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Supplemental Data

Prp43 Bound at Different Sites on the Pre-rRNA

Performs Distinct Functions in Ribosome Synthesis

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Figure S1: Prp43 crosslinks to cellular RNA. (A) Cells expressing HTP-tagged Prp43 or Rok1, or no tagged protein as control, were UV irradiated, tagged Prp43 and Rok1 were enriched on IgG sepharose (IgG eluate) and crosslinked RNA was trimmed and radiolabeled (labeled RNA). Proteins were separated by SDS PAGE and visualized by western blot (upper and middle panel) and crosslinked, radiolabelled RNA by autoradiography (lower panel). Crosslinked RNAs were then ligated to linkers, followed by RT-PCR and Solexa deep sequencing (B-E). Crosslinking with cells expressing Prp43-HTP was either performed *in vivo* (B) or *in vitro* (C). Untagged control cells were crosslinked *in vivo* (D). Identified sequences were sorted into categories shown in (E).

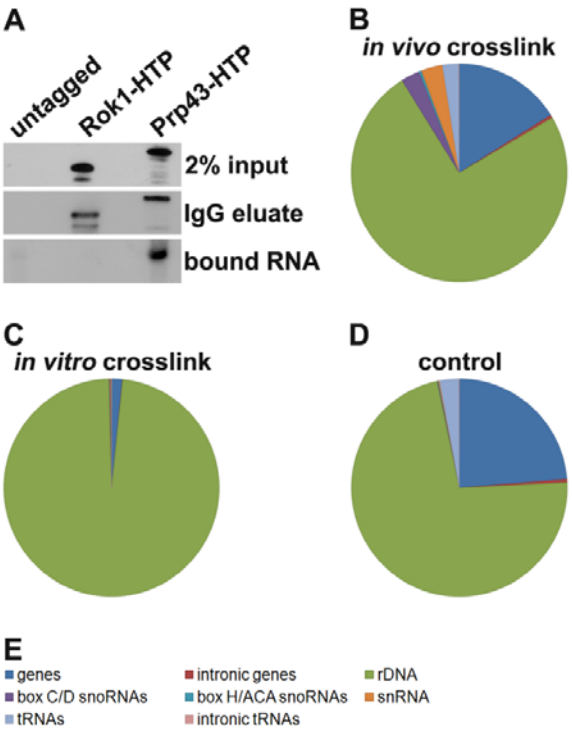


Figure S2: Analysis of snoRNA levels before and after depletion of Prp43.

Prp43 was depleted in the P_{tet}-*prp43* strain as described for Figure 4 and total RNA was isolated from cells followed by separation on a 10% acrylamide/8M urea gel and Northern transfer. snoRNAs were probed as indicated. No significant changes in the cellular levels of these snoRNAs were detected after Prp43 depletion.

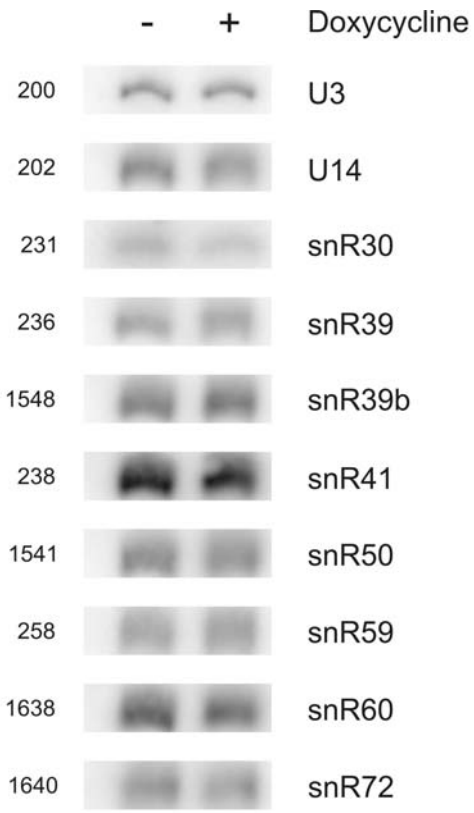


Figure S3: Analysis of Prp43 wild type and mutants for growth complementation.

A P_{tet}-*prp43* strain was transformed with plasmids expressing wild type Prp43 from its own promoter, the motif I mutant Prp43-T123A, motif III mutant Prp43-S247A, or with empty vector. (A, B) Growth was analyzed after spotting 10-fold dilutions of cultures on plates in the absence (permissive conditions; A) or presence (B) of doxycycline to repress chromosomal Prp43 expression. (C) Growth was followed in liquid culture for 24h after addition of doxycycline.

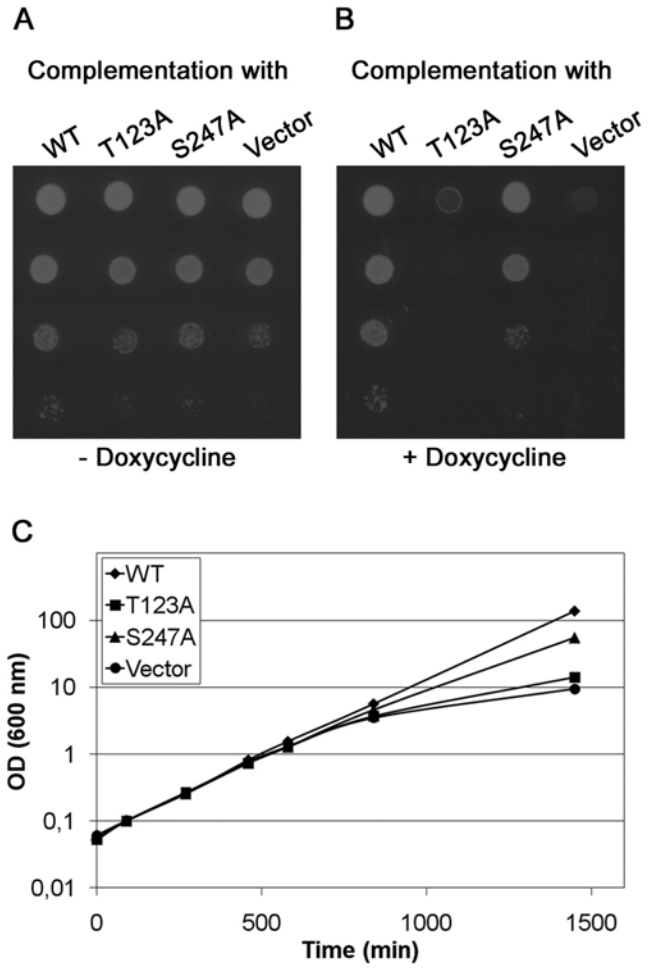
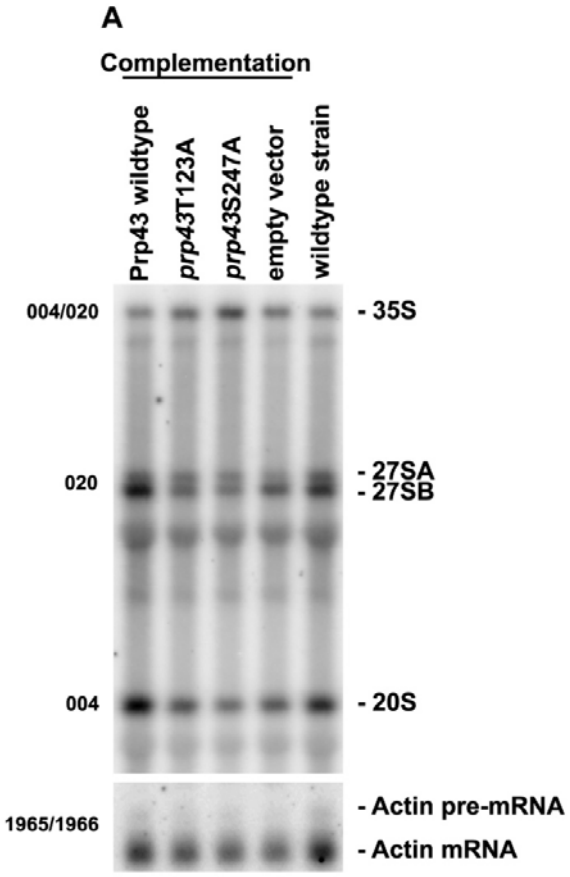


Figure S4: Northern blot analysis of pre-rRNA and actin mRNA levels in Prp43 mutant strains.

Genomic *PRP43* was depleted in cells expressing wildtype or mutant Prp43, or carrying empty vector. After 10 hours depletion cells were harvested, total RNA was isolated, separated on a 1.2% agarose gel followed by Northern transfer. The levels of several pre-ribosomal RNA intermediates and actin (*ACT1*) mRNA were probed as indicated (A). Levels of 27SB, 20S and actin mRNA were quantified (B). Expression of Prp43 mutants resulted in a slightly stronger reduction in pre-rRNA levels that in cells carrying the empty vector, whereas the opposite effect was observed on actin mRNA levels.



B

Quantification

95	50	33	78	84	27SB
128	77	67	89	109	20S
70	56	51	45	71	Actin mRNA

Table S1: Oligonucleotides used in this study.

Sequences of the oligonucleotides used as probes for Northern hybridisation or cloning of the *PRP43* gene including 465 nucleotides upstream and 446 nucleotides downstream of the open reading frame are shown.

No.	Sequence	Use
004	CGGTTTTAATTGTCCTA	pre-rRNA probe
020	TGAGAAGGAAATGACGCT	pre-rRNA probe
200	TTATGGGACTTGTT	U3a/b probe
202	TCACTCAGACATCCTAGG	U14 probe
231	CTAAGTTAAACTCGTCAACG	snR30 probe
236	GGTGATAAGTTACGACAGC	snR39 probe
238	GGGTTGTCGACATGTAGTTA	snR41 probe
258	GGTGATTAAACGACAGCATTGTCAAAGACTAGTCGA	snR59 probe
1396	ATATTACTCGAGATGCCAAGGAAGCGTACTGAGTACAG	FP Prp43 cloning
1397	ATTATTGCGGCCGCTGTAGTTCAAATTAGATAGTGG	RP Prp43 cloning
1541	GCTGCAAATTGCTACCTCTTTCA	snR50 probe
1548	TGCTAGTCACTTTTTGGAATGCC	snR39b probe
1638	CAATCAGTTGAACTATGCATCTTTGG	snR60 probe
1640	GATCAGACTGACGTGCAAATCATT	snR72 probe
1965	CAATAACCAAAGCAGCAAC	actin1 probe
1966	ATACCGGCAGATTCCAAACCC	actin1 probe

Table S2:

To generate the snoRNA hit table, we used a perl script for information from the novoalign output file (www.novocraft.com; version 2.04) to count the number of hits for each gene of interest from the total number of mapped reads.

Prp43 in vivo

----box CD snoRNAs----

snoRNA	hits	perc_hits
snR128	817	0.00084111
snR13	232	0.00023885
snR17a	715	0.0007361
snR17b	369	0.00037989
snR18	37	3.81E-05
snR190	267	0.00027488
snR24	406	0.00041798
snR38	62	6.38E-05
snR39	116	0.00011942
snR39B	136	0.00014001
snR4	219	0.00022546
snR40	664	0.0006836
snR41	45	4.63E-05
snR45	271	0.000279
snR47	403	0.00041489
snR48	0	0
snR50	83	8.54E-05
snR51	15582	0.01604186
snR52	663	0.00068257
snR53	13	1.34E-05
snR54	207	0.00021311
snR55	176	0.00018119
snR56	113	0.00011633
snR57	372	0.00038298
snR58	40	4.12E-05
snR59	250	0.00025738
snR60	1419	0.00146088
snR61	556	0.00057241
snR62	1	1.03E-06
snR63	282	0.00029032
snR64	383	0.0003943
snR65	0	0
snR66	18	1.85E-05
snR67	314	0.00032327
snR68	89	9.16E-05
snR69	155	0.00015957
snR70	130	0.00013384
snR71	143	0.00014722
snR72	2231	0.00229684
snR73	452	0.00046534
snR74	586	0.00060329
snR75	0	0
snR76	107	0.00011016
snR77	127	0.00013075
snR78	0	0
snR79	718	0.00073919
snR87	0	0

Control

----box CD snoRNAs----

snoRNA	hits	perc_hits
snR128	224	0.00047194
snR13	160	0.0003371
snR17a	0	0
snR17b	0	0
snR18	0	0
snR190	0	0
snR24	3	6.32E-06
snR38	0	0
snR39	0	0
snR39B	0	0
snR4	0	0
snR40	0	0
snR41	0	0
snR45	0	0
snR47	0	0
snR48	0	0
snR50	0	0
snR51	0	0
snR52	0	0
snR53	0	0
snR54	0	0
snR55	0	0
snR56	0	0
snR57	0	0
snR58	67	0.00014116
snR59	398	0.00083853
snR60	31	6.53E-05
snR61	0	0
snR62	0	0
snR63	0	0
snR64	0	0
snR65	0	0
snR66	0	0
snR67	0	0
snR68	0	0
snR69	0	0
snR70	0	0
snR71	0	0
snR72	0	0
snR73	0	0
snR74	0	0
snR75	0	0
snR76	0	0
snR77	0	0
snR78	0	0
snR79	0	0
snR87	0	0

Prp43 in vitro

----box CD snoRNAs----

snoRNA	hits	perc_hits
snR128	155	9.41E-05
snR13	10	6.07E-06
snR17a	254	0.00015415
snR17b	197	0.00011955
snR18	0	0
snR190	0	0
snR24	28	1.70E-05
snR38	0	0
snR39	0	0
snR39B	0	0
snR4	67	4.07E-05
snR40	47	2.85E-05
snR41	5	3.03E-06
snR45	9	5.46E-06
snR47	7	4.25E-06
snR48	0	0
snR50	0	0
snR51	90	5.46E-05
snR52	32	1.94E-05
snR53	19	1.15E-05
snR54	0	0
snR55	0	0
snR56	0	0
snR57	19	1.15E-05
snR58	15	9.10E-06
snR59	10	6.07E-06
snR60	18	1.09E-05
snR61	115	6.98E-05
snR62	3	1.82E-06
snR63	0	0
snR64	7	4.25E-06
snR65	0	0
snR66	0	0
snR67	36	2.18E-05
snR68	0	0
snR69	0	0
snR70	0	0
snR71	57	3.46E-05
snR72	198	0.00012016
snR73	73	4.43E-05
snR74	25	1.52E-05
snR75	0	0
snR76	232	0.0001408
snR77	0	0
snR78	0	0
snR79	14	8.50E-06
snR87	0	0

----box HACA snoRNAs----

snoRNA	hits	perc_hits
snR10	554	0.00057035
snR11	298	0.00030679
snR161	32	3.29E-05
snR189	10	1.03E-05
snR191	99	0.00010192
snR3	68	7.00E-05
snR30	990	0.00101922
snR31	135	0.00013898
snR33	494	0.00050858
snR34	0	0
snR35	196	0.00020178
snR36	11	1.13E-05
snR37	360	0.00037062
snR42	96	9.88E-05
snR43	2	2.06E-06
snR44	16	1.65E-05
snR46	0	0
snR49	3	3.09E-06
snR5	105	0.0001081
snR8	180	0.00018531
snR80	0	0
snR81	1	1.03E-06
snR82	234	0.00024091
snR83	453	0.00046637
snR84	40	4.12E-05
snR85	139	0.0001431
snR86	70	7.21E-05
snR9	26	2.68E-05

----box HACA snoRNAs----

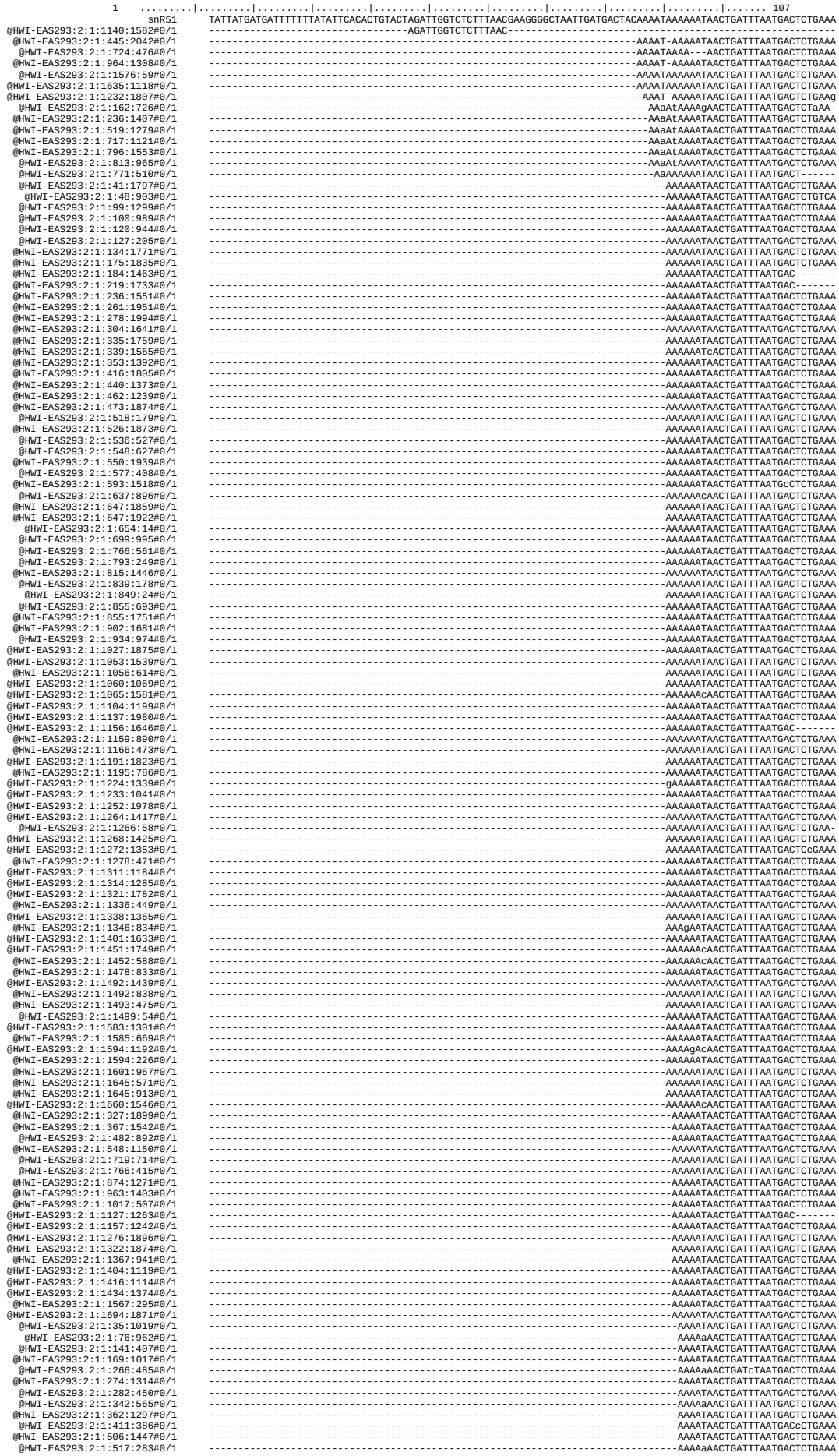
snoRNA	hits	perc_hits
snR10	0	0
snR11	0	0
snR161	0	0
snR189	0	0
snR191	0	0
snR3	4	8.43E-06
snR30	498	0.00104922
snR31	0	0
snR33	22	4.64E-05
snR34	1	2.11E-06
snR35	0	0
snR36	0	0
snR37	0	0
snR42	0	0
snR43	0	0
snR44	0	0
snR46	0	0
snR49	0	0
snR5	0	0
snR8	1	2.11E-06
snR80	0	0
snR81	0	0
snR82	1	2.11E-06
snR83	86	0.00018119
snR84	0	0
snR85	0	0
snR86	0	0
snR9	0	0

----box HACA snoRNAs----

snoRNA	hits	perc_hits
snR10	245	0.00014868
snR11	0	0
snR161	0	0
snR189	0	0
snR191	0	0
snR3	1	6.07E-07
snR30	118	7.16E-05
snR31	0	0
snR33	0	0
snR34	0	0
snR35	91	5.52E-05
snR36	0	0
snR37	13	7.89E-06
snR42	0	0
snR43	0	0
snR44	0	0
snR46	0	0
snR49	0	0
snR5	23	1.40E-05
snR8	0	0
snR80	15	9.10E-06
snR81	0	0
snR82	0	0
snR83	26	1.58E-05
snR84	0	0
snR85	0	0
snR86	32	1.94E-05
snR9	38	2.31E-05

Bohnsack *et al*., legend to supplemental sequence alignments

Alignments were generated using a perl script that uses the information from the novoalign output file (www.novocraft.com; version 2.04) to mark the location of the deletions and substitutions in the reads and to align them to the reference sequence. Each alignment file contains a maximum of 200 nucleotide sequences which the program randomly selected from the novoalign output file. Substitutions in sequences are indicated in lower case, whereas deletions are indicated as gaps. Alignments of snoRNA hits for snR51, snR60 and snR72 and alignments with sequences around the 5 major peaks on the rDNA found after *in vivo* or *in vitro* cross-linking are shown.


snR51
1 107
snR51 TATTATGATGATTTTTTTATATTCACACTGTACTAGATTGGTCTCTTTAACGAAGGGGCTAATTGATGACTACAAAATAAAAAATAACTGATTTAATGACTCTGAAA
@HWI-EAS293:2:1:1140:1582#0/1 AGATTGGTCTCTTTAAC
@HWI-EAS293:2:1:445:2042#0/1 AAAAT-AAAAATAACTGATTTAATGACTCTGAAA
@HWI-EAS293:2:1:724:476#0/1 AAAATAAAA---AACTGATTTAATGACTCTGAAA
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@HWI-EAS293:2:1:1053:1539#0/1 AAAAAATAACTGATTTAATGACTCTGAAA
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@HWI-EAS293:2:1:1060:1069#0/1 AAAAAATAACTGATTTAATGACTCTGAAA
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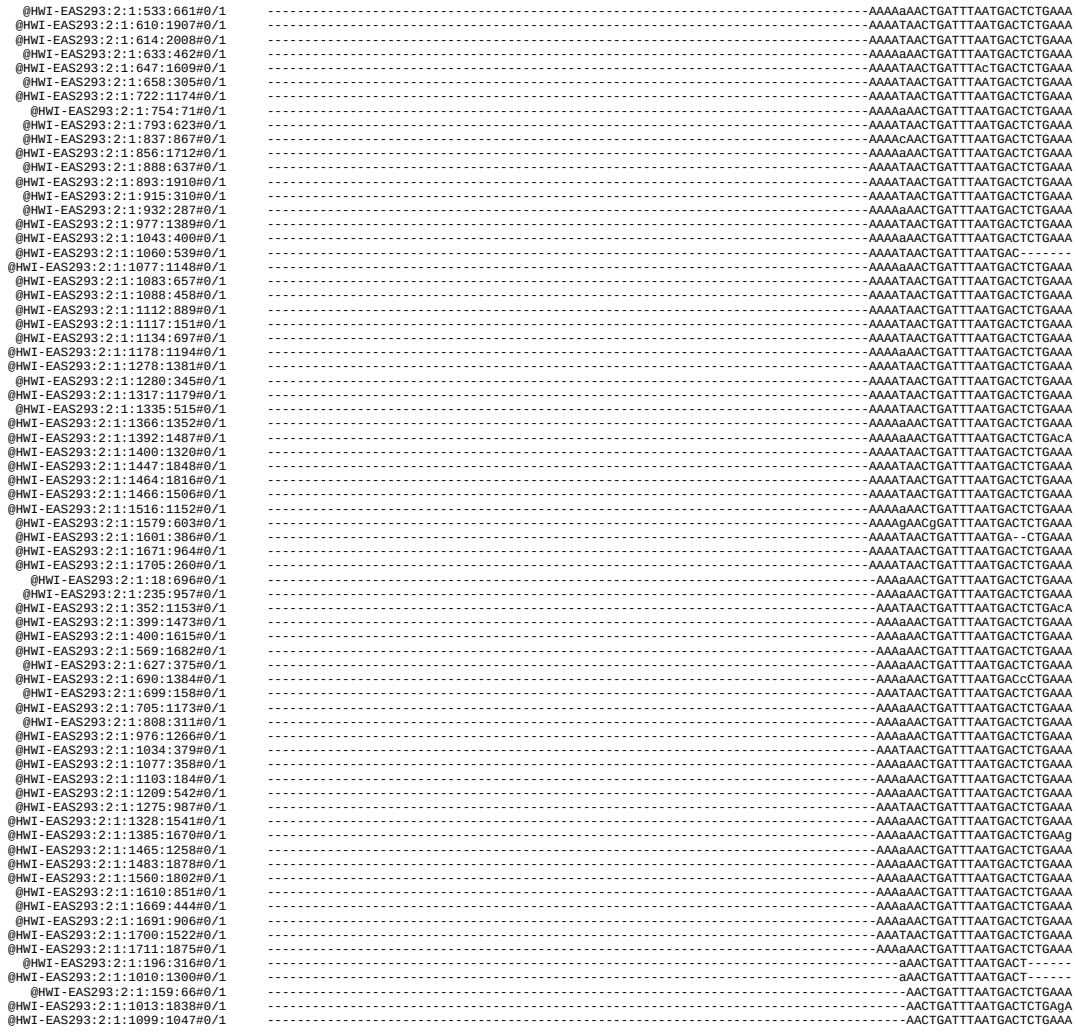
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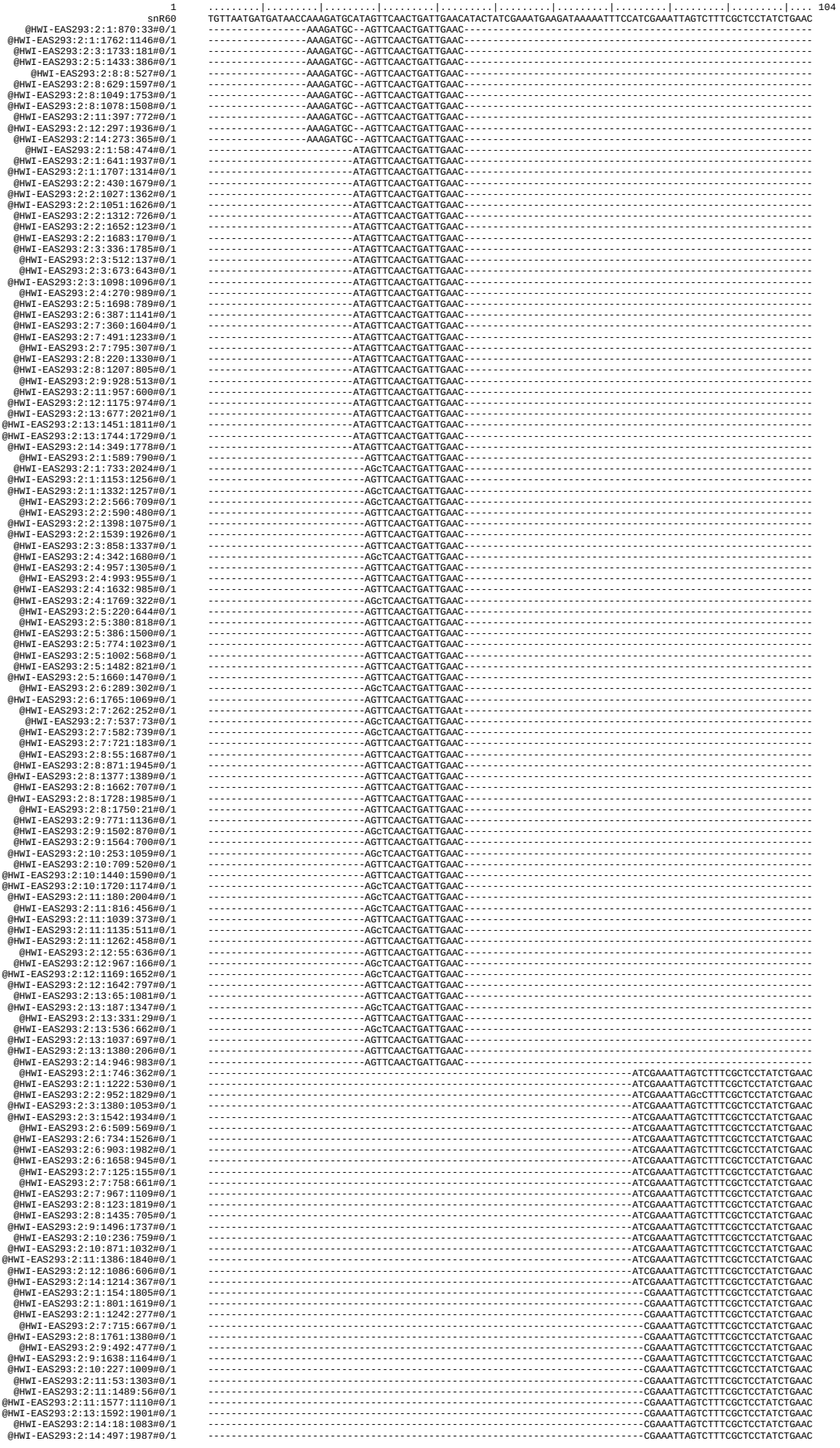
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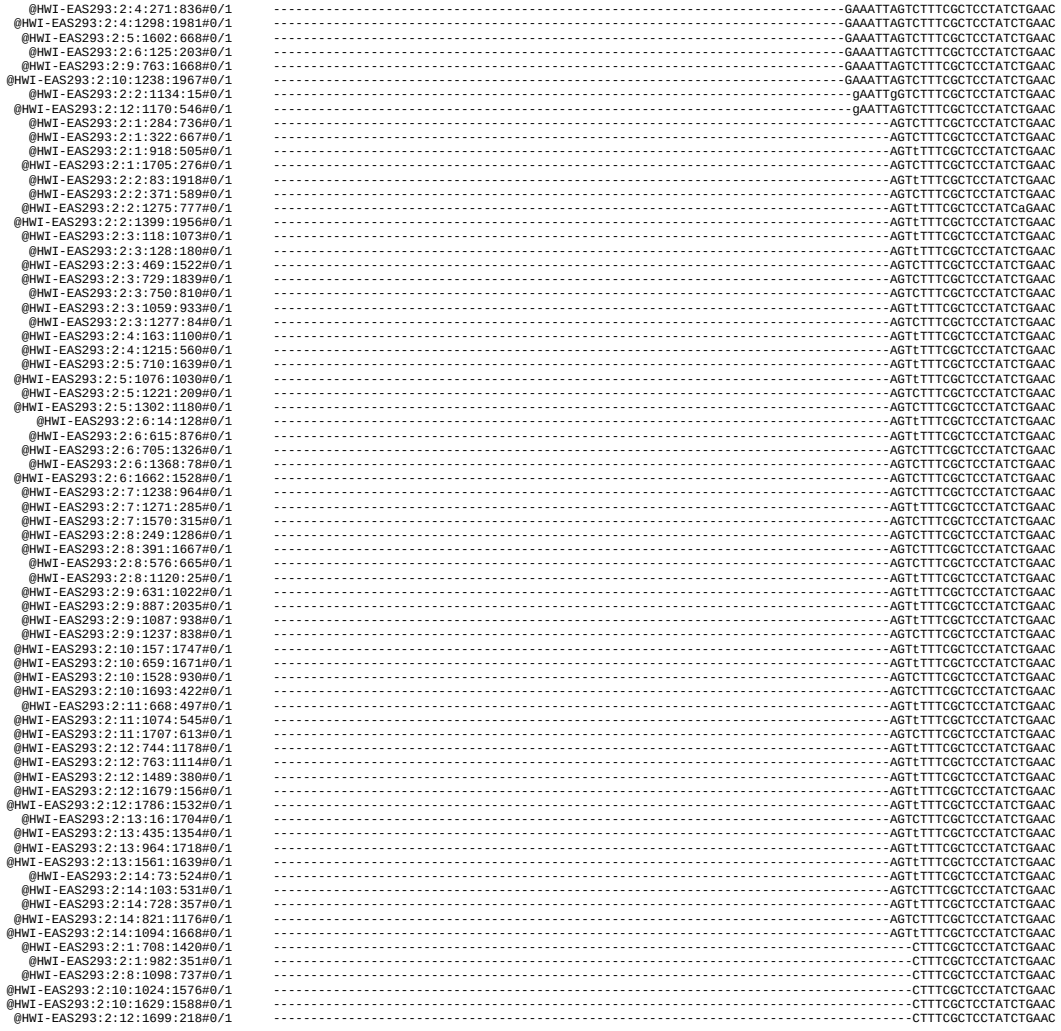
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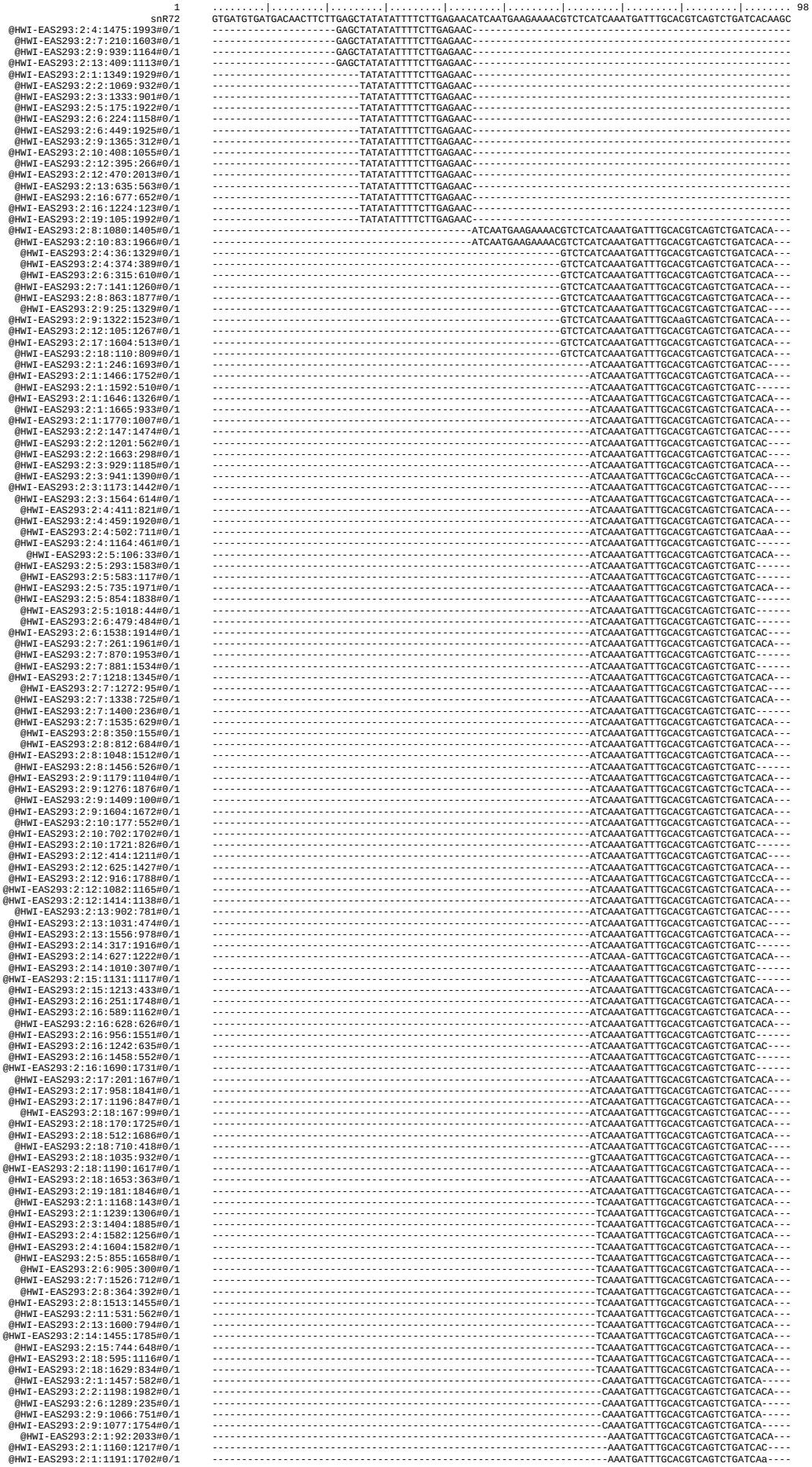
snR72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98

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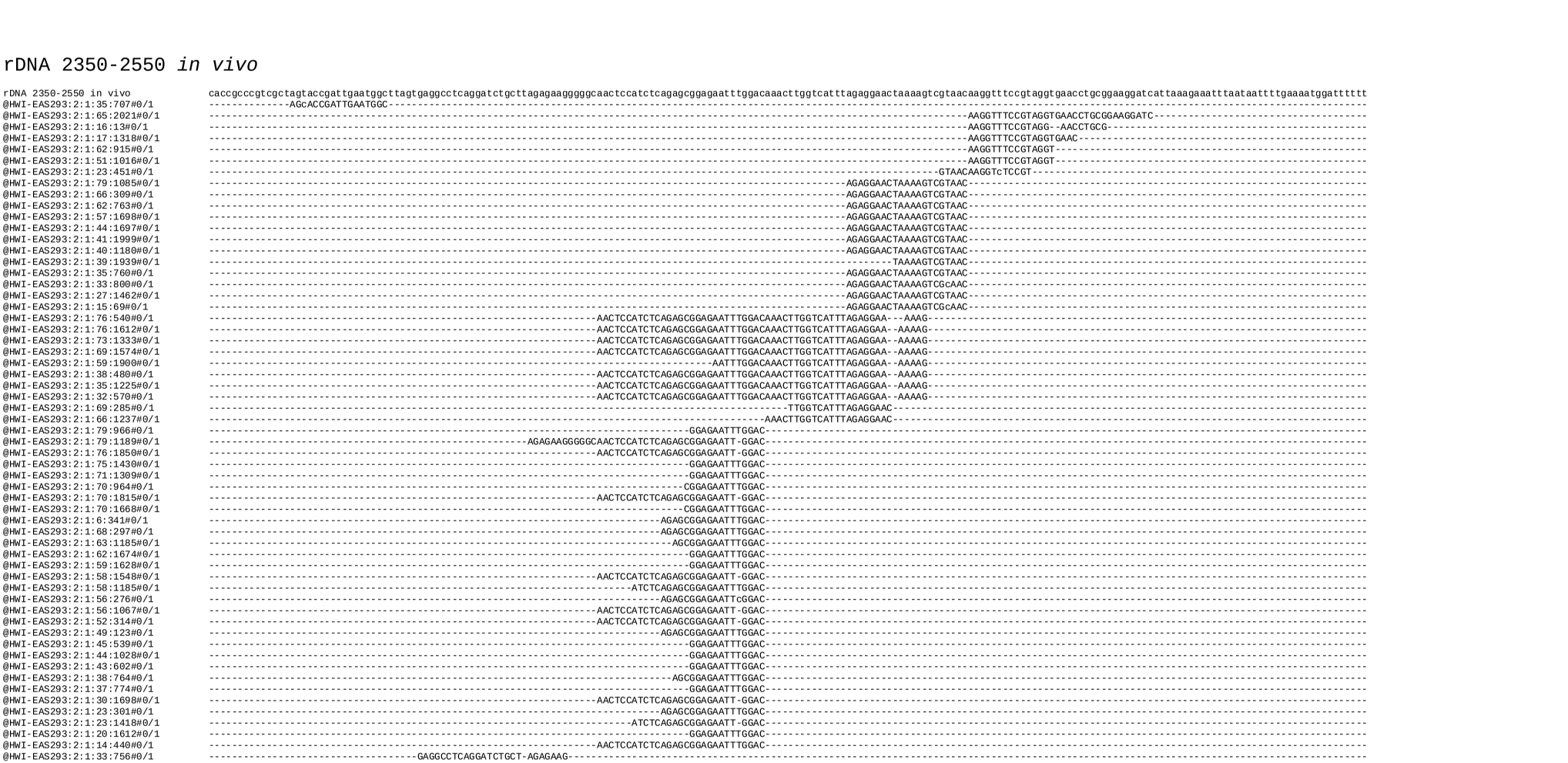

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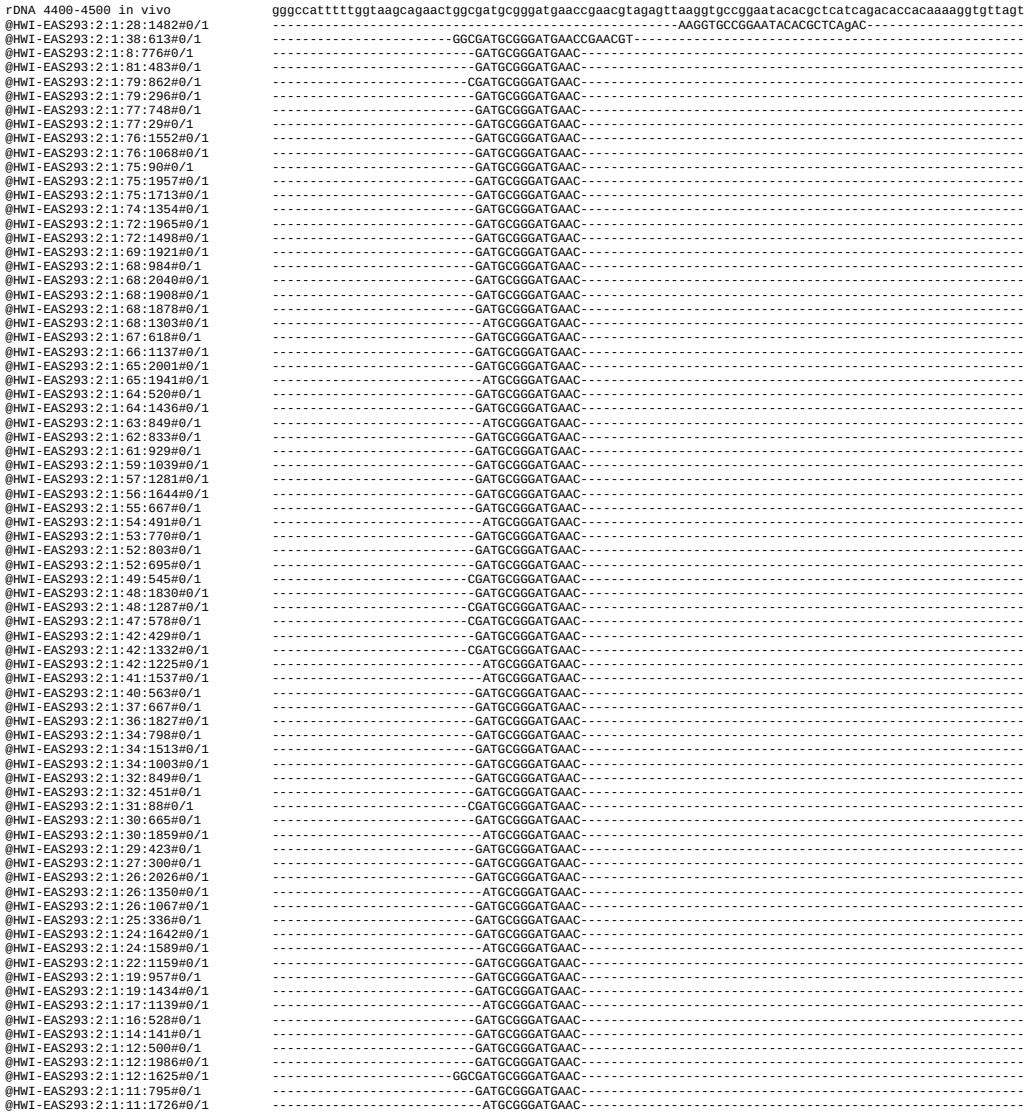

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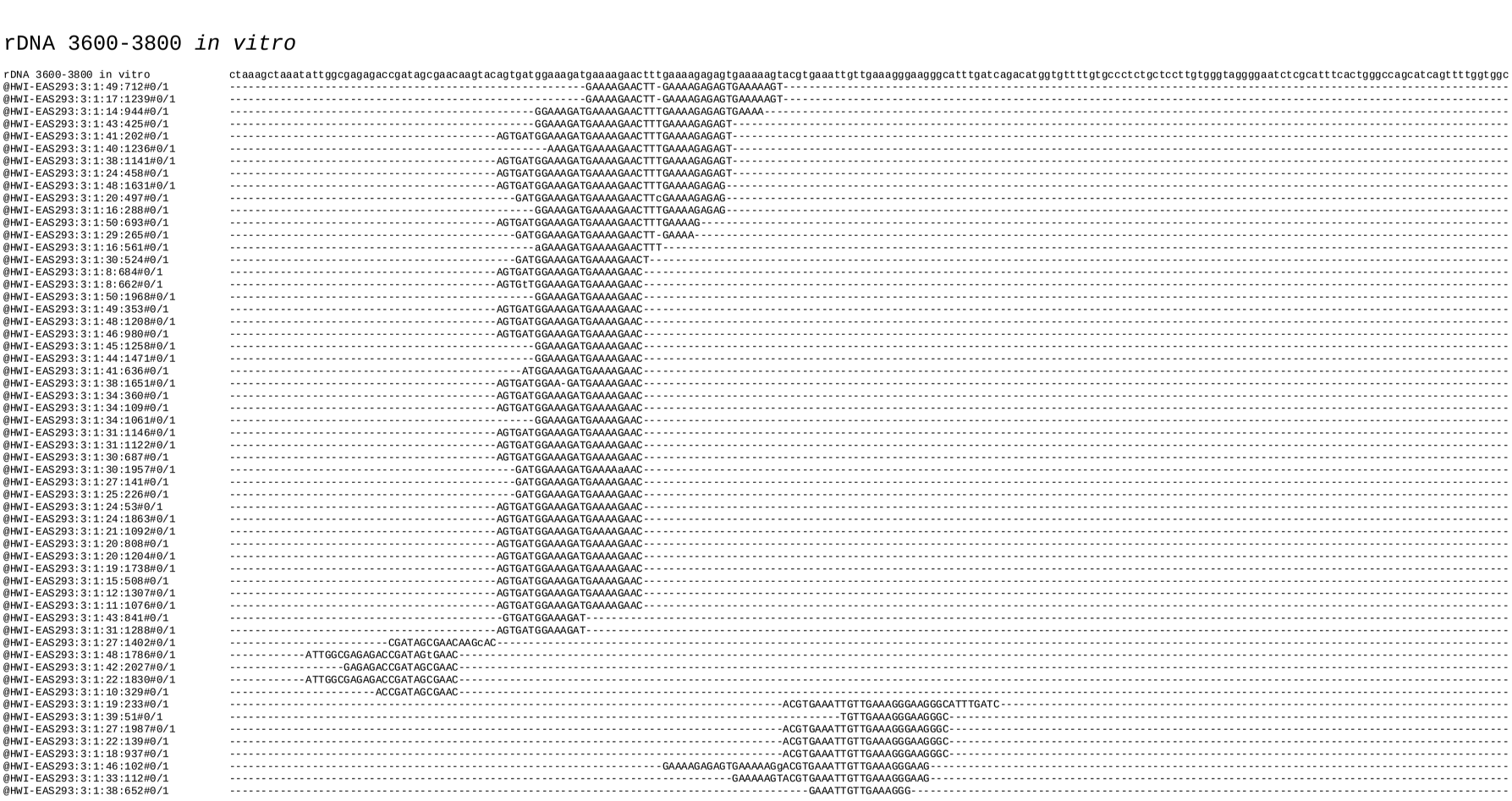

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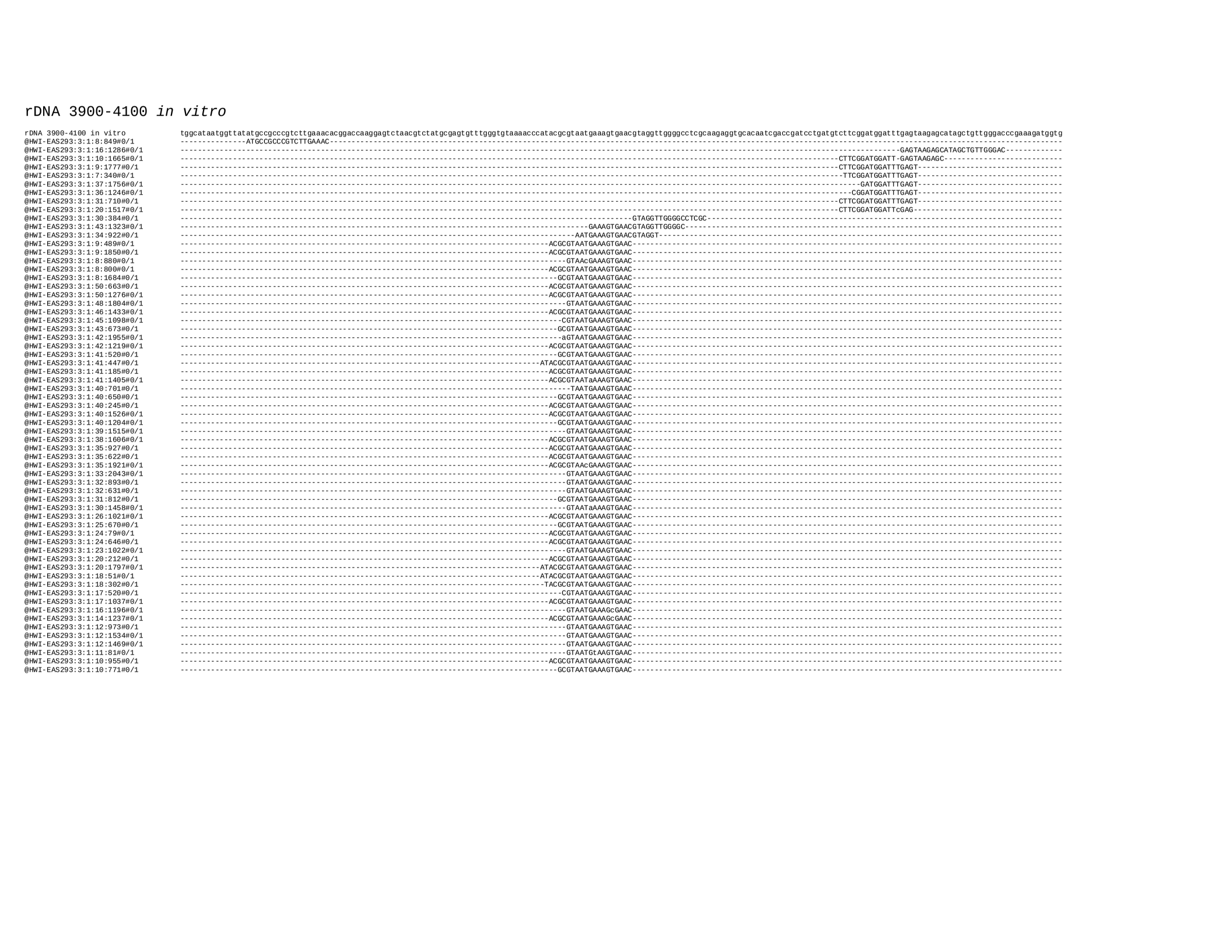

rDNA 5900-6000 *in vitro*