IN THE CRIMINAL DISTRICT COURT NO. 3
DALLAS COUNTY, TEXAS

EX PARTE	§	Writ No. W96-39973-J
	§	(Trial Court No. F96-39973-J)
DARLIE LYNN ROUTIER	§	

SECOND AFFIDAVIT OF DR. ELIZABETH A. JOHNSON

STATE OF CALIFORNIA

COUNTY OF VENTURA

Before me, the undersigned notary, on this day, personally appeared Dr. Elizabeth A. Johnson, a person whose identity is known to me. After I administered an oath to her, upon her oath, she stated as follows:

1. My name is Elizabeth A. Johnson. I am over twenty-one years of age and reside in Ventura County, California. I am fully competent to make this affidavit. The facts stated in this affidavit are true and correct and within my personal knowledge.

2. I received a B.S. degree in chemistry in 1982 from Wofford College in Spartanburg, SC and a Ph.D. degree in immunology in 1987 from the Medical University of South Carolina. My formal education also includes four years of post-doctoral training in the field of molecular biology and DNA analysis at the Medical University of SC and at MD Anderson Cancer Center in Houston, Texas.

3. I have been a forensic scientist for over eleven years. I was hired to establish the DNA laboratory within the Harris County Medical Examiner's Office in Houston, Texas in 1991, and I was the director of that laboratory. I personally implemented and validated both RFLP- (chemiluminescent detection) and PCR-based DNA analysis in this laboratory. I also assumed supervision of the serology laboratory in 1996 and performed evidence examinations and body fluid identification on items of physical evidence in numerous cases.

4. I was a Senior Forensic Scientist at Technical Associates, Inc., Ventura, California from February 1997 until May 2003 and performed and supervised both RFLP and PCR-based testing including DQA1, Polymarker, D1S80 and various STR multiplex systems in addition to performing evidence examinations and body fluid identification testing in numerous cases.

5. I am currently in private practice performing forensic science consultation.

6. I am a court qualified expert in the field of Criminalistics and DNA analysis, and I have been appointed by courts of various jurisdictions in California, Texas, and other states to consult on and/or perform PCR- and RFLP-based DNA typing in numerous criminal cases and have testified as an expert on both PCR- and RFLP-based DNA analysis for both the defense and prosecution.

7. I developed the multiplex system that allows the co-amplification of the Amelogenin gene in a single reaction with the six Polymarker loci and I have performed validation on this multiplex system. The manuscript describing this technique and its validation has been published by the peer reviewed *Journal of Forensic Sciences*, January, 1997. I am also published in the area of medical research.

EAJ

8. In addition to the qualifications listed above, I have presented numerous papers and posters at scientific meetings dealing with many of the techniques developed by the laboratories with which I have been employed. I regularly attend forensic science meetings and stay abreast of the current literature in this field. I am also a member of the American Academy of Forensic Sciences and the Association of DNA Analysts and Administrators. I routinely give lectures and presentations on the subject of forensic evidence examination and DNA analysis.

9. I have been retained by counsel for Darlie Lynn Routier in the above-captioned matter to provide analysis and opinions regarding forensic biological evidence collected in this case. My evaluation and recommendations include the suitability of samples to be tested for the first time or tested further by today's laboratory methods which have advanced significantly since 1996.

10. Several advances have been made in the area of forensic evidence testing since 1996 that could significantly improve the probative information obtain from an item of evidence and which are directly applicable to evidence in the Routier case. These advances include:

a) More sensitive chemical tests to detect the presence of seminal fluid or other male cells, thereby enabling DNA testing of the male material. Test cards are available today that are based on an antigen-antibody complex formation and are very similar to testing methods used in early pregnancy test strips. These tests (available from ABA and Seratec) are very sensitive and are capable of detecting dilutions of seminal fluid as low as 1:10,000. This is much more sensitive than testing methods commonly employed in 1996 such as acid phosphatase testing and cross-over immuno-electrophoresis. Additionally, methods to recover spermatozoa from a sample vary in efficiency, and I have many times detected sperm cells on a microscopic evaluation that were missed by previous testing at other laboratories.

b) DNA testing by short tandem repeats (STRs) has greatly increased the probative value of DNA testing. Testing performed in 1996 in this case utilized the DQ alpha, and D1S80 systems. While these systems employed the PCR technology and, in the case of the DQ alpha system was very sensitive, very limited information was obtained by this testing. Only one genetic region, or locus, was tested by the DQ alpha or D1S80 systems. Many people in the general population can have the same DNA type at any one given locus, so the power of discrimination of these systems was very low compared to today's testing methods. Most of the samples analyzed by SWIFS and Genescreen employed testing by one of these systems; only four samples were analyzed at SWIFS using the additional Polymarker system. Today's STR analysis methods make possible the determination of a DNA profile at 9 to 16 genetic loci in one amplification step, making it much easier to distinguish between individuals in a possible mixed DNA sample. Furthermore, sex determination is included in these analyses, and it is now possible to determine if a DNA sample originated from a male or female (or both in possible mixture situations).

c) Y chromosome testing is a type of STR testing that became available for forensic use around the year 2000. This DNA testing is a type of STR testing that targets regions only on the male-specific Y chromosome. Since males have an X and Y chromosome and females have two copies of the X chromosome this type of DNA analysis ignores any female DNA contribution and amplifies and detects DNA only from a male. It is extremely sensitive and can detect DNA from fewer than 10 male cells in a mixture with thousands of female cells. The Y chromosome DNA is present only in males and is inherited from the father. Therefore, all men with the same paternal lineage are expected to have the same Y chromosome DNA type. In cases such as this, the benefit of such testing would be to determine if male DNA from someone other than the

Routier male lineage were present in a sample, even if mixed with large amounts of Darlie Routier's DNA.

d) Mitochondrial (mt) DNA testing was used primarily at the Armed Forces DNA laboratory in 1996 for identification of remains but was not generally used in forensic casework until several years later. This type of testing is still limited in use, but it is performed at several private and government labs today. The primary use of mt testing is on single source samples such as hair shafts, bone, and teeth. Mt DNA is possessed by both males and females but is inherited only through the maternal line. Therefore, all relatives with a common maternal relative are expected to have the same mt DNA sequence. For cases such as this which involve multiple family members, its usefulness lies in the ability to exclude someone from the maternal family line as the source of the sample. It is useful for testing hairs which have no root (a root is required for testing by STR methods) since the mt DNA can be found in the hair shaft.

e) DNA "clean up" procedures are available today that were not in 1996. Inhibitors of the enzyme that drives the PCR amplification process are often encountered in forensic samples, and although some techniques were utilized in 1996 to overcome this problem, these techniques were not always utilized by all laboratories and when they were, success was variable. Technology has advanced considerably in this area, and several commercially available products are available today such as DNA IQ by Promega and Quiagen columns. Using a procedure such as these has proven to significantly improve amplification of extracted DNA that previously gave a "no result" due to the presence of inhibitors.

11. Given the advances in testing technology mentioned above, in my professional opinion additional testing should be conducted on the tube sock found in the alley for several reasons. First, an amylase mapping procedure could be performed on the sock to determine if

possible saliva stains exist which could then be excised and subjected to DNA typing. Second, several bloodstains are present on the sock and it appears that representative samples were not taken from each area of staining during initial DNA testing performed by the State. One sample, 5013-T6, gave no results on DNA testing by Genescreen. It is possible that this "no result "is due to the presence of inhibitors on the sock in this area and that performing an additional "clean up step" on remaining DNA extract from this sample may make it possible to determine a DNA profile.

12. In my professional opinion, additional examination and testing should be performed on Darlie Routier's night shirt for several reasons. It is extremely important to fully examine and evaluate the bloodstains on the nightshirt for indications of blood that might have originated from an attacker since perpetrators often cut themselves during the act of stabbing their victims. Several bloodstain samples appeared to have been selected at random and subjected to DNA testing at Genescreen. However, this testing was limited in nature in that it only utilized the D1S80 test which was not as sensitive as today's STR tests in detecting mixtures of DNA, nor was it as informative in power of discrimination. As expected, most of the bloodstains tested by Genescreen appear to originate from Darlie Routier. However, Y chromosome testing could reveal a mixture of DNA from an unrelated male perpetrator. Furthermore, it is necessary to determine whether additional bloodstains exist on the nightshirt that would support or refute the State's claims that the stains consistent with her son are consistent with cast off from Darlie stabbing him.

13. The sexual assault kit collected from Darlie Routier was determined presumably at an early screening stage to be negative for the presence of probative evidence. However, as mentioned above in paragraph 10a, laboratories often miss low quantities of semen and sperm

during an initial screening examination. I have personally detected seminal fluid and/or sperm on numerous items of evidence that was overlooked at many different laboratories. Furthermore, Y chromosome testing could make possible the detection of a few male cells mixed with large numbers of Ms. Routier's vaginal cells if oral copulation of her occurred, or if penetration without ejaculation occurred.

14. The knife (which was assumed to be the only murder weapon) and any samples collected from it in the form of swabbings or DNA extracts should be re-examined and re-tested. It is critical to establish if blood from Darlie, Damon, and Devon is on the knife and if the knife blade or handle especially contains any of the perpetrator's DNA. Again, STR testing, and especially Y chromosome testing, is a significant advancement that could be applied to this evidence to obtain much more information than was possible in 1996.

15. Testing of other items such as the tape lift of the bloody fingerprint, the limb hairs, and pubic hairs recovered from the living room can reveal whether any of these samples originated from a common source and whether that could be the same individual who is the source of the facial hair which has already been shown through DNA testing to be from a person unrelated to the Routier family. Testing of the fingerprint lift with Y chromosome testing could be especially useful in determining whether a male DNA profile is present that is unrelated to the Routier male lineage. Testing of any remaining DNA extract from the facial hair root with Y testing methods could then be compared to any data obtained from the fingerprint.

16. Lastly, SWIFS reported that no human DNA was obtained from the window screen, window and garage door outside and that no DQ alpha amplification product was obtained from the 31T1 carpet, 51 chair, or 28 dish towels. Testing of these items apparently stopped at that point. Samples that were once deemed to contain no human DNA quite often give results if the

DNA extracts are taken through the amplification process using a maximum input volume for the system used, and these DNA extracts should be amplified and the testing completed using STR testing. The three samples that were stopped in testing after the amplification process may have had no DQ alpha amplification product due to the presence of inhibitors in the samples, and successful typing results could be obtained it the DNA extracts are "cleaned up" as mentioned above or if new samples are taken from these items of evidence.

FURTHER, AFFIANT SAYETH NOT.

Elizabeth A. Johnson

SUBSCRIBED AND SWORN TO BEFORE ME, the undersigned Notary Public, on the 31st day of January, 2005.

HELEN C. JANOS
Comm. # 1305546
NOTARY PUBLIC - CALIFORNIA
Los Angeles County
My Comm Expires May 24, 2005

Notary Public, State of California
HELEN C. JANOS