Published in final edited form as:

DNA Repair (Amst). 2007 January 4; 6(1): 3–7.

DNA polymerase beta null mouse embryonic fibroblasts harbor a homozygous null mutation in DNA polymerase iota

Robert W. Sobol*

Department of Pharmacology, University of Pittsburgh School of Medicine & University of Pittsburgh Cancer Institute, Hillman Cancer Center, Pittsburgh, PA 15213-1863

Dear Editor

Prior to the explosion of gene targeting technology, considerable biochemical and cell-based experiments suggested a role for DNA polymerase β (pol β) in DNA repair. Since its initial discovery in 1971 [1], the enzyme that was to eventually be designated pol β [2,3] was unique in its enzymatic properties [4–6] as compared to the other newly characterized mammalian DNA polymerases alpha (pol α), gamma (pol γ) and delta (pol δ) [1,7]. Of the four eukaryotic DNA polymerases identified by 1977, pol β soon earned the moniker of ‘the’ DNA repair polymerase [8–11]. These early studies defined a role for pol β in repair using isolated nuclei or nuclear extracts, monitoring the incorporation of radioactive nucleosides following ultraviolet (UV) light-induced DNA damage [8–11]. A more definitive role for pol β in excision repair *in vitro* was demonstrated in 1983, in which complete removal of pyrimidine dimers in DNA was conducted by purified enzyme preparations of pol β , T4 denV (UV endonuclease) and HeLa AP endonuclease II [12]. Although it was subsequently shown that pol α can also carry-out gap-filling DNA synthesis in a base excision repair (BER) reaction [13], the evidence continued to mount in support of pol β acting as ‘the’ DNA repair polymerase in the nucleus, while pol γ participated in replication and repair in mitochondria [1]. Studies continued to identify a role for pol β in the repair of damage induced by many different DNA damaging agents, including bleomycin [14,15], UV-radiation [16], benzo[a]pyrene [17], methylmethane sulfonate [18], ionizing radiation [19], G-T mis-pairs [20] and uracil [21–24]. Several groups reported complete BER *in vitro* with pol β and additional purified proteins [22,23,25]. Although it was demonstrated in heterologous systems (*E. coli* and *Saccharomyces cerevisiae*) that pol β can conduct DNA replication and repair *in vivo* [26,27], it was not until a mouse gene knockout was made that the specificity of the repair conducted by pol β was defined [28].

Characterization of the pol β knockout mouse [29,30] and mouse embryonic fibroblasts (MEFs) deficient in pol β [28] clearly demonstrated a requirement for pol β in repair of alkylation and oxidative DNA damage [28,31] and provided a valuable resource to explore additional functions of pol β [32,33], to evaluate the impact of pol β on mutagenesis [34–38] and mechanisms of genotoxin-induced cell death [39–47], to investigate alternate or compensatory repair pathways in the absence of pol β [48–53] and to address structure-function relationships or protein partners of pol β *in vivo* [34,54,55]; among other studies too numerous to mention herein. The most definitive and reproducible endpoint that has been used to evaluate pol β

*To whom correspondence should be addressed: Robert W. Sobol, Hillman Cancer Center, University of Pittsburgh Cancer Institute, Research Pavilion, Suite 2.6, 5117 Centre Avenue, Pittsburgh, PA 15213-1863, Phone: 412-623-7764, Fax: 412-623-7761, e-mail: rws9@pitt.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

participation in repair *in vivo* is survival following DNA damage such as exposure to alkylating agents [28,55]. Surprisingly, it is the 5'dRP lyase function of pol β [56] that appears to be essential and sufficient for alkylating-agent resistance [55].

In the absence of pol β (in MEFs), cells are unable to efficiently repair the highly toxic 5'dRP moiety and therefore are hypersensitive to different types of alkylating agents such as methylmethane sulfonate, *N*-methyl-*N*-nitrosourea and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [28,36,42,44,55], the thymidine analog 5-hydroxymethyl-2' -deoxyuridine [40] as well as the therapeutic agent temozolomide [40,44]. Recently, it was also demonstrated that in addition to pol β , DNA polymerase λ (pol λ) and DNA polymerase ι (pol ι), as well as the mitochondrial polymerase pol γ have 5'dRP lyase activity and can function (*in vitro*) in the removal of the 5'dRP group that arises during BER [53,57–61]. This would suggest that pol ι and/or pol λ may participate in BER, possibly as a back-up to pol β or may function with unique lesion specificity, in-line with the proposition that the initiating lesion directs the repair sub-pathway [51,62]. Although MEFs deficient in either pol ι or pol λ are not hypersensitive to alkylating agents [44], the ability of either pol ι and/or pol λ to complement pol β *in vivo* has not been evaluated. Using MEFs isolated from pol λ knockout mice [63], a back-up role for pol λ was observed following oxidative damage, although the requirement for the 5'dRP activity of pol λ has not been determined [53,59]. To date, a targeted deletion or inactivation of the mouse POLI gene has not been reported. However, it was observed that mice derived from the 129 strain harbor a nonsense mutation in exon 2, rendering these mice deficient in pol ι expression [64].

The pol β knockout mouse [29] and hence the derived MEFs [28] were developed by gene-targeting using 129-derived embryonic stem cells. As such, there is the possibility that the pol β deficient cells may harbor a null mutation in pol ι in addition to the targeted null mutation in pol β . We have therefore evaluated all of the pol β deficient MEFs we have available for the presence of the nonsense mutation in the POLI gene. The PCR-based genotyping screen of codon 27 differentiates a wild-type allele (codon = TCG) from a null allele (codon = TAG) as this codon is part of a *TaqI* restriction enzyme site in the wild-type allele (*TaqI* recognition sequence = TCGA) but the *TaqI* site is destroyed in the null allele (TAGA) [64]. As shown in Figure 1 and described in Table I, one of the pol β null MEF cell lines (19tsA, clone 2B2), initially described in 1996 [28] and available from ATCC, also harbors a null mutation in pol ι . We have notified ATCC to change the description for this cell line and will point out, as indicated in Table I that a second matched pair of MEFs are available from ATCC each of which are WT for pol ι . The alkylation sensitivity of pol β deficient cells is not in question - several additional reports have confirmed this observation using the matched pairs listed in Table I that are WT for pol ι , including the 36.3 and 38.4 matched pair [36,55] and the 92TAG and 88TAG matched pair [42,44] as well as pol β deficiency mediated by RNA interference [44,65] and many other studies from other laboratories. It remains to be determined if these double null cells have an increased sensitivity to alkylating agents and most importantly, if complementation with mouse pol ι effects a change as well.

This same procedure can be utilized to check other MEF cell lines derived from mouse 129 strains. Note that in general, this should not be a significant problem, as most knockout lines are bred for several generations to the C57BL/6 strain before MEFs are developed. As indicated in Table I, only the 19tsA cell line harbors the 129 strain-derived POLI gene mutation. This cell line was developed from crossing an F1 generation and was not found in MEFs that were derived from later generations. In addition, as it is almost standard practice to breed newly developed knockout lines for a few generations to C57BL/6 before initiation of detailed experimental analysis, we do not foresee this as impacting any of the published whole animal studies with regard to the pol β +/- mice.

Acknowledgements

I would like to thank Sandy Schamus for assistance with the genomic DNA isolation and PCR analysis. Supported by a Research Scholar grant (RSG-05-246-01-GMC) from the American Cancer Society, grants from the Susan G. Komen Breast Cancer Foundation (Grant # BCTR0403276), NIH (1 R01 AG24364-01; P20 CA103730), the UPMC Health System Competitive Medical Research Fund, the Elsa U. Pardee Foundation and the University of Pittsburgh Cancer Institute to RWS.

References

1. Weissbach A. Eukaryotic DNA polymerases. *Annu Rev Biochem* 1977;46:25–47. [PubMed: 332062]
2. Weissbach A, Baltimore D, Bollum F, Gallo R, Korn D. Nomenclature of eukaryotic DNA polymerases. *Eur J Biochem* 1975;59:1–2. [PubMed: 1204601]
3. Weissbach A, Baltimore D, Bollum F, Gallo R, Korn D. Nomenclature of eukaryotic DNA polymerases. *Science* 1975;190:401–402. [PubMed: 1179222]
4. Ono K, Ohashi A, Tanabe K, Matsukage A, Nishizawa M, Takahashi T. Unique requirements for template primers of DNA polymerase beta from rat ascites hepatoma AH130 cells. *Nucleic Acids Res* 1979;7:715–726. [PubMed: 503843]
5. Tanabe K, Bohn EW, Wilson SH. Steady-state kinetics of mouse DNA polymerase beta. *Biochemistry* 1979;18:3401–3406. [PubMed: 465481]
6. Yoshida S, Yamada M, Masaki S. Novel properties of DNA polymerase beta with poly(rA).oligo(dT) template-primer. *J Biochem (Tokyo)* 1979;85:1387–1395. [PubMed: 457638]
7. Byrnes JJ, Downey KM, Black VL, So AG. A new mammalian DNA polymerase with 3' to 5' exonuclease activity: DNA polymerase delta. *Biochemistry* 1976;15:2817–2823. [PubMed: 949478]
8. Hubscher U, Kuenzle CC, Spadari S. Functional roles of DNA polymerases beta and gamma. *Proc Natl Acad Sci U S A* 1979;76:2316–2320. [PubMed: 287074]
9. Siedlecki JA, Szyszko J, Pietrzykowska I, Zmudzka B. Evidence implying DNA polymerase beta function in excision repair. *Nucleic Acids Res* 1980;8:361–375. [PubMed: 6252546]
10. Waser J, Hubscher U, Kuenzle CC, Spadari S. DNA polymerase beta from brain neurons is a repair enzyme. *Eur J Biochem* 1979;97:361–368. [PubMed: 467424]
11. Wawra E, Dolejs I. Evidences for the function of DNA polymerase-beta in unscheduled DNA synthesis. *Nucleic Acids Res* 1979;7:1675–1686. [PubMed: 503866]
12. Mosbaugh DW, Linn S. Excision repair and DNA synthesis with a combination of HeLa DNA polymerase beta and DNase V. *J Biol Chem* 1983;258:108–118. [PubMed: 6848490]
13. Mosbaugh DW, Linn S. Gap-filling DNA synthesis by HeLa DNA polymerase alpha in an in vitro base excision DNA repair scheme. *J Biol Chem* 1984;259:10247–10251. [PubMed: 6469963]
14. Seki S, Oda T. DNA repair synthesis in bleomycin-pretreated permeable HeLa cells. *Carcinogenesis* 1986;7:77–82. [PubMed: 2417738]
15. DiGiuseppe JA, Dresler SL. Bleomycin-induced DNA repair synthesis in permeable human fibroblasts: mediation of long-patch and short-patch repair by distinct DNA polymerases. *Biochemistry* 1989;28:9515–9520. [PubMed: 2482074]
16. Orlando P, Geremia R, Frusciante C, Tedeschi B, Grippo P. DNA repair synthesis in mouse spermatogenesis involves DNA polymerase beta activity. *Cell Differ* 1988;23:221–230. [PubMed: 3132329]
17. Ishiguro T, Otsuka F, Ochi T, Ohsawa M. Involvement of DNA polymerases in the repair of DNA damage by benzo[a]pyrene in cultured Chinese hamster cells. *Mutat Res* 1987;184:57–63. [PubMed: 3600683]
18. Park IS, Park JK, Koh HY, Park SD. DNA single stranded gaps formed during DNA repair synthesis induced by methyl methanesulfonate are filled by sequential action of aphidicolin- and dideoxythymidine sensitive DNA polymerases in HeLa cells. *Cell Biol Toxicol* 1991;7:49–58. [PubMed: 1905189]
19. Price A. The repair of ionising radiation-induced damage to DNA. *Semin Cancer Biol* 1993;4:61–71. [PubMed: 8513149]

20. Wiebauer K, Jiricny J. Mismatch-specific thymine DNA glycosylase and DNA polymerase β mediate the correction of G.T mispairs in nuclear extracts from human cells. *Proceedings of the National Academy of Science* 1990;87:5842–5845.
21. Dianov G, Price A, Lindahl T. Generation of single-nucleotide repair patches following excision of uracil residues from DNA. *Molecular and Cellular Biology* 1992;12:1605–1612. [PubMed: 1549115]
22. Nealon K, Nicholl ID, Kenny MK. Characterization of the DNA polymerase requirement of human base excision repair. *Nucleic Acids Res* 1996;24:3763–3770. [PubMed: 8871556]
23. Singhal RK, Prasad R, Wilson SH. DNA polymerase β conducts the gap-filling step in uracil-initiated base excision repair in a bovine testis nuclear extract. *J Biol Chem* 1995;270:949–957. [PubMed: 7822335]
24. Singhal RK, Wilson SH. Short gap-filling synthesis by DNA polymerase β is processive. *J Biol Chem* 1993;268:15906–15911. [PubMed: 8340415]
25. Kubota Y, Nash RA, Klungland A, Schar P, Barnes DE, Lindahl T. Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase β and the XRCC1 protein. *EMBO Journal* 1996;15:6662–6670. [PubMed: 8978692]
26. Blank A, Kim B, Loeb LA. DNA polymerase delta is required for base excision repair of DNA methylation damage in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 1994;91:9047–9051. [PubMed: 8090767]
27. Ohnishi T, Yuba S, Date T, Utsumi H, Matsukage A. Rat DNA polymerase β gene can join in excision repair of *Escherichia coli*. *Nucleic Acids Res* 1990;18:5673–5676. [PubMed: 2216761]
28. Sobol RW, Horton JK, Kuhn R, Gu H, Singhal RK, Prasad R, Rajewsky K, Wilson SH. Requirement of mammalian DNA polymerase- β in base-excision repair. *Nature* 1996;379:183–186. [PubMed: 8538772]
29. Gu H, Marth JD, Orban PC, Mossmann H, Rajewsky K. Deletion of a DNA polymerase β gene segment in T cells using cell type-specific gene targeting. *Science* 1994;265:103–106. [PubMed: 8016642]
30. Sugo N, Aratani Y, Nagashima Y, Kubota Y, Koyama H. Neonatal lethality with abnormal neurogenesis in mice deficient in DNA polymerase β . *EMBO Journal* 2000;19:1397–1404. [PubMed: 10716939]
31. Horton JK, Baker A, Berg BJ, Sobol RW, Wilson SH. Involvement of DNA polymerase β in protection against the cytotoxicity of oxidative DNA damage. *DNA Repair (Amst)* 2002;1:317–333. [PubMed: 12509250]
32. Esposito G, Texido G, Betz UA, Gu H, Muller W, Klein U, Rajewsky K. Mice reconstituted with DNA polymerase β -deficient fetal liver cells are able to mount a T cell-dependent immune response and mutate their Ig genes normally. *Proceedings of the National Academy of Science* 2000;97:1166–1171.
33. Gonda H, Sugai M, Katakai T, Sugo N, Aratani Y, Koyama H, Mori KJ, Shimizu A. DNA polymerase β is not essential for the formation of palindromic (P) region of T cell receptor gene. *Immunology Letters* 2001;78:45–49. [PubMed: 11470151]
34. Niimi N, Sugo N, Aratani Y, Koyama H. Genetic interaction between DNA polymerase β and DNA-PKcs in embryogenesis and neurogenesis. *Cell Death & Differentiation* 2005;12:184–191. [PubMed: 15647757]
35. Cabelof DC, Guo Z, Raffoul JJ, Sobol RW, Wilson SH, Richardson A, Heydari AR. Base excision repair deficiency caused by polymerase β haploinsufficiency: accelerated DNA damage and increased mutational response to carcinogens. *Cancer Res* 2003;63:5799–5807. [PubMed: 14522902]
36. Sobol RW, Watson DE, Nakamura J, Yakes FM, Hou E, Horton JK, Ladapo J, Van Houten B, Swenberg JA, Tindall KR, Samson LD, Wilson SH. Mutations associated with base excision repair deficiency and methylation-induced genotoxic stress. *Proceedings of the National Academy of Science* 2002;99:6860–6865.
37. Bennett SE, Sung JS, Mosbaugh DW. Fidelity of uracil-initiated base excision DNA repair in DNA polymerase β -proficient and -deficient mouse embryonic fibroblast cell extracts. *J Biol Chem* 2001;276:42588–42600. [PubMed: 11551933]

38. Poltoratsky V, Horton JK, Prasad R, Wilson SH. REV1 mediated mutagenesis in base excision repair deficient mouse fibroblast. *DNA Repair (Amst)* 2005;4:1182–1188. [PubMed: 15950550]
39. Ochs K, Sobol RW, Wilson SH, Kaina B. Cells deficient in DNA polymerase β are hypersensitive to alkylating agent-induced apoptosis and chromosomal breakage. *Cancer Res* 1999;59:1544–1551. [PubMed: 10197627]
40. Horton JK, Joyce-Gray DF, Pachkowski BF, Swenberg JA, Wilson SH. Hypersensitivity of DNA polymerase beta null mouse fibroblasts reflects accumulation of cytotoxic repair intermediates from site-specific alkyl DNA lesions. *DNA Repair (Amst)* 2003;2:27–48. [PubMed: 12509266]
41. Le Page F, Schreiber V, Dherin C, De Murcia G, Boiteux S. Poly(ADP-ribose) polymerase-1 (PARP-1) is required in murine cell lines for base excision repair of oxidative DNA damage in the absence of DNA polymerase β . *J Biol Chem* 2003;278:18471–18477. [PubMed: 12637553]
42. Sobol RW, Kartalou M, Almeida KH, Joyce DF, Engelward BP, Horton JK, Prasad R, Samson LD, Wilson SH. Base Excision Repair Intermediates Induce p53-independent Cytotoxic and Genotoxic Responses. *J Biol Chem* 2003;278:39951–39959. [PubMed: 12882965]
43. Horton JK, Stefanick DF, Naron JM, Kedar PS, Wilson SH. Poly(ADP-ribose) polymerase activity prevents signaling pathways for cell cycle arrest following DNA methylating agent exposure. *J Biol Chem* 2005;280:15773–15785. [PubMed: 15701627]
44. Trivedi RN, Almeida KH, Fornasaglio JL, Schamus S, Sobol RW. The Role of Base Excision Repair in the Sensitivity and Resistance to Temozolomide Mediated Cell Death. *Cancer Res* 2005;65:6394–6400. [PubMed: 16024643]
45. Cabelof DC, Raffoul JJ, Nakamura J, Kapoor D, Abdalla H, Heydari AR. Imbalanced base excision repair in response to folate deficiency is accelerated by polymerase beta haploinsufficiency. *J Biol Chem* 2004;279:36504–36513. [PubMed: 15218023]
46. Ochs K, Lips J, Proffittlich S, Kaina B. Deficiency in DNA polymerase β provokes replication-dependent apoptosis via DNA breakage, Bcl-2 decline and caspase-3/9 activation. *Cancer Res* 2002;62:1524–1530. [PubMed: 11888930]
47. Tomicic MT, Thust R, Sobol RW, Kaina B. DNA polymerase β mediates protection of mammalian cells against ganciclovir-induced cytotoxicity and DNA breakage. *Cancer Res* 2001;61:7399–7403. [PubMed: 11606369]
48. Biade S, Sobol RW, Wilson SH, Matsumoto Y. Impairment of proliferating cell nuclear antigen-dependent apurinic/apyrimidinic site repair on linear DNA. *J Biol Chem* 1998;273:898–902. [PubMed: 9422747]
49. Fortini P, Pascucci B, Parlanti E, Sobol RW, Wilson SH, Dogliotti E. Different DNA polymerases are involved in the short- and long-patch base excision repair in mammalian cells. *Biochemistry* 1998;37:3575–3580. [PubMed: 9530283]
50. Stucki M, Pascucci B, Parlanti E, Fortini P, Wilson SH, Hubscher U, Dogliotti E. Mammalian base excision repair by DNA polymerases delta and epsilon. *Oncogene* 1998;17:835–843. [PubMed: 9780000]
51. Fortini P, Parlanti E, Sidorkina OM, Laval J, Dogliotti E. The type of DNA glycosylase determines the base excision repair pathway in mammalian cells. *J Biol Chem* 1999;274:15230–15236. [PubMed: 10329732]
52. Dianov GL, Prasad R, Wilson SH, Bohr VA. Role of DNA polymerase β in the excision step of long patch mammalian base excision repair. *J Biol Chem* 1999;274:13741–13743. [PubMed: 10318775]
53. Braithwaite EK, Prasad R, Shock DD, Hou EW, Beard WA, Wilson SH. DNA polymerase lambda mediates a back-up base excision repair activity in extracts of mouse embryonic fibroblasts. *J Biol Chem* 2005;280:18469–18475. [PubMed: 15749700]
54. Kedar PS, Kim SJ, Robertson A, Hou E, Prasad R, Horton JK, Wilson SH. Direct interaction between mammalian DNA polymerase β and proliferating cell nuclear antigen. *J Biol Chem* 2002;277:31115–31123. [PubMed: 12063248]
55. Sobol RW, Prasad R, Evenski A, Baker A, Yang XP, Horton JK, Wilson SH. The lyase activity of the DNA repair protein β -polymerase protects from DNA-damage-induced cytotoxicity. *Nature* 2000;405:807–810. [PubMed: 10866204]
56. Matsumoto Y, Kim K. Excision of deoxyribose phosphate residues by DNA polymerase β during DNA repair. *Science* 1995;269:699–702. [PubMed: 7624801]

57. Bebenek K, Tissier A, Frank EG, McDonald JP, Prasad R, Wilson SH, Woodgate R, Kunkel TA. 5'-Deoxyribose phosphate lyase activity of human DNA polymerase iota in vitro. *Science* 2001;291:2156–2159. [PubMed: 11251121]
58. Prasad R, Bebenek K, Hou E, Shock DD, Beard WA, Woodgate R, Kunkel TA, Wilson SH. Localization of the deoxyribose phosphate lyase active site in human DNA polymerase iota by controlled proteolysis. *J Biol Chem* 2003;278:29649–29654. [PubMed: 12777390]
59. Braithwaite EK, Kedar PS, Lan L, Polosina YY, Asagoshi K, Poltoratsky VP, Horton JK, Miller H, Teebor GW, Yasui A, Wilson SH. DNA polymerase lambda protects mouse fibroblasts against oxidative DNA damage and is recruited to sites of DNA damage/repair. *J Biol Chem* 2005;280:31641–31647. [PubMed: 16002405]
60. Garcia-Diaz M, Bebenek K, Gao G, Pedersen LC, London RE, Kunkel TA. Structure-function studies of DNA polymerase lambda. *DNA Repair (Amst)* 2005;4:1358–1367. [PubMed: 16213194]
61. Garcia-Diaz M, Bebenek K, Kunkel TA, Blanco L. Identification of an intrinsic 5'-deoxyribose-5-phosphate lyase activity in human DNA polymerase lambda: a possible role in base excision repair. *J Biol Chem* 2001;276:34659–34663. [PubMed: 11457865]
62. Almeida, KH.; Sobol, RW. Increased Specificity and Efficiency of Base Excision Repair through Complex Formation. In: Siede, W.; Doetsch, PW.; Kow, YW., editors. *DNA Damage Recognition*. Marcel Dekker Inc; New York: 2005.
63. Bertocci B, De Smet A, Flatter E, Dahan A, Bories JC, Landreau C, Weill JC, Reynaud CA. Cutting edge: DNA polymerases mu and lambda are dispensable for Ig gene hypermutation. *J Immunol* 2002;168:3702–3706. [PubMed: 11937519]
64. McDonald JP, Frank EG, Plosky BS, Rogozin IB, Masutani C, Hanaoka F, Woodgate R, Gearhart PJ. 129-derived strains of mice are deficient in DNA polymerase iota and have normal immunoglobulin hypermutation. *Journal of Experimental Medicine* 2003;198:635–643. [PubMed: 12925679]
65. Polosina YY, Rosenquist TA, Grollman AP, Miller H. 'Knock down' of DNA polymerase β by RNA interference: recapitulation of null phenotype. *DNA Repair (Amst)* 2004;3:1469–1474. [PubMed: 15380102]

FIGURE 1.

PCR genotyping of mouse POLI codon 27 in pol β null and matched wild-type cell pairs. Genomic DNA isolated from each cell line was purified and characterized for the pol β codon 27 Wt and null allele as described [64]. Briefly, amplification was performed using flanking PCR primers that amplify an 88 bp fragment surrounding codon 27 in a volume of 25 μ l, with 250 μ M of each dNTP, 10 pmoles of each primer, and 1.25 units of AmpliTaq Gold DNA polymerase. After denaturation at 95°C for 10 min, the reaction mixture is subjected to 35 PCR cycles as follows: denaturation at 95°C for 0.5 min, annealing at 57°C for 0.5 min and primer extension for 1 min at 72°C, followed by a single primer extension step at 72°C for 10 min. The resulting fragment is then purified using a Qiagen PCR spin-column and the DNA is incubated in the presence of TaqI (NEB) at 65°C for 2 hours. The final products were then analyzed by electrophoresis using a 4% NuSieve 3:1 agarose gel in TBE. The amplicon (88 bp) containing a WT allele is digested to two fragments of 39 and 49 bp whereas the null allele (mut) remains as the undigested 88 bp fragment. The cell lines and corresponding genomic DNA used for lanes 1–10 are listed in Table I. M = DNA size markers; WT = DNA derived from C57BL/6 mice harboring a WT POLI codon 27; mut = DNA derived from c129 mice harboring a mutant POLI codon 27.

TABLE I

Cell lines screened for the POLI codon 27 mutation.

Lane #	Cell Line	ATCC Number	Overall Genotype regarding pol β and pol ι	Reference
1	16tsA (clone 1B5)	CRL-2307	WT	[28]
2	19tsA (clone 2B2)	CRL-2308	pol β null and pol ι mutant (null)	[28]
3	36.3	N/A*	WT	[36,55]
4	38 Δ 4	N/A	pol β null	[36,55]
5	50TA _g	N/A	pol β null	-
6	53TA _g	N/A	WT	-
7	88TA _g	CRL-2820	pol β null	[42,44]
8	92TA _g	CRL-2816	WT	[42,44]
9	369TA _g	N/A	pol ι mutant (null)	[44]
10	370TA _g	N/A	pol ι heterozygous (+/-)	-

* N/A = Not available from ATCC.