

Technical Addendum
A Very Technical explanation of process to supplement more general description
Suspect Inversion Center
Paul Vanouse, 2013

Suspect Inversion Center (SIC) is an open working laboratory where artist Paul Vanouse and assistant Kerry Sheehan recreate DNA evidence from the 1995 OJ Simpson murder trial: using the artist's own DNA. Since this process involves reverse-engineering the original image and recreating it by a combination of similar DNA Fingerprinting methods it is much more difficult than making a standard DNA image. The detailed, very technical process follows:

Phase I: Computational Procedures:

I. Measure original band locations and estimate original DNA sizes.

- A. Since DNA moves in a gel at a rate inversely proportional to its size, successive band locations denote different DNA sizes. A DNA ladder in the original image facilitates this estimation. See figure 1A.

II. Make a list of every single band size in every lane of the gel.

- A. Make an exhaustive list that includes all suspects, victims and even non-human size standards used as measurement standards for the original experiment (such as Lambda DNA digest). In the forthcoming steps I recreate DNA of these exact sizes, one at a time, by a technique called PCR amplification. See figure 2A.

III. Chose a region of actual (artist's) DNA to work with.

- A. Human DNA imaging actually works with only a small fraction of the total 3 billion base pairs in Human DNA. I chose to work with a region—the TPA gene, because of its significance in the history of DNA typing. Ref:
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC310683/>

IV. Obtain the DNA sequence for this region.

- A. There were two different ways to go about this:
 1. I could have this region of my own DNA sequenced by an external lab, but this would be expensive. But more importantly, I felt that it might undermine my hacker approach to DNA analysis and primer design.
 2. Instead, I downloaded a free, generic human genome from NCBI that is built from thousands of sequences of hundreds of anonymous individuals patched together (this was one of the achievements of the Human Genome Project). While most DNA forensic imaging relies on differences between individual DNA regions, most human DNA is conserved across the species. My working method is to assume similarity rather than difference of human DNA sequences needed for the subsequent phases described next. See ref:
http://useast.ensembl.org/Homo_sapiens/Location/View?g=ENSG00000104368;r=8:42032236-42065242

V. Design Primers to PCR amplify required DNA sizes.

A. Primers are like molecular bookends that hybridize to DNA regions when matched exactly to the source. They allow a researcher to use them like in-out points (to use a video metaphor). If these primers work correctly, one can amplify (create billions of copies) of this small region. Through this method and lots of tests with dozens of unique primers I recreated all DNA sizes required for my image reproduction. Ref: http://en.wikipedia.org/wiki/Polymerase_chain_reaction

VI. Myriad primer design issues effect the success of PCR amplification.

- A. Primer nucleotide sequences need to be matched exactly to the actual DNA region. Furthermore, they need to be long enough sequences not to match at more than one location. One primer sequence I use is: R-1H-tPA(pv), sequence: "GGTACAGCAATTCCAATGAGAAAACACAGC"
- B. Primer nucleotide sequences must not compliment one another too well, or they hybridize to themselves rather than the target DNA region. See figure 6A,B.
- C. The ratio of G/C to A/T base pairs within the primer and target sequence should be nearly 50%. Furthermore, there must be a predominance of G/C at the ends.
- D. The two different primers paired should have similar "annealing" temperatures, which are a product of both G/C concentrations and total length of sequence, if they are to amplify correctly. It's a tricky process, not all primers work. See figure 6A,B.

Phase II, wet phase:

VII. Design varied experiments.

- A. To complete the reproduction, I designed and ran over 60 PCR experiments, each with several individual band sizes attempted from 1kb-15kb in size. See figure 7A,B.
- B. Experimental parameters become very important when trying to amplify "large" DNA fragments, anything over 5 kb is considered large. See figure 7A,B.

VIII. Extract my own cheek cell DNA.

- A. This process is very simple, the only tricky part with this project is that DNA needs to be very fresh and extracted with no excess chemicals that might interfere with amplification. Thus, this procedure was completed within 24 hours of most PCR attempts. A very standard protocol follows. See figure 8A.

IX. Run PCR.

- A. This device thermocycles DNA tubes (also containing primers, taq enzyme, DMSO). See ref: http://en.wikipedia.org/wiki/Polymerase_chain_reaction
- B. This process involves three different temperature settings typically repeated in 30 cycles: Denaturation, Annealing and Extension. These choices depend upon the specific annealing temperatures of different primers and primer pairs, as well as the specific strain of Taq polymerase enzyme used. Each of the over 60 PCR experiments I ran took about a day to complete. See Figure 9B.

X. Test amplification outcomes using Gel Electrophoresis.

- A. Gel electrophoresis is the basis of most of my artwork for the last 12 years. For every gel that I run in public, there are dozens like this that I use to determine whether my experimental processes actually worked. See reference: http://en.wikipedia.org/wiki/Gel_electrophoresis
- B. It is important in this step to keep track of which experiments actually worked, and to estimate the concentration of DNA in each lane of successful amplification. For this, my assistant Kerry Sheehan and I developed a system of linked experimental data files that could be used to view previous gel images, to estimate the concentrations of DNA in tubes as well as the exact experimental conditions under which bands could be reproduced. See figure 10B.

XI. Design an insertion plan for creating the final image.

- A. Since there are 14 gel lanes with six to ten bands per lane, this means that over one hundred different small amounts from the amplification experiments need to be combined into the 14 different tubes, then inserted into 14 lanes of the gel before running. See figure 11A.

XI. Run the full electrophoresis gel.

- A. Pour gel, Insert DNA into all lanes, apply voltage, observe. See reference: http://en.wikipedia.org/wiki/Gel_electrophoresis
- B. Image the result through first staining with a chosen fluorescing compound, then standard photographic processes.
- C. This is precisely the image documented in the SIC documentation video. Nearly three years of process work was required to complete this image.

Continuations:

XII. I may chose to do a final Southern Blot procedure before declaring the reproduction process complete.

- A. This was just done at UC Irvine in March of 2013 with excellent results. This process was completed with the aid of Tom Fielder, scientist at UCI, and hasn't been fully documented yet. It was not attempted in the gallery due to needing to use radioactive probes. See reference: http://en.wikipedia.org/wiki/Southern_blot
- B. The original Simpson evidence was created through this process, but since my process involved reproducing the image of these other person's DNA I couldn't use the technique as originally invented and thus used a radically different procedure to get to this point.
- C. This procedure will not alter the placement of bands, but allows for a slightly different character to the marks owing to the specific way that x-ray film is used with the radioactive probes. See figure 12. See updates on: <http://www.paulvanouse.com/sic.html>

Figures:



Figure 1A: Determining band sizes from band positions in the DNA image.

SIZE	QUANTITY	WHERE	WHERE	WHERE	WHERE	WHERE
13000/14000		3	lane 7 (BOOT)	lane 15 (N BR)	lane 18 (K552)	
11500		1	lane 3 (TDS)			
11000		1	lane 7 (BOOT)			
10500		5	lane 5 (BUND)	lane 7 (BOOT)	lane 9 (FOYER)	lane 13 (O SIMP)
10180		2	lane 9 (FOYER)	lane 17 (R GOLDMAN)		
9500		1	lane 3 (TDS)			
9416		4	lane 5 (BUND)	lane 9 (FOYER)	lane 13 (O SIMP)	lane 15 (N BROWN)
9000		1	lane 3 (TDS)			
8900		5	lane 5 (BUND)	lane 7 (BOOT)	lane 9 (FOYER)	lane 13 (O SIMP)
8500		1	lane 3 (TDS)			
8144		1	lane 18 (K552)			
7500		1	lane 3 (TDS)			
7126		3	lane 7 (BOOT)	lane 15 (N BR)	lane 18 (K552)	
6200/6400		1	lane 3 (TDS)			
6000		2	lane 7 (BOOT)	lane 15 (N BROWN)		
5800		3	lane 7 (BOOT)	lane 15 (N BR)	lane 17 (R GOLDMAN)	
5500		2	lane 7 (BOOT)	lane 15 (N BROWN)		
5300		4	lane 5 (BUND)	lane 9 (FOYER)	lane 13 (O SIMP)	lane 18 (K552)
5090		3	lane 5 (BUND)	lane 9 (FOYER)	lane 13 (O SIMPSON)	
4700/4800		5	lane 5 (BUND)	lane 9 (FOYER)	lane 13 (O SIMP)	lane 17 (R GOLD)
4600		1	lane 17 (R GOLDMAN)			
4400		1	lane 18 (K552)			
3800		1	lane 17 (R GOLDMAN)			
3000		1	lane 3 (TDS)			
2027		1	lane 18 (K552)			
1700		3	lane 5 (BUND)	lane 9 (FOYER)	lane 13 (O SIMPSON)	
1636/1650		2	lane 3 (TDS)	lane 17 (R GOLDMAN)		

Figure 2A: Making a list of DNA band sizes in the gel.

KEY

>hg18_dna range=chr8:42151912-42184351 5'pad=0 3'pad=0

strand=+ repeatMasking=none

BOLD = F PRIMERS UNDERLINE BOLD = R PRIMERS (NEED RC)

Green highlighted = a probe from within should hybridize to one or more of the sequences.

Yellow highlight = probably an unstable region of high VNP count.

CAACTGTACTTTATGGATTAATTTGAATACATTCTTTAAAAACAGCTGCAAAAAAGGC
AGTCACAGGCAGGGAGTGGCATGTTAATTCGGCTTACTCAGGTCAAAATAAGTGCCTAG
GGCCAGCTACACACTGGGCCCCCAAATACAGAGTTGCTGTACAGAAGGGAGTGTGTG
CTGTGTGCTCCACAGCAATGCCAGCCCTCGTCCCTGTGCCTCTCTGCTCCCTGACAGC
ATTCCAACCTCCGTGGCCAGATGGACACAGACCCCTGGAGCCTCCGGAAGCATCACAG
CCTTGAGGGGGCGGCACACAGGGATCCTGCACACCAACCTGGCTGGGCGAAACGAAGACT
GCTCCACACAGCAGCAGCACACAGCAGAGCCCTCTCTTCATTGCATCCATGATTGCTTC
ACAGCGTCCCTTAAATTCGGAAGGAGAGAAAAACAGCTTGATCACGGGGTGGGAGAG
GTCCATGATGGCAACAGAGGAGGATAAGCCGCAATGTTGTCTGTCTGGCTTCCCTCCAG
TGCCAGCCCGCGGCAGAAACGCCAGACATGCATCTAGAAGAGGATTCTAGAAACTACA
TATTTTTTATTTGTTGCAAAATATATATAACAAAAATTTAC**CACCTTAACGATGTT****CAG**
TGCACAGTTCAGTGGCATTAAAGCACGTCATATTGCTGTGCGGCCGGCCGTCAACACCGC
CCATCTCCAGAACTTCTCCATCACCCCTAACTGAAACTCTGTAGCCATTAAGAATAAC
TCCCCATCCCCTTTTCCCAGCAGCCAGCTGCTACTTTCTGTCTGTATGAACCTGAC
TGCTCTACATACATCCTAGAAGTGAATGAGACAGTGTTCCTCTTTTTCGCACTGGTTT
GTTTCACTTTGCAAAATGCTTCAAGGTGCATCCATGCTGTAGCATGTCTTTTTTTTTTT
TTTCTTTTAAGATGGAGTCTCGCTCTGTGCGCCAGGCTGGAGTGCAGTGGTGTGATCTC
GGCTCACTACAAGCTCCGCTCCCGGGTTCATGCCATTCTCATGTCTCAGCCTCCTGAG
TAGCTGGGACTACAGGCGCTGCCACCACGCCCCGGCTAATTTTTGTATTTTTTAGTAGAG
ACGG**GGTTTCACCGTGTAGCCAGGATGGTCTCGATCTCCTGACCTCGTGATCCGCTCG**
CCTTGGCCTCCCAAAGTGGTGGGATTACAGGCGTGAGCCACCGCGCCCCCGCTGCCTTT
TTTTCTTTTTCTCTTCTTCTTCTTCTTCTTTTTTTTTTTTTTTTTTTTTGAGACTAGG
TCTCGCTCTGTATCCAGACTGGAGTGCAGTGGCATGAACATGGTTCAGTGCAGCCTTG
ACCCACCAGGCTCAAGAGATCCTCCTGCCTCAGCTTCCAGAGCTGGGACTTCAGGCAT
GTGCCACTGTGCTGGCTGAGAGAGAGAGAGAGAGAGAGAGAGAGTGTGTGTGTGTGTG
TG
GTCTCGAACTCCTGGGCACAAATGATCCTCCTTCTCAGCCTCCCAAAGTGTGGGATC
AGGCGTGTAGCCACCGCGCCAGCATTTTTCTCTTCTTTTTAAAGCTGAATAATACTCCA
CTGTGGGATAGAACACATTTTGTATTATCCATTGCTGTGCAATGCTGGGAGATAATTTT
GCCAGAGATTTCTGTGGGTGAAGGGCTAGACCATTTCTGAGACATCTTGGCAGCCCA
GAGACCACAGCGCTCTGGCTTGGTTTCTGGAAGGGAATTCCTGCCCTGGGCTTGACTA
GATGGAGCCGAGTCATGTCTTCTGGACTTCTGCAGCGGAAGCCATGGGATTTTATT
GCCAGCACATCATAGAGCCAGCCTGCCTTAGACTGCTCAGGTTCCCTCACCGCAGGATGG
AGCCTACTCCCTGCCTGAGGTTCTCCCACAAAGCAGAGGCCCCCAACCCAACCTCC
ACGCCCACCCCCCACCCTCTGCCAGAACATCTGTCTCCTTTTTTCATAGACTGCAGC
AGATCCGGCAATGTGCATATTTGCAGGAAGGAGGGGAGGCAAAATGATGTTTGGTCGGAA
GACTCAGATCTTCTGGACCCAGAGCCCAAGTTTCCATCTGCCTCTCCTCTGTGATCC
TGCCCTCGGCAGCTAAA

Paul Vanouse 3/23/13 12:07 AM

Comment [1]: R-1E-4RA(pv)

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Comment [2]: R-1E**-4RA(pv)

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Comment [3]: F-1J-4RA(pv)

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Comment [4]: F-1P*-4RA(pv)

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Comment [5]: R-1D-4RA(pv)

Figure 6 A,B,C: One excerpted page (14 total pages in actual DNA area) of annotated DNA sequence. Note, several primers are noted in bold and underline font and highlighted. This is how the primer design process worked and was annotated.

02/22/2011

medium sizes, with similar anneal temps--60ul volumes, titrating dmsol and magnesium.

EXP OJ 33			
MasterMix			
OJ32a (4.8k)		OJ32b (4.8k)	
sH2O	19.65	sH2O	18.75
5x Buffer	6	5x Buffer	6
Nucleotides	1.5	Nucleotides	1.5
DNA Template	1.2	DNA Template	1.2
Taq Ploymerase	0.45	Taq Ploymerase	0.45
Primer 1 (F1-tPA(7))	0.6	Primer 1 (F1-tPA(7))	0.6
Primer 2 (R1-tPA(10))	0.6	Primer 2 (R1-tPA(10))	0.6
Total Volume	30	DMSO	0.9
*good, lots of cupping, 3x brightness		Total Volume	30
		*good, lots of cupping, 3x brightness	
OJ32c (10.17k)		OJ32d (10.17k)	
sH2O	19.65	sH2O	18.75
5x Buffer	6	5x Buffer	6
Nucleotides	1.5	Nucleotides	1.5
DNA Template	1.2	DNA Template	1.2
Taq Ploymerase	0.45	Taq Ploymerase	0.45
Primer 1 (F2A-tPA(pv))	0.6	Primer 1 (F2A-tPA(pv))	0.6
Primer 2 (R1B-tPA(pv))	0.6	Primer 2 (R1B-tPA(pv))	0.6
Total Volume	30	DMSO	0.9
*nothing		Total Volume	30
		*good, lots of cupping, 3x brightness	
OJ32e (11.1k)		OJ32f (11.1k)	
sH2O	19.65	sH2O	18.75
5x Buffer	6	5x Buffer	6
Nucleotides	1.5	Nucleotides	1.5
DNA Template	1.2	DNA Template	1.2
Taq Ploymerase	0.45	Taq Ploymerase	0.45
Primer 1 (F2-tPA(1))	0.6	Primer 1 (F2-tPA(1))	0.6
Primer 2 (R1**x-tPA(pv))	0.6	Primer 2 (R1**x-tPA(pv))	0.6
Total Volume	30	DMSO	0.9
*nothing		Total Volume	30
		*good, lots of cupping, 3x brightness	

PCR Settings	temp	time	cycles
Denaturation	92	2	1 hold 26*
Denaturation	92	0.30	30 cycle 27*
Annealing	65	0.30	
Elongation	68	15.00	
final Elongation	68	15.00	1 hold 28*
cooling	4	30.00	1 hold 1*
method 29*			

Figure 7 A,B: A very successful experiment example showing one experiment with six different sample tests (a to f). There were over 60 experiments total, each with 4-12 individual sample tubes tested. Red results were added after each experiment ran.

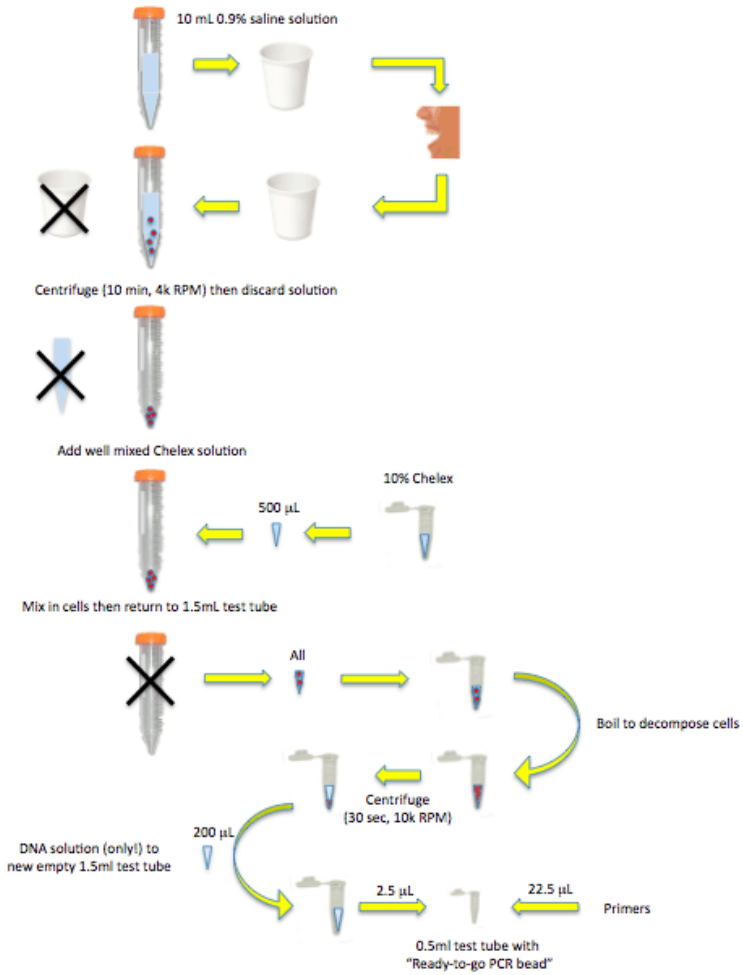


Figure 8A. Relatively simple and reliable DNA extraction protocol involving heating and centrifugation.

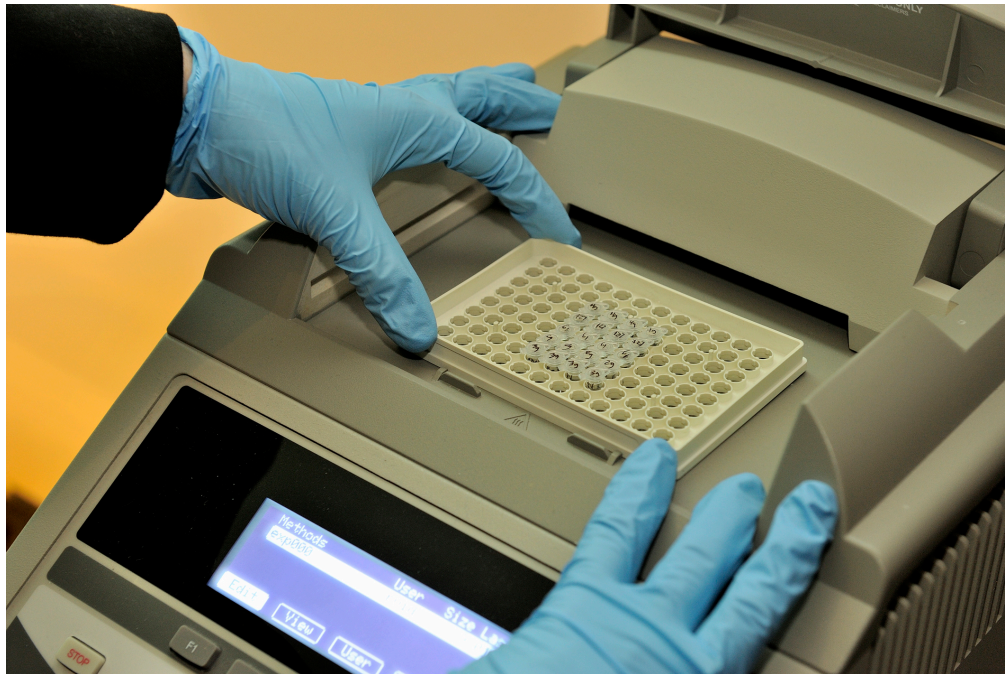


Figure 9B. Running PCR experiment in the thermocycler.

SIC Experiment #12 2011-02-22

SIC amplification in Berlin! This experiment tests smaller fragments first in 60ul volumes. We will be varying the amounts of DMSO and evaluating it's impact on the results.

SIC12a (4.8k) (ul)	SIC12b (4.8k) (ul)	SIC12c (10.17k) (ul)
sH2O 19.65	sH2O 18.75	sH2O 19.65
5x Buffer 6	5x Buffer 6	5x Buffer 6
Nucleotides 1.5	Nucleotides 1.5	Nucleotides 1.5
DNA Template 1.2	DNA Template 1.2	DNA Template 1.2
Taq Ploymerase 0.45	Taq Ploymerase 0.45	Taq Ploymerase 0.45
Primer 1 (F1-tPA(7)) 0.6	Primer 1 (F1-tPA(7)) 0.6	Primer 1 (F2A-tPA(pv)) 0.6
Primer 2 (R1-tPA(10)) 0.6	Primer 2 (R1-tPA(10)) 0.6	Primer 2 (R1B-tPA(pv)) 0.6
DMSO 0.9	DMSO 0.9	
Total Volume 30	Total Volume 30	Total Volume 30

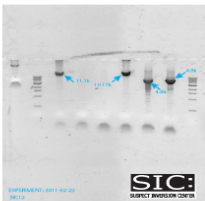
SIC12d (10.17k) (ul)
sH2O 18.75
5x Buffer 6
Nucleotides 1.5
DNA Template 1.2
Taq Ploymerase 0.45
Primer 1 (F2A-tPA(pv)) 0.6
Primer 2 (R1B-tPA(pv)) 0.6
DMSO 0.9
Total Volume 30

PCR Settings

PCR Settings	temp	time	cycles
Denaturation	92	02:00	1
Denaturation	92	00:30	30
Annealing	65	00:30	
Elongation	68	15:00	
final Elongation	68	15:00	1
Cooling	4	30:00	1

Results

4.8k great in both dms0 and non dms0 reactions, lots of cupping, 3 times as bright as needed (can use less in the gel), same with 10.17k with dms0 and 11.17k with dms0. 10.17k without dms0 and 11.1k without dms0 were unsuccessful.



SIC Experiment #13 2011-03-17

This experiment tests the newly designed primer pairs to accomplish fragment sizes of 1.7k, 2.2k, 3.8k, 4.6k, 5.1k, 5.3k, 5.8k, 6k, 7.1k.

SIC13a (7.1k) (ul)	SIC13b (6k) (ul)	SIC13c (5.8k) (ul)
sH2O 18.75	sH2O 18.75	sH2O 18.75
5x Buffer 6	5x Buffer 6	5x Buffer 6
Nucleotides 1.5	Nucleotides 1.5	Nucleotides 1.5
Primer 1 (F-1K*-tPA(pv)) 0.6	Primer 1 (F-1K*-tPA(pv)) 0.6	Primer 1 (F-1L*-tPA(pv)) 0.6
Primer 2 (R-1B-tPA(pv)) 0.6	Primer 2 (R-1E-tPA(pv)) 0.6	Primer 2 (R-1B-tPA(pv)) 0.6
DNA Template 1.2	DNA Template 1.2	DNA Template 1.2
DMSO 0.9	DMSO 0.9	DMSO 0.9
Taq Polymerase 0.45	Taq Polymerase 0.45	Taq Polymerase 0.45
Total Volume 30	Total Volume 30	Total Volume 30

SIC13d (5.5k) (ul)
sH2O 18.75
5x Buffer 6
Nucleotides 1.5
Primer 1 (F-1L*-tPA(pv)) 0.6
Primer 2 (R-1B-tPA(pv)) 0.6
DNA Template 1.2
DMSO 0.9
Taq Polymerase 0.45
Total Volume 30

PCR Settings

PCR Settings	temp	time	cycles
Denaturation	92	02:00	1
Denaturation	92	00:30	30
Annealing	65	00:30	
Elongation	68	15:00	
final Elongation	68	15:00	1
Cooling	4	30:00	1

Results

Good results for 2.2k, 3.8k, 5.1k, 5.3k, 5.5k, and 5.8k. 7.1k, 6.0k, 4.6k and 1.7k unsuccessful.

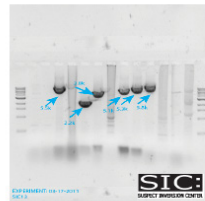


Figure 10B. Two examples of interlinked spreadsheets, part of a database of previous experiments, results, image documentation, etc., needed to organize. This is an advancement of the previous database used in figure 7AB. Some sample tubes were stored for years before we could make the final image. It was important to quantify amounts and confirm the purity of each sample.

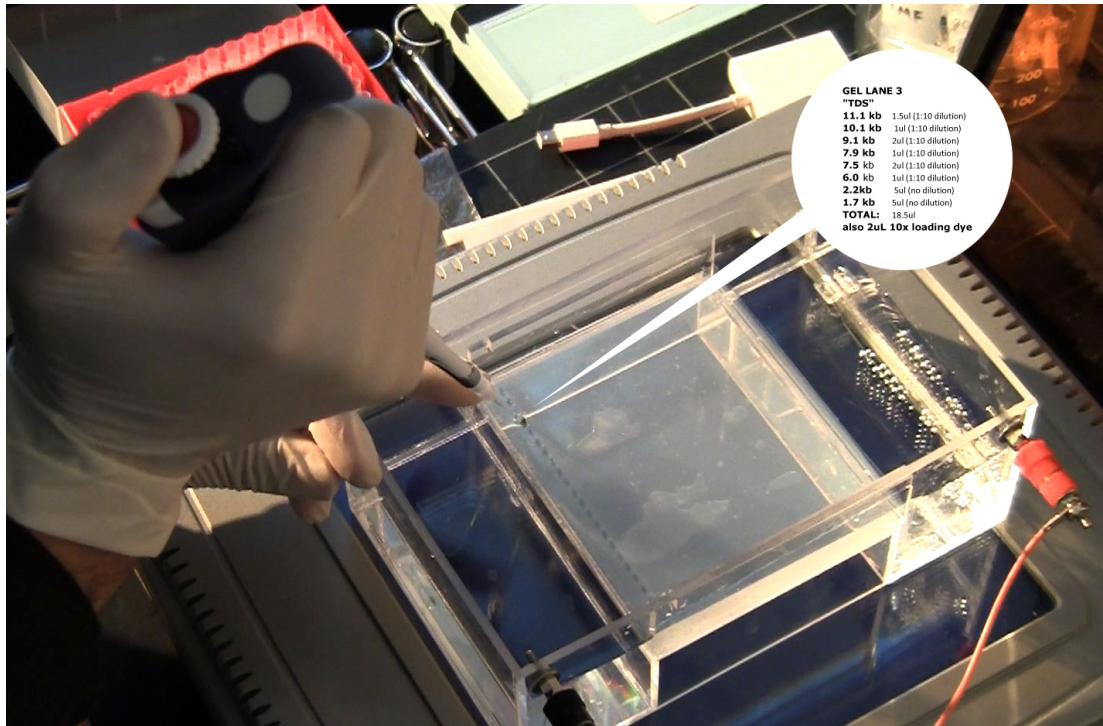


Figure 11A: Inserting DNA into gel. Each insert was itself composed of multiple samples mixed in exacting amounts as shown in bubble.

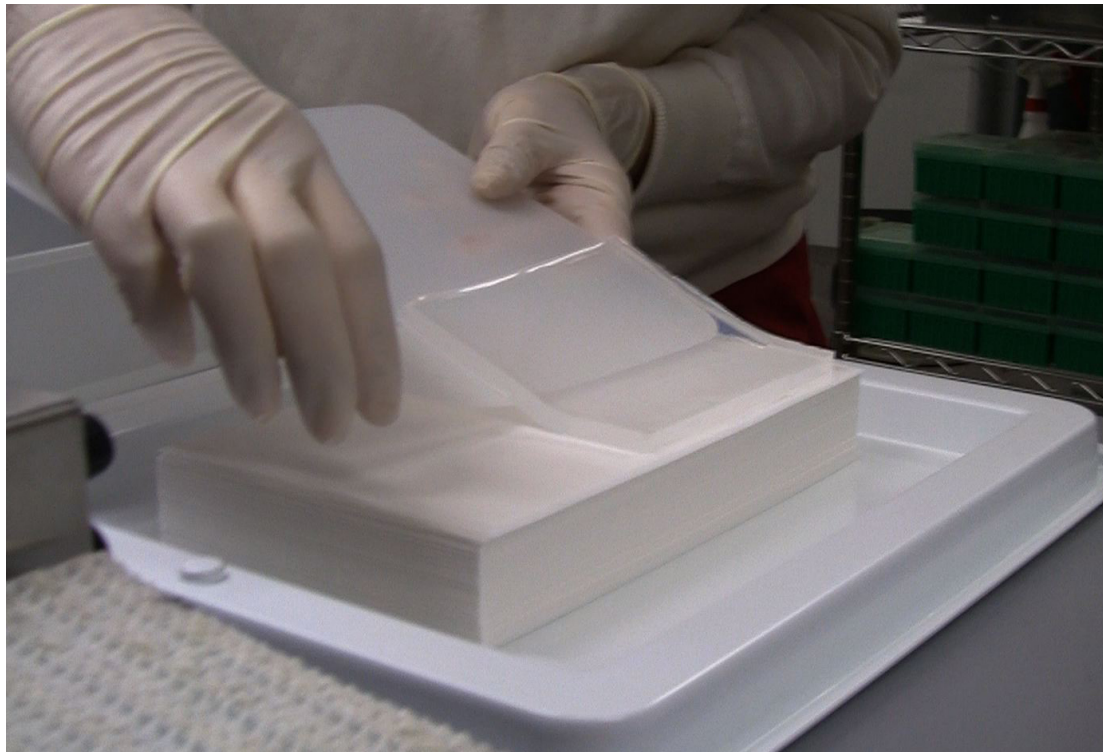


Figure 12: Beginning Southern Blot process transferring DNA from gel to membrane.