

# EXHIBIT 8

# Timeline of Events Surrounding Development and Use of miniSTR Loci for Forensic DNA Typing\*

<u>Date</u>	<u>Event</u>
1994	The Forensic Science Service in running degraded DNA samples from remains of victims of the Branch Davidian fire in Waco, Texas find that smaller STRs in their 4plex work better than the larger loci--see Whitaker et al. (1995) <i>BioTechniques</i> 18(4):670-677 and Lygo et al. (1994) <i>Int. J. Legal Med.</i> 107:77-89.
1996	GeneTrace Systems, a company in Menlo Park, California, begins work with shorter STR amplicons to perform rapid DNA typing using MALDI-TOF mass spectrometry; small PCR products are necessary to obtain successful results with MALDI-TOF
Sept 1997	John Butler and coworkers from GeneTrace submit a patent on mass spec typing and multiplexing using small STR amplicons; patent is granted July 18, 2000--see U.S. Patent 6,090,558
1997-1999	John Butler and coworkers from GeneTrace (funded by National Institute of Justice Grant 97-LB-VX-0003 to do this work) give numerous talks on TOF-MS STR typing with smaller PCR products; some of their work is published in <i>Int. J. Legal Med.</i> 112: 45-49 and <i>Profiles in DNA</i> 1999; 2(3):3-6; manuscripts are also published as part of the International Symposium on Human Identification in 1997 and 1998 as well as the <u>Second European Symposium on Human Identification in 1998.</u>
June 1998	Hermann Schmitter of the German BKA hears <u>Kathy Stephens from GeneTrace speak on small STRs and independently decides to try them on degraded DNA samples; the efforts of his laboratory lead to work with STR typing of telogen hair shafts--see Hellmann et al. (2001) STR typing of human telogen hairs-a new approach. <i>Int. J. Legal Med.</i> 114:269-273.</u>
	John Butler (now back at NIST) puts fluorescent dyes

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- Summer 2000 on his GeneTrace mass spec primers and demonstrates reliable STR typing using the ABI 310; this work is presented as a poster at the 11th International Symposium on Human Identification-- see Ruitberg CM and Butler JM "New Primer Sets for Y Chromosome and CODIS STR Loci"
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- Summer 2001 Bruce McCord (Ohio University) joins John Butler in an informal collaboration working on short STRs to aid analysis of degraded DNA samples
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- October 2001 The National Institute of Justice publishes John Butler's final report from his work at GeneTrace Systems including primer sequences for the short STR amplicons (see p. 24); this report is available at <http://www.ojp.usdoj.gov/nij/pubs-sum/188292.htm>
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- November 2001 After discussions with the World Trade Center Kinship and Data Analysis Panel (WTC KADAP), Dr. Robert Shaler, of the New York City Office of the Chief Medical Examiner (OCME) who is leading the efforts to identify victims of the WTC attacks using DNA testing, contacts John Butler at NIST and asks that efforts with short STRs be accelerated so that this technology may be used with aiding WTC victim identifications; the term "miniSTR" is coined at this time
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- December 2001- January 2002 John Butler and Bruce McCord (along with a postdoc named Yin Shen) work to develop five miniplex assays that incorporate all of the CODIS STR loci as well as D2S1338, Penta D, and Penta E; some concordance studies and initial validation studies are performed but the primary focus is on primer development, generation of allelic ladders and creation of Genotyper macros rather than assay optimization in terms of sensitivity; a summary of this work is later published in Sept 2003: J. Forensic Sci 48(5) 1054-1064.
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- John Butler speaks at SWGDAM (Quantico, VA) informing the attendees of research progress with miniSTRs; Mitch Holland of Bode Technology Group

January 16, 2002	is present and learns of this effort; John Butler meets with Bob Shaler and Howard Baum of NYC OCME to make decisions about which miniplex would be tried by NYC OCME
February 2002	"Big Mini" assay and protocols are supplied to NYC OCME and New York State Police in Albany for trial use; OCME work with Big Mini proves sensitive down to 0.5 ng of DNA template with the provided protocol of 28 cycle PCR and 1 U of TaqGold in 25 uL volumes
February 22, 2002	John Butler <u>speaks to the WTC KADAP</u> as part of their meeting in Albany, NY to inform the group of research progress with miniSTRs
March 2002	Bruce McCord and John Butler submit a proposal to the National Institute of Justice to further develop miniSTR assays; NIJ Grant 2002-IJ-CX-K007 is awarded and results in several publications: Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. <i>J. Forensic Sci.</i> 49(4): 733-740 and Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, <i>J. Forensic Sci.</i> 49(4): 859-860.
March 2002	Bob Shaler meets with Jim Schumm of the Bode Technology Group and discusses the possibility of Bode performing miniSTR testing; Bode is already testing bones from the WTC investigation with conventional STR methods
April 4, 2002	Jim Schumm contacts John Butler for primer sequences and information on miniplex work performed to-date; an Excel file and PowerPoint presentation are emailed from NIST to Bode to aid their efforts
Summer 2002	BodePlexes are developed by Jim Schumm and coworkers at the Bode Technology Group using NIST supplied information as well as the NIJ-published GeneTrace report; a majority of the PCR primers are kept the same but dye labels are switched to provide

different marker combinations; BodePlexes utilize 5X amount of TaqGold and 4-6 more PCR cycles to improve sensitivity over previous miniSTR work

November 22, 2002 Jim Schumm invites Bob Shaler and Howard Baum (OCME) along with John Butler (NIST) to visit the Bode Technology Group (Springfield, VA) in order to review their validation studies for the BodePlex 1 and BodePlex 2 assays that were intended for use on WTC samples; while PCR primer sequences were not revealed, it was admitted that a majority of them came from the NIST and GeneTrace information

Late 2002 - Summer 2003 BodePlex 1 (D13S317, D21S11, D7S820, D16S539, and CSF1PO) and BodePlex 2 (TPOX, FGA, D7S820, and D18S51) are run by the Bode Technology Group on the bone and tissue samples that are part of the WTC investigation--the use of miniSTR BodePlexes has been described in several publications: Schumm, J.W., Wingrove, R.S., Douglas, E.K. (2004) Robust STR multiplexes for challenging casework samples. *Progress in Forensic Genetics* ICS 1261:547-549, Holland, M.M., Cave, C.A., Holland, C.A. and Bille, T.W. Development of a quality, high throughput DNA analysis procedure for skeletal samples to assist with the identification of victims from the World Trade Center attacks. (2003) *Croatian Medical Journal*, 44:264-272.

September 2003 Butler, Shen, and McCord publish miniSTR primer sequences in *J. Forensic Sci* 48(5) 1054-1064 for 13 CODIS STRs and miniplex sets developed in late 2001 and early 2002

December 2003 Mike Coble begins work at NIST as NRC postdoc to develop new miniSTRs beyond the CODIS loci

February 2004 Mike Coble speaks at the American Academy of Forensic Sciences meeting in Dallas, TX about efforts at NIST to develop new miniSTR assays. Denise Chung, a graduate student from Bruce McCord's lab at Ohio University, speaks on their progress with miniSTRs

John Butler is invited to participate in the European DNA Profiling Group (EDNAP) and European Network

April 2004	of Forensic Science Institutes (ENFSI) meeting in Cyprus to help setup an interlaboratory test with Peter Gill on degraded DNA; this EDNAP/ENFSI study will involve conventional STR testing, miniSTR assays, and SNP assays on the same degraded DNA samples
July 2004	Publication of McCord lab work on miniSTRs: <i>J. Forensic Sci.</i> 49(4): 733-740 and <i>J. Forensic Sci.</i> 49(4): 859-860
September 2004	EDNAP/ENFSI study involving miniSTRs and SNPs is initiated--assay materials for <u>two miniSTR systems</u> are provided by NIST to all participating laboratories
January 2005	Mike Coble publishes his work with new miniSTR loci: Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. <i>J. Forensic Sci.</i> 50: 43-53.
September 2005	Mike Coble outlines work with new miniSTR loci at the International Society of Forensic Genetics (ISFG) meeting: "Characterization and performance of new miniSTR loci for typing degraded samples" [ <a href="#">.pdf</a> ]
October 2005	Applied Biosystems announces at the ENFSI meeting that they are working on a commercial kit involving miniSTR loci, which should be available in 2006
January 2006	Peter Gill, Lyn Fereday, Niels Morling, and Peter Schneider representing the <b>EDNAP and ENFSI groups propose that miniSTRs be adopted as the way forward to increase both the robustness and sensitivity of forensic DNA analysis.</b> Their recommendations are published in Gill et al. (2006) <i>Forensic Sci. Int.</i> 156:242-244 in an article entitled "The evolution of DNA databases--Recommendations for new European STR loci." They also recommend that three new miniSTR loci be adopted by European laboratories: D10S1248, D14S1434 (now replaced by D2S441), and D22S1045--all loci described by Coble and Butler in <i>J. Forensic Sci.</i> 50: 43-53. [see also <a href="http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm">http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm</a> ]

February 2006	Becky Hill presents a poster at the AAFS meeting in <u>Seattle, WA</u> on allele frequencies in U.S. populations found with 27 new miniSTR loci under development at NIST
February 2006	Applied Biosystems announces at the AAFS meeting in Seattle, WA efforts to develop a 9plex miniSTR kit (using their 5-dye and mobility modifier technology); this miniSTR kit is designed to recover information from the larger loci in their Identifiler kit and will amplify the following loci: D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA, and amelogenin
March 2006	Bruce McCord's group publishes their work using miniSTRs on skeletal remains: Opel KL, Chung DT, Drabek J, Tatarek NE, Jantz LM, McCord BR (2006) The application of miniplex primer sets in the analysis of degraded DNA from human skeletal remains. <i>J Forensic Sci.</i> 51(2): 351-356
October 2006	Becky Hill presents a poster at the Promega meeting in <u>Nashville, TN</u> on characterization of 26 miniSTR loci under development at NIST
October 2006	Applied Biosystems publishes information about their new <u>MiniFiler kit</u> in their marketing newsletter <u>Forensic News</u>
March 2007	Applied Biosystems releases their new <u>MiniFiler kit</u>
July 2007	NIST and Applied Biosystems co-publish an article examining concordance of allele calls between the MiniFiler kit and other commercial STR kits: Hill, C.R., Kline, M.C., Mulero, J.J., Lagace, R.E., Chang, C.-W., Hennessy, L.K., Butler, J.M. (2007) Concordance study between the AmpFISTR MiniFiler PCR Amplification Kit and conventional STR typing kits. <i>J. Forensic Sci.</i> 52(4): 870-873.

TO BE CONTINUED...

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\*It should be noted that additional work with small PCR products for STRs has been published that is not included on this timeline. See <http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm#Publications> for a fairly comprehensive listing of miniSTR references.

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