mass range make up the bulk of the dark matter and that the long tradition of considering neutrino cold dark matter should come to an end. Constraints can also be imposed from the LEP experiments on supersymmetric dark-matter candidates[17].

Finally we should mention some possible loopholes in our line of reasoning. The errors in the LEP and SLC measurements are still large, and future

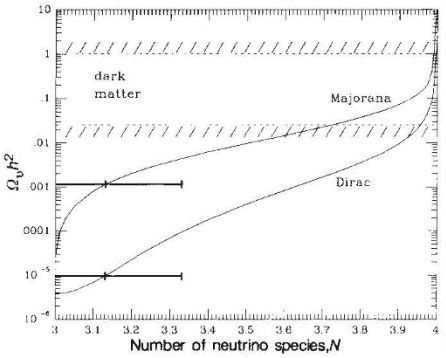


FIG. 2 Relic abundance of hypothetical fourth-generation neutrino as a function of the measured 'number' of neutrino species for the Dirac and Majorana cases. The data points are the combined LEP and SLC results. The region where the neutrino relic abundance is appropriate to supply the bulk of the dark matter ($\Omega_\nu = 0.1$ and $h = 0.5$ to $\Omega_\nu = 1$ and $h = 1$) is shown by dashed lines.

results could move the data point enough to make it consistent with the relict abundance limits. Note from Fig. 1, however, that to allow the canonical dark-matter neutrino mass of 5–10 GeV, N must be close to 4, a value several standard deviations away from the present measurement. Also note that, contrary to claims in the literature, the relict abundance of extremely massive neutrinos ($m_\nu \gg$ TeV) must again approach the critical density, if the neutrinos remain elementary particles[18]. The existence of such particles is, of course, not constrained by the LEP experiment and they may make up the dark matter. Perturbation theory breaks down long before this limiting mass is reached, however, so it is not clear that a theory with such heavy particles makes sense. Note that additional entropy generated in the early Universe could change the results of our relict abundance calculation, but in this case the abundance would be smaller than we claim, and neutrino cold dark matter would be even more unlikely.

KIM GRIEST
JOSEPH SILK
Center for Particle Astrophysics and Department of Physics and Astronomy, University of California, Berkeley, California 94720, USA

1. ALEPH collaboration: Decamp, D. *et al Phys. Lett.* B **231**, 519–529 (1989).
2. Opal collaboration: Akrawy, M. Z. *et al. Phys. Lett.* B **231**, 530–538 (1989).
3. Delphi collaboration: Aarnio, P. *et al. Phys. Lett.* B **231**, 539–547 (1989).
4. L3 collaboration: Adeva, B. *et al. Phys. Lett.* B. **231**, 509–518 (1989).
5. SLC results: Abrams, G. S. *et al. Phys. Rev. Lett.* **63**, 2173–2176 (1989).
6. Vysotskii, M. I., Dolgov, A. D. & Zeldovich, Ya. B. *J.E.T.P. Lett.* **26**, 188–190 (1977).
7. Lee, B. W. & Weinberg, S. *Phys. Rev. Lett.* **39**, 165–168 (1977).
8. Kolb, E. W. & Olive, K. A. *Phys. Rev.* D **33**, 1202–1203 (1986).
9. Scherrer, R. J. & Turner, M. S. *Phys. Rev.* D **33**, 1585–1589 (1986).
10. Srednicki, M. *et al. Nucl. Phys.* B **310**, 693–713 (1989).
11. Steigman, G. A. *Rev. Nucl. Part Sci.* **29**, 313–338 (1979).
12. Aguilar-Benitez, M. *et al. Phys. Lett.* B **204**, 1–486 (1988).
13. Griest, K. & Seckel, D. *Nucl. Phys.* B **283**, 681–705 (1987); B **296**, 1034–1036 (1988).
14. Griest, K. & Sadoulet, B. in *Dark Matter in the Universe* (eds Galeotti, P. & Schramm, D. N.) (Kluwer, Dordrecht, The Netherlands, 1989).
15. Ahlen, S. P. *et al. Phys. Lett.* B **195**, 603–608 (1987).
16. Caldwell, D. O. *et al. Phys. Rev. Lett.* **61**, 510–513 (1988).
17. Barbieri, R. *et al. Phys. Lett.* B **195**, 500–506 (1987).
18. Griest, K. & Kamionkowski, M. Preprint FERMILAB-Pub-89/205-A (1989).

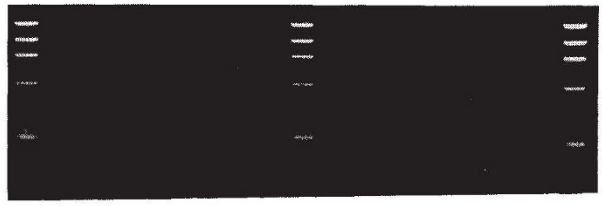
Shedding light on PCR contamination

SIR—The most pernicious problem plaguing the widely used technique of polymerase chain reaction (PCR) is contamination of reagents with previously amplified material[1–3]. Care is essential in the routine handling of amplified samples, and heroic efforts are required if PCR from single cells is to be safely performed[3,4]. The following experiment illustrates how treatment with ultraviolet light directly before adding the template DNA can eliminate most sources of contamination.

Between 3 and 30,000 pg of target DNA was added to 20 μl PCR mix, which was then irradiated for 5 or 20 minutes with a combination of 254- and 300-nm bulbs and subsequently amplified with 30 cycles of PCR. As shown in the figure, even when the quantity of DNA was 10^5-fold (5 min) or 10^6-fold (20 min) more than the minimum quantity of DNA that we routinely detect with PCR[5], no amplified product was seen. DNA added after irradiation of the PCR mix could still be amplified, indicating that irradiation of the PCR mix does not compromise the ability of the reagents to mediate efficient amplification. Irradiation with the 300-nm transilluminator alone, though considerably less effective than if the 254-nm bulb is also used, should still be adequate for the levels of contamination that routinely occur in practice.

In additional experiments, concentrated stock solutions of buffer (×10), oligonucleotide primers (×10), deoxytriphosphates (×7), or *Taq* polymerase (×40) were contaminated with 1.5 pg μl^{-1} of target DNA and irradiated for 5 minutes with 254- and 300-nm ultraviolet light. In each case, the contaminant could not be amplified whereas 0.015 pg μl^{-1} of target DNA added after irradiation was efficiently amplified.

It was initially surprising that the 5-min ultraviolet treatment reduced the efficiency of amplification from double-stranded DNA by more than 100,000-fold but preserved the functional integrity of the single-stranded oligonucleotide primers in the PCR mix. However, this seems generally to be the case because 5-min irradiation of four additional unrelated pairs of oligonucleotide primers did not reduce the intensity of the product amplified from 250 ng of human genomic DNA.



Effect of ultraviolet irradiation on PCR. A standard protocol[6] was used to prepare 20-μl PCR mixes, containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.1% (w/v) gelatin, 200 μM each dNTP and 0.1 μM of each of two PCR primers that amplify a 750-base pair region of human factor IX complementary DNA. Linearized factor IX plasmid (6-kilobases in length) was added so that the amount of target DNA was as indicated below. The samples were irradiated for 5 (lanes 2–8) or 20 min, (lanes 10–16) and then denatured for 10 min. *Taq* polymerase (0.5 U) was added, and 30 cycles of PCR performed[6]. Lanes: S — standards, *Hin*dIII digestion of φX174; 1, 9 — no DNA and no ultraviolet; 2, 10 — no DNA; 3, 11 — 3 pg DNA; 4, 12 — 30 pg target DNA; 5, 13 — 300 pg target DNA; 6, 14 — 3,000 pg target DNA; 7, 15 — 30,000 pg DNA; 8, 16 — ultraviolet irradiation before addition of 3 pg non-irradiated target DNA (positive control).
Method of irradiation. The 20-μl samples in a clear 0.5-ml polypropylene microcentrifuge tube were placed in direct contact with a glass platform of the Fotodyne 1000 transilluminator. Irradiation was performed with 254- and 300-nm ultraviolet bulbs in the standard instrument configuration.

In additional experiments, concentrated stock solutions of buffer (×10), oligonucleotide primers (×10), deoxytriphosphates (×7), or *Taq* polymerase (×40) were contaminated with 1.5 pg μl^{-1} of target DNA and irradiated for 5 minutes with 254- and 300-nm ultraviolet light. In each case, the contaminant could not be amplified whereas 0.015 pg μl^{-1} of target DNA added after irradiation was efficiently amplified.

It was initially surprising that the 5-min ultraviolet treatment reduced the efficiency of amplification from double-stranded DNA by more than 100,000-fold but preserved the functional integrity of the single-stranded oligonucleotide primers in the PCR mix. However, this seems generally to be the case because 5-min irradiation of four additional unrelated pairs of oligonucleotide primers did not reduce the intensity of the product amplified from 250 ng of human genomic DNA.

GOBINDA SARKAR
STEVE S. SOMMER
Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55905, USA

1. Kwock, S. *Amplifications* **2**, 4 (1989).
2. Williams, J.F. *BioTechniques* **7**, 762–766 (1989).
3. Gibbs, R.A. & Chamberlain, J.S. *Genes Dev.* **3**, 1095–1098 (1989).
4. Li, H. *et al. Nature* **335**, 414–417 (1988).
5. Sommer, S.S., Cassady, J., Sobell, J.L. & Bottema, C.D.K. *Proc. Mayo Clinic* **64**, 1361–1372 (1989).
6. Sarkar, G. & Sommer, S.S. *Science* **244**, 331–334 (1989).

Scientific Correspondence
Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of less than 500 words and five references.