The *Dlx5* locus was targeted first in R1 ES cells using a neomycin resistance cassette. Three positive clones were expanded and underwent targeting of the *Dlx6* locus using a puromycin resistance cassette. Genomic DNA was isolated from ES cells and digested using standard protocols. To assay recombination of the 5’ homology arm of the *Dlx5* and *Dlx6* vectors, DNA was digested with Bgl II or Hind III, respectively. Recombination of the 3’ homology arm of the *Dlx5* vector was assayed by digestion with Xma I. To determine if the targeting of the *Dlx5* and 6 loci occurred *in cis* or *in trans*, DNA was digested with Not I. Digests were electrophoresed on 1% or 0.5% agarose gels, transferred to charged nitrocellulose membrane, and hybridized with radioactive labeled probe using standard protocols. All probes were located outside of the regions of homology and were isolated from 129SV genomic clones by restriction digestion and gel purification. The *Dlx5* 5’ probe was a 200 bp Spe I/Xho I fragment that detects a 5.5 kb band in wild type versus a 8.3 kb band if targeting occurs correctly; the *Dlx6* 5’ probe was a 780 bp EcoR V/Bgl I fragment that detects a 6.4 wild band versus a 7.9 kb targeted band. A 750 bp BamH I/Spe I probe was used to monitor recombination of the *Dlx5* 3’ homology arm as well as to assay allele targeting of both vectors; after Xma I digestion a 7.0 kb band is seen for non targeting while a targeted chromosome has a 8.4 kb band. Digestion with Not I detects a doublet of 21 kb if the targeting of both vectors occurs *in trans*, or two bands of 18kb and 24 kb if targeting occurred *in cis*. Recombination of the 3’ homology arm of *Dlx6* was assayed by PCR with one primer located in the puromycin resistance cassette and the three different primers located outside of the region of homology, generating 3.1, 3.4 and 3.9 kb fragments. PCR was accomplished using a protocol that increased extension time by 30 second per cycle after the first ten cycles and allowed a 10 second denaturation period for all cycles.