In Nucleic Acids Res. [REF], we issued a correction for our article, *Computational identification of non-coding RNAs in Saccharomyces cerevisiae by comparative genomics* (Nucleic Acids Res. 31: 4119-4128, 2003). This paper reported eight new noncoding RNA genes in yeast, named RUF1 through RUF8, for “RNA of Unknown Function.” We subsequently found that we cannot verify the transcription of three of the eight RUF loci (RUF4, RUF6, RUF7); the originally published results for these three RNAs appear to be experimental artifacts. Another locus, RUF8, is in fact a coding mRNA composed of three exons, and has recently been independently identified as SUS1, a protein component of the SAGA complex and the nuclear pore-associated mRNA export machinery (Rodriguez-Navarro et al., Cell 116: 75-86, 2004).

We reconfirmed transcription of RUF1, RUF2, RUF3, and RUF5 by Northerns with strand-specific riboprobes, and by additional RACE-PCRs. Coordinates for the ends of RUF3 and RUF5 were corrected. Coordinates for the ends of RUF1 and RUF2 were reconfirmed, and updated to the new genomic coordinates of the *Saccharomyces* Genome Database (SGD), as shown in Table 1.

<table>
<thead>
<tr>
<th>RNA</th>
<th>Chromosome</th>
<th>Old Coordinates</th>
<th>Revised Coordinates</th>
<th>Size</th>
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<tr>
<td>RUF1</td>
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<td>1493029 - 1492480</td>
<td>1493014 - 1492465</td>
<td>550</td>
</tr>
<tr>
<td>RUF2</td>
<td>7</td>
<td>316786 - 317053</td>
<td>316789 - 317056</td>
<td>268</td>
</tr>
<tr>
<td>RUF3</td>
<td>13</td>
<td>626239 - 626669</td>
<td>626348 - 626653</td>
<td>306</td>
</tr>
<tr>
<td>RUF5</td>
<td>8</td>
<td>212379 - 213153</td>
<td>212410 - 213119 (Major ends)</td>
<td>710</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>212350 - 213153 (Minor ends)</td>
<td>804</td>
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</table>

Table 1. Old and revised coordinates for RUF1, RUF2, RUF3, and RUF5. The sizes given are calculated from the revised coordinates. Compare with table 2 in the original NAR paper.

**RUF1 and RUF2**

The changes to RUF1 and RUF2 only reflect annotation changes at SGD. Additional RACE-PCR sequencing confirmed the original ends.

Figures 1A, 1B, 1C, and 1D show Northern blots for RUF1 and RUF2. Figures 1A and 1C show Northern blots probed with end-labeled oligo probes. Figure 1B is a Northern blot probed with a body-labeled 580 nucleotide (nt) (1493014 to 1492436) riboprobe and figure 1D is a Northern blot probed with a body-labeled 271 nt (316789 to 317059) riboprobe. RUF2 shows two products, of about 270 and 280 nt on the riboprobe Northern; RACE-PCR only showed one 5’ and one 3’ end, consistent with the 268 nt major transcript we annotate.

**RUF3**

The revised annotation in the 5’ end of RUF3 is a substantial change. The change to the 3’ end of RUF3 is minor, and reflecting data from 11 more RACE-PCR sequences at this end.

An oligo probe was used in the Northern blot published in the original paper (Figure 1E). The oligo used to identify RUF3 in the screen is now known to be upstream of the major transcript (Figure 2) and therefore did not reveal the major 306 nt transcript. Upon hybridizing with a long riboprobe spanning the locus (Figure 2) the primary 306 nucleotide transcript was obvious (Figure 1F). A longer exposure of the same blot revealed higher bands (Figure 1G lane 2) that roughly agree with the sizes seen in the initial oligo-probed Northern blot (Figure 1E); we interpret these larger RNAs as probable precursors and/or processing byproducts.
Figure 1. Northern blots for RUF1, RUF2, RUF3, RUF5, and RUF8 (SUS1). Figures 1A, 1C, 1E, 1H, and 1J are reproductions of figures 1F, 1G, 1H, 1I, and 1M from the original NAR paper. These Northern were probed with an end-labeled oligo probe. For these Northern, lane 1 is the size indicated for the 100 base pair ladder size marker (lane 2). Lanes 3-7 contain total RNA from the following growth conditions (all grown at 30°C to mid-log except where noted): lane 3, minimal media; lane 4, GALactose; lane 5, heat shock 30 min at 37°C in YDP; lane 6, saturated YPD growth; lane 7, YPD. Lane 8 in the estimated size of each transcript. Figures 1B, 1D, 1F, 1G, 1I, and 1K are Northern probed with a long body-labeled RNA probe. For 1B, 1D, 1F, 1G, and 1K, lane one is total RNA from a saturated growth in YPD, lane 2 in total RNA from a mid-log growth in YPD, lane 3 is size standards, and lane 4 indicates the size of selected standards. For 1I, lane 1 is total RNA from a saturated growth in YPD, lane 2 is size standards, and lane 3 indicates the size of selected standards.
**RUF5**

The changes to both the 5’ and 3’ end of RUF5 reflect a large number of new RACE sequencing reads for both ends (15 new ends for the 5’ end, 10 new ends for the 3’ end). In general, both ends are more scattered than in the other RNAs examined. For both the 5’ and 3’ end, there was a major end that corresponded to the majority of the sequences and a minor end that corresponded to a smaller number of sequences. In addition to these, there were a few outliers for each end. The major and minor ends are listed in Table 1.

RUF5 is in a tandem array in the genome that includes CUP1, a copper metallothionein; it overlaps CUP1 in an antisense orientation. Given that the RUF5 locus exists in multiple loci in a tandem repeat, the heterogeneity we observe could possibly result from heterogeneity in the tandemly duplicated loci, but more probably reflects some heterogeneity in RNA transcription and/or processing. Our RACE-PCR sequences are all identical to the published genome sequence, indicating that it is likely that all the tandem loci are identical.

The Northern blot for RUF5 shows a smear that is typical of poly(A)+ RNAs, but could also reflect the heterogeneity we observed in the RACE sequencing reads. This is most easily seen in figure 1I (which was a lower percentage gel run longer than the Northern in 1H). Figure 1I is a blot probed with a 750 nt (212431 to 213180) riboprobe and figure 1H is a blot probed with an end-labeled oligo probe.

**RUF8**

RUF8, which we called a “possible ncRNA,” is in fact a three exon transcript for a protein gene (Paul Cliften, personal communication). As we stated in the paper, we saw protein-coding features in the RNA but they were not statistically significant. Had we predicted and accounted for the splicing, the protein-coding features would have been scored as significant and we would have classified it as a probable mRNA, but we did not consider the possibility of a multiply spliced gene in yeast.

RUF8 has recently been independently identified as a 96aa protein, a component of the SAGA complex and the nuclear pore-associated mRNA export machinery (Rodriguez-Navarro et al., 2004) that has been named SUS1. Our analysis of the gene structure of SUS1 generally agrees with this work. However, we see an approximately 500 nt transcript on Northern while they see an approximately 320 nt transcript (Figure 1 J,K). We performed 3’ RACE with a gene-specific primer in the first exon, which allowed us to see all of the processed forms of SUS1 as well as the true 3’ end of the transcript. Of six recovered sequences, 2 corresponded to the expected form with both introns removed (figure 3B), 2 had only intron 2 removed (figure 3D), one was completely unspliced (figure 3E) and one was an alternatively-spliced transcript (figure 3C). Five of the 6 sequenced products had an approximately 164 nt 3’ UTR (the sequencing read on the sixth stopped before the end of the UTR). The expected spliced form with both introns removed and a 164 nt 3’ UTR gives a size of 455. This size with a poly(A) tail could easily give a 500 nt transcript, in agreement with what we see on the Northern. Although the size of the exon-skipped transcript (Figure 3C, 315 nt) is consistent with the 320 nt transcript reported by Rodriguez-Navarro, they report it as the full three-exon transcript, not two; we have not yet accounted for the difference between these results and the shorter transcript seen by Rodriguez-Navarro et. al. It is also not clear if the alternatively-spliced (exon-skipped) transcript we see is biologically significant.
Conclusion

Both Genbank and the Saccharomyces Genome Database have been updated to reflect these changes. RUF4, RUF6, RUF7, and RUF8 have been withdrawn, and the annotations for RUF1, RUF2, RUF3 and RUF5 have been updated. We regret the errors in our work, and we apologize for any inconvenience that our erroneous conclusions have caused.

Materials and Methods

With the exception of the in vitro transcription of RNA probes, all the methods described above were the same as the original NAR paper. In vitro transcriptions were performed with Ambion’s MAXIscript T7 transcription kit in the following reaction: 1 µg template DNA (PCR product), 2 µL 10X MAXIscript buffer, 1 µL ATP, 1 µL CTP, 1 µL GTP, 0.1 µM UTP, 5 µL 800 Ci/mmol α-32P UTP, 2 µL T7 polymerase, H2O to 20 µL, 37°C, 1 hr.