Oligoarray Comparative Genomic Hybridization-Mediated Mapping of Suppressor Mutations Generated in a Deletion-Biased Mutagenesis Screen

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ABSTRACT Suppressor screens are an invaluable method for identifying novel genetic interactions between genes in the model organism Caenorhabditis elegans. However, traditionally this approach has suffered from the laborious and protracted process of mapping mutations at the molecular level. Using a mutagen known to generate small deletions, coupled with oligoarray comparative genomic hybridization (aCGH), we have identified mutations in two genes that suppress the lethality associated with a mutation of the essential receptor tyrosine kinase rol-3. First, we find that deletion of the Bicaudal-C ortholog, bcc-1, suppresses rol-3-associated lethality. Second, we identify several duplications that also suppress rol-3-associated lethality. We establish that overexpression of srap-1, a single gene present in these duplications, mediates the suppression. This study demonstrates the suitability of deletion-biased mutagenesis screening in combination with aCGH characterization for the rapid identification of novel suppressor mutations. In addition to detecting small deletions, this approach is suitable for identifying copy number suppressor mutations, a class of suppressor not easily characterized using alternative approaches.

Suppressor screening is an efficient way to generate mutations in developmentally associated genes. In the model organism Caenorhabditis elegans, suppressor mutations have given insight into a number of gene functions, facilitating the elucidation of complex developmental mechanisms and pathways (Dorfman et al. 2009; Galvin et al. 2011; Rohlfing et al. 2011; Schumacher et al. 2005; Singh and Han 1995). The time and expense required to identify mutations generated in such screens are, however, a major limiting factor in the value of these approaches (Barbazuk et al. 1994; Fay and Johnson 2006; Jørgensen and Mango 2002). Alternative strategies to generate and rapidly map suppressor mutations are therefore required.

The advent of high-resolution molecular methods for mapping mutations has, to a certain extent, replaced the need for slower traditional approaches. A number of such methods are available. Principal among these are whole-genome sequencing (WGS) and oligoarray comparative hybridization (aCGH). WGS has been applied to mutant identification in C. elegans and shown to be suitable for detecting a variety of DNA lesions (Chu et al. 2012; Filbott et al. 2010; Hiller et al. 2008; Hobert 2010; Sarin et al. 2008; Shen et al. 2008), although methods to reliably identify small insertion/deletions (indels) and duplications are still being refined (Smith 2011). Additionally, although WGS is becoming more cost-effective, intensive and specialized bioinformatic analysis is required for mutation identification and validation. However, aCGH analysis, a technique for querying genome alterations to a high resolution (Dhami et al. 2005; Selzer et al. 2005), is a viable alternative approach that does not require specialized bioinformatic analysis. Several C. elegans—specific aCGH platforms have been developed and have been shown to be effective for rapidly identifying novel single-gene deletions (Maydan et al. 2007; O’Meara et al. 2009), as well as large duplications and deficiencies (Jones et al. 2007; Lipinski et al. 2011; Maydan et al. 2010). The use of aCGH circumvents the need for time-consuming genetic

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mapping methods and is therefore a suitable method for identifying novel mutations generated in suppressor screens.

The creation of genomic lesions suitable for high-resolution mapping with aCGH requires the use of a mutagen capable of creating small deletions. The generation of single-gene deletion mutations (also known as knockout mutations) is routinely performed in C. elegans (Barstead and Moerman 2006; Filibotte et al. 2010; Gengyo-Ando and Mitani 2000; Moerman and Barstead 2008). UV-TMP is the mutagen of choice for generating knockout mutations as it has been shown to generate a high frequency of deletions with an average detectable size of 10 kb, in addition to a significant number of small indels (Flibotte et al. 2010; Gengyo-Ando and Mitani 2000; Kage-Nakadai et al. 2012; Liu et al. 1999; Yandell et al. 1994).

Here we describe the use of a UV-TMP mutagenesis screen for the generation of suppressors of a mutation in the essential receptor tyrosine kinase (RTK), rol-3. We then use aCGH to rapidly map the lesions. In all cases, we detect the suppressive lesion, defining two novel loci that suppress rol-3–associated lethality.

MATERIALS AND METHODS

Strains and genetics

Maintenance and handling of C. elegans were performed as previously described (Brenner 1974). Worms were cultured at 20°C, unless otherwise stated. All strains are derivatives from Bristol N2 wild-type animals. Strains used were Bristol N2 wild-type, rol-3(s1040), sDp31(s3067), sDp32(s3068), sDp33(s3069), sDp34(s3070), sDf149(s3072), rol-3(s1040); sEx2693, bcc-1(tm3821).

Transgenic arrays

sEx2693 is a transgenic array comprising the fosmid WRM0262cC02, which contains the genomic region of T06D8.1/srap-1.

UV-TMP mutagenesis and suppressing screening

The suppressor screen strategy was modified from that previously reported (Barbazuk et al. 1994). The mutagenesis method used for this rol-3 suppressor screen was modified from (Flibotte et al. 2010). A mixed stage population of BC3129, rol-3(s1040), was harvested at the permissive temperature of 15°C, and the worm suspension was incubated in 2 μg/ml TMP in the dark for 1 hr. Worms were then exposed to 90 sec of UV irradiation at 340 microwatts per square centimeter. After treatment, 50 gravid adults were transferred to 10 cm plates seeded with OP50 and cultured at the permissive temperature (15°C) for one generation (7–10 days) before being shifted to the restrictive temperature of 20°C. Animals were grown for a further 7–10 days. A single animal was isolated from each plate and maintained at the restrictive temperature (20°C) to ensure that the suppressor mutation was retained. We estimate that at least 500,000 chromosomes were screened in this analysis.

aCGH data analysis of UV-TMP suppressors

aCGH was performed using a whole-genome C. elegans array designed with overlapping 50-mer probes targeting annotated exons and micro-RNAs (Maydan et al. 2007). aCGH sample preparation, hybridization, and analysis were done as previously described (Maydan et al. 2007). Copy number aberrations were detected by visual inspection using the SignalMap browser software (Roche Nimblegen Inc., Madison, WI).

Molecular identification of deficiency breakpoints in s3071

PCR amplification across the region of the breakpoint in the strain rol-3(s1040), sDf149(s3072) was performed using the appropriate nested primers (available upon request), and purified products were sent for sequencing at Macrogen (Macrogen, Seoul, Korea).

RNAi analysis

Generation of dsRNA: RNA interference (RNAi) experiments were performed by injection as previously described (Sonnichsen et al. 2005).

RNAi injection against deletion and duplication suppressor gene candidates: An annealed dsRNA targeting N7.7, bcc-1, T06D8.1, T06D8.5, and F37H8.5 was injected directly into the syncytial gonad of mutant strains to be tested. Injected animals were recovered for 16 hr at 20°C in order to lay any eggs present in utero prior to injection and were then transferred individually onto fresh NGM agar plates maintained at restrictive temperature (20°C). Evidence of suppression or disruption of suppression was assessed after 7–10 days of growth.

Suppression of rol-3(s1040) lethality with transgenic arrays containing srap-1

DNA prepared from the fosmid clones WRM0625cC02, WRM0625bF10, and WRM0635dC04 was injected directly into young adult rol-3(s1040) animals raised at permissive temperature of 15°C. Fosmid DNA was injected at a concentration of 10 ng/μl with 80 ng/μl dpy-5 carrier DNA. Injected animals were maintained at the restrictive temperature of 20°C to select for suppression. Suppression was assayed by screening for viable progeny after 7–10 days of growth.

Microscopy and image processing

Analysis of mutant and GFP transgenic animals was performed using a ZEISS Stemi SVC11 dissecting microscope with GFP filters and a Zeiss Axioskop with 10×/0.25, 40×/0.65 and 60×/0.85 objective lenses. All pictures were taken using QCapture software (QImaging) with a QImaging digital camera mounted on the Zeiss Axioskop. Images were processed using Photoshop CS4 (Adobe).

| Table 1 | aCGH mapping data for duplications and deletions in the UV-TMP–derived suppressor strains |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Designation     | Allele          | Chr             | Left (bp)       | Right (bp)      | Size (kb)       | Type            |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| sDp31           | s3067           | II              | 10054547        | 15242654        | 5188            | dup             |
| sDp32           | s3068           | II              | 10905657        | 11229193        | 324             | dup             |
| sDp33           | s3069           | II              | 10905657        | 11229193        | 324             | dup             |
| sDp34           | s3070           | ND              |                 |                 |                 |                 |
| sDf149          | s3071           | II              | 10643051        | 11289830        | 647             | dup             |
| sDf31           | s3072           | IV              | 11087657        | 11092473        | 5               | del             |
| sDf35           | s3073           | ND              |                 |                 |                 |                 |
| sDp25           | s3074           | II              | 11187892        | 11983055        | 795             | dup             |

bp, base pair coordinate; Chr, chromosome, del, deletion; dup, duplication; ND, not determined.
RESULTS AND DISCUSSION

Suppressors of rol-3 generated by UV-TMP harbor deletions and duplications that can be detected using aCGH

The fully penetrant, temperature-sensitive lethality of a hypomorphic mutation in rol-3(s1040) animals (Johnsen and Baillie 1991) lends itself to the isolation of extragenic suppressors. Previously, eight suppressor mutations defining the two loci srl-1 and srl-2 (suppressor of rol-3 lethality) were generated (Barbazuk et al. 1994). Using traditional genetic methods, these suppressors were mapped to chromosomes II and III, respectively. However, further mapping was complicated by the lack of obvious phenotypes in the single mutant animals and a complex inter se complementation between specific alleles of srl-1 and srl-2 (Barbazuk et al. 1994).

Given the relative ease of screening for suppressors of rol-3, we undertook to generate de novo suppressor mutations that might be suitable for detection by aCGH. Approximately 500,000 UV-TMP--
treated genomes were screened, yielding eight suppressors that we have designated s3067–s3074 (Table 1). Although the temperature-dependent larval lethality associated with the s1040 allele is suppressed in these strains, animals still exhibit the characteristic adult left-handed rolling (LRol) phenotype (data not shown).

We analyzed six of the eight suppressor strains using aCGH. Of these, only s3072 contains an obvious deletion, located on chromosome IV (Figure 1, A and B). PCR and sequencing of the deleted region in s3072 animals confirmed the presence of a 6266 bp deletion and 1064 bp insertion from an intergenic region located on chromosome II (Figure 1C). This indel, which we have designated sDf149, is a deficiency that disrupts two predicted genes. sDf149 deletes the complete coding region of the predicted ORF M7.7 and the majority of the gene bcc-1, including the ATG start site. M7.7 is an uncharacterized and poorly conserved putative protein kinase. bcc-1 is an ortholog of the mRNA stabilizing protein Bicaudal-C (Eckmann et al. 2002).

**Disruption of bcc-1 is responsible for suppression of rol-3 lethality**

To determine which of the two genes disrupted by sDf149 is responsible for the suppression of rol-3, we attempted to phenocopy the suppression using RNAi. rol-3(s1040) animals are 100% inviable when raised at the restrictive temperature of 20°, arresting at an early larval stage of development (Table 2) (Johnsen and Baillie 1991). To assess suppression by RNAi, rol-3(s1040) animals raised at the permissive temperature of 15° were injected with dsRNA targeting either M7.7 or bcc-1. These animals were then cultured at the restrictive temperature of 20°, and suppression was assayed by the presence of adults in the progeny of the injected animals. Injection of rol-3(s1040) adults with dsRNA targeting bcc-1, however, resulted in approximately 10% of the progeny developing to the adult stage (Table 2). This result demonstrates that the suppression is due specifically to disruption of bcc-1. To provide further support for this result, we obtained a mutant allele of bcc-1, tm3821, in which a 517 bp region comprising exon 6 is deleted (Figure 1B). This deletion should lead to truncation of the predicted protein by creating a premature stop codon (Figure 1D).

| Table 2 RNAi of suppressor gene candidates in rol-3(s1040) animals maintained at 20° |
|------------------------------------------|--------|--------|--------|--------|--------|
| Phenotype | Mock⁴ | bcc-1 | M7.7 | Phenotype |
| Lvl       | >500  | 88    | >300  | Lvl    |
| Adult     | 0     | 8     | 0     | adult  |
| % Suppression | 0   | 9     | 0     | % Suppression |

The progeny of 3–10 injected worms was scored. ⁴ A placebo of injection buffer containing no dsRNA was injected as a negative control.
rol-3(s1040) animals that are also mutant for bcc-1(tm3821) are viable when grown at the restrictive temperature of 20°C (41.5%) (Table 1). Together, these data establish that disruption of bcc-1 alone is sufficient to suppress rol-3 associated lethality.

Five of the suppressors characterized by aCGH contain duplications of chromosome II

The genomes of the five remaining suppressors analyzed with aCGH (s3067, s3068, s3069, s3071, and s3074) do not contain any obvious deletions. However, all genomes contain relatively large duplications of chromosome II. We have designated these duplications sDp31–sDp35 (Figure 2 and Table 1). In addition to several duplicated regions of chromosome II, the suppressor strain s3067 contains a duplication of 0.6 Mb of DNA from the left end of chromosome V (Figure 2A and Table 1). The duplications sDp32 and sDp33 appear to be identical in size; however, based on the probe data, the duplication is present at a higher copy number in sDp32 (Figure 2B).

Duplications of the predicted mucin T06D8.1 suppress rol-3 lethality

The prevalence of duplications in the suppressed animals suggested that their insertion into the genome might have disrupted the function of a specific gene, giving rise to the suppression. Alternatively, extra copies of a gene, or genes, present in the duplicated regions might be responsible for the suppression. That the five duplications identified by aCGH are not randomly distributed across the genome but overlap the same region of chromosome II suggested that the latter hypothesis was more likely. The genomic region common to the duplications is around 40 kb in size and contains only three complete ORFs: T06D8.1, a predicted mucin; T06D8.3, a predicted lipid phosphate

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Table 3 Fosmids used for transgenic suppression of rol-3(s1040) animals maintained at 20°C

| Fosmid       | Genomic Region     | Gene Target | rol-3(s1040) Viability at 20°C |
|--------------|--------------------|-------------|-------------------------------|
| WRM0613dF11  | II: 11190820–11222798 | T06D8.1     | Yes                           |
| WRM0625bf10  | II: 11169946–11207249 | F37H8.5     | No                            |
| WRM0635dC04  | II: 11217009–11252193 | T06D8.3     | No                            |

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Figure 3 Overexpression of the predicted mucin SRAP-1 (T06D8.1) suppresses rol-3 lethality. (A) A schematic showing the region common to all duplication-containing suppressor strains. (B) Fosmids used for transgenic suppression experiments are shown below the gene models. (C) The predicted structure of SRAP-1. Duf (Mult), multiple copies of a domain of unknown function; PAN_AP, PAN Apple domain; PAN_1, PAN1 domain; sp, signal sequence.
Table 4. RNAi against suppressor candidates in duplication suppressed animals maintained at 20°C.

| Strain          | % Viability of Strains Targeted by RNAi |
|-----------------|----------------------------------------|
| Bristol N2      | Mock                                    |
| rol-3(s1040); 5| T06D8.1: 100                           |
| rol-3(s1040); 5| T06D8.3: 100                           |
| rol-3(s1040); 5| F37H8.5: 100                           |

The progeny of 3–10 injected worms was scored.

The C. elegans homolog of gamma-interferon–inducible lysosomal thioreductase (Figure 3A).

To determine whether one of the three duplicated genes is responsible for the suppression, we attempted to increase the copy number of individual candidate genes by introducing gene-specific fosmids as transgenic arrays (Figure 3B). We injected DNA directly into rol-3(s1040) animals raised in the permissive temperature of 15°C and screened the progeny of these animals for viability at the restrictive temperature of 20°C. Using this approach, we found that only one fosmid that encompasses the complete coding region of T06D8.1 (WRM0626c02) is capable of rescuing rol-3–associated lethality (sEx2693). Transgenic arrays composed of fosmids containing either of the adjacent genes, T06D8.3 or F37H8.5, were unable to confer suppression (Figure 3B and Table 3).

It is likely that overexpression of T06D8.1 mediates the suppression of rol-3. To provide further support for this finding, we surmised that knockdown of T06D8.1 by RNAi in suppressed rol-3 mutant strains would abrogate suppression, leading to targeted lethality. Injection of dsRNA targeting T06D8.1 in wild-type animals does not result in lethality (Table 4). However, RNAi targeting T06D8.1 in suppressor strains containing the duplications sDp33 and sDp35 completely abolishes suppression (Table 4). This effect is specific to T06D8.1, because introduction of dsRNA targeting the two other candidate genes, T06D8.3 and F37H8.5, does not disrupt the suppression. Together, these data demonstrate that suppression of rol-3 in the duplication-containing strains is due specifically to the presence of extra copies of T06D8.1. Furthermore, abrogation of the suppression by RNAi knockdown reveals that the suppression is due to overexpression of T06D8.1 (Table 4). T06D8.1 encodes a predicted mucin similar to the serine-rich adhesion molecule SraP from Staphylococcus aureus (McKay et al. 2003). We have renamed this gene spr-1 (for serine rich adhesion protein-like).

CONCLUSIONS

This study describes a straightforward approach for the rapid identification of de novo suppressor mutations. Using a deletion-based mutagenesis screen combined with high-resolution aCGH mapping, we have identified two novel loci that suppress the lethality associated with a temperature-sensitive mutation of the essential RTK rol-3. These two loci represent the first suppressors of this gene to be identified at the molecular level. The approach we have described does not require complex sample preparation or specialized informatics analysis beyond the scope of standard laboratory techniques. Additionally, and perhaps most significantly, this approach can be used to rapidly characterize copy number suppressors to a high resolution. This is something that is not easily achieved using alternative approaches, such as WGS. The use of aCGH opens up the possibility of tailoring suppressor screens for the isolation of dominant suppressors that can be quickly assayed for the presence of duplications. Investigations of this type will facilitate a better understanding of the consequences of altering expression levels of genes that function in important biological pathways.

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LITERATURE CITED

Barbazuk, W. B., R. C. Johnsen, and D. L. Baille, 1994 The generation and genetic analysis of suppressors of lethal mutations in the Caenorhabditis elegans rol-3(V) gene. Genetics 136: 129–143.

Barstead, R. J., and D. G. Moerman, 2006 C. elegans deletion mutant screening. Methods Mol. Biol. 351: 51–58.

Brenner, S., 1974 The genetics of Caenorhabditis elegans. Genetics 77: 71–94.

Chu, J. S., R. C. Johnsen, S. Y. Chua, D. Tu, M. Dennison et al., 2012 Allelic ratios and the mutational landscape reveal biologically significant heterozygous SNVs. Genetics 190: 1225–1233.

Dhani, P., A. J. Coffey, S. Abbas, J. R. Vermesch, J. P. Dumanski et al., 2005 Exon array CGH: detection of copy-number changes at the resolution of individual exons in the human genome. Am. J. Hum. Genet. 76: 750–762.

Dorfman, M., J. E. Gomes, S. O’Rourke, and B. Bowerman, 2009 Using RNA interference to identify specific modifiers of a temperature-sensitive, embryonic-lethal mutation in the Caenorhabditis elegans ubiquitin-like Nedd8 protein modification pathway E1-activating gene rfl-1. Genetics 182: 1035–1049.

Eckmann, C. R., B. Kraemer, M. Wickens, and J. Kimble, 2002 GLD-3, a bicaudal-C homolog that inhibits FBF to control germline sex determination in C. elegans. Dev. Cell 3: 697–710.

Fay, D., and W. Johnson, 2006 Genetic mapping and manipulation: Chapter 10-Suppressor mutations (February 17, 2006), WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.99.1, http://www.wormbook.org.

Filibotte, S., M. L. Edgley, I. Chaudhry, J. Taylor, S. E. Neil et al., 2010 Whole-genome profiling of mutagenesis in Caenorhabditis elegans. Genetics 185: 431–441.

Galvin, B. D., D. P. Denning, and H. R. Horvitz, 2011 SPK-1, an SR protein kinase, inhibits programmed cell death in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 108: 1998–2003.

Gengyo-Ando, K., and S. Mitani, 2000 Characterization of mutations induced by ethyl methanesulfonate, UV, and trimethylpsoralen in the nematode Caenorhabditis elegans. Biochem. Biophys. Res. Commun. 269: 64–69.

Hillier, L. W., G. T. Marth, A. R. Quinlan, D. Dooling, G. Fewell et al., 2008 Whole-genome sequencing and variant discovery in C. elegans. Nat. Methods 5: 183–188.

Hobert, O., 2010 The impact of whole genome sequencing on model system genetics: get ready for the ride. Genetics 184: 317–319.

Johnsen, R. C., and D. L. Baille, 1991 Genetic analysis of a major segment [LGV(left)] of the genome of Caenorhabditis elegans. Genetics 129: 735–752.

Jones, M. R., J. S. Maydan, S. Filibotte, D. G. Moerman, and D. L. Baille, 2007 Oligonucleotide Array Comparative Genomic Hybridization (oaCGH) based characterization of genetic deficiencies as an aid to gene mapping in Caenorhabditis elegans. BMC Genomics 8: 402.

Jorgensen, E. M., and S. E. Mango, 2002 The art and design of genetic screens: caenorhabditis elegans. Nat. Rev. Genet. 3: 356–369.
Kage-Nakadai, E., H. Kobuna, O. Funatsu, M. Otori, K. Gengyo-Ando et al.,
2012 Single/low-copy integration of transgenes in Caenorhabditis elegans
using an ultraviolet trimethylpsoralen method. BMC Biotechnol. 12: 1.
Lipinski, K. J., J. C. Farslow, K. A. Fitzpatrick, M. Lynch, V. Katju et al.,
2011 High spontaneous rate of gene duplication in Caenorhabditis elegans.
Curr. Biol. 21: 306–310.
Liu, L. X., J. M. Spoerke, E. L. Mulligan, J. Chen, B. Reardon et al.,
1999 High-throughput isolation of Caenorhabditis elegans deletion
mutants. Genome Res. 9: 859–867.
Maydan, J. S., S. Flibotte, M. L. Edgley, J. Lau, R. R. Selzer et al.,
2007 Efficient high-resolution deletion discovery in Caenorhabditis elegans
by array comparative genomic hybridization. Genome Res. 17:
337–347.
Maydan, J. S., A. Lorch, M. L. Edgley, S. Flibotte, and D. G. Moerman,
2010 Copy number variation in the genomes of twelve natural isolates
of Caenorhabditis elegans. BMC Genomics 11: 62.
McKay, S. J., R. Johnsen, J. Khattra, J. Asano, D. L. Baillie et al.,
2003 Gene expression profiling of cells, tissues, and developmental stages of the
nematode C. elegans. Cold Spring Harb. Symp. Quant. Biol. 68: 159–169.
Moerman, D. G., and R. J. Barstead, 2008 Towards a mutation in every
gene in Caenorhabditis elegans. Brief. Funct. Genomics Proteomics 7:
195–204.
O’Meara, M. M., H. Bigelow, S. Flibotte, J. F. Etchberger, D. G. Moerman
et al., 2009 Cis-regulatory mutations in the Caenorhabditis elegans
homeobox gene locus cog-1 affect neuronal development. Genetics 181:
1679–1686.
Rohling, A. K., Y. Miteva, L. Moronetti, L. He, and T. Lamitina, 2011 The
Caenorhabditis elegans mucin-like protein OSM-8 negatively regulates
osmosensitive physiology via the transmembrane protein PTR-23. PLoS
Genet. 7: e1001267.
Sarin, S., S. Prabhu, M. M. O’Meara, I. Pe’er, and O. Hobert,
2008 Caenorhabditis elegans mutant allele identification by whole-genome sequencing. Nat. Methods 5: 865–867.
Schumacher, B., M. Hanazawa, M. H. Lee, S. Nayak, K. Volkmann et al.,
2005 Translational repression of C. elegans p53 by GLD-1 regulates
DNA damage-induced apoptosis. Cell 120: 357–368.
Selzer, R. R., T. A. Richmond, N. J. Pofahl, R. D. Green, P. S. Eis et al.,
2005 Analysis of chromosome breakpoints in neuroblastoma at sub-
kilobase resolution using fine-tiling oligonucleotide array CGH. Genes Chromosomes Cancer 44: 305–319.
Shen, Y., S. Sarin, Y. Liu, O. Hobert, and I. Pe’er, 2008 Comparing platforms for C. elegans mutant identification using high-throughput whole-genome sequencing. PLoS ONE 3: e4012.
Singh, N., and M. Han, 1995 sur-2, a novel gene, functions late in the let-60
ras-mediated signaling pathway during Caenorhabditis elegans vulval
induction. Genes Dev. 9: 2251–2265.
Smith, H. E., 2011 Identifying insertion mutations by whole-genome sequenc-
ing. Biotechniques 50: 96–97.
Sonnichsen, B., L. B. Koski, A. Walsh, P. Marschall, B. Neumann et al.,
2005 Full-genome RNAi profiling of early embryogenesis in Caeno-
rhabditis elegans. Nature 434: 462–469.
Yandell, M. D., L. G. Edgar, and W. B. Wood, 1994 Trimethylpsoralen
induces small deletion mutations in Caenorhabditis elegans. Proc. Natl.
Acad. Sci. USA 91: 1381–1385.

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