INQUIRY & INVESTIGATION

Forensic DNA Banding Patterns: How to Simulate & Explain DNA Fingerprinting in a Classroom with No Budget

DOUG CHRISTENSEN

Abstract

Understanding how DNA banding patterns in a gel can aid in the conviction or exoneration of suspects and be utilized for positive identification of biological fathers in paternity cases can be intimidating. In reality, the logistics and technology used in such cases are rather straightforward. This exercise is designed for use in high school environments as a stand-alone paper lab and as a primer to an actual wet-lab experience, if available. Upon conclusion of the experience, students will have confidence in understanding how DNA fingerprinting can constitute strong evidence in forensic analysis.

Key Words: *DNA fingerprinting; primer design; forensics; paternity testing.*

What is the language of DNA bands, and how can scientists use them to help place criminals behind bars? How can DNA analysis result in chairs being thrown on the *Jerry Springer Show*? The truth is that the language of DNA banding patterns is simple to learn and fun to teach. Best of all, you can fully indulge in teaching and learning this language in your classroom, even if you have no molecular equipment and little or no funding.

Here, I provide informed faculty with a simulation of DNA fingerprinting that can stand on its own as a solid instructional tool or be utilized as an introduction to a wet lab that includes actual DNA fingerprint analysis. I describe how DNA fingerprinting, through the polymerase chain reaction (PCR), is carried out in a wet lab and provide a dry-lab simulation of the critical steps of the process. Upon completing the dry lab, students will be able to utilize their unique chromosome amplification products to generate gel banding patterns. This technique was presented to secondary science teachers from around the state of Nebraska in a 2-hour workshop for the Rural Academic Secondary School Science Partnership. Most participants found it very useful (see conclusions) and felt that they could duplicate the exercise on their own. The most tenuous component of this experience will certainly be how well the instructor understands the structure of DNA, the use of PCR, and why individuals possess DNA that varies slightly in content because of random mutations and errors during DNA synthesis that result in variable-number tandem repeats (VNTRs). Although PCR (Dieffenbach & Dveklser, 2003) and VNTR amplification have been around for years (Nakamura et al., 1987; Ali & Wallace, 1988; Weber & May, 1989), the general concepts of their use in fact finding are not widely taught. Each instructor can decide how deeply to discuss these methods with students. Technical descriptions of VNTRs and PCR can be found in a wide range of textbooks and journals, but I encourage the use of simple animations (see Resources) for those who wish to provide some modest detail of PCR and VNTRs to their introductory students. Even with limited knowledge in these areas, this exercise is designed to be useful in teaching how unique DNA banding patterns are deciphered in evidence-based forensics cases. It will challenge students to understand how DNA priming is critical to this step, and it will illuminate why properly designed primers can reveal real differences about the DNA that each individual's cells harbor.

The truth is that the language of DNA banding patterns is simple to learn and fun to teach.

○ Materials for Each Student

- Simulated chromosome sets (see Appendix)
- Investigative questions (see below)

○ Exercise Design

The Appendix consists of three pairs of simulated chromosomes, represented as chromosome 1 (two versions), chromosome 2 (two versions), and chromosome 3 (two versions). Notice that each chromosome varies only in the number of VNTRs that are present. The VNTR is underlined a single time. The underlined pattern of nucleotides then repeats several times, and it varies on each version of the chromosome. For example, on chromosome 1, the VNTR pattern of **ccttaacgat** is present either 9 times or 21 times while the rest of

The American Biology Teacher, Vol. 75, No. 9, pages 682–691. ISSN 0002-7685, electronic ISSN 1938-4211.
DOI: 10.1525/abt.2013.75.9.10

the chromosome sequence is identical. Each student should be given two chromosome 1 sequences. It is important to note that in human populations, certain versions of a chromosome are more prominent then others. If you have 20 students, you should make 40 copies of chromosome 1, but you might have 30 of those copies containing 9 repeats of the VNTR while only 10 copies contain the 21 repeat version (as an example). In other words, just as students receive two copies of chromosome 1, one from their father and one from their mother, in this exercise students may have identical 9-repeat versions for both copies, some will have 9-repeat and 21-repeat versions, and some may draw both 21-repeat versions.

The second and third chromosomes are set up in the same fashion, although the VNTRs vary in length and sequence pattern. It is recommended that when making copies of chromosomes, you mimic real conditions by altering the ratio of each chromosome the students have access to. In the end, each student will have obtained two copies each of chromosomes 1, 2, and 3, each pair representing one chromosome from the student's mother and one from the father. In this practice, we are limiting the experience to just three chromosomes with two VNTR variations per chromosome. In reality, VNTRs often exist in more than two variations per chromosome site and, thus, could be represented by more than two versions of each chromosome if the instructor so chooses. Also, the diversity in chromosome populations would only be dramatically increased when utilizing 23 pairs of chromosomes (the actual number of pairs that human cells contain) with multiple VNTRs possible per chromosome. Finally, the chromosomes provided in this exercise are displayed only in single-stranded format. Explain to each student that DNA is double stranded and that we must envision every G (guanine) interacting with a C (cytosine) and every A (adenine) interacting with a T (thymine). The chromosomes are not labeled with 5′ and 3′ ends. The relevance of this detail and its impact on primer design can be elaborated on if the instructor chooses to do so; this requires a mere substitution of a 5′ label at each primed area designated with an asterisk. In an effort to prevent limitations in instructor use, the specifics of 5′–3′ directionality are not elaborated on in this exercise and are not required to conceptually grasp the general role of VNTRs in unique DNA pattern recognition.

Detection of Variation among the Student Chromosome Population through Simulated PCR Reactions

Oligonucleotides are the "eyes" of the reaction. These are short segments of DNA that "find" the specific region of DNA you are interested in amplifying and anneal to it on the basis of G-C and A-T interactions via hydrogen bonding. Remember that there are >3 billion nucleotides in nearly every single cell in your body, and generally we are only interested in ~1000 of those nucleotides for analysis. That means that the oligonucleotides (or "primers") need to seek out the specific 0.00003% of the genome we are targeting. In this case, as in "real life" DNA fingerprinting, we are interested in making more copies of a segment of DNA that varies on each chromosome, and not in making more copies of DNA that is the same on each chromosome. This is accomplished by designing "primers" that stick to the DNA that immediately flank the VNTR region. Basically, we want to "bookend" the VNTR region with two primers. One primer will anneal to the top strand of DNA (sequence provided in the Appendix) while the other will anneal to the bottom strand of DNA (sequence of the strand not shown in the Appendix). An example utilizing chromosome 1 is presented in Figure 1, showing both strands of DNA. Note that directionality is incorporated into the primer with an asterisk and that for formatting and space reasons, the actual simulated chromosomes (Appendix) show only the single top strand sequence, while the second strand can be assumed. Directionality will be important in both proper formulation of the primer and grading of primer design. The underlined region depicts the start of the VNTR, or the sequence we want to bookend with our primers. Primers will be designed that are exactly 10 nucleotides long and, thus, should adhere to the portions of DNA shown in bold and stimulate the generation of DNA directionally as indicated with arrows. This is achieved by ensuring that the asterisk is placed on the side of the primer that will not be a DNA synthesis point (the 5′ end) but will instead allow new DNA to be generated on the downstream end of the primer.

Using the segment in Figure 1, a correct set of primers can be designed for this sequence. The first is *-gtggtctact, which would adhere to the first bold region and directionally prime toward the

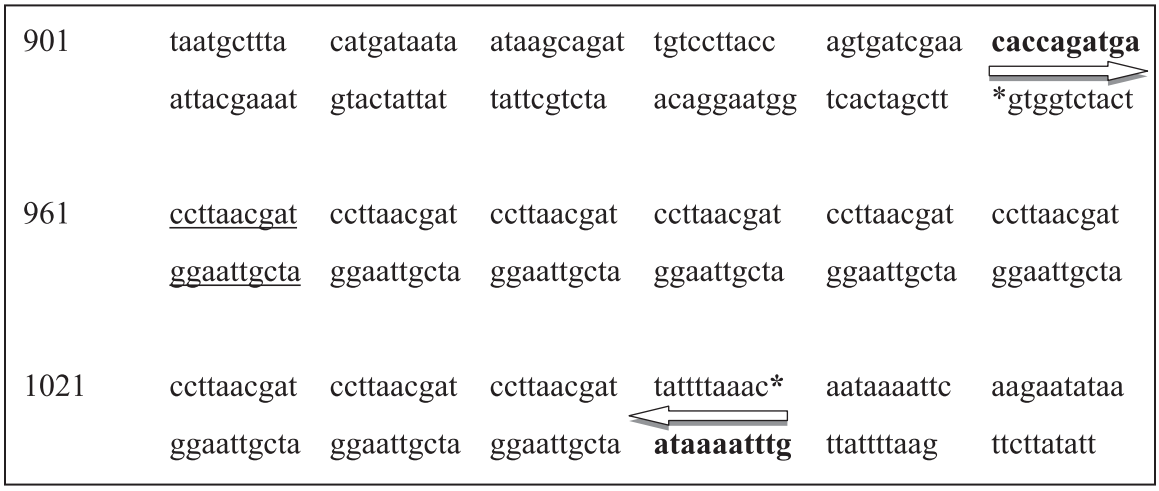

Figure 1. Primer design is critical to this exercise. Each set of chromosomes will require a set of two primers that bookend the VNTRs as shown here. One primer must anneal to the top strand of DNA and allow for the generation of a new bottom strand toward the VNTR. The other primer must anneal to the bottom strand of DNA and allow for generation of a new top strand toward the VNTR.

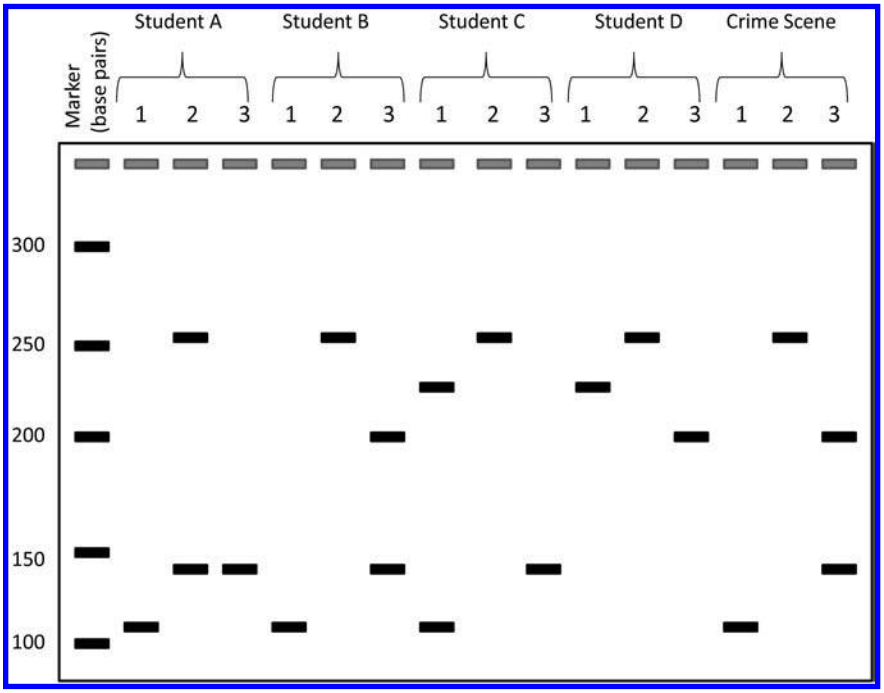

Figure 2. Example of a banding pattern that may result from four students and crime scene DNA.

VNTR as indicated with an arrow. This primer would allow for, and become part of, a newly synthesized strand of DNA complementary to the top strand shown in Figure 1. The second necessary primer is *caaattttat, which would anneal to the second bold section and would prime and become a part of the newly synthesized bottom strand of DNA being generated toward the VNTR. This particular priming scenario (when utilized with PCR) would result in the generation of newly synthesized DNA that is 110 total nucleotides in length (denoted between the asterisks above). It is important to note that the exact same primer set would generate DNA on both versions of chromosome number 1 but the total length would vary. Thus, a student who had obtained two copies of a single version of the chromosome would generate only a single 110-nucleotide DNA fragment. If a student had obtained two variations of chromosome 1, then two sizes of newly synthesized DNA would be generated via PCR and, thus, two different-sized DNA bands would ultimately be visualized (see Figure 2).

The following facts will benefit students at this point:

1. The number of PCR reactions needed for this exercise is dictated by the number of primer sets utilized to detect variability. In this case, students would set up three independent PCR reactions, each using an individual primer set designed for chromosome 1, chromosome 2, or chromosome 3.
2. The template DNA would be the entire set of chromosomes that each student possesses. The primers will have access to all chromosomes but will only properly prime complementary DNA. Only the amplified region of DNA will be visible in the detection portion of the lab because of its relatively high concentration (see below). The concentration of chromosomal DNA utilized as template for amplification is exceptionally low. As a result, the template DNA (chromosomes) is simply not visualized in later steps.

○ Visualization of the Amplified VNTR Regions of DNA

Any simple demonstration of how large and small fragments of DNA can be easily separated by running the samples through an agarose gel could be applied here (for animation link, see Resources). A large chalkboard can be used to simulate an agarose gel including loading wells and a standard DNA marker (for an example of a completed gel, see Figure 2). The students will have three PCR products. In each reaction, their chromosomal DNA will have been utilized as template with primers specific to chromosome 1, chromosome 2, and chromosome 3 (designed by each student). As a result, the gel will require three loading lanes for each student. The VNTR-amplified region utilizing chromosome 1 primers would be "loaded" in one of the lanes. Follow suit for VNTR regions for chromosomes 2 and 3.

Now that each student has determined the size of the VNTR (and flanking primed region) of their amplified DNA, they are asked to come to the board and draw a band that represents the particular size fragment(s) they would generate from chromosome 1 (first well), chromosome 2 (second well), and chromosome 3 (third well). Alternatively, students are asked to physically cut out the amplified region of each simulated chromosome sequence and tape them to the board as they would expect them to appear after a simulated gel run. Suggested gel migration could be set at 1 cm/10 base pairs, resulting in band migrations from 14 to 26 cm (140 or 260 base pairs, respectively) when using the attached chromosomes. This alternative approach may lead to the impression that PCR results in a cutting of DNA, however, so reinforcement of DNA amplification may be necessary. Also, it should be reiterated at this point that any additional chromosome regions (such as a fourth VNTR) would substantially enhance the overall pattern of DNA banding that each student has obtained. The instructor may choose to have the "crime scene" DNA results placed in the last three lanes of the gel. This will allow the students to make decisions on who may and may not be eliminated as a suspect in this simulated forensic investigation.

○ Instructions to Instructors (based on 20-student classroom)

1. Photocopy the chromosomes (Appendix). Each student will need two copies of each chromosome. It is recommended that copies of one version of each chromosome outnumber the other version at various ratios, ranging from 20% and 80% to 40% and 60%. Shuffle chromosome 1 copies together so that each version is represented throughout the copies. Separate this shuffled set into two equal piles, representing the version of the chromosome donated by the father and the mother, respectively. Repeat for chromosomes 2 and 3.
2. Instruct students to gather a chromosome 1 from the "father" pile and one from the "mother" pile. Repeat for chromosomes 2 and 3.

3. Teach students to design a primer set for each chromosome. The primers should be designed with the following specific instructions (primarily for ease in grading):
   a. Primers must be 10 nucleotides long (in reality, primers are typically 14–20 nucleotides long).
   b. Primers must be designed to specifically amplify the VNTR region of the chromosome only as near as possible (with no unnecessary bases included). (Note: this step may not be as straightforward as it appears; many students will likely design primers that anneal to the VNTR itself instead of the immediate flanking region. The negative result of this is that the primer would allow for amplification of VNTR regions from various locations within the VNTR and would not generate a single specific fragment of DNA. As a result, the only properly designed primers will anneal to the 10 nucleotides that bookend the VNTR region. It is suggested that you allow students to draw this conclusion on their own.
   c. Primers need to be "capped." This means that the primer needs to be assigned directionality. This is really accomplished through 5′ and 3′ ends of the primer; however, some instructors may lack a comfort level in discussing this detail. As a result, it is suggested that primer simply be designed with an asterisk on the end to which no new DNA will be added (represents the 5′ end).
   d. Once primers are designed, each student needs to determine the exact size of DNA fragment(s) they would yield from a PCR utilizing primer sets 1, 2, and 3.
   e. Once the exact sizes of fragments have been calculated, students are asked to display where the amplified VNTR DNA would yield a band on the simulated gel (drawn on a chalkboard). This will demonstrate the variable patterns that can be achieved by your class given even a limited number (3 chromosomes) of VNTR regions.
   f. Students are asked to complete the following investigative questions (a brief answer is shown below each).

○ Investigative Questions

Exercises & Discussions

1. **What is the sequence of each set of primers that would be utilized with these chromosome sets?**

   Chromosome 1 Primer Set: *-GTGGTCTACT and *-CAAATTTTAT [* represents 5′ end, for those comfortable with these details]

   Chromosome 2 Primer Set: *-TAAGTCTCAG- and *-CGCCACCTCA

   Chromosome 3 Primer Set: *-ATTGATGTGT and *-ACCGATCCCA

2. **Would you expect mutations to occur at an equal rate in and outside of gene regions? Would this result in an equal accumulation of mutations within and outside of genes in a population?**

   Each nucleotide has an equal chance of being altered or mutated. Genes, however, make up a small percentage of our entire genome, so genes themselves are less often affected by random changes. Another factor is that when a gene is altered, it often results in a broken protein. If that protein is important to the cell, then the cell with a broken version of the protein is weakened or dies, and thus the mutation is not part of future cells. The end result is that although mutations take place at an equal frequency among the nucleotides in our DNA, they accumulate to a greater extent outside of genes.

3. **Assume that a PCR was carried out on a VNTR of chromosome 7 utilizing DNA from a mother and her child. The mother's reaction resulted in two bands whereas the child's reaction yields just one. Is this normal? (Explain your answer.)**

   Yes, both the mother and the child would possess two chromosome 7's. In this case, the region of chromosome 7 that was amplified in the mother must have varied in length on each of her chromosomes. The child, on the other hand, inherited two chromosome 7's (one from Mom and one from Dad), but each contained a VNTR region of the same length.

4. **Would it be normal for the child's band to be a different size than both of the mother's bands?**

   No, the child would inherit one of his chromosome 7's from the mom, so the size of one of the mom's chromosome 7's and the child's should match.

5. **Your chromosomes would result in how many bands and of what size (exactly), assuming that you utilize the primer sets of your design.**

   Varies from person to person, with the following potential sizes for each chromosome (remember, students must include the flanked VNTR region *and* primer because the primer would become a part of the amplified product).

   Chromosome 1 potential sizes: 110 and/or 230 nucleotides

   Chromosome 2 potential sizes: 140 and/or 260 nucleotides

   Chromosome 3 potential sizes: 140 and/or 200 nucleotides

6. **In this exercise, only three VNTR regions were utilized (with two variations at each site). If examined together, how many pattern variations could occur? If a fourth VNTR region was added (with two variations), how many pattern variations could occur?**

   Assuming that only two VNTRs exist for each chromosome pair, we get three potential patterns for each chromosome (large bands, small bands or a large and small band). As a result, with three VNTR regions on three chromosomes, we could obtain $(3 \times 3 \times 3) = 27$ variations. With four chromosome VNTR regions we would get $(3 \times 3 \times 3 \times 3) = 81$ variations.

7. **The following gel (Figure 3) shows the banding pattern of VNTRs for a child, the child's mother, and three potential fathers. Which potential father (if any) seems more likely to be the actual father on the basis of these results?**

   Potential Father A: Could be the father.

   Potential Father B: Could not be the father. Evidence for chromosome 2 VNTR region indicates that father B could only donate a 340-base-pair VNTR region, which is not present in the child's genetic make-up.

   Potential Father C: Could not be the father. The child's genetic make-up includes a chromosome 3 VNTR of 750 bases. This segment of DNA was not donated by the child's known mother. Therefore, the child would have had to receive it from the father. Father C's genetics preclude this possibility.

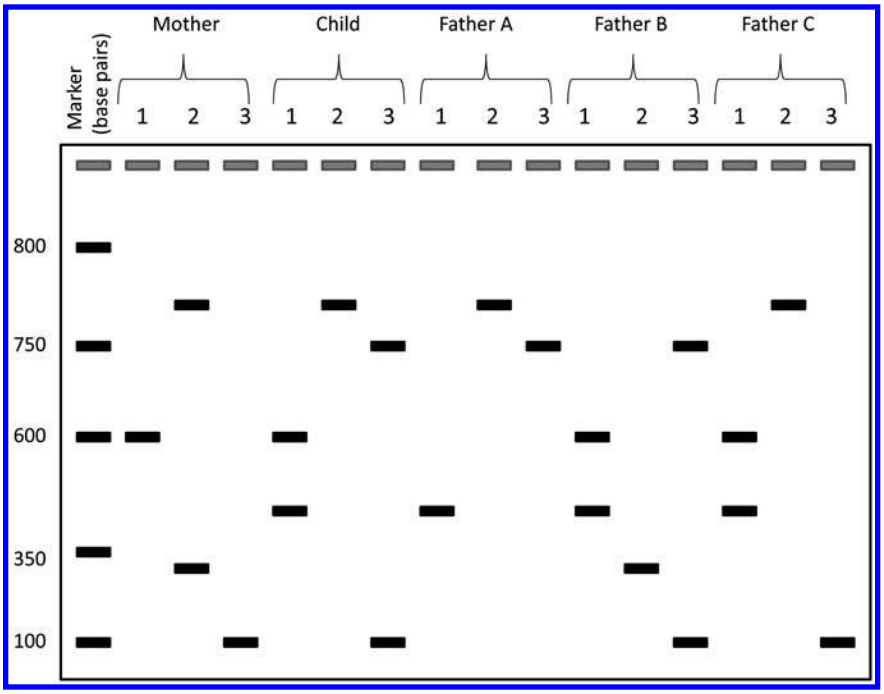

Figure 3. Banding pattern for question 6.

Conclusion

This activity was presented to 15 high school science teachers (physical and biological science areas) from Nebraska. Although most of the teachers were not educated in molecular techniques, on a scale of 1 to 5 (best), 93% of the participants gave a rating of 4 or better to the following statements: (A) I learned why "bands" in a gel are so meaningful; (B) The activity will improve the way I teach science; and (C) I learned ways to improve student understanding of science. Despite the diverse science background of these teachers, 67% (rating of 4 or better) felt they could comfortably perform this activity in their own classroom with no support.

Acknowledgments

Thanks to Barb Engebretsen and Gustavo Zardenta for internal review of this article. Library access was aided by the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427).

References

Ali, S. & Wallace, R.B. (1988). Intrinsic polymorphism of variable number tadem repeat loci in the human genome. *Nucleic Acids Research*, *16*, 8487–8496.

Dieffenbach, C. & Dveksler, G. (2003). *PCR Primer: A Laboratory Manual, 2nd Ed.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, T., Culver, M. & others. (1987). Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science*, *235*, 1616–1622.

Weber, J.L. & May, P.E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics*, *44*, 388–396.

Resources

PCR animation
http://www.dnalc.org/resources/animations/pcr.html

An interactive overview of VNTRs
http://www.rvc.ac.uk/review/dna_1/4_VNTRs.cfm

Gel electrophoresis animation
http://www.dnalc.org/resources/animations/gelelectrophoresis.html

DOUG CHRISTENSEN is Professor of Biology at Wayne State College, 210G Carhart Science, 1111 N. Main, Wayne, NE 68787. E-mail: dochris1@wsc.edu.

Appendix

Chromosome 1

1	TTTTGTTTTT	GTTTTTAACC	ACTTTTCCAC	AAGAAAAGAT	GCTATCGAAT	CTCTTGATTA
61	ACAGAATTTA	TCATCTTTTC	CACAAATTGT	GGAAAACTTA	TGTCCACATT	GTGGACTCAT
121	CTTTTTTCAC	CTGTGGAAAA	CTTTCTCAAT	TTATGGTAAA	ATTTTCTTAT	TACAATCTTG
181	ATAGGAGTAC	ACTATGACAG	AAAATGAACA	AATTTTTTGG	AATAGGGTTT	TAGAACTTGC
241	ACAAAGTCAA	TTAAAGCAAG	CGACATTTGA	TTTTTTCGTT	TCAGATGCTA	AATTATTGAA
301	AGTTGAAGGA	AATATTGCGA	CTATCCTTCT	TGATGATATG	AAAAAATTAT	TTTGGGAAAA
361	AAATTTACAG	CCTGTTGTTC	TAACAGCTGG	ATTCGAGGTC	TTTAATACAG	AAATTTCAAT
421	TGAGTATGTT	TTTGAAGAAA	CTCAATCCAC	ATCAAACAGC	CCACAAATTT	CTCAGAATAA
481	AACTGCAGAA	CTTGCAACAG	AAACACTTCC	TTTTGTGCAA	AATGATCTTA	ATCCAAAATA
541	TAGTTTTGAT	AACTTTGTGA	TTGGTGATGA	AAATCGTTGG	GCTTTTACAG	CATCCGTTTC
601	TGTTGCAGAC	CTTCCTGGAA	CAACCTATAA	TCCTCTCTTT	ATTTATGGAG	GACCCGGTTT
661	AGGAAAGACC	CACCTTCTAA	ATGCCATTGG	TAACTCTGTT	TTAGCAAGCA	ATCCTAAAGC
721	TCGTATAAAA	TATATTTCTG	CCGAAAATTT	TATTAATGAA	TTTGTTGTCC	ACATTAGACT
781	ACAAAATATG	GATGAGCTAA	AGAAGAAATT	TCGAAATTTA	GATTTACTAT	TAATTGATGA
841	TATTCAATCT	TTAGCCAAAA	AAAGTTTAGC	TGCCACTCAA	GAAGAATTTT	TCAACACCTT
901	TAATGCTTTA	CATGATAATA	ATAAGCAGAT	TGTCCTTACC	AGTGATCGAA	CACCAGATGA
961	**CCTTAACGAT**	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT
1021	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	TATTTTAAAC	AATAAAATTC	AAGAATATAA
1081	TTATAGTTTT	CTCTCTGAAA	CAATTGAATA	CTTAGCTGGA	CAGTTCGACT	CCAACGTCCG
1141	TGATCTAGAA	GGTGCCCTAA	AAGATATCAG	TCTTGTGGCT	AATTTTAAAA	AACTTGATGT
1201	TATTACTGTT	GAAGTTGCAG	CAGAAGCTAT	CAGGGCAAGA	AAGCAAGATA	GCAGTCCTAA
1261	GATGATTGTT	ATCCCTATTG	AAGATATCCA	AAAACAGGTT	GGAAAATTTT	ATGGCGTAAC
1321	TGTTAAAGAA	ATCAAATCAA	CCAAGAGAAC	ACAAAACATT	GTTTTAGCAC	GCCAAGTTGG
1381	CATGTATTTA	GCACGTGAAA	TGACAGATAA	CAGTCTTCCA	AAAATCGGAA	AGGAATTTGG
1441	AGGGCGAGAT	CATTCAACCG	TTCTACATGC	CTATAATAAA	ATCAAAAATA	TGTTAGCACA
1501	GGATGACAGT	CTAAAAATAG	AAATGGAAAC	GATTAAAAAT	AAGATAAAAT	AAATCCTGTG
1561	TATAAGATAA	AGAAAACGTA	TTATTTATCC	ACAAGTTGTG	AACAATCTTT	AAGACTGATG
1621	AAATATAAGA	TTGTTATACT	TATCCTCACT	ATACACAAAA	CCTACTACCA	CTACTAACTT
1681	ATTACTTATA	AAATAAAGGA	GTTTTCTATG	ATAAAATTTT	CAATTAATAA	AGTTTTTTTC
1741	TTACAAGCCT	TAAATGCTAC	CAAGCGAGCT	ATTAGTTCTA	AAAATGCTAT	TCCTATTCTT
1801	TCTAGTTTAA	AAATTGAAGT	GAATTCTCAG	TCCATTACTT	TAACAGGCTC	TAATGGACAA
1861	ATTTCTATTG	AAAATACGAT	TTCAGCTGAA	GAAGAAAATG	CTGGACTATT	GGTTACTTCT

Chromosome 1

1	TTTTGTTTTT	GTTTTTAACC	ACTTTTCCAC	AAGAAAAGAT	GCTATCGAAT	CTCTTGATTA
61	ACAGAATTTA	TCATCTTTTC	CACAAATTGT	GGAAAACTTA	TGTCCACATT	GTGGACTCAT
121	CTTTTTTCAC	CTGTGGAAAA	CTTTCTCAAT	TTATGGTAAA	ATTTTCTTAT	TACAATCTTG
181	ATAGGAGTAC	ACTATGACAG	AAAATGAACA	AATTTTTTGG	AATAGGGTTT	TAGAACTTGC
241	ACAAAGTCAA	TTAAAGCAAG	CGACATTTGA	TTTTTTCGTT	TCAGATGCTA	AATTATTGAA
301	AGTTGAAGGA	AATATTGCGA	CTATCCTTCT	TGATGATATG	AAAAAATTAT	TTTGGGAAAA
361	AAATTTACAG	CCTGTTGTTC	TAACAGCTGG	ATTCGAGGTC	TTTAATACAG	AAATTTCAAT
421	TGAGTATGTT	TTTGAAGAAA	CTCAATCCAC	ATCAAACAGC	CCACAAATTT	CTCAGAATAA
481	AACTGCAGAA	CTTGCAACAG	AAACACTTCC	TTTTGTGCAA	AATGATCTTA	ATCCAAAATA
541	TAGTTTTGAT	AACTTTGTGA	TTGGTGATGA	AAATCGTTGG	GCTTTTACAG	CATCCGTTTC
601	TGTTGCAGAC	CTTCCTGGAA	CAACCTATAA	TCCTCTCTTT	ATTTATGGAG	GACCCGGTTT
661	AGGAAAGACC	CACCTTCTAA	ATGCCATTGG	TAACTCTGTT	TTAGCAAGCA	ATCCTAAAGC
721	TCGTATAAAA	TATATTTCTG	CCGAAAATTT	TATTAATGAA	TTTGTTGTCC	ACATTAGACT
781	ACAAAATATG	GATGAGCTAA	AGAAGAAATT	TCGAAATTTA	GATTTACTAT	TAATTGATGA
841	TATTCAATCT	TTAGCCAAAA	AAAGTTTAGC	TGCCACTCAA	GAAGAATTTT	TCAACACCTT
901	TAATGCTTTA	CATGATAATA	ATAAGCAGAT	TGTCCTTACC	AGTGATCGAA	CACCAGATGA
961	**CCTTAACGAT**	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT
1021	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT
1081	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT
1141	CCTTAACGAT	CCTTAACGAT	CCTTAACGAT	TATTTTAAAC	AATAAAATTC	AAGAATATAA
1201	TTATAGTTTT	CTCTCTGAAA	CAATTGAATA	CTTAGCTGGA	CAGTTCGACT	CCAACGTCCG
1261	TGATCTAGAA	GGTGCCCTAA	AAGATATCAG	TCTTGTGGCT	AATTTTAAAA	AACTTGATGT
1321	TATTACTGTT	GAAGTTGCAG	CAGAAGCTAT	CAGGGCAAGA	AAGCAAGATA	GCAGTCCTAA
1381	GATGATTGTT	ATCCCTATTG	AAGATATCCA	AAAACAGGTT	GGAAAATTTT	ATGGCGTAAC
1441	TGTTAAAGAA	ATCAAATCAA	CCAAGAGAAC	ACAAAACATT	GTTTTAGCAC	GCCAAGTTGG
1501	CATGTATTTA	GCACGTGAAA	TGACAGATAA	CAGTCTTCCA	AAAATCGGAA	AGGAATTTGG
1561	AGGGCGAGAT	CATTCAACCG	TTCTACATGC	CTATAATAAA	ATCAAAAATA	TGTTAGCACA
1621	GGATGACAGT	CTAAAAATAG	AAATGGAAAC	GATTAAAAAT	AAGATAAAAT	AAATCCTGTG
1681	TATAAGATAA	AGAAAACGTA	TTATTTATCC	ACAAGTTGTG	AACAATCTTT	AAGACTGATG
1741	AAATATAAGA	TTGTTATACT	TATCCTCACT	ATACACAAAA	CCTACTACCA	CTACTAACTT
1801	ATTACTTATA	AAATAAAGGA	GTTTTCTATG	ATAAAATTTT	CAATTAATAA	AGTTTTTTTC

Chromosome 2

1	CAGAAGTCGC	GGCGCTGGAC	AAATCGAGTT	GGTCCTCGGG	CGCGGCAGAG	GTTCTTGTTG
61	TGGCAGCGTC	CGGCTTCAGA	CGGTTCGCGC	CGGCATTGGC	AGCTGGCTTG	GAAACCGACG
21	AGGCGGCTTG	GCTCGTGGAA	AGTCGAAATG	GACCGTAAAT	TTGCATCTTG	ACGCGTGCTC
81	CCGGGCGGAA	AGCGGAATTG	ACAACCGGAT	GACATACCGG	ACCTTCGTGG	CCCATTTGGC
241	ATGTCGATCG	ATCGACAAAA	GTTTGAAAAG	GATTGTGGGT	GCGACAACCG	ATCCGTCATG
301	GGAGTGCGCT	CGGGGCGCAT	CCAATCAGGG	AACTCTCGAC	TCGGTTATCA	GCGTTGCAAT
361	CCTTGTCCCA	GGAATCAATC	CGTCAGAATC	GGTGGAGACT	TCGCAAGCGC	AGTTTCGAGA
421	ACACGTCTGA	TATCGGCCTG	AATCGCCGAT	GGCCTGCAGT	CTCATTTGAA	TTCTGAGCAA
481	AAGTTCTGGG	CAAAATTCTG	TTCCGTCACA	CTATCAATCG	CGTGAAACCG	CCGGCAAGGT
541	CAACGCAGCC	AAAAAAATTG	GCTTTTGCAA	ACGAATTGAT	TCAAGCCTCA	CGAGTTGCCA
601	TCCGTGACCG	GTTTCTCGAC	TCCCAAAACA	CCTGACCGGA	TCTTTACCGT	CCCGTGACAG
661	TTGGGTGTGA	AACGATCGGG	TTTCACATCC	CGCCGCCGGA	GTTTTCCACT	CACGCTCCGG
721	GGGATGGGCA	GTTTGGTCCA	GCTACCGGCT	TGCCCGAAAG	GTACTGGCAA	GGGACGATGT
781	TGATCCTCCT	CGTTTTCCAT	TCGCAATCCT	CATCGCCGCA	ACGGTAATCT	CGGATCCCCG
841	ATGACTACGA	ATTCAGAGTC	**CTCCGCTCCC**	**CCGACGGCGA**	CTCCGCTCCC	CCGACGGCGA
901	CTCCGCTCCC	CCGACGGCGA	CTCCGCTCCC	CCGACGGCGA	CTCCGCTCCC	CCGACGGCGA
961	CTCCGCTCCC	CCGACGGCGA	ACTCCACCGC	CAATGTATGC	CTGGTCGTCC	TCGCCGCCGG
1021	CAAGGGAACT	CGCATGAACA	GCGAACTTCC	CAAGGTCCTG	TGCCCTGTAG	TCGATCGGCC
1081	CATGATTCAC	TTTGTCCTGG	ACGCCGCCGA	CAAAGCTGGC	ATTCAGAAAA	AAATCGCGGT
1141	GGTGGGCTAC	GAAGCGGATC	TGGTGCGAAA	AGAGCTCCAA	ACCCGCGGCG	ATGATTCGCT
1201	GACCTTTGCC	GAGCAAACCG	AGCAGCTAGG	AACGGGGCAT	GCCGTTCAAA	GGTGTCGCGA
1261	CCAGCTGGCC	GGCCACGAAG	GCCTCACTCT	CGTCATCGCT	GGTGACTCCC	CGCTGATTCA
1321	ACCATCCAGC	CTGATCAAGC	TGATCGAGCA	TTTCCAAGCC	ACCCGTCCGG	CGTTGCTGCT
1381	TGGTACTTTG	ACCAAAGACG	ATCCGACGGG	CCTGGGCCGA	ATCGTGCGAA	ACGAAAGCGG

Chromosome 2

1	CAGAAGTCGC	GGCGCTGGAC	AAATCGAGTT	GGTCCTCGGG	CGCGGCAGAG	GTTCTTGTTG
61	TGGCAGCGTC	CGGCTTCAGA	CGGTTCGCGC	CGGCATTGGC	AGCTGGCTTG	GAAACCGACG
21	AGGCGGCTTG	GCTCGTGGAA	AGTCGAAATG	GACCGTAAAT	TTGCATCTTG	ACGCGTGCTC
81	CCGGGCGGAA	AGCGGAATTG	ACAACCGGAT	GACATACCGG	ACCTTCGTGG	CCCATTTGGC
241	ATGTCGATCG	ATCGACAAAA	GTTTGAAAAG	GATTGTGGGT	GCGACAACCG	ATCCGTCATG
301	GGAGTGCGCT	CGGGGCGCAT	CCAATCAGGG	AACTCTCGAC	TCGGTTATCA	GCGTTGCAAT
361	CCTTGTCCCA	GGAATCAATC	CGTCAGAATC	GGTGGAGACT	TCGCAAGCGC	AGTTTCGAGA
421	ACACGTCTGA	TATCGGCCTG	AATCGCCGAT	GGCCTGCAGT	CTCATTTGAA	TTCTGAGCAA
481	AAGTTCTGGG	CAAAATTCTG	TTCCGTCACA	CTATCAATCG	CGTGAAACCG	CCGGCAAGGT
541	CAACGCAGCC	AAAAAAATTG	GCTTTTGCAA	ACGAATTGAT	TCAAGCCTCA	CGAGTTGCCA
601	TCCGTGACCG	GTTTCTCGAC	TCCCAAAACA	CCTGACCGGA	TCTTTACCGT	CCCGTGACAG
661	TTGGGTGTGA	AACGATCGGG	TTTCACATCC	CGCCGCCGGA	GTTTTCCACT	CACGCTCCGG
721	GGGATGGGCA	GTTTGGTCCA	GCTACCGGCT	TGCCCGAAAG	GTACTGGCAA	GGGACGATGT
781	TGATCCTCCT	CGTTTTCCAT	TCGCAATCCT	CATCGCCGCA	ACGGTAATCT	CGGATCCCCG
841	ATGACTACGA	ATTCAGAGTC	**CTCCGCTCCC**	**CCGACGGCGA**	CTCCGCTCCC	CCGACGGCGA
901	CTCCGCTCCC	CCGACGGCGA	CTCCGCTCCC	CCGACGGCGA	CTCCGCTCCC	CCGACGGCGA
961	CTCCGCTCCC	CCGACGGCGA	CTCCGCTCCC	CCGACGGCGA	CTCCGCTCCC	CCGACGGCGA
1021	CTCCGCTCCC	CCGACGGCGA	CTCCGCTCCC	CCGACGGCGA	CTCCGCTCCC	CCGACGGCGA
1081	CTCCGCTCCC	CCGACGGCGA	ACTCCACCGC	CAATGTATGC	CTGGTCGTCC	TCGCCGCCGG
1141	CAAGGGAACT	CGCATGAACA	GCGAACTTCC	CAAGGTCCTG	TGCCCTGTAG	TCGATCGGCC
1201	CATGATTCAC	TTTGTCCTGG	ACGCCGCCGA	CAAAGCTGGC	ATTCAGAAAA	AAATCGCGGT
1261	GGTGGGCTAC	GAAGCGGATC	TGGTGCGAAA	AGAGCTCCAA	ACCCGCGGCG	ATGATTCGCT
1321	GACCTTTGCC	GAGCAAACCG	AGCAGCTAGG	AACGGGGCAT	GCCGTTCAAA	GGTGTCGCGA
1381	CCAGCTGGCC	GGCCACGAAG	GCCTCACTCT	CGTCATCGCT	GGTGACTCCC	CGCTGATTCA

Chromosome 3

1	ATTTAAATCC	AGAGGTGCTT	GCATCGCCAG	CCAATGGAAA	ATCTGCAGGG	GGTTCTTTGG
61	CCGTCGAGTT	GATGGAGTAC	AATAGGGGCG	TTATTTTGTT	CTGAAGTGCT	TTCCCAGCGA
121	GCCAAAAGAT	GAAAGCCGTT	GCCATCGCTG	AGTTTGGCGA	GCCCGATGTC	TTGGTGCTAC
181	GGCAGGTACC	CGAGCCGCAG	ATTGAAGCTG	CCGATGAGGT	GAAAATTCAA	CTGCGGGCAG
241	CCAGTGTCAA	CCCCATTGAT	ACCAAGCTGC	GACAGCGGGG	CACGTTTTTT	CCCGATCGCC
301	GGCCAGCGAT	TCTTGGCTGT	GATGGTGCTG	GCGTAGTCGT	GGCCGTGGGT	GCAGCAGTAC
361	GGCGCTTTCG	CGTAGGCGAT	GAGGTCTATT	TCTGCTATGG	CGGCCTGGGC	GATCGCGGTG
421	GCTGTTACGC	CGAGTATGCA	GTTGTGCCGG	AAGCGGCTGT	TGCCCACAAG	CCCAAAACCC
481	TCTCCTTTAT	TCAGGCAGCC	GCCTTGCCCT	TGGCCGTCAT	TACCGCCTGG	GAAGCCTTGG
541	GCGATCGCGG	GGCTGTCCCA	CCGTTGAATG	TCCTCTCTAC	GGCAAAAACC	GTTCTCATTC
601	ACGCCGGCGC	GGGCGGGGTG	GGGCATCTGG	CCATTCAATT	AGCGCGGCGC	GCAGGGGCCC
661	AGGTGGCCAC	CACCATTAGT	TCACCGGCAA	AAGCCCAATT	TACAGAAGCC	TTGGGGGCAA
721	CCCTCGCCAT	TAACTACACA	**ACAACCGACT**	**GGGTGCAAGC**	**GGTTTTAGAT**	ACAACCGACT
781	GGGTGCAAGC	GGTTTTAGAT	ACAACCGACT	GGGTGCAAGC	GGTTTTAGAT	ACAACCGACT
841	GGGTGCAAGC	GGTTTTAGAT	ACCCTAGCCA	CCCTCTTGGA	ACCTGCAGTC	GATACCCCAT
901	GGAAAATTGC	CCGCCAACGC	AATCTCTTGA	TCCAACTGAC	CCTAATGCTC	ACCCCTCAAC

Chromosome 3

1	ATTTAAATCC	AGAGGTGCTT	GCATCGCCAG	CCAATGGAAA	ATCTGCAGGG	GGTTCTTTGG
61	CCGTCGAGTT	GATGGAGTAC	AATAGGGGCG	TTATTTTGTT	CTGAAGTGCT	TTCCCAGCGA
121	GCCAAAAGAT	GAAAGCCGTT	GCCATCGCTG	AGTTTGGCGA	GCCCGATGTC	TTGGTGCTAC
181	GGCAGGTACC	CGAGCCGCAG	ATTGAAGCTG	CCGATGAGGT	GAAAATTCAA	CTGCGGGCAG
241	CCAGTGTCAA	CCCCATTGAT	ACCAAGCTGC	GACAGCGGGG	CACGTTTTTT	CCCGATCGCC
301	GGCCAGCGAT	TCTTGGCTGT	GATGGTGCTG	GCGTAGTCGT	GGCCGTGGGT	GCAGCAGTAC
361	GGCGCTTTCG	CGTAGGCGAT	GAGGTCTATT	TCTGCTATGG	CGGCCTGGGC	GATCGCGGTG
421	GCTGTTACGC	CGAGTATGCA	GTTGTGCCGG	AAGCGGCTGT	TGCCCACAAG	CCCAAAACCC
481	TCTCCTTTAT	TCAGGCAGCC	GCCTTGCCCT	TGGCCGTCAT	TACCGCCTGG	GAAGCCTTGG
541	GCGATCGCGG	GGCTGTCCCA	CCGTTGAATG	TCCTCTCTAC	GGCAAAAACC	GTTCTCATTC
601	ACGCCGGCGC	GGGCGGGGTG	GGGCATCTGG	CCATTCAATT	AGCGCGGCGC	GCAGGGGCCC
661	AGGTGGCCAC	CACCATTAGT	TCACCGGCAA	AAGCCCAATT	TACAGAAGCC	TTGGGGGCAA
721	CCCTCGCCAT	TAACTACACA	**ACAACCGACT**	**GGGTGCAAGC**	**GGTTTTAGAT**	ACAACCGACT
781	GGGTGCAAGC	GGTTTTAGAT	ACAACCGACT	GGGTGCAAGC	GGTTTTAGAT	ACAACCGACT
841	GGGTGCAAGC	GGTTTTAGAT	ACAACCGACT	GGGTGCAAGC	GGTTTTAGAT	ACAACCGACT
901	GGGTGCAAGC	GGTTTTAGAT	ACCCTAGCCA	CCCTCTTGGA	ACCTGCAGTC	GATACCCCAT