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THE PCR REVOLUTION

The invention of the polymerase chain reaction (PCR) won the Nobel Prize in Chemistry in 1994 and remains one of the most important scientific discoveries of the twentieth century. More than 50,000 researchers in the United States use PCR, and this is reflected in the thousands of publications using this technology. In this book, Professor Stephen A. Bustin, a world-renowned PCR expert, has gathered contributions that describe in detail the latest innovations and the overall impact of PCR on many areas of molecular research. The book contains personal reflections, opinions, and comments by leading authorities on the many applications of PCR and how this technology has revolutionized their respective areas of interest. This book conveys the ways in which PCR has overcome many obstacles in life science and clinical research and also charts the PCR's development from time-consuming, low throughput, nonquantitative procedure to today's rapid, high throughput, quantitative super method.

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The PCR Revolution

BASIC TECHNOLOGIES AND APPLICATIONS

Edited by

Stephen A. Bustin

The Institute of Cell and Molecular Science, Barts
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Foreword

Russell Higuchi

Advances in science and technology come at an ever increasing pace. What was state of the art can be obsolete in just a few years. The old saw "The work that earns a Nobel prize today will be a graduate thesis ten years from now" may underestimate the rate of change. The hard-earned accomplishments of scientists and technologists can be made to seem trivial well within the time frame of a career. Faced with this, what do scientists celebrate as their accomplishments?

In the Stephen Sondheim play *Sunday in the Park with George*, about the pioneering artist, George Seurat, the character Dot says that the only things worth "passing on" to posterity are "children and art." I agree wholeheartedly with the children part but I wondered about how broadly art could be defined in this context. As evidenced by university colleges still organized around "Arts and Sciences" – a holdover I believe from classical uses of the terms – science and art did not used to be so far apart. I believe that beautiful, inventive thinking can be art, and good science is full of beautiful, inventive thinking – such as PCR.

When I first heard of PCR, I thought – art. When I later joined Cetus and heard from Kary Mullis his idea to use a thermostable enzyme – art. When Kary proposed the Hot Start (a bit arcane but to someone who was now an aficionado) – art.

Nonetheless, I did find myself thinking, "PCR – five years and something even better will come along." More than twenty years later, however, that has not proven to be the case, as PCR has matured and, as evidenced by the chapters in this book, is still increasingly useful. Part of that is due to real-time PCR, which I was the first to put into practice, and which is well covered in this book.

However, something better will definitely come along. We already have highly parallel sequencing of clonally amplified single DNA molecules with a throughput of a human genome a week. We are close to true single-molecule sequencing of a human genome a day. If ways can be found to parse this enormous throughput cost effectively among large numbers of samples, why use PCR and a probe to guess at what sequences might be in a sample when you can know everything that is there and at what frequency?

So the question: If its use is so transient, can it be art? I think so. With art in general, "usefulness" is not a measure of its import. How and why it comes

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into existence is. Hence the import of books like this, in which, unlike in journal articles, the how and why are told.

Lastly, I was pleased to see a chapter here on ancient DNA and PCR. The late Allan Wilson and I reported the first recombinant DNA cloning and sequencing of ancient DNA.[1] It was through trying to make this more efficient that I first learned PCR.[2] In referring to this work, a hard-core biochemist I knew said, "this is not science, it's art." Now I like to think it was a bit of both.

REFERENCES

1. Higuchi R, Bowman B, Freiberger M, Ryder OA, Wilson AC. DNA sequences from the quagga, an extinct member of the horse family. *Nature* 1984;**312**: 282–284.
2. Pääbo S, Higuchi RG, Wilson AC. Ancient DNA and the polymerase chain reaction. The emerging field of molecular archaeology. *Journal of Biological Chemistry* 1989;**264**: 9709–9712.

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Preface

We live in an age in which hyperbole has become so pervasive that it is difficult to find apt expressions for something truly exceptional. Furthermore, impatience, haste, and short attention span seem to be added hallmarks of our times, inviting technological bandwagon effects that briefly promise the earth, but then cannot deliver because the technologies were either conceived in haste without proper regard for technical and biological concerns or are superseded by the next technological "revolution."

The polymerase chain reaction (PCR) has been around a long time now: certainly as US Patent 4,683,202 since 1987, as a practical tool since 1985,[1] and as a theoretical proposition since 1971.[2] A Google search for "polymerase chain reaction" throws up more than 1.3×10^7 results, roughly the same number as a search for "monoclonal antibody," the other wonder technology in the molecular arsenal. Its conceptual clarity, practical accessibility, and ubiquitous applicability have made PCR the defining technology of our molecular age, with a three-letter abbreviation as distinctive as that of deoxyribonucleic acid (DNA). It has even made it to Hollywood, where the re-creation of dinosaurs in *Jurassic Park* was accomplished using PCR technology. The concept is so perfectly simple that the elemental scheme remains unchanged since its inception: two oligonucleotide primers that define converging sequences on opposite strands of a DNA molecule, a DNA polymerase, dNTP building blocks, and a series of heating and cooling cycles. This prompts the selection and enormous amplification of specific DNA sequences; consequently, the needle-in-a-haystack stumbling block is magically recast as a solution that creates a haystack made up of needles.

In contrast, the detection of amplification products has undergone, and continues to undergo, pronounced changes that have led the technology into new contexts and uses. The most dramatic, and dare I say revolutionary, innovation has been the invention of real-time quantitative PCR (qPCR), which in a flash has addressed many of the practical limitations associated with legacy, gel-based PCR.[3] This adaptation allows detection of the accumulation of amplified DNA in real time after each amplification cycle. At its simplest, a qPCR experiment uses legacy PCR protocols, with the simple addition of a DNA intercalating dye, most commonly SYBR Green.[4] When complexed with double-stranded DNA, the

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dye absorbs blue light (λ_{max} = 488 nm) and emits green light (λ_{max} = 522 nm) that is easily detected using a qPCR instrument that combines a thermal cycler with a fluorimeter.[5] At its most complex, qPCR can use a number of fluorescent dye–labeled probes to detect and quantify multiple targets in the same tube.[6] Certainly, qPCR has been a requisite for the translation of PCR from pervasive research technology to practical process and has propelled progress in every branch of the life sciences, from agriculture to zoology; indeed it has created and sustains whole new sectors.

So, how to describe this technology and do it justice? Luckily, PCR speaks for itself through the vast numbers of applications, settings, and achievements that are unthinkable without this simple technique. This book attempts to tell the story of the PCR and to shine the light on some of the scientific advances that would never have happened without it. It presents personal views of authors from a wide range of backgrounds, pursuing an eclectic mixture of interests but united in their appreciation of the key role played by the PCR in their individual pursuits. Contributors include giants of the PCR field: Carl Wittwer, the "father" of qPCR instrumentation[5,7–9] as well as the pacesetter behind numerous practical qPCR innovations[4,10–39]; Mickey Williams, one of the original "Taqmen" and major contributor to further modifications[40–42]; Fred Kramer and Sanjay Tyagi, inventors of the ingenious molecular beacons and variants, as well as practical applications for them[43–50]; and Michael Pfaffl, major contributor and instigator of the drive toward more reliable and appropriate target quantification.[51–59] The essays of these exceptional individuals are complemented by a range of contributions that focus on practical applications of the technology. These range from Susan Burchill, who has explored the clinical potential of this technology,[60–73] to the wonderful world of ancient DNA research.[74–83] Their stories are all about the impact PCR has had on these and many other areas of molecular research. They aim to convey a flavor of the obstacles faced by life science and clinical researchers and how the unique properties of the PCR have been instrumental in overcoming these limitations. The book also aspires to chart the development of this technique from a time-consuming, low throughput, nonquantitative procedure to today's rapid, high throughput quantitative super method. Reading through these chapters clarifies just how phenomenally powerful this technology is.

Sadly, something as commanding as this technology is also open to abuse and inappropriate use – which is also addressed within these pages. This is particularly distressing when it involves peoples' health and highlights the need for constant vigilance when interpreting PCR-derived data. Opportunely, the year 2009 sees the publication of the first set of guidelines for researchers publishing PCR-based results.[84]

Tempus fugit and PCR continues to evolve. I hope that this snapshot and the associated reflections of a group of individuals who have lived this technology will help to inspire others, and make them reflect that while a technology might be great, it is the individuals who craft and struggle with it that make it truly extraordinary.

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REFERENCES

1. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985;**230**:1350–1354.
2. Kleppe K, Ohtsuka E, Kleppe R, Molineux I, Khorana HG. Studies on polynucleotides. XCVI. Repair replications of short synthetic DNAs as catalyzed by DNA polymerases. *J Mol Biol* 1971;**56**:341–361.
3. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Biotechnology (NY)* 1993;**11**:1026–1030.
4. Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP. Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques* 1997;**22**:130–138.
5. Wittwer CT, Ririe KM, Andrew RV, David DA, Gundry RA, Balis UJ. The LightCycler: a microvolume multisample fluorimeter with rapid temperature control. *Biotechniques* 1997;**22**:176–181.
6. Weller SA, Elphinstone JG, Smith NC, Boonham N, Stead DE. Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. *Appl Environ Microbiol* 2000;**66**:2853–2858.
7. Wittwer CT, Fillmore GC, Hillyard DR. Automated polymerase chain reaction in capillary tubes with hot air. 1989;**17**:4353–4357.
8. Wittwer CT, Fillmore GC, Garling DJ. Minimizing the time required for DNA amplification by efficient heat transfer to small samples. *Anal Biochem* 1990;**186**:328–331.
9. Wittwer CT, Garling DJ. Rapid cycle DNA amplification: time and temperature optimization. *Biotechniques* 1991;**10**:76–83.
10. Weis JH, Tan SS, Martin BK, Wittwer CT. Detection of rare mRNAs via quantitative RT-PCR. 1992;**8**:263–264.
11. Ririe KM, Rasmussen RP, Wittwer CT. Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal Biochem* 1997;**245**:154–160.
12. Lay MJ, Wittwer CT. Real-time fluorescence genotyping of factor V Leiden during rapid-cycle PCR. 1997;**43**:2262–2267.
13. Ririe KM, Rasmussen RP, Wittwer CT. Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal Biochem* 1997;**245**:154–160.
14. Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP. Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques* 1997;**22**:130–131, 4–8.
15. Bernard PS, Ajioka RS, Kushner JP, Wittwer CT. Homogeneous multiplex genotyping of hemochromatosis mutations with fluorescent hybridization probes. 1998;**153**:1055–1061.
16. Bernard PS, Lay MJ, Wittwer CT. Integrated amplification and detection of the C677T point mutation in the methylenetetrahydrofolate reductase gene by fluorescence resonance energy transfer and probe melting curves. *Anal Biochem* 1998;**255**:101–107.
17. Morrison TB, Weis JJ, Wittwer CT. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *Biotechniques* 1998;**24**:954–962.
18. Bernard PS, Ajioka RS, Kushner JP, Wittwer CT. Homogeneous multiplex genotyping of hemochromatosis mutations with fluorescent hybridization probes. *Am J Pathol* 1998;**153**:1055–1061.
19. Bernard PS, Pritham GH, Wittwer CT. Color multiplexing hybridization probes using the apolipoprotein E locus as a model system for genotyping. 1999;**273**:221–228.
20. Elenitoba-Johnson KS, Bohling SD, Wittwer CT, King TC. Multiplex PCR by multicolor fluorimetry and fluorescence melting curve analysis. *Nat Med* 2001;**7**:249–253.
21. Wittwer CT, Herrmann MG, Gundry CN, Elenitoba-Johnson KS. Real-time multiplex PCR assays. *Methods* 2001;**25**:430–442.

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22. Bernard PS, Wittwer CT. Real-time PCR technology for cancer diagnostics. *Clin Chem* 2002;**48**:1178–1185.
23. Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ. High-resolution genotyping by amplicon melting analysis using LCGreen. *Clin Chem* 2003;**49**:853–860.
24. Gundry CN, Vandersteen JG, Reed GH, Pryor RJ, Chen J, Wittwer CT. Amplicon melting analysis with labeled primers: a closed-tube method for differentiating homozygotes and heterozygotes. *Clin Chem* 2003;**49**:396–406.
25. Palais R, Wittwer CT. Mathematical algorithms for high-resolution DNA melting analysis. *Methods Enzymol* 2009;**454**:323–343.
26. Zhou L, Errigo RJ, Lu H, Poritz MA, Seipp MT, Wittwer CT. Snapback primer genotyping with saturating DNA dye and melting analysis. *Clin Chem* 2008;**54**:1648–1656.
27. Seipp MT, Pattison D, Durtschi JD, Jama M, Voelkerding KV, Wittwer CT. Quadruplex genotyping of F5, F2, and MTHFR variants in a single closed tube by high-resolution amplicon melting. *Clin Chem* 2008;**54**:108–115.
28. Gundry CN, Dobrowolski SF, Martin YR, Robbins TC, Nay LM, Boyd N, et al. Base-pair neutral homozygotes can be discriminated by calibrated high-resolution melting of small amplicons. *Nucleic Acids Res* 2008;**36**:3401–3408.
29. Elenitoba-Johnson O, David D, Crews N, Wittwer CT. Plastic versus glass capillaries for rapid-cycle PCR. *Biotechniques* 2008;**44**:487–488, 90, 92.
30. Sundberg SO, Wittwer CT, Greer J, Pryor RJ, Elenitoba-Johnson O, Gale BK. Solution-phase DNA mutation scanning and SNP genotyping by nanoliter melting analysis. *Biomed Microdevices* 2007;**9**:159–166.
31. Reed GH, Kent JO, Wittwer CT. High-resolution DNA melting analysis for simple and efficient molecular diagnostics. *Pharmacogenomics* 2007;**8**:597–608.
32. Dames S, Pattison DC, Bromley LK, Wittwer CT, Voelkerding KV. Unlabeled probes for the detection and typing of herpes simplex virus. *Clin Chem* 2007;**53**:1847–1854.
33. Dames S, Margraf RL, Pattison DC, Wittwer CT, Voelkerding KV. Characterization of aberrant melting peaks in unlabeled probe assays. *J Mol Diagn* 2007;**9**:290–296.
34. Margraf RL, Mao R, Wittwer CT. Masking selected sequence variation by incorporating mismatches into melting analysis probes. *Hum Mutat* 2006;**27**:269–278.
35. Palais RA, Liew MA, Wittwer CT. Quantitative heteroduplex analysis for single nucleotide polymorphism genotyping. *Anal Biochem* 2005;**346**:167–175.
36. Chou LS, Meadows C, Wittwer CT, Lyon E. Unlabeled oligonucleotide probes modified with locked nucleic acids for improved mismatch discrimination in genotyping by melting analysis. *Biotechniques* 2005;**39**:644, 6, 8 passim.
37. Zhou L, Myers AN, Vandersteen JG, Wang L, Wittwer CT. Closed-tube genotyping with unlabeled oligonucleotide probes and a saturating DNA dye. *Clin Chem* 2004;**50**:1328–1335.
38. Reed GH, Wittwer CT. Sensitivity and specificity of single-nucleotide polymorphism scanning by high-resolution melting analysis. *Clin Chem* 2004;**50**:1748–1754.
39. Margraf RL, Page S, Erali M, Wittwer CT. Single-tube method for nucleic acid extraction, amplification, purification, and sequencing. *Clin Chem* 2004;**50**:1755–1761.
40. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res* 1996;**6**:986–994.
41. Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. *Genome Res* 1996;**6**:995–1001.
42. Winer J, Jung CK, Shackel I, Williams PM. Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. *Anal Biochem* 1999;**270**:41–49.
43. Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization. *Nat Biotechnol* 1996;**14**:303–308.
44. Tyagi S, Bratu DP, Kramer FR. Multicolor molecular beacons for allele discrimination. 1998;**16**:49–53.
45. Kostrikis LG, Tyagi S, Mhlanga MM, Ho DD, Kramer FR. Spectral genotyping of human alleles. *Science* 1998;**279**:1228–1229.

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978-0-521-88231-6 - The PCR Revolution: Basic Technologies and Applications
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More information

46. Vet JA, Majithia AR, Marras SA, Tyagi S, Dube S, Poiesz BJ, Kramer FR. Multiplex detection of four pathogenic retroviruses using molecular beacons. 1999;**96**:6394–6399.
47. Bonnet G, Tyagi S, Libchaber A, Kramer FR. Thermodynamic basis of the enhanced specificity of structured DNA probes. 1999;**96**:6171–6176.
48. Marras SA, Kramer FR, Tyagi S. Multiplex detection of single-nucleotide variations using molecular beacons. 1999;**14**:151–156.
49. Tyagi S, Marras SA, Kramer FR. Wavelength-shifting molecular beacons. *Nat Med* 2000;**18**:1191–1196.
50. El-Hajj HH, Marras SA, Tyagi S, Shashkina E, Kamboj M, Kiehn TE, et al. Identification of mycobacterial species utilizing sloppy molecular beacon probes. *J Clin Microbiol* 2009 (in press).
51. Fleige S, Pfaffl MW. RNA integrity and the effect on the real-time qRT-PCR performance. *Mol Aspects Med* 2006;**27**:126–139.
52. Fleige S, Walf V, Huch S, Prgomet C, Sehm J, Pfaffl MW. Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. *Biotechnol Lett* 2006;**28**:1601–1613.
53. Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations. *Biotechnol Lett* 2004;**26**:509–515.
54. Tichopad A, Didier A, Pfaffl MW. Inhibition of real-time RT-PCR quantification due to tissue-specific contaminants. *Mol Cell Probes* 2004;**18**:45–50.
55. Stahlberg A, Kubista M, Pfaffl M. Comparison of reverse transcriptases in gene expression analysis. *Clin Chem* 2004;**50**:1678–1680.
56. Tichopad A, Dilger M, Schwarz G, Pfaffl MW. Standardized determination of real-time PCR efficiency from a single reaction set-up. *Nucleic Acids Res* 2003;**31**:e122.
57. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* 2002;**30**:e36.
58. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;**29**:e45.
59. Pfaffl MW, Hageleit M. Validities of mRNA quantification using recombinant RNA and recombinant DNA external calibration curves in real-time RT-PCR. *Biotechnol Lett* 2001;**23**:275–282.
60. Viprey VF, Lastowska MA, Corrias MV, Swerts K, Jackson MS, Burchill SA. Minimal disease monitoring by QRT-PCR: guidelines for identification and systematic validation of molecular markers prior to evaluation in prospective clinical trials. *J Pathol* 2008;**216**:245–252.
61. Viprey VF, Corrias MV, Kagedal B, Oltra S, Swerts K, Vicha A, et al. Standardisation of operating procedures for the detection of minimal disease by QRT-PCR in children with neuroblastoma: quality assurance on behalf of SIOPEN-R-NET. *Eur J Cancer* 2007;**43**:341–350.
62. Beiske K, Ambros PF, Burchill SA, Cheung IY, Swerts K. Detecting minimal residual disease in neuroblastoma patients – the present state of the art. *Cancer Lett* 2005;**228**:229–240.
63. Patel K, Whelan PJ, Prescott S, Brownhill SC, Johnston CF, Selby PJ, Burchill SA. The use of real-time reverse transcription-PCR for prostate-specific antigen mRNA to discriminate between blood samples from healthy volunteers and from patients with metastatic prostate cancer. *Clin Cancer Res* 2004;**10**:7511–7519.
64. Riley RD, Heney D, Jones DR, Sutton AJ, Lambert PC, Abrams KR, et al. A systematic review of molecular and biological tumor markers in neuroblastoma. *Clin Cancer Res* 2004;**10**:4–12.
65. Burchill SA, Perebolte L, Johnston C, Top B, Selby P. Comparison of the RNA-amplification based methods RT-PCR and NASBA for the detection of circulating tumour cells. *Br J Cancer* 2002;**86**:102–109.

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66. Burchill SA, Kinsey SE, Picton S, Roberts P, Pinkerton CR, Selby P, Lewis IJ. Minimal residual disease at the time of peripheral blood stem cell harvest in patients with advanced neuroblastoma. *Med Pediatr Oncol* 2001;**36**:213–219.
67. Burchill SA, Selby PJ. Molecular detection of low-level disease in patients with cancer. *J Pathol* 2000;**190**:6–14.
68. Keilholz U, Willhauck M, Rimoldi D, Brasseur F, Dummer W, Rass K, et al. Reliability of reverse transcription-polymerase chain reaction (RT-PCR)-based assays for the detection of circulating tumour cells: a quality-assurance initiative of the EORTC Melanoma Cooperative Group. *Eur J Cancer* 1998;**34**:750–753.
69. Wyld DK, Selby P, Perren TJ, Jonas SK, Allen-Mersh TG, Wheeldon J, Burchill SA. Detection of colorectal cancer cells in peripheral blood by reverse-transcriptase polymerase chain reaction for cytokeratin 20. *Int J Cancer* 1998;**79**:288–293.
70. Keilholz U, Willhauck M, Scheibenbogen C, de Vries TJ, Burchill S. Polymerase chain reaction detection of circulating tumour cells. EORTC Melanoma Cooperative Group, Immunotherapy Subgroup. *Melanoma Res* 1997;7 Suppl **2**:S133–141.
71. Pittman K, Burchill S, Smith B, Southgate J, Joffe J, Gore M, Selby P. Reverse transcriptase–polymerase chain reaction for expression of tyrosinase to identify malignant melanoma cells in peripheral blood. *Ann Oncol* 1996;**7**:297–301.
72. Johnson PW, Burchill SA, Selby PJ. The molecular detection of circulating tumour cells. *Br J Cancer* 1995;**72**:268–276.
73. Burchill SA, Bradbury MF, Pittman K, Southgate J, Smith B, Selby P. Detection of epithelial cancer cells in peripheral blood by reverse transcriptase-polymerase chain reaction. *Br J Cancer* 1995;**71**:278–281.
74. Knapp M, Rohland N, Weinstock J, Baryshnikov G, Sher A, Nagel D, et al. First DNA sequences from Asian cave bear fossils reveal deep divergences and complex phylogeographic patterns. *Mol Ecol* 2009 (in press).
75. Hofreiter M. DNA sequencing: mammoth genomics. *Nature* 2008;**456**:330–331.
76. Krause J, Unger T, Nocon A, Malaspinas AS, Kolokotronis SO, Stiller M, et al. Mitochondrial genomes reveal an explosive radiation of extinct and extant bears near the Miocene-Pliocene boundary. *BMC Evol Biol* 2008;**8**:220.
77. Gilbert MT, Jenkins DL, Gotherstrom A, Naveran N, Sanchez JJ, Hofreiter M, et al. DNA from pre-Clovis human coprolites in Oregon, North America. *Science* 2008;**320**:786–789.
78. Meyer M, Briggs AW, Maricic T, Hober B, Hoffner B, Krause J, et al. From micrograms to picograms: quantitative PCR reduces the material demands of high-throughput sequencing. *Nucleic Acids Res* 2008;**36**:e5.
79. Rohland N, Hofreiter M. Ancient DNA extraction from bones and teeth. *Nat Protoc* 2007;**2**:1756–1762.
80. Rompler H, Dear PH, Krause J, Meyer M, Rohland N, Schoneberg T, et al. Multiplex amplification of ancient DNA. *Nat Protoc* 2006;**1**:720–728.
81. Leonard JA, Rohland N, Glaberman S, Fleischer RC, Caccone A, Hofreiter M. A rapid loss of stripes: the evolutionary history of the extinct quagga. *Biol Lett* 2005;**1**:291–295.
82. Hofreiter M, Lister A. Mammoths. *Curr Biol* 2006;**16**:R347–348.
83. Hofreiter M, Poinar HN, Spaulding WG, Bauer K, Martin PS, Possnert G, Pääbo S. A molecular analysis of ground sloth diet through the last glaciation. *Mol Ecol* 2000;**9**:1975–1984.
84. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* 2009;**55**:611–622.

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