Unniraman *et al.* reply:

The generation of *Igh* switch region–*Myc* (*Igh-Myc*) translocations is thought to be a primary step in the development of mouse plasmacytomas[1,2]. Ramiro *et al.* recently reported the detection of *Igh-Myc* translocations in wild-type but not AID-deficient (*Aicda*$^{-/-}$) *Il6*-transgenic mice using a long template PCR assay with primers specific for *Myc* and *Igh* C_μ (ref. 3). In contrast, we detected equal frequencies of translocations in unmanipulated wild-type and *Aicda*$^{-/-}$ mice[4] using a consensus primer PCR assay that takes advantage of primers that hybridize to multiple locations in S_μ and S_α (ref. 5).

The consensus primer PCR assay is more sensitive[2,5,6], whereas the long template PCR assay allows detection of the two reciprocal translocation products in mice in which translocation-bearing cell populations have been triggered to expand[2,7–9]. Before expansion, cells containing these translocations are rare, making them difficult to detect in duplicate assays of the same DNA sample[4–6]. Translocations are also detected in strains of mice that are resistant to plasmacytomagenesis, suggesting that resistance in these strains arises at a later step in the disease process, probably during expansion of translocation-bearing cell populations[5,6]. Although translocations have been detected in unmanipulated wild-type mice using both the long template and consensus primer PCR assays, they are more readily detected using the latter assay[2,5,6,10,11]. Ramiro *et al.* now report that *Igh-Myc* junction products can be amplified from wild-type kidney and the gut of *Rag1*$^{-/-}$ mice with the consensus primer PCR assay. These data contrast with our finding that no products could be obtained from brain or kidney with this assay[4]. We have now evaluated 30 gut and 5 spleen DNA samples obtained from five C57BL/6 *Rag1*$^{-/-}$ mice using the consensus primer PCR assay (**Fig. 1**). None of these 35 samples, assayed in duplicate, yielded a positive signal in any reaction (**Fig. 1a** and **c**, lanes 1–16, and **d**, lanes 1–3, and data not shown). In addition, 25 BALB/c brain and kidney samples, assayed once in parallel, were uniformly negative (**Fig. 1b**, lanes 5–16, and data not shown). In the same set of reactions, DNA samples from untreated mice (**Fig. 1b**, lanes 1–4, **d**, lanes 5–8, and data not shown) that had a positive score in previous assays yielded a positive signal at the expected frequency (6 positive results in 15 reactions; **Fig. 1b**, lane 4, and **d**, lane 7, and data not shown), including four samples from untreated *Aicda*$^{-/-}$ mice (**Fig. 1b**, lane 4, and data not shown).

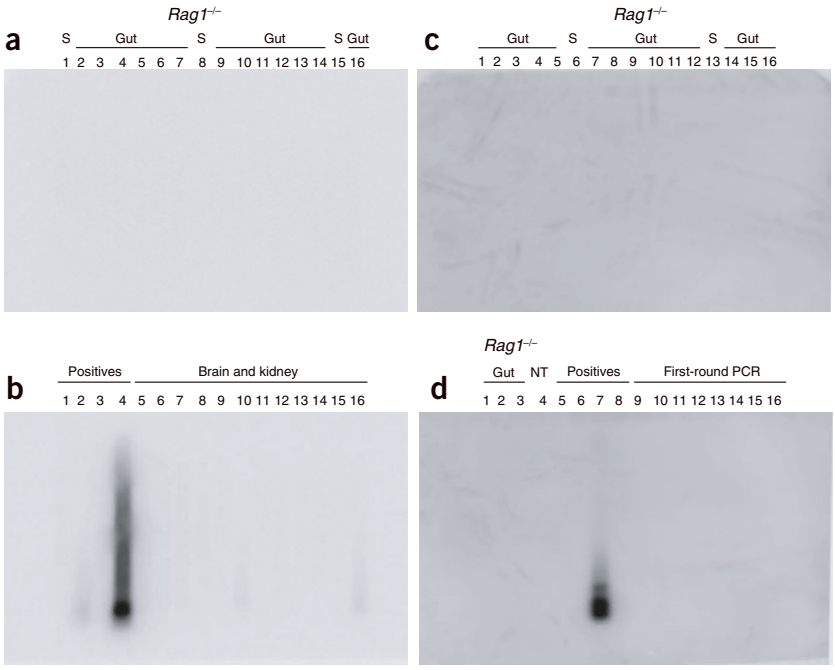

Figure 1 PCR and Southern blot assays of samples from *Rag1*$^{-/-}$ mice as well as positive and negative controls. Reactions were done as reported[4]. All reactions were done at the same time and data represent parts of the same gels that were split only for the convenience of labeling. Data are representative reactions with DNA from 30 gut and 5 spleen (S) samples from five *Rag1*$^{-/-}$ mice (**a**, lanes 1–16, **c**, lanes 1–16 and **d**, lanes 1–3). Data also include representative reactions done in parallel with eight kidney and four brain samples from *Aicda*$^{+/+}$, *Aicda*$^{-/-}$ and BALB/c mice (**b**, lanes 5–16) and six gut and two Peyer's patch samples from unmanipulated *Aicda*$^{+/+}$, *Aicda*$^{+/-}$ and *Aicda*$^{-/-}$ mice (**b**, lanes 1–4 and d5-8), as well as eight representative products (from three *Rag1*$^{-/-}$ samples and three previously positive and two brain and kidney samples) after the first 40 cycles of PCR (**d**, lanes 9–16). NT, no template. Membranes were exposed to a phosphorimager screen for 16 h.

Although we do not have a ready explanation for the discrepancy between our results and those of Ramiro *et al.*, we note that the consensus primer PCR assay is very sensitive and that variations in reaction conditions might result in the generation of artifacts. The only previously reported artifact arising from consensus primer PCR assays was the amplification of switch-switch products[5], but our assay does not detect such products because of our inclusion of a hybridization step with a *Myc*-specific probe. We did detect faint smears in some samples that were less than 2% as strong as the positive signals (for example, **Fig. 1b**, lanes 2, 10 and16). However, we have never succeeded in cloning any *Igh-Myc* junctions from such reactions and therefore have consistently assigned them negative scores. Our inability to detect positive signals from many brain and kidney samples or from tissues from *Rag1*$^{-/-}$ mice, together with our published data[4], provide molecular evidence for the existence of *Igh-Myc* translocations in wild-type and *Aicda*$^{-/-}$ mice. It will now be necessary to develop alternative types of assays capable of detecting rare translocation-positive cells.

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