Amplifying *Igh* translocations

To the editor:

PCR has been used to amplify chromosome translocation breakpoints between *Myc* and *Igh* switch regions in B cell tumors and mouse tissues[1–4]. However, the *Igh* switch region is both long and redundant and this is a particularly challenging part of the genome to amplify accurately. To assay for *Igh-Myc* translocations Unniraman *et al.*[5] used primers specific for *Myc* combined with consensus primers that hybridize in multiple locations in *Igh* S_μ and S_α switch region repeats and did 80 cycles of nested amplification followed by Southern blotting for detection[5]. When we did this assay using DNA extracted from lymph nodes of interleukin 6 (*Il6*)-transgenic mice, which have *Igh-Myc* translocations, we found that multiple species were present in ethidium bromide–stained gels and that some of the minor bands hybridized with internal *Myc*-specific probes (data shown). In contrast, nested PCR with a combination of *Myc* and *Igh* constant region–specific primers produced discrete bands in ethidium bromide–stained gels that also hybridized with the same internal *Myc*-specific probe[1–4] (data shown). Using the 80-cycle consensus primer PCR assay, Unniraman *et al.*[5] were able to amplify *Igh-Myc* hybrid DNA from 30% of the gut samples from activation-induced deaminase (AID)–deficient mice but not from kidney or brain samples. The tissue samples that were positive were reproducibly positive only 40% of the time[5]. Nevertheless, cloning showed that some of the amplicons were hybrid molecules of *Myc* and *Igh*[5]. Unniraman *et al.*[5] suggest that these amplicons come from B cells that experienced AID-independent *Igh-Myc* translocations and that these cell populations were expanded by pristane injection in an unknown but AID-dependent way. However, these amplicons are also found in mouse strains that fail to develop pristane-induced plasmacytoma and are therefore not predictive of plasmacytoma development[1].

To determine whether B cells are required for the production of these IgH-Myc amplicons using consensus primers, we assayed 40 gut samples from 10 C57BL/6 recombination activating gene 1–deficient (*Rag1*$^{-/-}$) mice, 40 gut samples from 10 BALB/c mice and 10 kidney samples from BALB/c mice. We found that 20% of the C57BL/6 *Rag1*$^{-/-}$ gut samples, 52% of the BALB/c gut samples and 60% of the BALB/c kidney samples were positive in this assay, although there was substantial variability between samples, including differences in the pattern and intensity of hybridization (**Fig. 1**). Cloning showed that amplicons produced with the consensus primers from *Rag1*$^{-/-}$ gut and BALB/c kidney samples had unique *Igh-Myc* hybrid sequences (data not shown), but these amplicons could not be detected with the specific primers in the same samples. We caution that amplicons produced using consensus *Igh* switch primers may or may not reflect the existence of a chromosome translocation and that in our experience, such amplicons are not dependent on the presence of mature B cell DNA.

Almudena R Ramiro, Mila Jankovic & Michel C Nussenzweig

The Rockefeller University, Howard Hughes Medical Institute, Laboratory of Molecular Immunology, New York, New York 10021, USA.
e-mail: nussen@mail.rockefeller.edu

1. Muller, J.R., Jones, G.M., Janz, S. & Potter, M. *Blood* **89**, 291–296 (1997).
2. Janz, S., Muller, J., Shaughnessy, J. & Potter, M. *Proc. Natl. Acad. Sci. USA* **90**, 7361–7365 (1993).
3. Muller, J.R., Potter, M. & Janz, S. *Proc. Natl. Acad. Sci. USA* **91**, 12066–12070 (1994).
4. Muller, J.R., Jones, G.M., Potter, M. & Janz, S. *Cancer Res.* **56**, 419–423 (1996).
5. Unniraman, S., Zhou, S. & Schatz, D.G. *Nat. Immunol.* **5**, 1117–1123 (2004).

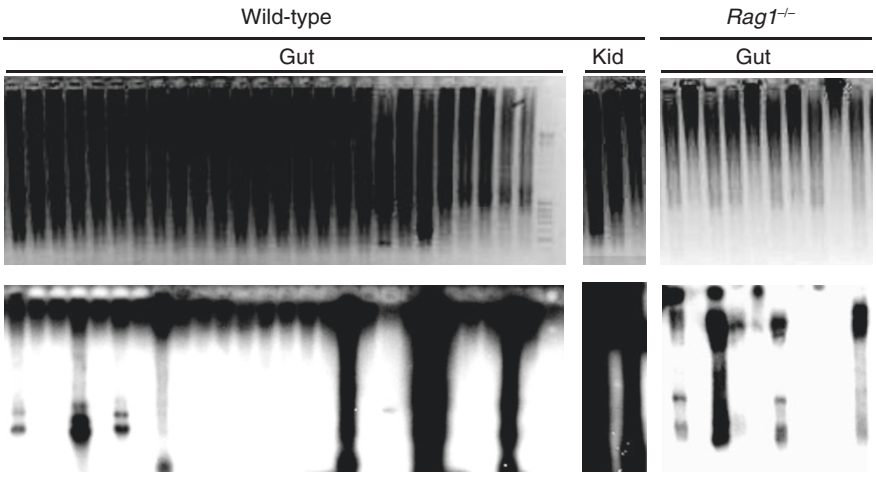

Figure 1 *Igh-Myc* PCR and Southern blotting. Top, ethidium bromide–stained agarose gels of PCR of BALB/c gut and kidney (Kid) and *Rag1*$^{-/-}$ gut tissue samples using primers that hybridize with *Igh* switch region repeats and unique sequences in *Myc*[1]. Bottom, Southern blots of the same samples using the internal *Myc* oligonucleotide as a probe[5], after a 24-hour exposure. The *Myc*-hybridizing bands are not necessarily the prominent bands in the ethidium bromide gels. PCR and Southern blots were done as described[5].