the interpretation of the profile employing genetic analysis software for each sample. The PowerPlex® Fusion System amplifies human DNA at up to 22 locations on autosomal chromosomes and 3 locations (Amelogenin X, Y, and DYS391) on the sex chromosomes.

32) These locations on the DNA are known as "locus" if singular and "loci" if plural. Loci serve as a "points of comparison" to determine whether or not two DNA profiles match. At each locus, specialized genetic analysis software (*e.g.*, GeneMapper® IDX, GeneMarker®, OSIRIS, etc.) analyzes the detected signal (called "peaks") that meet laboratory predefined criteria for height, size and shape.

33) These peaks represent different variants of the DNA sequence that is present at each locus. These variants are called alleles and are designated by a numerical value (e.g. 7, 9). Using the Promega PowerPlex® Fusion System, each person present in a sample typically contributes one peak (if homozygous 7, 7) and two peaks (if heterozygous 7, 9) per locus. Allele numbers will generally vary between humans, hence, their utility in human identification.

34) These alleles (peaks) are identified, measured and labeled with such descriptors as peak height (in Relative Fluorescence Units, or "RFUs"). Using system-defined reference points (known as allelic ladders), the genetic analysis software also labels the peaks at each locus.

35) Human alleles are designated by numerical values called "allele numbers," and the relative peak height is measured in RFU. Using the Globalfiler® kit as an example, at locus D3S1358, an individual could have two peaks (e.g., a heterozygote) at allele positions 15 and 16, with a peak height of 1,624 and 917 RFU, respectively. The same individual at locus CSF1PO could have one peak at position 11 with a height of 360 RFU. As is the case with some low level DNA samples, this individual did not produce results at the TPOX locus, also known as complete "dropout". Figure 1 below illustrates these points along with several of the salient features associated with autosomal DNA profiles that are discussed herein (the use of the Globalfiler kit here is for demonstrative purposes only).

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36) Figure 1. AmpFLSTR® Globalfiler® PCR Amplification Kit, blue channel.

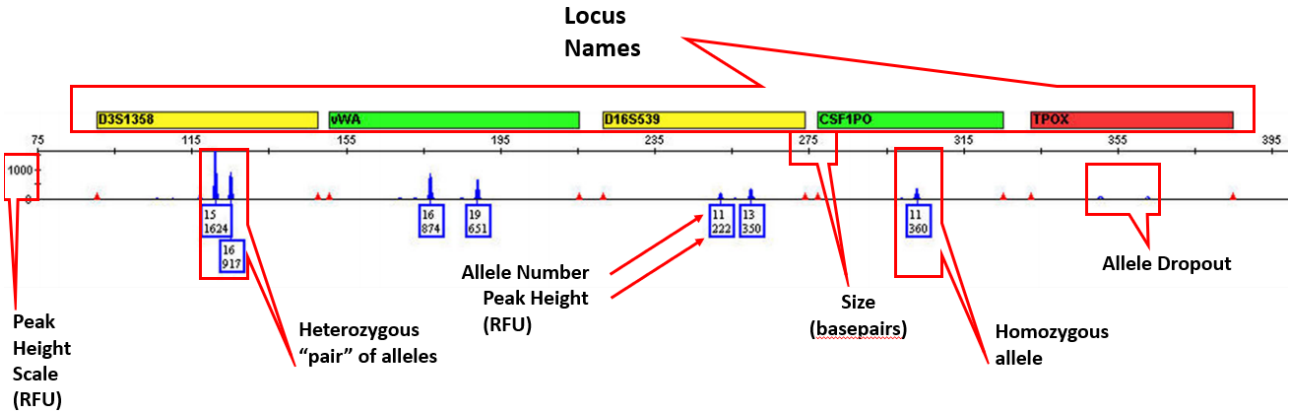

Figure 1: Example of a portion of a female DNA profile generated with the AmpFLSTR® Globalfiler® PCR Amplification Kit. Shown are five loci from the blue channel. Key elements of DNA profile, such as locus names, allele designations, peak height(s), and scales have marked accordingly.

37) When using the Promega PowerPlex® Fusion System, a "match" between a known autosomal DNA profile *(e.g.*, a reference profile from a person of interest) and the autosomal DNA profile developed from an item of evidence (*i.e.*, a questioned sample) requires that the profiles be identical at each locus. In other words, at each of the up to 22 loci analyzed (excluding X, Y chromosome markers), the allele numbers for the reference profile must be exactly the same as the allele number for the questioned profile. **If at any one or more of the 22 loci analyzed the allele numbers of the reference autosomal DNA profile differs from the allele number of the questioned autosomal DNA profile, that means that the reference profile cannot be the source of the DNA obtained from the item of evidence.** In such cases, the reference profile is said to be excluded as the source of the questioned DNA profile.

38) Partial/Incomplete DNA profiles occur when alleles are obtained for only a portion of the loci analyzed (see Figure 1, "Allele Dropout"). There are several reasons why only a partial profile might be obtained from an item of evidence. The amount of DNA recovered for testing may be "limiting" (*i.e.*, low quantity of DNA recovered); the DNA may be partially degraded (*i.e.*, chemically broken down) and/or the user-defined interpretation criteria *(e.g.*, analytical threshold) may result in an artificial loss of allelic data at some points of comparison. The interpretation of DNA matches based on **partial profiles, therefore, must be viewed with caution**. On the one hand, it is possible that missing allelic data could further confirm a potential match between a reference and questioned profile. Conversely, missing data could exclude an individual who appears to match based on a partial/incomplete profile.

39) The interpretation of a DNA profile is followed by statistical analysis that provides mathematical weight for the conclusion. The Likelihood Ratio is the preferred model. LR programs (calculations) are generally based on two models: 1) a fully continuous model that considers

more data within the electropherogram (*e.g.* allele call, allelic peak height value, peak height balance, stutter values, drop-in, drop-out, number of contributors to the sample etc.), and 2) a semi-continuous model, which are limited to fewer parameters (*e.g.* allele call and population frequencies for each allele, number of contributors to sample, drop-in, and drop out) but does not consider peak height values or stutter. The higher the LR the stronger the evidence.

Fundamentals for Establishing an Analytical Threshold for the Detection of Allelic Data

40) The purpose of an analytical threshold is to facilitate the identification of DNA peaks that represent actual alleles as opposed to instrumental noise[5]. In the published 2017 SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories, sections 1.1 addresses the need for laboratories to develop a scientifically sound analytical threshold for the detection of interpretable peaks in a DNA profile. These sections specifically state:

41) *"1.1 Analytical threshold: The analytical threshold should be based on signal-to-noise analyses of internally derived empirical data. An analytical threshold defines the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise. Because the analytical threshold is based upon a distribution of noise values, it is expected that occasional, non-reproducible noise peaks may be detected above the analytical threshold. Usage of an exceedingly high analytical threshold increases the risk of allelic data loss."* [6]

42) *"1.6 Non-allelic peaks: Because forensic DNA typing characterizes STR loci using PCR and electrophoretic technologies, some data that result from this analytical scheme may not represent actual alleles that originate in the sample. It is therefore necessary, before the STR typing results can be used for comparison purposes, to identify any potential non-allelic peaks. Non-allelic peaks may be PCR products (e.g., stutter, non-template dependent nucleotide addition, and non-specific amplification product) or instrumental artifacts (e.g., spikes, raised baseline, and incomplete spectral separation resulting in pull-up or bleed-through)."*

43) *"1.6.1 The laboratory must establish criteria based on empirical data (obtained internally or externally), and specific to the amplification and detection systems used, to address the interpretation of non-allelic peaks. These guidelines should address identification of nonallelic peaks and the uniform application, across all loci of a DNA profile, of the criteria to identify non-allelic peaks."*

44) *"1.6.1.1 In general, the empirical criteria are based on qualitative and/or quantitative characteristics of peaks. As an example, dye artifacts and spikes may be distinguished from allelic*

[5] Instrument noise is the ever present background electronic signal originating from the detection instrument hardware itself and should not be confused with artifacts and other forms of non-allelic data.

[6] SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories – APPROVED 01/12/2017 Rev 07/13/2021.

peaks based on morphology and/or reproducibility. Stutter and non-template dependent nucleotide addition peaks may be characterized based on size and amplitude relative to an allelic peak."

45) It is my professional opinion that the color (dye channel) specific thresholds are scientifically sound and should be based on generally accepted methods in the relevant scientific DNA community.

46) It is important to note that as of this writing, **there is no published standard that dictates how to determine or apply an analytical threshold**.[7] Rather, published guidelines and peer-reviewed publications provide several examples of how to determine an analytical threshold derived empirical data from each laboratory. Therefore, laboratories routinely apply different analytical thresholds for the same DNA typing chemistry and/or instrumentation based on 1) data acquired through requisite internal validation studies conducted by the laboratory, and/or 2) adjustments made based internal laboratory policy.

47) Furthermore, the application of various Probabilistic Genotyping systems, TrueAllele®, STRmix™, DNA View Mixture Solution, etc. may also employ different methods for applying an analytical threshold. They may range from as low as 1 RFU for all color channels; use color specific thresholds; or, they may employ one global RFU value for all thresholds in all colors based on laboratory derived empirical data. Here, each peak that is detected above the analytical threshold is given a mathematical "weight" and is incorporated into the final Likelihood Ratio (LR) calculation. These methods are also generally accepted in the relevant scientific community.

48) Various data sets obtained through internal validation used to assess empirically derived analytical threshold(s) may differ slightly from run to run on the same instrument, AND, may also vary slightly from instrument to instrument. In some cases, laboratories may decide to apply one global analytical threshold to all instruments within the lab/system (*e.g*. as documented in the ISP Lab Biology/DNA Casework Analytica Methods, issued 12/09/2022 – page 73/105 – 4.2.2 – *Peak Amplitude Threshold was empirically established as 75 rfu in all colors. Peaks below 75 rfu are deemed inconclusive.*). This practice may employ either the lowest or highest analytical threshold or may even employ the average across all of the instruments in the laboratory. Ultimately, the laboratory must decide which procedures are best suited for its operations based on internal validation.

49) Naturally, the application of different analytical thresholds to the same data set may result in the detection of more or less DNA peaks (data). The higher the analytical threshold, the fewer low-level peaks will be detected. Naturally, differences in allele detection between two data sets may result in differing opinions on whether a DNA profile is partial of full at all queried loci, number of contributors, and/or the potential source of the DNA. This is especially true when low level DNA peaks are present in the profile in question, particularly in mixed DNA profiles. Such

[7] A standard is high authority document, such as the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories, that specifies a requirement for compliance. Guidelines are generally lower in authority and represent "best practice" recommendations from the scientific community.

differences may be subtle, or they can be substantive depending on the complexity of the DNA profile. Generally speaking, the more complete the data set, the higher the confidence in the interpretation of the profile.

50) The analytical threshold is set within the analysis software (GeneMarker, GeneMapper IDX, OSIRIS, or other similar software) as a user-defined value such that the **software will not recognize (as a potential allele) any DNA peaks that fall below the analytical threshold**. For example, when employing a 50 RFU analytical threshold in the blue channel for the data set in Figure 3, no DNA peaks having a height of less than 50 RFU were detected by the analytical software. However, with the use of a validated analytical threshold of 29 RFU, the software recognizes alleles at multiple loci (see Figure 2 on the next page).

51) Figure 2. Visual representation of the impact of using two different thresholds for the same data

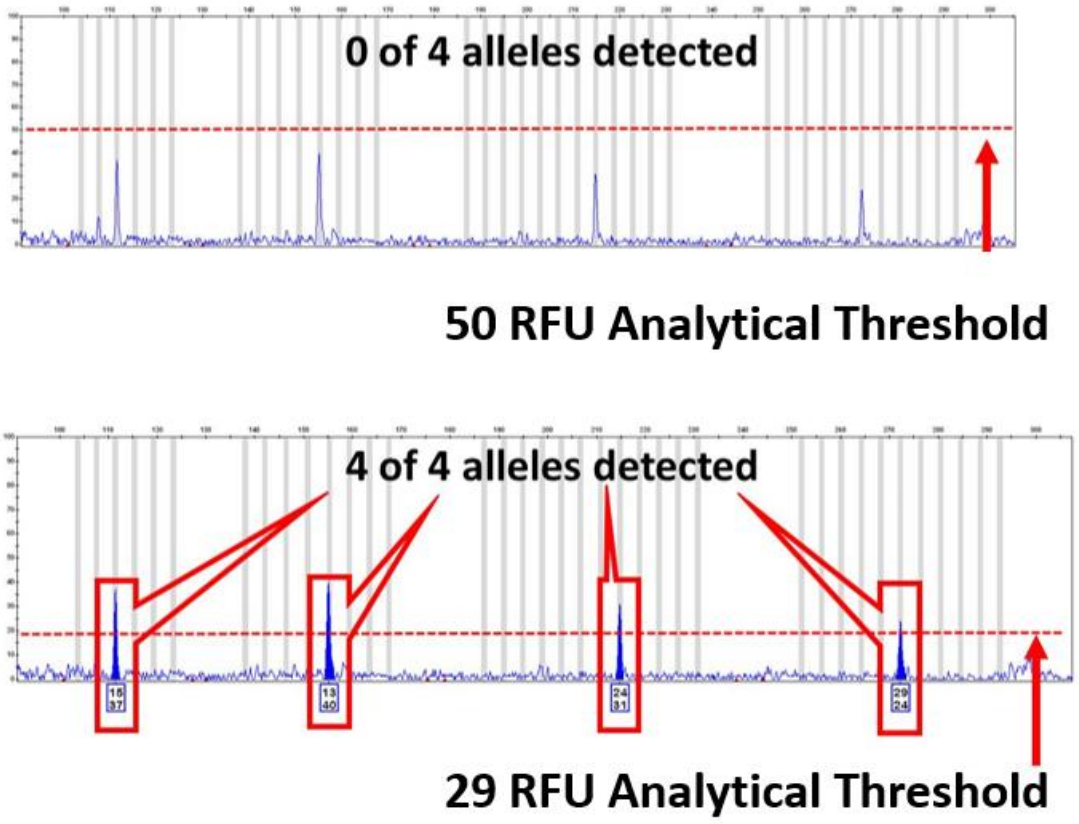

Figure 2: The application of different analytical thresholds are demonstrated with the same data set. (Top) Failure to detect DNA alleles occurs when employing a 50 RFU analytical threshold. (Bottom) Reanalysis of the same data using a lower detection threshold, but that falls within the validated range of values for the blue color channel derived through internal validation, allows for the detection of four DNA peaks. Note that in both the top and bottom panels, the instrument noise level remains well below the analytical threshold. (Peak labels show allele number followed by peak height in RFUs). **This example does not rely upon data from this case and is only intended to demonstrate the impact of using a higher analytical thresholds when low-level data are present.** The application of an analytical threshold should be based on data from internally derived validation studies by the testing laboratory.

52) Among the various methods for determining an analytical threshold, the average noise signal +3 Standard Deviations (known as Limit of Detection, LOD) and average noise signal +10 Standard Deviations (also known as the Limit of Quantitation, LOQ) are routinely discussed in the relevant scientific literature. LOQ is routinely employed by testing laboratories and reflects a confidence level greater than 99.99%.

53) Application of an analytical threshold above the mathematically derived values obtained through internal laboratory validation may reflect internal policies for how to apply an analytical threshold.

54) A concern associated with lower analytical thresholds is the presence of artifacts which might be mistaken for alleles. Artifacts are signal "peaks" that are clearly discernable from instrument noise, but which are not true DNA alleles. Some artifacts can be readily distinguished from true alleles based on their shape and/or location within the analytical window. True allelic DNA peaks have a distinctive narrow symmetrical shape and position within the electropherogram (*i.e.*, within bins). Many artifacts are readily identified because they lack these requisite characteristics which trained forensic scientists rely upon when interpreting DNA profiles regardless of the analytical threshold being used. For example, electronic "spikes" are artifacts that are much narrower than true allelic peaks. Furthermore, they often occur in the exact same position in more than one color channel. Dye blobs are another type of artifact, but generally only appear in a single color channel. Dye blobs are readily distinguished artifacts because they are wider than true DNA peaks, and they are reproducible even in samples without any DNA.

55) A second class of artifacts are those that do mimic the shape and position of true allelic DNA. These artifacts, however, are also distinguishable from true DNA alleles. For example, "stutter" is an artifact that has the same peak shape and position as true allelic DNA. However, stutter is readily identifiable because it is a byproduct of the DNA analysis process and only occurs adjacent to a much higher authentic allelic peak (Figure 3). The stutter rates for each DNA technology are published prior to sue, and the instrument software includes specific filters to ensure that stutter artifacts are not labelled as authentic alleles. Yet another artifact is "spectral pull-up" which is an optical byproduct of multi-color STR analysis. It is ready distinguished by the presence of a much higher corresponding peak in a different color channel. Here too, the DNA analysis software readily flags such peaks as non-allelic artifacts for editing.

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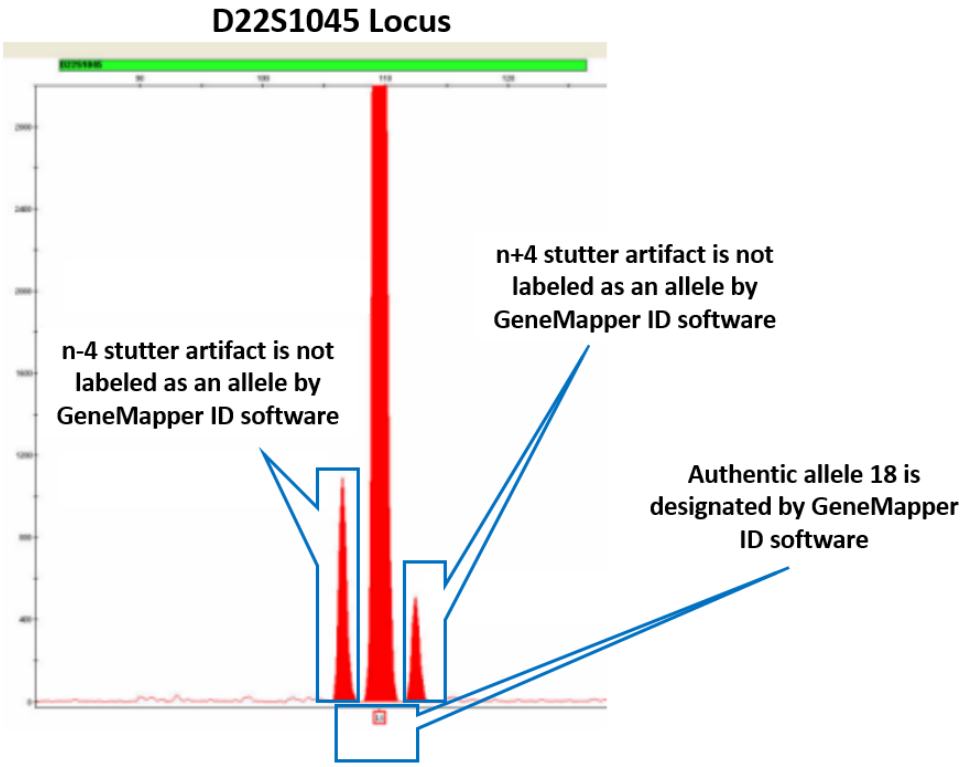
56) Figure 3 – Common artifacts such as n-4 and n+4 stutter



57) **Figure 3:** Example of a stutter peak at locus D22S1045. The stutter peak has the shape and position consistent with an allelic peak, but is recognized as a non-allelic artifact because of its location adjacent to a much larger authentic 18-allele peak (tall peak in the middle). Here, the GeneMapper software recognizes this small peak as an expected artifact and does not designate it as an allele.

Opinion in Regard to DNA Analysis Conducted by ISP Lab and Cybergenetics

58) The following describes my review of the DNA testing in the matter of Idaho v. Bryan C. Kohberger. As part of my analysis, I have been provided materials from the Latah District Attorneys Office (William W. Thompson, Jr.) relevant to this matter for my review.

As part of the forensic investigation, I have been asked to address the following three questions:

1. What is the definition of inclusion, exclusion, and inconclusive as it applies to forensic DNA analysis - and why do laboratories set different ranges/criteria for their protocols/purposes?
2. What are the substantive differences between the analysis conducted by the ISP Lab employing the STRmix™ software and the subsequent analysis of the ISP test results conducted by Cybergenetics employing the TrueAllele® software for Item 13.1?
3. What is my independent opinion regarding the ISP Lab DNA results for Item 13.1 as it pertains to the complexity of the DNA mixture obtained and conclusion reached?

59) **Question #1**: The interpretation of a DNA profile typically involves comparisons between an item of evidence (questioned/evidentiary) and a person of interest (reference/known). The purpose of comparing questioned and known profiles is to determine whether a DNA profile of interest may either be "included" as a potential contributor (also known as fail to exclude), or "excluded" as potential contributor. In some cases, it may be the opinion of the analyst that the comparison is inconclusive. To better understand these terms, we reference the SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories (2017 – revised in 2021).

- ***Inclusion****: a conclusion for which an individual cannot be excluded as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other).*
- ***Exclusion:*** *a conclusion that eliminates an individual as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other).*
- ***Inconclusive****: a determination that no conclusion (i.e., inclusion/exclusion) can be drawn from the comparison of a reference sample to suitable data. This could also result from statistical analyses that fail to provide sufficient support for an inclusion or exclusion.*

60) Furthermore, the National Institute of Justice (NIJ) provides further guidance on the mean of "exclusion" or "non-match" in their Principles of Forensic DNA for Officer of the Court (June 20, 2023). This guidelines states:

- ***Exclusion or Non-match:*** *When comparing a known sample to an evidence sample, the donor of the known is excluded as a source of the evidence if the profiles are different. This can either be referred to as an exclusion or a non-match .*

  When an individual is excluded as the source of DNA, it does not necessarily mean the individual was not involved. For example, a true perpetrator who left no detectable biological material will be excluded as a source of DNA.

61) Here, the NIJ publication provides an important consideration to the term "excluded". First, the term exclusion means the DNA profile for a questioned sample (evidence) is not consistent with the known DNA profile from a person of interest (reference sample). Second, this means that either the known DNA profile is not present in the sample, OR, that there is insufficient data on which to support inculpatory conclusion. Based on the testing conducted, it may be not be possible to distinguish between these two alternatives. This is especially true in the case of mixed DNA profiles.

62) When addressing the potential for "inconclusive" results, the SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories (2017) section XVII. (3.1.3) states:

- The laboratory shall establish guidelines for inclusionary, exclusionary, and inconclusive determinations based on comparisons of DNA typing results from known samples to both single-source and mixed evidentiary samples.

63) Referencing the ISP Biology/DNA Casework Analytical Methods ((12/09/2022) section 4.8 – Conclusions, the laboratory clearly defines the range in which the term "inconclusive" is appropriate. These values were derived based on empirical data developed by the testing laboratory through internal validation of the STRmix™ prior to its use in forensic casework. It reads as follows:

- *STRmix validation data shows that occasionally a known non-contributor can result in an LR > 1 and a true contributor an LR < 1. The range of LRs for which this may occur was observed to be between 0.001 and 1,000 for PP16 or PP16HS data and between 0.01 and 100 for Fusion data during internal validation. For this reason STRmix comparisons in which the LR is between 0.001 and 1,000 for PP16 or PP16HS data and* ***between 0.01 and 100 for Fusion data will be reported as inconclusive****. LRs > 1,000 for PP16 or PP16HS data and > 100 for Fusion data will be reported as an inclusion. LRs < 0.001 for PP16 or PP16HS data and < 0.01 for Fusion data will be reported as an exclusion.*

64) Therefore, the numerical range of the term "inconclusive" was clearly defined in the ISP protocols based on internally derived validation data, and was correctly used by the ISP Lab in this case. The decision to use an inconclusive range for reporting LRs varies between laboratories. Some scientists may reasonably disagree with the application of an inconclusive range when reporting LRs, while others readily accept it. Each laboratory sets their procedures, which they must follow. While somewhat conflicting, SWGDAM also published the Recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios in 2018. Recommendation 3.1 states the following:

- *A likelihood ratio appropriately conveys the weight of the evidence and should not be reported as inconclusive based on its magnitude.*
- *… As with RMPs or CPIs, likelihood ratios should not be deemed inconclusive to mitigate a potential risk of adventitious support.*
- *Similarly, any analysis that provides Limited Support for Hd, no matter how close to 1, should be reported as support for Hd rather than as inconclusive.*

65) *In toto*, it is ultimately up to the ISP Laboratory management to decide on the appropriate policies and procedures (as defined in their quality management system) to determine how each technology will be employed by the laboratory. The decision to employ a buffer "zone" above and below of LR of 1 is not uncommon.

66) **Question #2:** Before we can address the differences between the ISP Lab STRmix™ analysis and that of Cybergenetics employing the TrueAllele® software, it is important to note that the relevant scientific community (professional societies, leadership organizations, technical working groups, etc.) provide clear written guidance on the need for validating any commercial Probabilistic Genotyping software utilizing laboratory derived data prior to its use in forensic casework.

67) Specifically, this applies to the use of fully continuous Probabilistic Genotyping models, where laboratory derived values (signal to noise considerations, common stutter phenomena, allele peak height balance, artifact formation, etc.) should be evaluated prior to its use in casework. While ISP Lab fulfilled this requirement when they validated the STRmix™ program using their own data, similar studies were not conducted by Cybergenetics when employing TrueAllele® software with ISP derived validation data. Such studies are not consistent with best practice recommendations, but they also provide greater confidence in the application of these programs.

68) I have provided a brief summary of some of the relevant standards and guidelines that are regularly discussed in the forensic science community.

Recommendations of Scientific Working Group on DNA Analysis Methods (SWGDAM)

69) The SWGDAM Guidelines for the Validation of Probabilistic Genotyping systems (consistent with reference [12] in Cybergenetics report) states the following:

70) *4. Internal Validation*
Internal validation of probabilistic genotyping software system is the accumulation of test data within the laboratory *to demonstrate that the established parameters, software settings, formulae, algorithms and functions perform as expected. In accordance with QAS, internal validation data may be shared by all locations in a multi-laboratory system.*

71) *Depending on the features and capabilities of the probabilistic genotyping system, some DNA typing results may or may not be determined to be suitable for such analysis. To identify data features (e.g., minimum quality requirements, number of contributors) that render a profile appropriate or inappropriate for probabilistic genotyping,* ***the laboratory should test data across a range of characteristics that are representative of those typically encountered by the testing laboratory.*** *Data should be selected to test the system's capabilities and to identify limitations.* ***In particular, complex mixtures and low-level contributors should be evaluated thoroughly during internal validation****, as the data form such samples generally help to define the software's limitations, as well as sample and/or data types which may potentially not be suitable for computer analysis. In addition, some exclusions may be evident without the aid of probabilistic software."*

72) The SWGDAM PGM Validation guidelines further go on to state that:

73) *"4.1* ***The laboratory should test the system using representative data generated in-house with the amplification kit, detection instrumentation and analysis software used for casework.*** *Additionally, some studies may be conducted by using artificially created or altered input files to further assess the capabilities and limitations of the software."*

74) *"4.1.3 Variable DNA typing conditions (e.g., any **variations in the amplification and/or electrophoresis parameters used by the laboratory** to increase or decrease the detection of alleles and/or artifacts)"*

75) It is generally accepted in the relevant scientific community that certain parameters can only be achieved by looking at specific data sets that originate from the testing laboratory employing their procedures, equipment, instrumentation, staff, and other environmental working conditions. A subset of these types of requirements are listed below.

- *"4.1.7.1 – Allele and locus drop-out"*
- *"4.1.7.2 DNA degradation"*
- *"4.1.7.3 Inhibition"*
- *"4.1.8 Allele drop-in"*

76) In summary, the SWGDAM PGM Validation Guidelines provides rigorous guidance for forensics science laboratories who wish to employ probabilistic genotyping systems in forensic casework. The guidelines are written by forensic scientists who conduct these studies and understand the importance of internal validation prior to its use in the field. It is well understood, and industry norm, that each lab must conduct internal validation studies employing data from their own laboratory that captures subtle nuances from their processes prior to their use in forensic casework. Based on the materials provided through discovery, I was not able to find any indication that Idaho State Police Forensic Laboratory internal validation studies had been requested or employed by Cybergenetics to verify/validate the functionality of TrueAllele® with ISP Lab data. Such a study, even if in brief, would allow the user (and anyone else reviewing/auditing the work performed) to assess the influence of any lab specific parameters prior to its use in forensic casework. In contrast, these studies were successfully completed by the ISP Lab prior to the implementation of the STRmix™ software, consistent with industry norms.

Recommendations of the International Society of Forensic Genetics (ISFG)

77) The International Society of Forensic Genetics has also published recommendations on this topic. Specifically, The DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods[8] states:

78) "*Estimates of drop-out and drop in probabilities should be based on the validations studies that are representative of the method used."*

[8] Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. P Gill, L Gusmão, H Haned, WR Mayr, N Morling… - Forensic Sci Int Genet, 2012

79) The DNA commission goes on to clarify that:

80) "*The introduction of software solution to interpret DNA profiles must be accompanied by a validation process ensuring conformity with existing laboratory procedures.* ***Validation studies should be carried out to characterize drop-out and drop-in probabilities bearing in mind that these will differ between processes****…"*

81) *"Internal laboratory policies are necessary in order to address the quality of the data that will be required to attempt a comparative interpretation."*

82) ***In summary, each forensic laboratory has its own infrastructure to support their forensic service testing capabilities. These include people, laboratory space, instruments, protocols, reagents, and other -in-house preparations that may differ from one lab to another. It is common, and generally accepted, that data from one lab will show differences in sensitivity and reproducibility compared to another. The differences between one process and another are captured via validation, thereby ensuring that the laboratory is working with protocols that are representative of the samples tested in forensic casework.***

Standards of the Organization for Scientific Area Committees (OSAC)

83) The OSAC biology subcommittee published the Standard for Validation of Probabilistic Genotyping Systems in 2020[9]. The OSAC lexicon defines the term internal validation under section 3.4 as the:

84) *The acquisition of test data within the laboratory to verify the functionality of the system, the accuracy of the statistical parameters, the appropriateness of analytical and statistical parameters, and the determination of limitations of the system.*

85) Furthermore, Section 4 – Requirements, spells of the specific standards that are needed for compliance. Several critical excerpts regarding developmental and internal validations demonstrate the need for lab specific validations studies prior to use in forensic casework:

86) *"4.1 The laboratory shall validate a probabilistic genotyping system prior to its use for casework samples in the laboratory."*

87) *"4.1.1 Validations shall include both developmental and internal studies.* ***Developmental validation may be conducted by the manufacturer/developer of the application or another laboratory/agency. Developmental validation shall not replace internal validation****."*

[9] Standard for Validation of Probabilistic Genotyping Systems, ASB Approved December 2019, ANSI Approved July 2020

88) *"4.1.2 Developmental validation studies shall address the following: accuracy, sensitivity, specificity, and precision. These studies shall include case-type profiles of known composition that represent (in terms of number of contributors, mixture ratios, and total DNA template quantities) the range of scenarios that would likely be encountered in casework. Studies shall not be limited to pristine DNA samples but shall also include compromised DNA samples (e.g., low template, degraded, and inhibited samples)."*

89) *"4.1.3 Internal validation studies shall address the following: accuracy, sensitivity, specificity, and precision.* ***These studies shall include internally generated case-type profiles of known composition that represent (in terms of number of contributors, mixture ratios, and total DNA template quantities) the range of actual casework samples intended for analysis with the system at the laboratory. Studies shall not be limited to pristine DNA samples but shall also include compromised DNA samples (e.g., low template, degraded, and inhibited samples).*** *The internal validation shall not exceed the scope of the conditions tested in the developmental validation. Case type profiles that fall outside the range of conditions explored in developmental validation shall require additional developmental validation studies. See Annex A."*

90) *"4.1.4* ***Internal validation studies shall include evaluating user input parameters that vary run to run. The effects of artifacts (e.g., stutter) and parameters that relate to the statistical algorithm (e.g., run time parameters for the software system that can vary from system to system) shall also be evaluated.*** *The parameters may vary depending upon the approach or intended use of the software. Therefore, the specific parameters to be tested shall be determined by the laboratory."*

91) In summary, the data generated from each laboratory will vary slightly depending on a variety of factors which are reflective of the unique processes that laboratory may employ. In fact, a review of multiple validations studies from different laboratories conducted by the same manufacturer revealed slight differences in signal to noise data. Such differences can ultimately culminate in slightly different Analytical Thresholds depending on the models employed. Forensic practitioners readily recognize these differences, and therefore thoroughly validate each technology prior to use in casework. Likewise, the use of multiple amplification systems in a lab (aka, DNA technologies, chemistries, or STR kits) would raise questions pertaining sample to sample comparisons when different technologies were used. For example, a trained analyst can readily see the differences in kit sensitivity and the frequency of artifact formation between the PP16HS and the PowerPlex Fusion technologies. Therefore, one would not anticipate seeing the same peak heigh values or stutter rates between these two kits. The differences between one process and another is captured via validation and generally does not allow for a "once size fits all approach" when considering the measurable differences between one technology and another.

92) Referencing the Idaho State Police Forensic Laboratory Biology/DNA Casework Analytical Methods manual issued on 12/09/2022, the STR Interpretation Guidelines and Statistical Analyses (BI-212), section 1.1 states the following:

- *… Software programs are available to the forensic scientist as tools to aide in their interpretation. STRmix™ is a software program that applies a fully continuous approach to DNA profile interpretation.* ***It standardizes the analysis of profiles in the laboratory by using estimates of variance of results derived from validation data****. DNA analysis is a comparative test and STRmix™ provides statistical weight to comparisons by calculating Likelihood Ratios.*

93) Therefore, the application of different probabilistic genotyping systems which consider different user defined criteria including the applications of different analytical thresholds to the same data set(s), I would reasonably anticipate different results may be obtained between these two systems, especially when analyzing complex DNA profiles (e.g. partial profiles, degraded DNA, inhibition, and/or complex mixtures of varying proportions) as observed in the DNA profile from Item 13.1.

94) As previously stated, the application of a lower analytical threshold to the same data set may result in the detection of additional low level peaks (alleles), particularly when minor contributors and degradation are anticipated. The detection of more (or less in the case of higher thresholds) data can influence different opinion(s) regarding the interpretation of a DNA profile. The more peaks that are detected, the more substantive those differences may be.

95) For example, for Item 13.1, the application of a single global analytical threshold of 75 rfu for all color-channels by the ISP Lab resulted in the detection of 61 alleles across 22 comparable loci (excluding X, Y chromosome markers). At one locus, D18S51, six unique alleles were detected (based on maximum allele count alone, this would be consistent with a mixture of at least 3 contributors).**I agree with this conclusion when employing a global 75 rfu AT.**

96) A similar analysis by Cybergenetics employing TrueAllele® and a global analytical threshold of 1 rfu (with a peak height cutoff of 15 rfu) resulted in the detection of approximately 140 potential alleles across 22 comparable loci (excluding X, Y chromosome markers. Three loci (D1S1656, D18S51, and D21S11) were consistent with a mixture of at least 3 individuals, and one locus (D18S51) was consistent with a mixture of at least 4 individuals. With consideration to victim(s) reference genotypes at D18S51, this is consistent with a mixture of at least 4 contributors). **With the application of a lower AT, I agree with this conclusion based on the inferred genotypes reported. The mixture is consistent with a mixture of 4 contributors.**

97) Comparing the results obtained from the interpretation of the DNA profile developed by the ISP Lab for Item 13.1 to the analysis conducted by Cybergenetics, the difference(s) in interpretation may be attributed to the following factors:

- The analytical threshold (ISP Peak Amplitude Threshold vs. Cybergenetics Peak Height Cutoff) of 75 rfu v. 15 rfu for the interpretation of allelic data.

- Potential differences in the application of forward and reverse stutter parameters (e.g. those obtained by ISP Lab through internal validation, vs. those published for the Promega PowerPlex® Fusion System[10], vs. a probability based model).
- The method employed for estimating number of contributors (maximum allele count at D18S51) vs. potential mixture deconvolution employing expected contributors (e.g. victim(s) relative contributions to the major and mid-level alleles at D18S51).
- Potential differences in the estimated proportion of victim(s) DNA as a major and/or secondary contributor(s), particularly at locus D18S51, the remaining alleles in the mixture.

98) These factors (and potentially others), either individually or collectively, may account for different opinions regarding 1) the potential number of contributors to the mixture observed from Item 13.1, and 2), reporting the mathematical weight of the evidence (expressed as a likelihood ratio) calculated by both the ISP Lab and Cybergenetics, respectively. **Reporting the LR = 0.0469 by ISP as "inconclusive" falls within their validated inconclusive range (0.01 and 100 for Fusion data) and is in accordance with ISP Lab protocols.** Ultimately, both results (when considering the guidance from SWGDAM for reporting likelihood ratios) provide support for the proposition that Bryan Kohberger is not a contributor to the mixed DNA profile obtained from Item 13.1 (albeit, with different mathematical weights).

99) **Question #3:** In order to conduct an independent analysis of the results obtained by the ISP Lab for Item 13.1, color specific analytical thresholds were determined (and validated) based on the data provided by the ISP Lab and reported in Figure 4 below. These thresholds are suitable for the interpretation of the raw electronic data provided in this case.

Figure 4. Analytical Threshold Validation Conducted from Idaho State Lab PP Fusion Data

Dye Channel	Blue (6-FAM™)	Green (VIC™)	Yellow (NED™)	Red (TAZ™)
Sample ID				
LVRB3_112022JEM	19	21	34	25
LVRB1_112022JEM	20	23	35	25
LVRB3_112022JEM_REINJ	20	24	37	27
LVRB3_112022JEM_RESET_REINJ	16	19	31	21
NEG_CTRL	22	23	35	26
Average + 10SD (LOQ)	**19**	**22**	**34**	**25**

Figure 4: Summary of results for minimum threshold data from the ISP Lab data from Negative Control and Reagent Blank Control samples provided for review. Minimum AT Threshold (RFU) values are reported as average + 10

[10] Developmental validation of the PowerPlex® Fusion System for analysis of casework and reference samples: A 24-locus multiplex for new database standards. Forensic Science International: Genetics 12(2014) 69-76

Standard Deviations (also known as Limit of Quantitation, LOQ). The Average LOQ reported in the table were used for the analysis of the data in this case. Note, data sets from individual runs demonstrate slight variations in LOQ observed (plus or minus 5 RFU), as expected.

100) My findings here are based upon my analysis and interpretation of the ISP Laboratory DNA test results employing the Promega PowerPlex® Fusion System employing the OSIRIS analysis software for: Item 13.1 (sample from body of Madison Mogen); Item 11 (reference sample from Madison Mogen); Item 14 (reference sample from Kaylee Goncalves); and Item 108 (reference sample from Bryan Kohberger) are described below. Additional items were tested by the ISP Lab but not reviewed or discussed herein.

101) The ISP Labs' DNA quantitation results for the DNA extract generated from Item 13.1 were reviewed and analyzed to assess the total yield of human and male DNA. DNA quantitation data provide a useful guide when assessing DNA mixtures that may contain disproportionate quantities of female and male DNA. The following results were observed:

- Autosomal quantity (ng/µl) = 0.506
- Y-Chromosome quantity (ng/µl) = 0.007
- **Sample 13.1 contains approximately 1.3% male DNA**
- NOTE: One diploid cell contains approximately 0.006pg of nuclear DNA

102) Based on the DNA quantitation results observed, the ISP Lab subsequently performed a dilution of the DNA extract generated from Item 13.1 (5µl of DNA extract (0.506ng/µl) plus 20.3µl TE Buffer – final concentration 0.1ng/µl. Amp volume 15µl, total quantity of DNA amplified 1.5ng human DNA of which 20pg male DNA).

103) Interpretation of the ISP Lab DNA results for Item 13.1 employing the validated color specific thresholds resulted in the detection of a full DNA profile, consistent with a mixture of at least 4 contributors, at least one of whom is male. The following comparisons were made:

- Madison Mogen cannot be excluded as a potential contributor[11]
- Kaylee Goncalves cannot be excluded as a potential contributor[12]
- Bryan Kohberger was excluded as a potential contributor to the mixed DNA profile

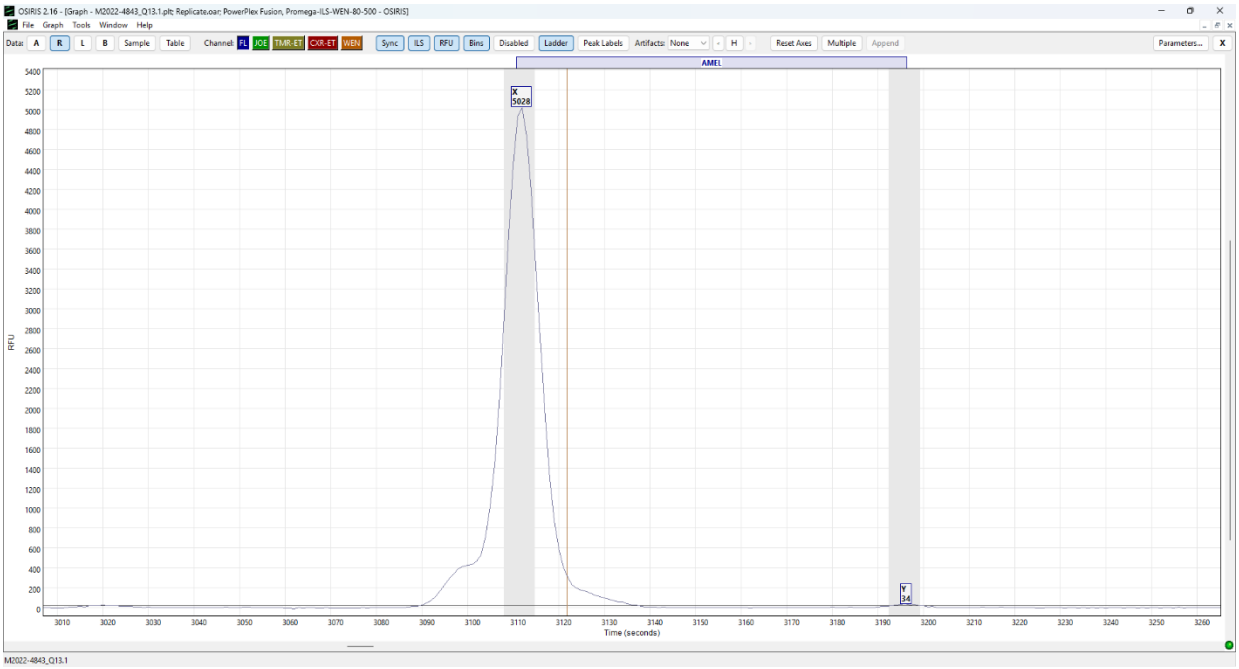
104) Based on the initial qualitative assessment of the DNA profiles obtained from Item 13.1, the known reference DNA profiles from Madison Mogen and Kaylee Goncalves were used to estimate the relative mixture proportions observed. This analysis was conducted based on the relative peak values (rfu) across multiple loci in the mixed DNA profile. The following mixture proportions were calculated:

[11] Given the nature of the evidence, a match statistic was not calculated. A statistic can be calculated, if requested.
[12] Given the nature of the evidence, a match statistic was not calculated. A statistic can be calculated, if requested.

- Madison Mogen ~ 83%
- Kaylee Goncalves ~ 12%
- Unknown contributors ~ 5%
    a. Unknown male(s) DNA component at Amelogenin (X, Y): ~ 1%
    b. This result was consistent with the information gained from the DNA quantitation assay for Y-chromosome DNA as summarized in paragraph 101 above. Approximately 1% of the DNA in this sample is of male origin.

- NOTE: Based on the quantity of DNA amplified for Item 13.1 (1.5ng total human DNA, of which approximately 20pg is male DNA), **this quantity of DNA is consistent with 3-4 diploid male cells. The number of minor male contributors cannot be determined based on lack of results at DYS391.** I would therefore anticipate that the minor male DNA component to the mixture is barely above the detection threshold (with high levels of allele drop-out, *e.g.* missing data) for the PowerPlex® Fusion system, resulting in a partial DNA profile from any minor contributor(s). Other factors, such as the presence of potentially degraded DNA, would further limit the detection of any minor alleles. (See Figure 5, Amelogenin results, and male specific locus DYS391, below). Due to low quantity and/or quality of male DNA component to Item 13.1, no results were observed at locus DYS391.

Figure 4. DNA Results at Sex Determination Locus Amelogenin, and Male Specific DYS391



Locus: Amelogenin X, Y result. Note relative differences in X and Y chromosome detection.

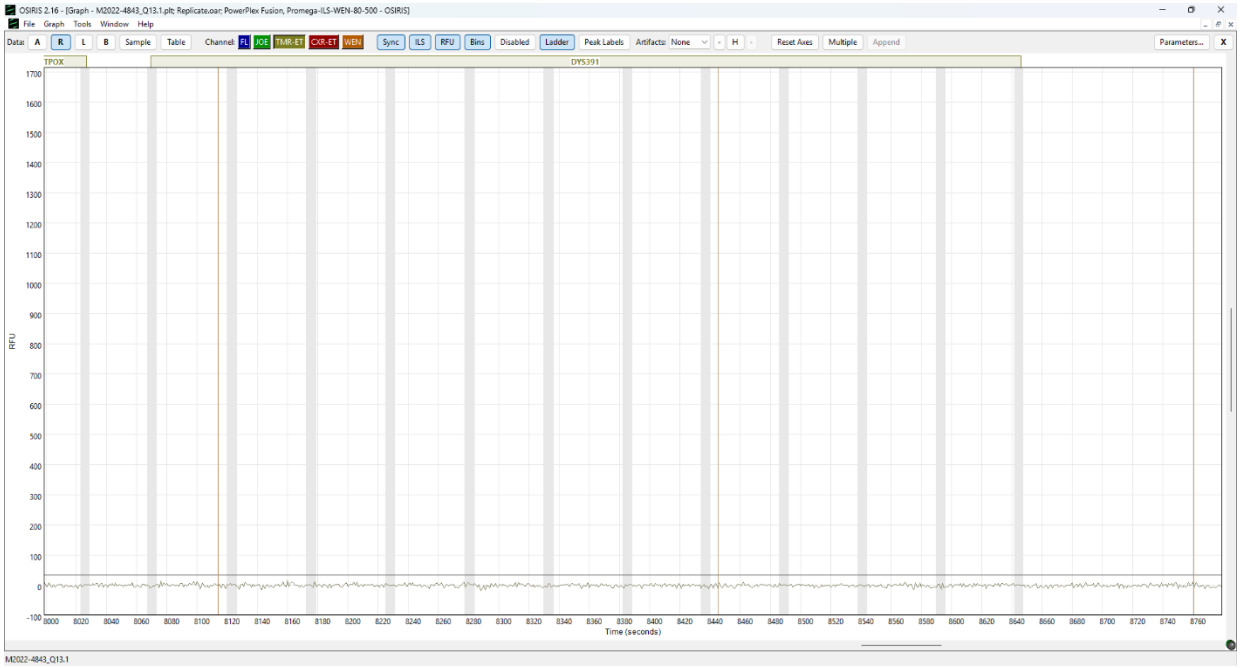


Figure 5: Screen capture of the DNA results obtained from Item 13.1. Top image, results obtained at the sex determination locus Amelogenin (note relative proportions of female (X) to male (Y) DNA in the sample, consistent with ~1% male). Bottom image, results at male specific locus DYS391. No peaks were detected.

105) Due to the complexity of the mixed DNA profile (relative number of contributors and uneven mixture proportions, degraded DNA, etc), the results obtained for Item 13.1 at locus D18S51 were particularly useful for assessing the number of contributors in the mix, see Figure 5 below.

Figure 4. DNA Results at Locus D18S51

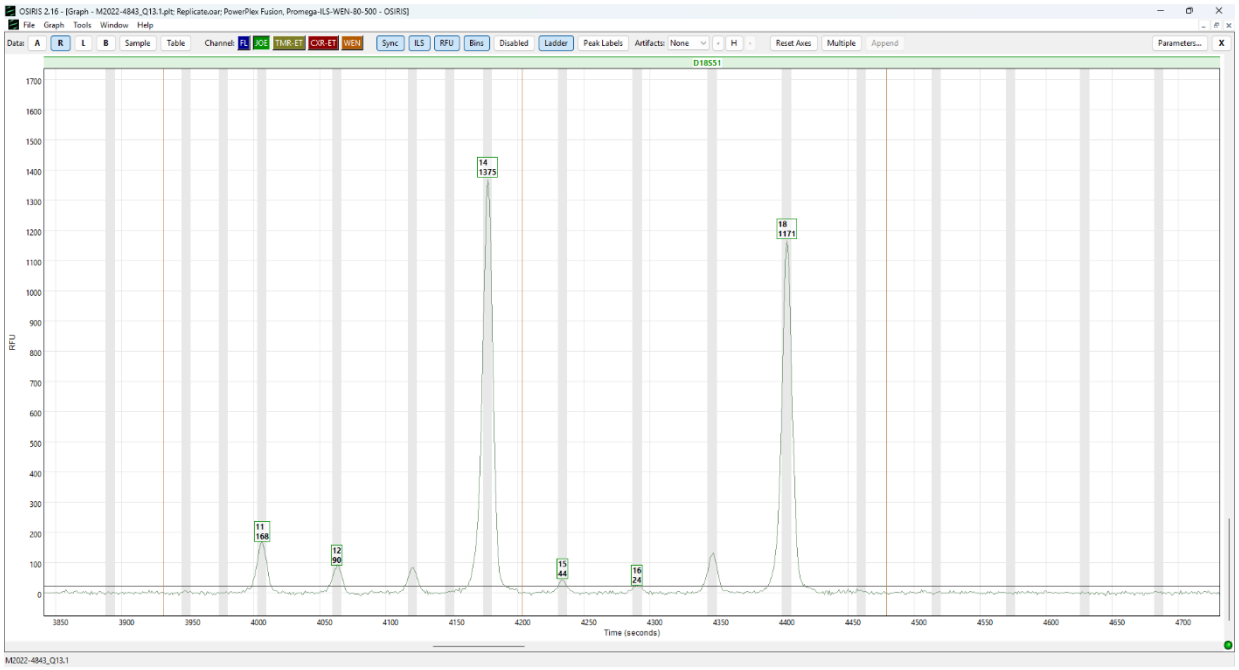


Figure 5: Screen capture of the DNA results obtained from Item 13.1. DNA results (peaks) at positions "13" and "17" fell below the published stutter threshold for n-4 (back stutter) and were therefore not called as alleles. See Promega PowerPlex® Fusion Developmental validation study for stutter values. Under the assumption of allele sharing (based on relative peak heights) at positions "11", "14", and "18" between Madison Mogen and Kaylee Goncalves, alleles "12", "15", and "16" provide support for proposition that this is a mixture of at least 4 contributors. Consideration for allele "15" at not being N+4 stutters was based on the lack of forward stutter for the "18" allele. Based on the peak heights observed, these peaks heights are consistent minor contributors.

106) Based on the interpretation of the mixed DNA profile observed from Item 13.1, including the results observed at locus D18S51 (and others), Bryan Kohberger was excluded as a potential contributor to the mixed DNA profile. These results are consistent with the findings reported by Cybergenetics group gwhen employing the TrueAllele® software. As part of my analysis, I employed the LRmix Studio software[13] for calculating Likelihood Ratios.

107) A match between Item 13.1(the body sample from Madison Mogen) and Bryan Kohberger is:

- 5 thousand times *less* probable than a coincidental match to an unrelated African American person,
- 68 thousand times *less* probable than a coincidental match to an unrelated Caucasian person, and
- 101 thousand times *less* probable than a coincidental match to an unrelated Hispanic person

108) **In summary,** it is my professional opinion that:

109) The discovery materials provided by the Idaho State Police Forensic Laboratory and the Cybergenetics group were appropriate for this type of review.

110) The definitions provided for included, excluded, and inconclusive are based on generally accepted sources in the field of forensic science.

111) The ISP Laboratory internal validations studies should have been incorporated into the analysis performed by Cybergenetics. Such studies may help bridge the gap in LRs between STRmix™ and TrueAlle® in this case. In the absence of such studies, a direct comparison between these two figures is not meaningful and merely reflects the difference between two systems employing different analytical approaches.

112) I agree with assessment from Cybergenetics regarding the number of contributors to Item 13.1. The DNA mixture obtained from Item 13.1 is consistent with a mixture of at least 4 individuals, at least one of whom is male. Madison Mogen and Kaylee Goncalves *cannot be excluded* as potential contributors to the mixture.

113) Based on the quantitative testing conducted by the ISP Laboratory, approximately 1% of the total DNA yield in Item 13.1 is male DNA. These results are further supported by the STR results at several loci. The male DNA component analyzed on consistent with approximately 3 to 4 cells.

[13] The LRmix Studio software is a semi-continuous system for calculating Likelihood Ratios. The only user limited set of user defined parameters (probability of drop-in). The revised NIST Allele Frequency Database was employed for calculating LR (Cau, AA, His). The most conservative number was reported.

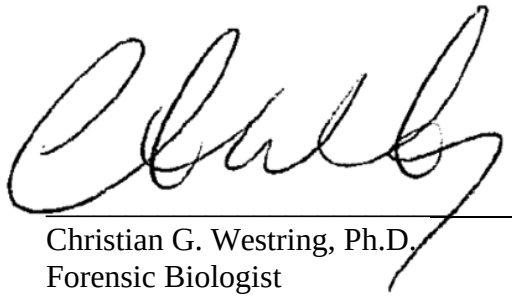
114) The ISP Laboratory concluded that the analysis was *inconclusive* with regards to Bryan Kohberger, in accordance with their published SOPs. A policy to apply an LR "threshold" on 0.01 to 100 is balanced, and neither favors one side over the other (Hp vs Hd).

115) Considering the SWGDAM scale of verbal qualifiers for reporting Likelihood Ratios, the reciprocal of the LR of 0.046 (1/0.046 = 25) calculated by ISP provides *limited* support for the defense proposition. This reflects the probability of the DNA evidence being 25 times likely if the DNA originated from an unknown, unrelated individual (Hd) than if it originated from Bryan Kohberger (Hp).

116) Based on my independent analysis of the data generated by the ISP Laboratory, Bryan Kohberger was excluded as potential contributor to the mixed DNA sample obtained from the body swab collected from Madison Mogen (Item 13.1). Ann LR was calculated for the DNA mixture relative to Bryan Kohberger but was not necessary based on the qualitative assessment of the alleles detected relative to Bryn Kohberger. The LR calculated provides further support for Hd.

117) If any additional information becomes available, I reserve my right to amend this report.

I declare under penalty of perjury pursuant to the law of the State of Idaho that the foregoing is true and correct.



04/05/2025

Christian G. Westring, Ph.D. Date
Forensic Biologist

END OF REPORT

LATAH COUNTY PROSECUTOR'S OFFICE
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IN THE DISTRICT COURT OF THE FOURTH DISTRICT OF THE

STATE OF IDAHO, IN AND FOR THE COUNTY OF ADA

STATE OF IDAHO, Plaintiff, V. BRYAN C. KOHBERGER, Defendant.	Case No. CR01-24-31665 PARTIES' PROPOSED STIPULATIONS OF FACT

COMES NOW the State of Idaho and Defendant, Bryan C. Kohberger, to submit the proposed stipulations of fact as provided below.

STIPULATIONS OF FACT

PARTIES' PROPOSED STIPULATION OF FACT NO. 1

An FBI lab tested samples taken from (i) Defendant Bryan C. Kohberger's apartment at 1630 Northeast Valley Road, Apartment G201, Pullman, Washington, (ii) Defendant Bryan C. Kohberger's parents' residence at 119 Lamsden Drive, Albrightsville, Pennsylvania, and (iii)

Defendant Bryan C. Kohberger's 2015 white Hyundai Elantra. None of the samples tested by the FBI lab from these locations showed a link to the residents of 1122 King Road, Moscow, Idaho.

PARTIES' PROPOSED STIPULATION OF FACT NO. 2

A DNA sample taken from underneath a fingernail of Madison Mogen contained a mixture of DNA from three individual contributors. Madison Mogen and Kaylee Goncalves were identified as two of the three contributors to the mixture. Defendant, Bryan C. Kohberger, was excluded as a contributor to the mixture.

DATED this __ day of May 2025.

_____

WILLIAM W. THOMPSON, JR.
Latah County Prosecuting Attorney
ASHLEY JENNINGS
Senior Deputy Prosecuting Attorney
JOSHUA D. HURWIT
Special Deputy Prosecuting Attorney

_____

ANNE C. TAYLOR
ANNE TAYLOR LAW, PLLC
Attorney for Defendant

CERTIFICATE OF DELIVERY

I hereby certify that true and correct copies of the PARTIES' PROPOSED STIPULATIONS OF FACT were served on the following in the manner indicated below:

Anne Taylor
Attorney at Law
PO Box 2347
Coeur d'Alene, ID 83816
info@annetaylorlaw.com

☐ Mailed
☒ E-filed & Served / E-mailed
☐ Faxed
☐ Hand Delivered

Dated this ___ day of May 2025.