Mutagenesis of diploid mammalian genes by gene entrapment

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ABSTRACT

The present study describes a genome-wide method for biallelic mutagenesis in mammalian cells. Novel poly(A) gene trap vectors, which contain features for direct cloning vector–cell fusion transcripts and for post-entrapment genome engineering, were used to generate a library of 979 mutant ES cells. The entrapment mutations generally disrupted gene expression and were readily transmitted through the germline, establishing the library as a resource for constructing mutant mice. Cells homozygous for most entrapment loci could be isolated by selecting for enhanced expression of an inserted neomycin-resistance gene that resulted from losses of heterozygosity (LOH). The frequencies of LOH measured at 37 sites in the genome ranged from $1.3 \times 10^{-5}$ to $1.2 \times 10^{-4}$ per cell and increased with increasing distance from the centromere, implicating mitotic recombination in the process. The ease and efficiency of obtaining homozygous mutations will (i) facilitate genetic studies of gene function in cultured cells, (ii) permit genome-wide studies of recombination events that result in LOH and mediate a type of chromosomal instability important in carcinogenesis, and (iii) provide new strategies for phenotype-driven mutagenesis screens in mammalian cells.

INTRODUCTION

The number and diversity of genes identified by the mammalian genome projects suggests that considerable biology remains to be characterized on a molecular level and has provided the impetus for developing genome-wide strategies to characterize gene functions important in normal and disease processes. Tagged sequence mutagenesis uses gene trap vectors to disrupt genes in cultured cells combined with rapid, DNA sequence-based screens to characterize the disrupted genes at the nucleotide level. The approach has been widely used to disrupt genes in mouse embryonic stem (ES) cells (1–3). Gene trapping has also been used, but to a far lesser extent, to identify genes responsible for recessive phenotypes in somatic cells (4–9).

Mutagenesis of mammalian cells is hindered by the fact that the autosomal genome is diploid and consequently, most entrapment mutations are recessive. The problem is circumvented by gene-based studies in ES cells where selected mutations can be transmitted through the mouse germline and subsequently bred to a homozygous state. However, gene inactivation in somatic cells requires pre-existing hemizygosity or spontaneous loss of heterozygosity; thus, even with strategies to enhance the recovery of loss-of-function mutations (4,5,7,10,11), gene trap mutagenesis has seen only limited use in phenotype-driven screens.

Mammalian cells heterozygous at a given locus undergo spontaneous conversion to a homozygous state by loss of heterozygosity (LOH) at frequencies of $\sim 10^{-5}$ per cell (12–15). Homozygous mutant cells can be selected based on phenotypes caused by gene dosage effects. For example, mutations involving the insertion of a neomycin-resistance gene (Neo) may be converted to a homozygous state simply by selecting for clones that survive in higher concentrations of G418 (16). Levels of neomycin resistance correlate with levels of Neo gene expression (17). Mitotic recombination, which appears to be the preferred mechanism for spontaneous LOH (13,14,18,19), generates cells homozygous for the inserted Neo genes, and the increase in Neo gene copy number allows moderately resistant cells to acquire higher levels of antibiotic resistance. However, unlike targeted mutations, LOH has not been reliably achieved with mutations induced by gene trapping. A major problem stems from variations in Neo gene expression that can result, e.g. when the entrapment cassette is expressed from different cellular promoters. In addition, some gene trap vectors use a ‘corrected’ version of the Neo gene that induces higher levels of antibiotic resistance than a widely used ‘mutant’ gene (20,21). Cells expressing the corrected Neo gene may be insufficiently G418-sensitive to select for LOH events (22). We reasoned that gene trap
mutations generated by a poly(A) trap (23–25) and that incorporate the less active Neo gene might be reproducibly converted to homozygosity, if the resulting mutant clones expressed similar, moderate levels of neomycin resistance. In the present study, new poly(A) trap vectors were developed in which gene trapping selects for inserted Neo sequences that splice to the 3' ends of cellular genes. The vectors have additional features that permit vector–cell fusion transcripts to be directly cloned in *Escherichia coli* and that allow genes and chromosomes tagged by gene trapping to be engineered by DNA site-specific recombinases (26–31). The vectors are suitable for large-scale mutagenesis of mouse ES cells, and we show that most mutations selected from a stem cell library can be converted to a homozygous state following selection for higher levels of drug resistance. The ease and efficiency of obtaining homozygous entrapment mutations will (i) facilitate genetic studies of gene function in cultured cells, (ii) permit genome-wide studies of recombination events that result in LOH and mediate a type of chromosomal instability important in carcinogenesis, and (iii) provide new strategies for phenotype-driven mutagenesis screens in mammalian cells.

**RESULTS AND DISCUSSION**

GTRx.x gene trap vectors (Figure 1a, Supplementary Data) function as 3' gene [or poly(A)] traps (23–25,32). The virus inserts a Neo gene throughout the genome, and selection for G418 resistance generates clones in which Neo sequences splice to 3' distal exons of cellular genes. The Neo gene in GTR1.x vectors was expressed from the promoter of the gene encoding RNA polymerase II (Pol2) (33), or in GTR2.x vectors from the PGK promoter (34). Expression of the

Figure 1. (a) Structures of the GTR retrovirus gene trap vectors. Expression of an intron-containing Neo gene (5' Neo + 3' Neo) carried by the GTR1.0 poly(A) trap vector selects for inserts in which the Neo gene, expressed from the promoter of the RNA polymerase 2 gene (Pol2), splices to downstream exons of cellular genes. Transcripts of occupied cellular genes splice to a 3' exon [consisting of the 3' end of a puromycin-resistance gene (3' Puro), an internal ribosome entry site (IRES), a lacZ reporter and a polyadenylation site (PA)], disrupting their expression. A wild-type loxP site (loxP, left of 3' Puro) and mutant loxP sites (lox 5171, on either side of the 3' Neo exon) allow the body of the provirus to be replaced by other sequences by Cre-mediated cassette exchange. An RNA instability sequence (MI, flash symbol) increases the specificity of gene entrapment by reducing the levels of unspliced Neo transcripts. The positions of the provirus long terminal repeats (3' and 5' LTRs) are also indicated. Viruses lacking either the message instability sequence (pGTR1.3) or the lox 5171 in the Neo intron (GTR1.2) or both elements (GTR1.1) have also been constructed. GTR1.4–1.7 are identical to GTR1.0–1.3 except they contain an enhanced green fluorescence (EGFP) reporter instead of lacZ. GTR2.0–2.3 are identical to GTR1.0–1.3 except Neo is expressed from the PGK promoter. Some elements are not drawn to scale to enhance clarity. (b) Direct cloning of 3' RACE products. Gene entrapment by GTR vectors generates clones in which the Neo gene (white boxes) is expressed from transcripts that splice to downstream exons of cellular genes (black boxes). The intron and NotI endonuclease cleavage site in the Neo coding sequence ensure that only recombinant plasmids that contain cDNA inserts amplified from spliced Neo-cell fusion transcripts can give rise to kanamycin-resistant *E.coli.*
occupied cellular genes is disrupted by a 3' exon consisting of sequences from the 3' end of a puromycin-resistance gene, an internal ribosome entry site (IRES) and a reporter protein [either a nuclear \(\beta\)-galactosidase (lacZ; GTR1.0–GTR1.3) or enhanced green fluorescent protein (EGFP; GTR1.4–GTR1.7)]. Wild-type and mutant GTR1.0 and GTR1.3 contain an additional \(\text{lox}P\text{SI71}\) site located in a synthetic intron inserted into the \(\text{Neo}\) gene (Supplementary Figure 1c). The 3' \textit{Puro} segment provides the 3' end of a split puromycin-resistance gene and, when used in combination with the 5' end of the gene, is designed to select for Cre-mediated inter- and intra-chromosomal recombination events, as has been described using a split \(\text{Hprt}\) gene (30,31). The split \(\text{Neo}\) gene in GTR1.0 and GTR1.3 can also be used for this purpose if the 3' \(\text{Neo}\) exon is first deleted via recombination at the \(\text{lox}S171\) sites.

The \(\text{Neo}\) gene was engineered by inserting an intron and a NotI cleavage site by site-directed mutagenesis; neither modification affected the protein coding sequence (Supplementary Data and Supplementary Figure 1c). These features allow 3' RACE products from spliced fusion transcripts to be cloned directly in \(\text{E.coli}\) (Figure 1b). Briefly, fusion transcripts amplified by 3' RACE are cleaved with NotI and ligated to a plasmid (pSCV) containing \(\text{Neo}\) sequences upstream of the NotI site under the control of a strong bacterial promoter. Bacterial clones containing the desired RACE products are then selected on kanamycin plates. All steps in the process (expansion and cryopreservation of neomycin-resistant ES clones, RNA extraction, 3' RACE, and DNA sequencing) were performed in a 96-well format. Fusion transcripts were cloned from 70–80% of ES cells grown in a single well without using nested PCR or highly competent \(\text{E.coli}\), equivalent to the efficiency of cloning 3' RACE products manually (28).

3' RACE products from 979 ES cell clones were cloned, sequenced and compared against the mouse genome, EST and RefSeq databases using MultiBlaster, a relational database for performing BLAST searches on large numbers of DNA sequences (36). A total of 141 sequences contained repetitive DNA or were derived from probable sister clones (colonies from the same dish with identical 3' RACE products). Of the remaining 838 sequences, 822 returned significant matches with sequences in the mouse genome (MM). Most were derived from transcribed regions of the genome, as 568 matched sequences represented in the Expressed Sequence Tag (EST) database; 505, 371 and 337 matched entries for genes in the UniGene, RefSeq and Mouse Genome Informatics (MGI) databases, respectively (Figure 2). The UniGene, RefSeq and MGI matches correspond to genes with increasing degrees of characterisation/annotation. Nearly all matches had \(P\)-values <10\(^{-50}\), and none was >10\(^{-20}\). Only 31% of the 3' RACE products matched mouse genomic sequences (MGSCv3) for which there was no information to suggest that the provirus had inserted into a previously identified gene or transcription unit. Some of these inserts may reflect the presence of either new genes or additional exons that would extend the boundaries of adjacent transcription units. However, many probably correspond to cryptic or orphan 3' exons, as suggested by

![Figure 2](https://academic.oup.com/nar/article-abstract/34/20/e139/3100469)
in some cases by widespread examples of anti-sense transcripts in genes, spliced ESTs that are not incorporated into the structural models of nearby genes and abundant cytoplasmic poly(A)-containing RNAs that do not match annotated gene sequences. For comparison, 56% of cytoplasmic polyadenylated RNAs do not contain annotated exon or intron sequences (37).

The GTR vectors preferentially targeted the last intron and expressed fusion transcripts that spliced to a single downstream exon (Figure 2b). This provides further evidence that poly(A) traps select for fusion transcripts that evade nonsense-mediated decay (28,38). However, the preference was less pronounced, as 29% of the inserts in well-characterized genes were in upstream introns.

As with other gene trap vectors, gene targeting was not random (2,39): 15% of the unique UniGene sequences were targeted multiple times in clones derived from independent culture dishes, representing potential preferred sites for virus integration and/or gene trap selection. These include 7 inserts in 160010M07Rik by different GTR vectors and 11 inserts in CDC57 (4933434G05Rik), all by GTR2.3. The overall accumulation of gene trap inserts into unique UniGene clusters (Figure 2c) as a function of the total ES cell clones analyzed (including sister clones and non-informative 3′ RACE products with repetitive sequences) is similar to that reported for other gene trap vectors (39); however, the number of mutations analyzed is too small to predict eventual genome coverage (2,40).

Clones from the entrapment library were highly germline competent as all 10 entrapment loci that have been tested to date were readily transmitted through the germline (data not shown). While detailed analysis of the mutant mice is outside the scope of the present study, the GTR vectors appeared to be effective mutagens: 3 of 9 inserts into annotated genes induced obvious phenotypes when bred to a homozygous state. Specifically, an insert into Pfdn1 produced similar defects in eye development to those described for a Hesx1 homozygous state. An insert into Dymeclin induced obvious phenotypes when bred to a homozygous state (20). Nevertheless, the intrinsic neomycin sensitivity of cells with GTR2.3 proviruses was insufficient to select for potential LOH events that occur at per-cell frequencies of $\sim 10^{-5}$ (12-16,19). Levels of neomycin resistance correlate with levels of Neo gene expression (17), and relatively low levels of resistance are necessary in order to select for LOH events (22). Therefore, use of the Pol2 promoter, which is four times weaker than the PGK promoter (28), appears to be an important variable with regard to the reliable selection of potential homozygous mutant entrapment clones.

We tested whether homozygous mutants could be selected from heterozygous gene trap clones and examined the influence of chromosomal location on the frequencies of LOH. A total of 37 randomly selected clones with mutations (Supplementary Figure 2 and Supplementary Table 1) induced by the GTR1.3 vector were placed in media containing 2.0 mg/ml G418 to select potential clones that had undergone spontaneous LOH. The frequencies of resistance to 2.0 mg/ml G418 ranged from 1.3 $\times$ 10$^{-5}$ to 1.2 $\times$ 10$^{-4}$. Thus, all 37 clones with inserted GTR1.3 proviruses expressed degrees of neomycin sensitivity that are required in order to select for homozygous mutations generated by gene targeting (16,19). However, clones containing the GTR2.3 vector (in which Neo is expressed from the PGK promoter) displayed far greater resistance to G418 (Supplementary Table 1), even at 3.0 mg/ml G418 (data not shown). All of the GTR vectors were constructed using a ‘mutant’ Neo gene known to induce lower levels of antibiotic resistance than a ‘corrected’ variant (20). Nevertheless, the intrinsic neomycin sensitivity of cells with GTR2.3 proviruses was insufficient to select for potential LOH events that occur at per-cell frequencies of $\sim 10^{-5}$ (12-16,19). Levels of neomycin resistance correlate with levels of Neo gene expression (17), and relatively low levels of resistance are necessary in order to select for LOH events (22). Therefore, use of the Pol2 promoter, which is four times weaker than the PGK promoter (28), appears to be an important variable with regard to the reliable selection of potential homozygous mutant entrapment clones.
Genotypic analysis of 12 different mutants induced by GTR1.3 confirmed that a significant proportion (82% overall) of the colonies surviving in 2.0 mg/ml G418 had undergone LOH (Figure 4). Cells homozygous for all 12 entrapment loci tested were recovered at frequencies ranging from 40% to 100% of the high G418-resistant colonies selected from each clone. The 12 entrapment clones were randomly selected based on the availability of flanking sequence probes and primers capable of distinguishing the wild-type and occupied alleles. Since entrapment clones generated by GTR1.3 produced colonies resistant to 2.0 mg/ml G418 at similar frequencies (Supplementary Table 1), and a high proportion of the high G418-resistant colonies analyzed from each tested clone had undergone LOH, we conclude that cells homozygous for most mutations in the gene trap library can be selected in 2.0 mg/ml G418.

In contrast, we have encountered only one GTR1.3 entrapment clone for which homozygous mutant ES cells could not be isolated. These experiments (Cao et al., unpublished) were performed as part of a separate study to characterize a mutation in \( Pfdn1 \) (prefoldin), a chaperone that assists in the folding of cytoskeletal proteins (43). Briefly, cells heterozygous for the \( Pfdn1 \) mutation formed colonies in 2.0 mg/ml G418 at frequencies (5.6 \( \times \) 10\(^{-6}\)) more than 10-fold lower than observed with other GTR1.3 entrapment clones and similar to the frequencies observed with ES cells heterozygous for a targeted mutation in \( Ssrp1 \) (data not shown), which encodes a chromatin remodeling protein essential for ES cell viability (44). Moreover, the colonies arising in high G418 were small, and the cells could not be propagated further. Therefore, prefoldin appears to be required for the clonal outgrowth of ES cells, accounting for the failure to isolate homozygous mutant cells.

The frequency of colony formation in high G418 increased with increasing distance from the centromere (Figure 5). The \( R^2 \) by linear regression analysis was 0.54 for all genes in aggregate (Figure 5a) and 0.78 for the eight loci on chromosome 4 considered separately (Figure 5b). The influence of chromosome position was observed despite many potential variables that could influence the induction or recovery of clones with LOH [e.g. levels of \( Neo \) expression, DNA sequence effects on recombination, or clonal variation in the entrapment vector inserted in or near (*) the following genes: \( Ttc18 \) (a), \( Rbm4 \) (b), \( IL8Ra* \) (c), \( M11t10 \) (d), \( Cradd \) (e), \( 1700012G11Rik \) (f), \( Rnf7 \) (g), \( LOC240669 \) (h), \( Hess1 \) (i), \( 1810030N24Rik \) (j), \( Dscr1l1 \) (k) and \( Xrcc5 \) (l). Bands corresponding to the wild-type allele migrate more slowly in all cases except (b) than the allele disrupted by gene entrapment. LOH is indicated by the absence of the wild-type allele in cells selected in high G418 media.
plating efficiencies ranging from 30% to 50% (data not shown). The chromosome position effect suggests that mitotic recombination plays a significant role in spontaneous LOH, consistent with previous observations involving Neo genes inserted by gene targeting in ES cells (19) and the APRT gene in other cell types (13,14,18).

GTR1.3 represents the first insertional mutagen capable of biallelic mutagenesis of mammalian genes. However, like other early-generation poly(A) traps, the vector preferentially targets 3' introns, reflecting selection for fusion transcripts that escape nonsense-mediated decay (28,38). This interferes with the mutagenesis of a subset of genes that lack significant protein coding sequence in the last exon and may limit genome coverage (40). Inclusion of an IRES in the poly(A) trap cassette greatly enhances the recovery of inserts positioned throughout genes and increases the expression of fusion transcripts that append multiple, downstream exons (38). The IRES also appears to decrease the proportion of trapping events that splice to cryptic 3' exons, probably by increasing the size of the region within genes compatible with gene trap selection. Alternatively, small, non-coding poly(A) trap cassettes may be incorporated into a 5' gene trap vector (40).

The ease and efficiency of obtaining homozygous entrapment mutations will enhance the utility of mutant ES cell libraries in several ways. First, cells deficient in any gene of interest (assuming the gene is expressed in ES cells and is not required for cell viability) can be readily obtained for biochemical and metabolic studies of gene function, without the time or expense of introducing the mutation into the germline. As expected the Xrcc5-deficient Chinese hamster ovary (CHO) cell line (4) and a radiosensitive CHO cell line (Xrs5) defective in Ku86 were exposed to increasing doses of γ-irradiation, and cell survival was measured in a clonogenic assay.

Figure 5. The frequency of presumptive LOH at sites throughout the genome increases with distance from the centromere. A total of 37 ES cell clones, each containing a single GTR1.3 gene trap vector, were placed in media containing 2.0 and 0.3 mg/ml G418. The frequency of colony formation (Presumptive LOH) in the higher concentration of G418 (normalized to the number of colonies at the lower concentration) is plotted against the distance of each mutation from the centromere. The average values for three independent experiments are plotted for all 37 entrapment loci (a) and for all 8 inserts located on chromosome 4 (b). Linear regression analyses of the two groups produced R-squared values of 0.54 and 0.78, respectively. The SD values, which were 106% of the average values, have been omitted for clarity.

Figure 6. Loss of Xrcc5 expression in homozygous entrapment clones. (a) RNA was isolated from the parental AC1 ES cells (lane 1), the heterozygous Xrcc5 entrapment mutant before selection in 2.0 mg/ml G418 (lane 2) and clones isolated by selection in 2.0 G418 (lanes 3–6) and northern blot analysis was performed using Xrcc5 (downstream of exon 1 cloned by 3' RACE) and β-actin specific probes (upper and lower panels, respectively). Neo-Xrcc5 fusion transcripts in heterozygous and homozygous mutant cells are generated by splicing of the Neo sequences to exon 2 of the Xrcc5 gene. (b) Radiation sensitivity of Xrcc5 heterozygous and homozygous mutant cells. Parental ES cells (1), heterozygous Xrcc5 entrapment mutant (2) homozygous Xrcc5 entrapment mutants homozygous Xrcc5 entrapment mutants (3, 5, 6) a control Xrcc6-deficient Chinese hamster ovary (CHO) cell line (4) and a radiation-sensitive CHO cell line (Xrs5) defective in Ku86 were exposed to increasing doses of γ-irradiation, and cell survival was measured in a clonogenic assay.
(Figure 6a). Third, the ability to select homozygous mutant cells will provide an early assessment of whether the disrupted genes or chromosome deletions engineered post-entrapment are required for cell viability. Fourth, allelic imbalance is a common manifestation of chromosome instability in human cancers, which may harbor >10,000 regions of LOH per cell (45). The source of this genome-wide LOH is unknown and unfortunately, frequencies of LOH at specific sites are typically measured at relatively few loci (e.g., Tk, Apri, Hprt or cell surface antigens) where gene inactivation confers a selectable phenotype. Entrapment ES cell clones provide resources to study factors, such as carcinogens (46), localized elements in the genome or mutations in genes required for genome maintenance, that influence the frequencies of LOH at many sites throughout the genome.

Finally, LOH involving GTR poly(A) traps will assist phenotype-driven mutagenesis screens in mammalian cells (6,7,47). Mutagens incorporating Pol2Neo as an LOH selection cassette, should facilitate the recovery of homozygous mutants by combining selection for high G418 resistance together with strategies that enhance the frequencies of LOH (7,46,47). Alternatively, since LOH involving inserted Neo resistance genes extend across large chromosome regions (19), stem cell clones containing inserted GTR1.3 vectors can be used to enhance the recovery of homozygous recessive mutants located on the same chromosome as the gene trap vector. This provides an alternative to the use of site-specific recombinases to induce mitotic recombination (10,11), eliminating the need to insert recombinase target sequences at allelic sites in the genome.

**METHODS**

**Gene trapping**

AC1 mouse ES cells were derived from an explanted 129svJ blastocyst cultured on feeder layers of irradiated mouse embry fibroblasts and were cultured as described previously (48).

Construction of GTR poly(A) trap vectors is described in the Supplementary Data. Retroviruses were prepared by transfecting GTR plasmids into Phoenix Eco cells by calcium phosphate coprecipitation. Virus production by individual transfecting GTR plasmids into Phoenix Eco cells by calcium

**Isolation of flanking genomic DNA**

Flanking genomic DNA sequences were cloned by inverse PCR (49) or by ligation-mediated PCR (50) modified by the addition of a C3 spacer (Integrated DNA Technologies) to the NlaIII minus adaptor to block the amplification of fragments via adaptor primers alone. Briefly, genomic DNA was (i) digested with NlaIII, ligated to a 1:1 mixture of NlaIII plus (5'-AGATATCGAATCCTAATAGGGGCGCCCTTTAAAGG-GACCATG-3') and minus (5'-Phos-GCCCTTAACGCGGAG-C3 spacer) strand adaptors, (ii) digested with PstI to prevent amplification of sequences from the 5'-LIR and (iii) subjected to two rounds (30 cycles each) of PCR using nested primers to the LTR and adaptor sequences.

First round PCR, LTR: 5'-GCTAGCGTCCAAAAACCACT- CAGGTGG-3'

Adaptor: 5'-GGAATCGAATCCTAATAGGGGCGCCCTTTAAAGG-GACCATG-3'

Second round PCR, LTR: 5'-CCAAACCTACTACAGGT- GGTCTTTC-3'

Adaptor: 5'-AGGGTCCCGTTAAGGGAAC-3'

Regions of flanking genomic DNA downstream of the provirus in the Trc18, Rho4, M11t10, Cradd, l7000/2G1/Rik, Rnf7, LOC240669, Hesx1 and Dscrl1 genes or near the IL8Ra gene were sequenced, and the sequences have been submitted to the GenBank GSS database (accession numbers, DX977440–DX977449, respectively). The sequences of primers used for genotyping are available on request.
Selection and analysis of LOH

Serially diluted cells were plated in triplicate onto 150 mm plates and allowed to attach overnight. Subsequently, unattached cells were removed and selection media containing either 0.0, 0.3 or 2.0 mg/ml G418 was added to each dish. After 12 days, the number of colonies surviving in each dish was counted, and the frequency of colony formation at 2.0 mg/ml G418 was determined by dividing the number of colonies obtained from 0.3 mg/ml G418 selection to that obtained from 2.0 mg/ml G418 selection.

The genotypes of clones surviving in 2.0 mg/ml G418 were determined by Southern blot and PCR analysis. For Southern blot analysis, 5 μg of endonuclease-cleaved DNA was fractionated on 0.9% (w/v) agarose gels and hybridized to probe genomic DNA sequences adjacent to the entrapment vector. For PCR analysis, 200 ng of genomic DNA was amplified using two primers complementary to genomic DNA located on either side of the site of provirus insertion and one primer specific for the entrapment vector.

Loss of Cradd expression in homozygous mutant cells was determined by western blot analysis using a rabbit polyclonal antibody against a region containing Leu-97 of the human CRADD/RAIDD protein (catalog number 4899, Cell Signaling Technology, Inc.)

Analysis of Xrcc5 entrapment clones

Serially diluted cells were plated in triplicate onto 150 mm plates and allowed to attach overnight. Subsequently, cells were irradiated in culture medium at a dose rate of 3 Gy/min (200 kV, 4 mA, 0.78 mm Al). Colonies were counted at 12 days after irradiation, and the percentage of surviving cells was determined relative to the numbers of colonies from untreated cells.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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