Novel functional microRNAs from virus-free and infected *Vitis vinifera* plants under water stress.

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Supplementary Methods

5'-RACE

The degradation fragments resulting from miRNA cleavage were analysed by 5'-RACE. The RNA from free and GRSPaV-infected 'Bosco' leaves was extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA), and was treated with DNase I (Life Technologies, Carlsbad, CA, USA), incubated with a RNA adaptor (Table S1) and 5U of T4 RNA ligase (Life Technologies) following the manufacturer's instructions. The RNA was reverse-transcribed using random nonamers (Sigma-Aldrich) and M-MLV reverse transcriptase (Life Technologies). First-round and nested PCR analyses were performed using a universal forward primer and gene-specific reverse primers (Supplementary Table S2). The nested products were gel-purified, cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA), and ten independent clones from each product were sequenced using a Big-Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Life Technologies), and analysed using a 3130 Genetic Analyser capillary sequencer (Applied Biosystems, Life Technologies).

qRT-PCR analysis

The quantification of miRNA expression by qRT-PCR was carried out following the protocol of Shi and Chiang (2005) with some modifications. Two μg of RNA extracted from GRSPaV-free or infected leaves collected under WW, WS and SWS conditions were treated with DNaseI (Life Technologies) and polyadenylated using the Poly(A) Tailing Kit (Life Technologies) following the manufacturer's instructions. The RNA was precipitated by ethanol and reverse-transcribed with M-MLV reverse transcriptase (Life Technologies) and 0.5 μg poly(T) adapter (Supplementary Table S2).

For the amplification of all miRNAs and housekeeping genes, a universal 3′-adapter reverse primer was used (Supplementary Table S2) and the forward primer was designed based on the specific miRNA sequence. For some primers containing high GC content at the 3′ end, one or two A residues were added to the 3′-end to facilitate binding to the target site (Supplementary Table S2).

The relative expression was calculated based on the comparative C_t ($2^{-\Delta\Delta Ct}$) method as described by Livak and Schmittgen (2001). The PCR mix (10 µL) contained 5 µL PowerSYBR Green master mix (Applied Biosystems, Life Technologies), 0.25 µM of each primer and 1 µL of cDNA diluted 1:100 or 1:250, based on initial miRNA concentrations. Cycling conditions for all primer pairs consisted of initial denaturation at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s, 58 °C for 20 s and 60 °C for 40 s. The 5.8S rRNA and U6, which have been extensively used with the grapevine and other species as reference in qRT-PCR and northern hybridisations for sRNA analysis, were used as housekeeping genes. The geometric mean of their expression ratios was used as the normalisation factor in all samples. Accumulation of miRNAs was expressed as the mean and standard deviation calculated for three biological replicate.

Ecophysiological measurements

Transpiration rates (E) and stomatal conductance (g_s) were measured by two approaches. Firstly, we estimated E and g_s via pot weight. Daily E was calculated as the difference between two consecutive weights, and the daily grams of water loss per plant were converted into mmol H_2O m^{-2} s^{-1}. The g_s was then calculated by dividing E by the environmental vapour-pressure deficit on the basis of the air temperature and relative humidity that was recorded every day during the experiment (Figure S2A). Secondly, leaf gas-exchange parameters (P_n, E, g_s, c_i) were measured by the GFS-3000 portable gas-exchange fluorescence system (Walz Heinz GmbH, Effeltrich, Germany) on two different mature leaves per plant, on each experimental day (setting: cuvette temperature 27 °C, [CO_2], photosynthetic photon flux density (PPFD) and Rh were those of the greenhouse environmental conditions).

The Ψsoil was calculated following the equation obtained by pressure-plate analysis (Richards and Ogata, 1961): $\Psi soil = 3e^{-10.9\theta}$, where θ represents the soil volumetric water content, calculated as described by Hochberg *et al*. (2013). The bulk density (ρ) of the peat substrate used was 0.383 kg L^{-1}.The weight of the sealed pots was measured at the end of each day (6 p.m.) using an electronic balance.

The enumeration of stomata and cells was determined on two leaves per six plants for infected and GRSPaV-free plants. Two leaf imprints were collected in the WW condition and three counts for each imprint were performed using an optical microscope with a 10-XT objective (Nikon Eclipse 55i, Tokyo, Japan).

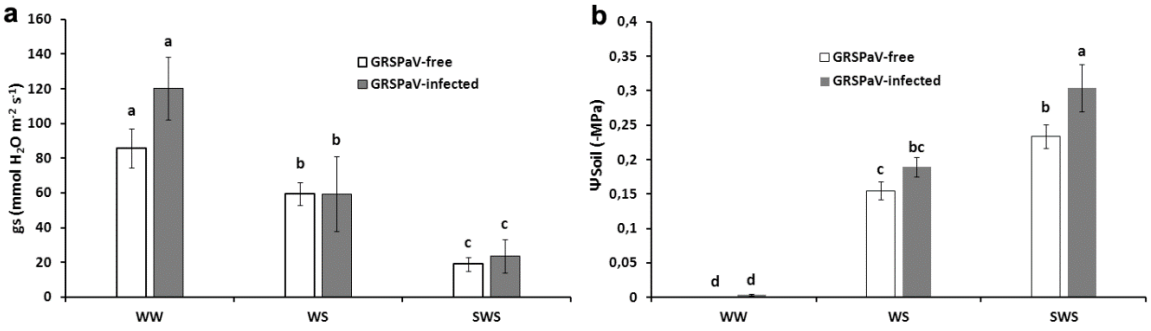
At the end of the experiments, dry weight of the root was quantified by eradicating, washing and drying the roots in oven at 70°C until reaching of constant root weigh. Roots of minor orders were separated manually from main roots after drying.

Supplementary References

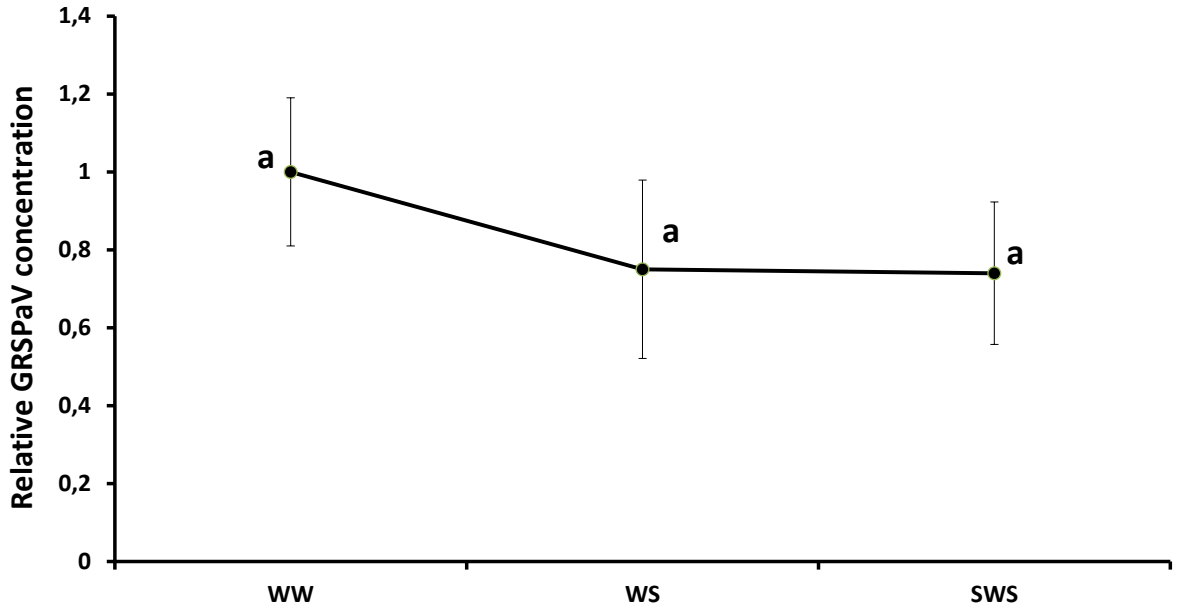
Livak, K.J. & Schmittgen, T,D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25,** 402- 408 (2001).

Hochberg, U., Degu, A., Fait, A. & Rachmilevitch S. Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. *Physiol*. *Plantarum* **147,** 443–452 (2013).

Supplementary Figures



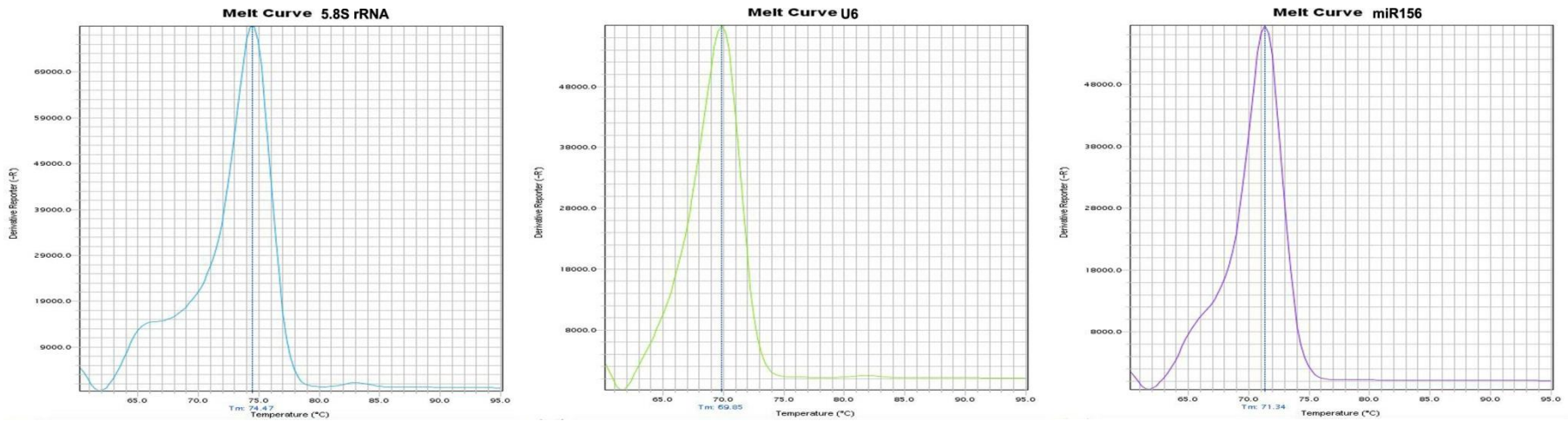
Supplementary Figure S1. a) stomatal conductance (g_s) measured and b) relative water stem potential (Ψstem) during the sampling time for small RNAs analysis. Bars represent the mean ± standard error (n=6). Different letters indicate significant difference among the means ($p \leq 0.05$). WW, well watered condition; WS, water stress condition; SWS severe water stress condition.

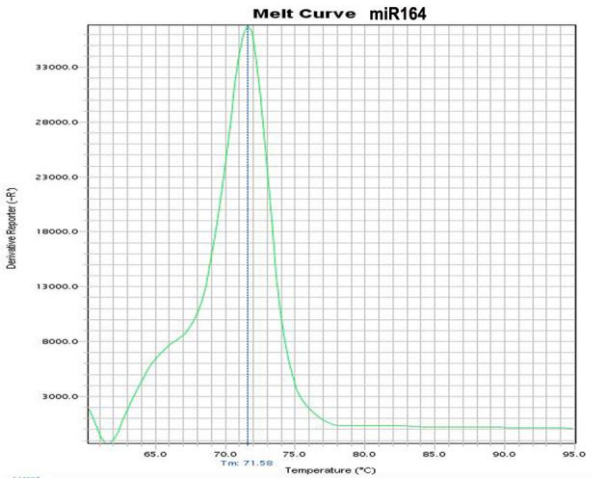


Supplementary Figure S2.Quantification of *Grapevine rupestris stem pitting-associated virus* (GRSPaV) RNA in 'Bosco' leaf as determined by qRT PCR. Samples were collected under well watered (WW), water stress (WS) and severe water stress (SWS) conditions. qRT-PCR signals were normalised to actin and ubiquitin levels. Data are presented as mean ± standard deviation of six plants and three technical replicates; different letters denote significant differences at $p \leq 0.05$.

Supplementary Fig. S3: Validation of qRT-PCR for quantitation of microRNAs (miRNAs). a) dissociation curves of qRT-PCR amplification and b) qRT-PCR products resolved on ethidium bromide-stained 5% agarose gel of all miRNAs and novel miRNA candidates analysed.

a

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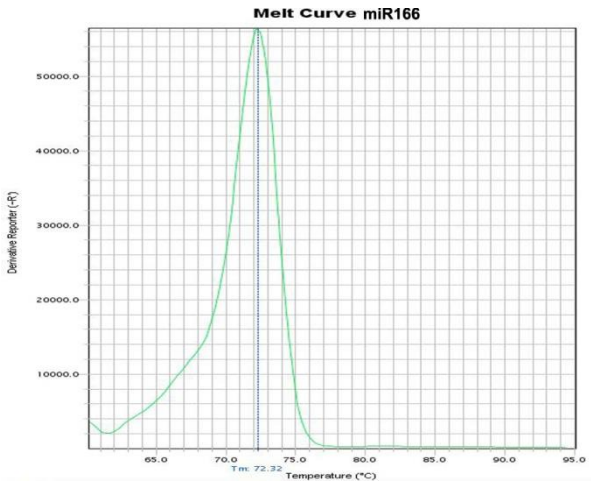
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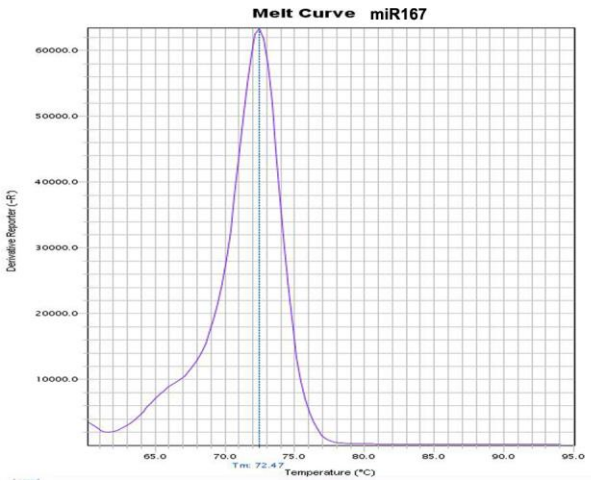
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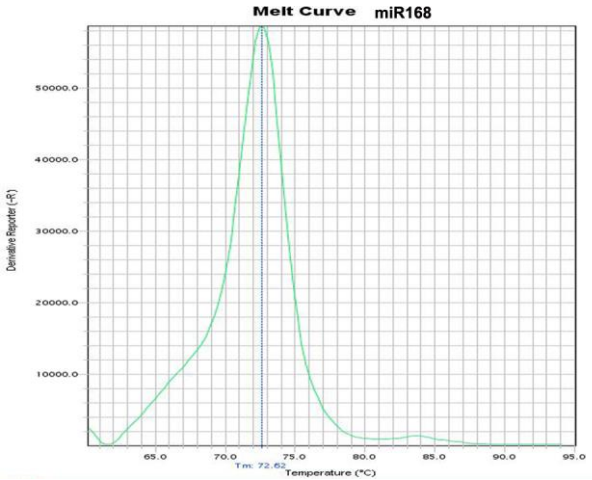
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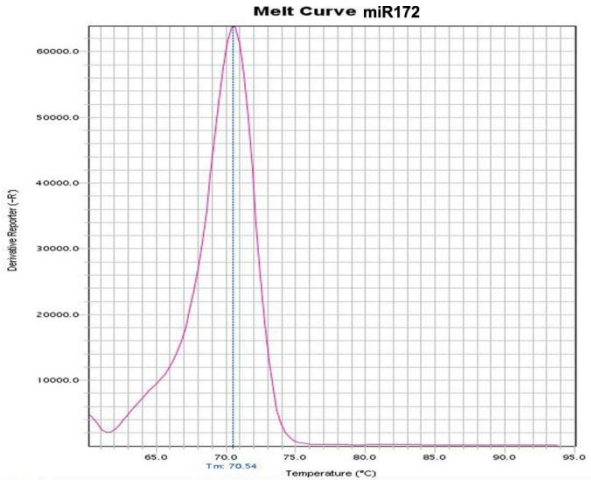
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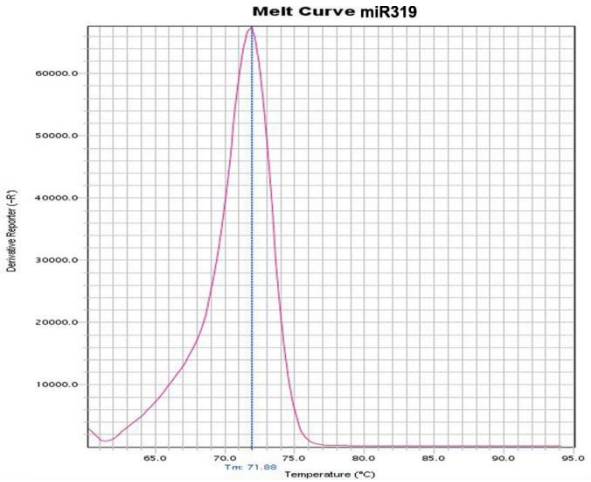
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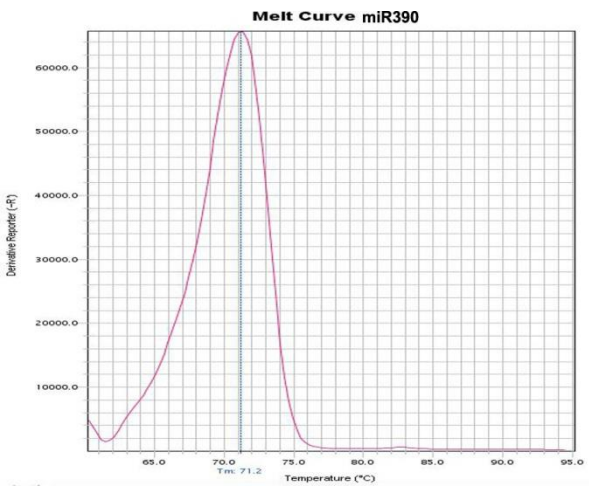
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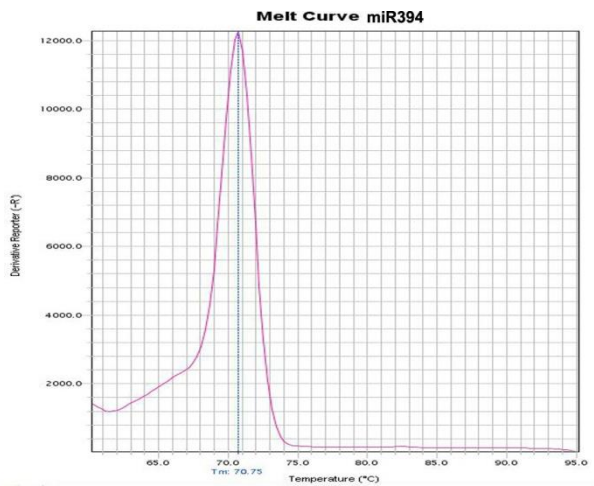
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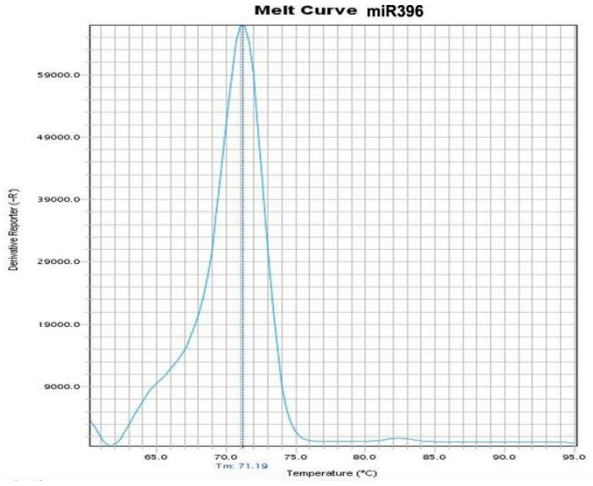
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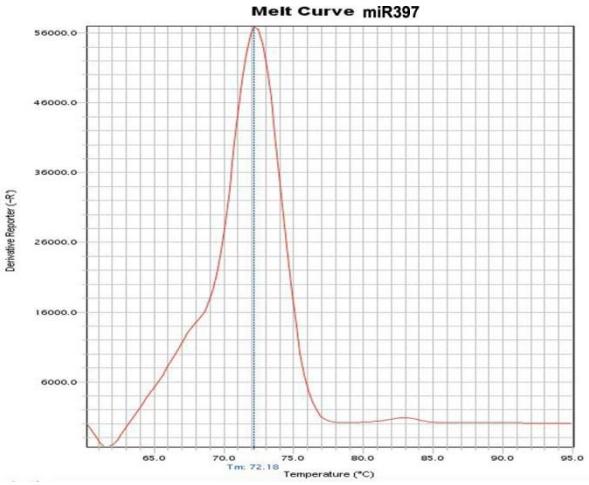
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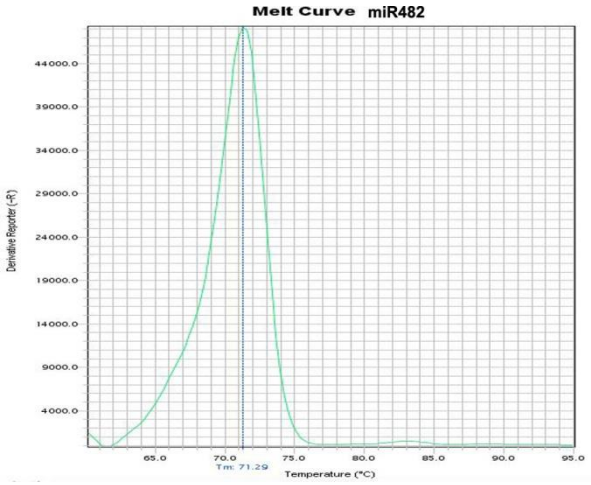
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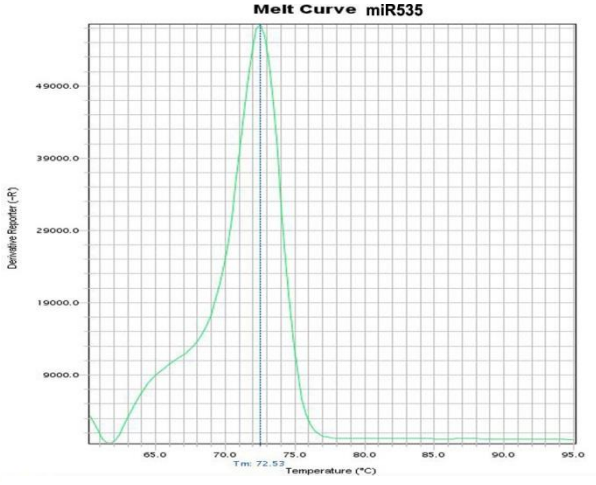
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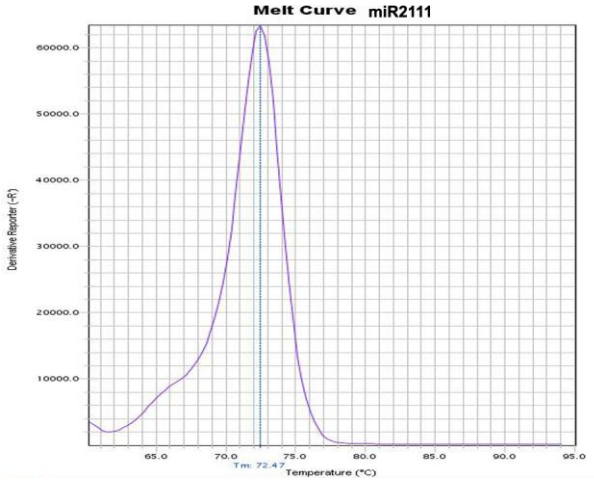
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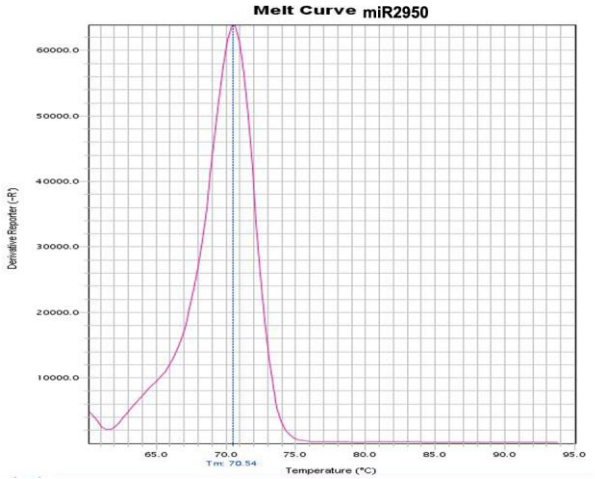
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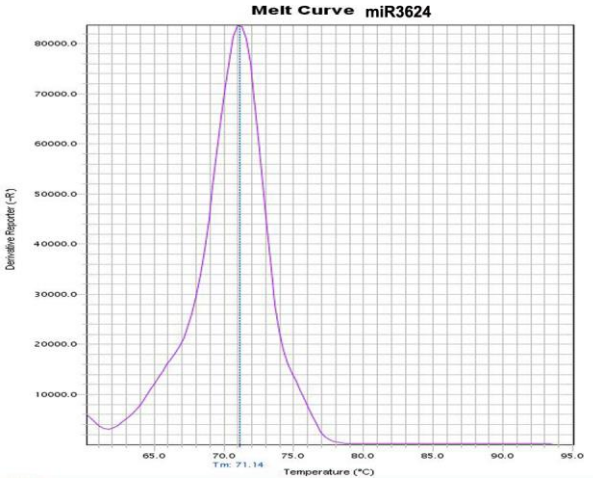
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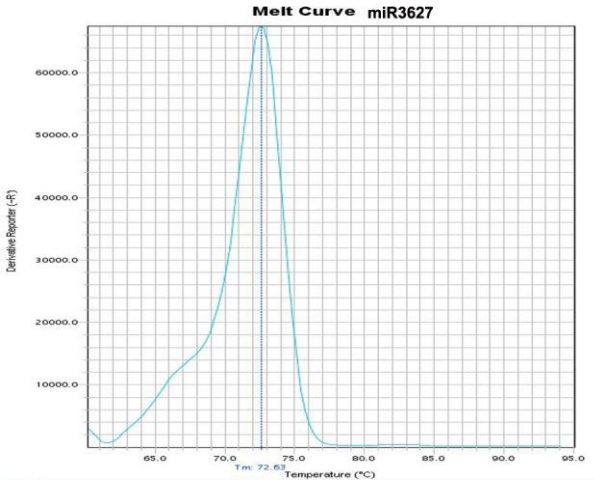
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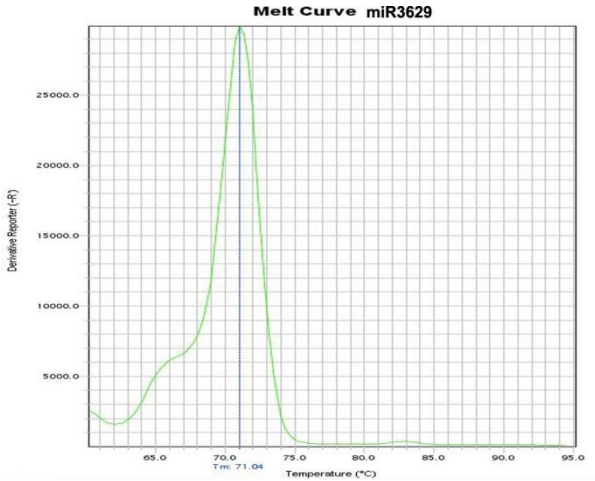
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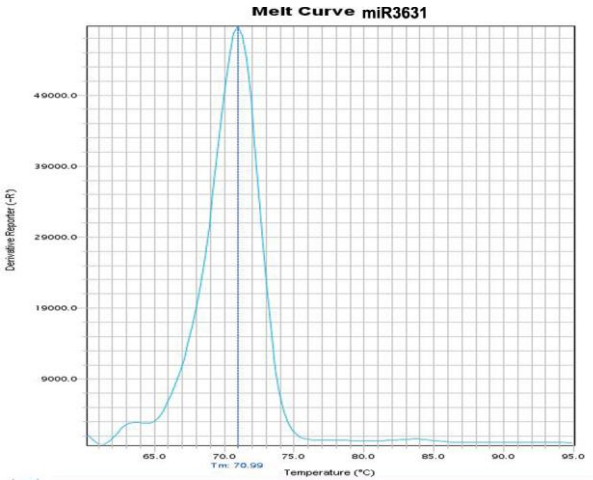
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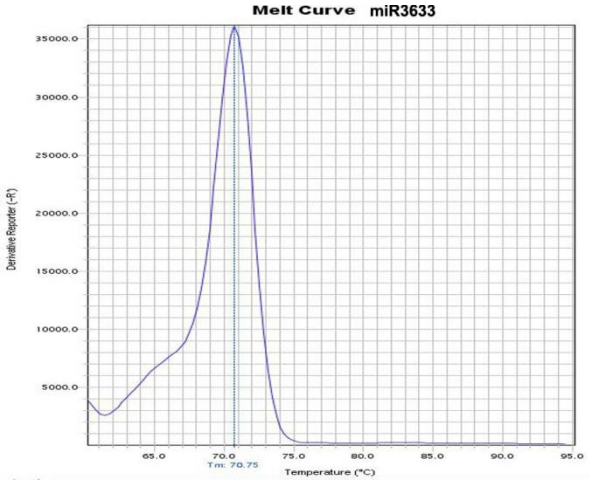
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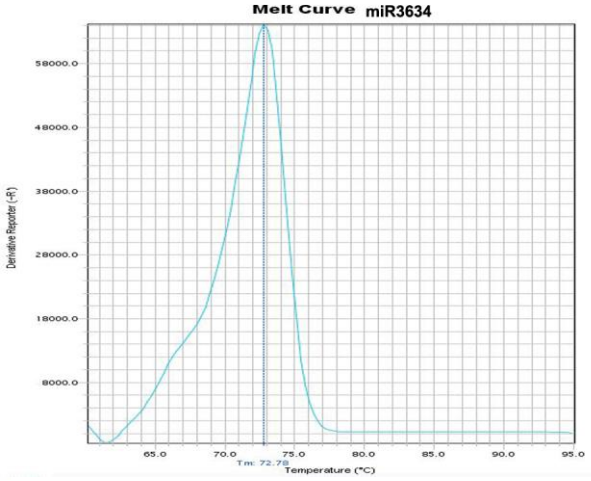
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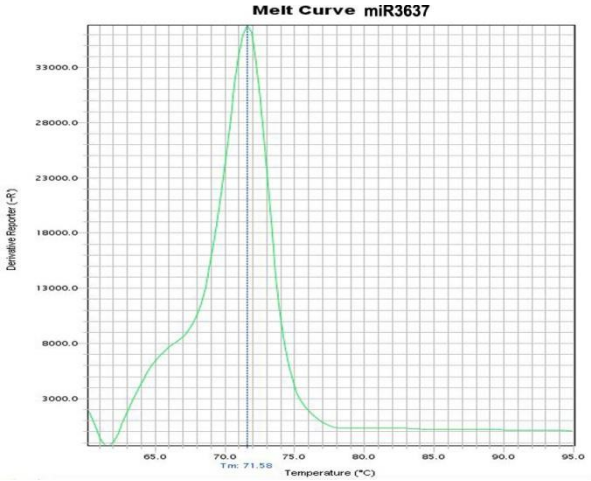
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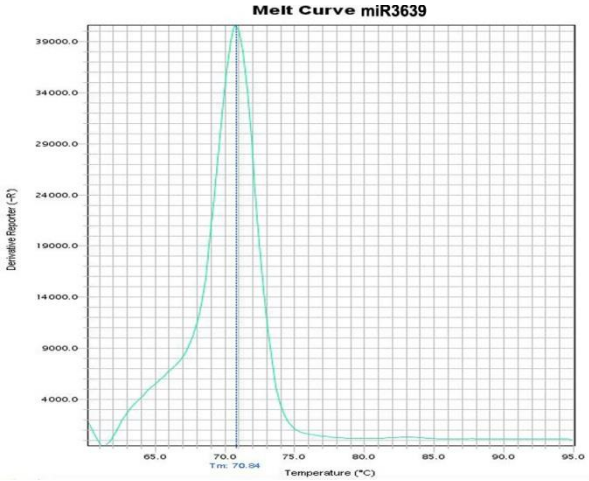
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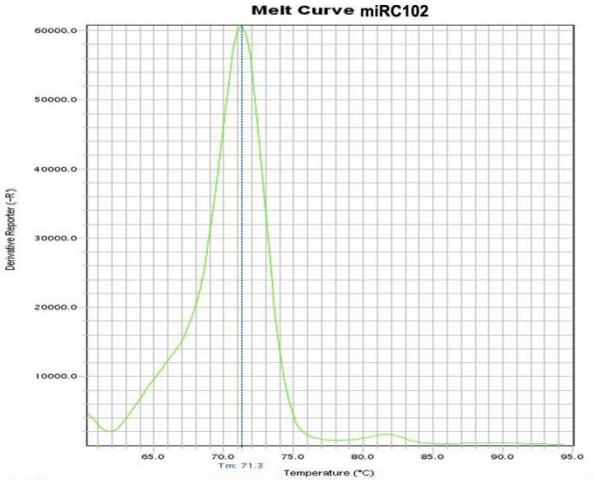
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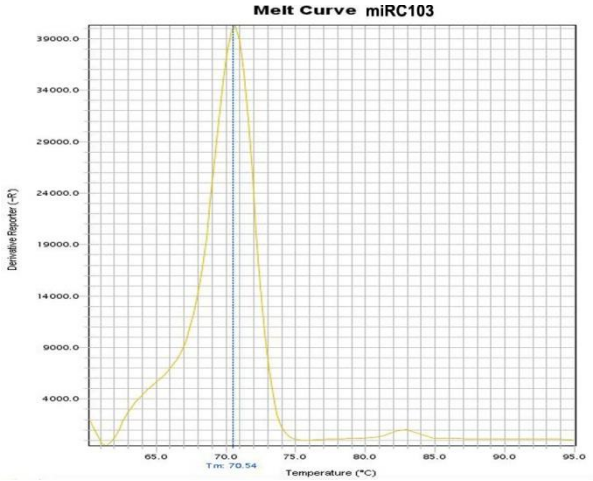
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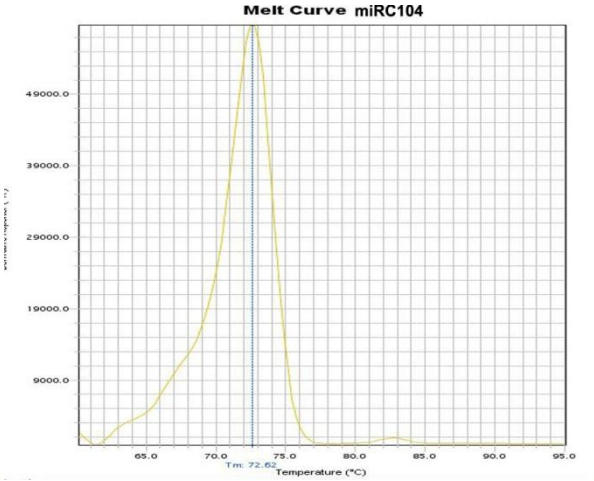
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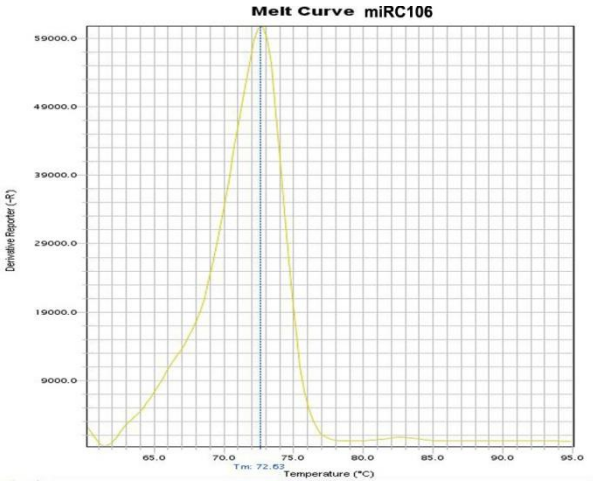
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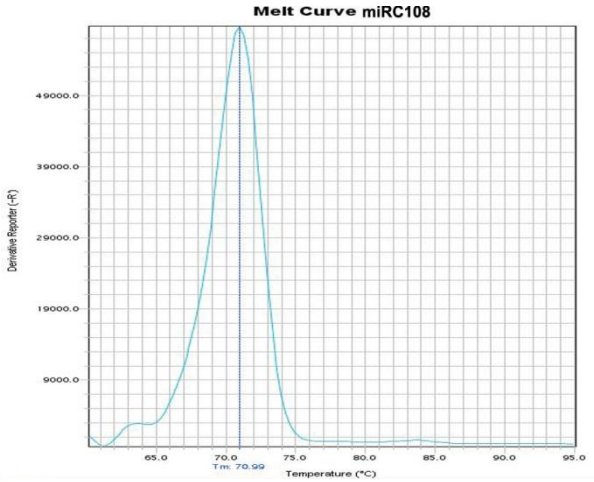
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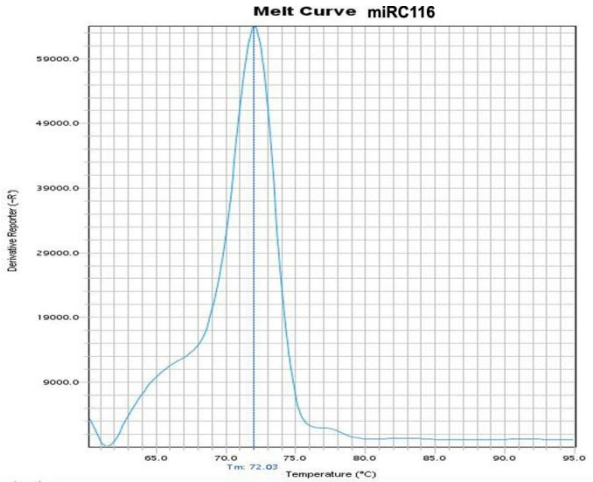
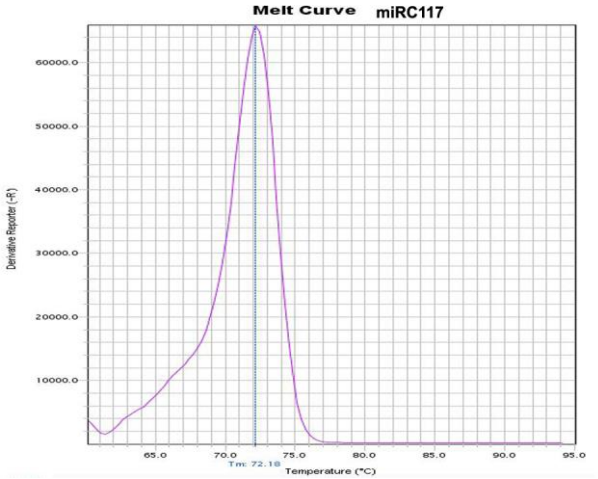
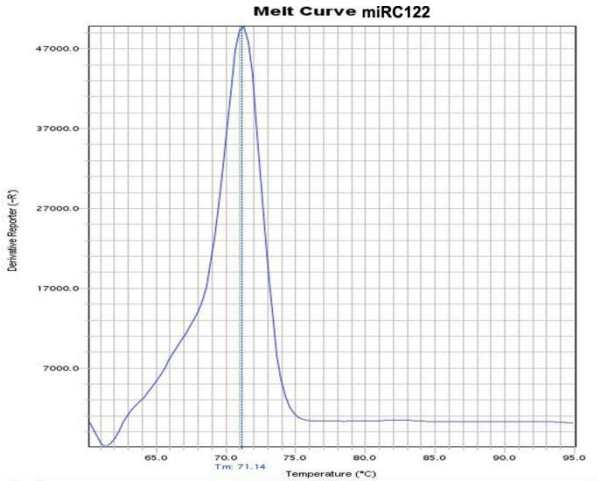
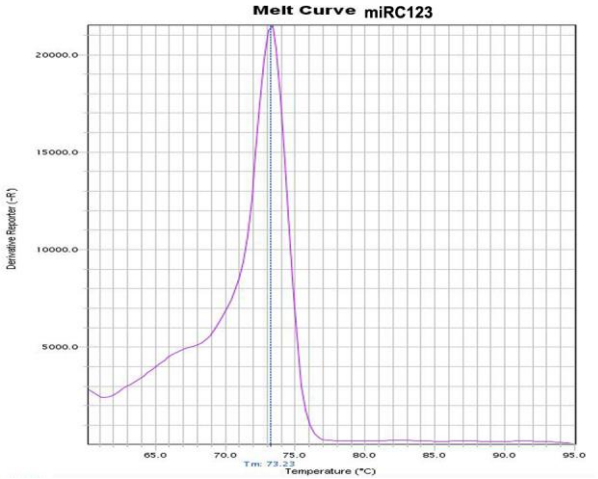
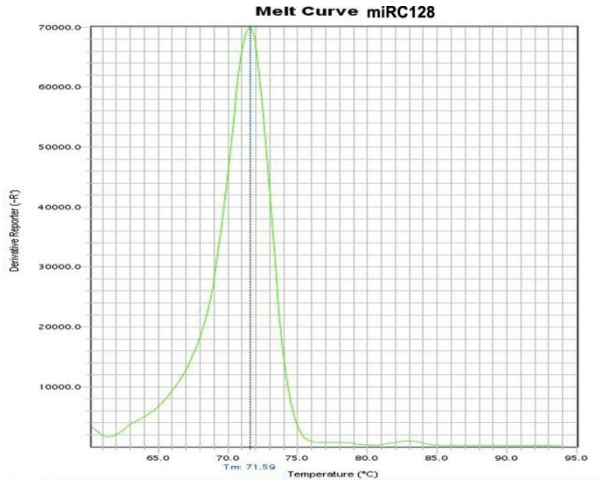
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 70.99
Temperature (°C)

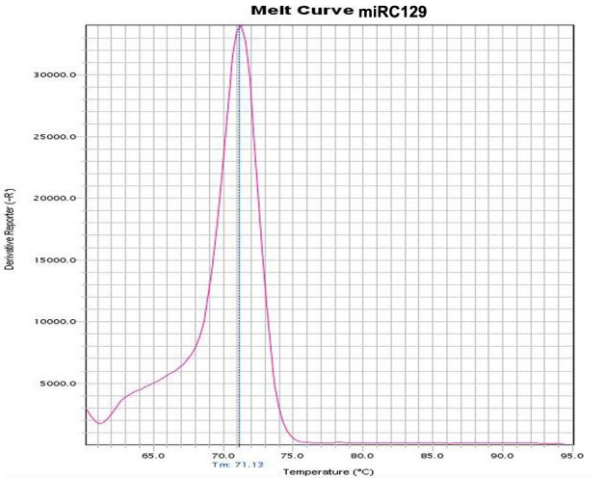

Melt Curve miRC116
Derivative Reporter (-R')
59000.0
49000.0
39000.0
29000.0
19000.0
9000.0
65.0
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 72.03
Temperature (°C)

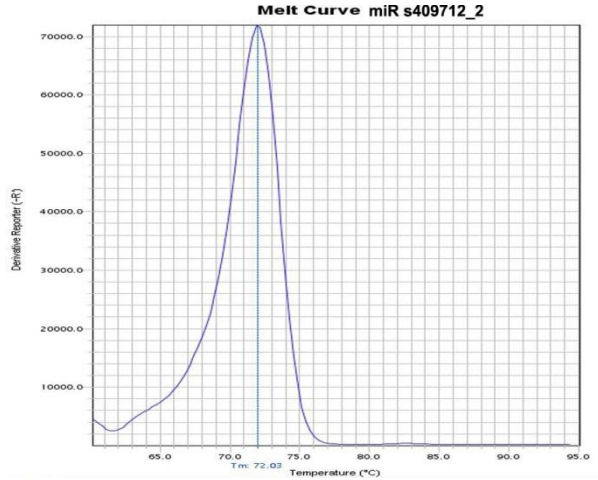

Melt Curve miRC117
Derivative Reporter (-R')
60000.0
50000.0
40000.0
30000.0
20000.0
10000.0
65.0
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 72.18
Temperature (°C)


Melt Curve miRC122
Derivative Reporter (-R')
47000.0
37000.0
27000.0
17000.0
7000.0
65.0
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 71.14
Temperature (°C)


Melt Curve miRC123
Derivative Reporter (-R')
20000.0
15000.0
10000.0
5000.0
65.0
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 73.23
Temperature (°C)


Melt Curve miRC128
Derivative Reporter (-R')
70000.0
60000.0
50000.0
40000.0
30000.0
20000.0
10000.0
65.0
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 71.59
Temperature (°C)


Melt Curve miRC129
Derivative Reporter (-R')
30000.0
25000.0
20000.0
15000.0
10000.0
5000.0
65.0
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 71.13
Temperature (°C)


Melt Curve miR s409712_2
Derivative Reporter (-R')
70000.0
60000.0
50000.0
40000.0
30000.0
20000.0
10000.0
65.0
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 72.03
Temperature (°C)

b

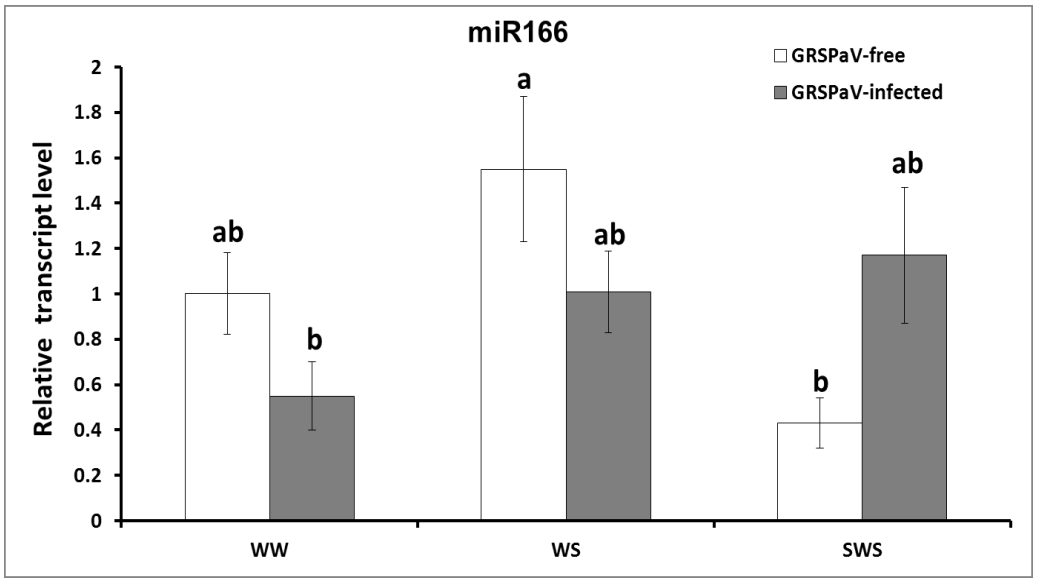
10 bp ladder
miRC128
miRC129
miR s4097l2_2

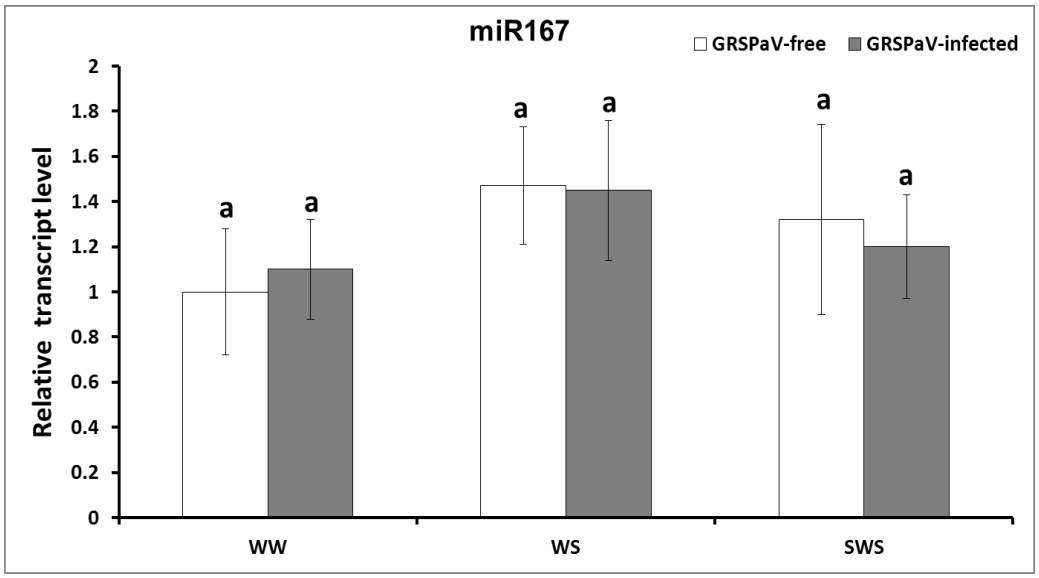
10 bp ladder
U6
5.8s rRNA
miR394
miR397
miR3627
miR3629
miR3631
miR3633
miR3634
miR3637
miR3639
miRC116
miRC117

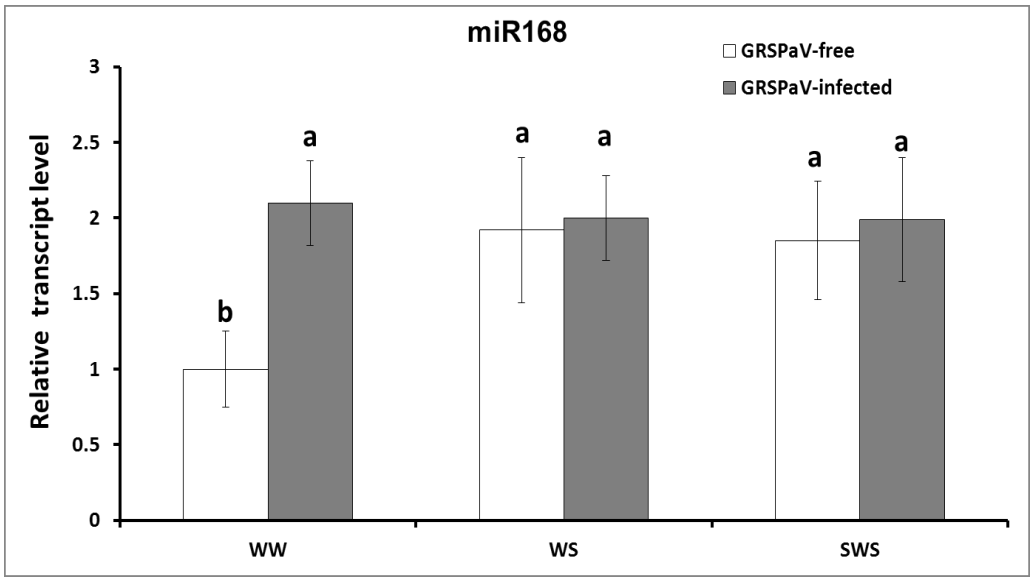
10 bp ladder
miR156
miR164
miR166
miR167
miR168
miR390
miR396
miR482
miR535
miR2111
miR2950
miR3624
10 bp ladder

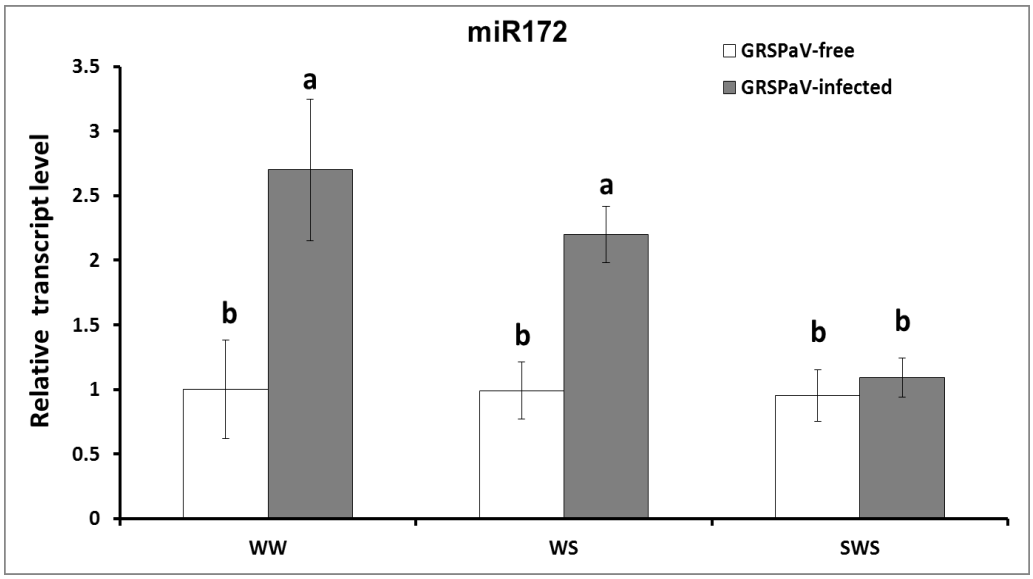
10 bp ladder
miRC102
miRC103
miRC104
miRC106
miRC108
miRC122
miRC123
10 bp ladder

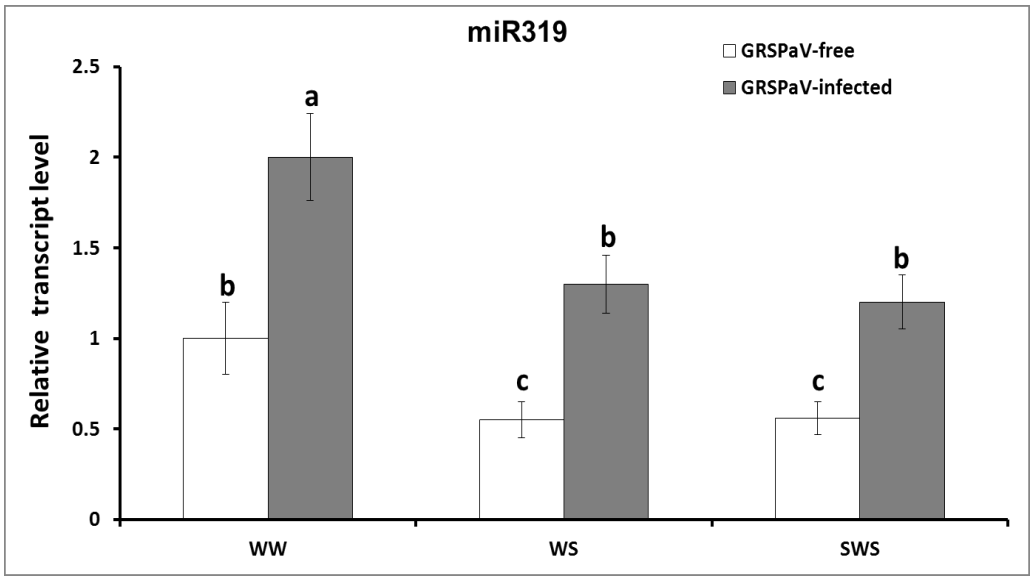
Supplementary Figure S4. Relative expression level of miR166, miR167, miR168, miR172, miR319, miR390, miR394, miR397, miR482, miR535, miR2111, miR2950, miR3624, miR3627, miR3629, miR3631, miR3633, miR3634, miR3637 and miR3639 in *Grapevine rupestris stem pitting-associated virus* (GRSPaV)-free and infected 'Bosco' leaf as determined by qRT-PCR. Samples were collected under: well watered (WW), water stress (SW), severe water stress (SWS) conditions. qRT-PCR signals were normalised to U6 and 5.8 rRNA. Data are presented as mean ± standard deviation of three biological replicates; different letters denote significant differences at $p \leq 0.05$.

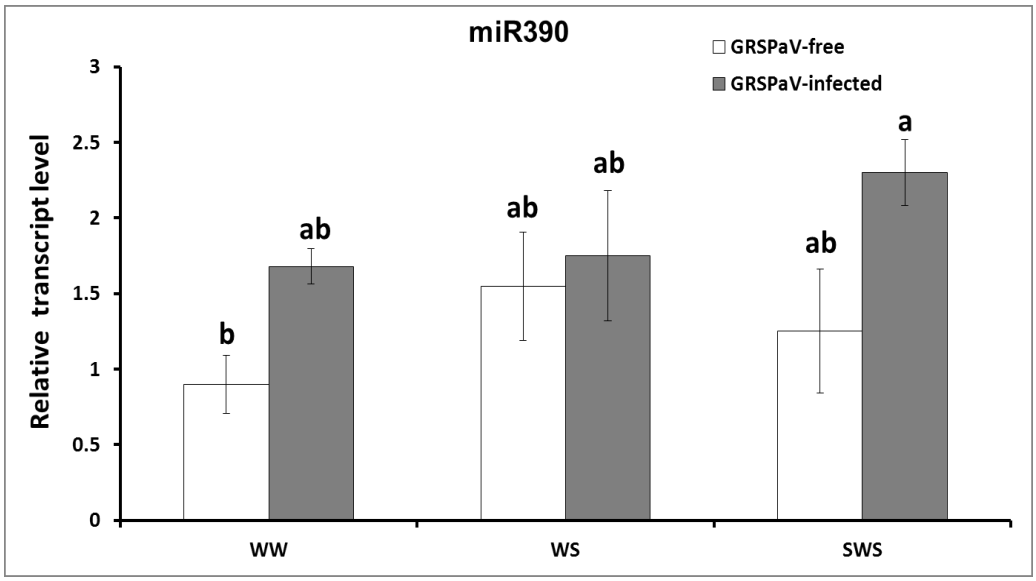


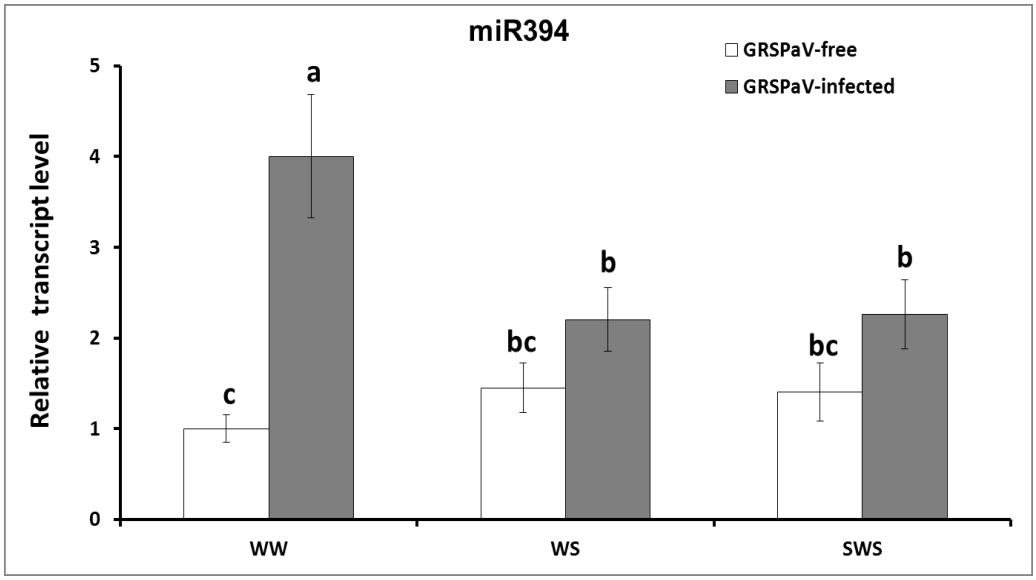


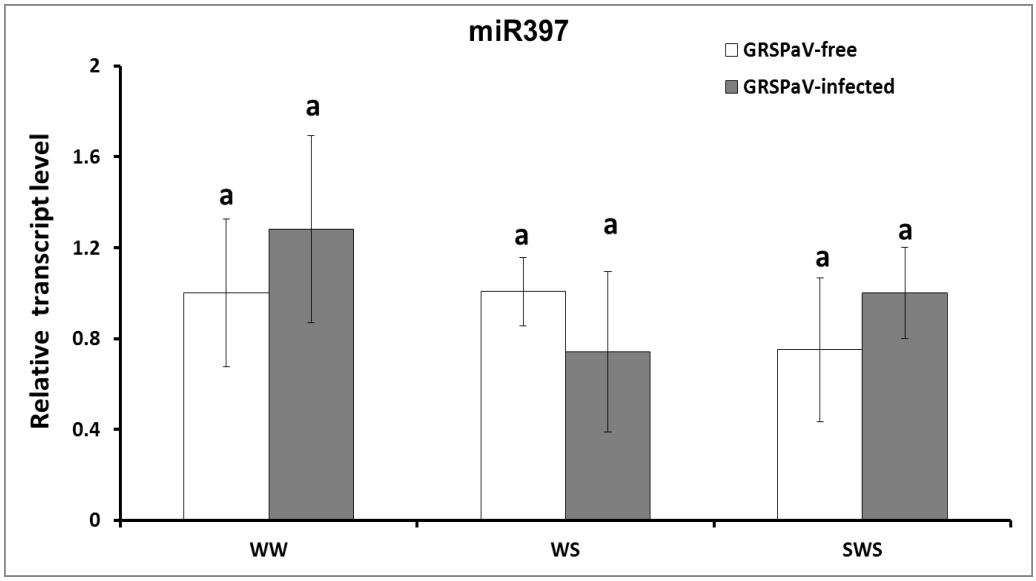

miR168
GRSPaV-free
GRSPaV-infected
Relative transcript level
3
2.5
2
1.5
1
0.5
0
a
a
a
b
a
a
WW
WS
SWS

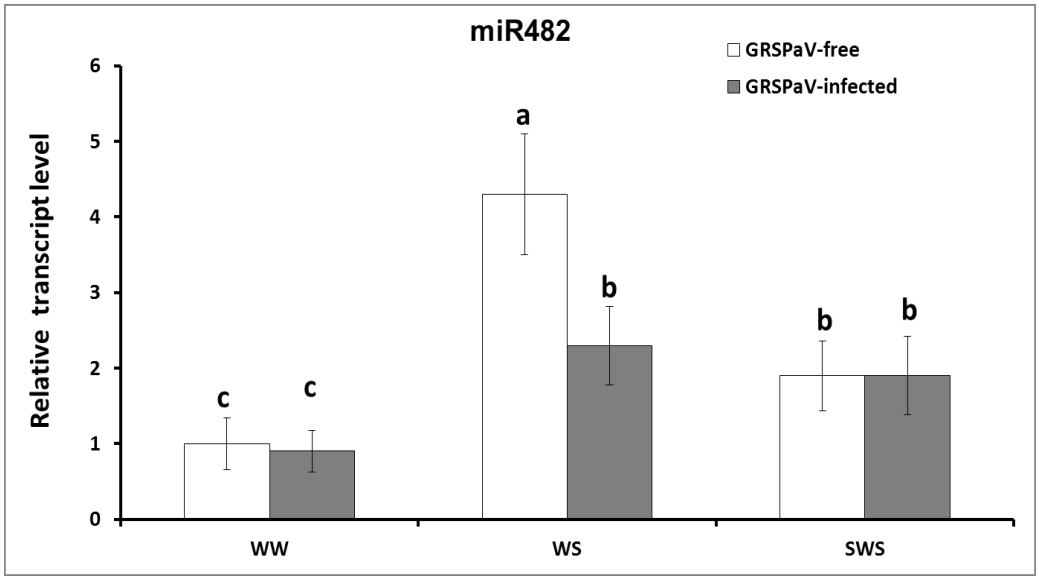

miR172
GRSPaV-free
GRSPaV-infected
Relative transcript level
3.5
3
2.5
2
1.5
1
0.5
0
a
a
b
b
b
b
WW
WS
SWS

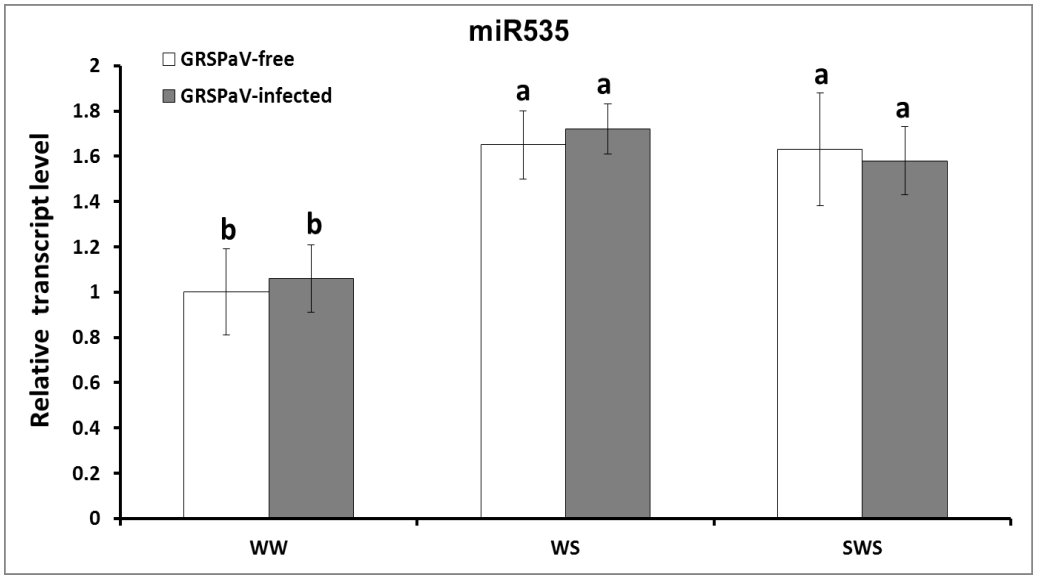

miR319
GRSPaV-free
GRSPaV-infected
Relative transcript level
2.5
2
1.5
1
0.5
0
a
b
b
c
b
c
WW
WS
SWS

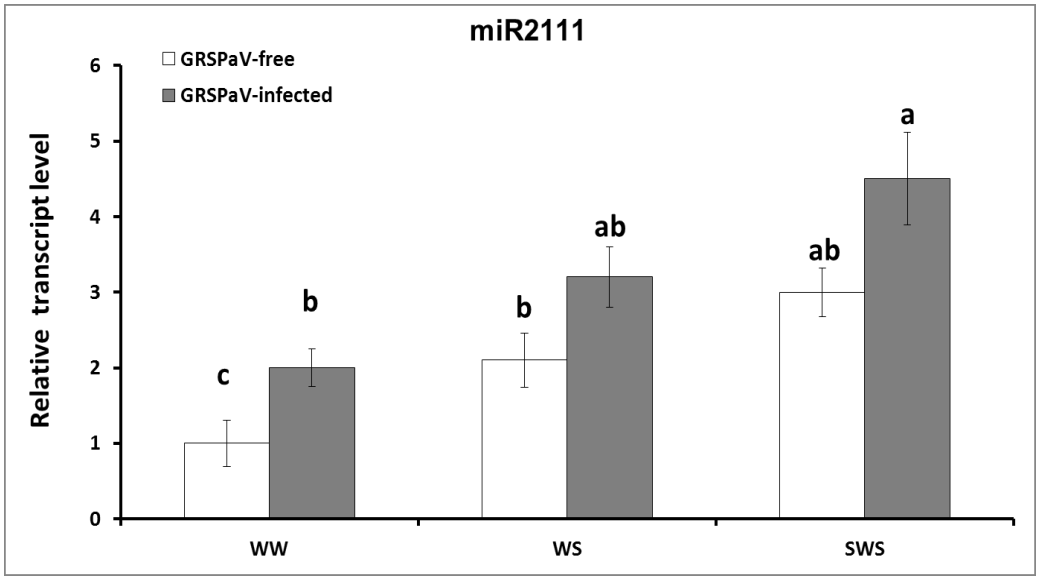

miR390
GRSPaV-free
GRSPaV-infected
Relative transcript level
3
2.5
2
1.5
1
0.5
0
b
ab
ab
ab
ab
a
WW
WS
SWS

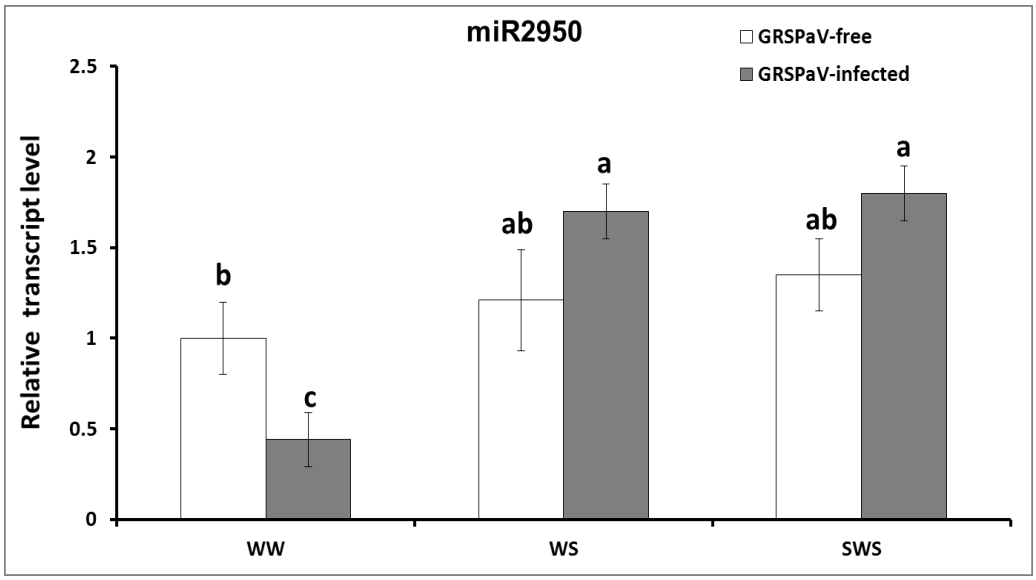

miR394
GRSPaV-free
GRSPaV-infected
Relative transcript level
5
4
3
2
1
0
c
a
bc
b
bc
b
WW
WS
SWS

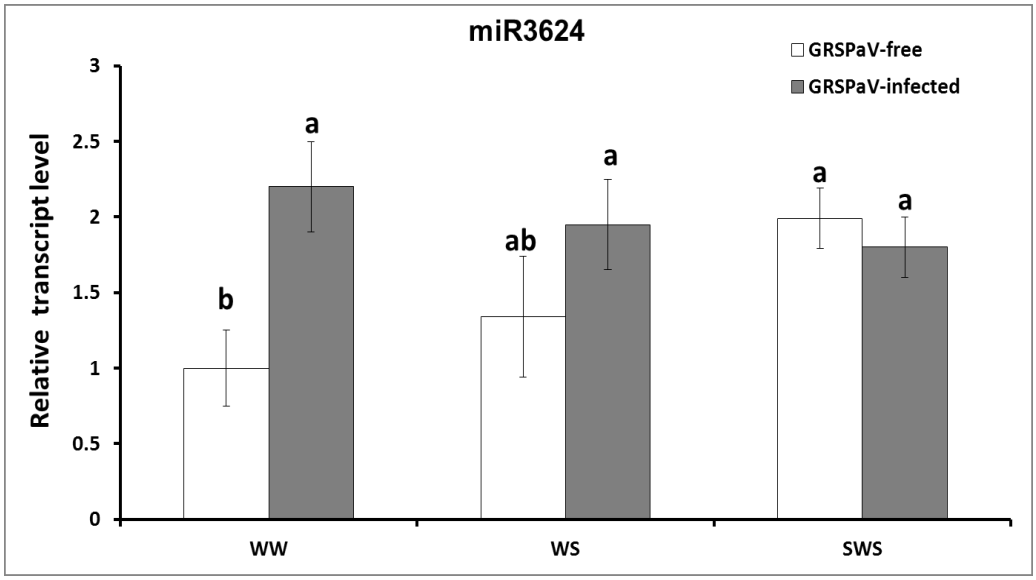

miR397
GRSPaV-free
GRSPaV-infected
Relative transcript level
2
1.6
1.2
0.8
0.4
0
a
a
a
a
a
a
WW
WS
SWS

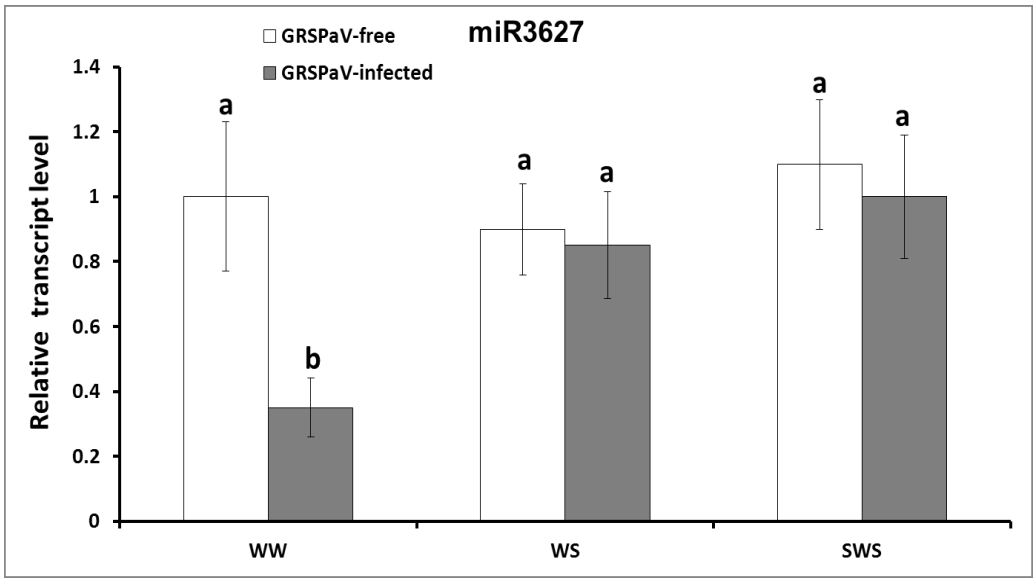

miR482
GRSPaV-free
GRSPaV-infected
Relative transcript level
0
1
2
3
4
5
6
c
c
a
b
b
b
WW
WS
SWS

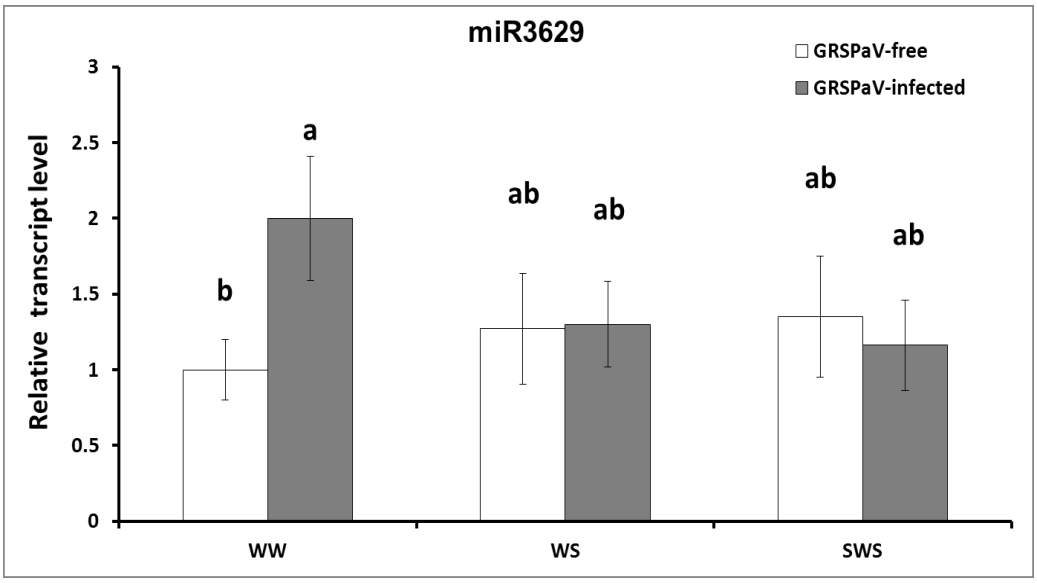

miR535
GRSPaV-free
GRSPaV-infected
Relative transcript level
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
b
b
a
a
a
a
WW
WS
SWS

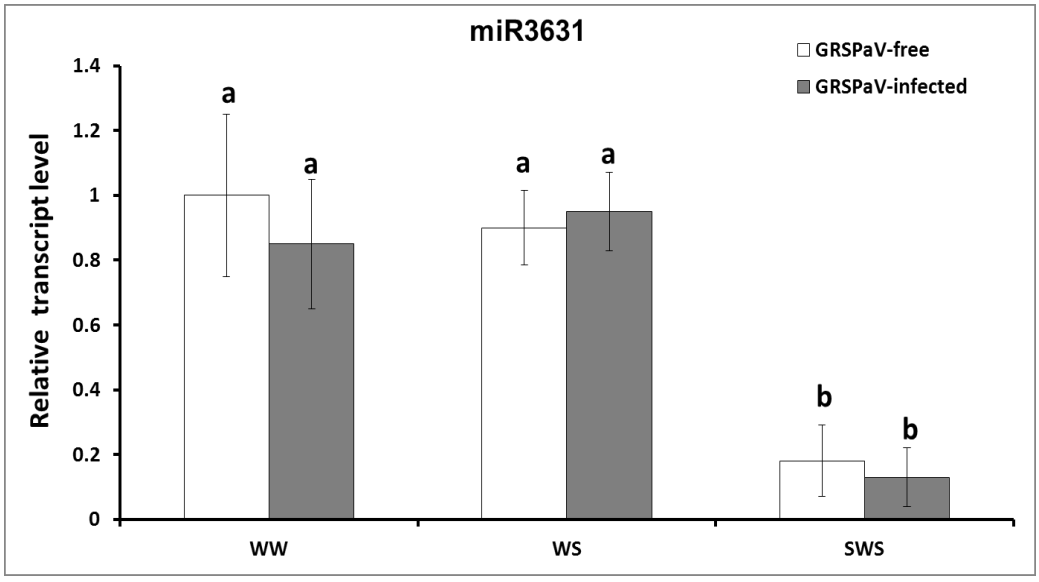

miR2111
GRSPaV-free
GRSPaV-infected
Relative transcript level
0
1
2
3
4
5
6
c
b
b
ab
ab
a
WW
WS
SWS

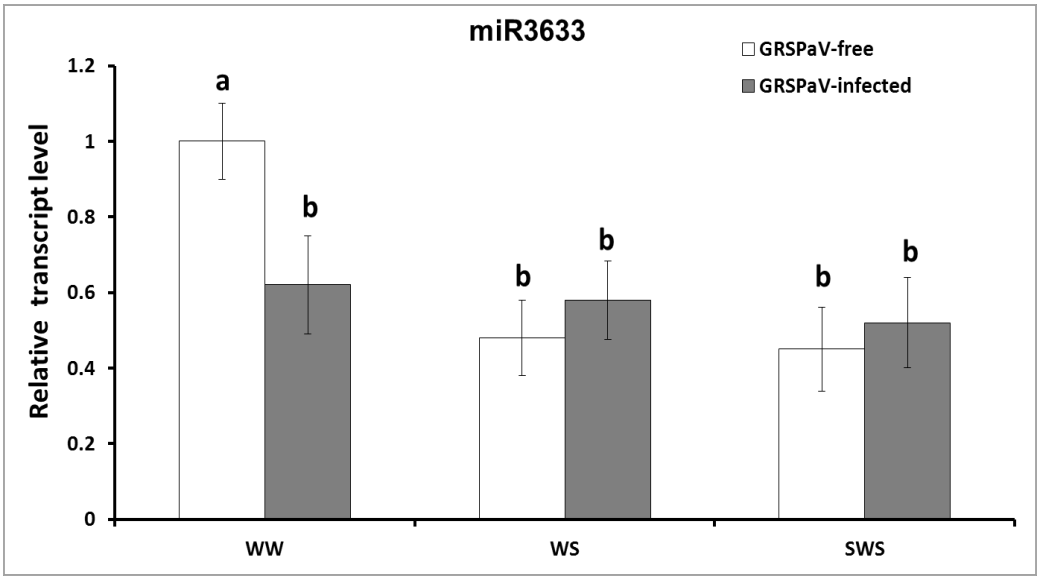

miR2950
GRSPaV-free
GRSPaV-infected
Relative transcript level
2.5
2
1.5
1
0.5
0
b
c
ab
a
ab
a
WW
WS
SWS

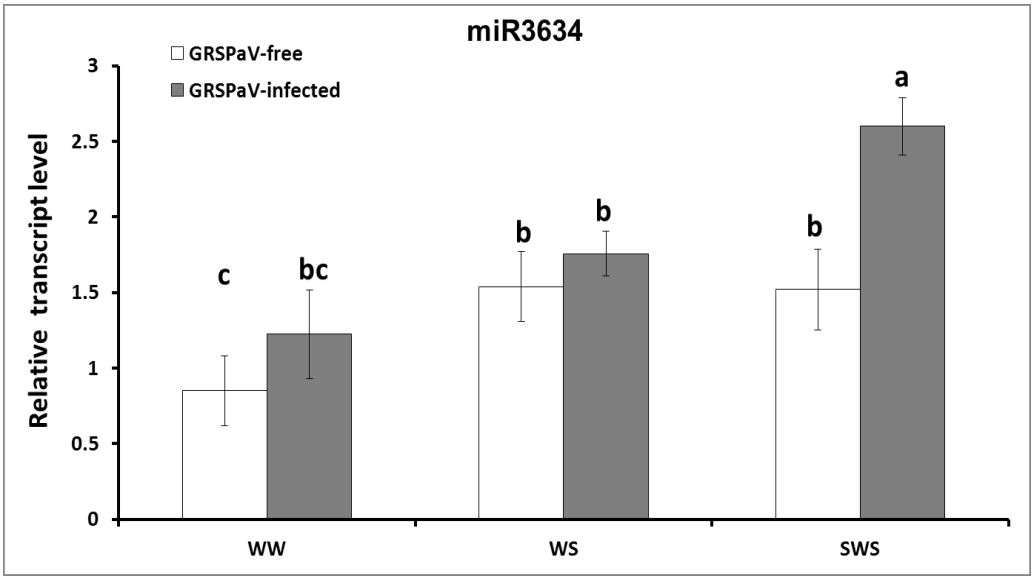

miR3624
GRSPaV-free
GRSPaV-infected
Relative transcript level
3
2.5
2
1.5
1
0.5
0
b
a
ab
a
a
a
WW
WS
SWS

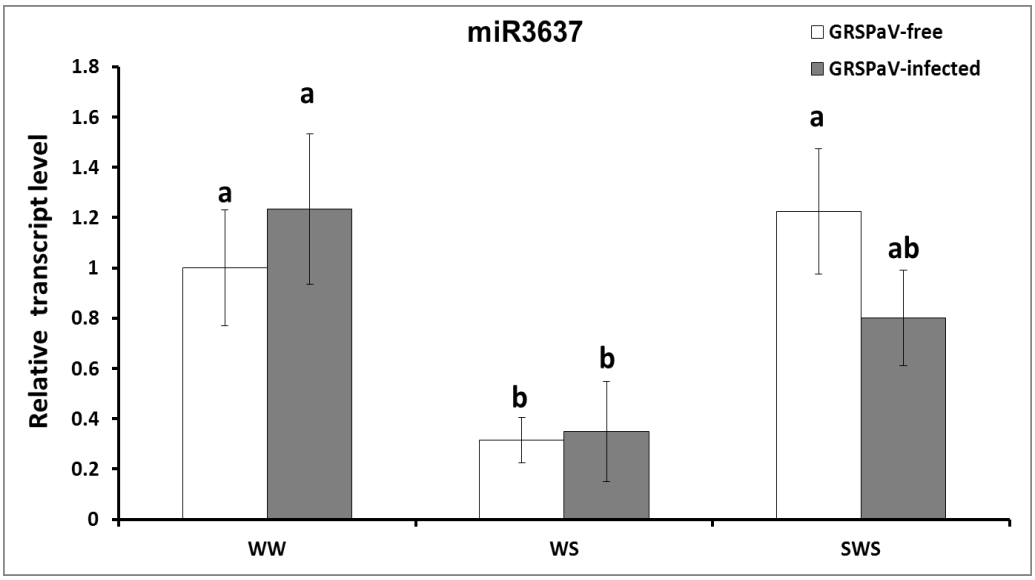

miR3627
GRSPaV-free
GRSPaV-infected
Relative transcript level
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
a
a
a
a
WW
WS
SWS

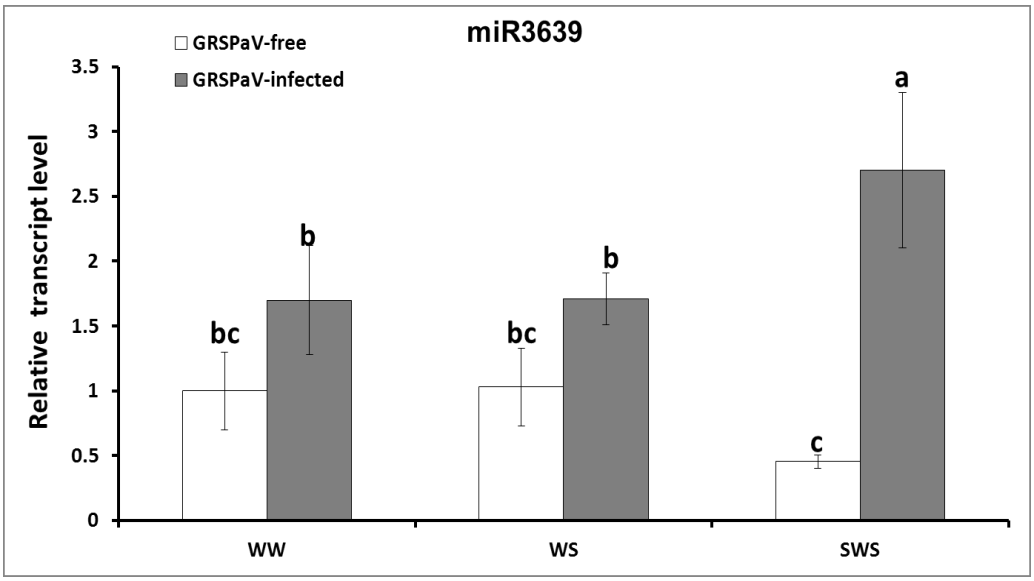

miR3629
GRSPaV-free
GRSPaV-infected
Relative transcript level
3
2.5
2
1.5
1
0.5
0
a
b
ab
ab
ab
ab
WW
WS
SWS


miR3631
GRSPaV-free
GRSPaV-infected
Relative transcript level
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
a
a
b
b
WW
WS
SWS


miR3633
GRSPaV-free
GRSPaV-infected
Relative transcript level
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
b
b
WW
WS
SWS


miR3634
GRSPaV-free
GRSPaV-infected
Relative transcript level
3
2.5
2
1.5
1
0.5
0
c
bc
b
b
b
a
WW
WS
SWS


miR3637
GRSPaV-free
GRSPaV-infected
Relative transcript level
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
b
b
a
ab
WW
WS
SWS


miR3639
GRSPaV-free
GRSPaV-infected
Relative transcript level
3.5
3
2.5
2
1.5
1
0.5
0
bc
b
bc
b
c
a
WW
WS
SWS

Supplementary Figure S5. Predicted secondary structures of new and putative grapevine specific miRNAs. In red and violet are indicated the miRNA and the miRNA*, respectively. "(" and "." indicate paired and unpaired bases, respectively. Within the mature miRNA "<" and "-" indicate paired an unpaired bases, respectively. Within the miRNA*, "{" and "=" indicate paired an unpaired bases, respectively.

116

TTCTCACCGTTTCTCAGC_chr9/3184798-3184818
CTGTTTGCTTGCTGAGAAACTGGGGGGAAAATGAAAGAAAACTATAAATCTTTTCAACATGTTCGAGTTGGAGTGGCAACTGTGACAATCCGGATTTTTTTTTTCTTCTTCTCACCGTTTCTCAGCCAACAAATGGAGAAC
(((((((...(({{{{{{{{={{={={{{={==((((((((.....(((((.((((((.....))))))((.((....)).)).....))))))))))))))--->>>>>->>>>>>>>>>>>...)))))))))).))

117

TAGAACCAAGGGCACCCT_chr9/5984686-5984706
TCTTAGAACCAAGGGCACCCTTAATCCTTCCCCGACTTCGATTCCGATATAAGGTATCAATGGGACCCATTGGTTCAAGAAAGA
<<<<-<<<<<<<<<<--<<<-....((((.....(((....)))...))))).....))).))).))))))))))))))

TAGAACCAAGGGCACCCT_chr14/19624264-19624284
TTTCTTAGAACCAAGGGCACCCTTAATATGTCCCTGACTTCAATTCCTAGGTGAGGTATCAATGGGACCCATTGGTTTGAGAATGAAG
.(<<<<<<<-<<<<<<<--<<-<(.((((.((((((.....)))).)).)))).)).))..))).))))))))))).))))

122

TGGGACTACATGTGTTACA_chr3/17925351-17925372
CCGCACCAACACACGTATTATTAAAGTGTTACACATGTAGTGCCATCATATGAAGAAAAGTGTATGATTTTTTTAATCAATCAGTTCTTTATATTATGGGACTACATGTGTTACACTTTGATGACATGTGGGTTAGTGTGGACCGG
((((((.(((.(((((.((((((((((((={{{{{{{{{{{={{{{={{{((((((((...((..(((((.....)))))..)).)))))))))>->>>>->>>>>>>>>>>->>>))))))))).))))).))).))))))))))

123

CAAGGTCGAGGATTGACT_chr14/14721702-14721722
AACAAACTCTTATACCAAGGTCGAGGATTGACTTCAATACTATTTGTAATGACCCCCTCCTAGCCAATAGGGGAAAAAGTTCTAAAGGCACAAGGGTTAATCATTCTTAGATTTGACAATATCTACATGGTTGAGAATTAGTCGTTACGAATGATATGAGAGTCAAACCTCAACCTTGGTATGAGAGTTTGTTTGTCCTT
((((((((((((<<<<<<<<<-<<<<-<<<<<<(..(((.((((((((((((.((((....(((..(((((.....)))))..)))...))))((((((((...(((((.....)))))).))))))))).....))))))))))))).))))..))){{{{={{{{={{{{{{{{{{{))))))))))))).))))

128

AGTCAATCCAAACAAGGTC_chr9/21823140-21823161
GTTTGGATTGACTTCTGAAAGGCTAAAAGCTCTTATTAAGCTTAAATAAGAGCTTTTGGCTTCTCAGAAGTCAATCCAAACAAGGT
((((((((((((((((((.((((((((((((((((((.....)))))))))))))))))))))).))>>>>>>>>>>>>>>>>->>>>

129

GTCTTTCTCTGTATAAGC_chr5/6820744-6820764
TCCATGCCATTTCATTACACAGAGAGATGACGGTGGAGATTTGTCTCTATTGCTCTCTACCGTCTTTCTCTGTATAAGCAGATGGAGTGGATCTT
(((((.((((((..{{{={{{{{{{{{={{{{{{{((((.....)))))>>>>>>>>>>>>>>>->>>--)))))).)))))))))

409712_2

TGTTTTAAAGTCGTGAGGG_chr10/4096605-4096626
TGTAGATGTGTTTTAAAGTCGTGAGGGGCCCTTTGGGTCCAAAGCAGACAATATCTACACGATTGGGAACGAGTCATTACAAATGGT
(((((<<-<<<<<---<<<<<<<--<<((((...))))))).....(((.....))).)))))))..))))).)))..)))))..)))

TGTTTTAAAGTCGTGAGGG_chr14/24304140-24304161
TGGACCCTCATGACTTTAAAATGCATCTACATAACTAAGAAAAGTTCATTGCATATACAGTGTTAGGAACTTTTCCCCTTTACCCTAATTATGTAGATGTGTTTTAAAGTCGTGAGGGTCCATTGA
((((((((((((((((((((((((((((((((((.((.(((((((((...((((.....))))...))))))))).....)).))))))))>>>>>>>>>>>>>>>>>>>>>>))))))))

TGTTTTAAAGTCGTGAGGG_chr19/20397809-20397830
ATCTTGTAGATGTGTTTTAAAGTCGTGAGGGTTAAGTCATTACAGATGGTATTAAAGTCGATCCTCGACCCCAATGTGGAGGTTTATTTGGTCCTATGAGGGGTGTTTGTAATATCCCACATTGGATGAGAGAGAAAATTACTTAGACTATATATGTATGAACTCCTCTTAACTTTGTAGACGTGTTTTAAAGTTATGAG
(..(((.((<--<<<<<<<<<<<---<<<<<.....((((.(((.(((((....((((....((((.((.....)).))))....(((.((((.(((.(((((((.....)))))))).))).)))).))).....)))).)))))...)))))))..))))))..))))))..))))))..)))

miRC104

```
miRC104              1  cugaacucucucccucau  18
                        ||||||||||||||||||
mes-miR1446          3  cugaacucucucccucau  20

miRC104              1  cugaacucucucccuca  17
                        |||||||||||||||||
ptc-miR1446a         3  cugaacucucucccuca  19
```

miRC108

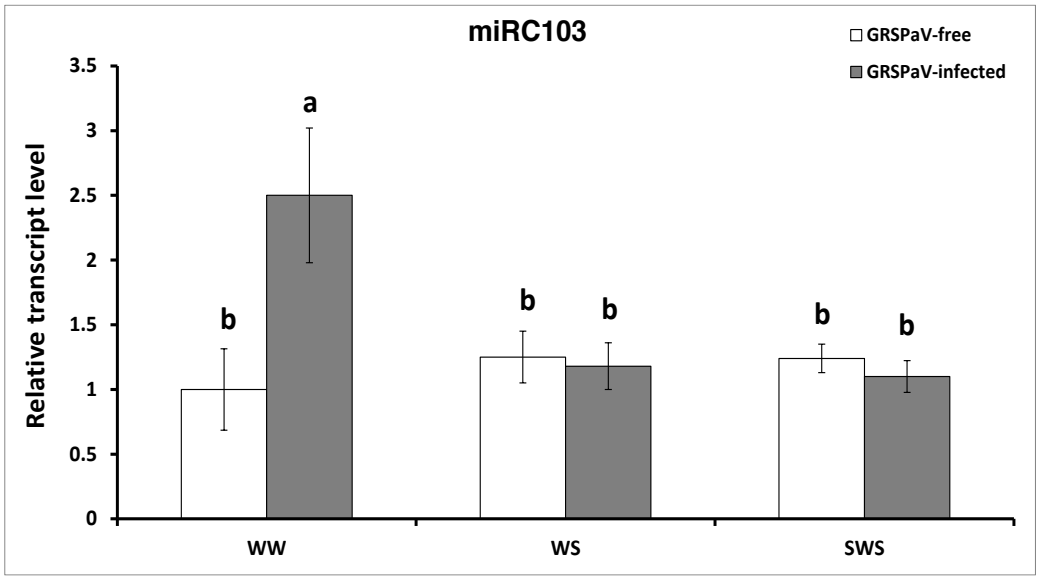
```
miRC108              5  uuggaugauaau  16
                        ||||||||||||
stu-miR7982a         4  uuggaugauaau  15

miRC108              5  uuggaugauaau  16
                        ||||||||||||
stu-miR7982b         4  uuggaugauaau  15
```

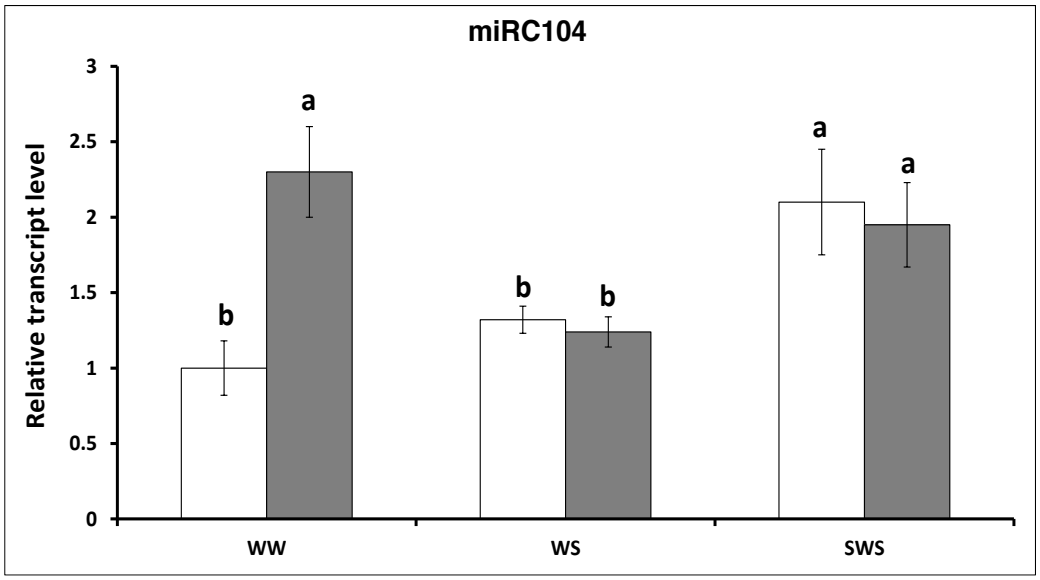
miRC129

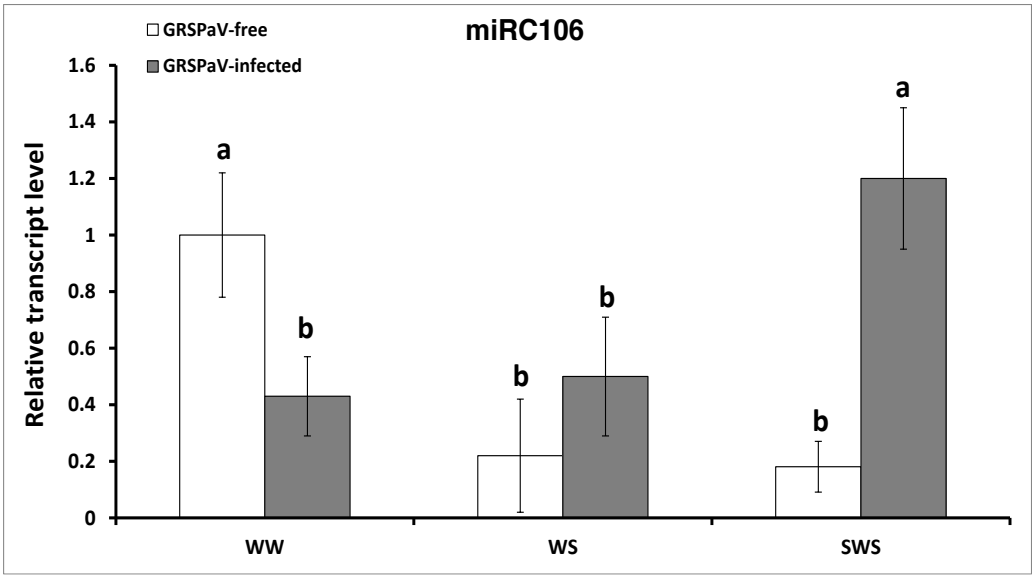
```
miRC129              2  ccgucuuucucuguauaa  19
                        ||||  |||||  |||||||
ppe-miR7122a-3p      2  ccguguuucuuuguauaa  19

miRC129              1  accgucuuucucuguauaagc  21
                        ||  |  |||||||||||||  |
stu-miR7122-3p       1  acagcguuucucuguauaacc  21
```

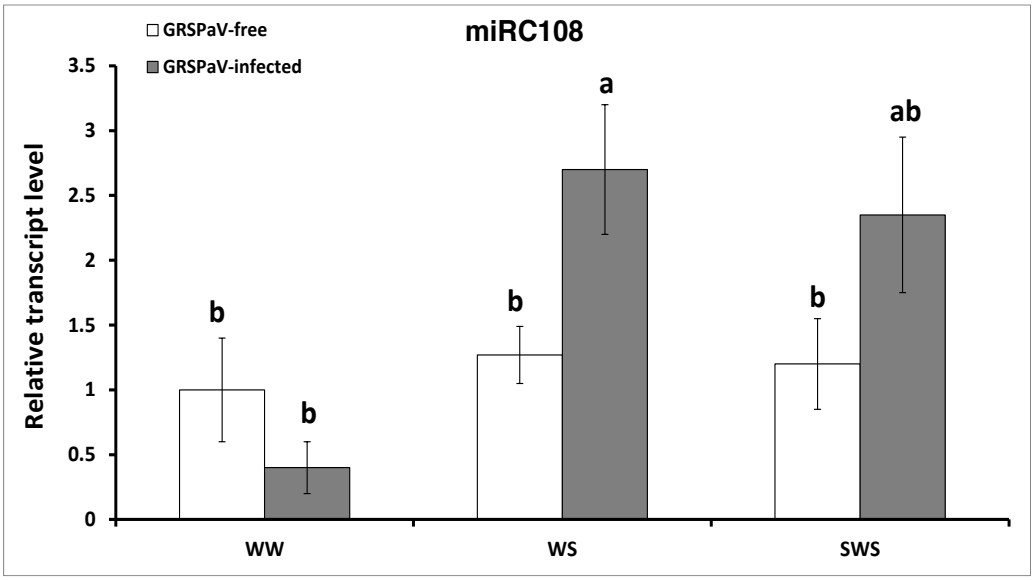
Supplementary Figure S6. BLASTN against selected mature miRNA in miRBASE.

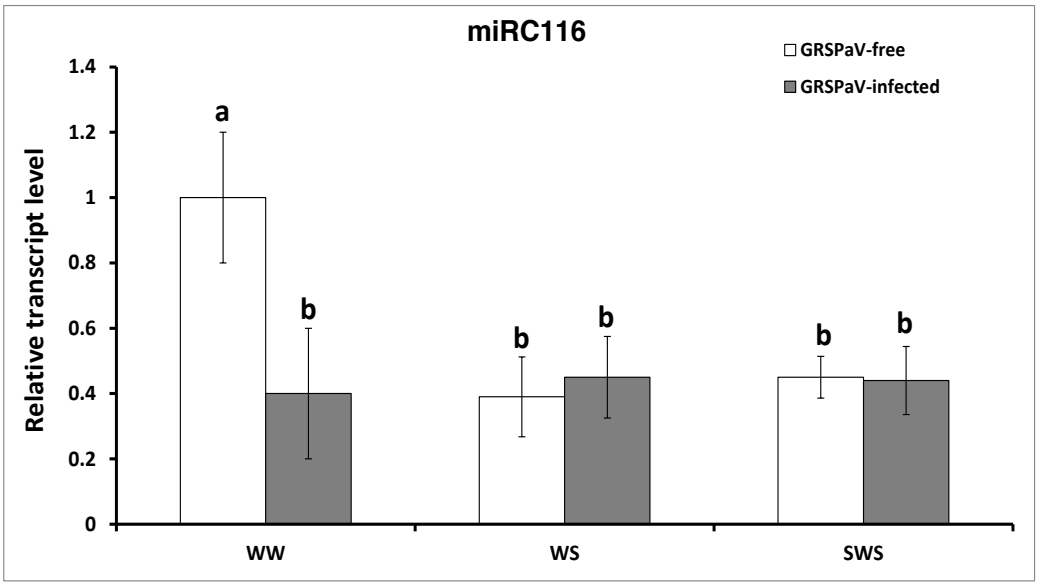
Supplementary Figure S7. Relative expression level of miRC103, miRC104, miRC106, miRC108, miRC116, miRC117, miRC122, miRC123 and miRC128 in *Grapevine rupestris stem pitting-associated virus* (GRSPaV) -free and infected 'Bosco' leaf as determined by qRT-PCR. Samples were collected under: well watered (WW), water stress (SW), severe water stress (SWS) conditions. qRT-PCR signals were normalised to U6 and 5.8 rRNA levels. Data are presented as mean ± standard deviation of three biological replicates; different letters denote significant differences at $p \leq 0.05$.

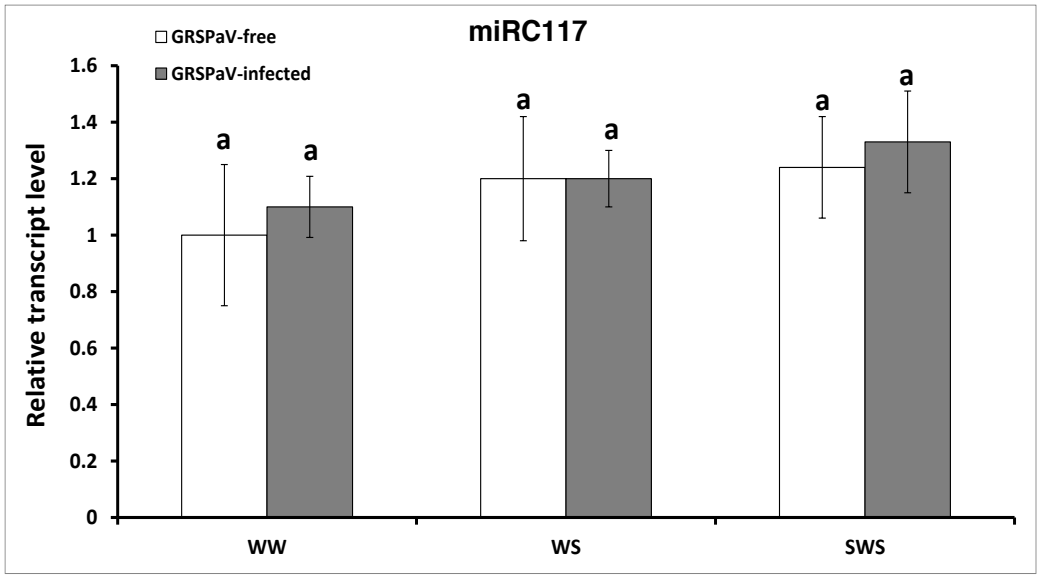


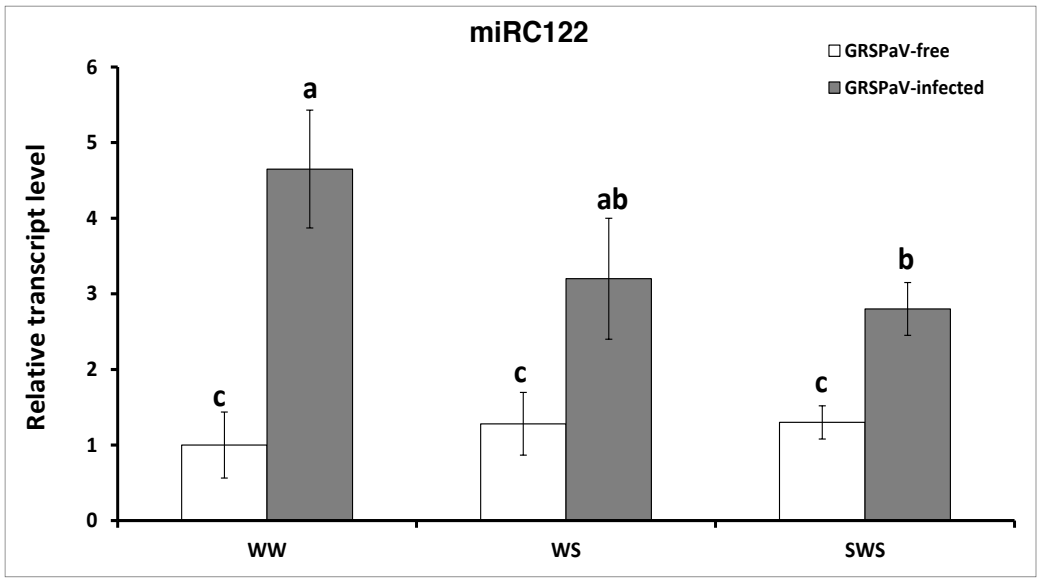


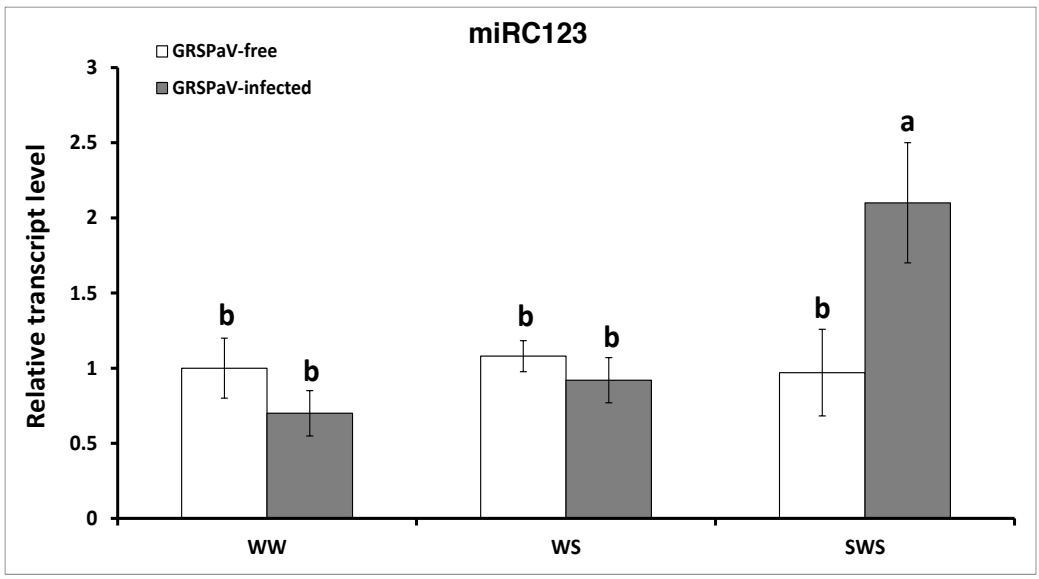

miRC106
GRSPaV-free
GRSPaV-infected
Relative transcript level
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
b
a
WW
WS
SWS

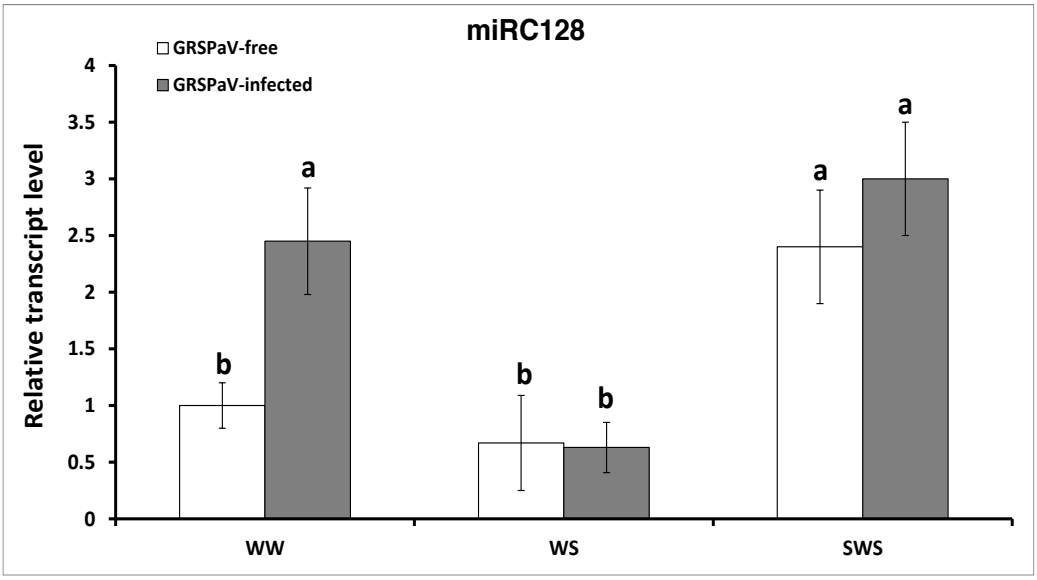

miRC108
GRSPaV-free
GRSPaV-infected
Relative transcript level
3.5
3
2.5
2
1.5
1
0.5
0
b
b
b
a
b
ab
WW
WS
SWS

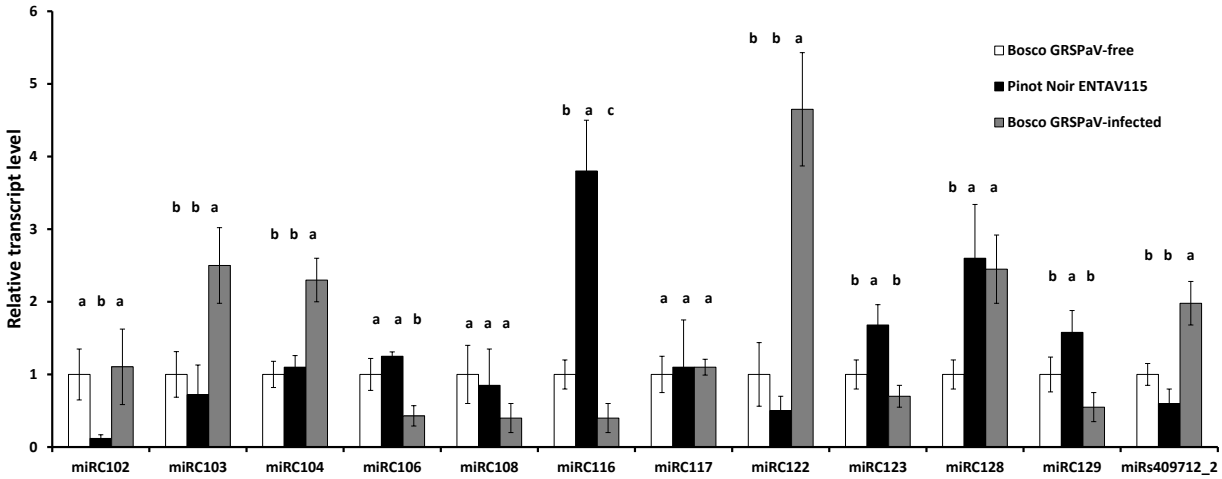

miRC116
GRSPaV-free
GRSPaV-infected
Relative transcript level
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
b
b
WW
WS
SWS


miRC117
GRSPaV-free
GRSPaV-infected
Relative transcript level
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
a
a
a
a
WW
WS
SWS


miRC122
GRSPaV-free
GRSPaV-infected
Relative transcript level
6
5
4
3
2
1
0
c
a
c
ab
c
b
WW
WS
SWS

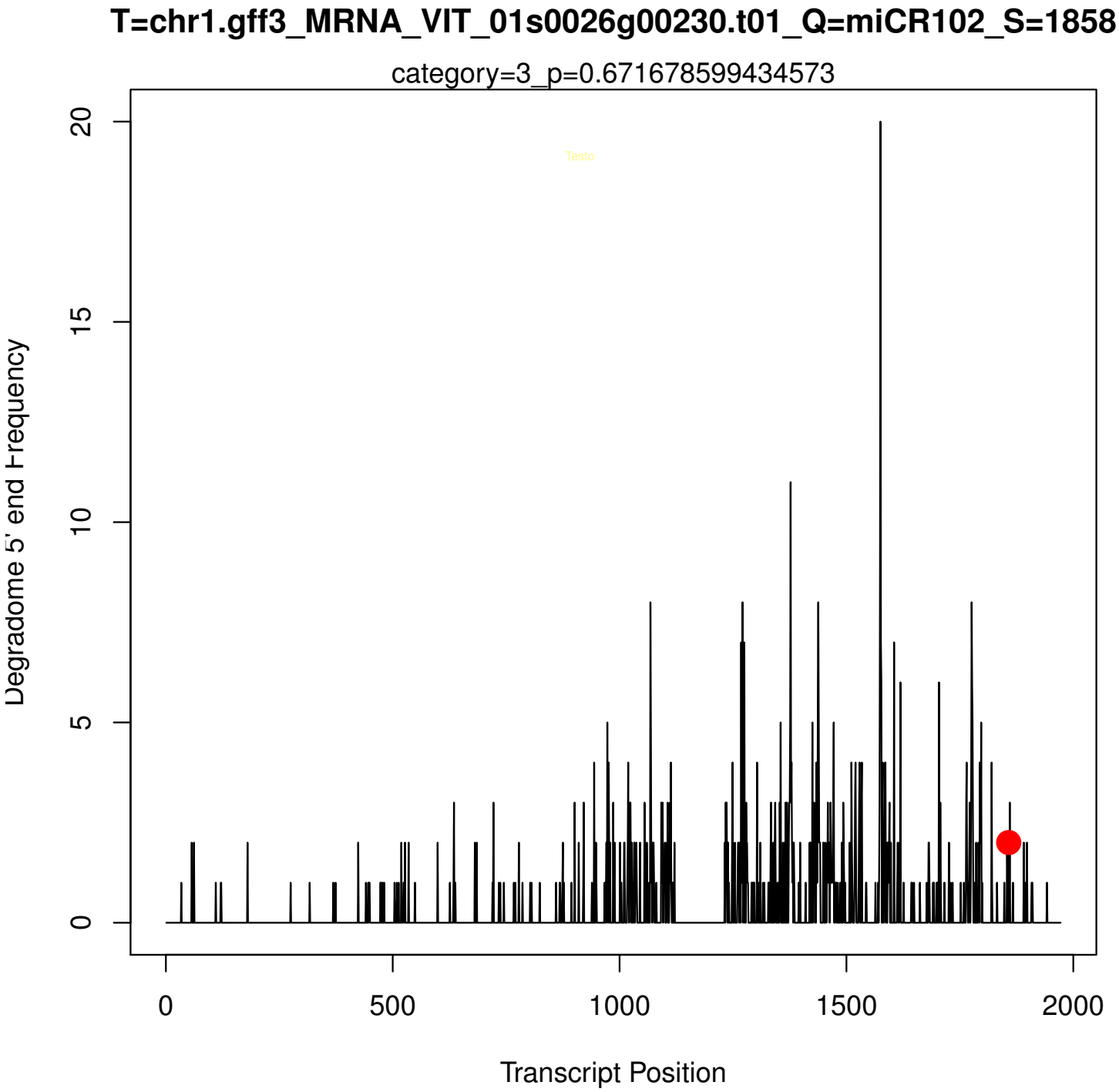

miRC123
GRSPaV-free
GRSPaV-infected
Relative transcript level
3
2.5
2
1.5
1
0.5
0
b
b
b
b
b
a
WW
WS
SWS

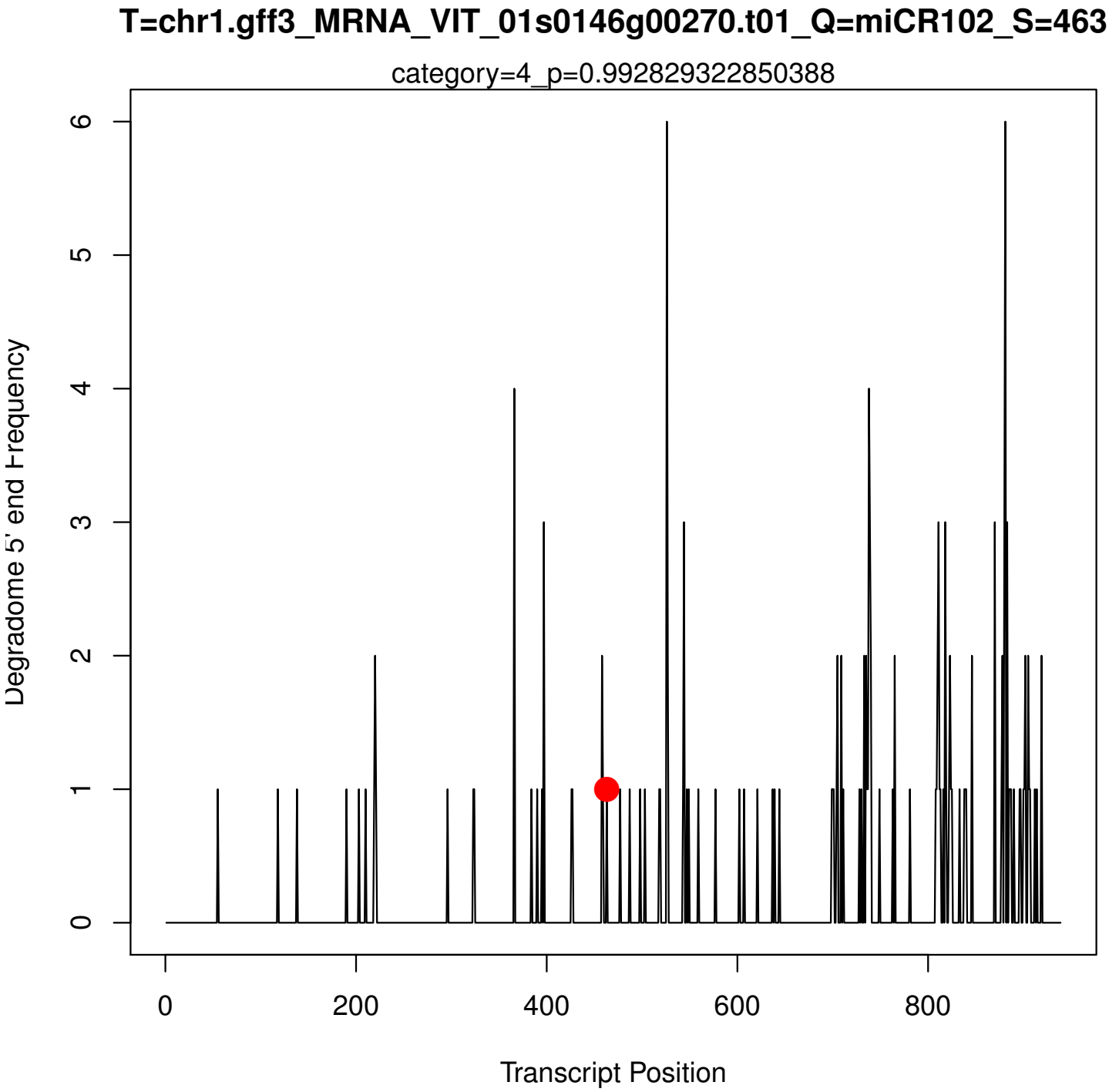

miRC128
GRSPaV-free
GRSPaV-infected
Relative transcript level
4
3.5
3
2.5
2
1.5
1
0.5
0
a
b
b
b
a
a
WW
WS
SWS



Supplementary Figure S8. Relative expression levels of all novel miRNA candidates in *Grapevine rupestris stem pitting-associated* (GRSPaV)-free, GRSPaV-infected 'Bosco' leaves and in 'Pinot noir' ENTAV115 leaves as determined by qRT-PCR. Samples were collected under well watered (WW) conditions. qRT-PCR signals were normalised to U6 and 5.8 levels. Data are presented as mean ± standard deviation of three biological replicates; different letters denote significant differences at $p \leq 0.05$.

Supplementary Figure S9. T-plots for targets of grapevine-specific microRNAs (miRNAs).

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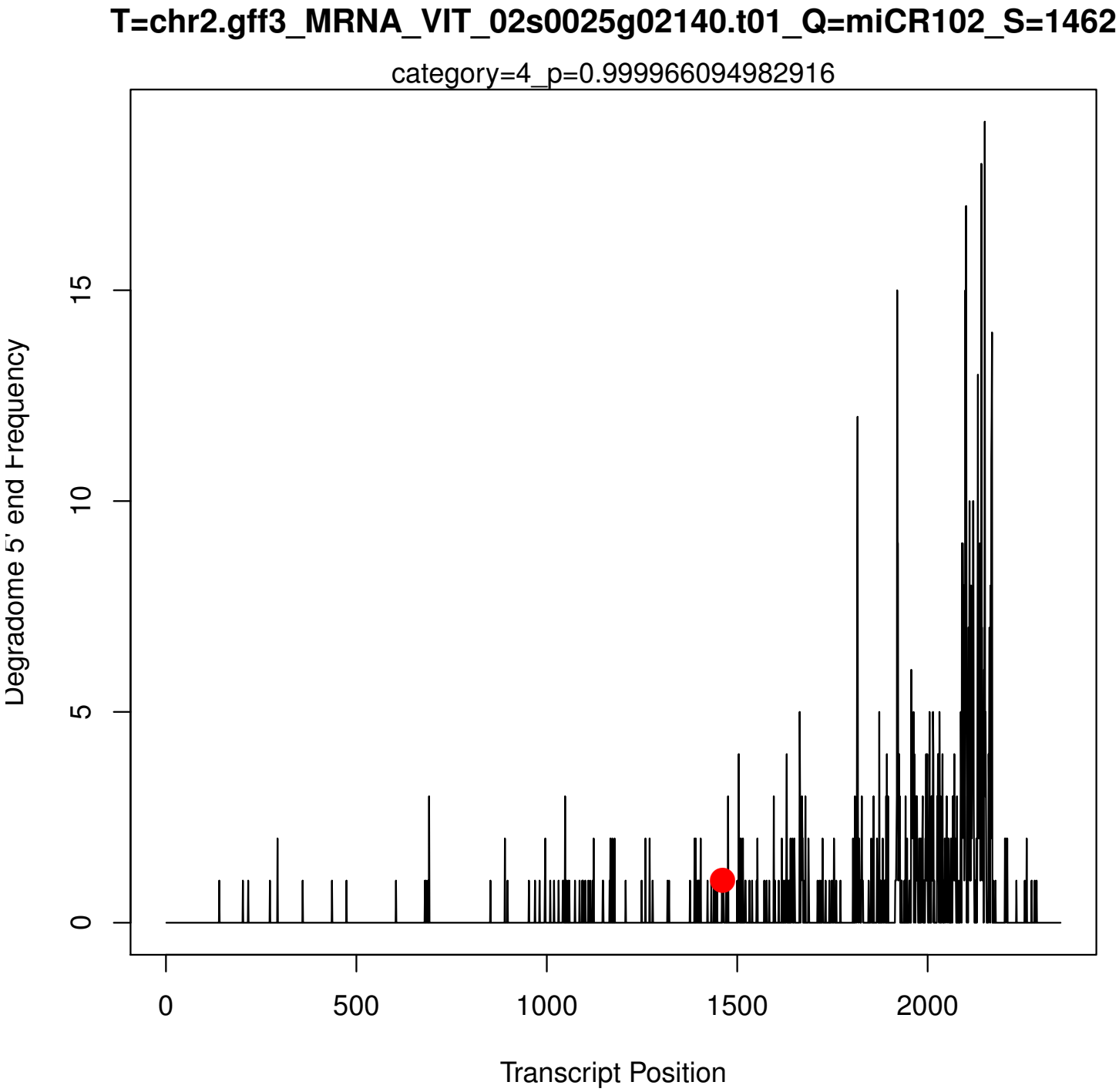
1
2
3
4
5
6
0
200
400
600
800
Transcript Position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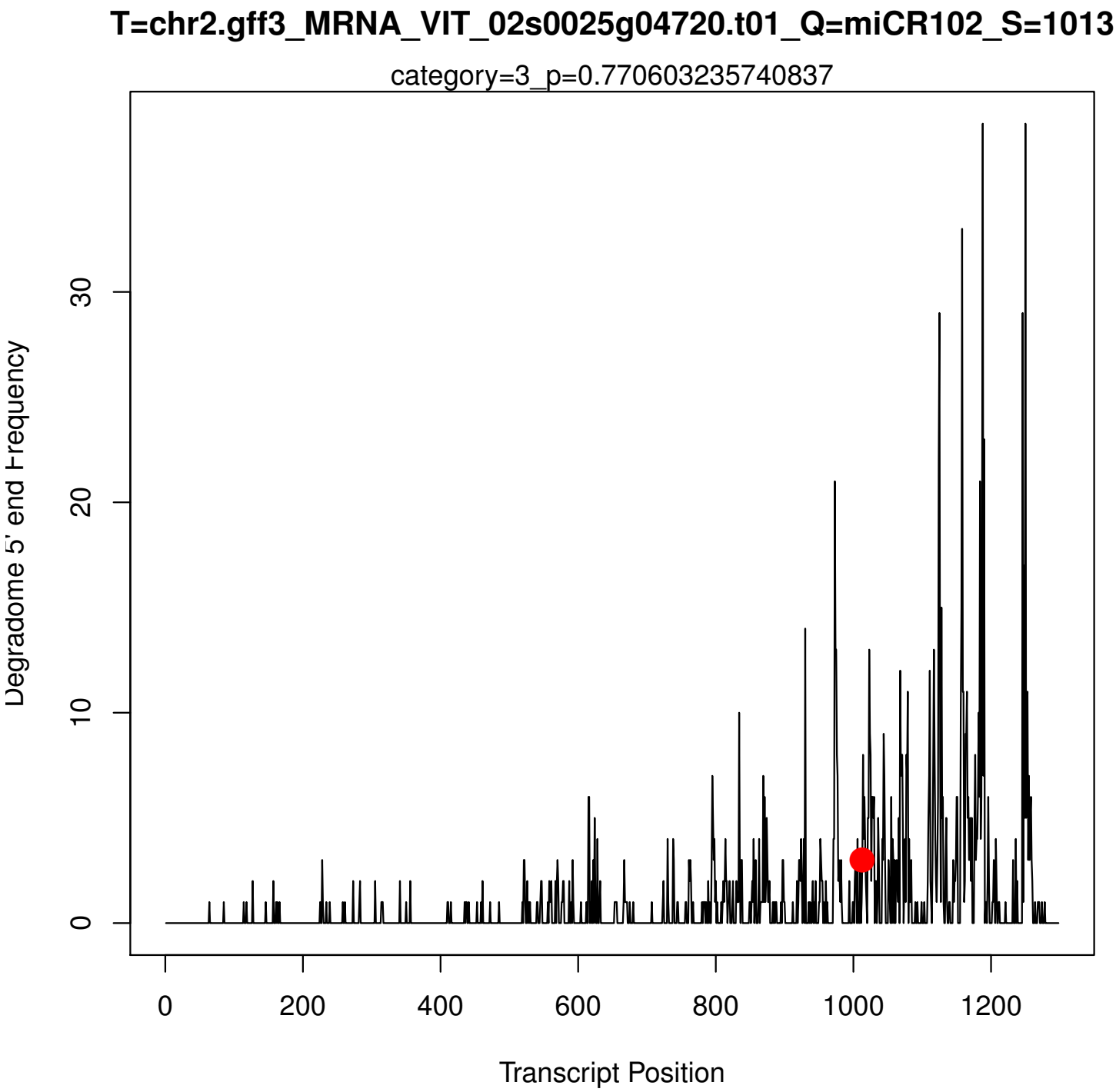
10
15
0
500
1000
1500
2000
Transcript Position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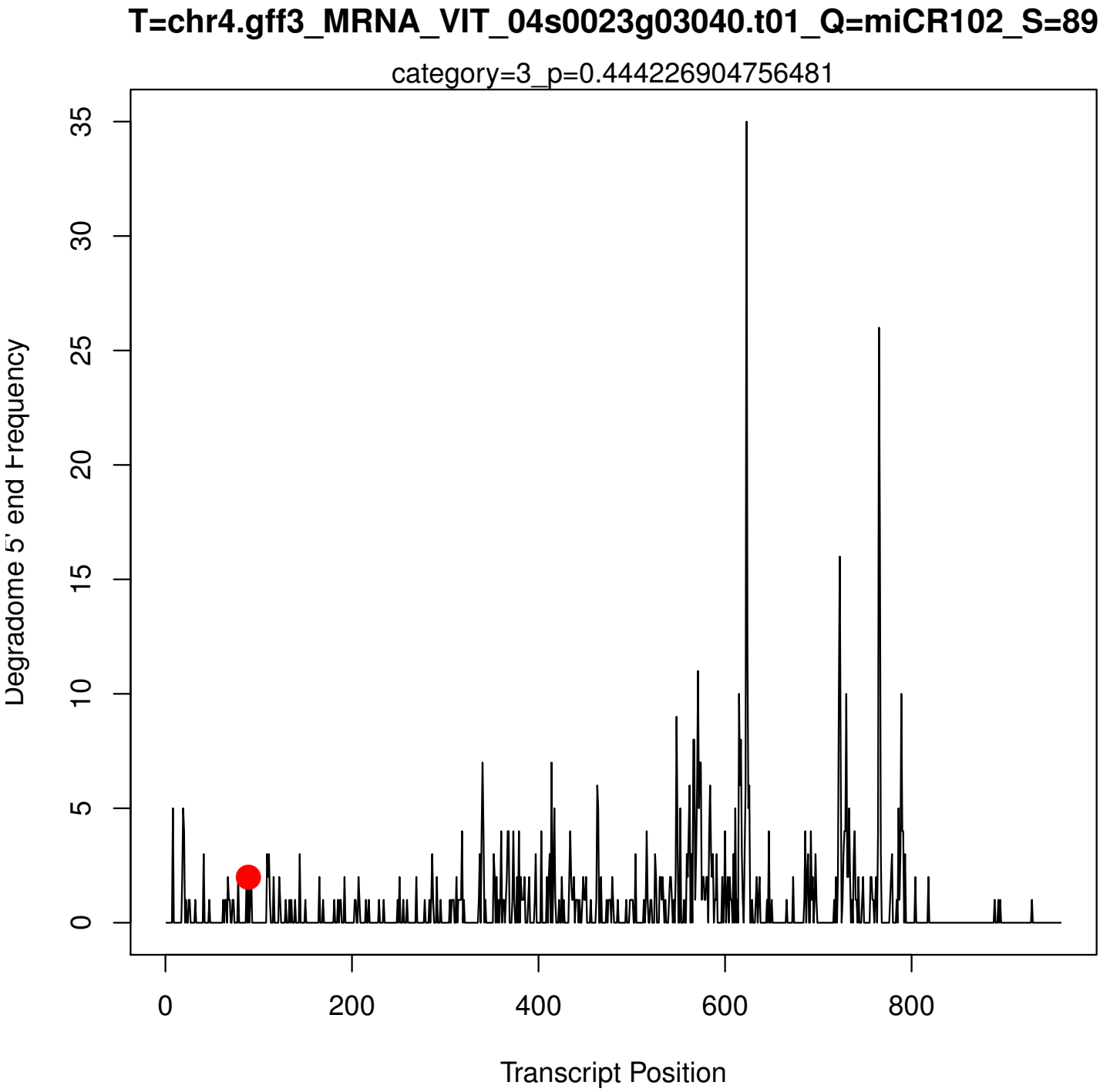
30
0
200
400
600
800
1000
1200
Transcript Position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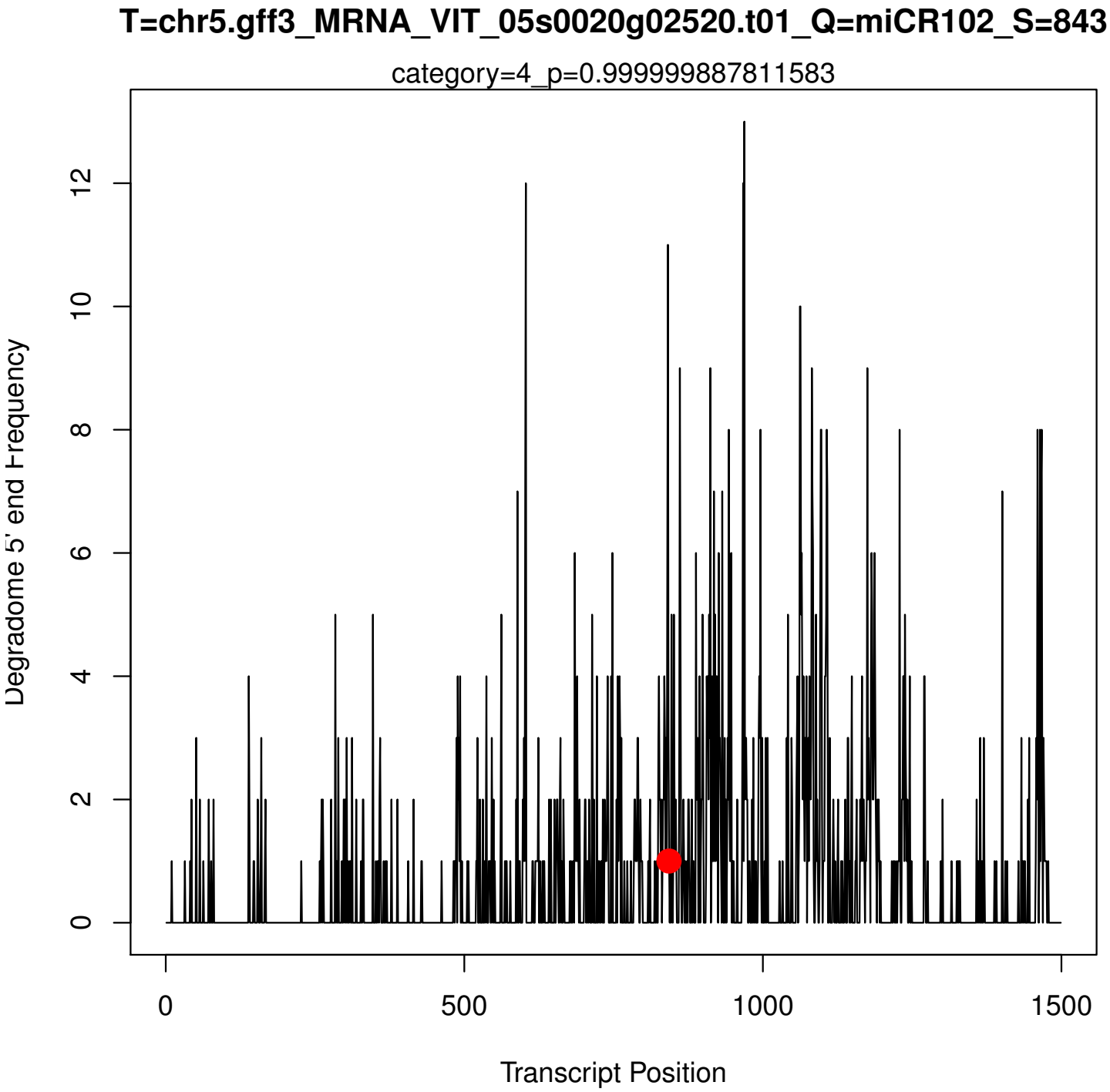
10
15
20
25
30
35
0
200
400
600
800
Transcript Position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2
4
6
8
10
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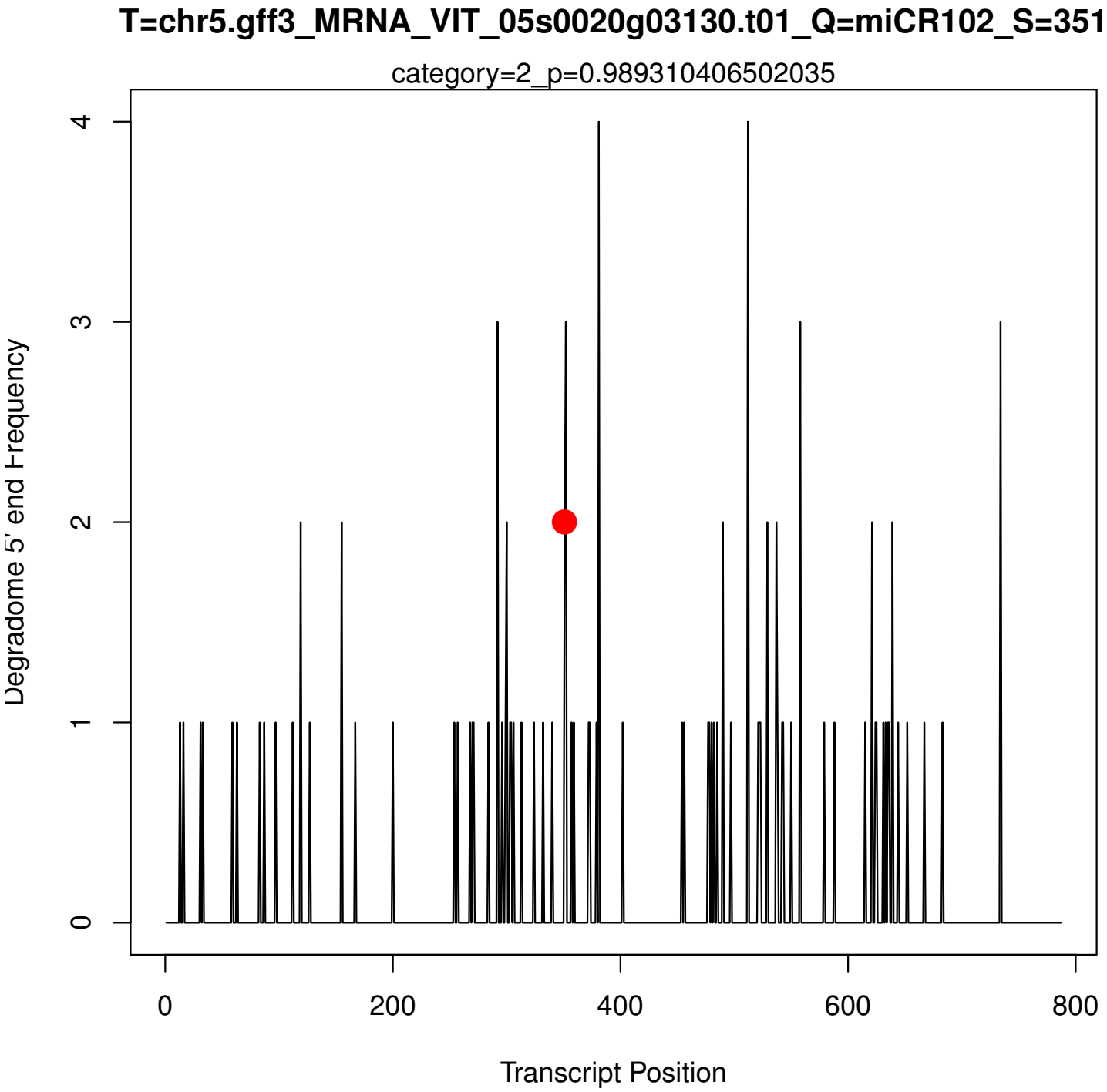
0
500
1000
1500
Transcript Position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3
4
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400
600
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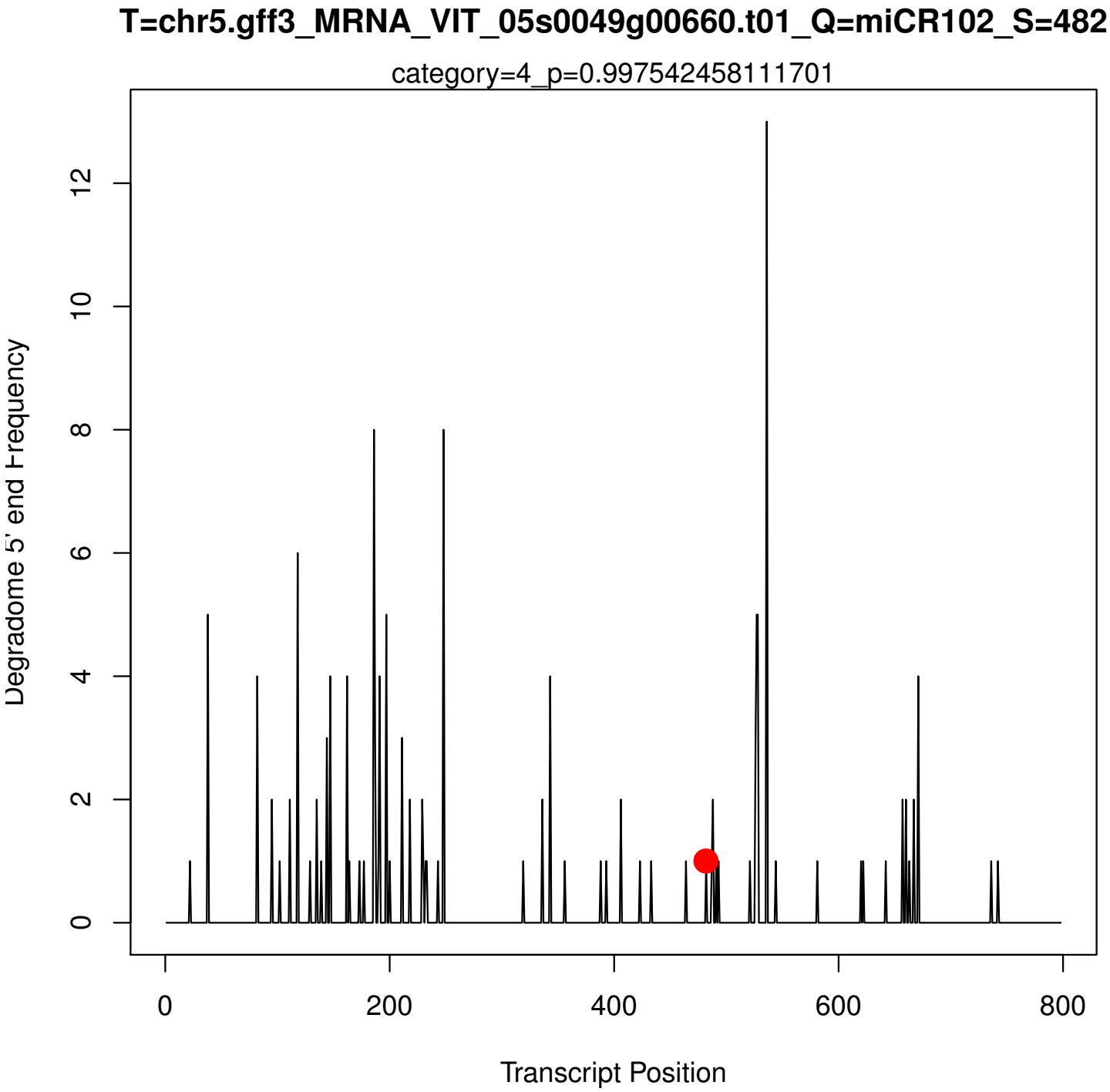
Transcript Position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6
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10
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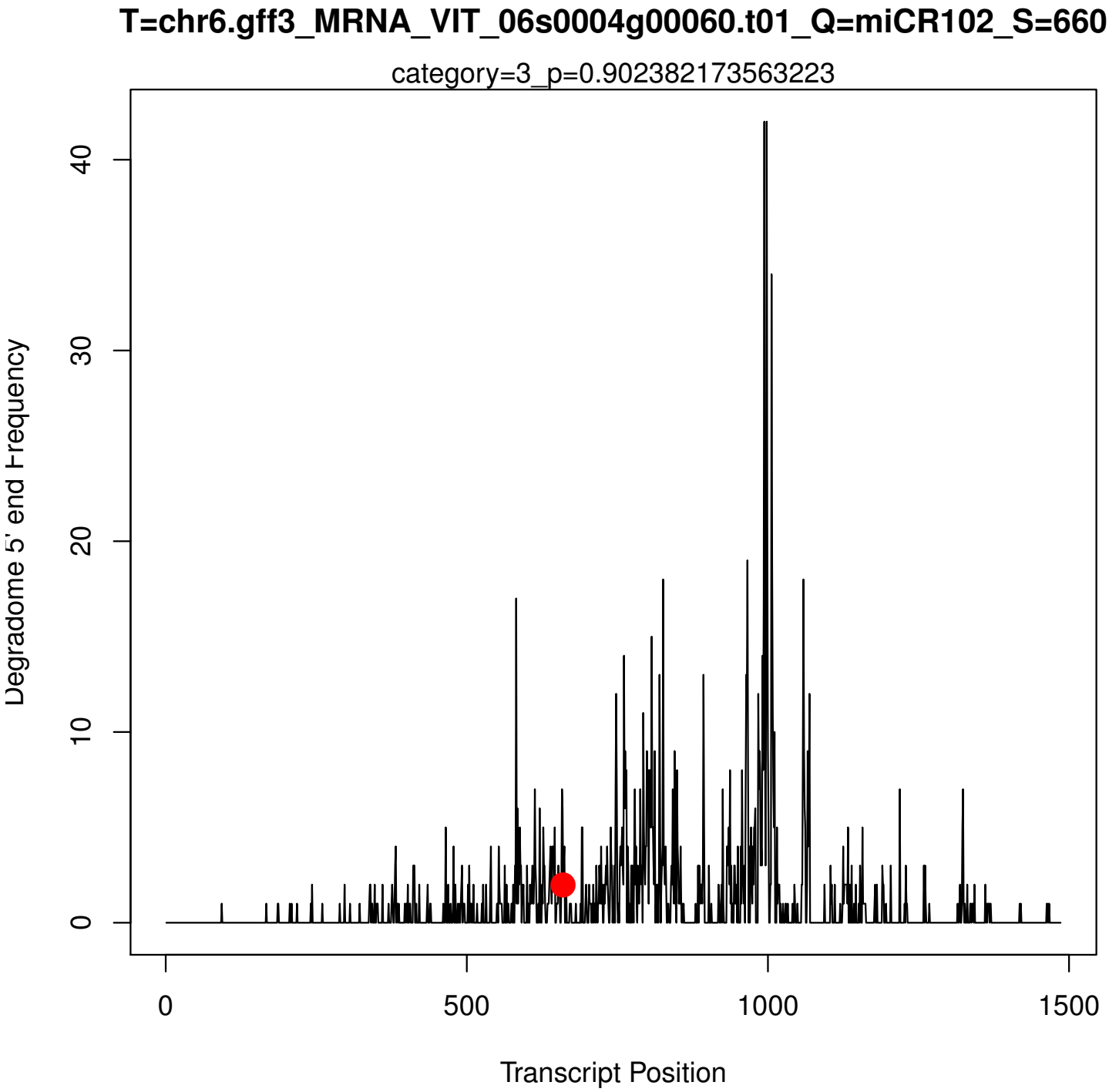
Transcript Position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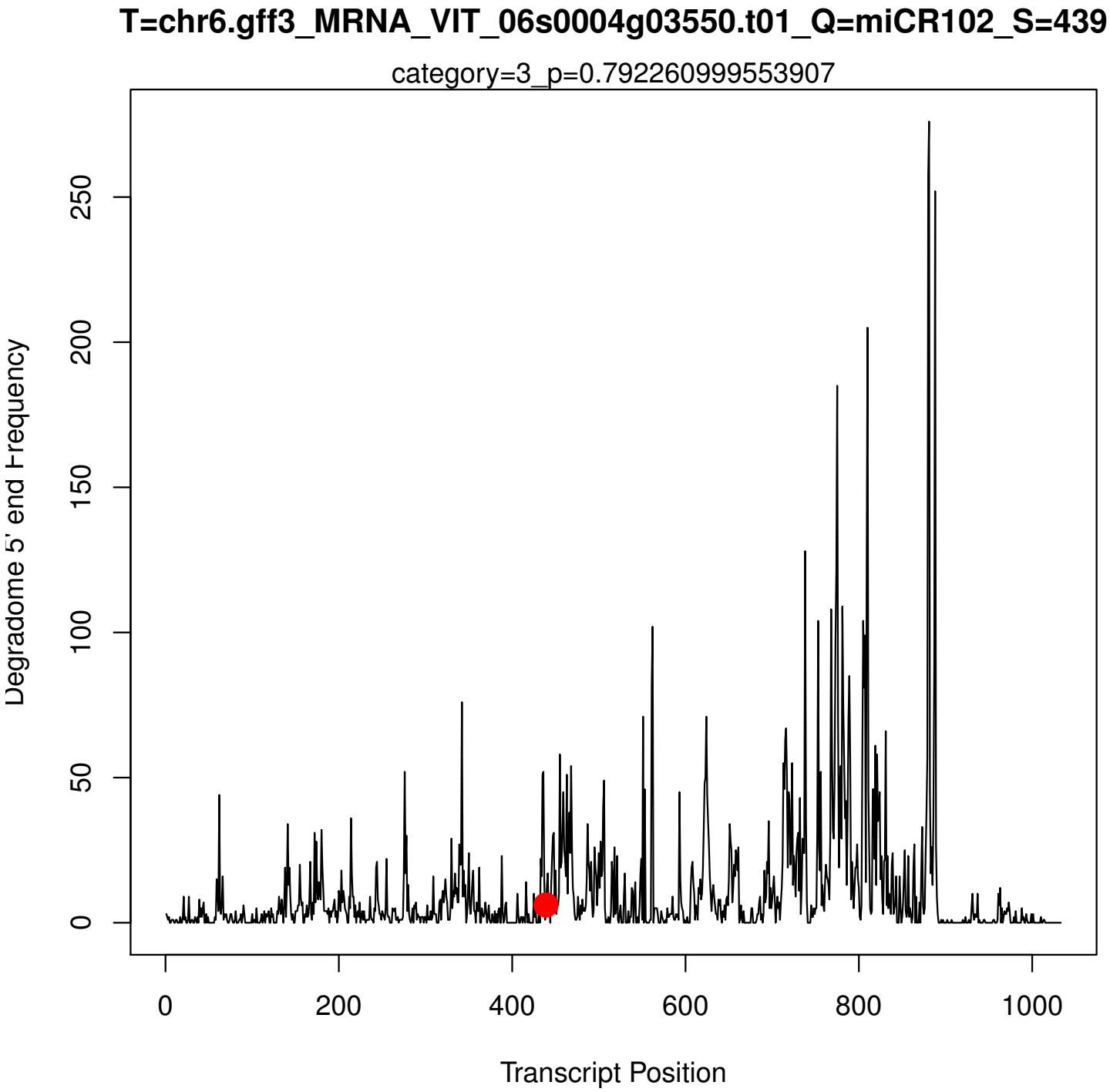
Transcript Position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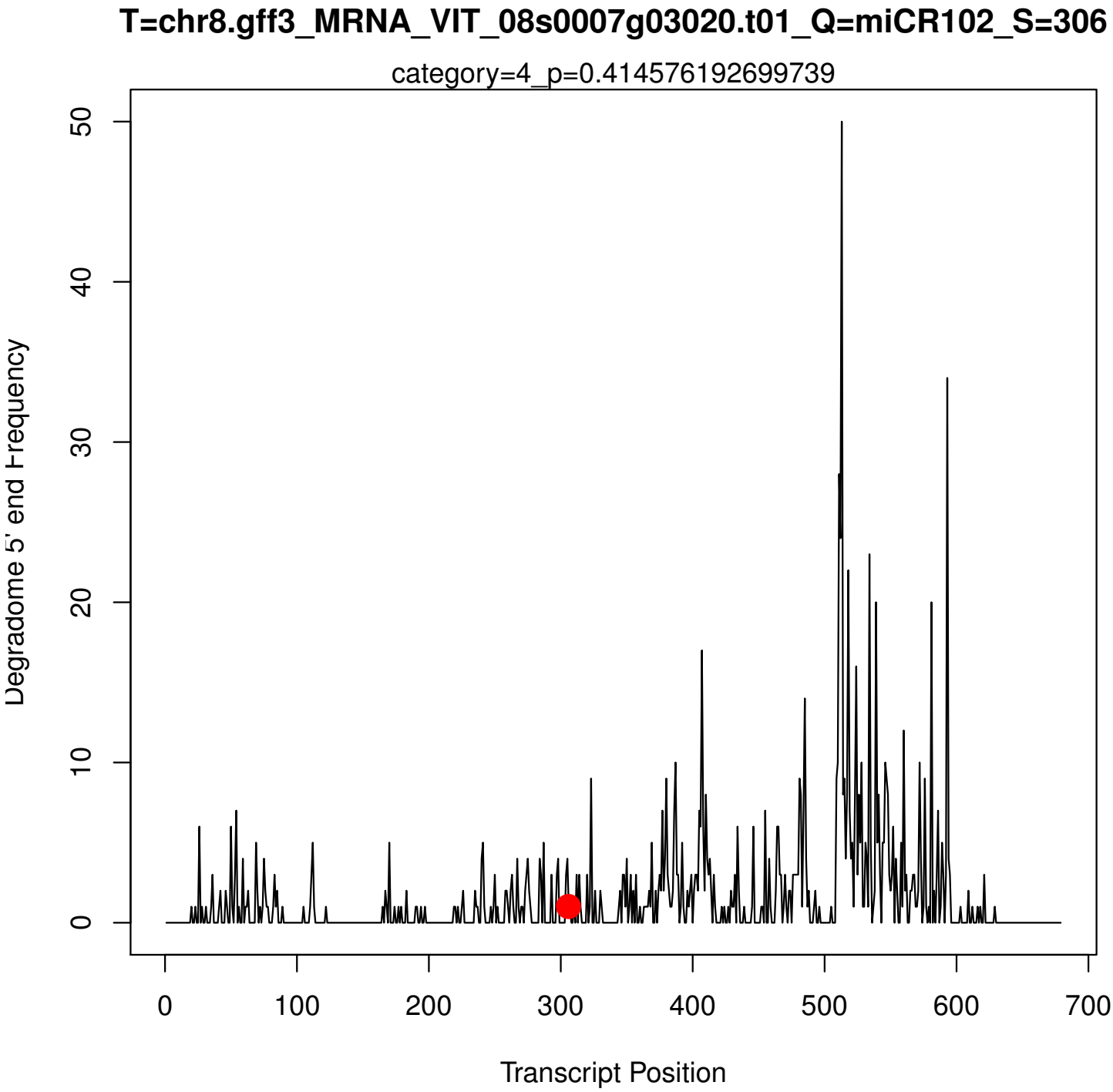
150
200
250
0
200
400
600
800
1000
Transcript Position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500
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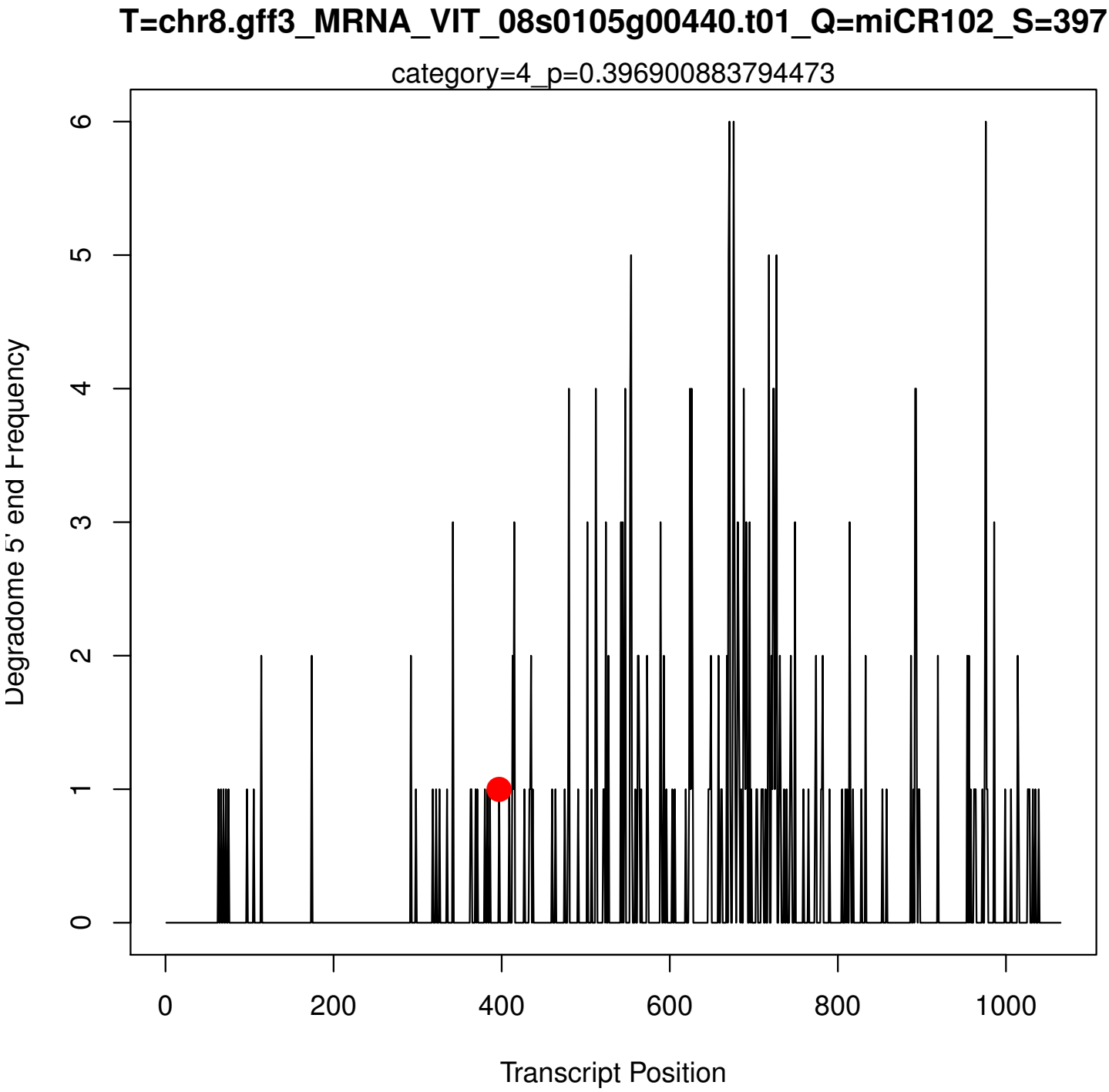
700
Transcript Position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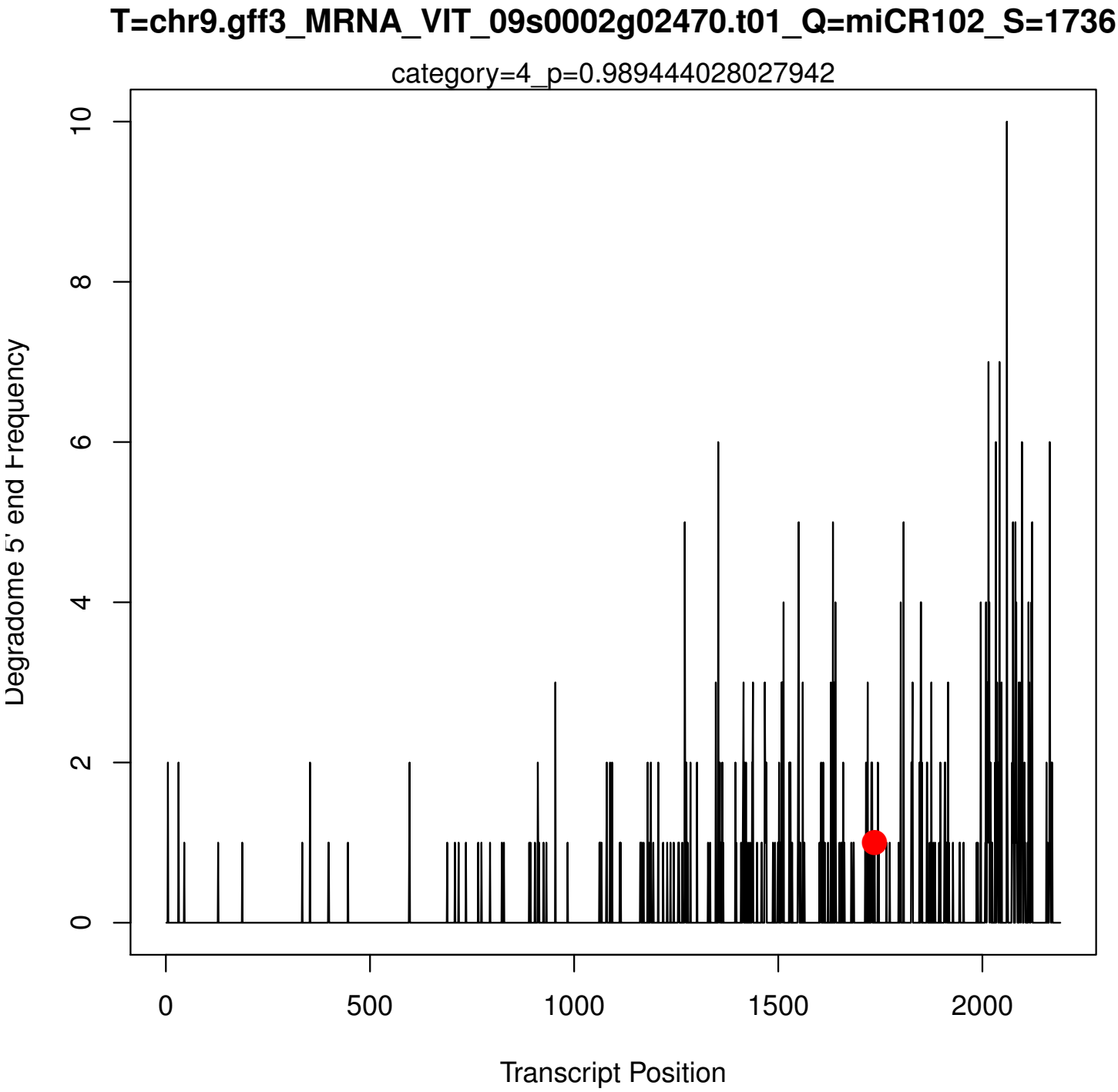
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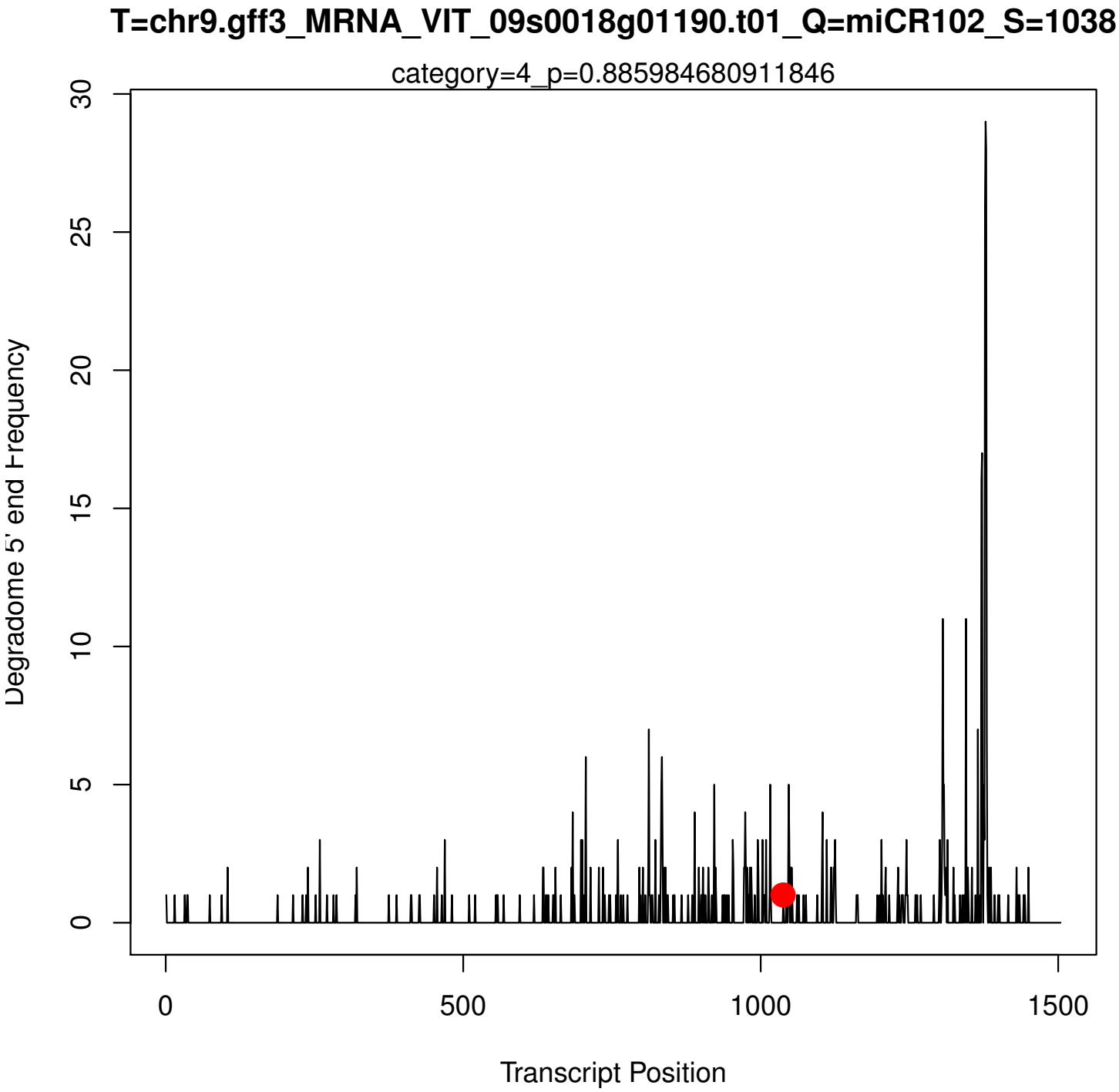
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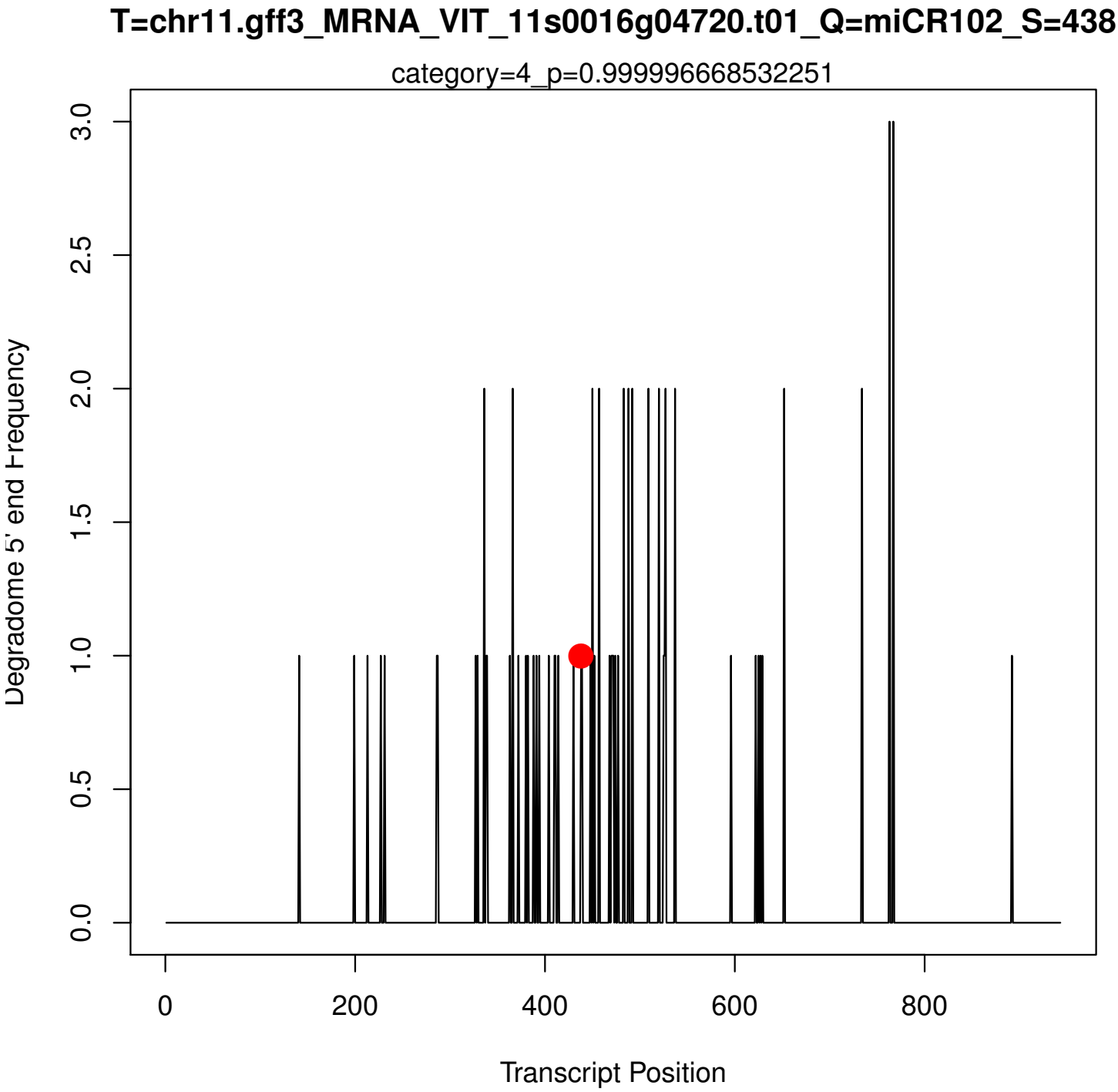
Transcript Position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400
600
800
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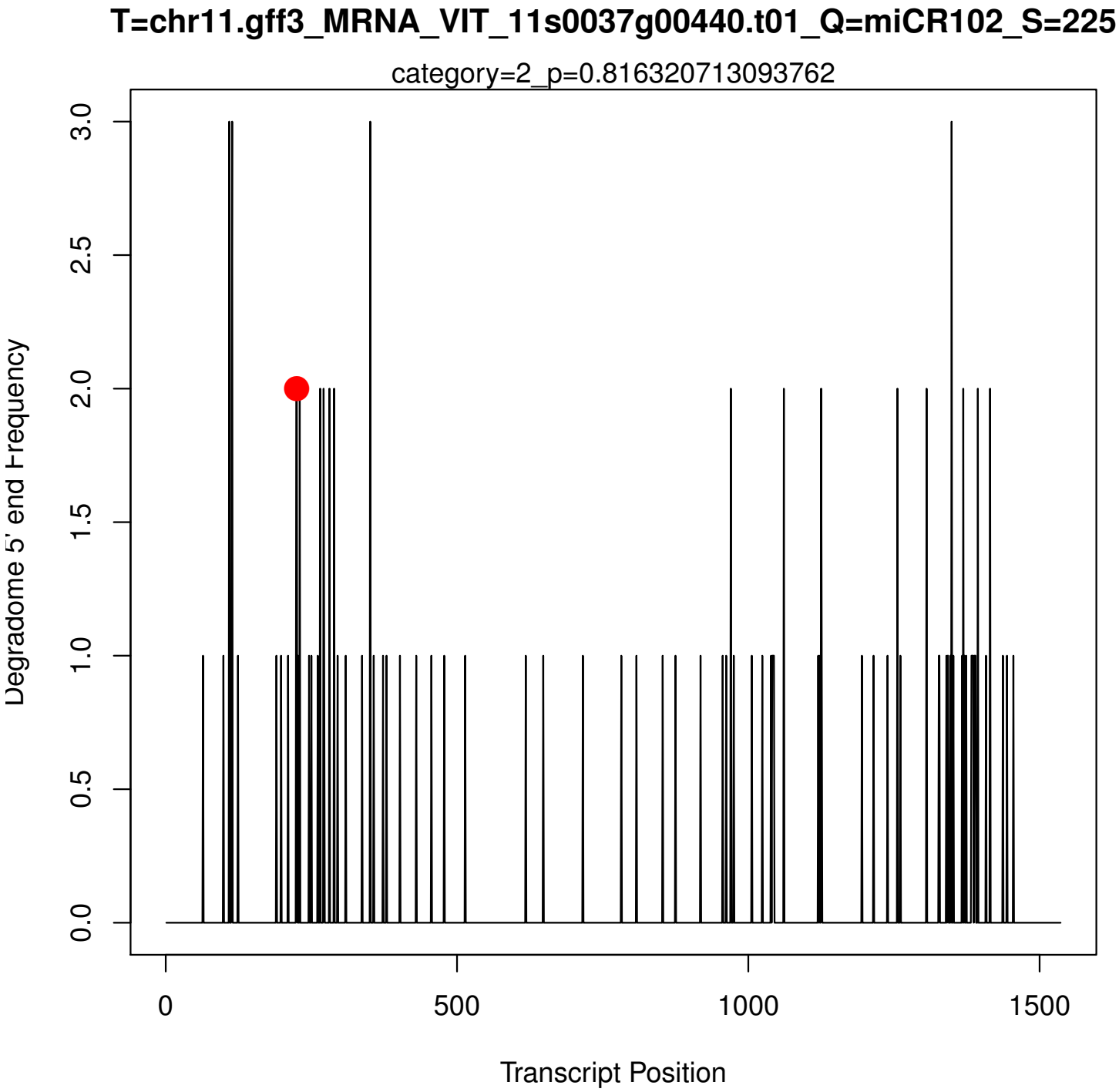
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1.5
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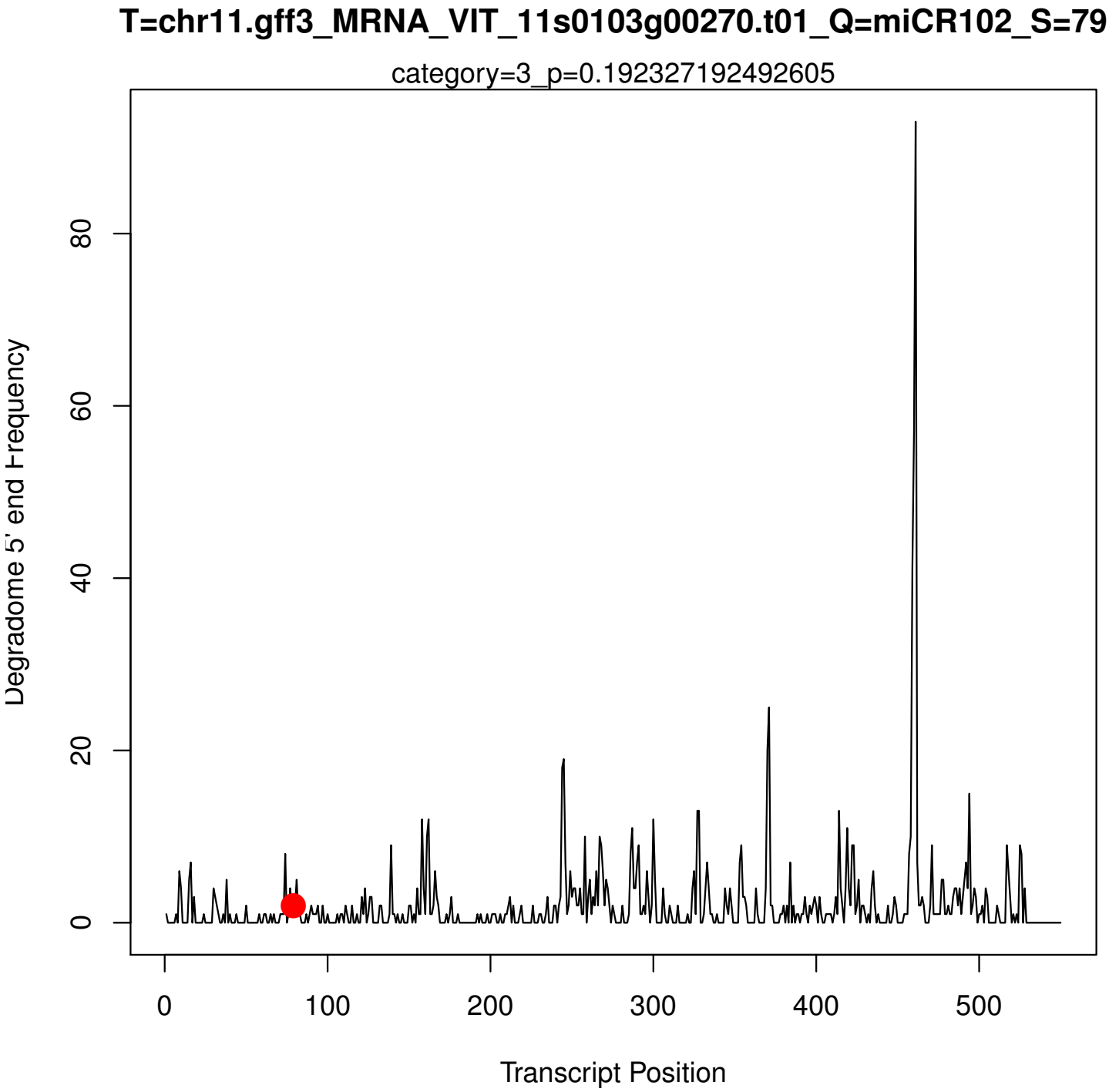
1000
1500
Transcript Position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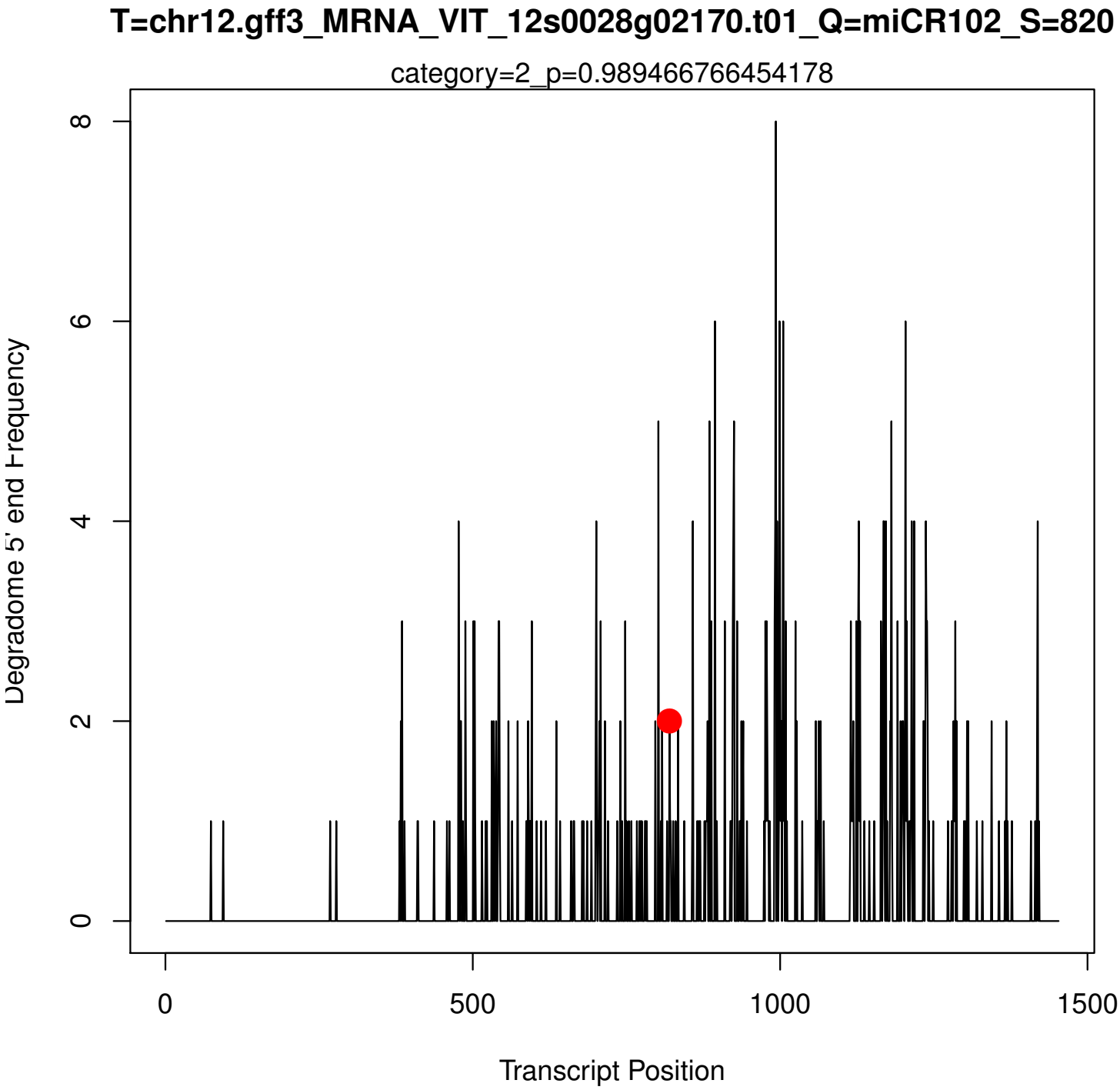
Transcript Position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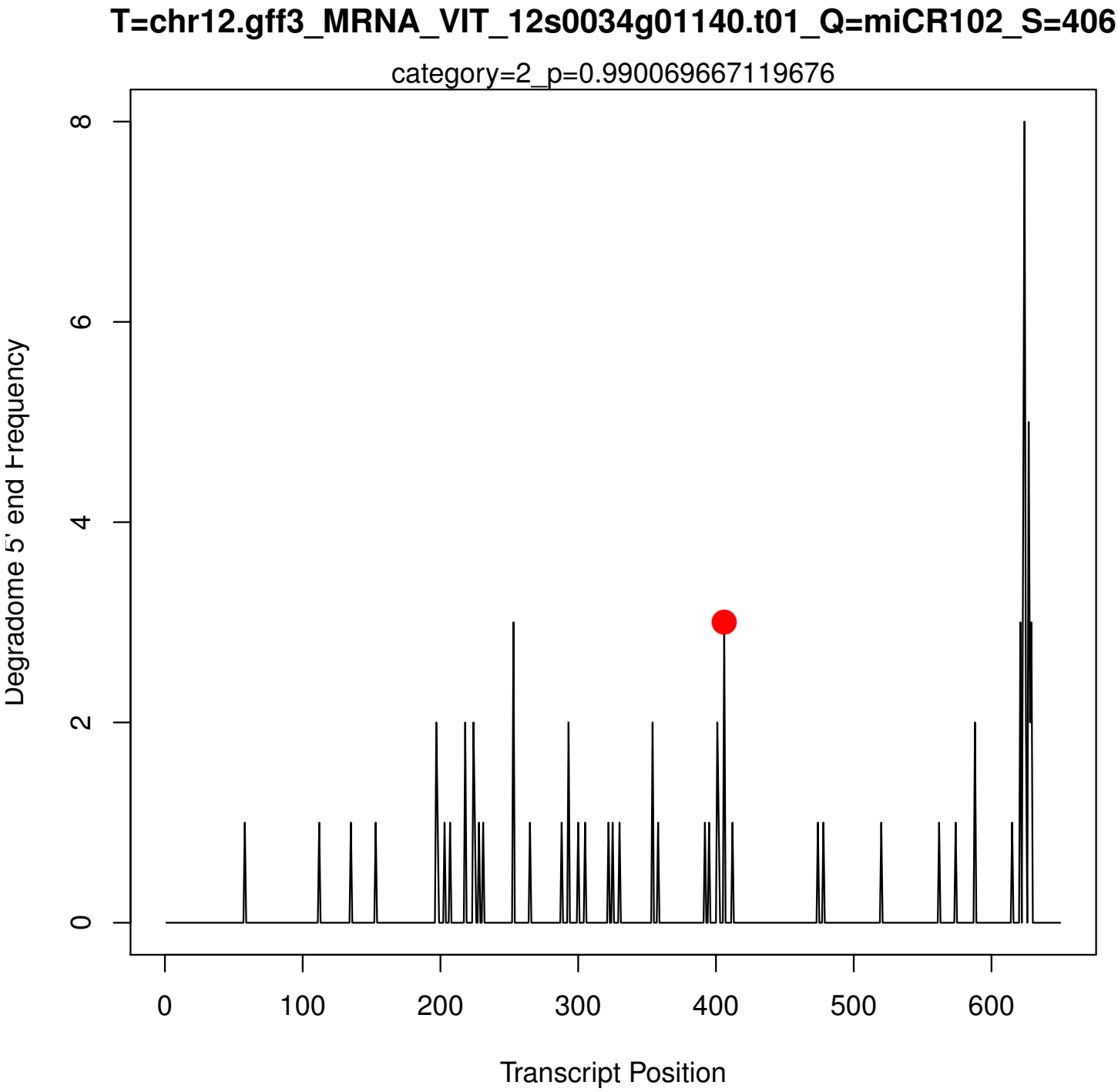
Transcript Position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4
6
8
0
100
200
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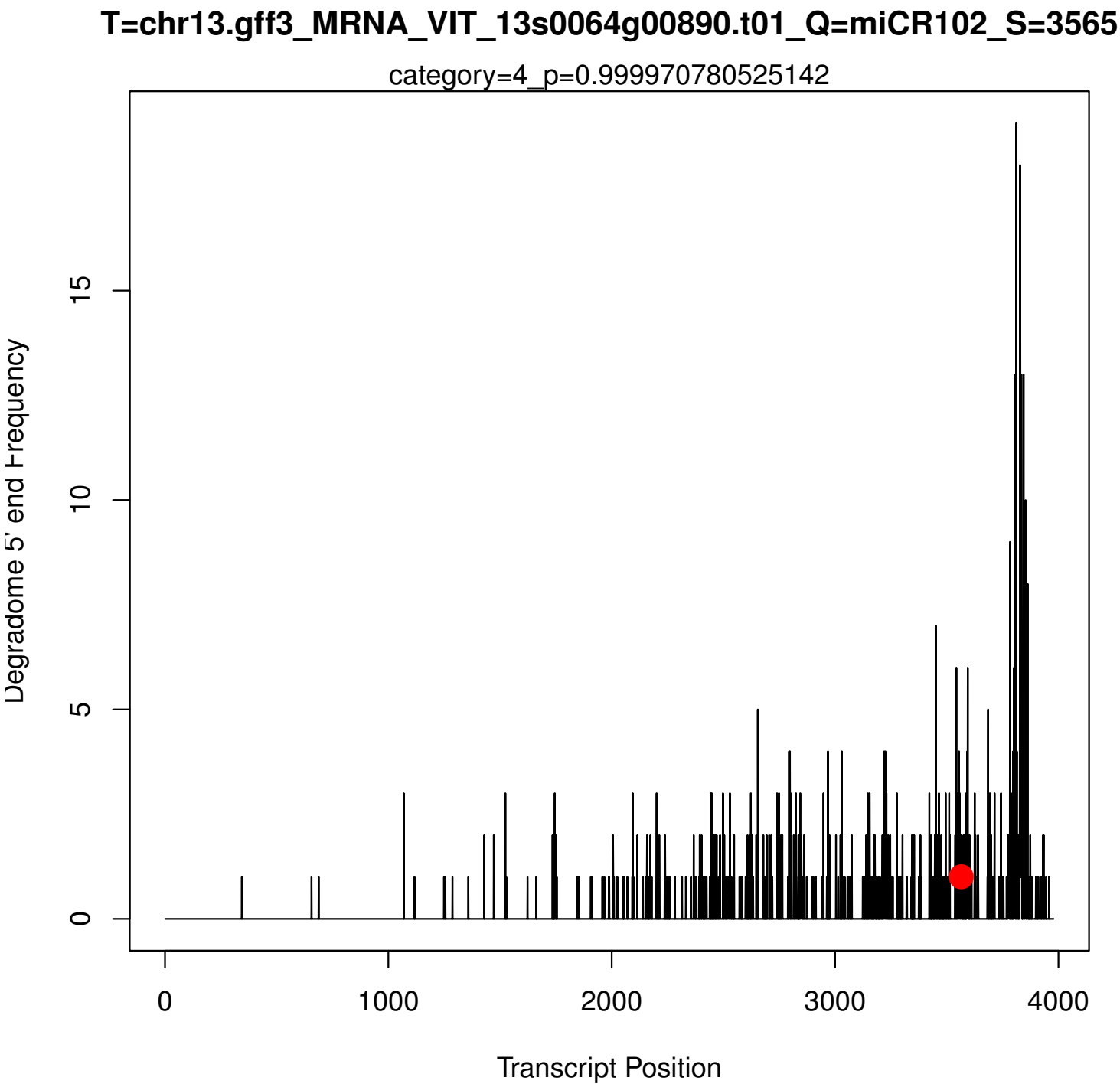
500
600
Transcript Position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10
15
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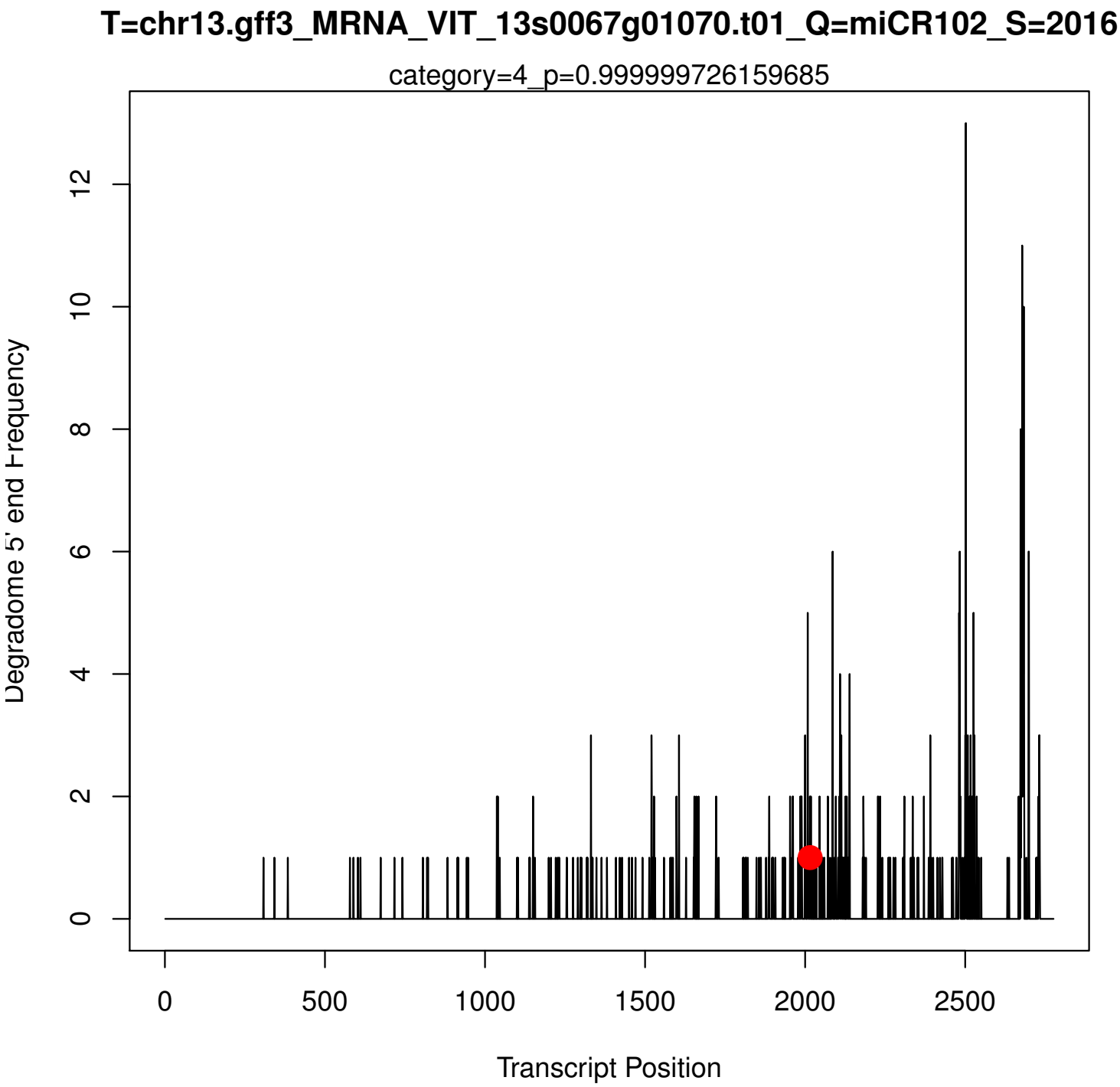
3000
4000
Transcript Position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0
2
4
6
8
10
12
0
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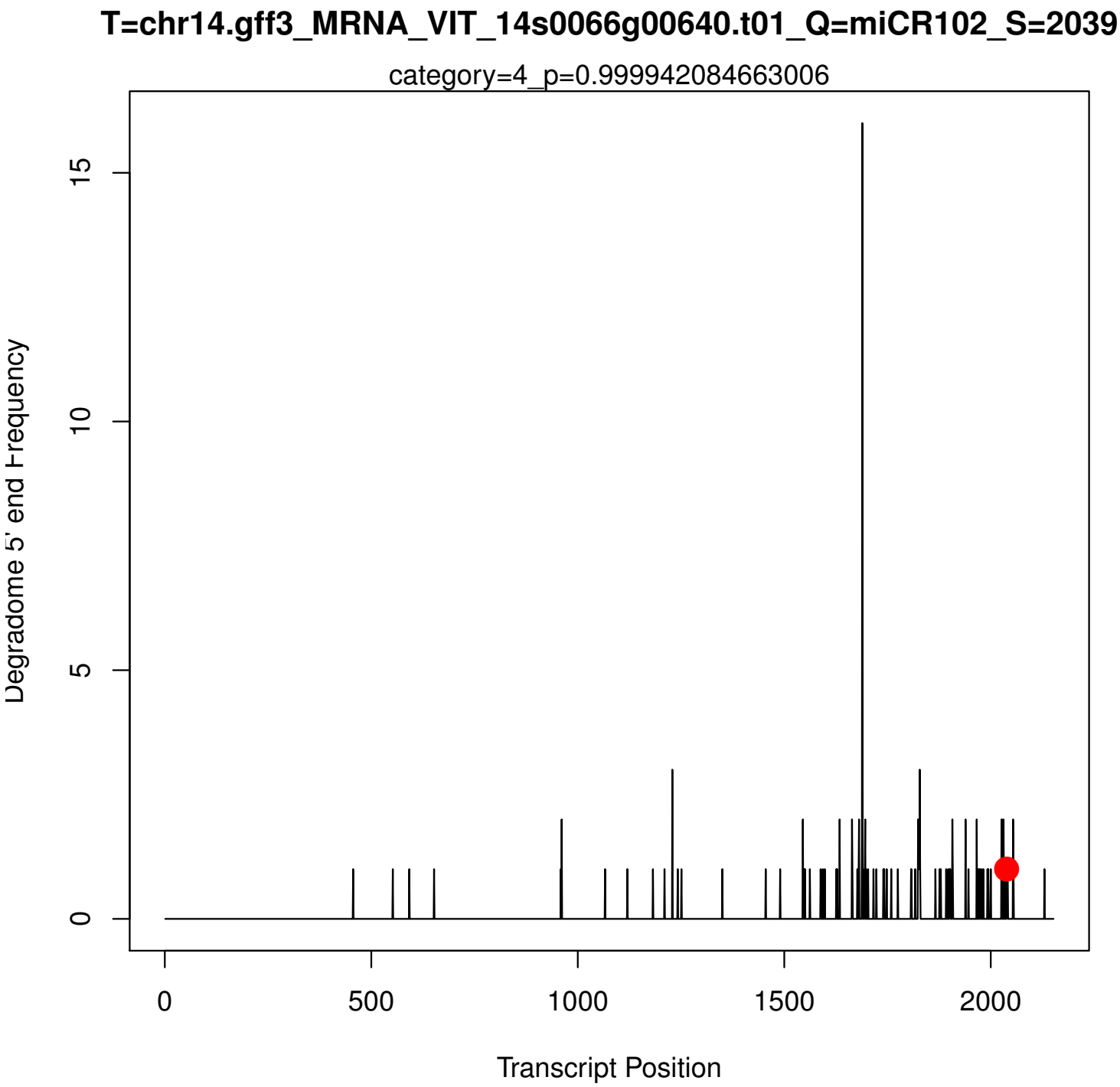
1000
1500
2000
2500
Transcript Position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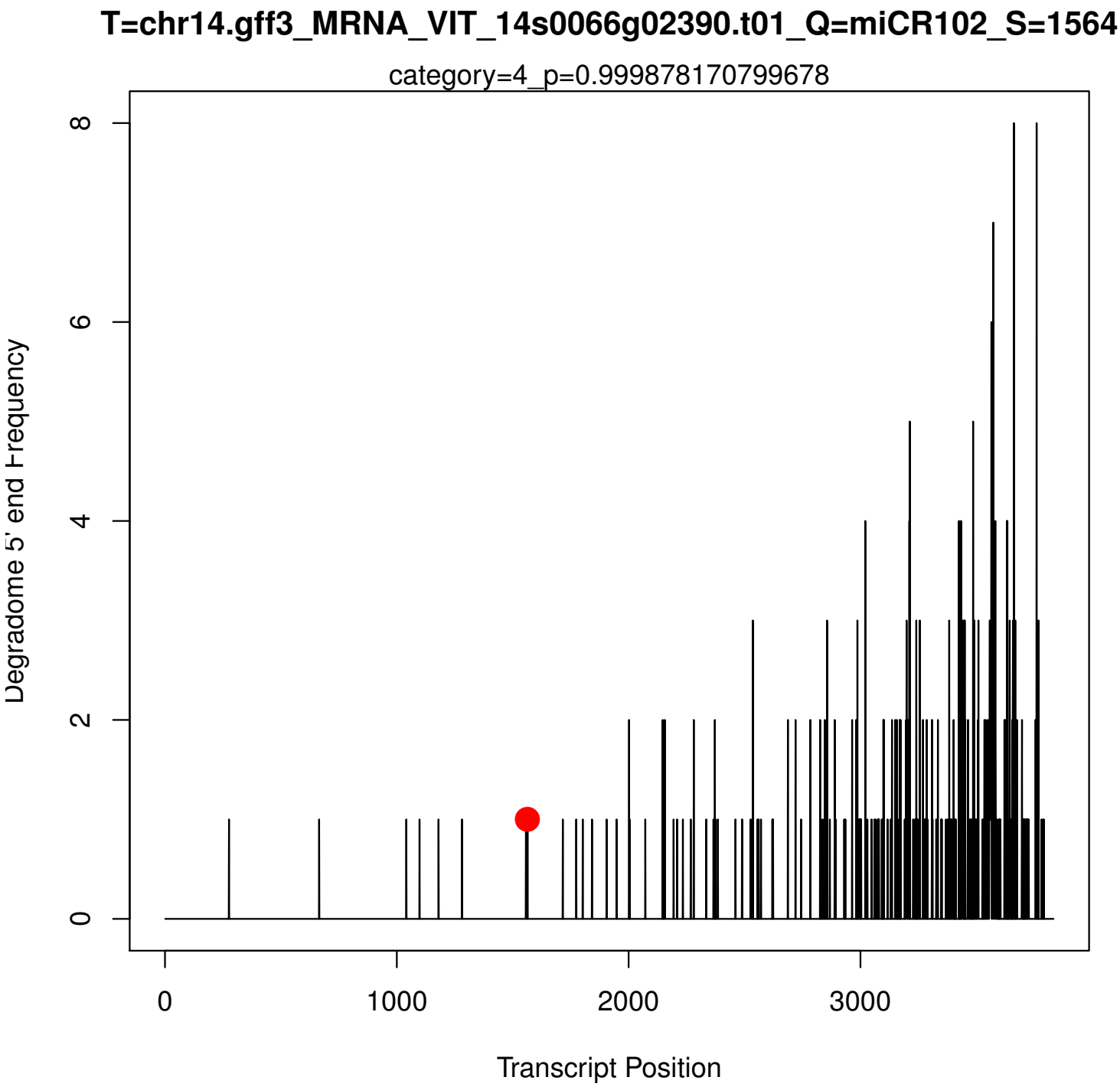
Transcript Position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6
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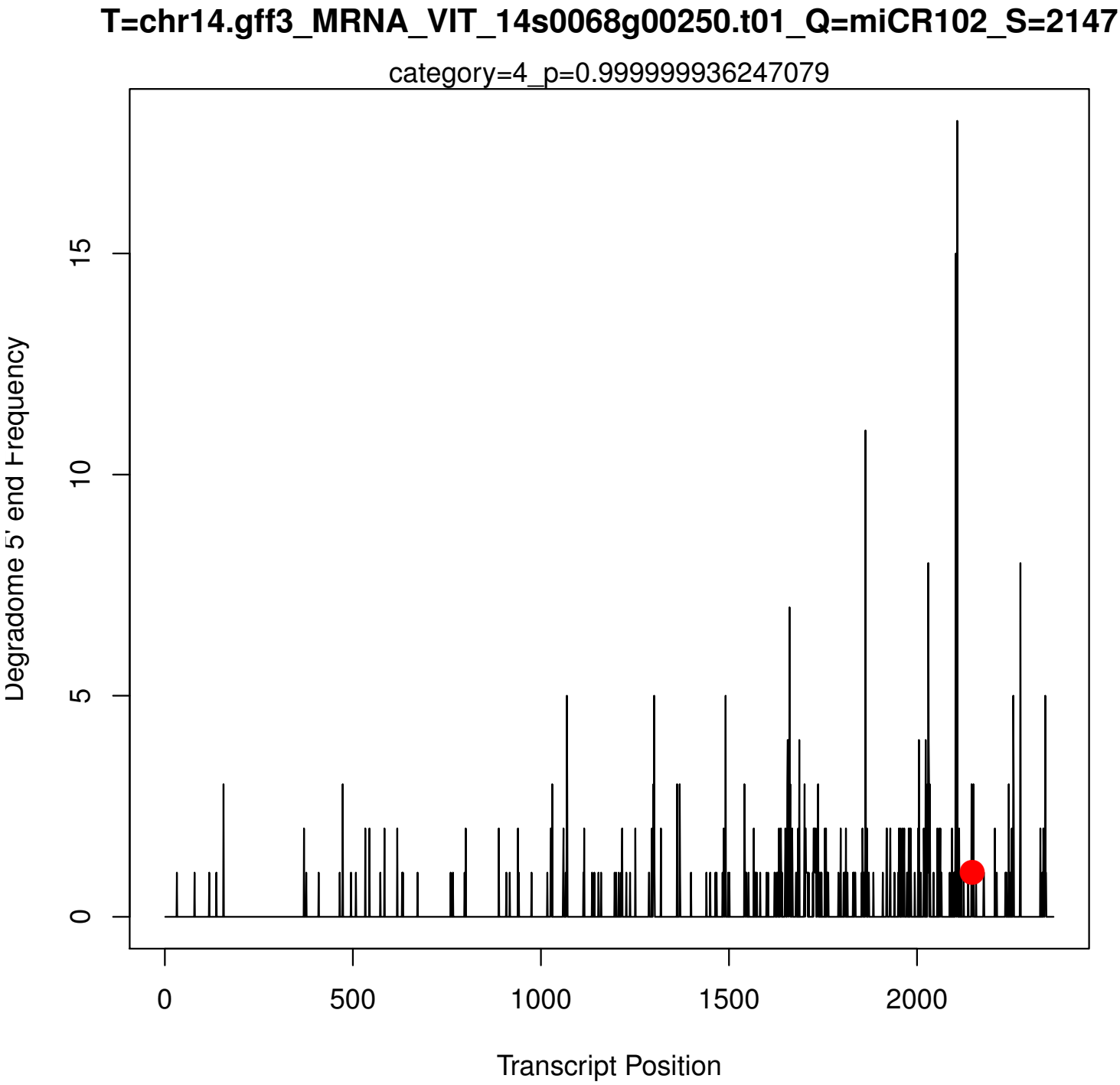
1000
2000
3000
Transcript Position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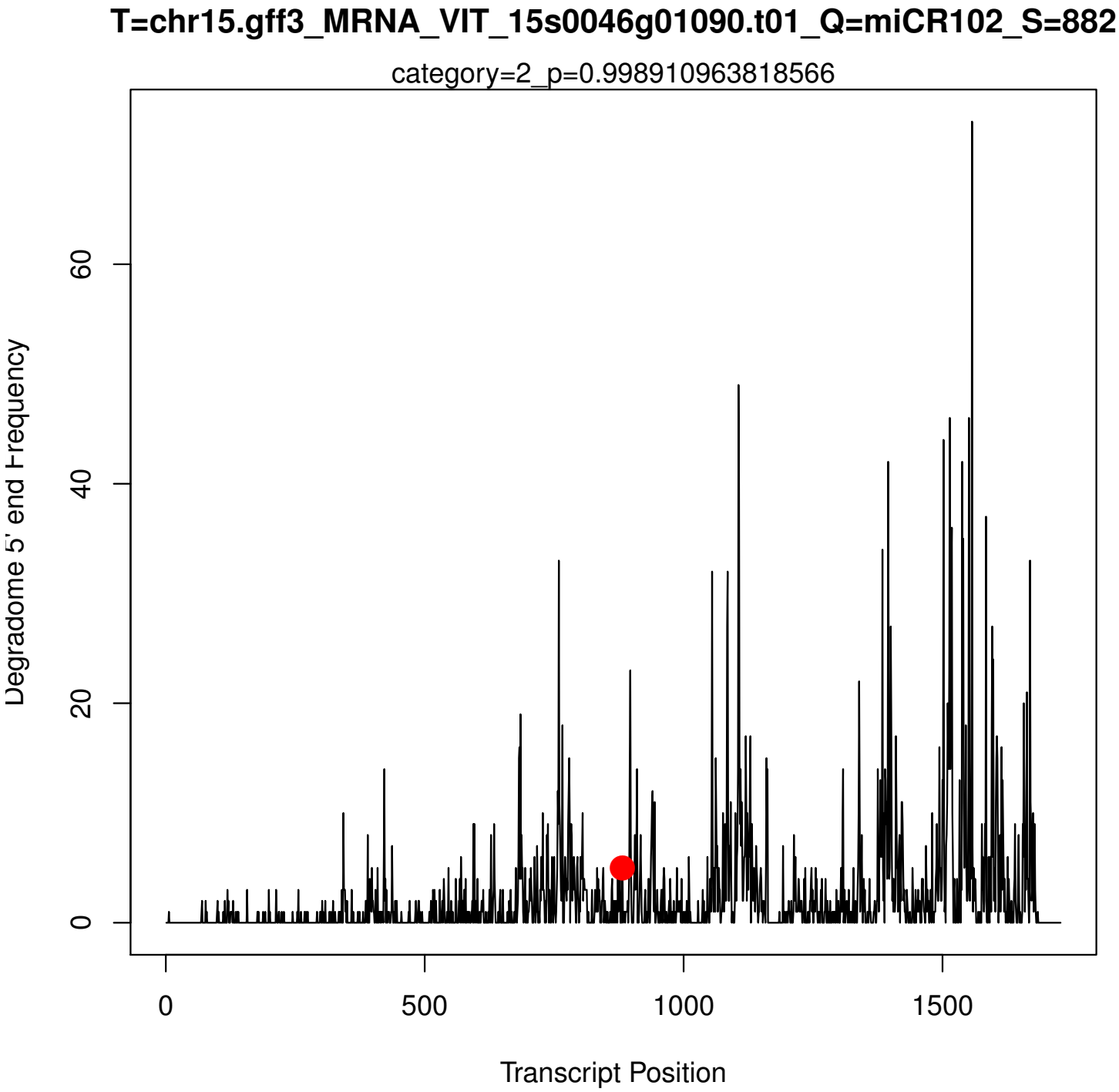
Degradome 5' end Frequency
0
5
10
15
0
500
1000
1500
2000
Transcript Position


T=chr15.gff3_MRNA_VIT_15s0046g01090.t01_Q=miCR102_S=882
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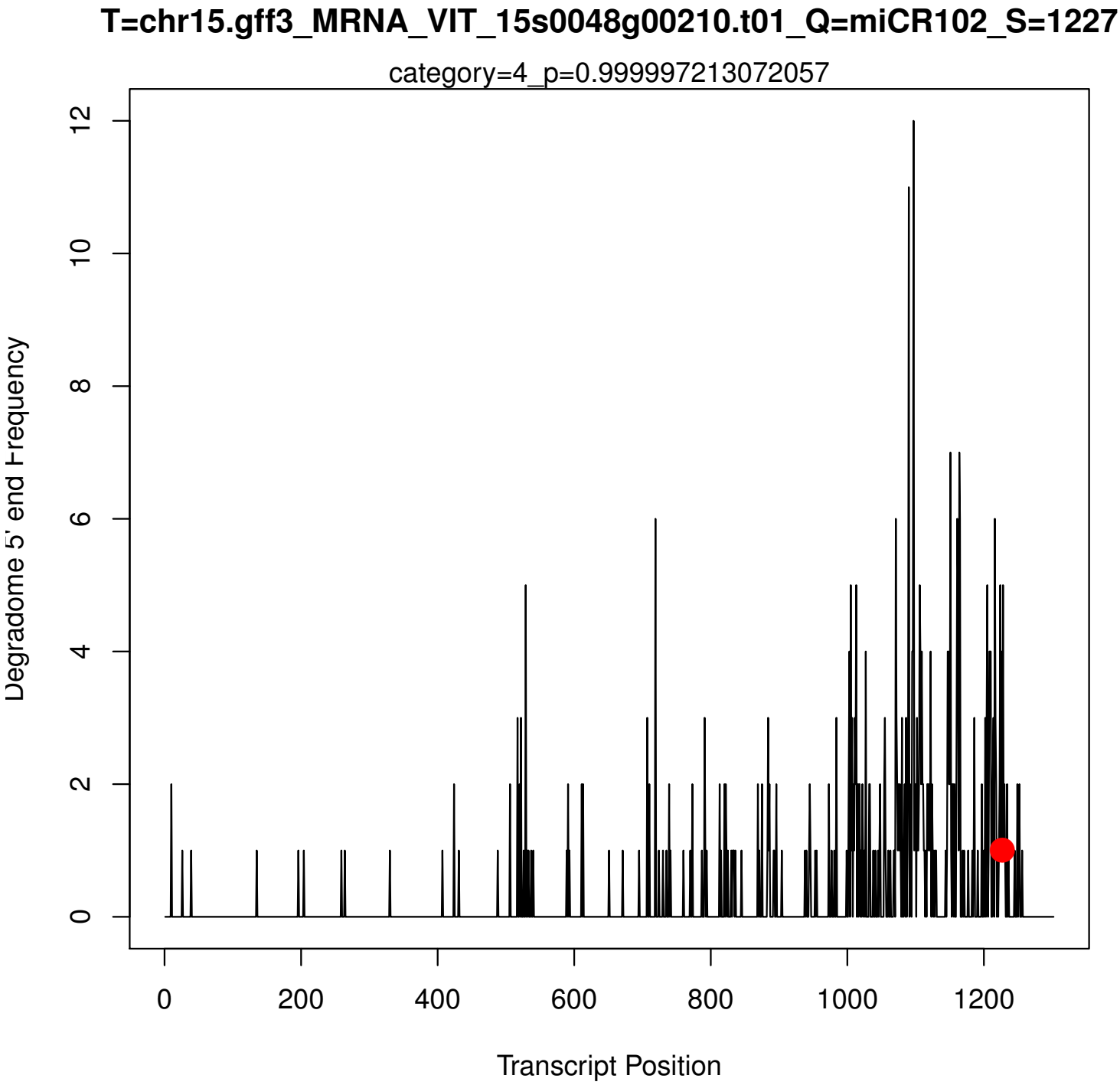
Transcript Position


T=chr15.gff3_MRNA_VIT_15s0048g00210.t01_Q=miCR102_S=1227
category=4_p=0.999997213072057
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200
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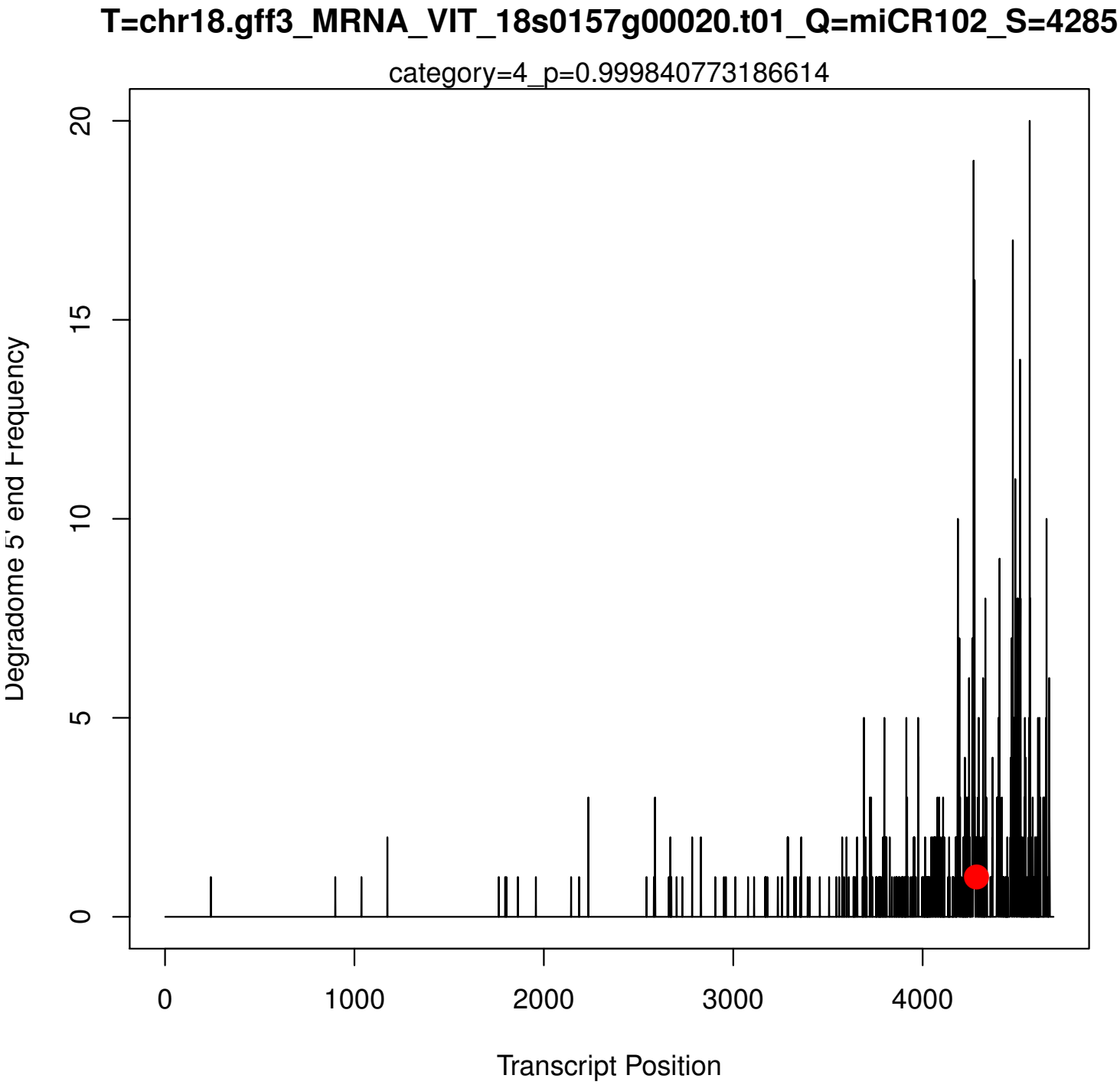
1200
Transcript Position


T=chr18.gff3_MRNA_VIT_18s0157g00020.t01_Q=miCR102_S=4285
category=4_p=0.999840773186614
Degradome 5' end Frequency
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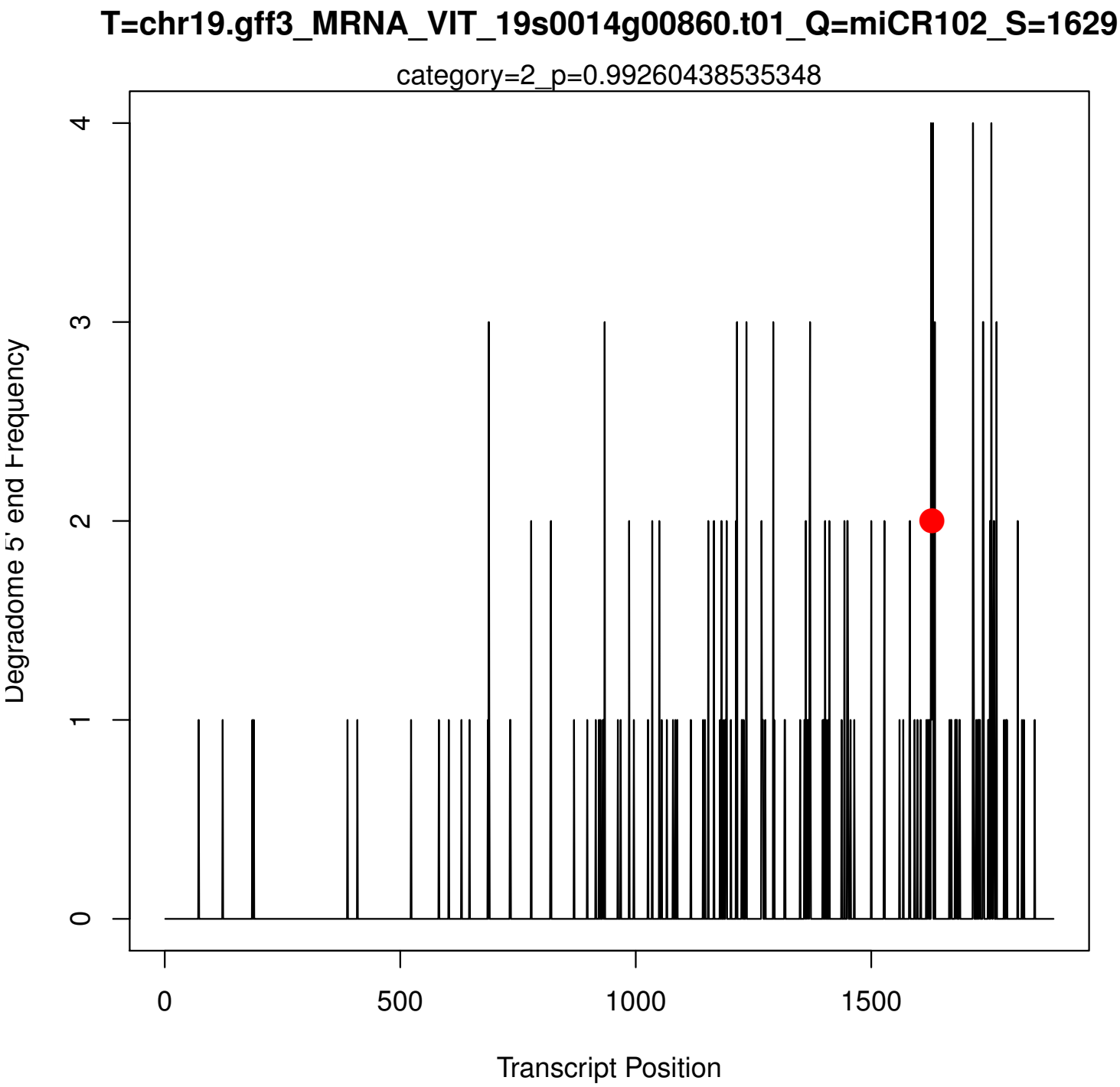
Transcript Position


T=chr19.gff3_MRNA_VIT_19s0014g00860.t01_Q=miCR102_S=1629
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3
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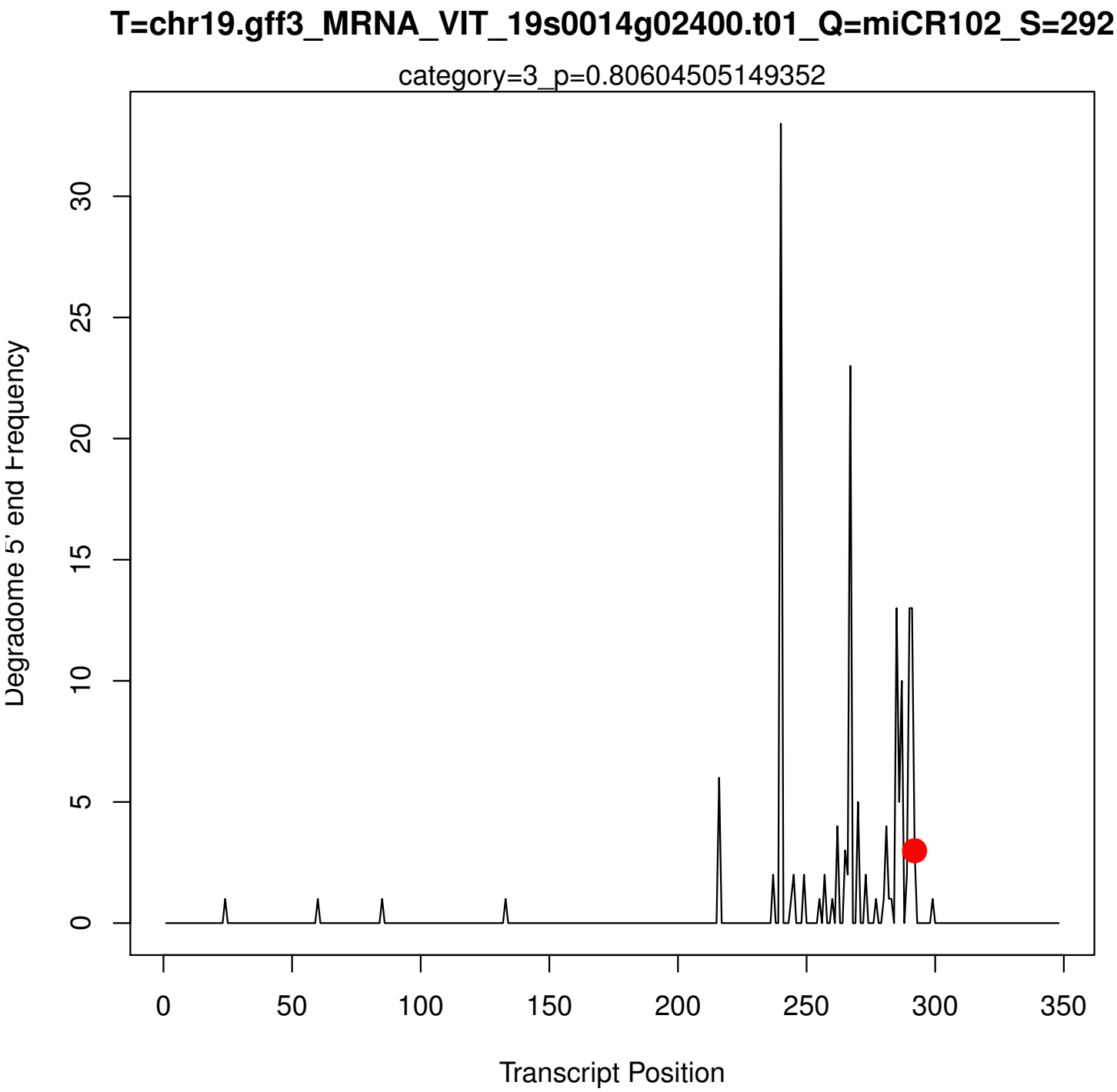
Transcript Position


T=chr19.gff3_MRNA_VIT_19s0014g02400.t01_Q=miCR102_S=292
category=3_p=0.80604505149352
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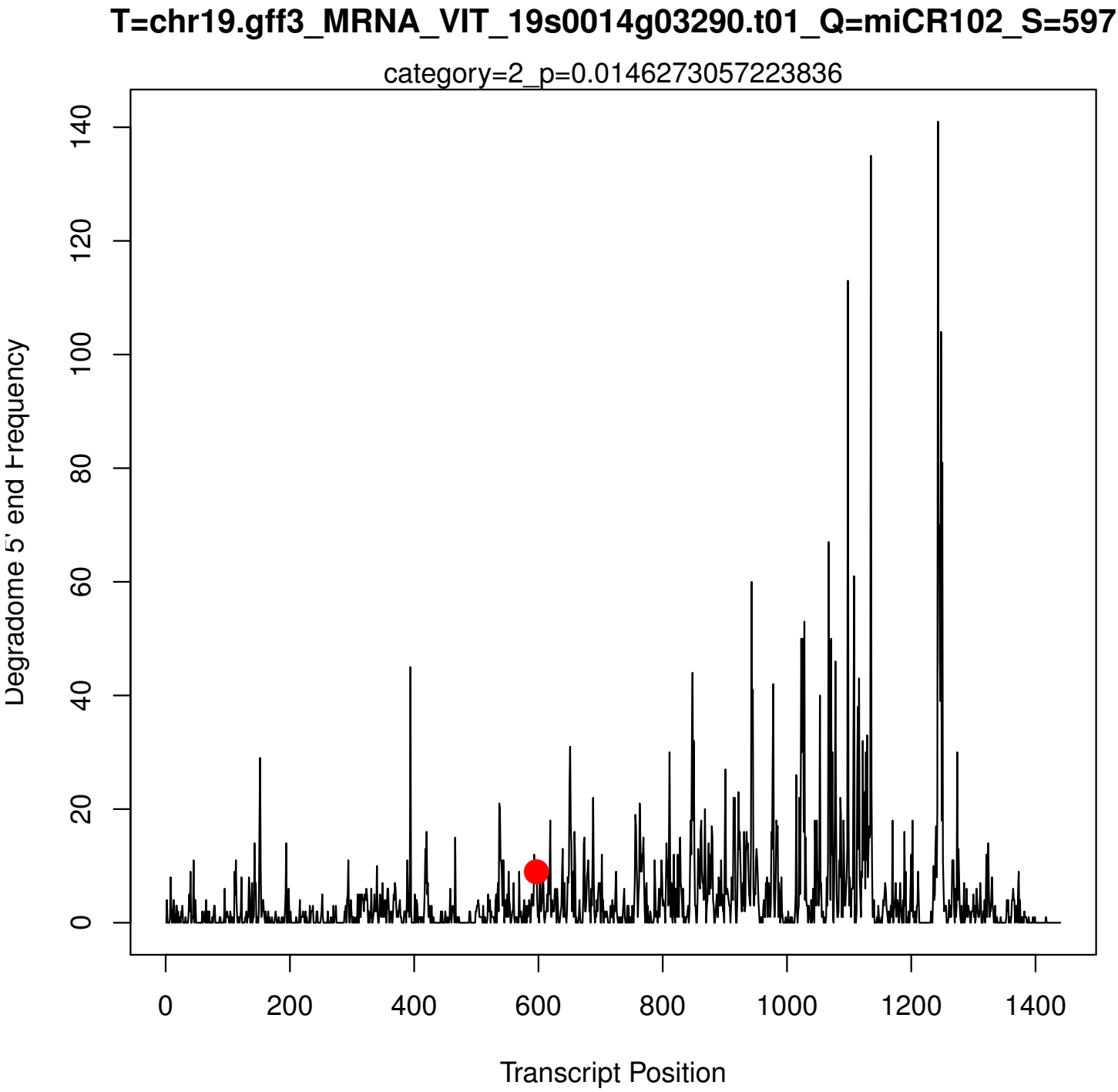
Transcript Position


T=chr19.gff3_MRNA_VIT_19s0014g03290.t01_Q=miCR102_S=597
category=2_p=0.0146273057223836
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400
600
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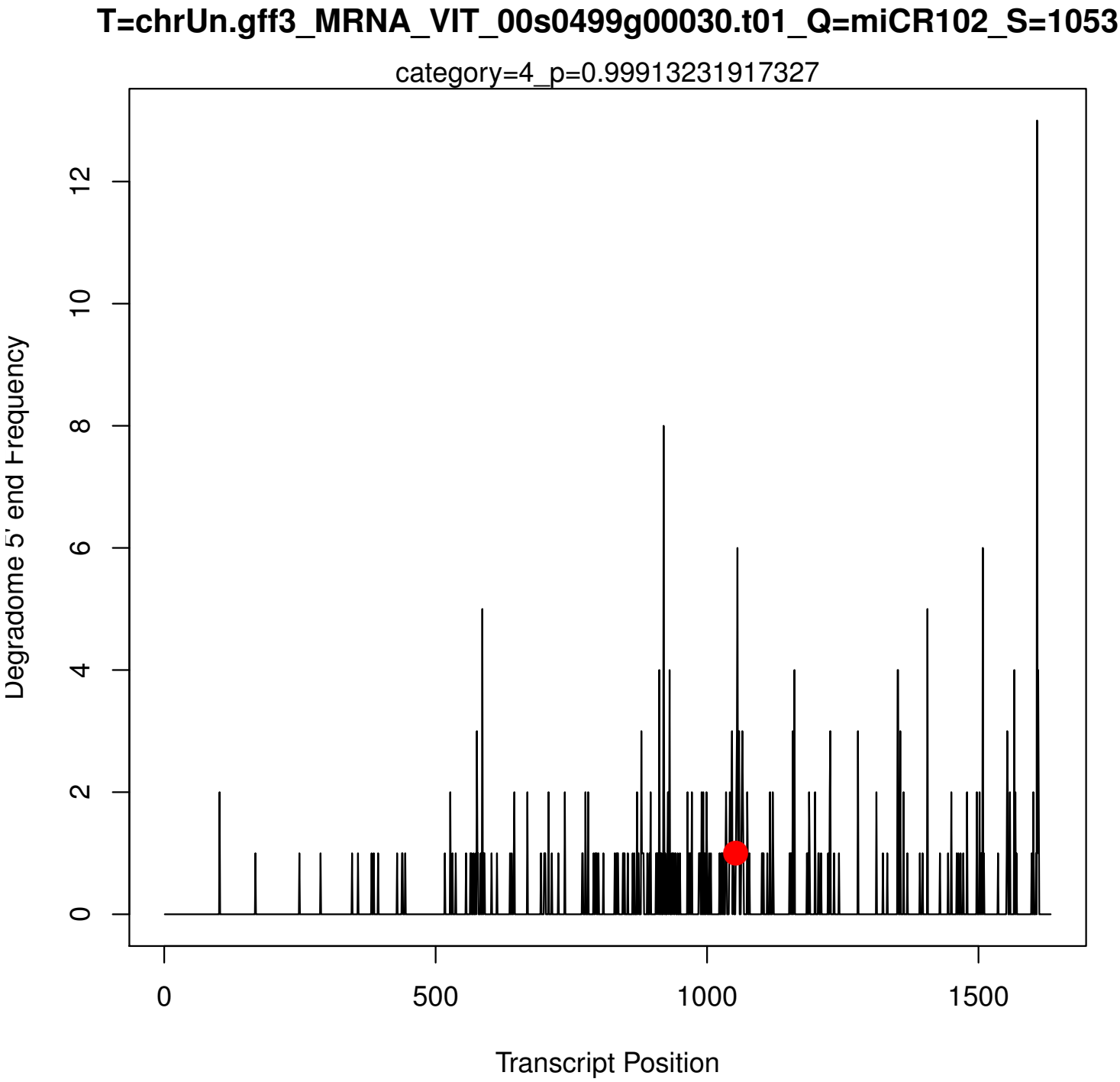
1000
1200
1400
Transcript Position


T=chrUn.gff3_MRNA_VIT_00s0499g00030.t01_Q=miCR102_S=1053
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Degradome 5' end Frequency
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6
8
10
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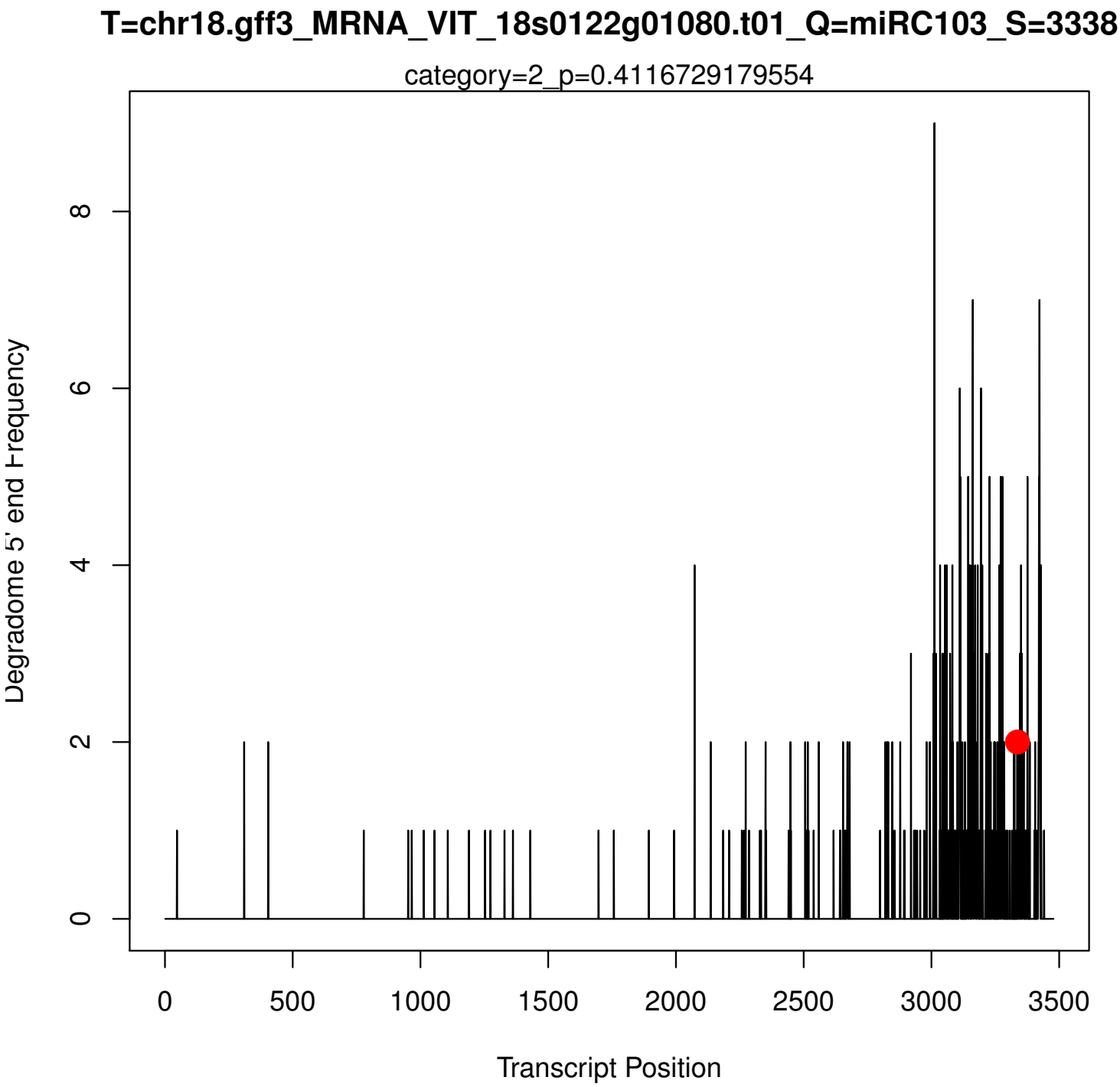
Transcript Position


T=chr18.gff3_MRNA_VIT_18s0122g01080.t01_Q=miRC103_S=3338
category=2_p=0.4116729179554
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2
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6
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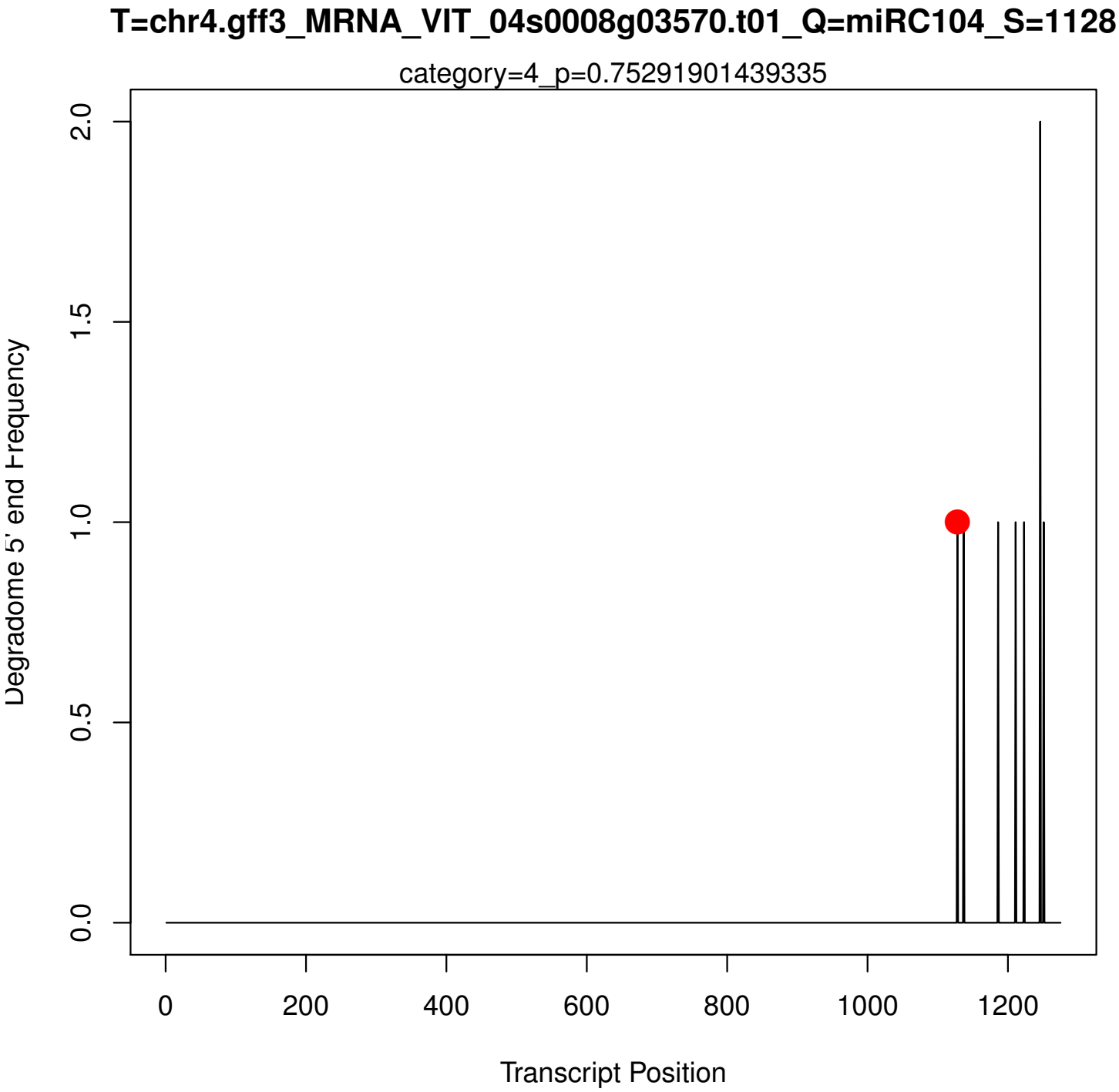
3500
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0008g03570.t01_Q=miRC104_S=1128
category=4_p=0.75291901439335
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0
200
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600
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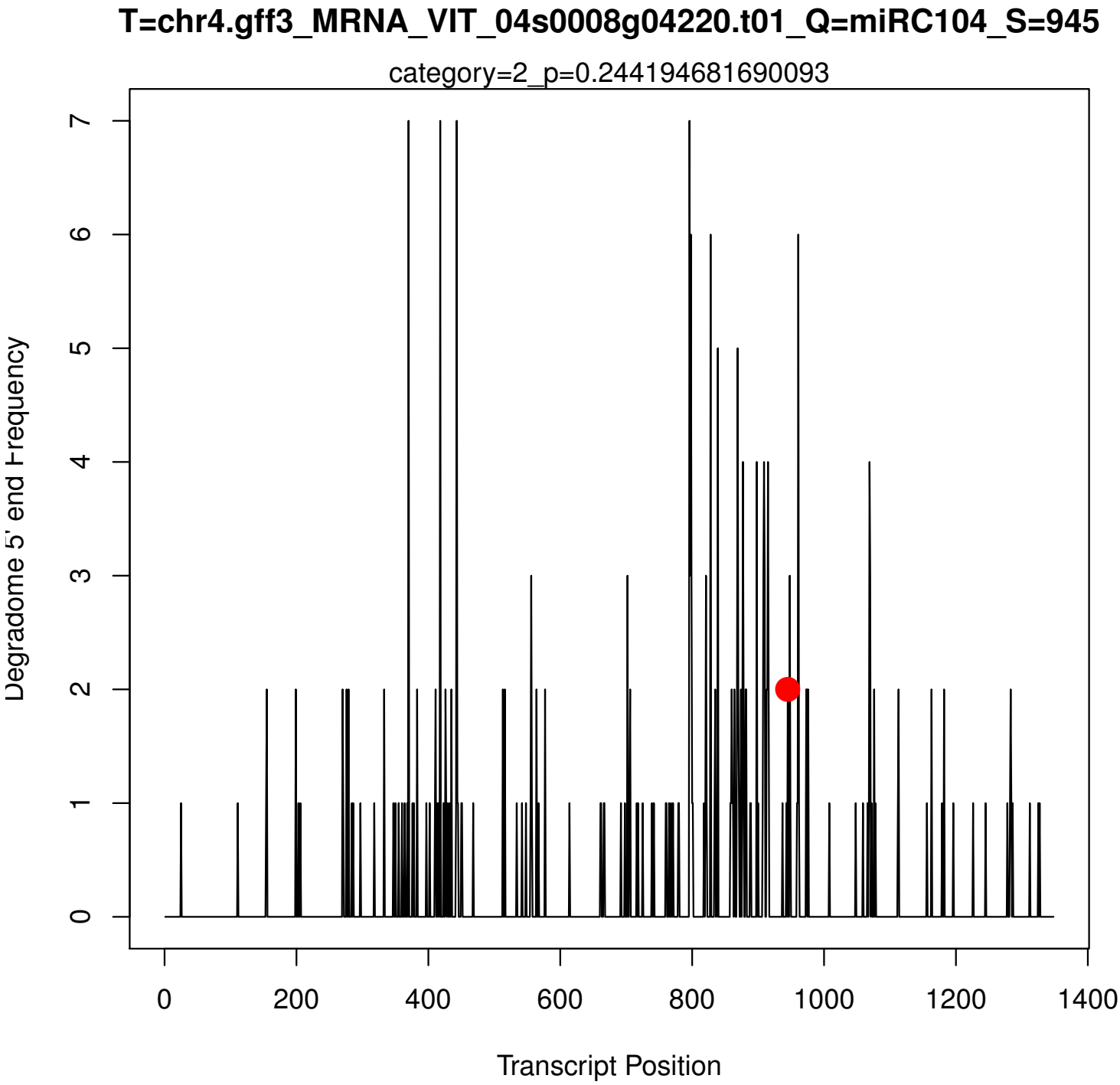
1000
1200
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0008g04220.t01_Q=miRC104_S=945
category=2_p=0.244194681690093
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2
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4
5
6
7
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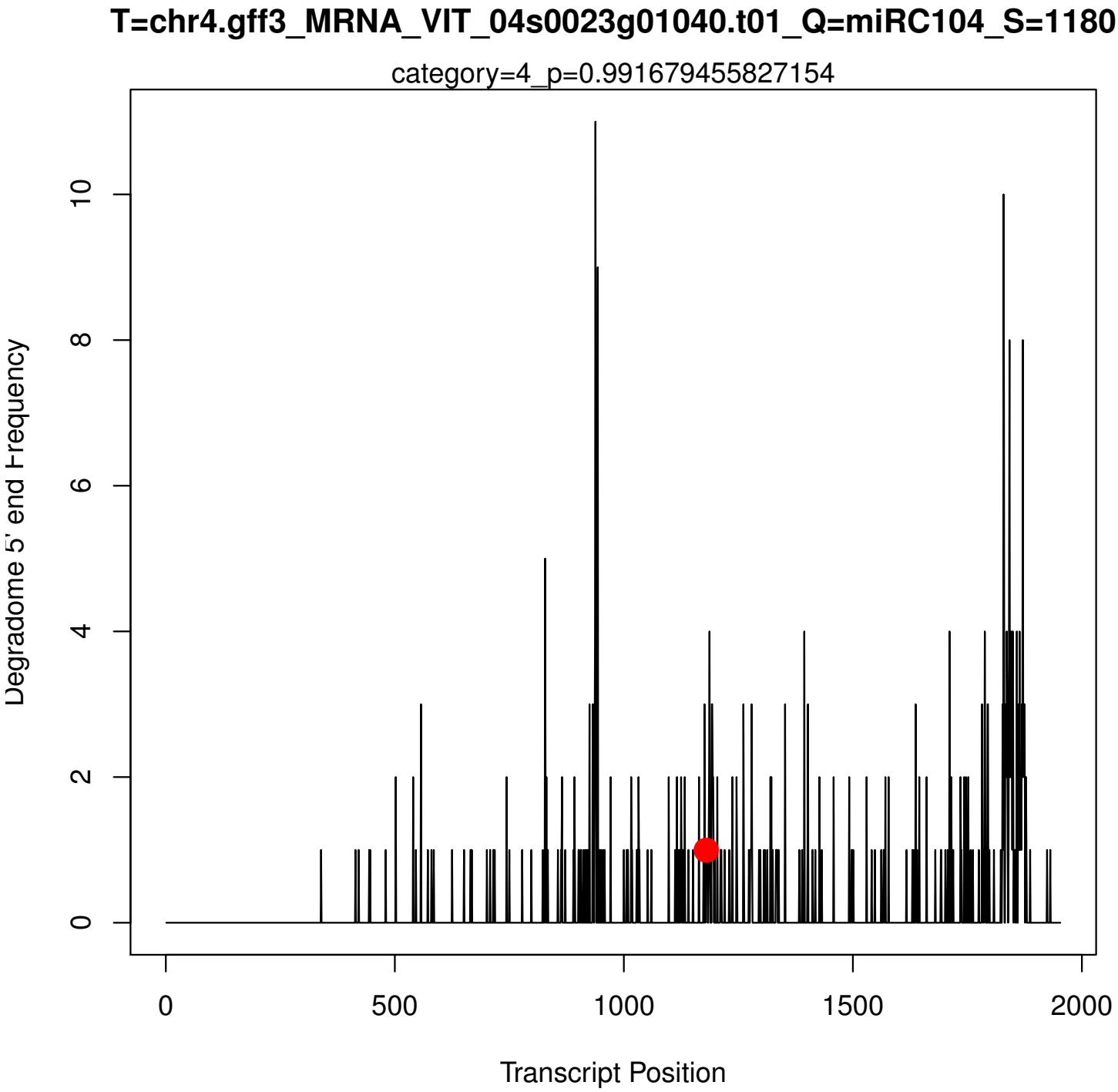
1400
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0023g01040.t01_Q=miRC104_S=1180
category=4_p=0.991679455827154
Degradome 5' end Frequency
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4
6
8
10
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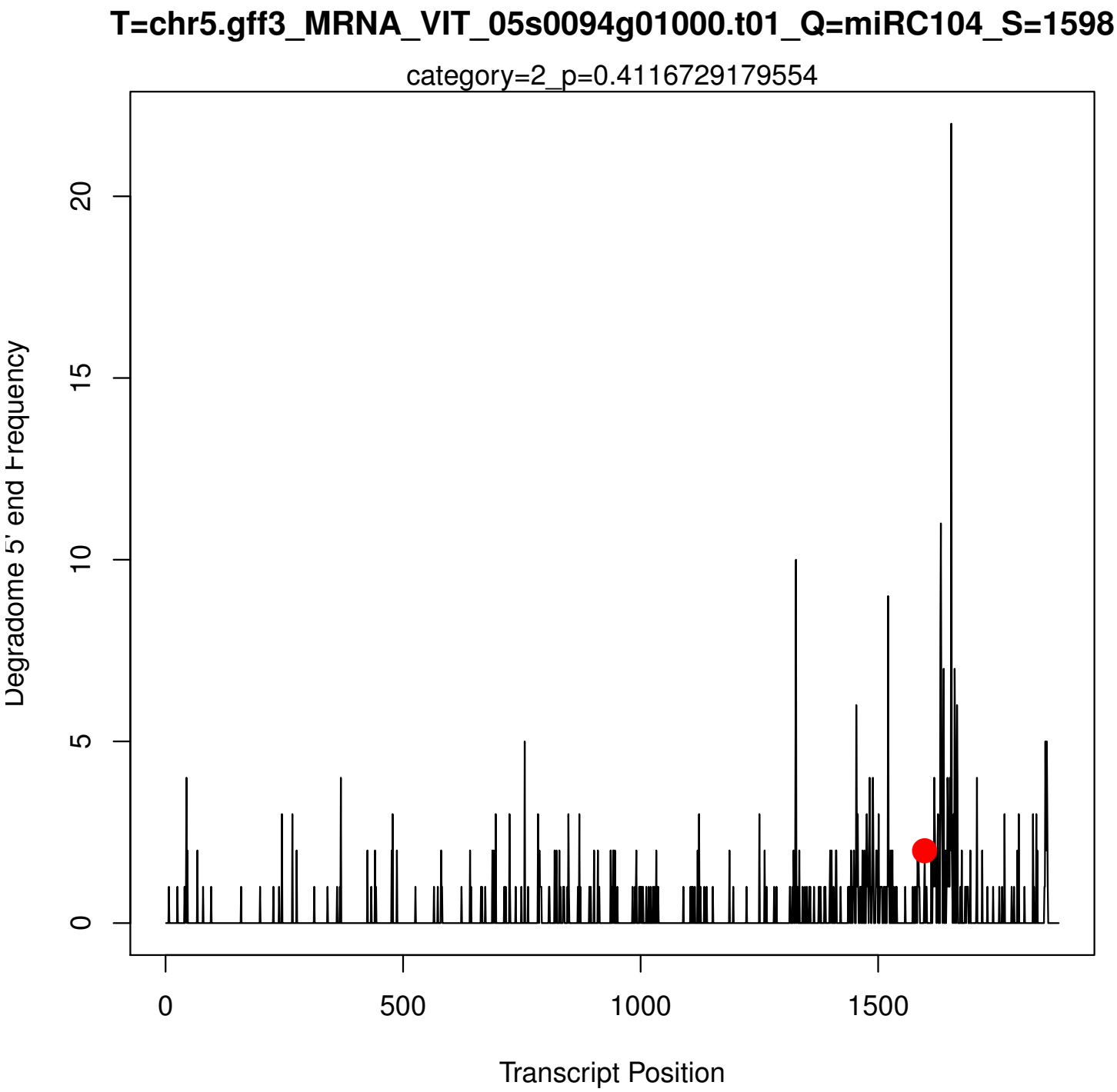
1500
2000
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0094g01000.t01_Q=miRC104_S=1598
category=2_p=0.4116729179554
Degradome 5' end Frequency
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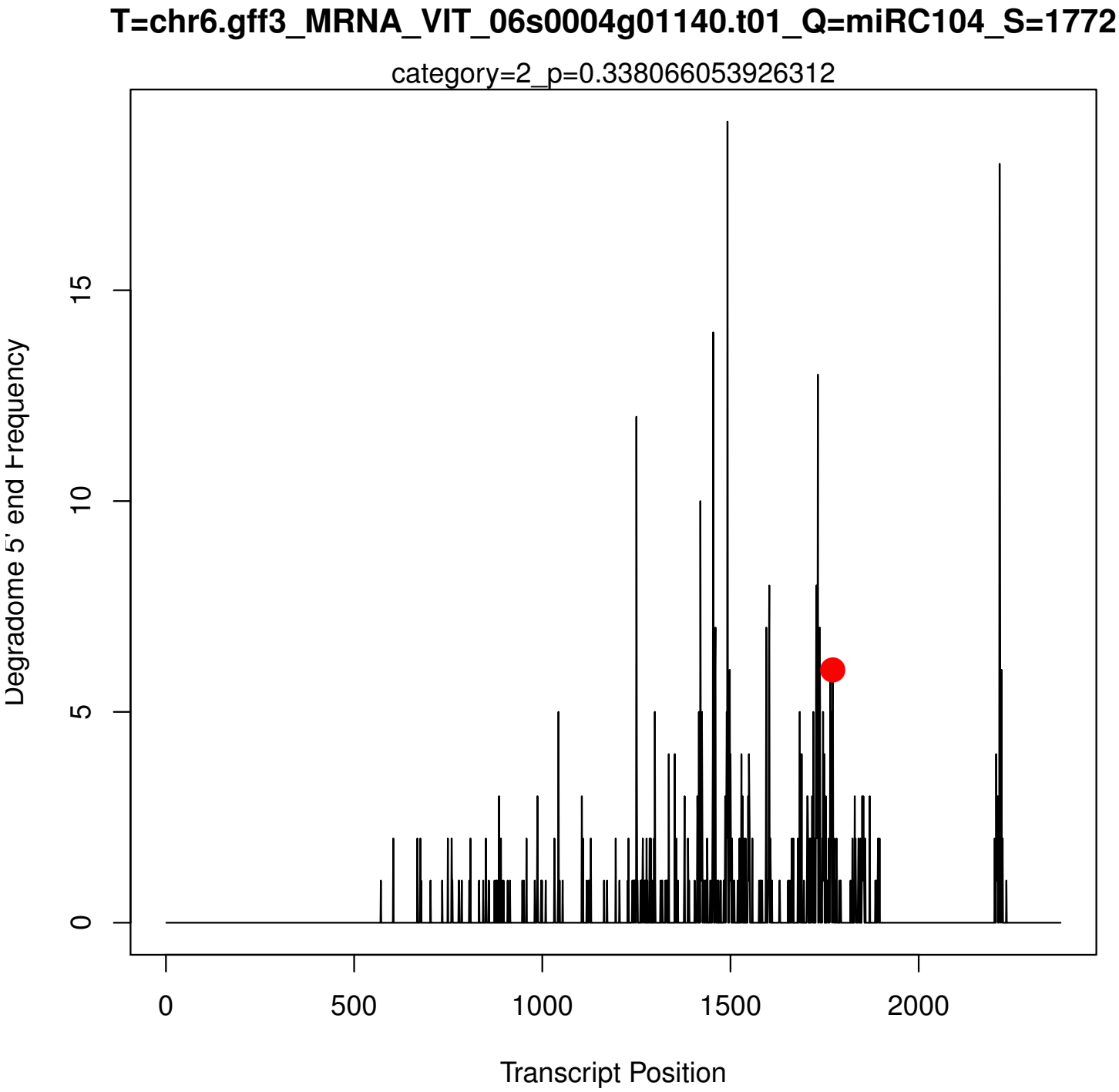
1000
1500
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0004g01140.t01_Q=miRC104_S=1772
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Degradome 5' end Frequency
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15
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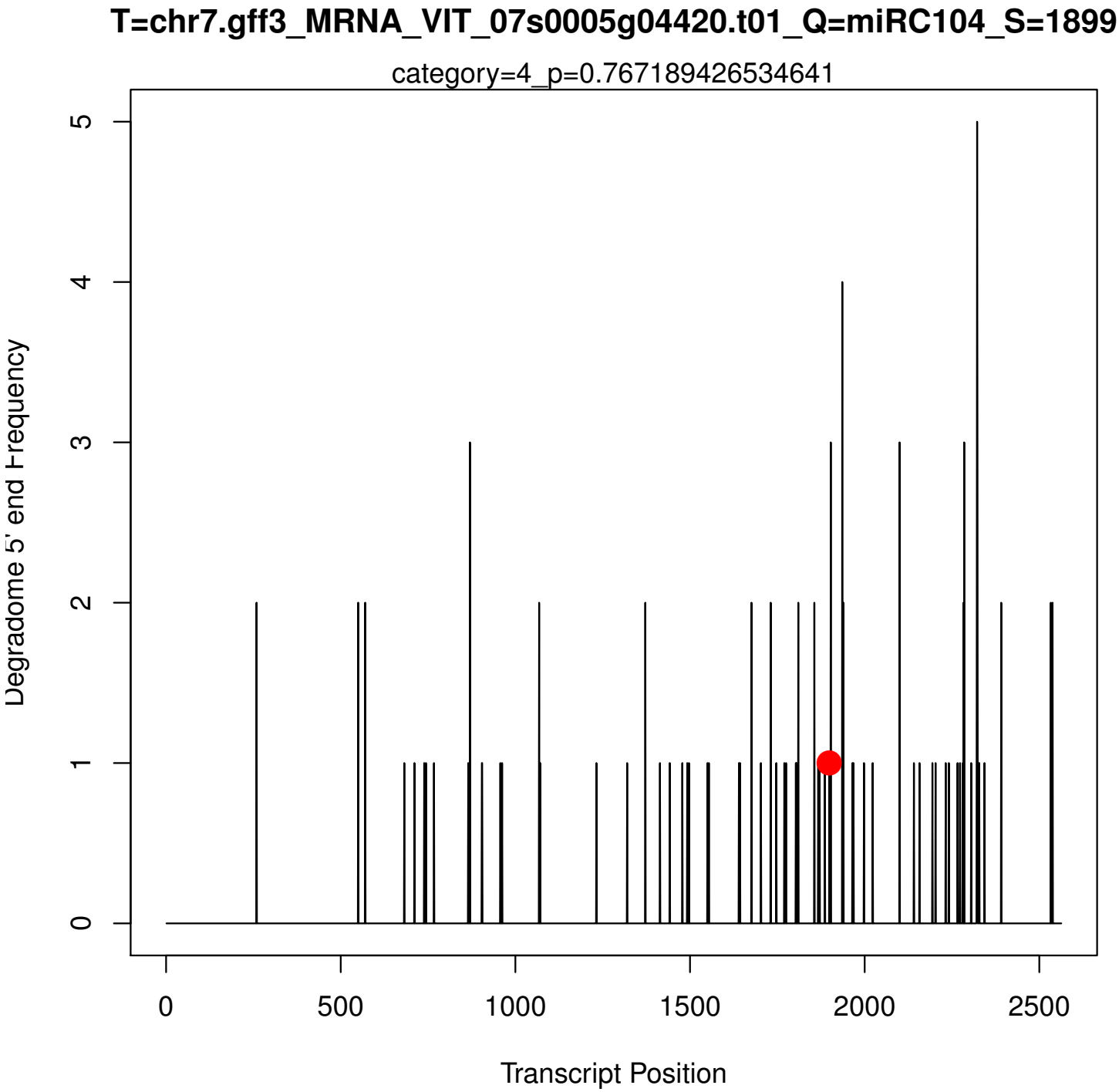
500
1000
1500
2000
Transcript Position


T=chr7.gff3_MRNA_VIT_07s0005g04420.t01_Q=miRC104_S=1899
category=4_p=0.767189426534641
Degradome 5' end Frequency
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1
2
3
4
5
0
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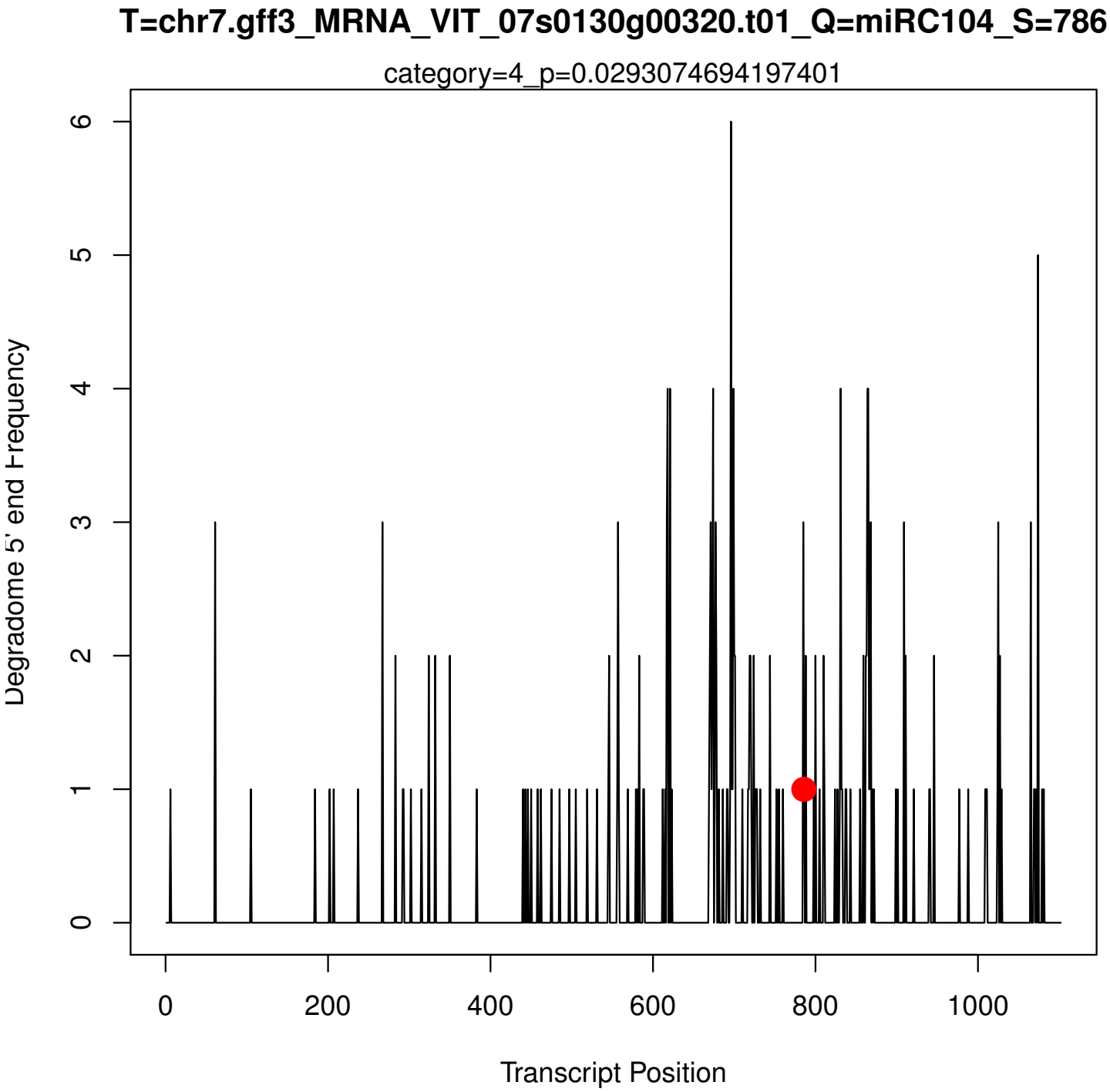
1000
1500
2000
2500
Transcript Position


T=chr7.gff3_MRNA_VIT_07s0130g00320.t01_Q=miRC104_S=786
category=4_p=0.0293074694197401
Degradome 5' end Frequency
0
1
2
3
4
5
6
0
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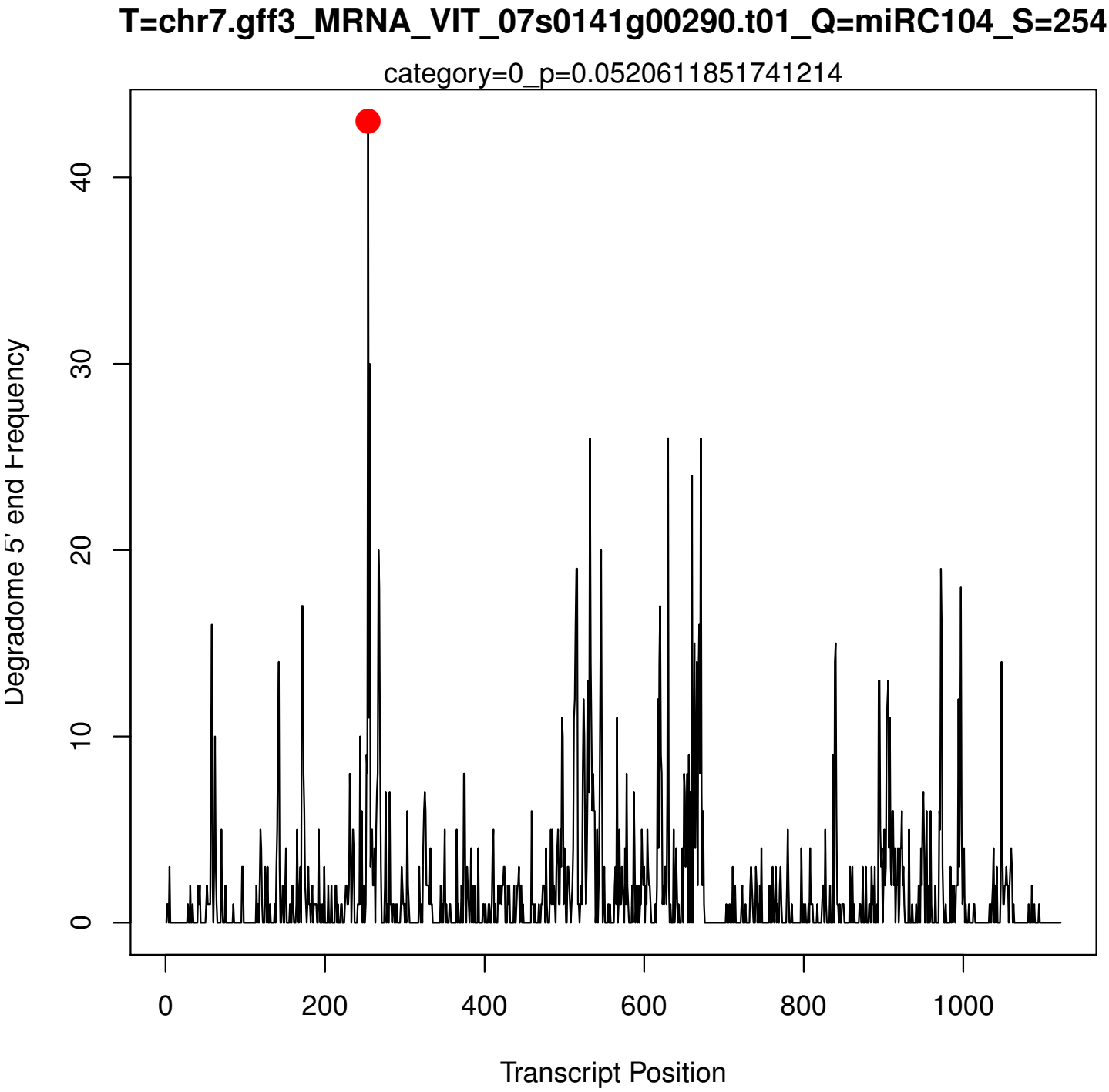
400
600
800
1000
Transcript Position


T=chr7.gff3_MRNA_VIT_07s0141g00290.t01_Q=miRC104_S=254
category=0_p=0.0520611851741214
Degradome 5' end Frequency
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10
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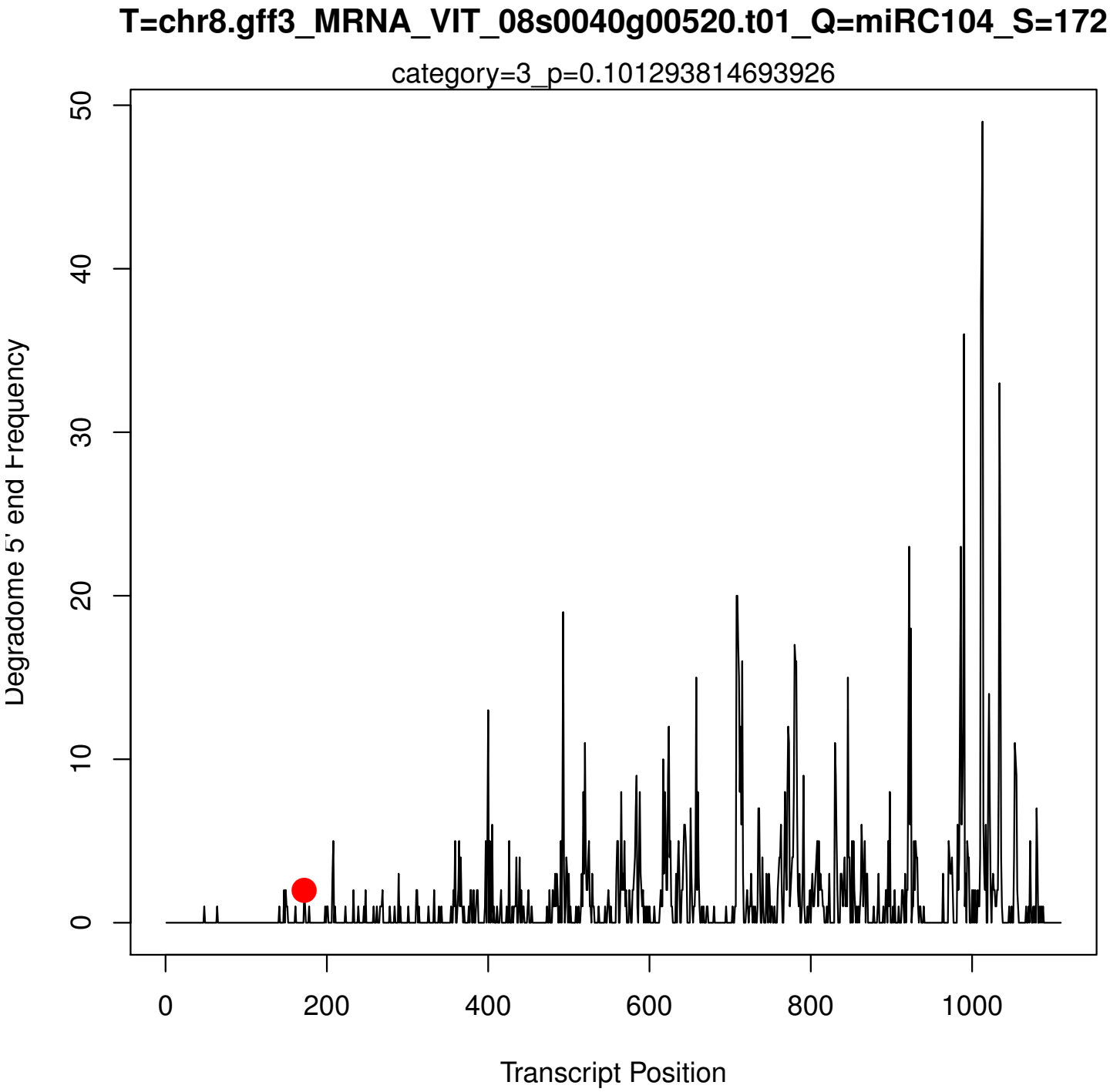
0
200
400
600
800
1000
Transcript Position


T=chr8.gff3_MRNA_VIT_08s0040g00520.t01_Q=miRC104_S=172
category=3_p=0.101293814693926
Degradome 5' end Frequency
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0
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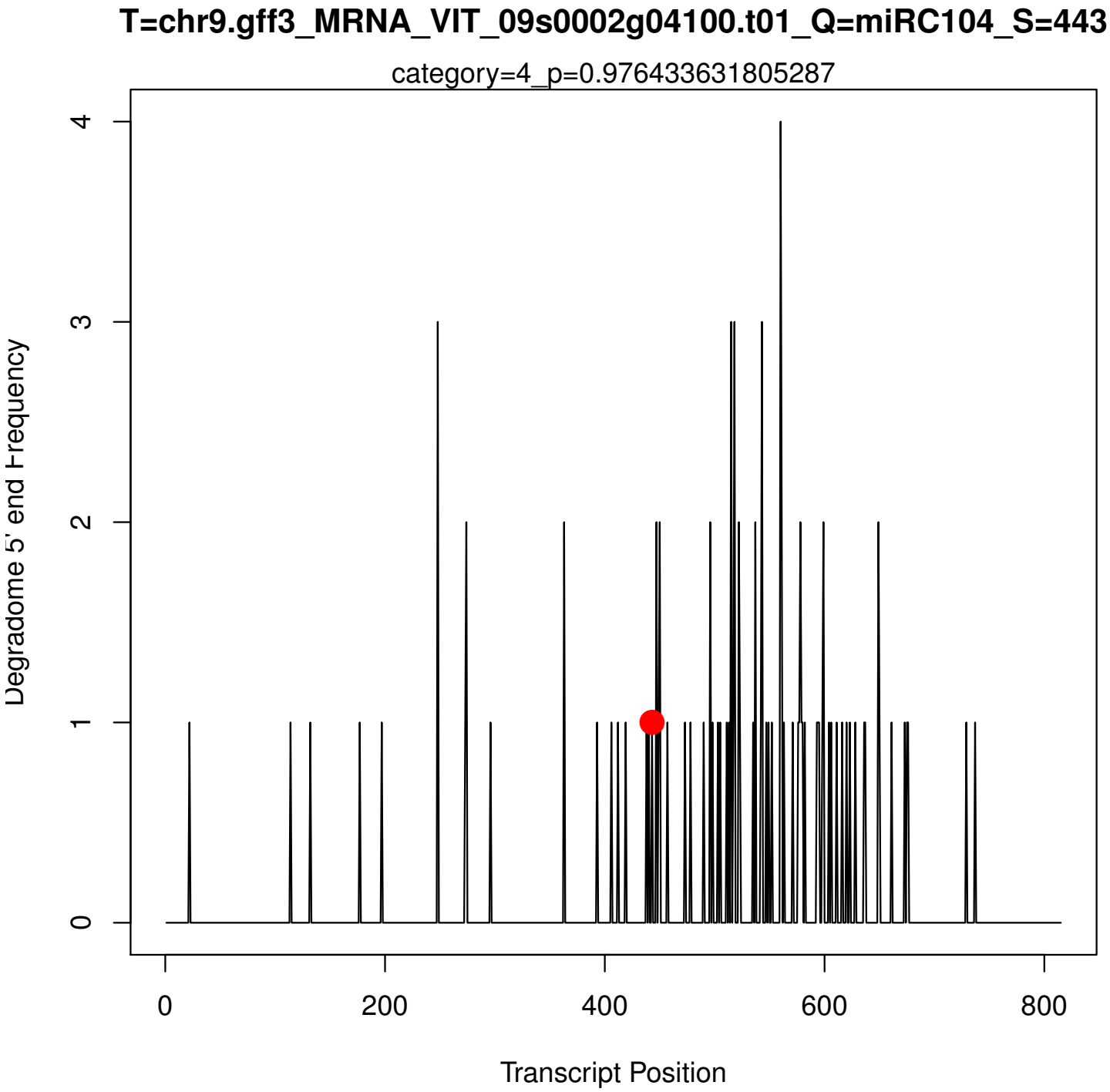
400
600
800
1000
Transcript Position


T=chr9.gff3_MRNA_VIT_09s0002g04100.t01_Q=miRC104_S=443
category=4_p=0.976433631805287
Degradome 5' end Frequency
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4
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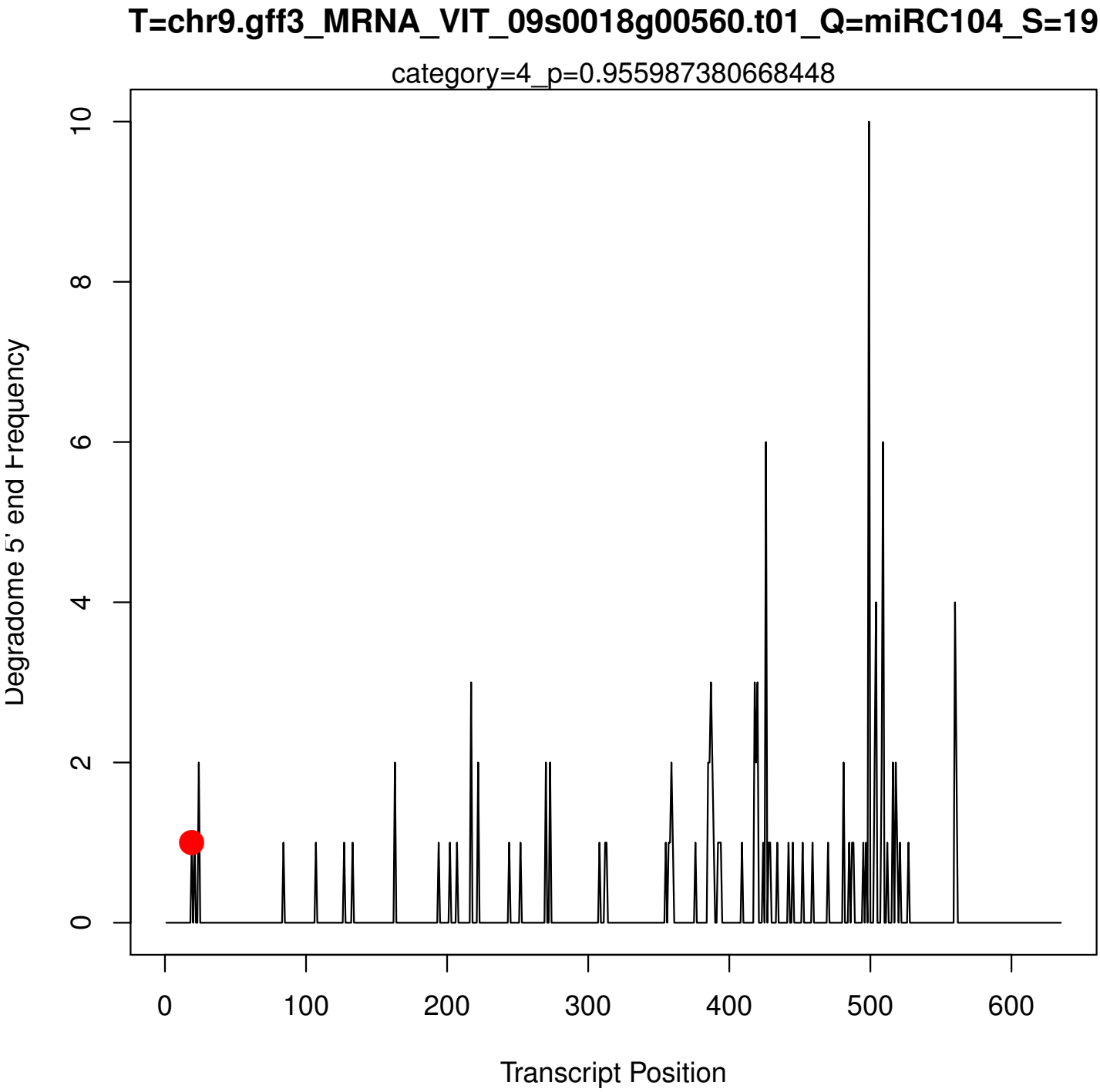
400
600
800
Transcript Position


T=chr9.gff3_MRNA_VIT_09s0018g00560.t01_Q=miRC104_S=19
category=4_p=0.955987380668448
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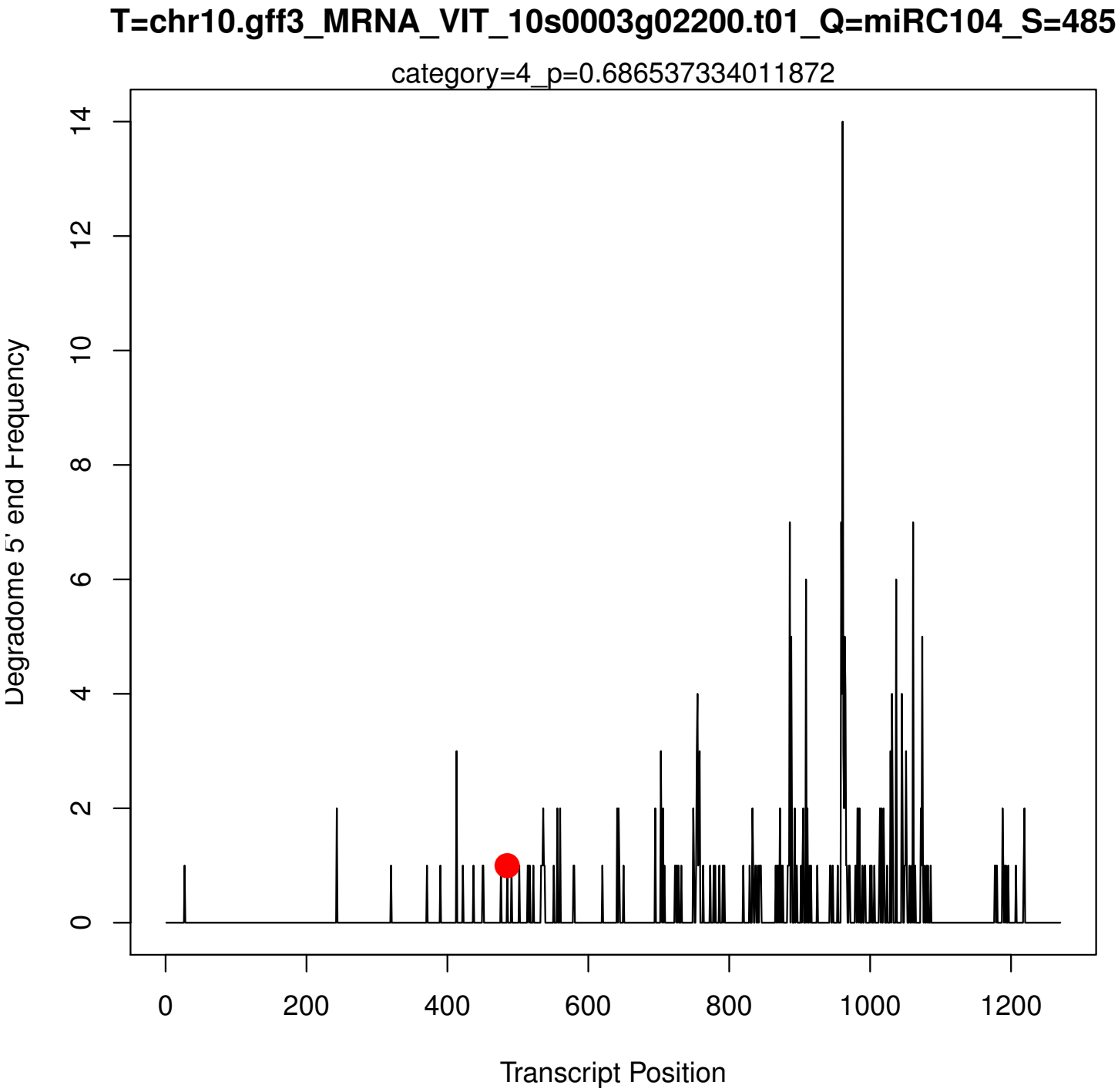
100
200
300
400
500
600
Transcript Position


T=chr10.gff3_MRNA_VIT_10s0003g02200.t01_Q=miRC104_S=485
category=4_p=0.686537334011872
Degradome 5' end Frequency
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6
8
10
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200
400
600
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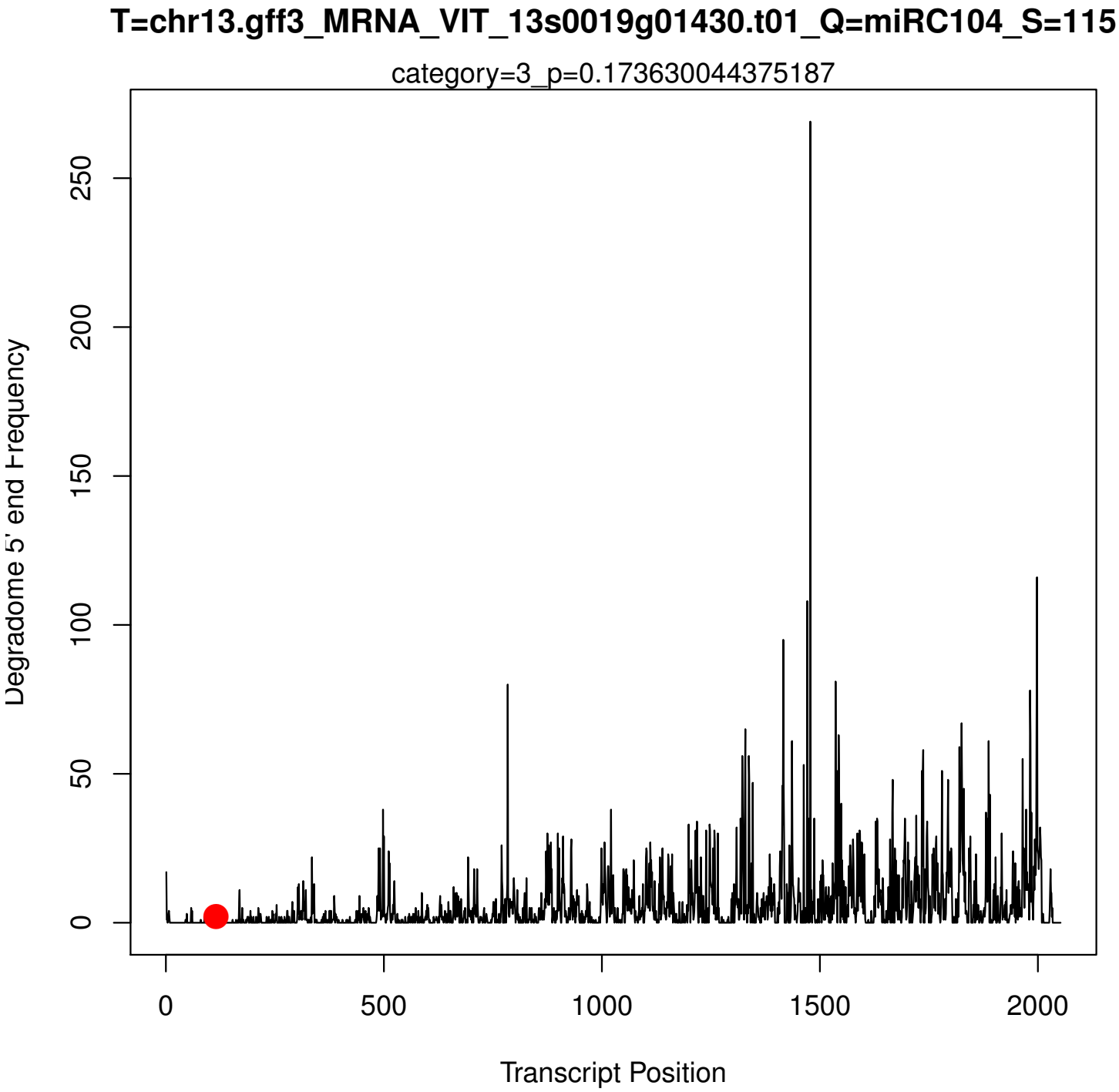
1000
1200
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0019g01430.t01_Q=miRC104_S=115
category=3_p=0.173630044375187
Degradome 5' end Frequency
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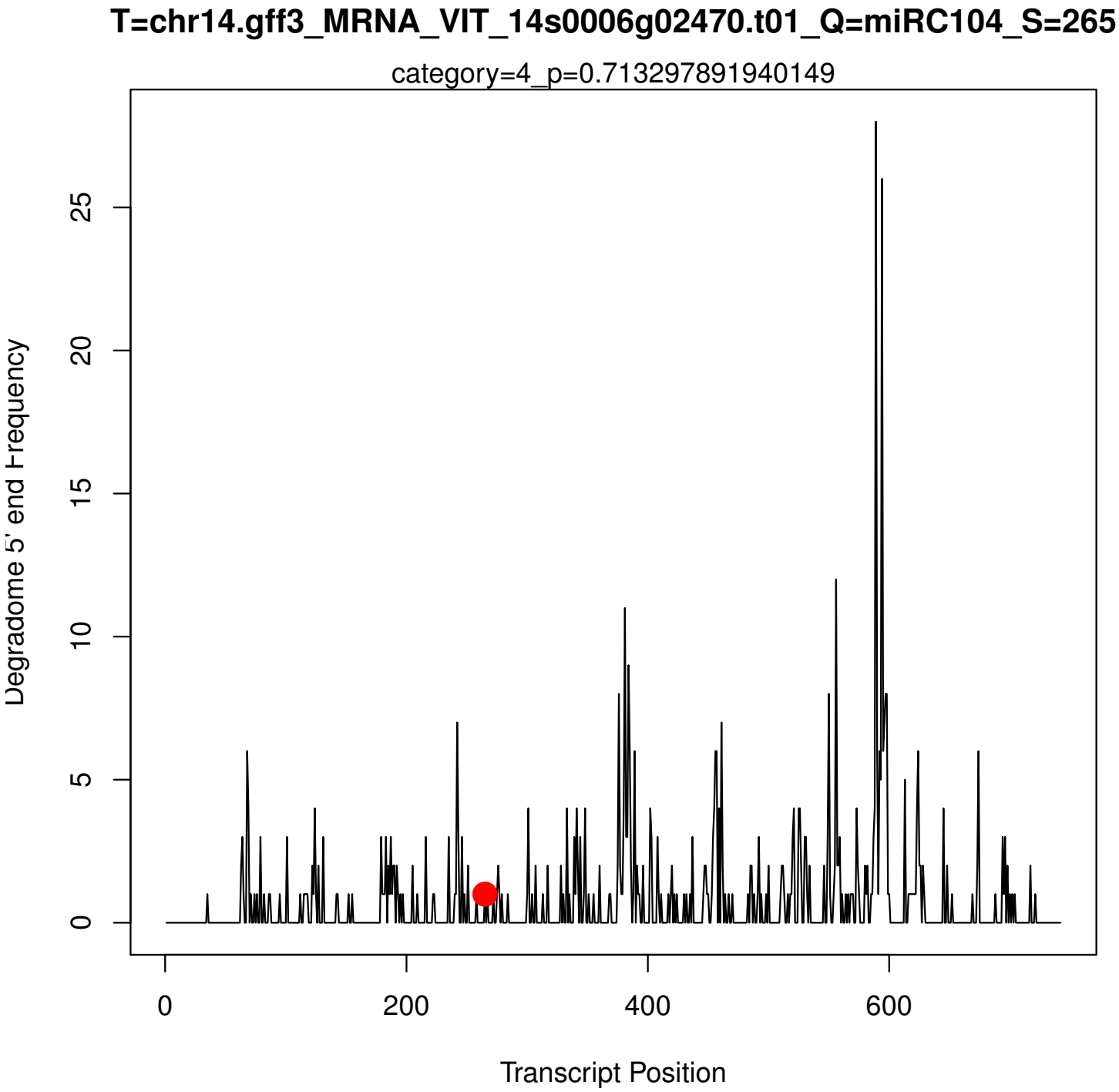
150
200
250
0
500
1000
1500
2000
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0006g02470.t01_Q=miRC104_S=265
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25
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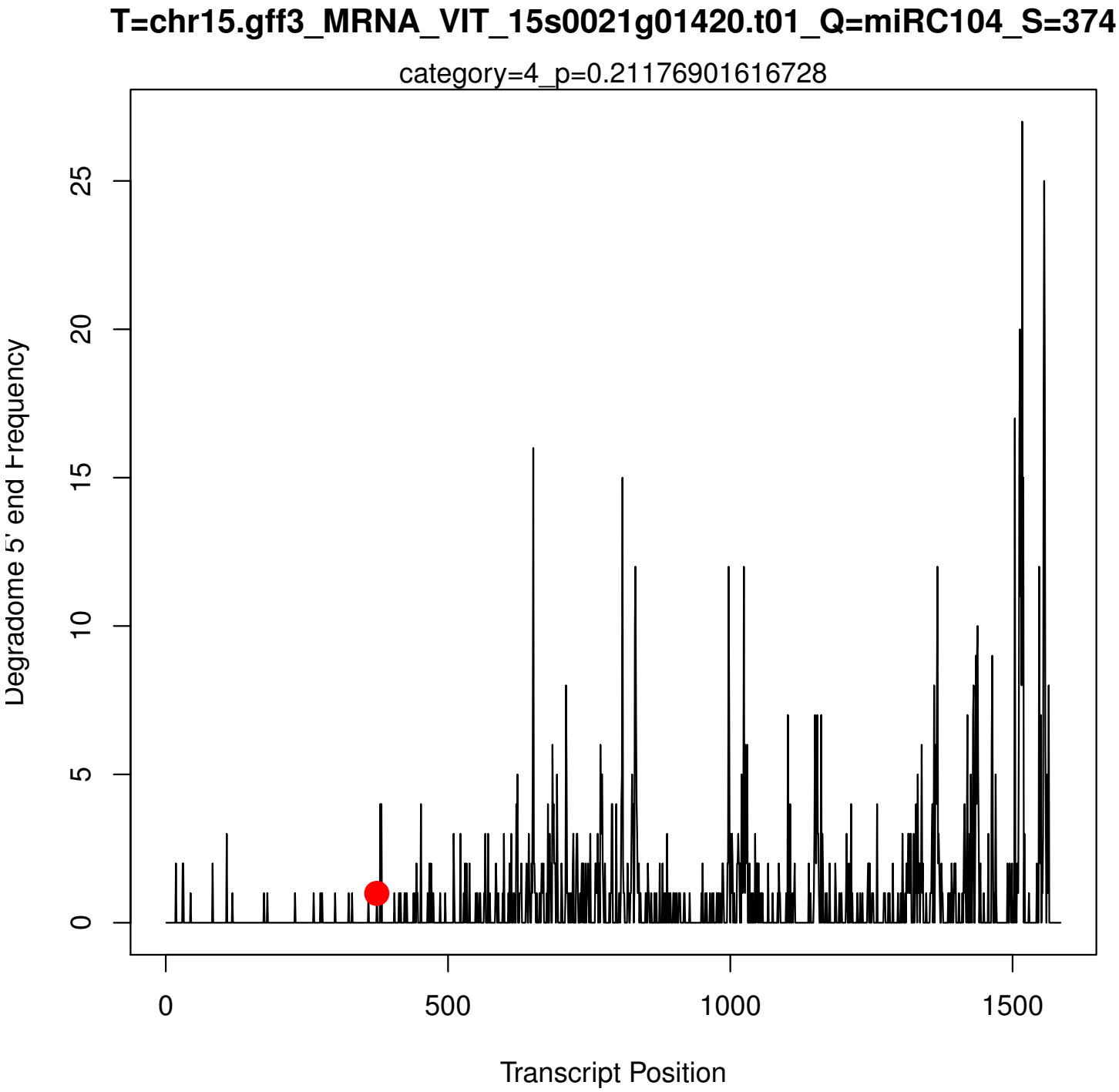
400
600
Transcript Position


T=chr15.gff3_MRNA_VIT_15s0021g01420.t01_Q=miRC104_S=374
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20
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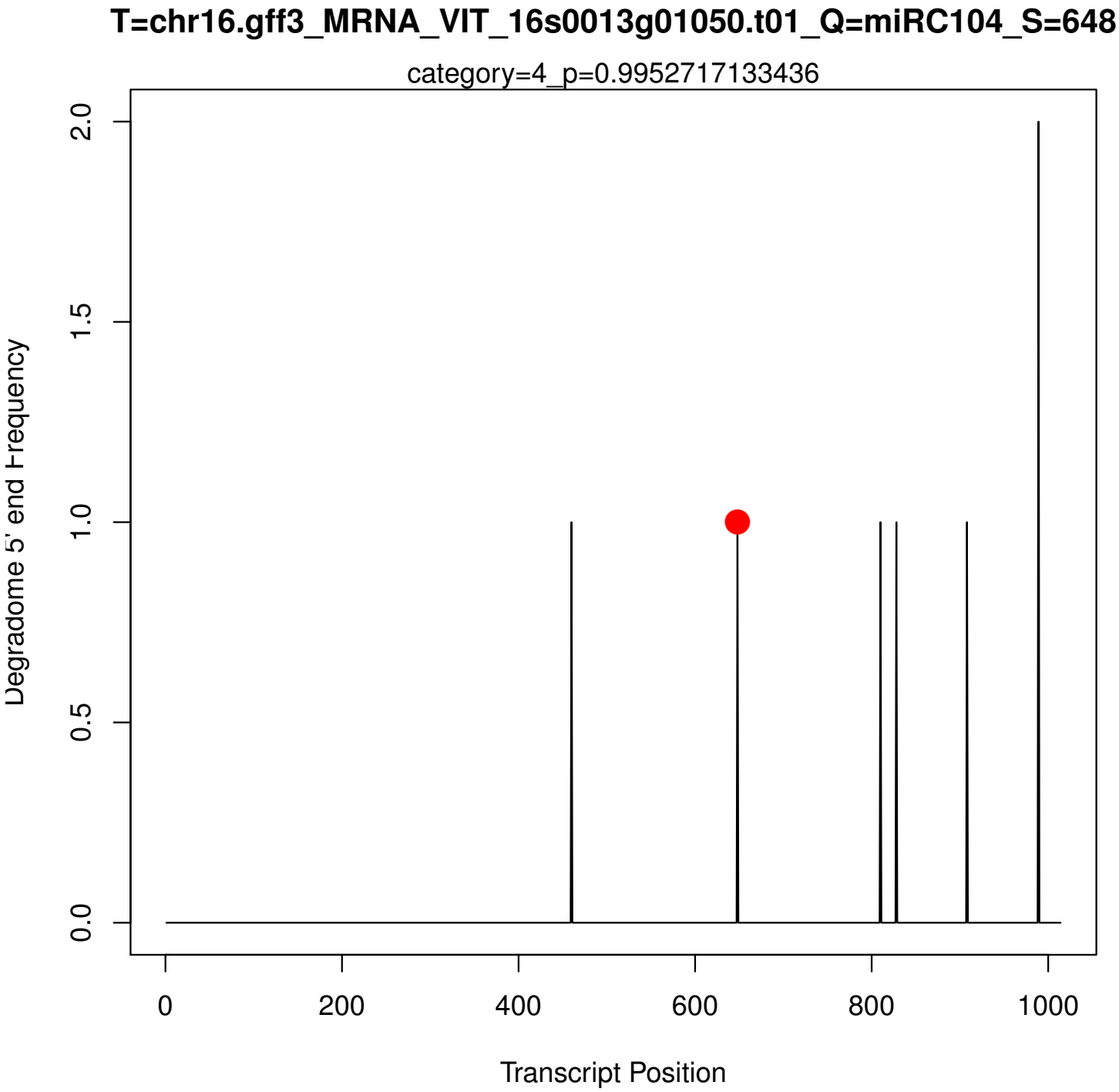
1000
1500
Transcript Position


T=chr16.gff3_MRNA_VIT_16s0013g01050.t01_Q=miRC104_S=648
category=4_p=0.9952717133436
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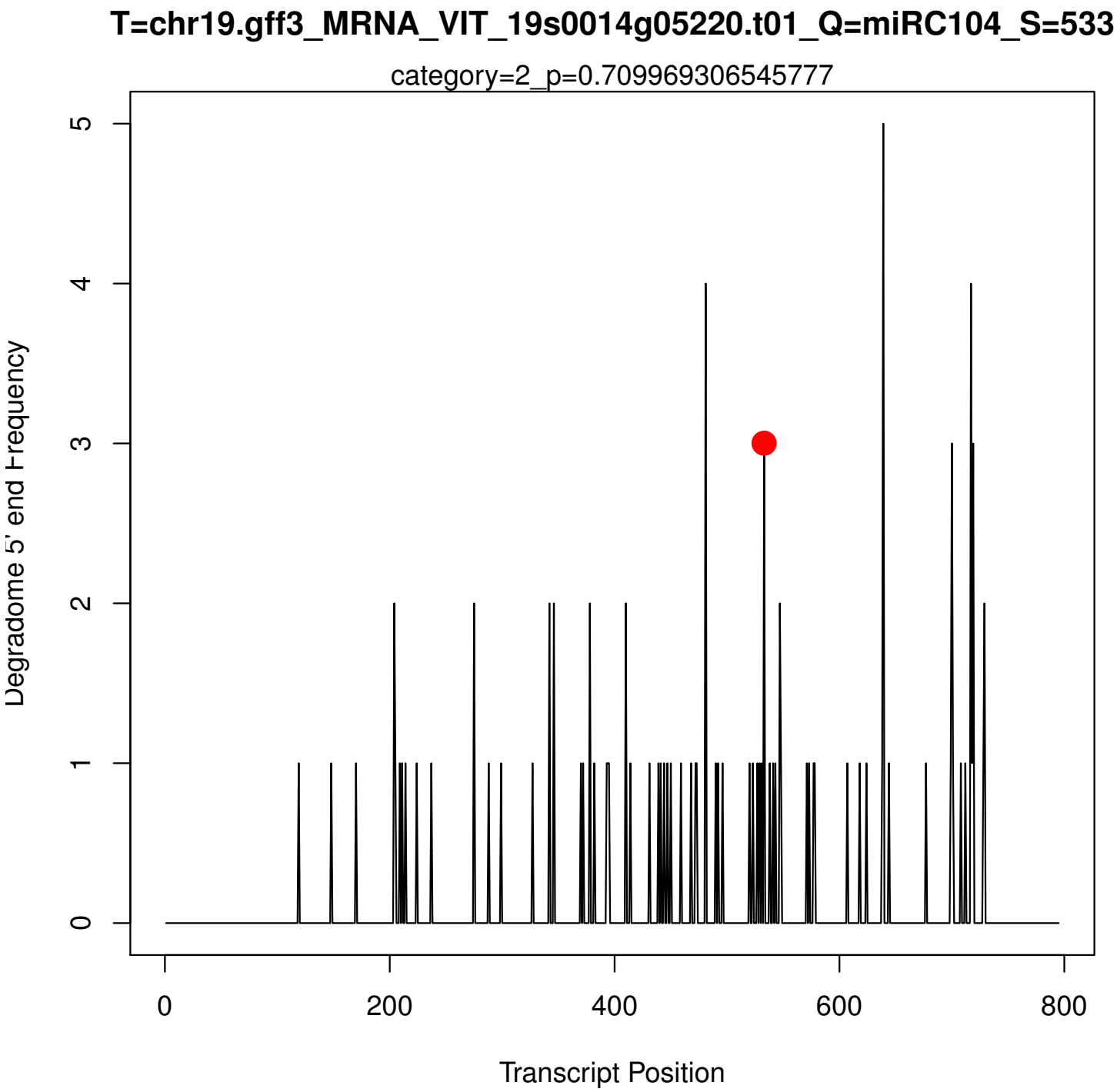
400
600
800
1000
Transcript Position


T=chr19.gff3_MRNA_VIT_19s0014g05220.t01_Q=miRC104_S=533
category=2_p=0.709969306545777
Degradome 5' end Frequency
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3
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5
0
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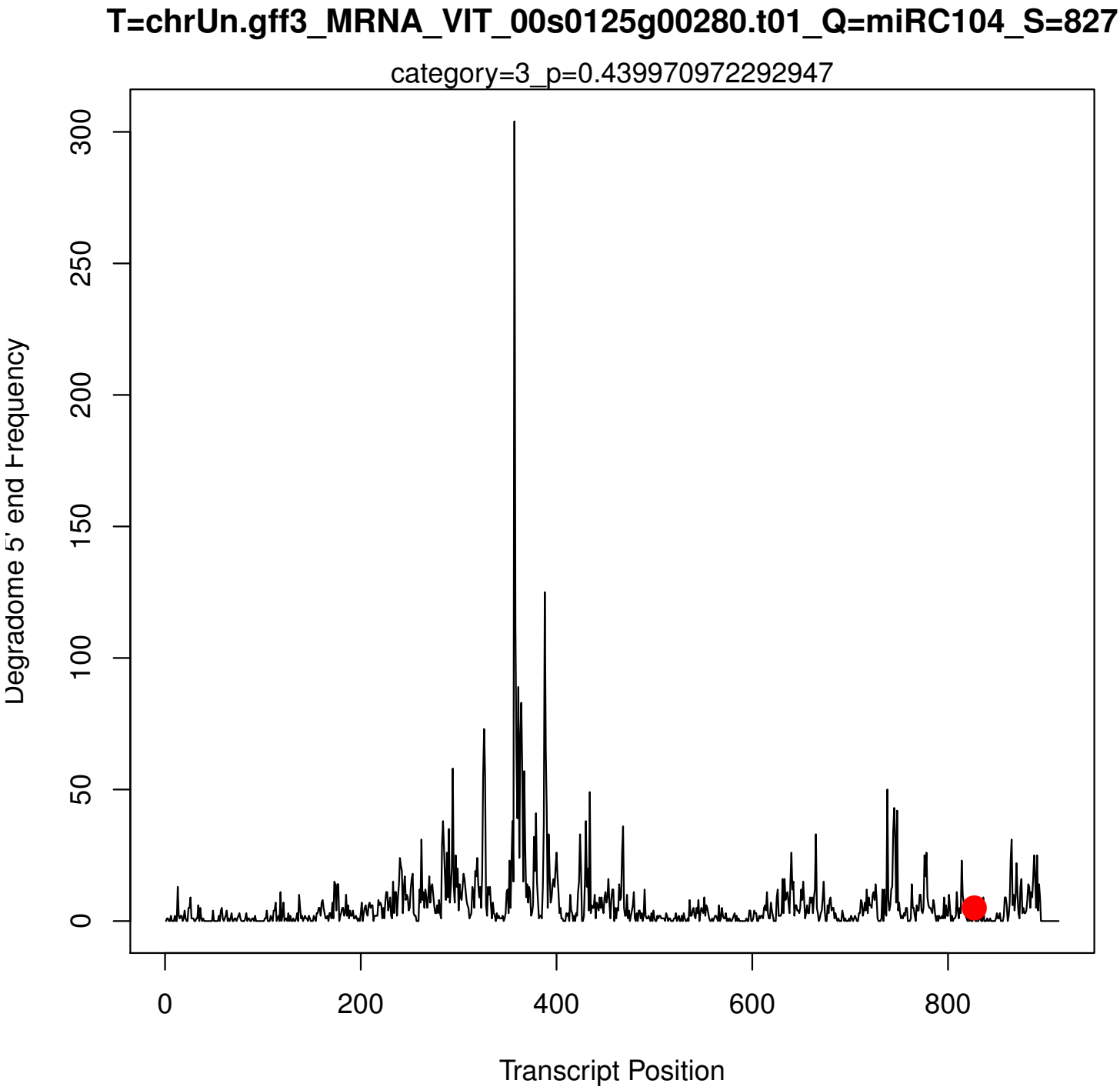
400
600
800
Transcript Position


T=chrUn.gff3_MRNA_VIT_00s0125g00280.t01_Q=miRC104_S=827
category=3_p=0.439970972292947
Degradome 5' end Frequency
0
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300
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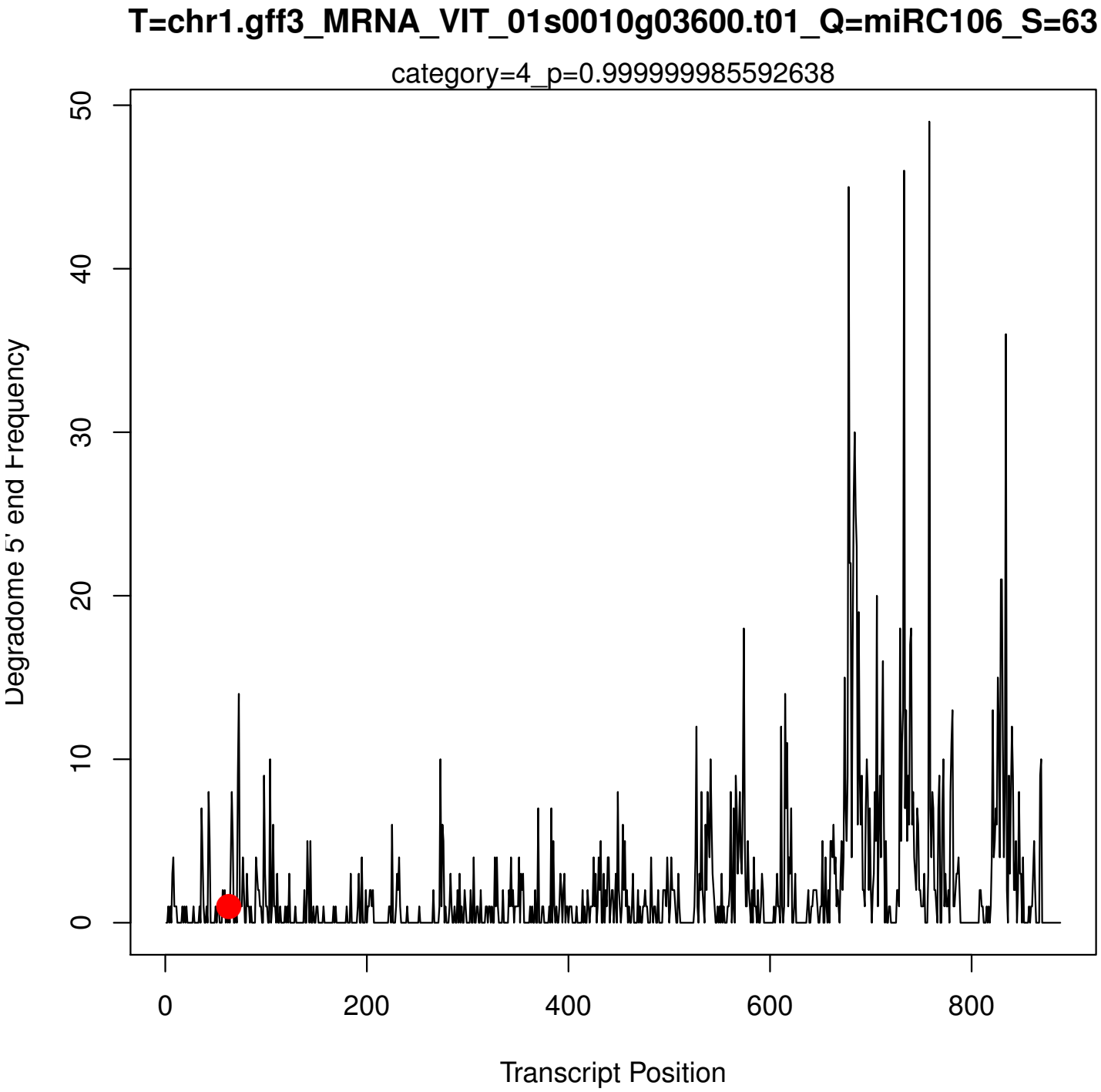
200
400
600
800
Transcript Position


T=chr1.gff3_MRNA_VIT_01s0010g03600.t01_Q=miRC106_S=63
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Degradome 5' end Frequency
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50
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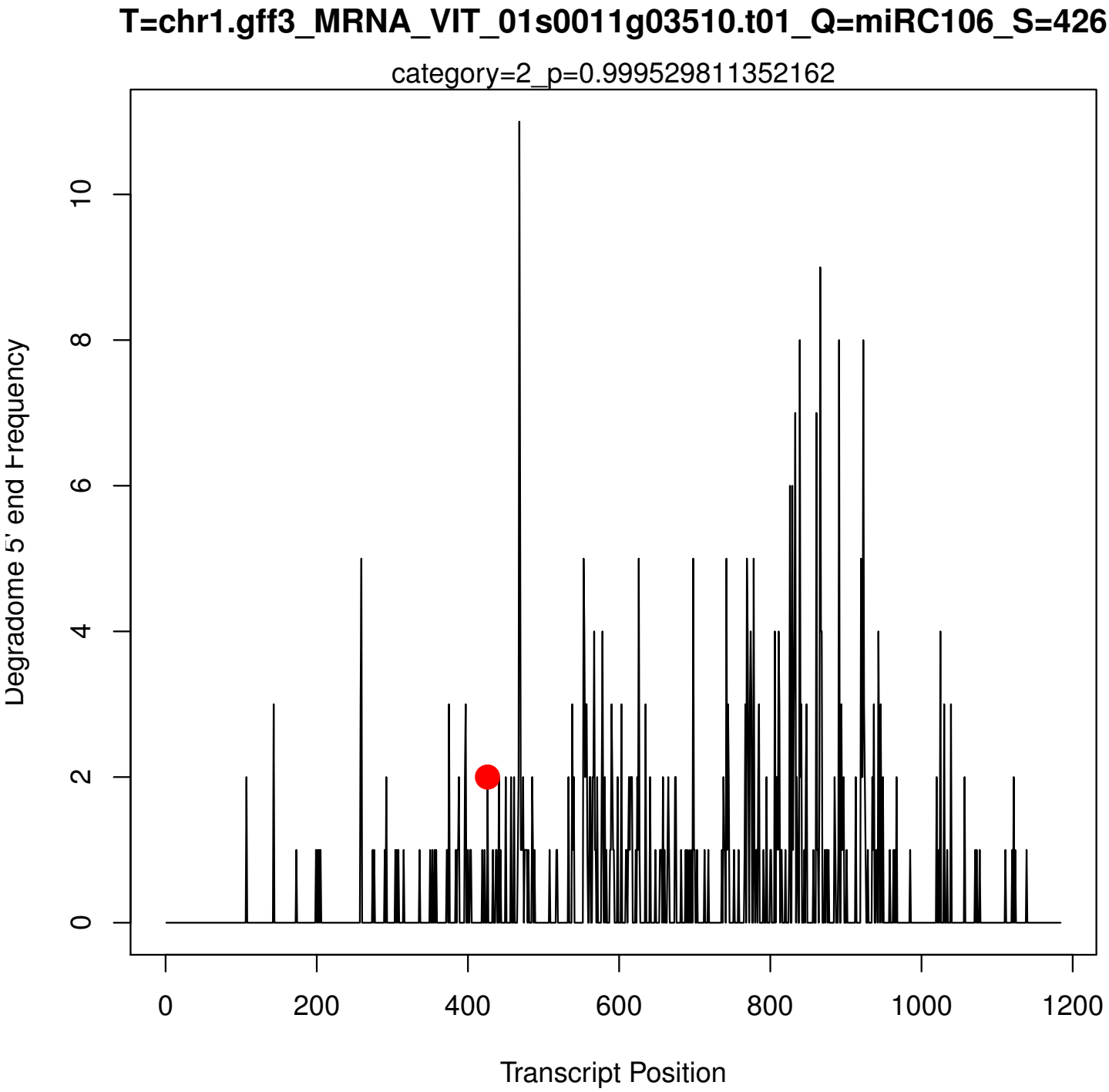
200
400
600
800
Transcript Position


T=chr1.gff3_MRNA_VIT_01s0011g03510.t01_Q=miRC106_S=426
category=2_p=0.999529811352162
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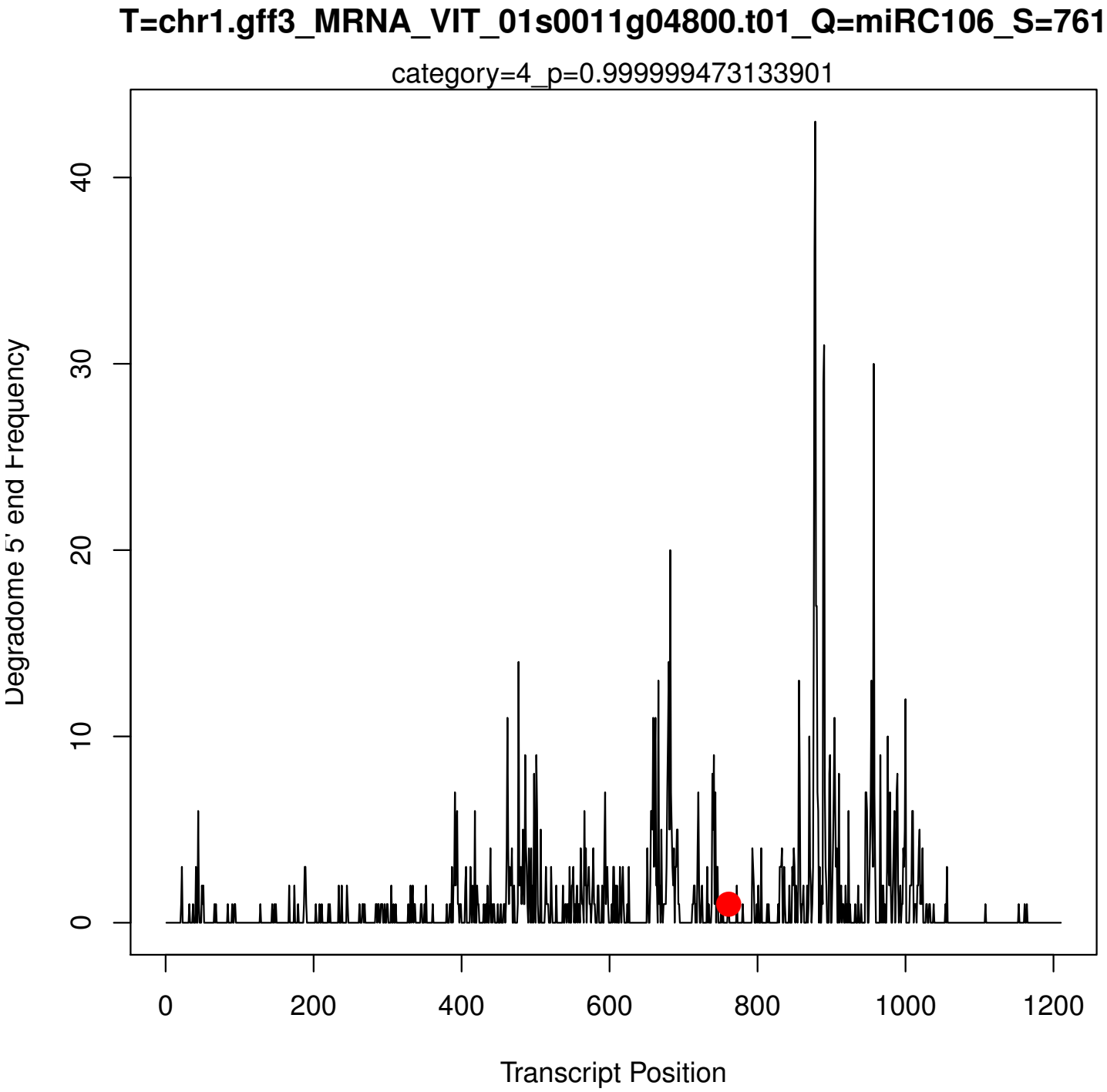
1000
1200
Transcript Position


T=chr1.gff3_MRNA_VIT_01s0011g04800.t01_Q=miRC106_S=761
category=4_p=0.999999473133901
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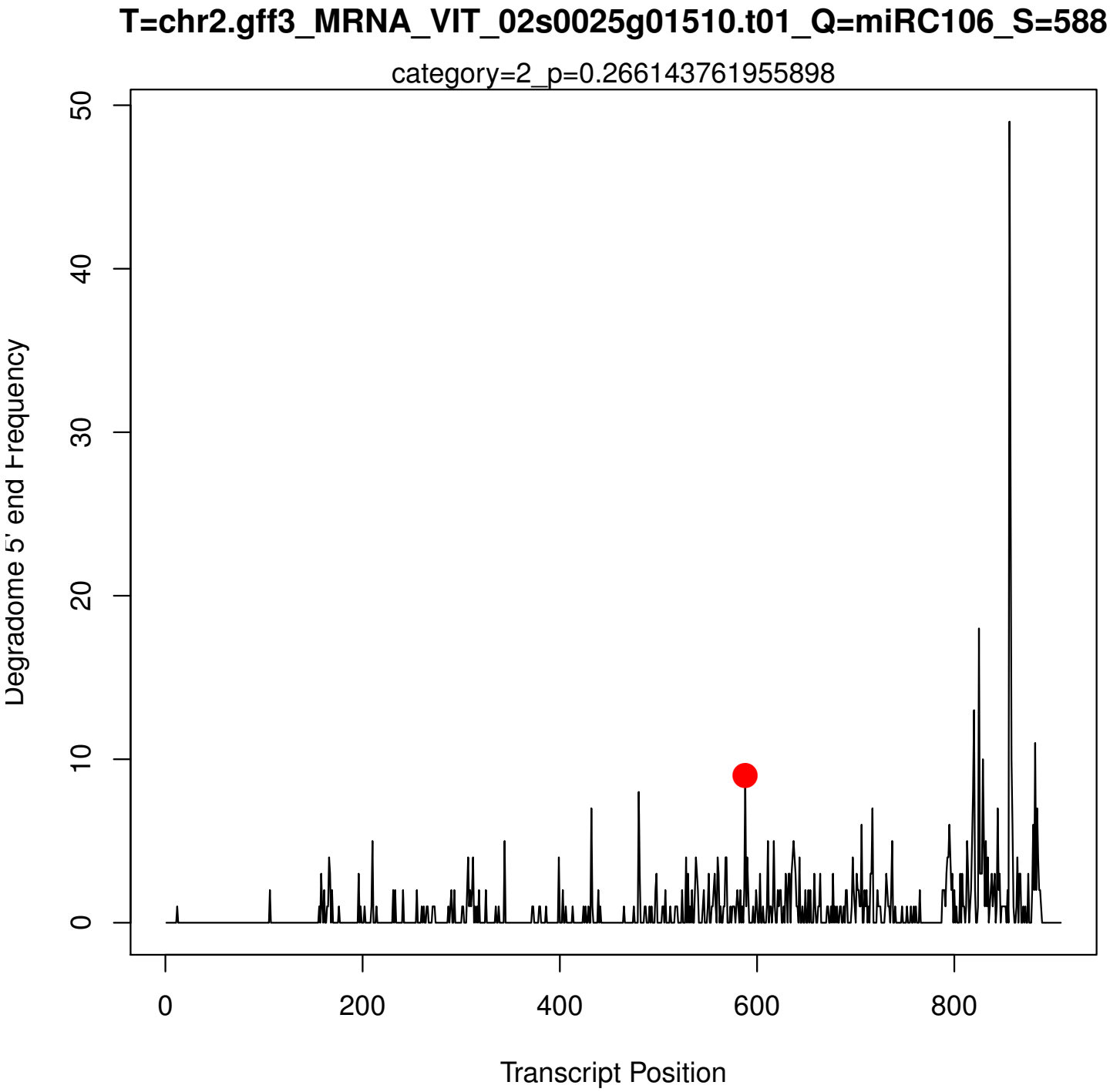
1000
1200
Transcript Position


T=chr2.gff3_MRNA_VIT_02s0025g01510.t01_Q=miRC106_S=588
category=2_p=0.266143761955898
Degradome 5' end Frequency
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50
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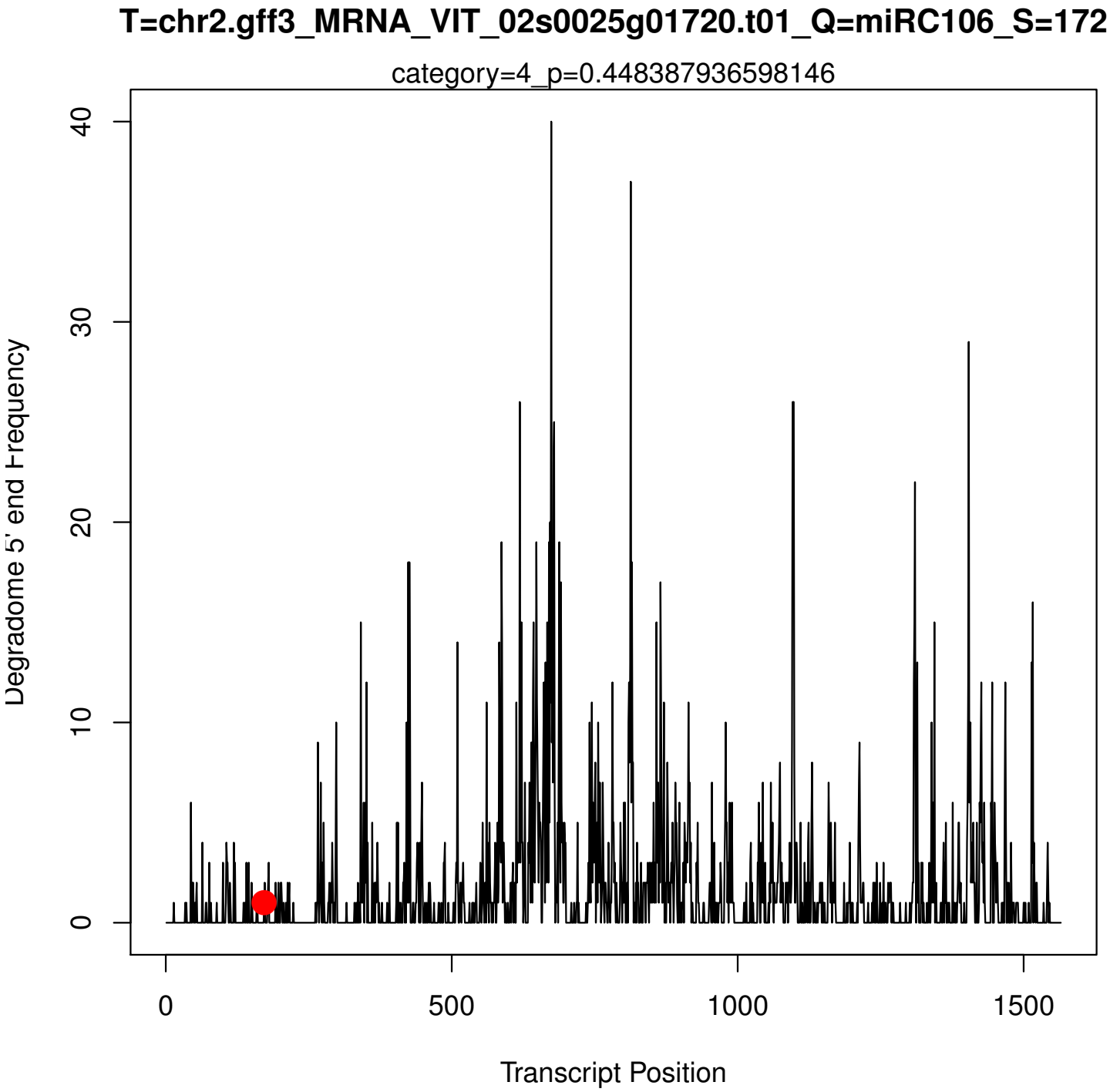
600
800
Transcript Position


T=chr2.gff3_MRNA_VIT_02s0025g01720.t01_Q=miRC106_S=172
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Degradome 5' end Frequency
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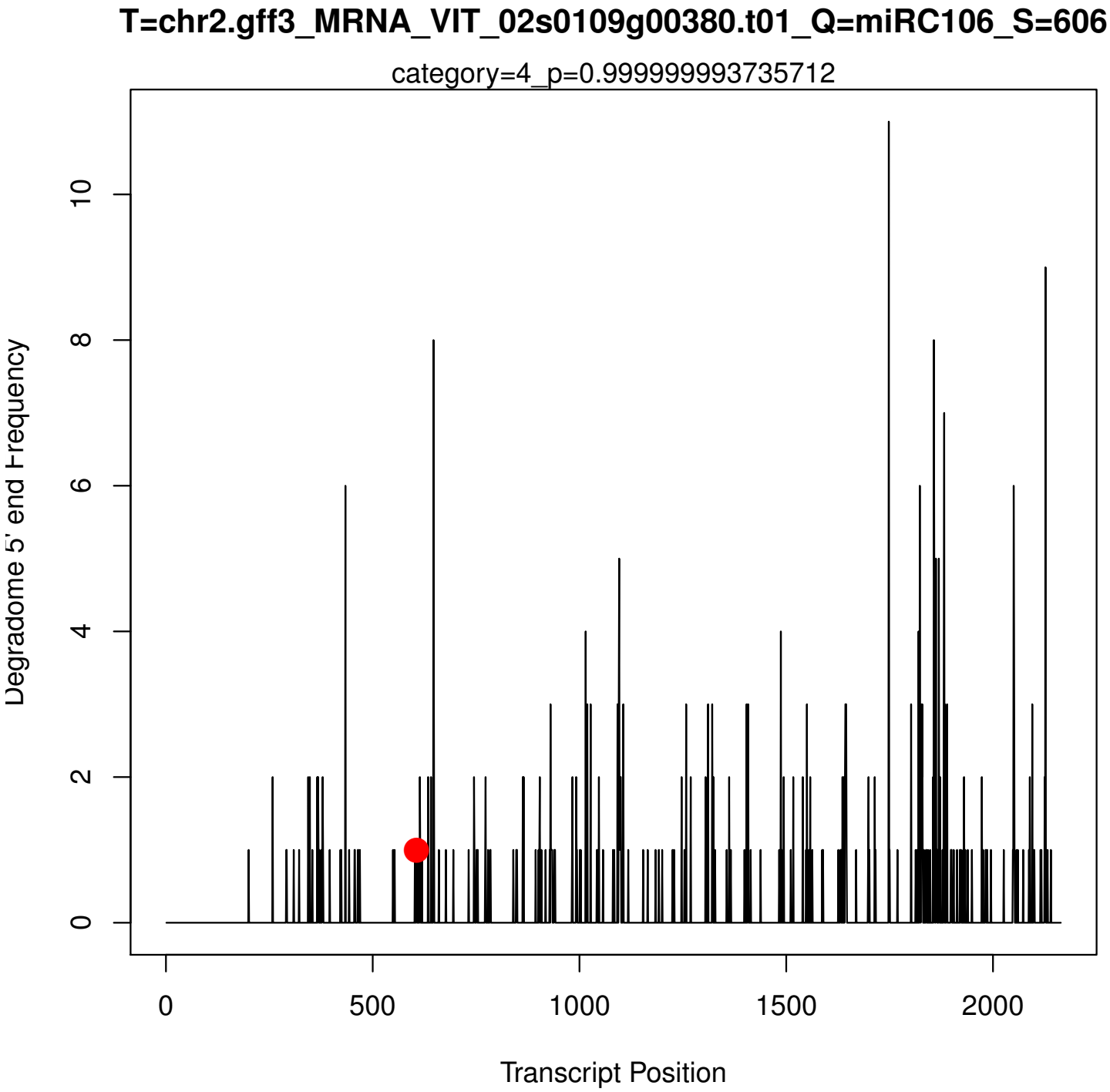
1000
1500
Transcript Position


T=chr2.gff3_MRNA_VIT_02s0109g00380.t01_Q=miRC106_S=606
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Degradome 5' end Frequency
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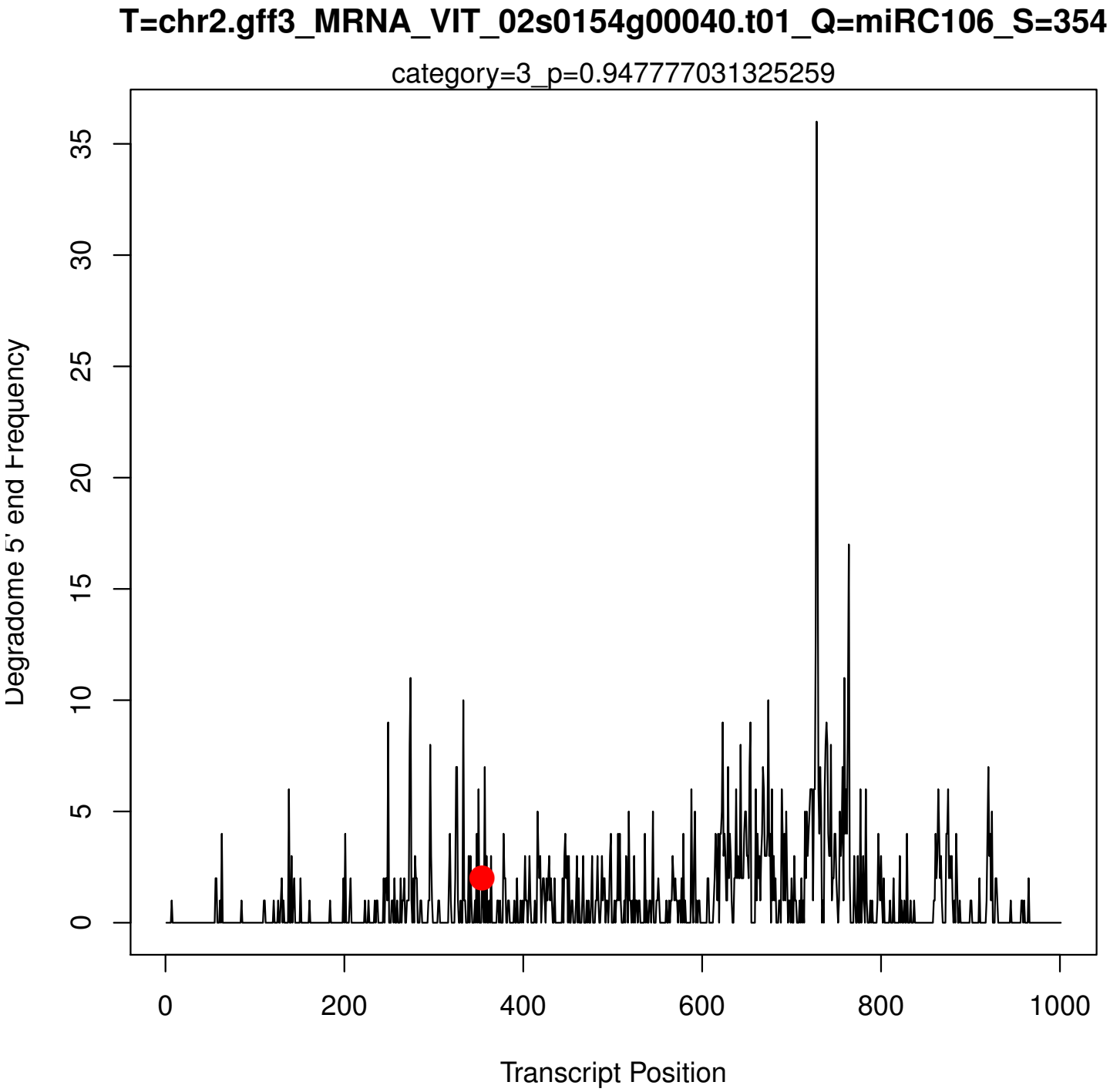
1000
1500
2000
Transcript Position


T=chr2.gff3_MRNA_VIT_02s0154g00040.t01_Q=miRC106_S=354
category=3_p=0.947777031325259
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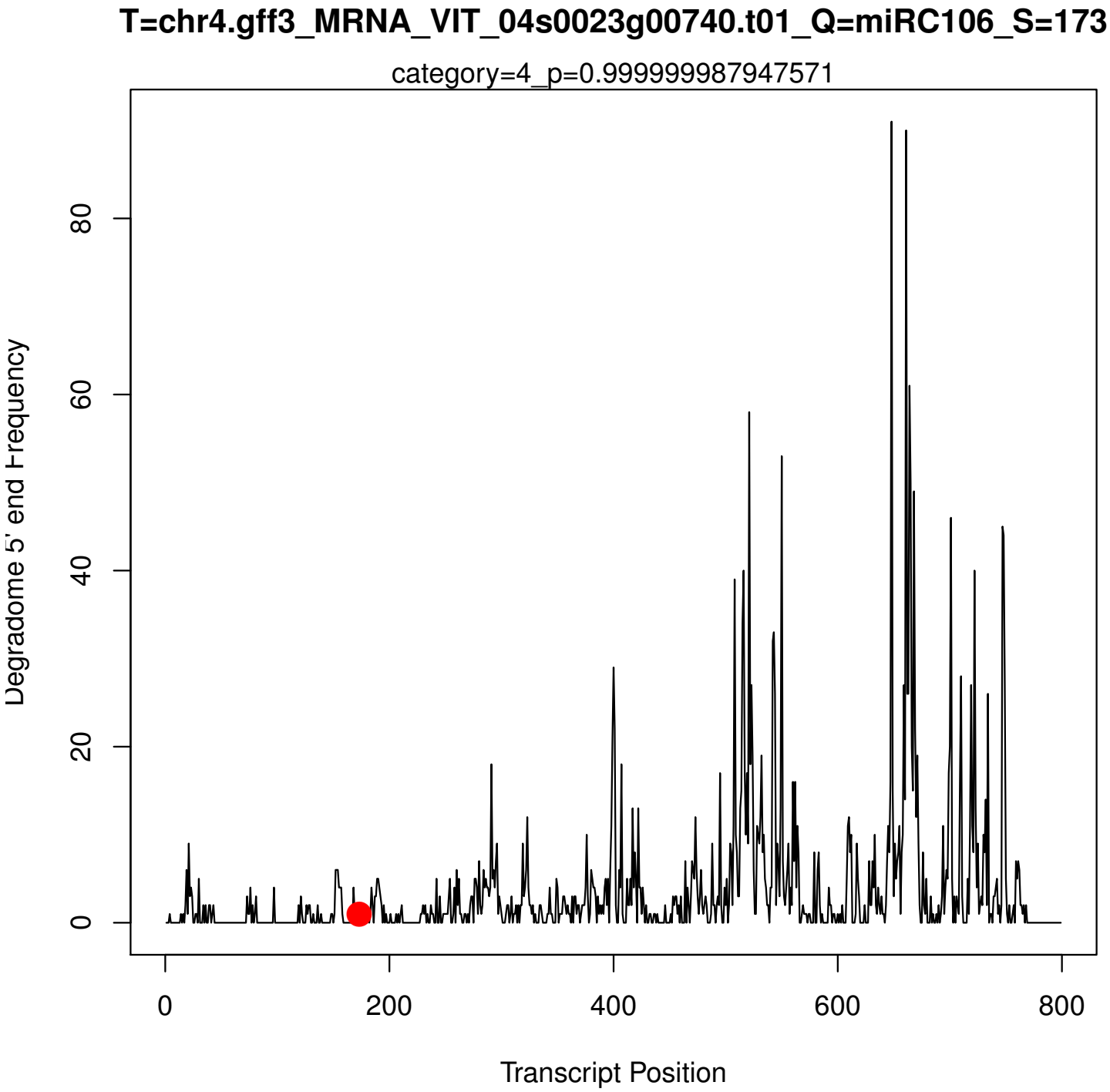
800
1000
Transcript Position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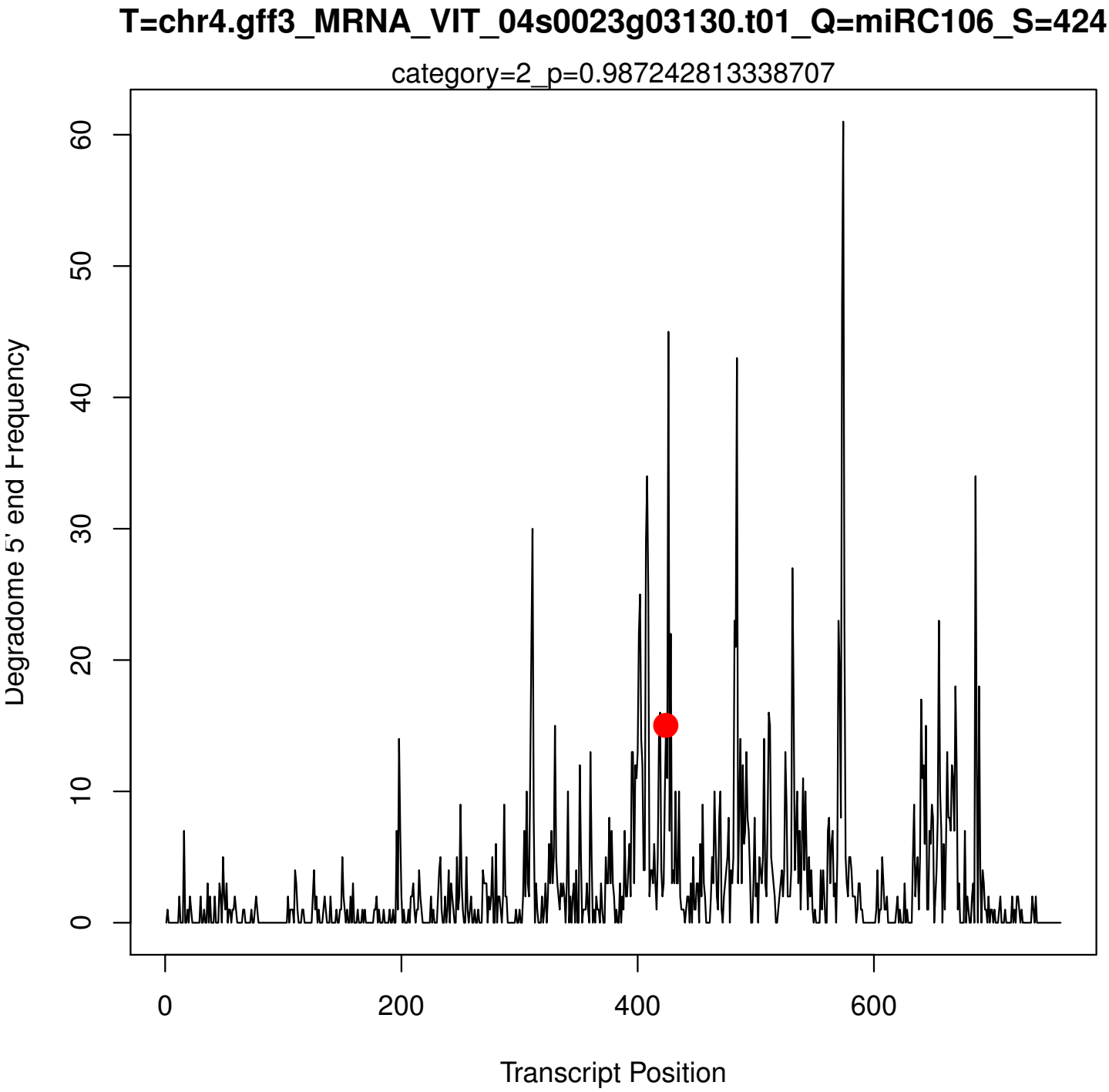
Transcript Position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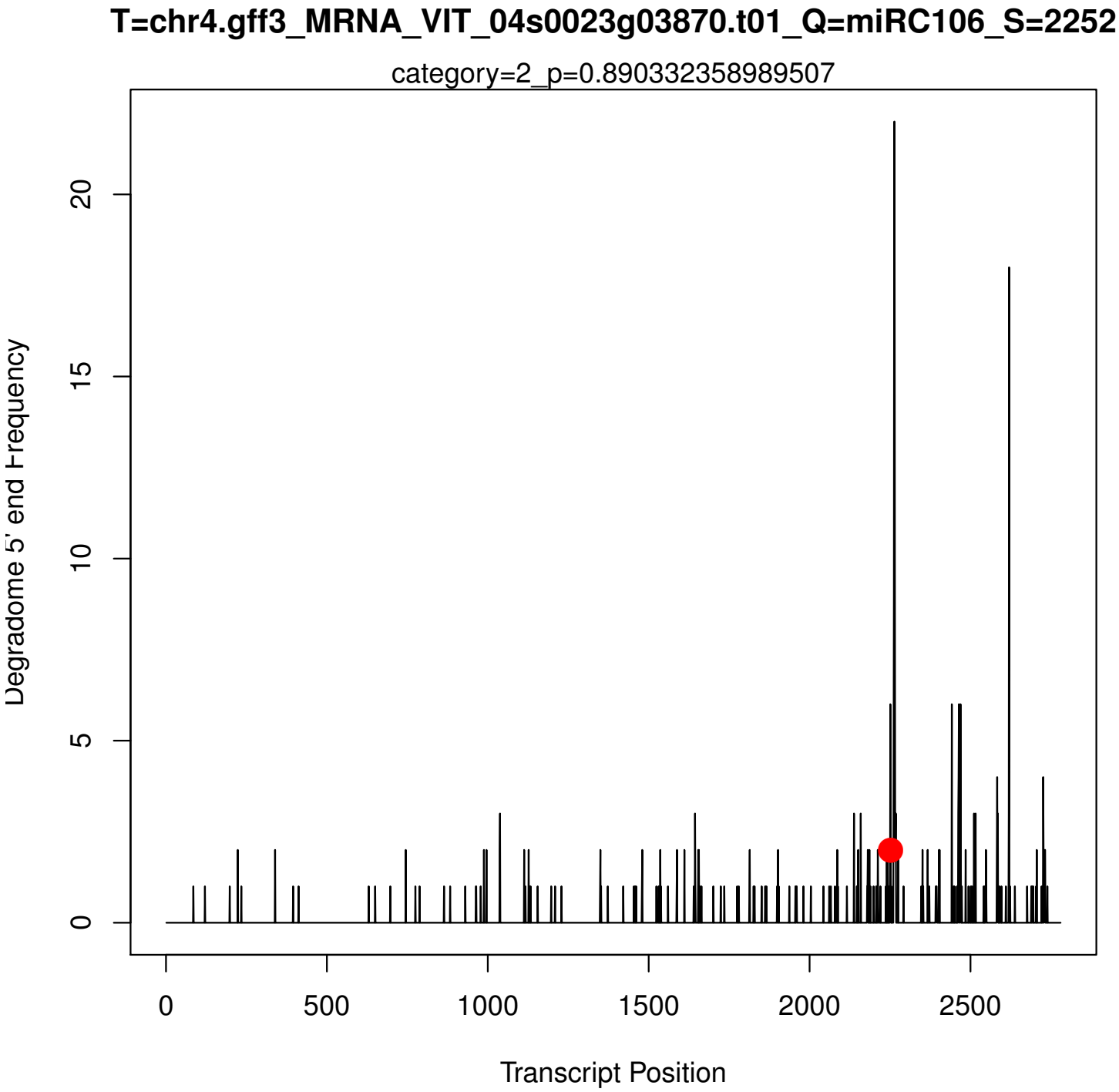
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0023g03870.t01_Q=miRC106_S=2252
category=2_p=0.890332358989507
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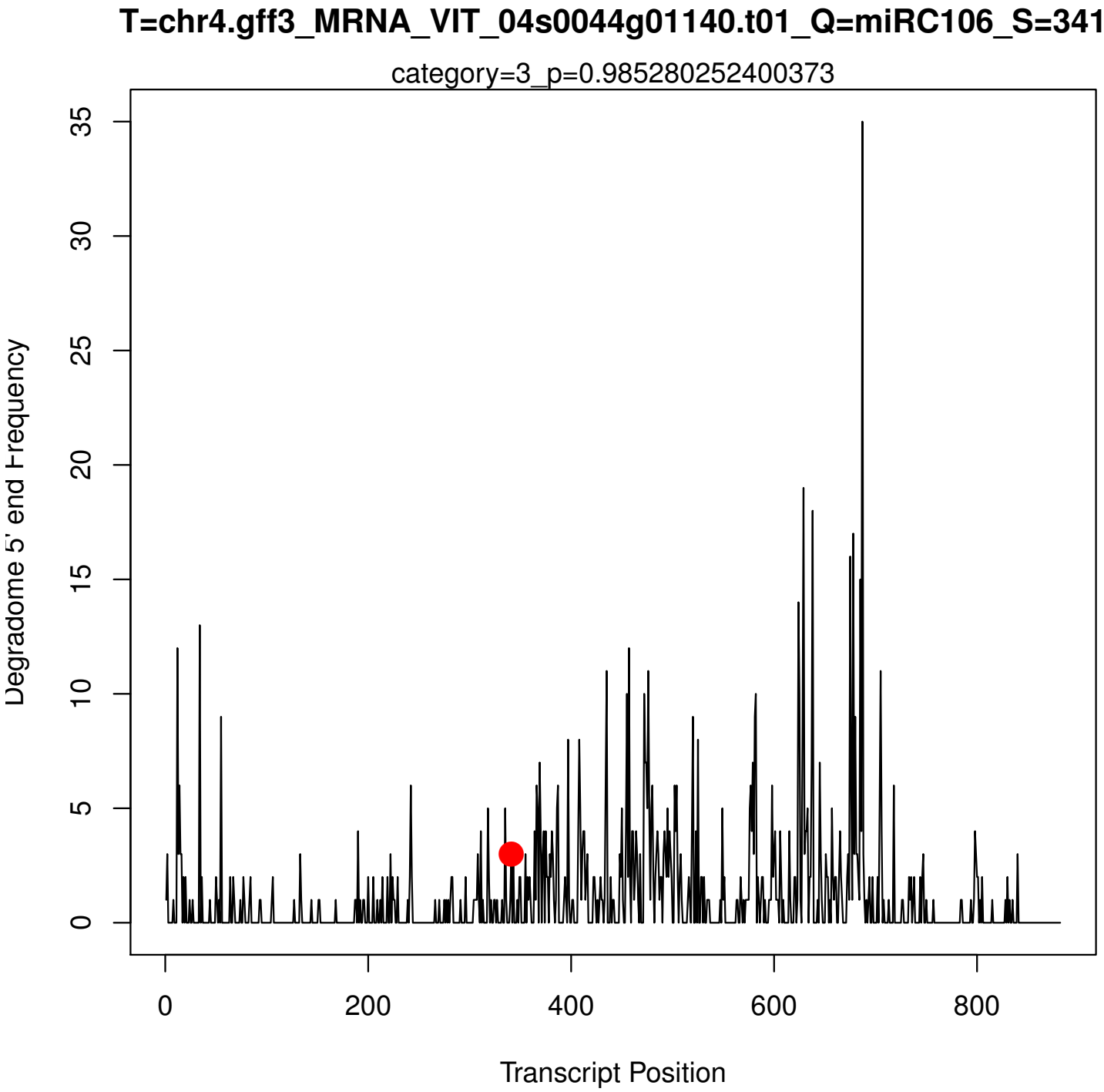
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0044g01140.t01_Q=miRC106_S=341
category=3_p=0.985280252400373
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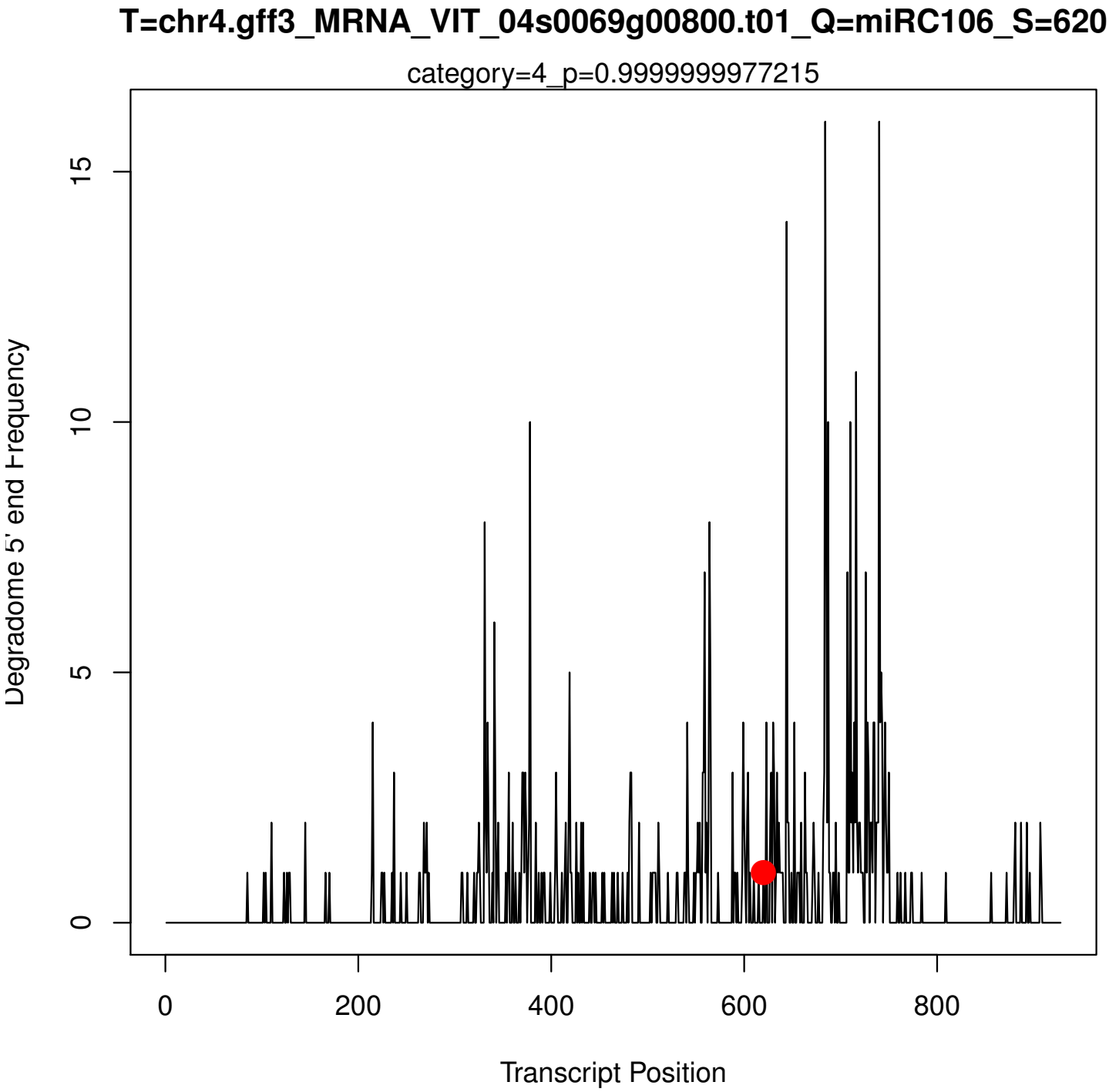
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0069g00800.t01_Q=miRC106_S=620
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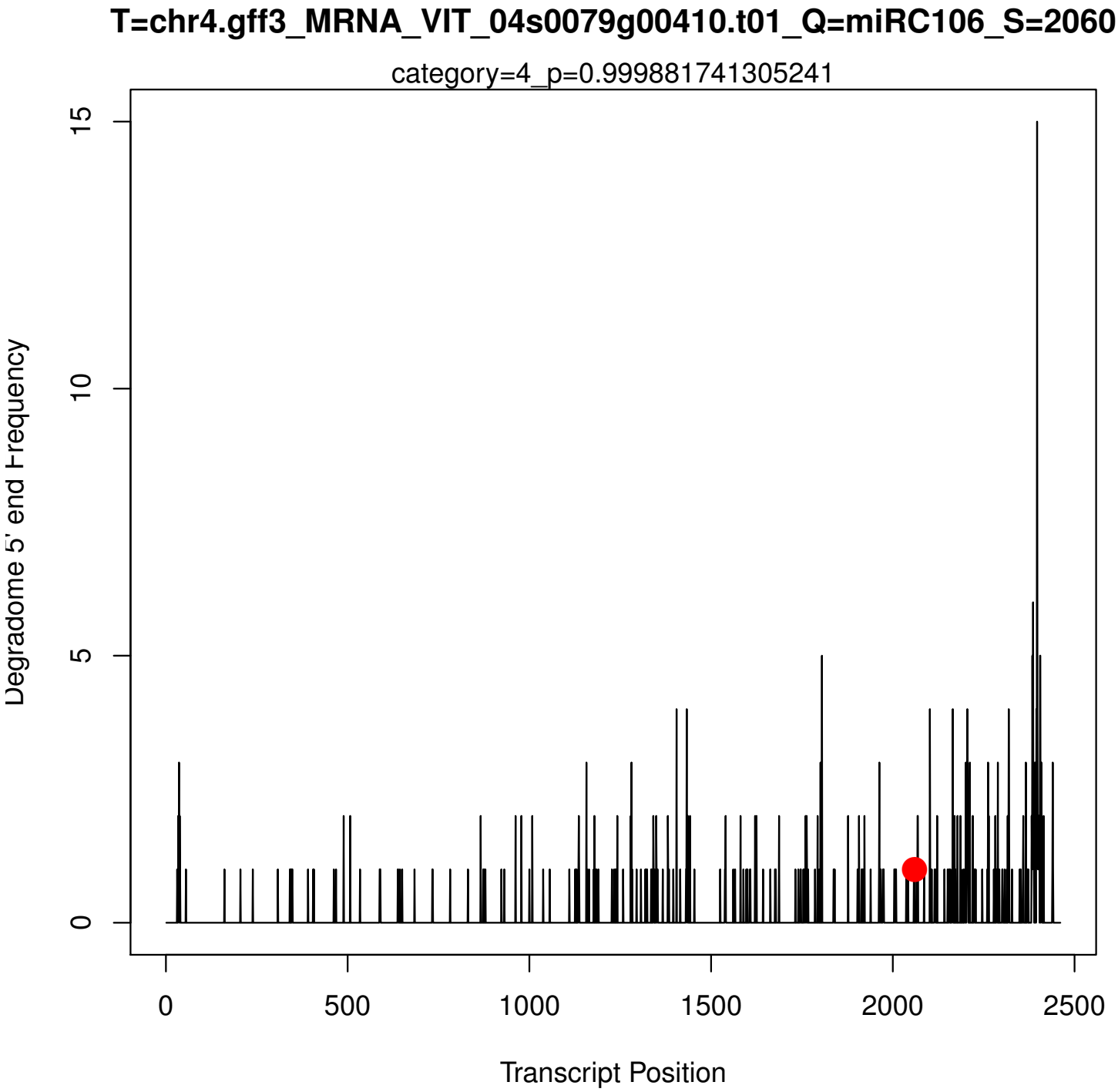
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0079g00410.t01_Q=miRC106_S=2060
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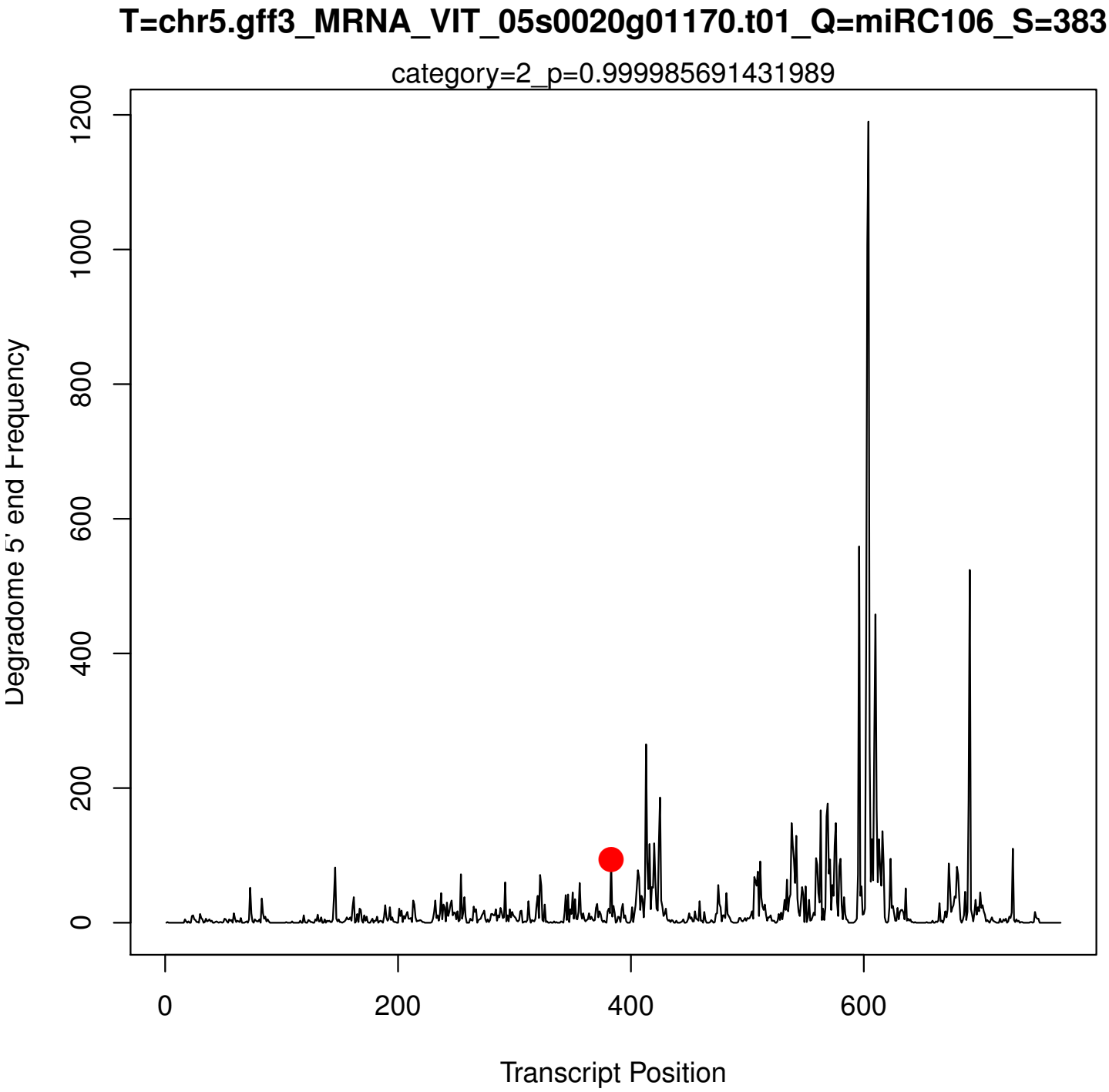
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0020g01170.t01_Q=miRC106_S=383
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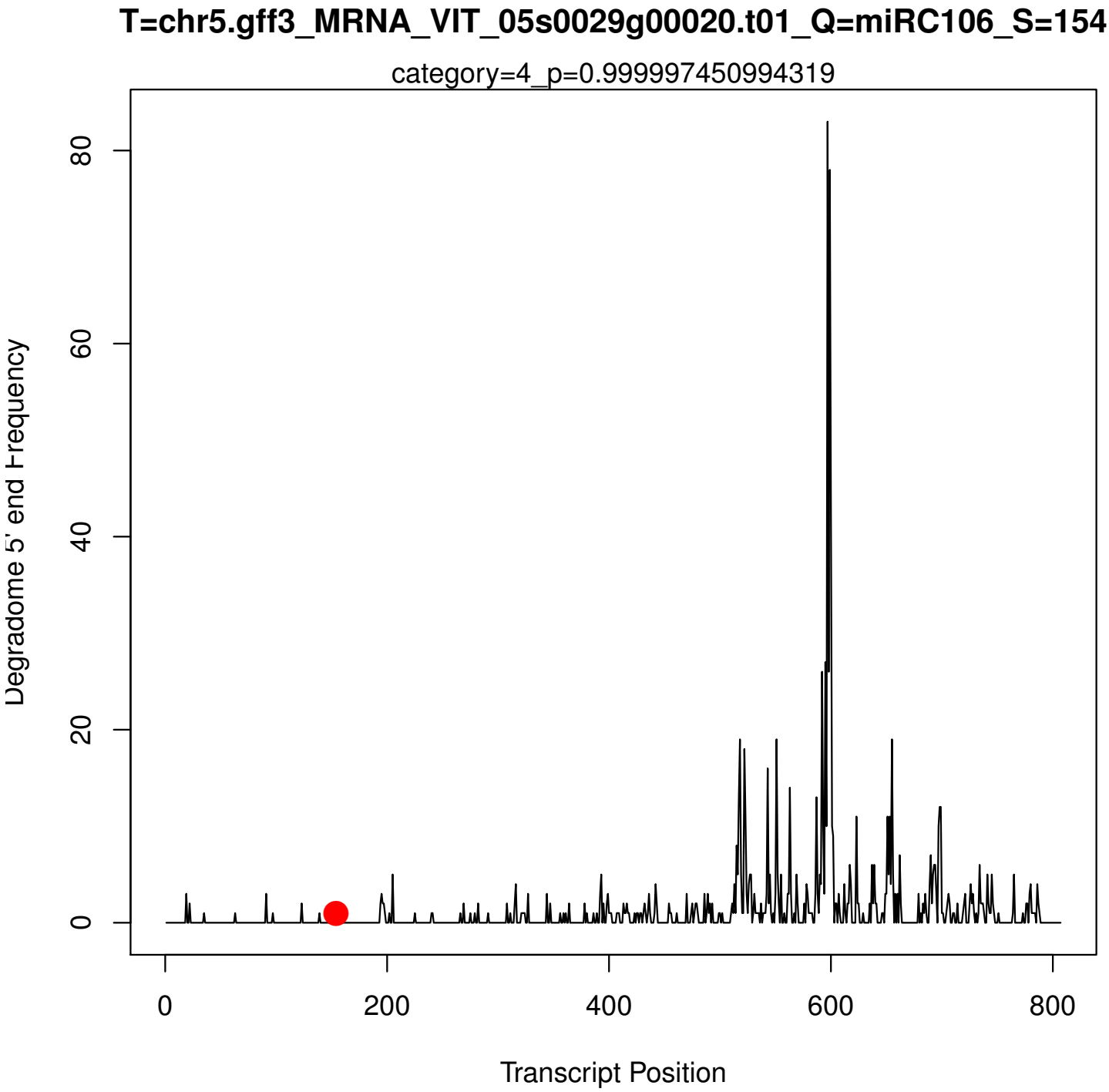
Transcript Position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category=4_p=0.999997450994319
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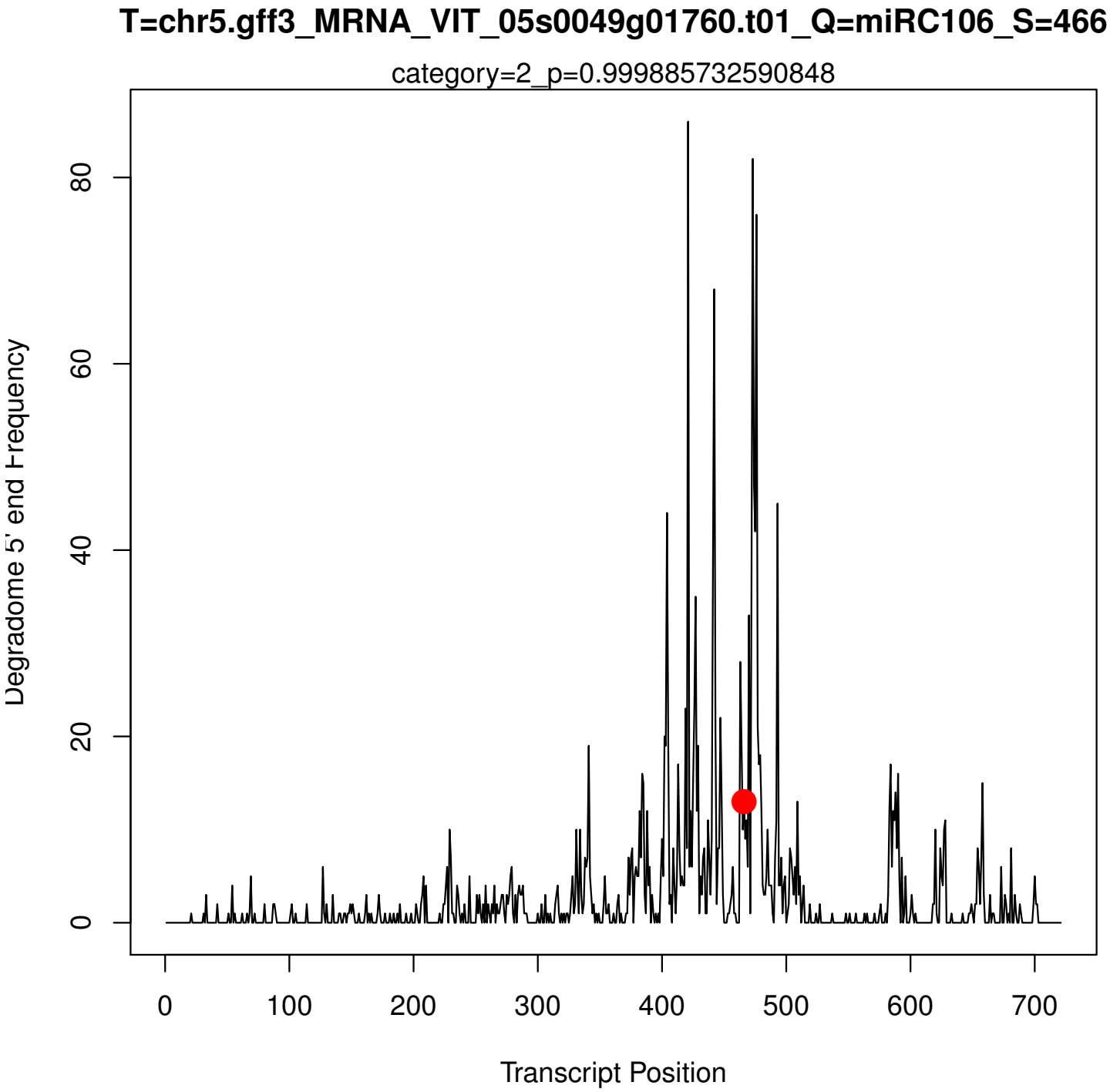
800
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0049g01760.t01_Q=miRC106_S=466
category=2_p=0.999885732590848
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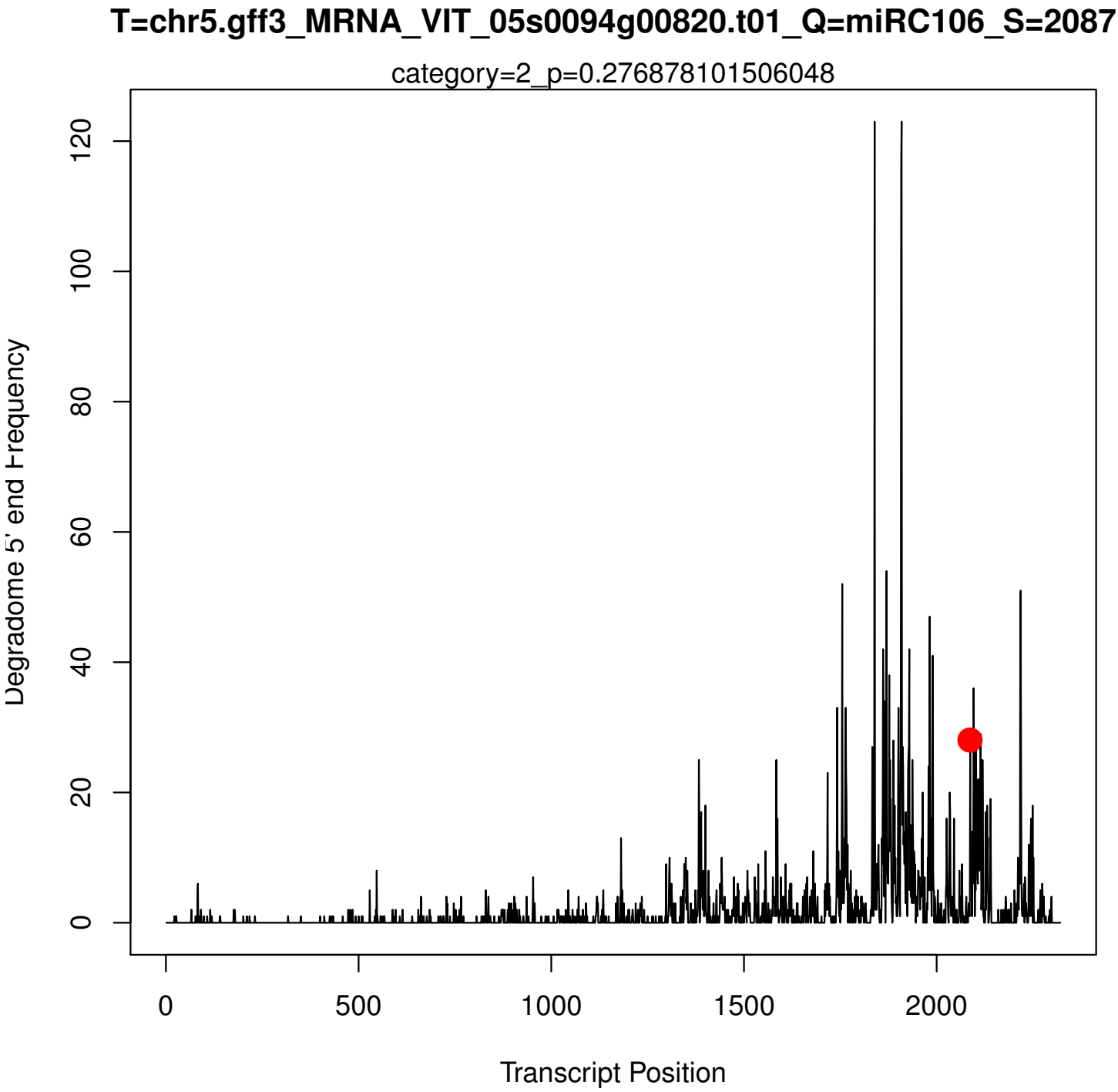
200
300
400
500
600
700
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0094g00820.t01_Q=miRC106_S=2087
category=2_p=0.276878101506048
Degradome 5' end Frequency
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120
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500
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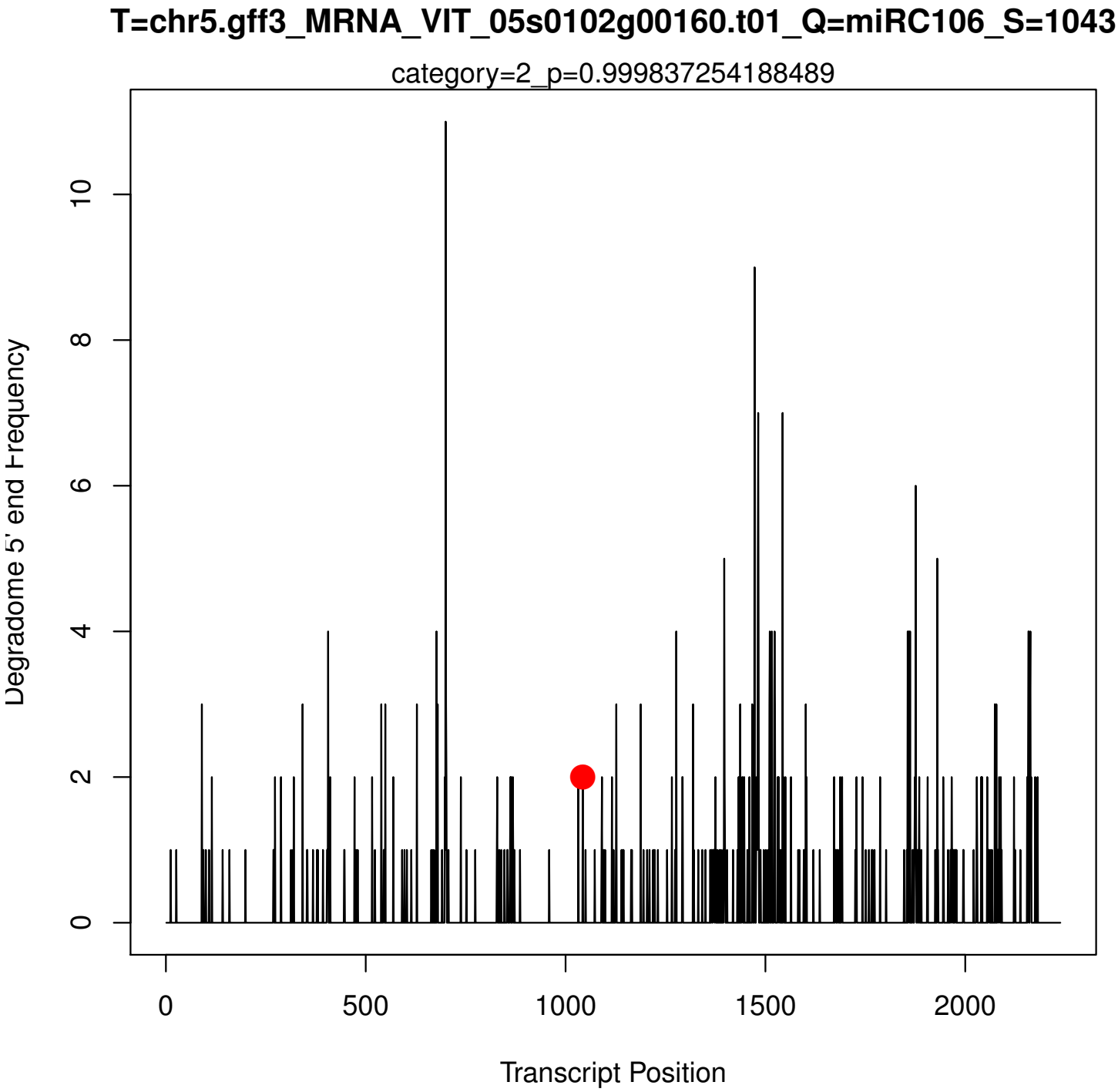
1500
2000
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0102g00160.t01_Q=miRC106_S=1043
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Degradome 5' end Frequency
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4
6
8
10
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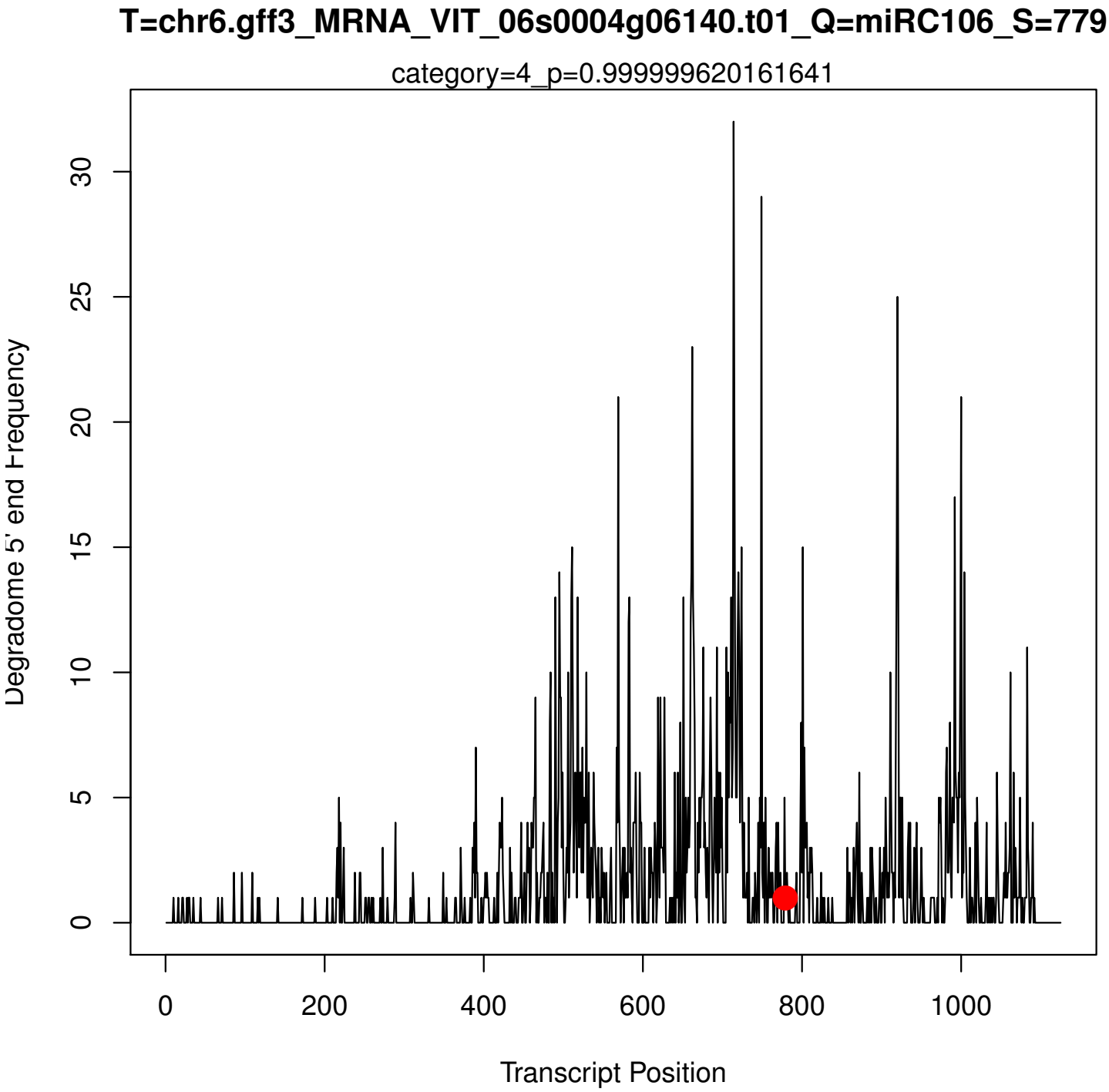
1500
2000
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0004g06140.t01_Q=miRC106_S=779
category=4_p=0.999999620161641
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10
15
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0
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400
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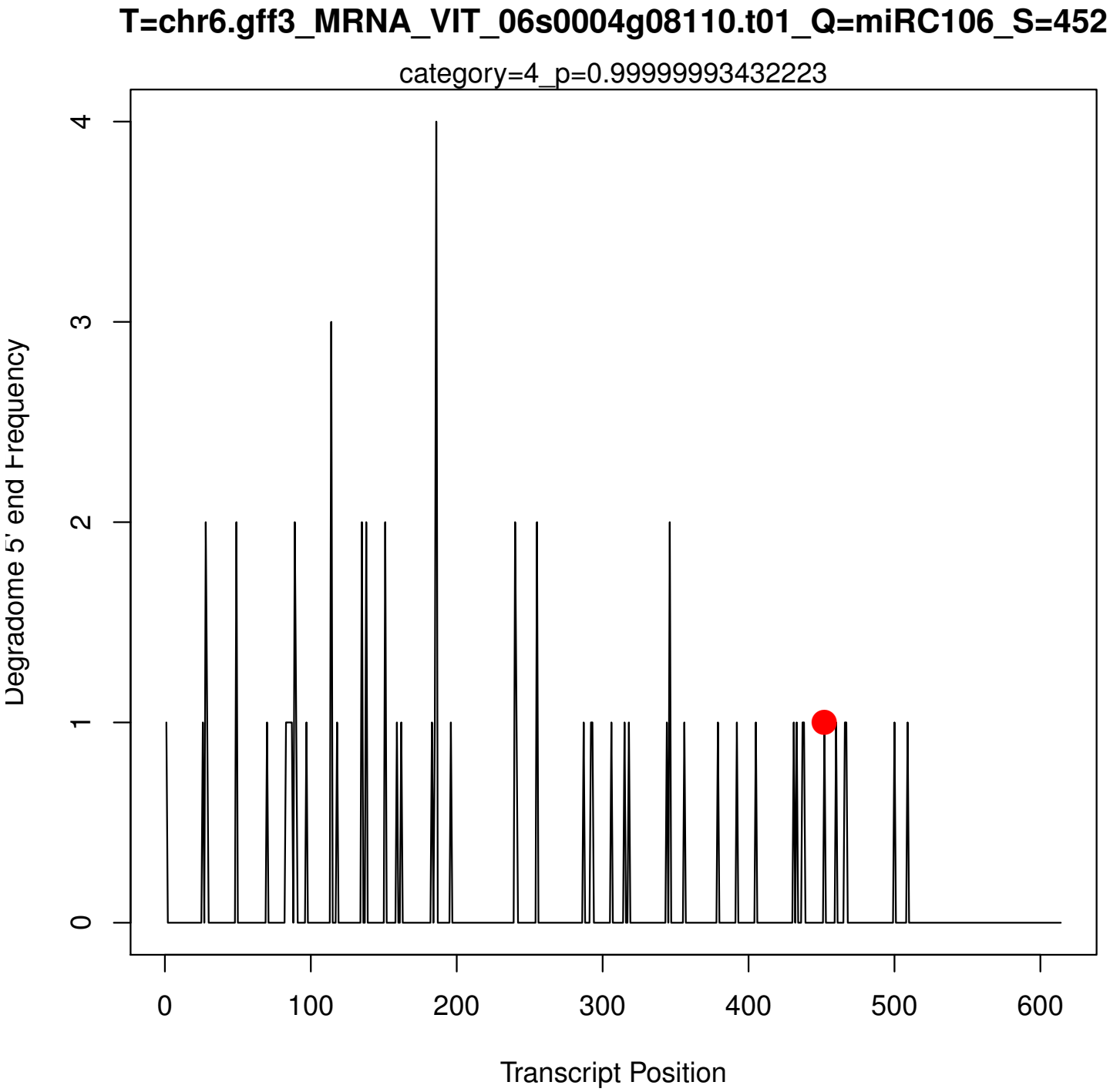
800
1000
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0004g08110.t01_Q=miRC106_S=452
category=4_p=0.99999993432223
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3
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500
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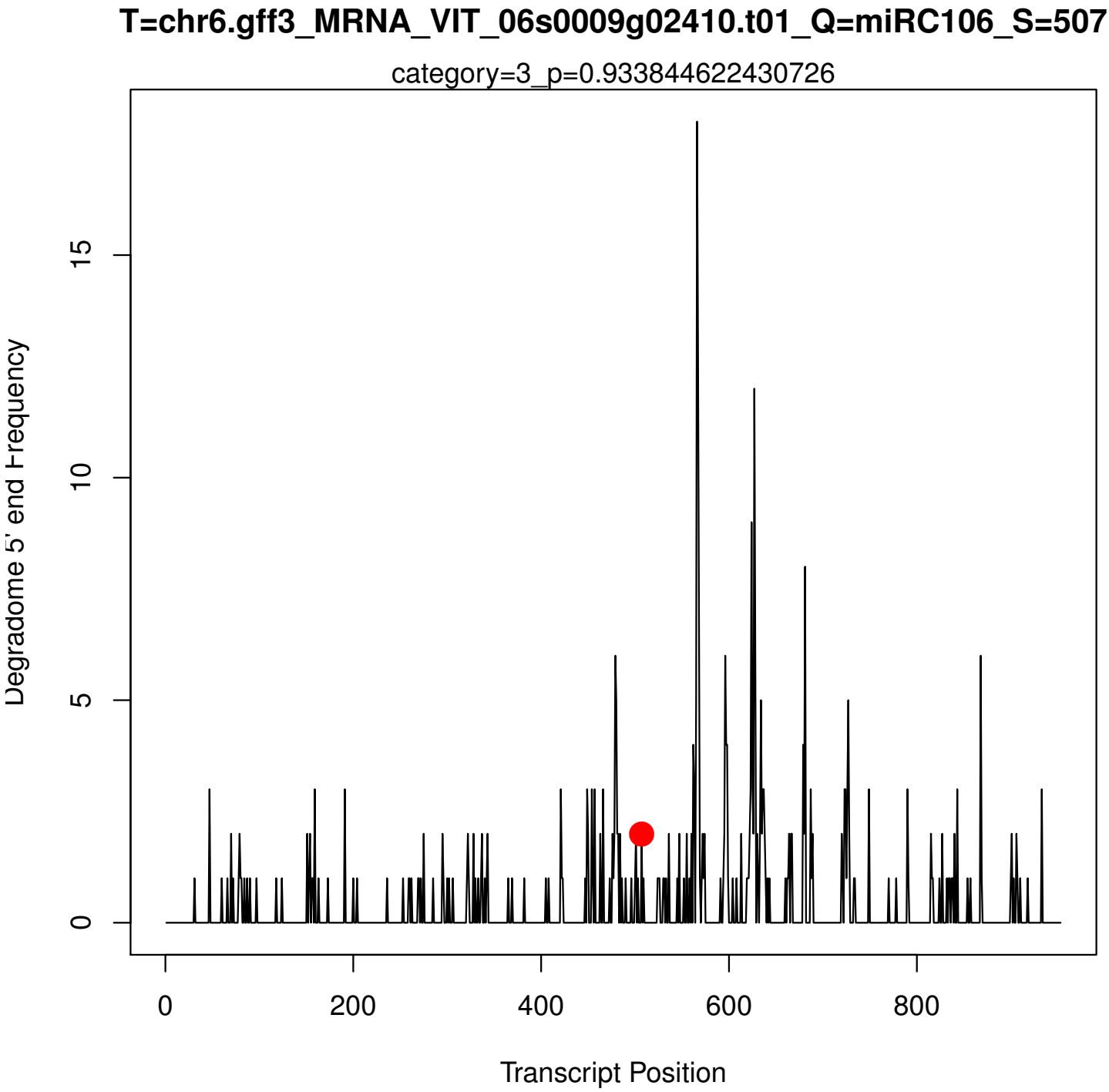
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0009g02410.t01_Q=miRC106_S=507
category=3_p=0.933844622430726
Degradome 5' end Frequency
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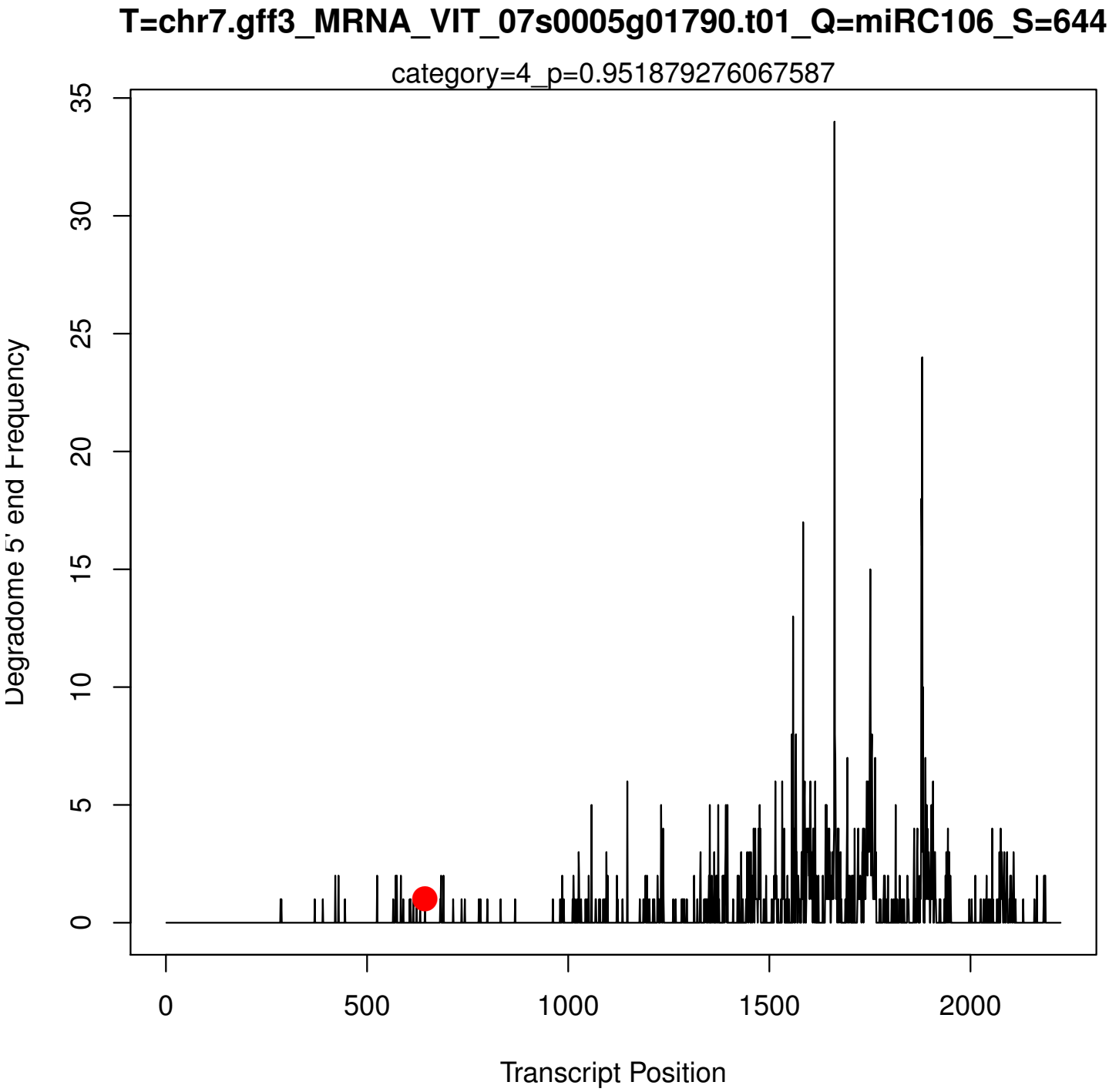
600
800
Transcript Position


T=chr7.gff3_MRNA_VIT_07s0005g01790.t01_Q=miRC106_S=644
category=4_p=0.951879276067587
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35
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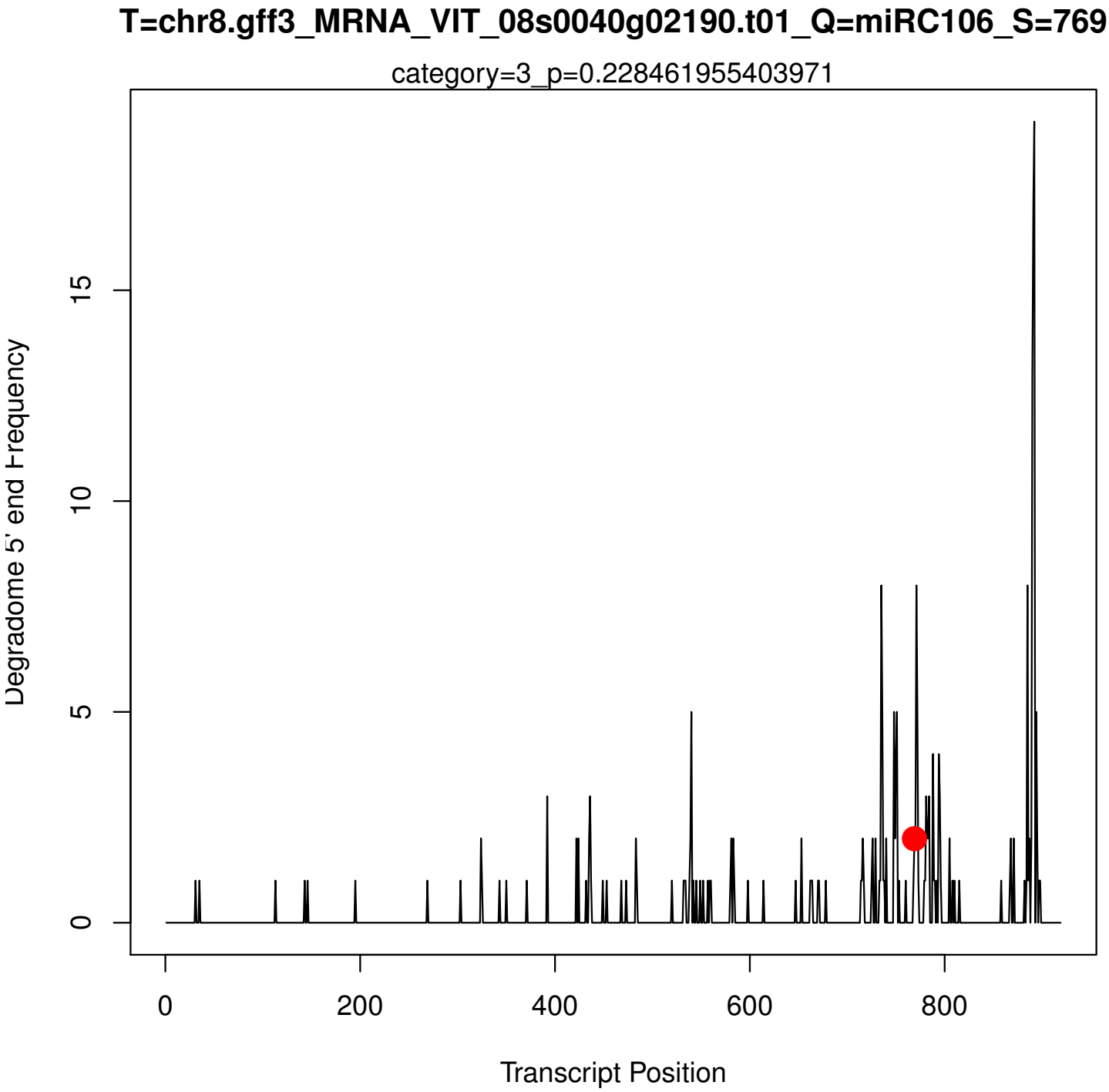
1500
2000
Transcript Position


T=chr8.gff3_MRNA_VIT_08s0040g02190.t01_Q=miRC106_S=769
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0
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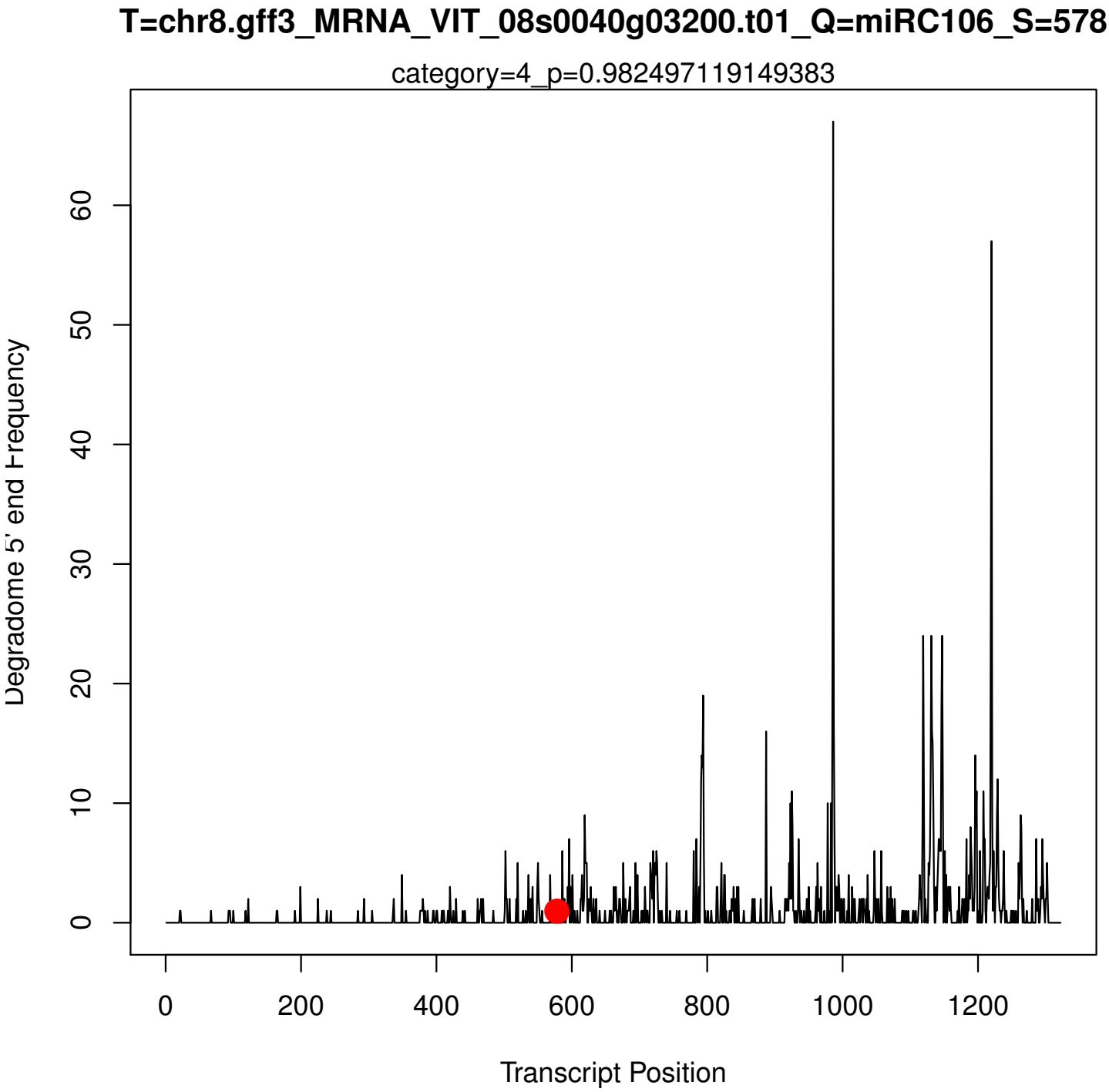
400
600
800
Transcript Position


T=chr8.gff3_MRNA_VIT_08s0040g03200.t01_Q=miRC106_S=578
category=4_p=0.982497119149383
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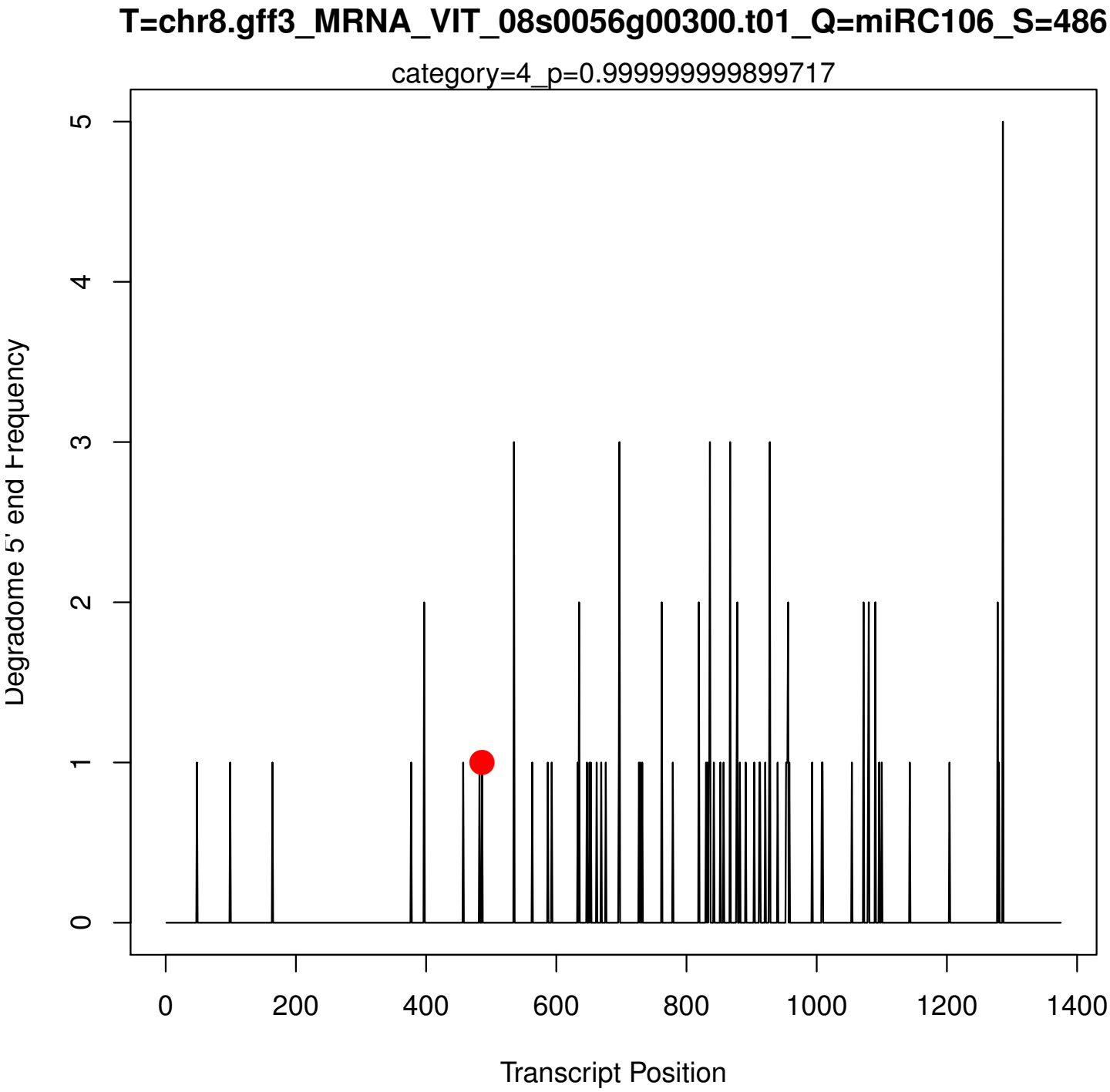
Transcript Position


T=chr8.gff3_MRNA_VIT_08s0056g00300.t01_Q=miRC106_S=486
category=4_p=0.999999999899717
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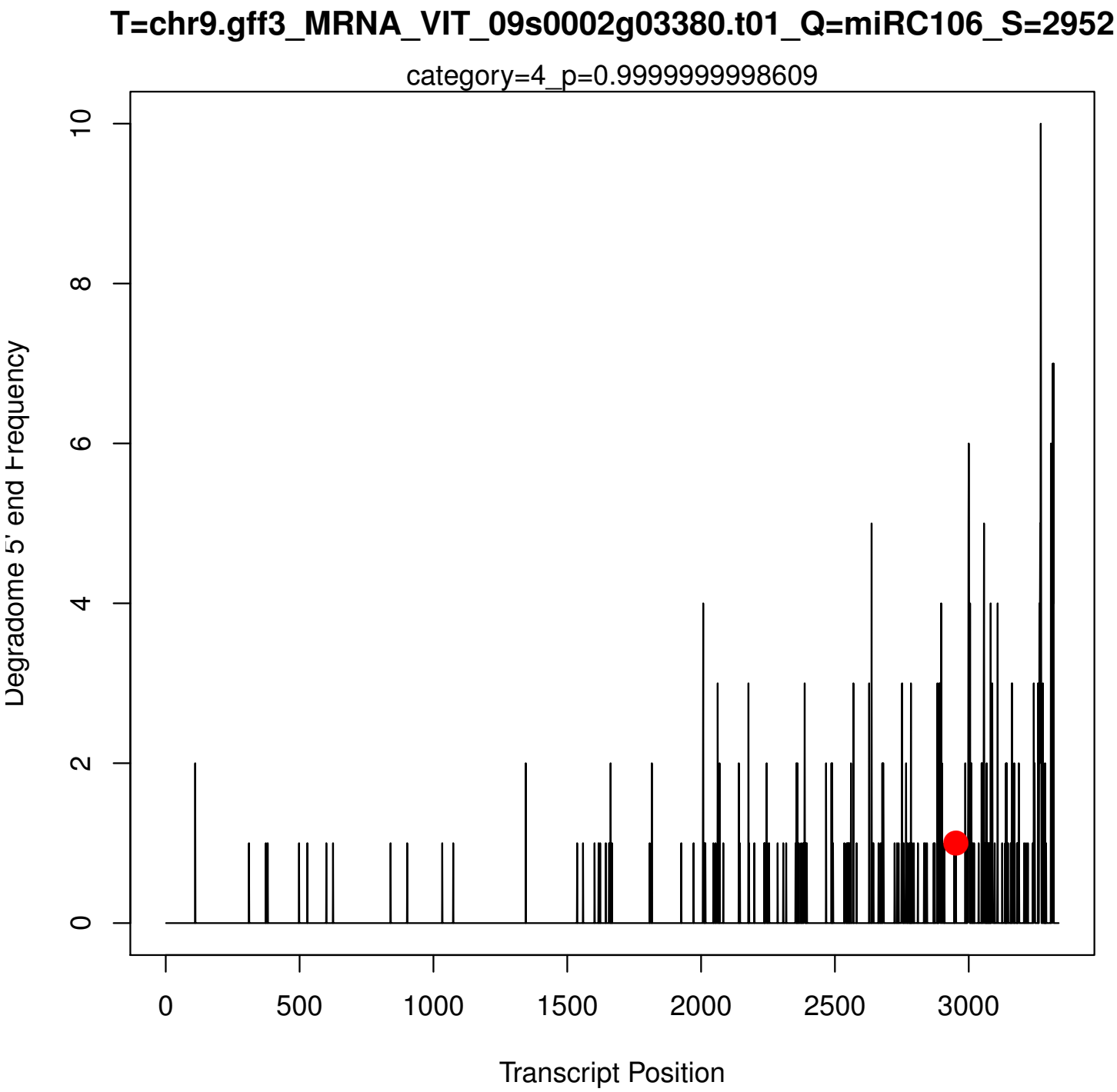
1400
Transcript Position


T=chr9.gff3_MRNA_VIT_09s0002g03380.t01_Q=miRC106_S=2952
category=4_p=0.9999999998609
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1500
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2500
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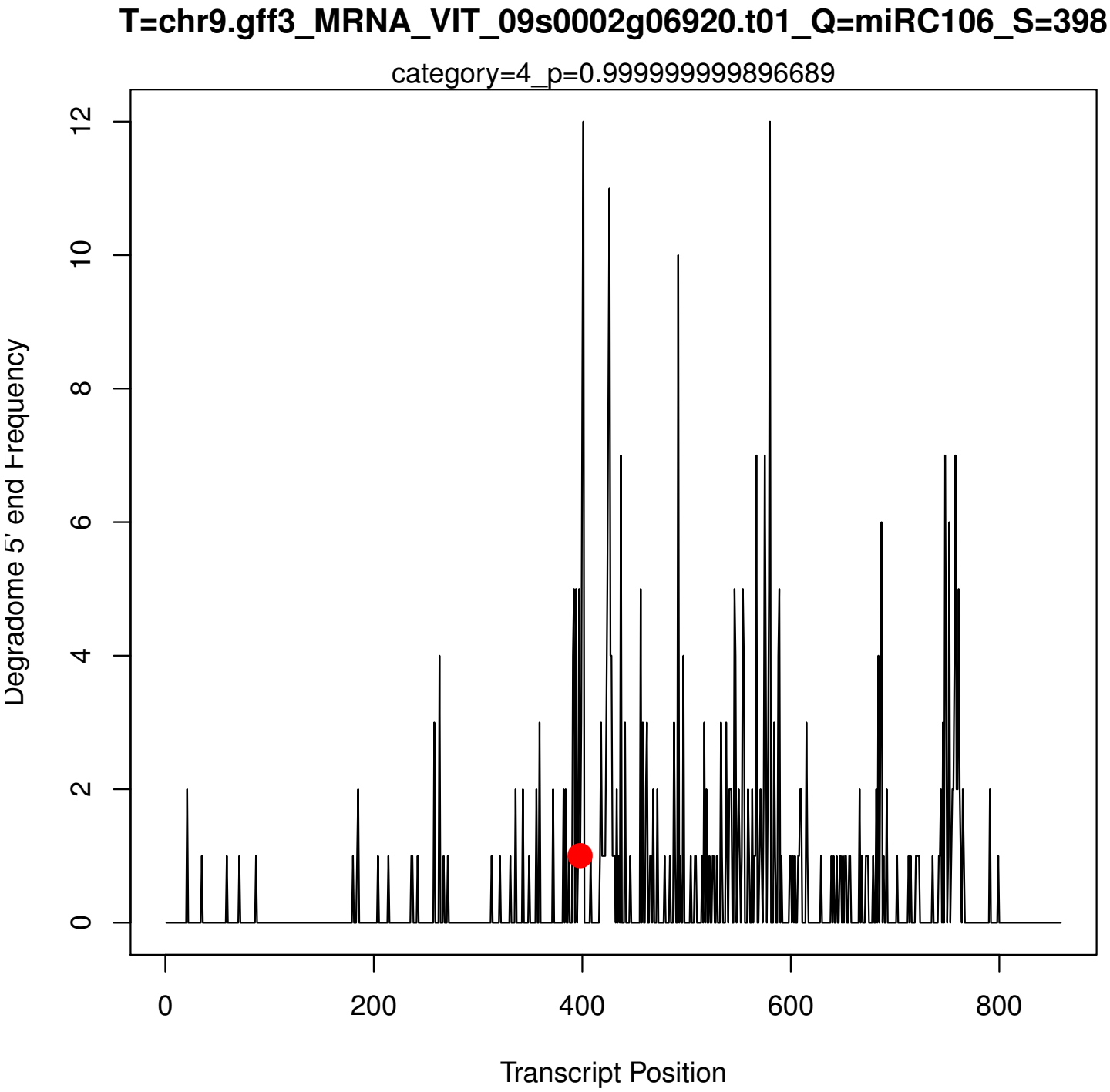
Transcript Position


T=chr9.gff3_MRNA_VIT_09s0002g06920.t01_Q=miRC106_S=398
category=4_p=0.999999999896689
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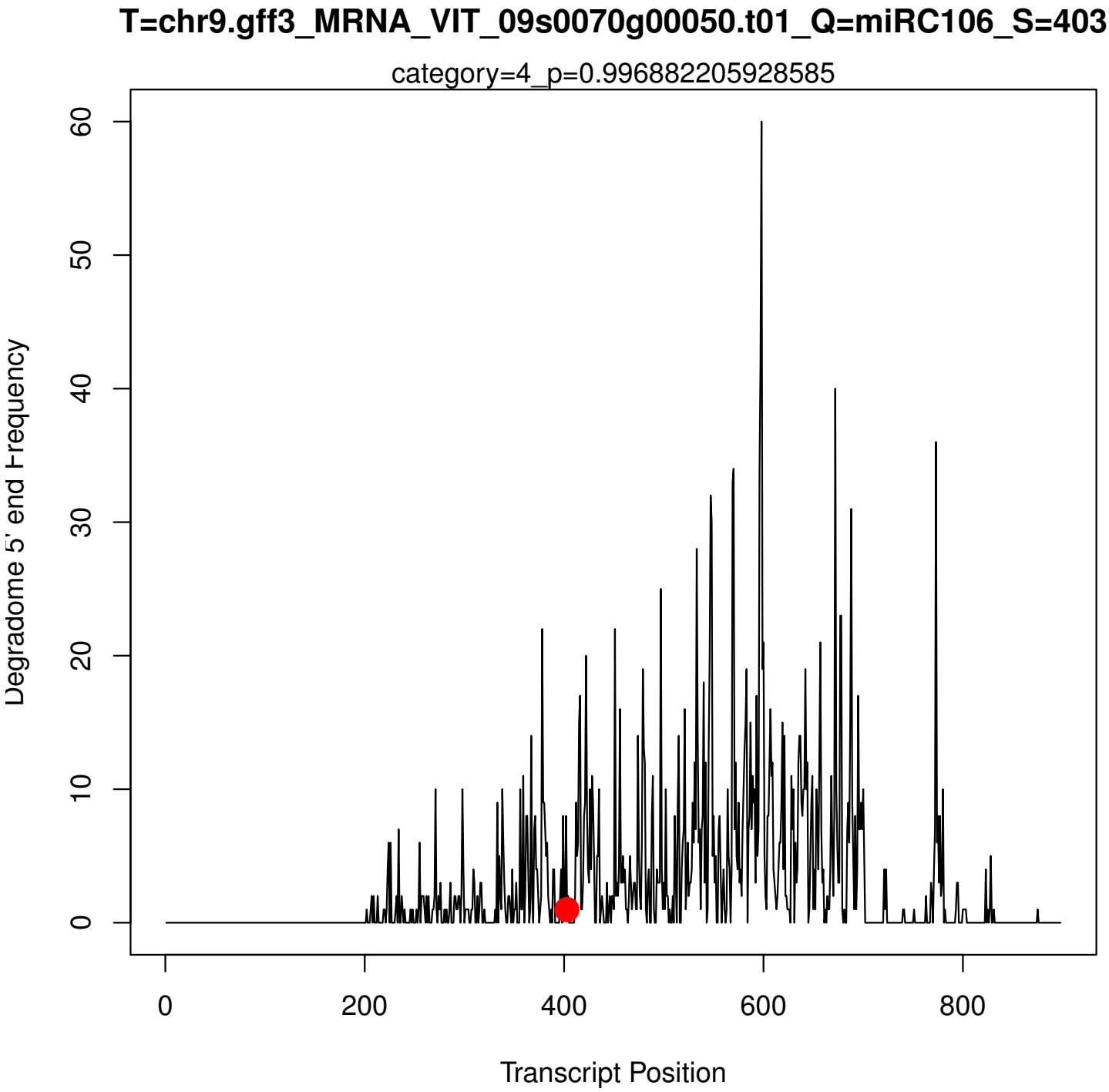
600
800
Transcript Position


T=chr9.gff3_MRNA_VIT_09s0070g00050.t01_Q=miRC106_S=403
category=4_p=0.996882205928585
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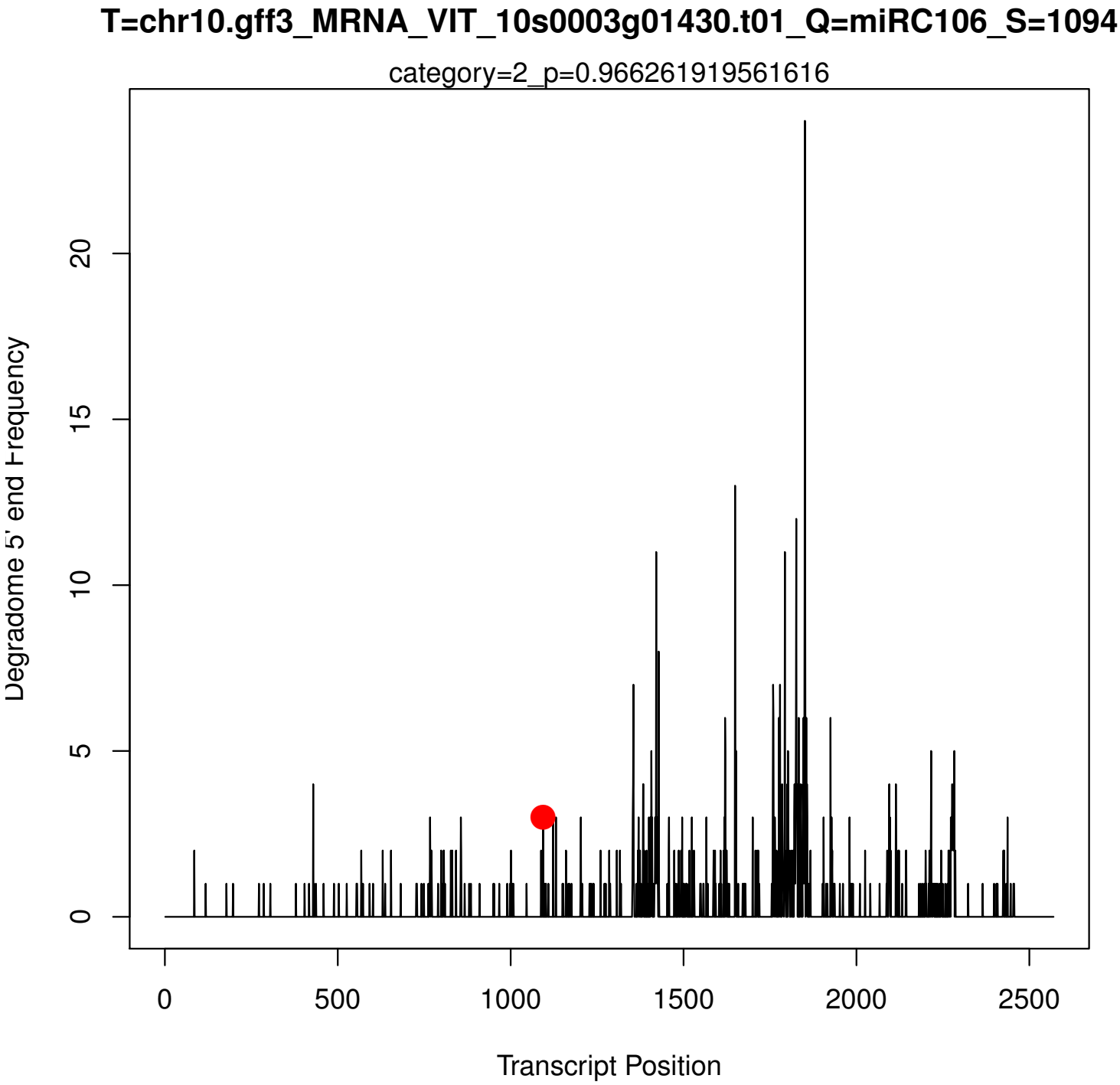
600
800
Transcript Position


T=chr10.gff3_MRNA_VIT_10s0003g01430.t01_Q=miRC106_S=1094
category=2_p=0.966261919561616
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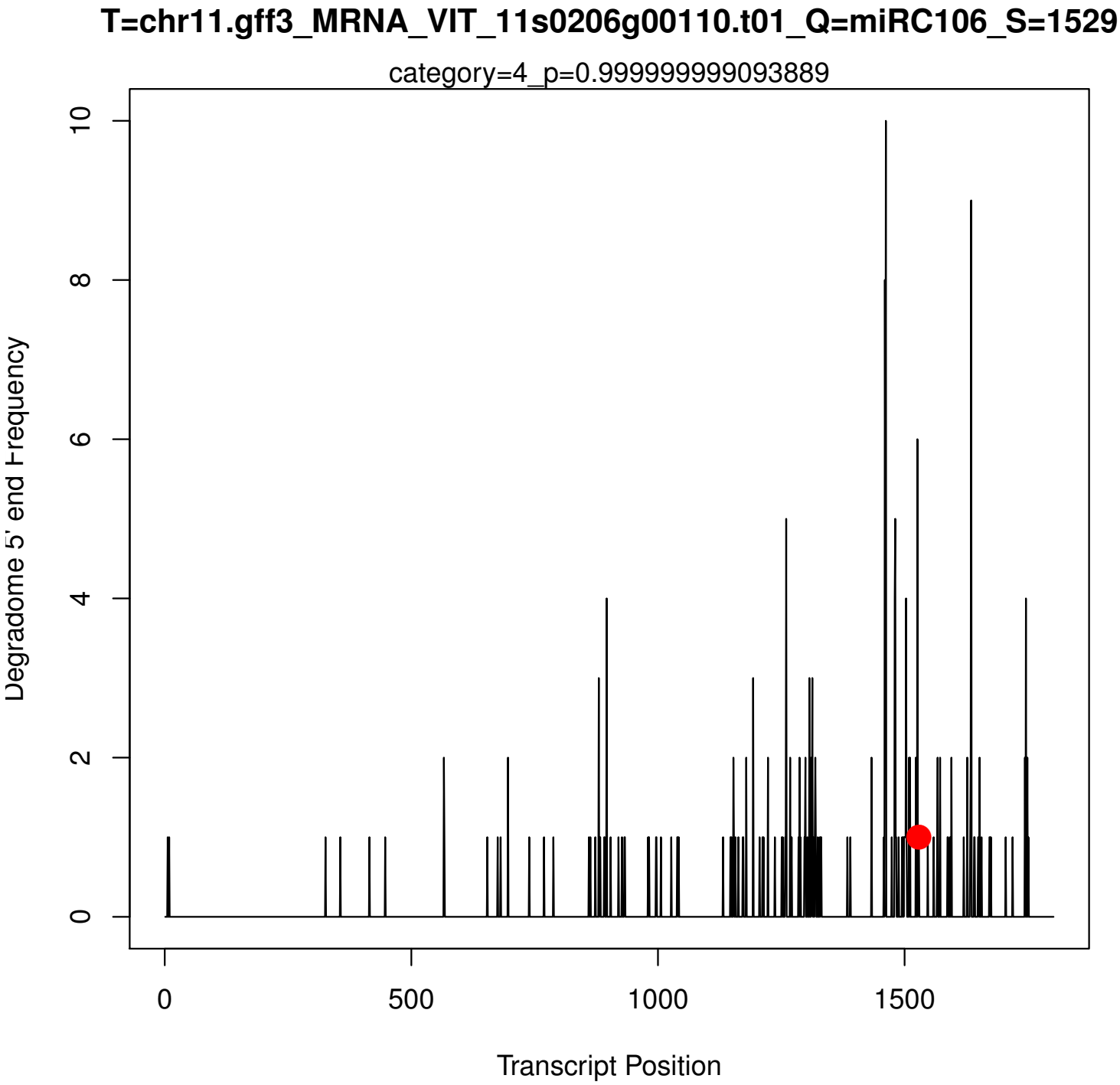
Transcript Position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8
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1000
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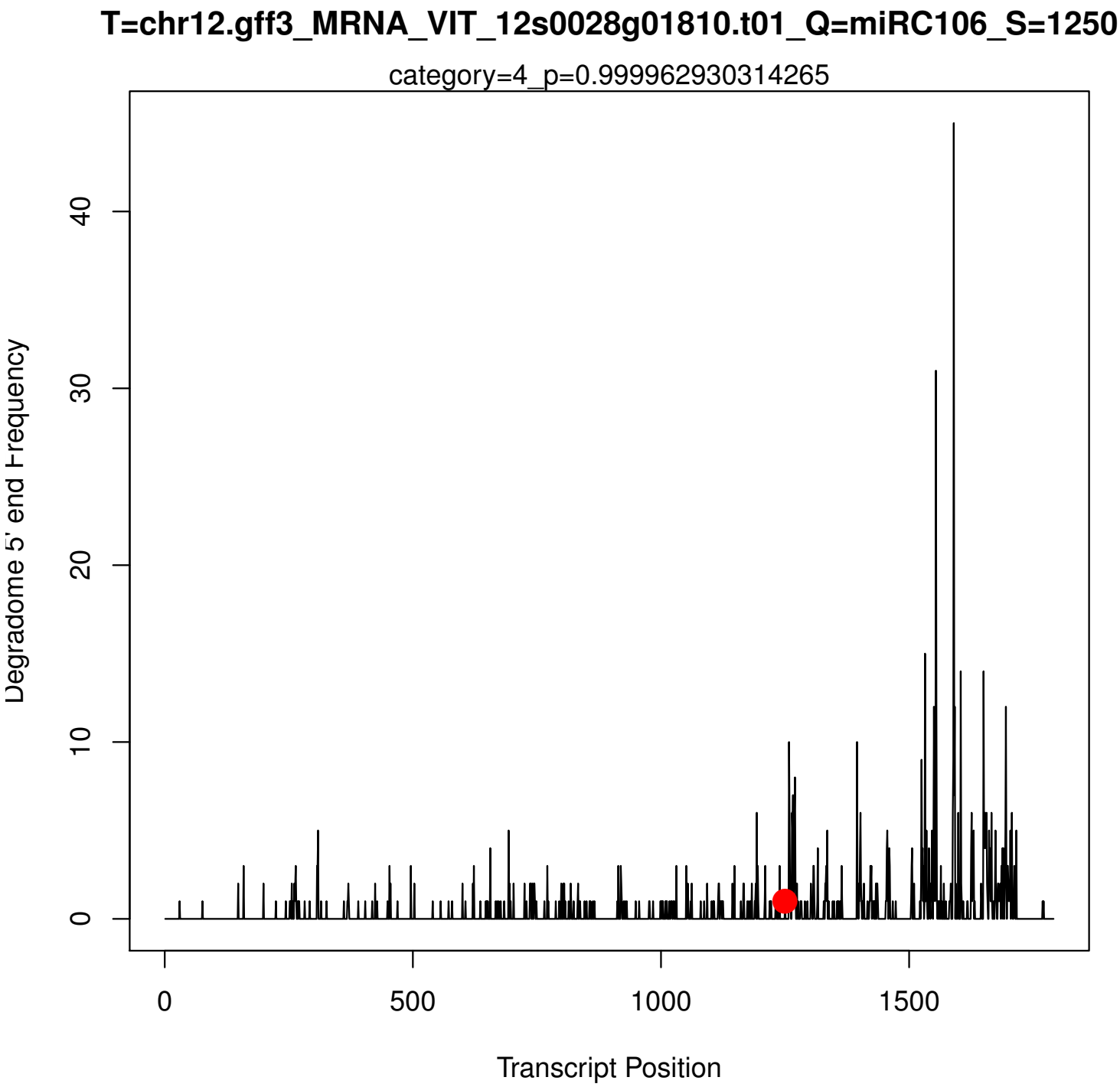
Transcript Position


T=chr12.gff3_MRNA_VIT_12s0028g01810.t01_Q=miRC106_S=1250
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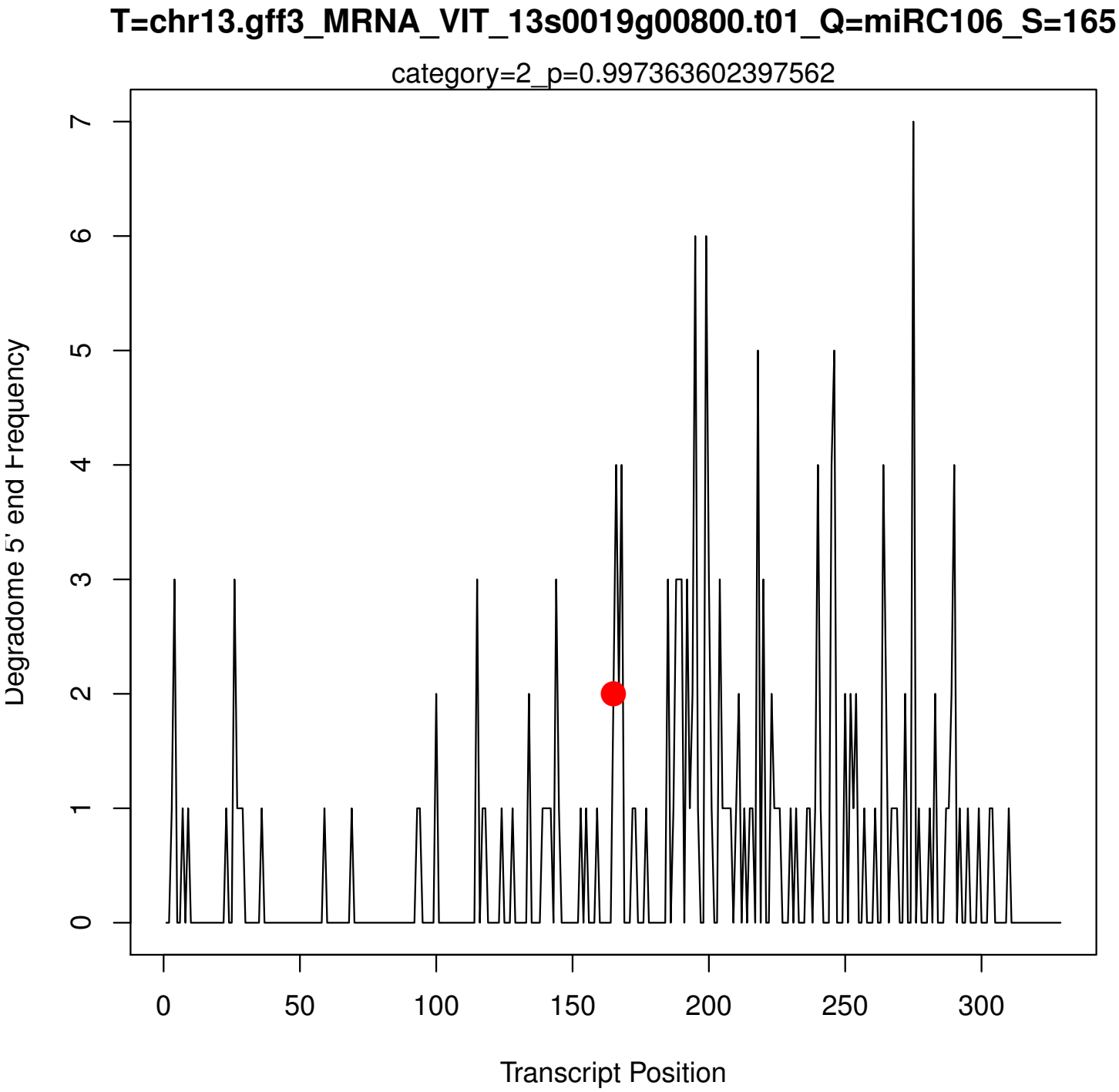
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0019g00800.t01_Q=miRC106_S=165
category=2_p=0.997363602397562
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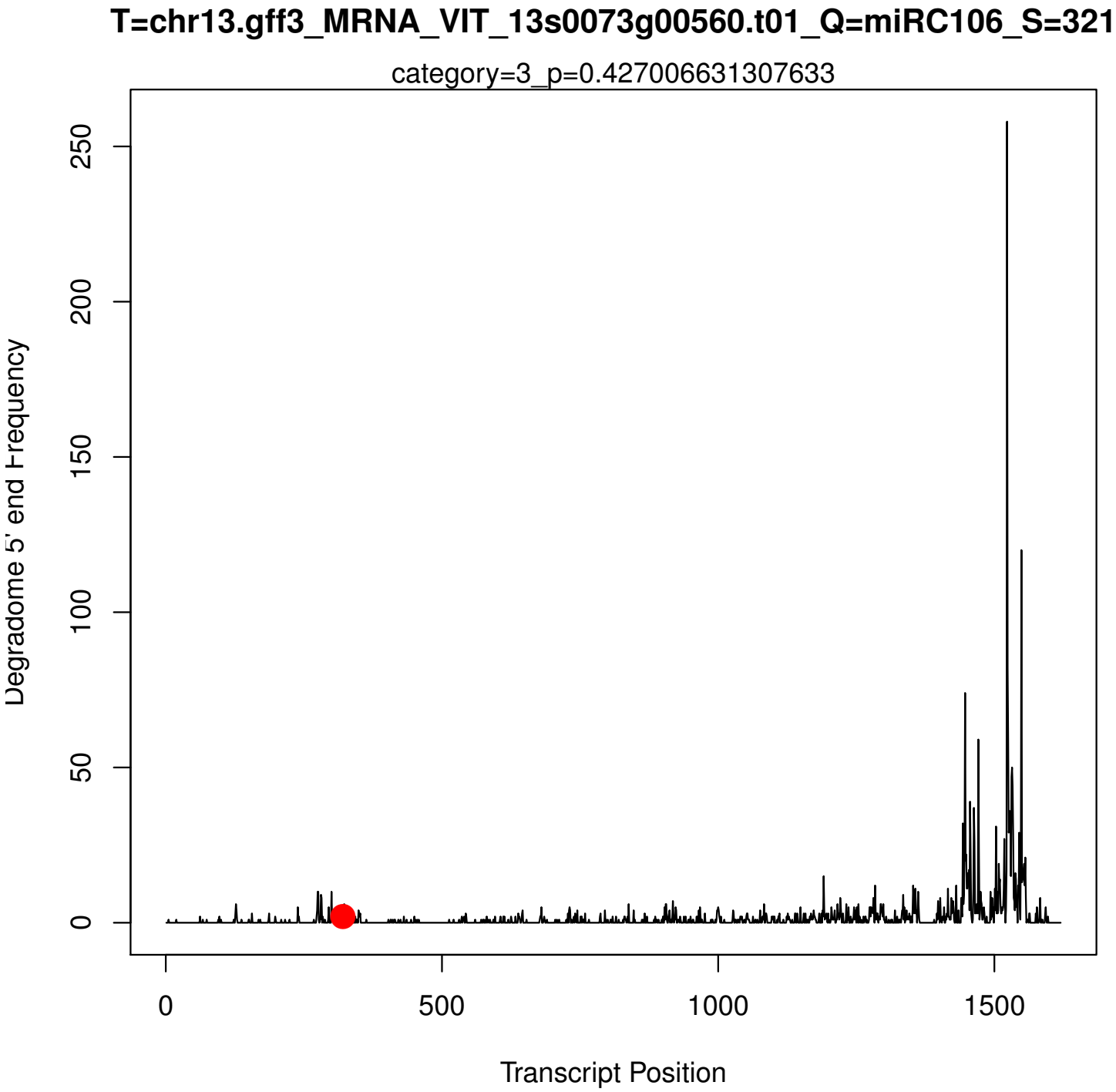
250
300
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0073g00560.t01_Q=miRC106_S=321
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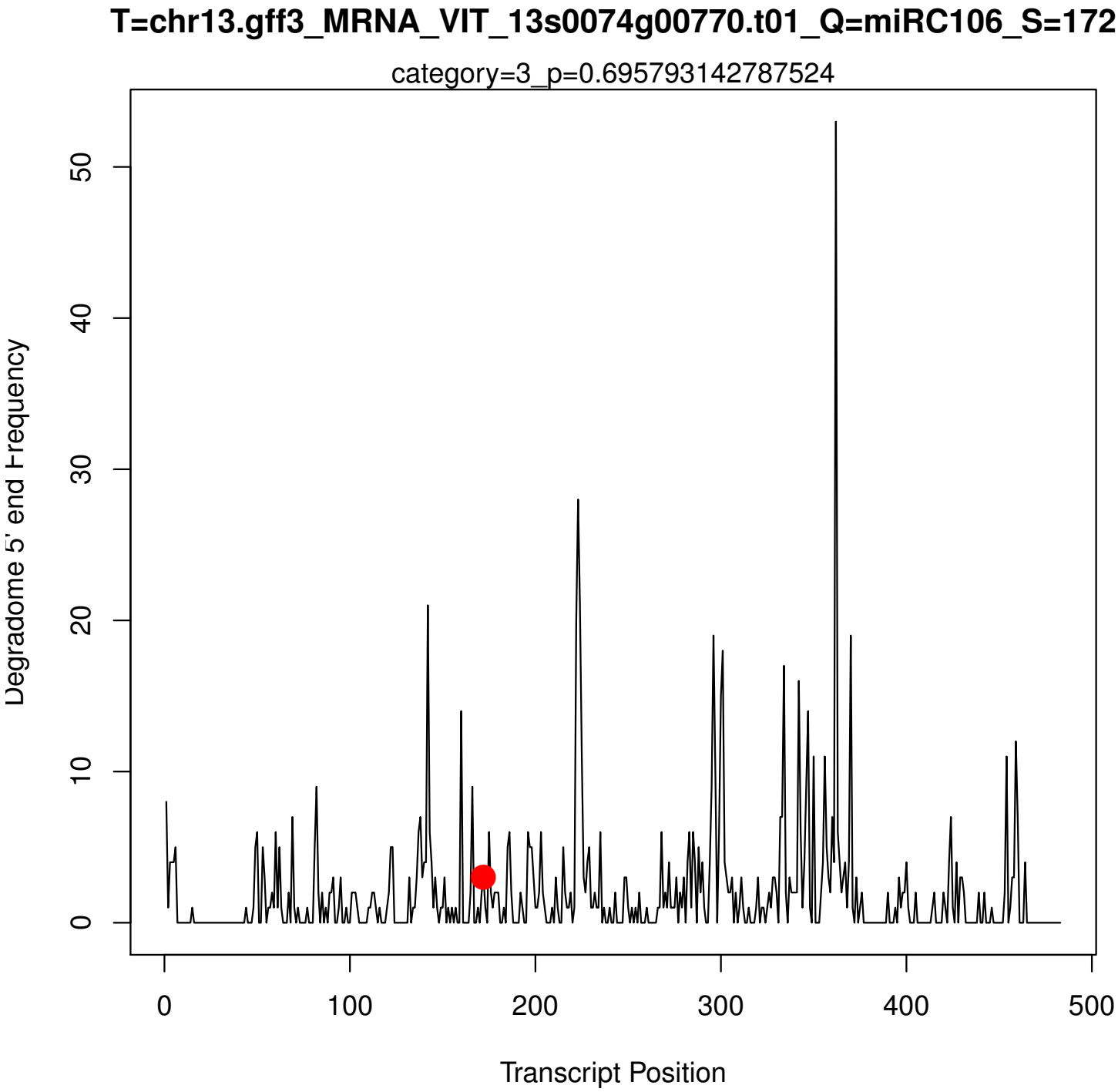
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0074g00770.t01_Q=miRC106_S=172
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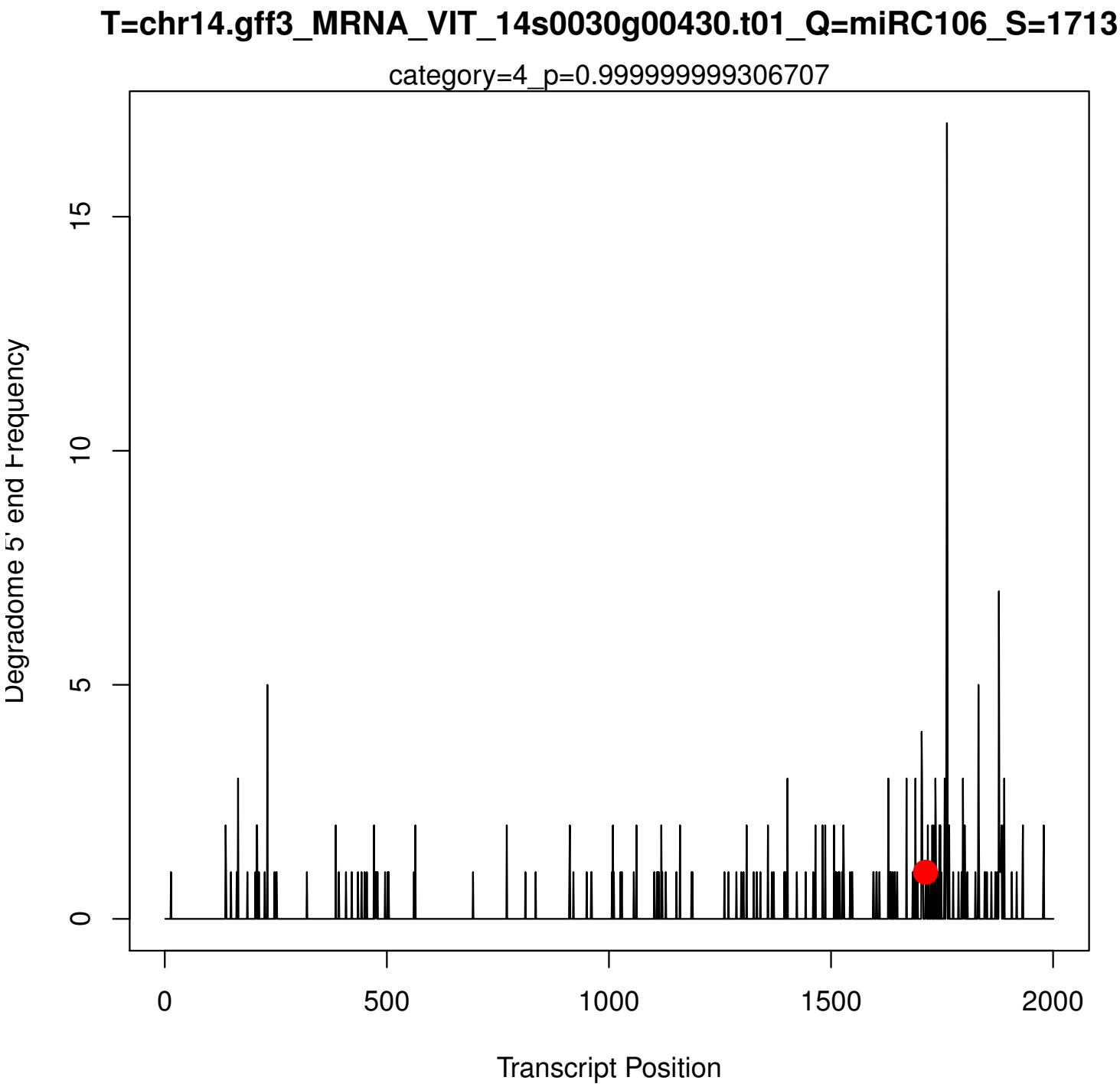
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0030g00430.t01_Q=miRC106_S=1713
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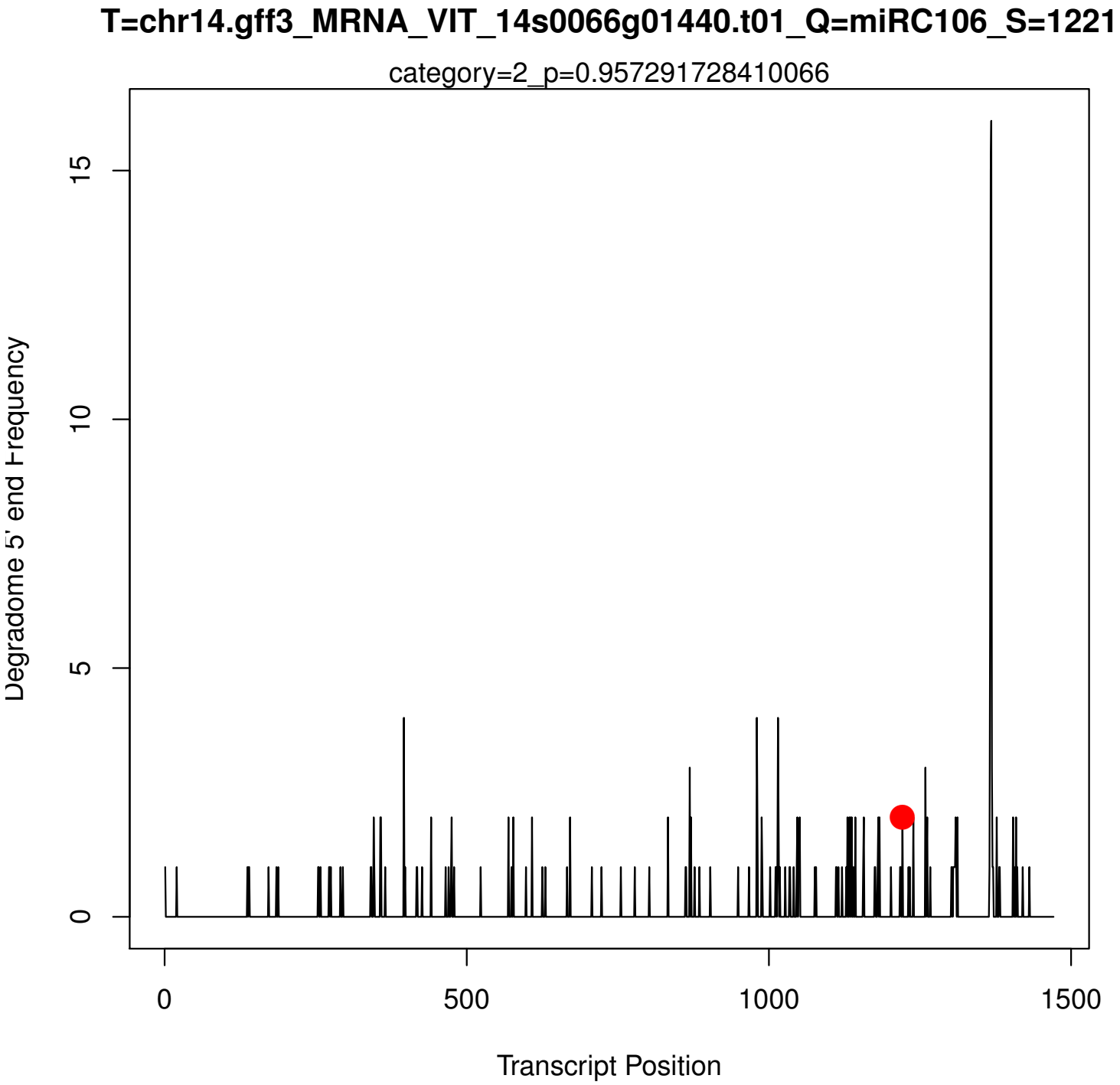
2000
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0066g01440.t01_Q=miRC106_S=1221
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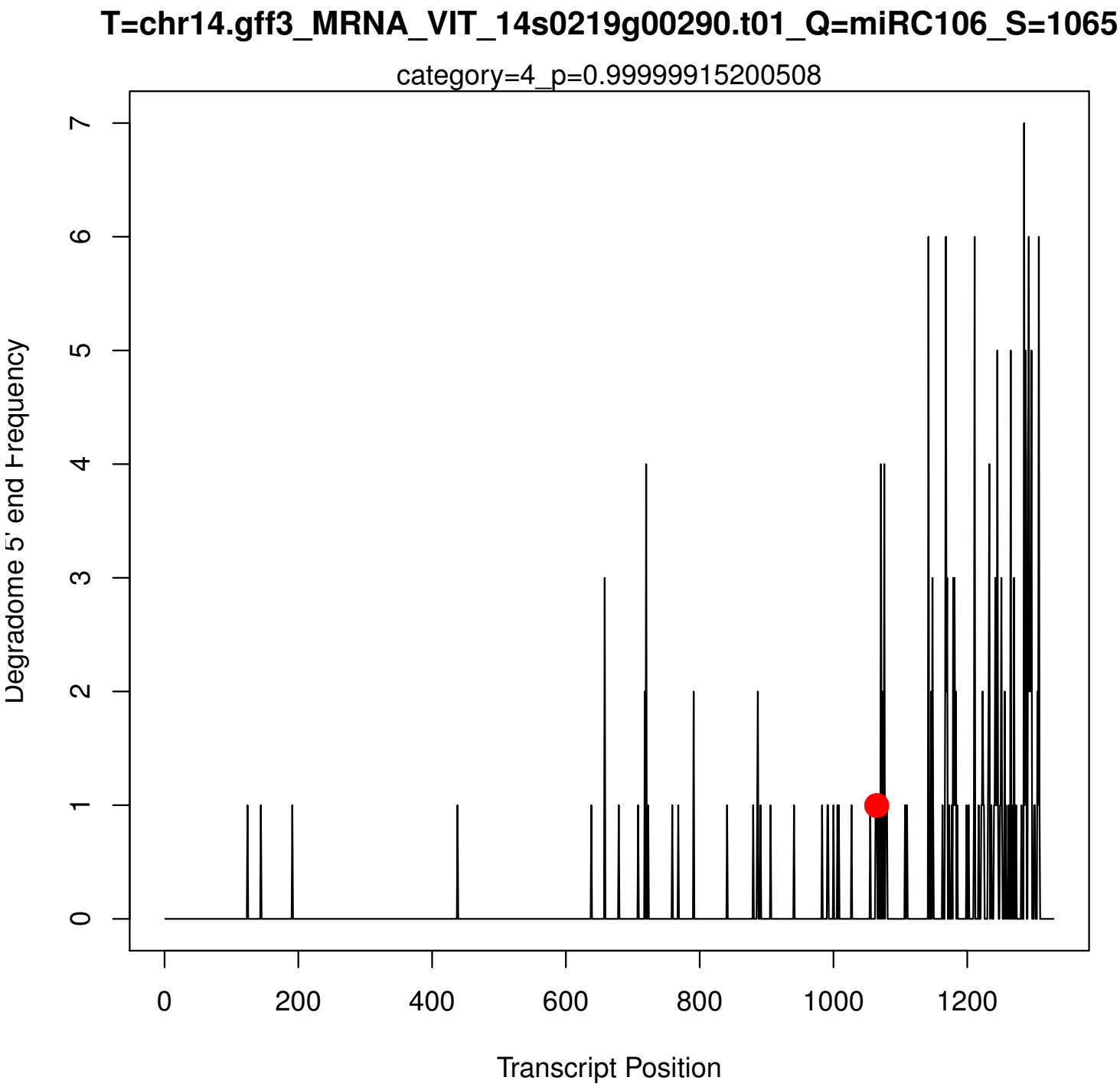
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0219g00290.t01_Q=miRC106_S=1065
category=4_p=0.99999915200508
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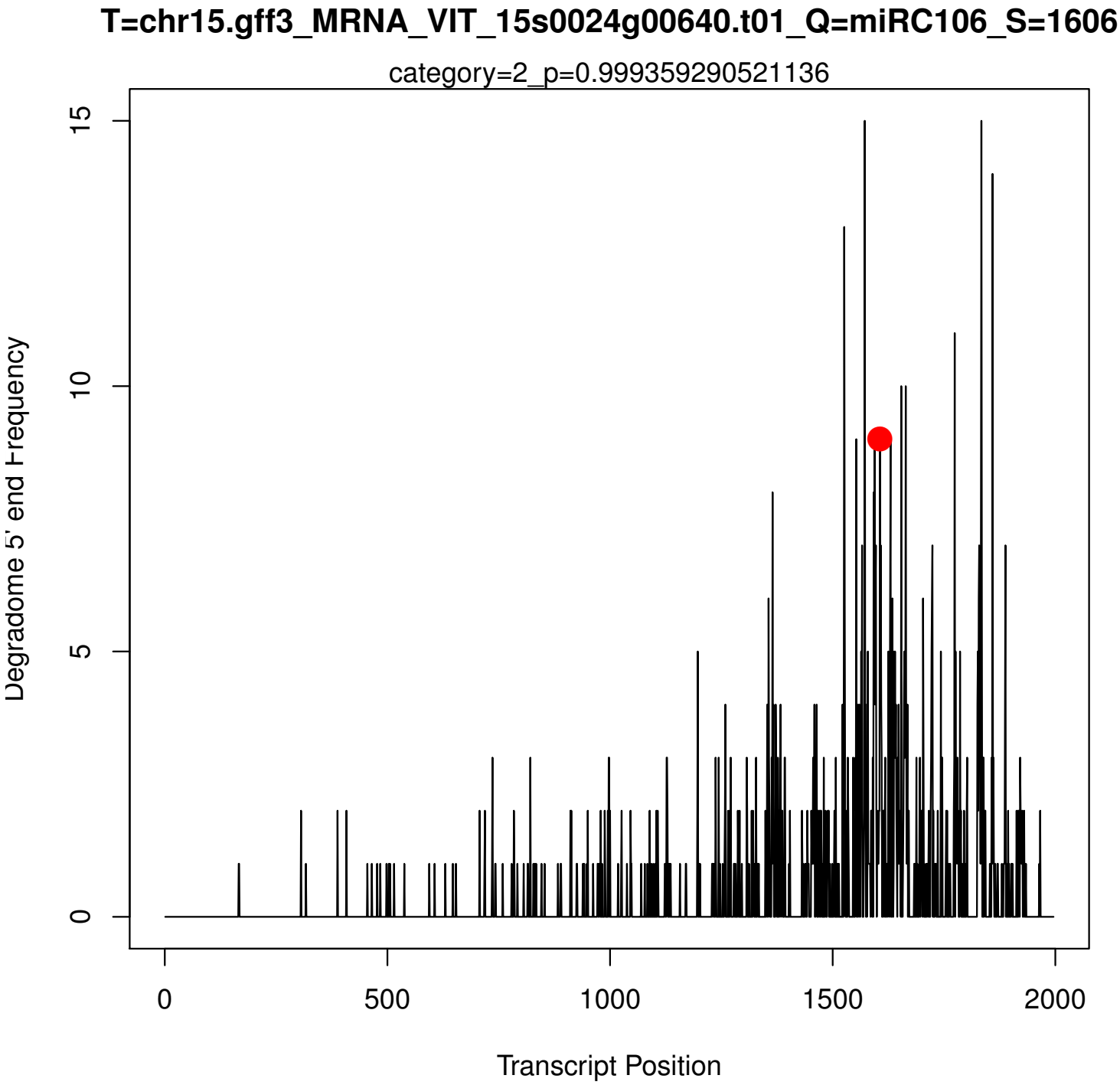
1200
Transcript Position


T=chr15.gff3_MRNA_VIT_15s0024g00640.t01_Q=miRC106_S=1606
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Degradome 5' end Frequency
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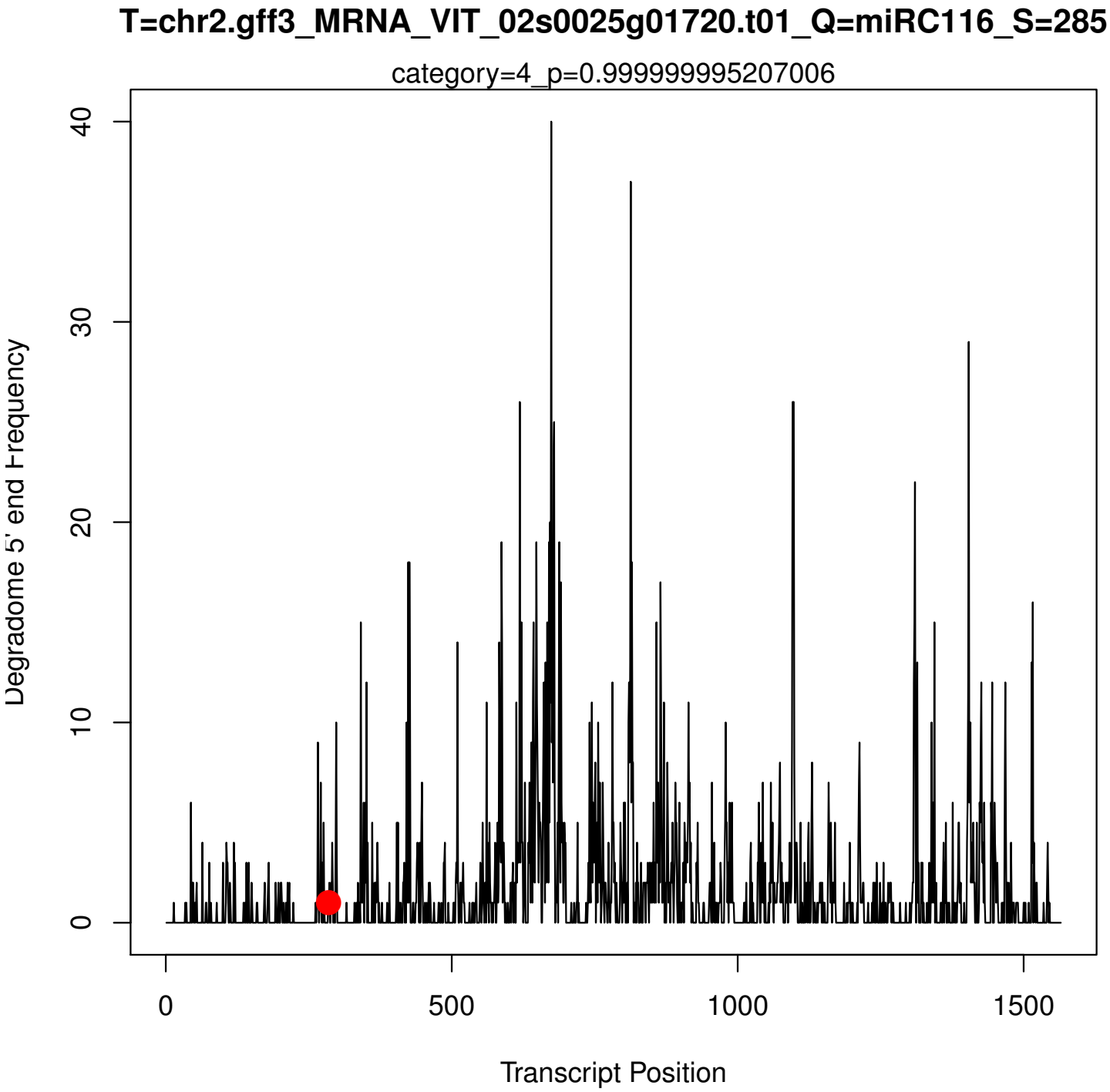
1500
2000
Transcript Position


T=chr2.gff3_MRNA_VIT_02s0025g01720.t01_Q=miRC116_S=285
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40
0
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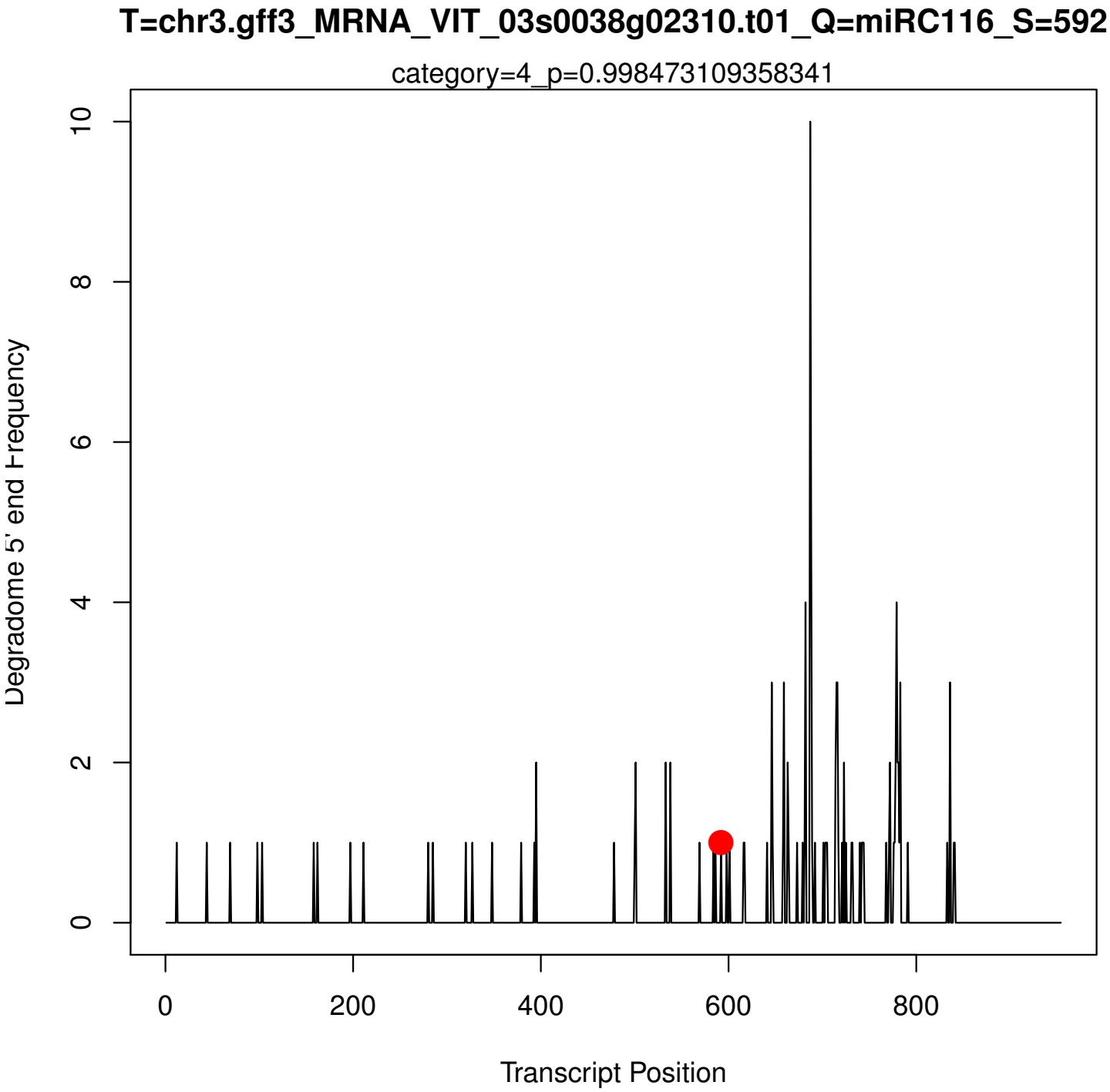
1000
1500
Transcript Position


T=chr3.gff3_MRNA_VIT_03s0038g02310.t01_Q=miRC116_S=592
category=4_p=0.998473109358341
Degradome 5' end Frequency
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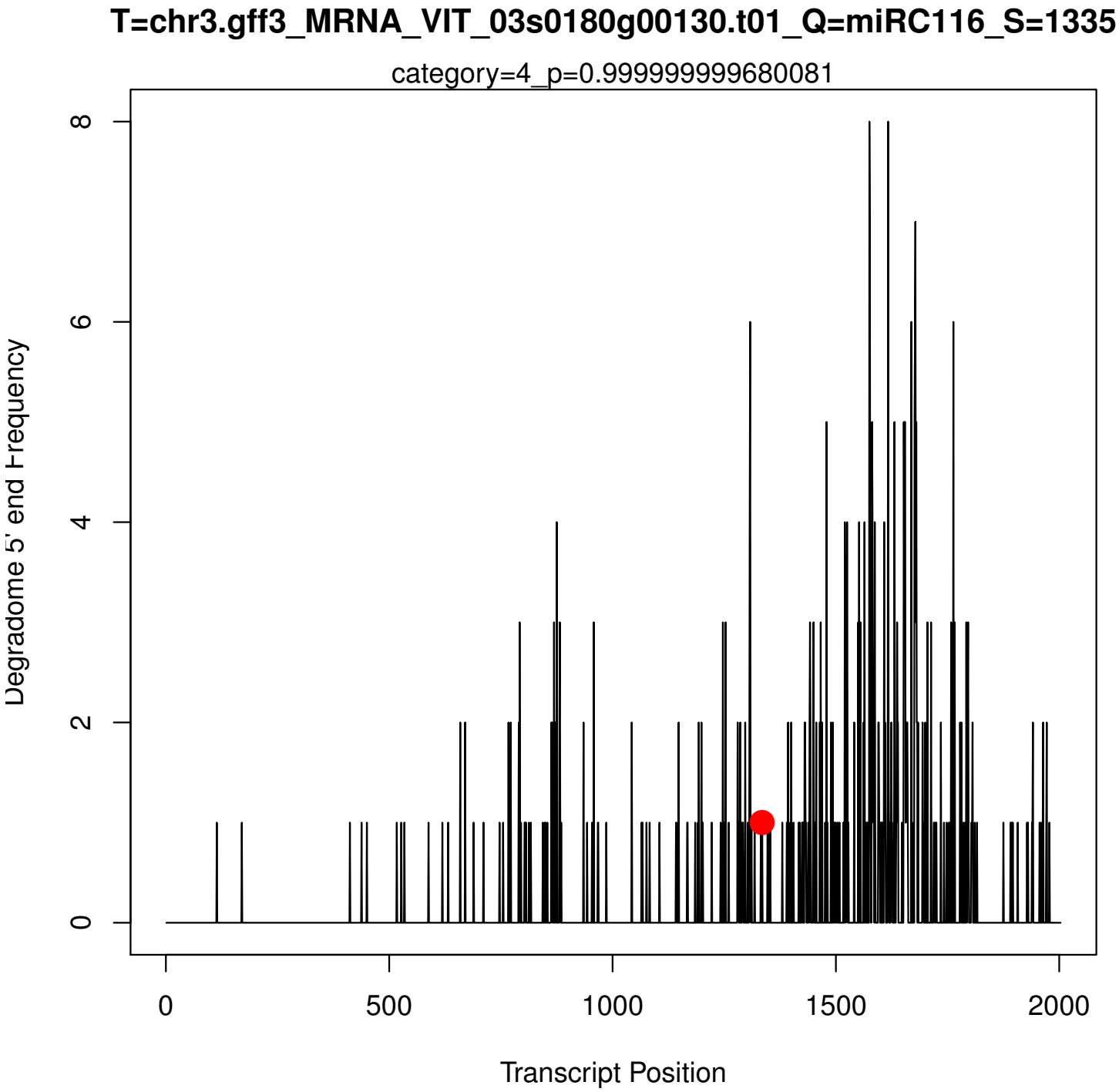
4
6
8
10
0
200
400
600
800
Transcript Position


T=chr3.gff3_MRNA_VIT_03s0180g00130.t01_Q=miRC116_S=1335
category=4_p=0.999999999680081
Degradome 5' end Frequency
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2
4
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8
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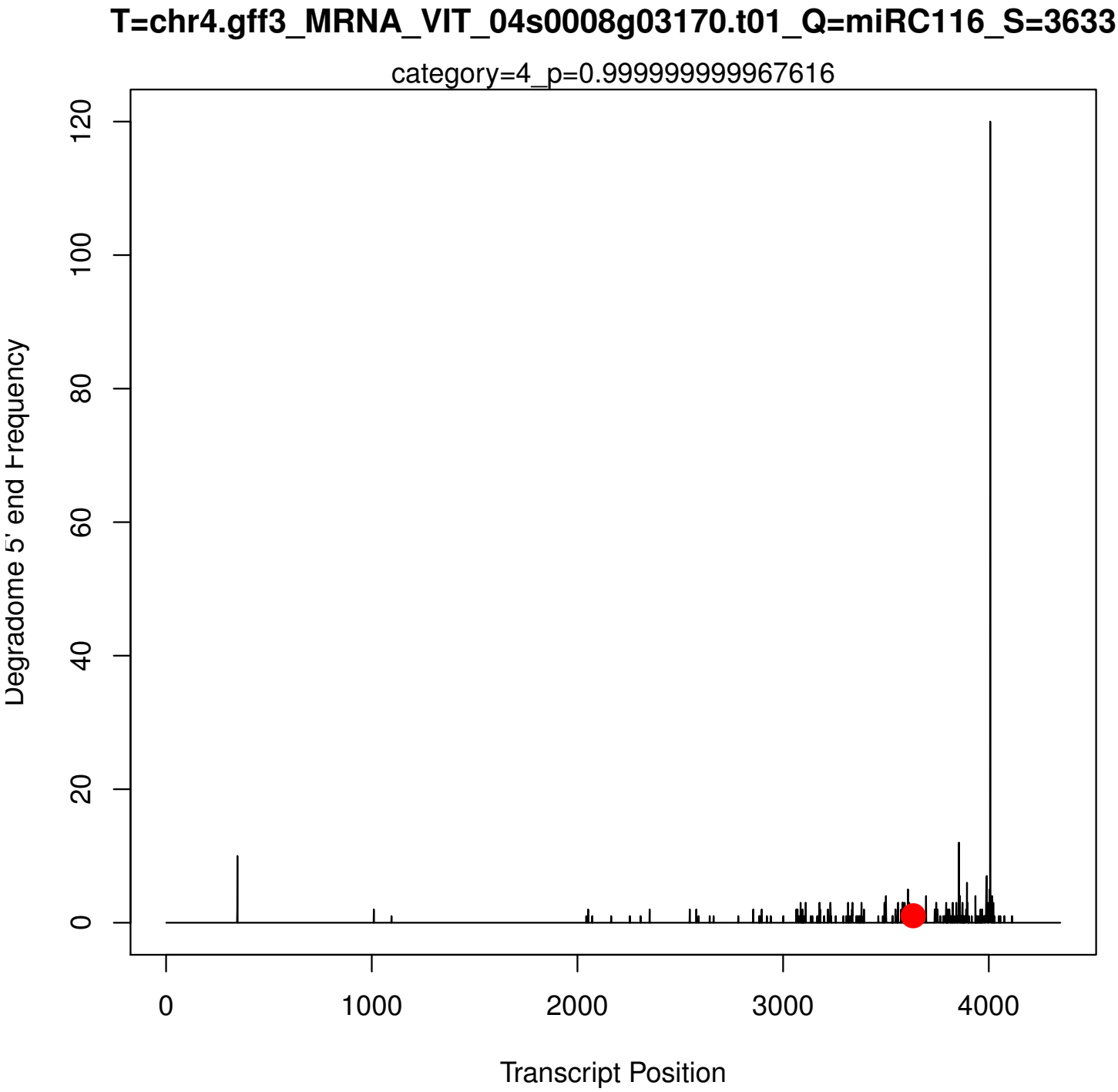
Transcript Position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category=4_p=0.999999999967616
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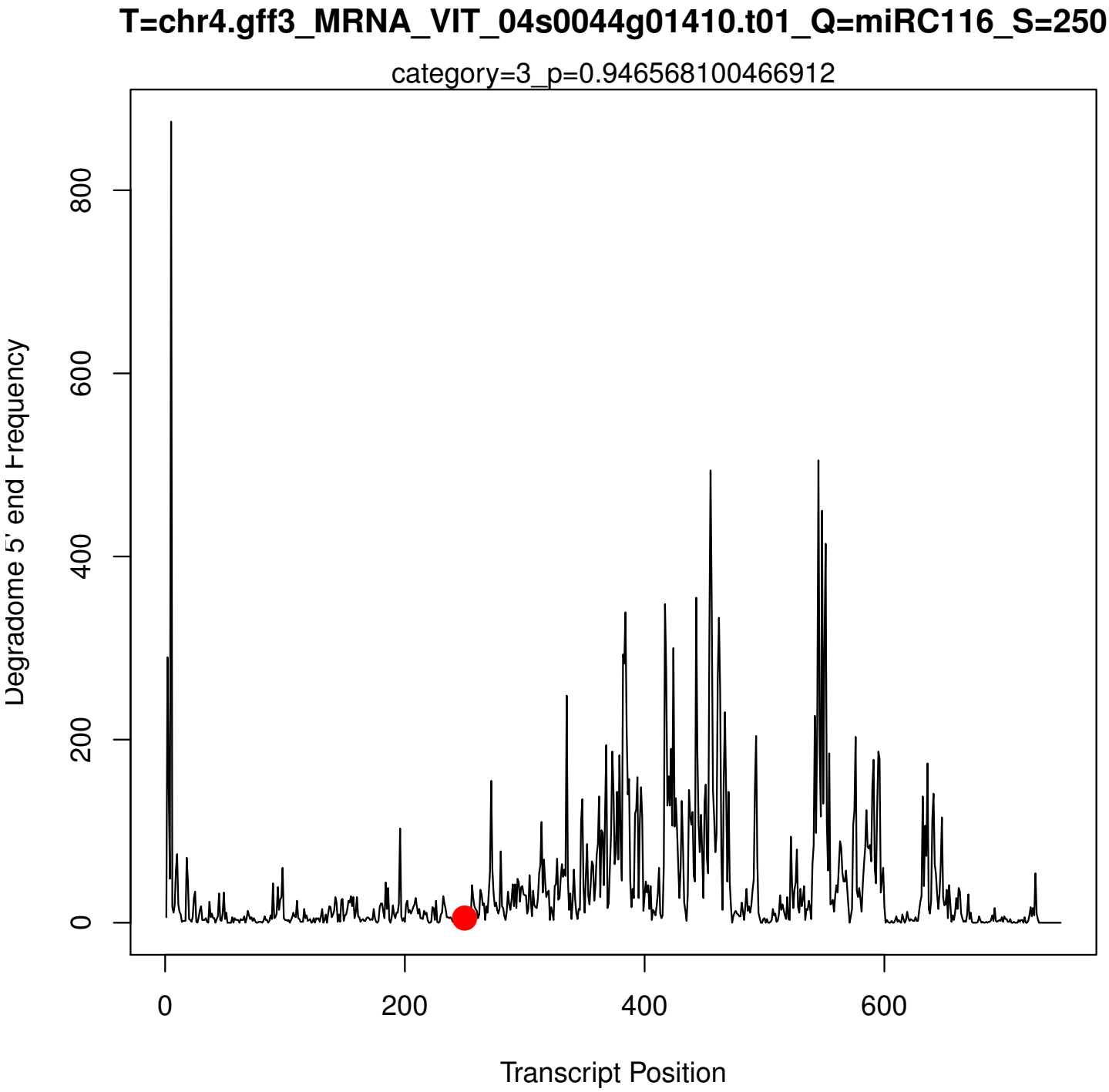
T=chr4.gff3_MRNA_VIT_04s0044g01410.t01_Q=miRC116_S=250
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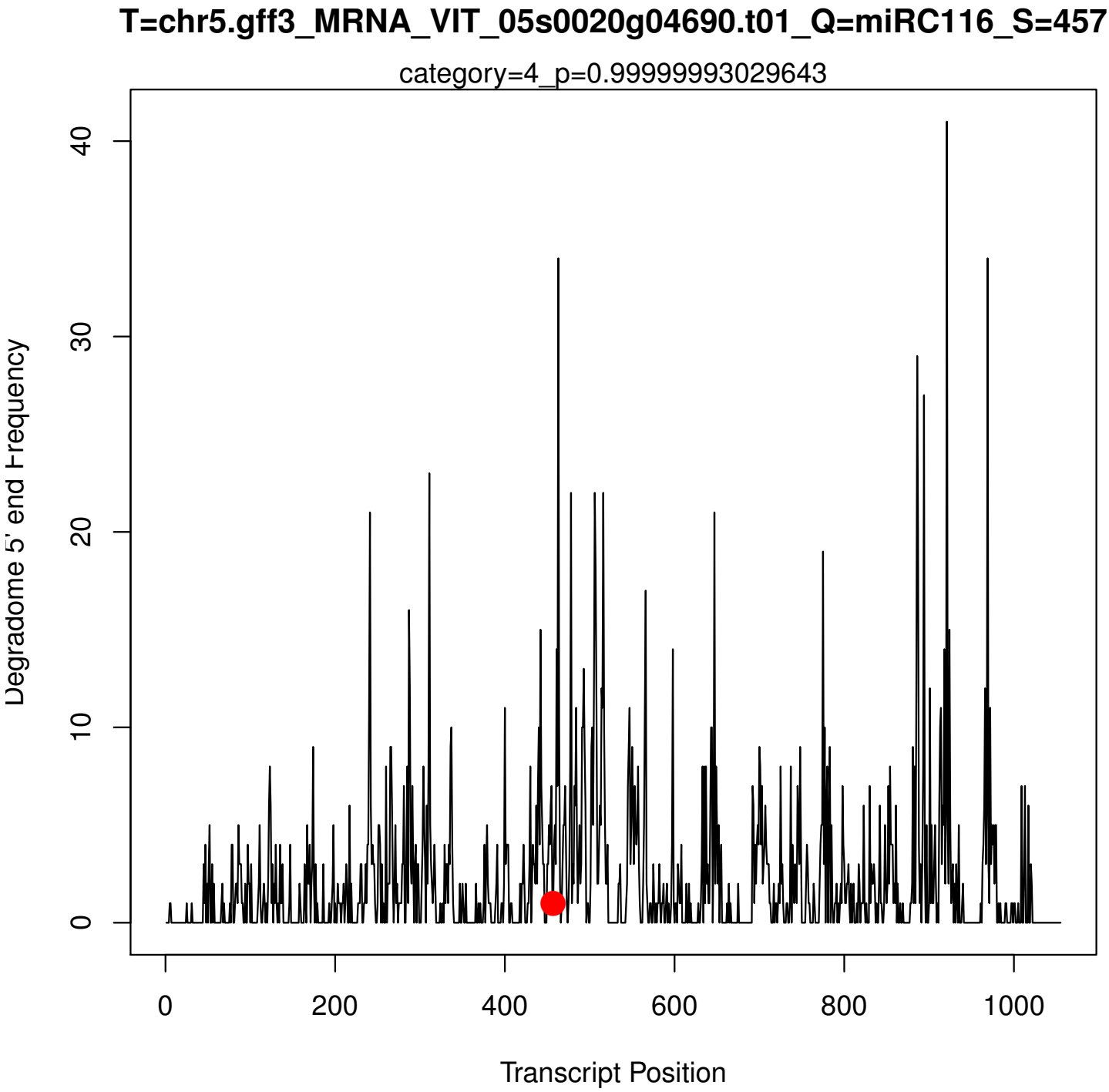
Transcript Position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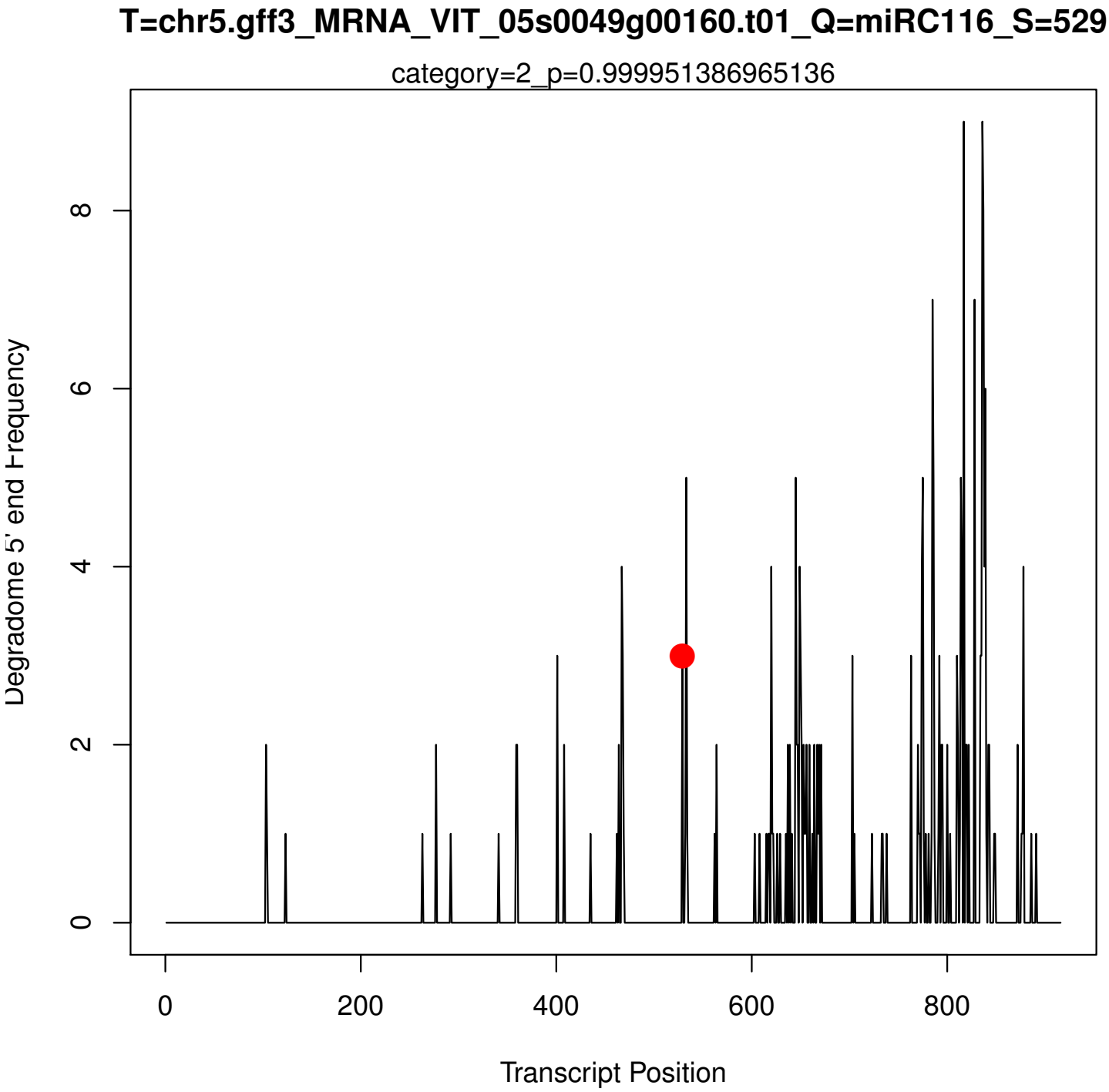
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0049g00160.t01_Q=miRC116_S=529
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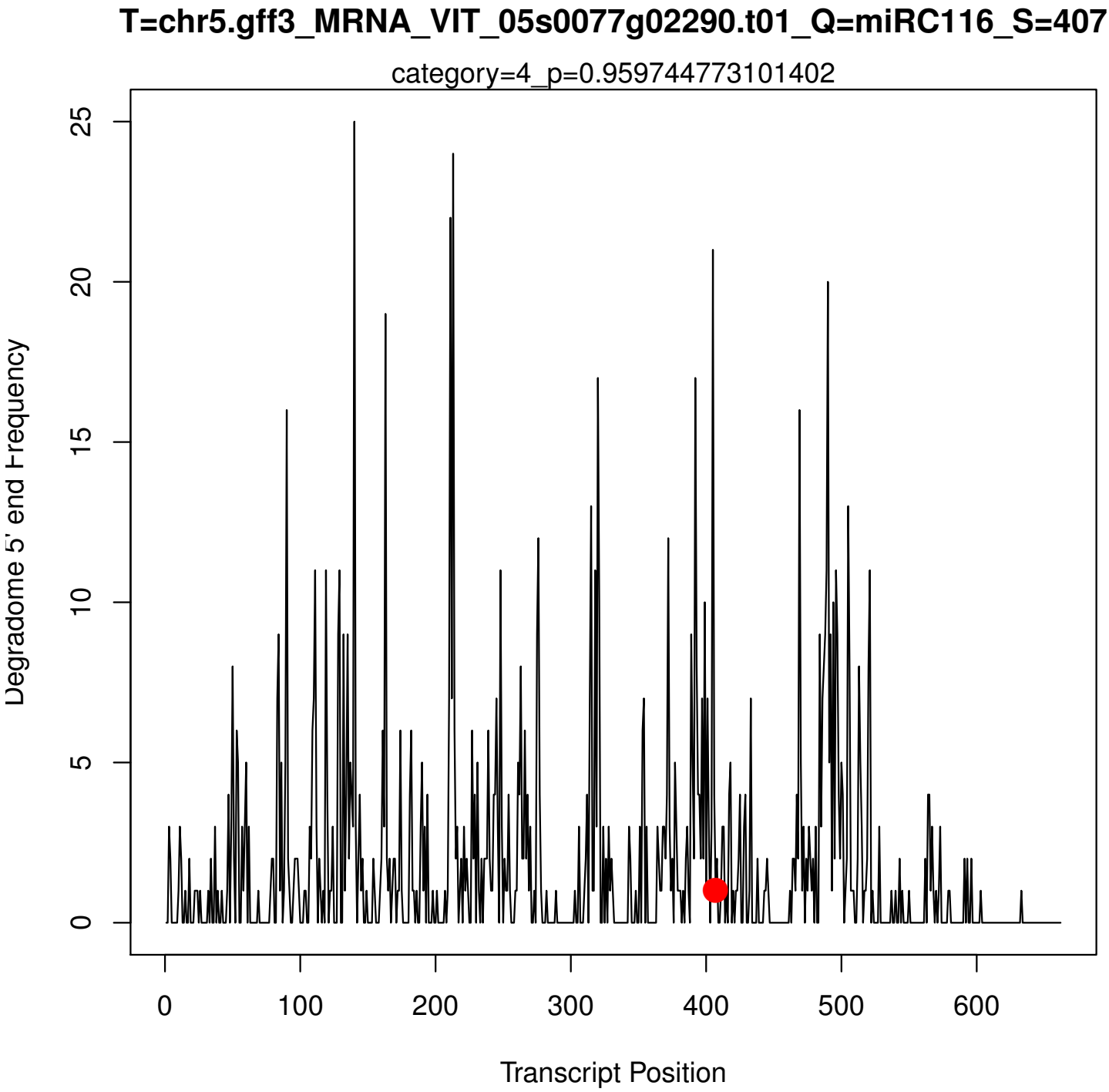
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0077g02290.t01_Q=miRC116_S=407
category=4_p=0.959744773101402
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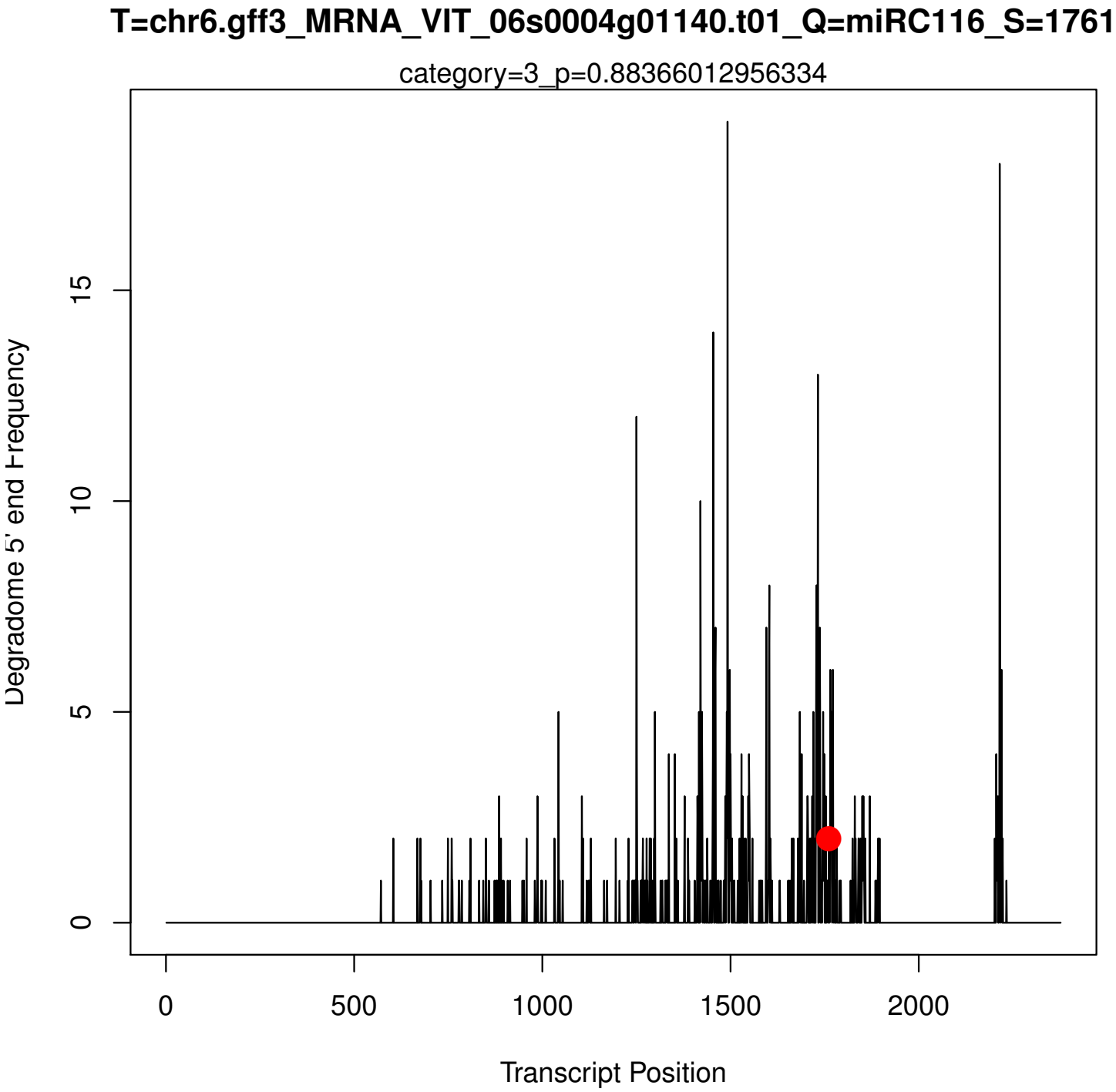
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0004g01140.t01_Q=miRC116_S=1761
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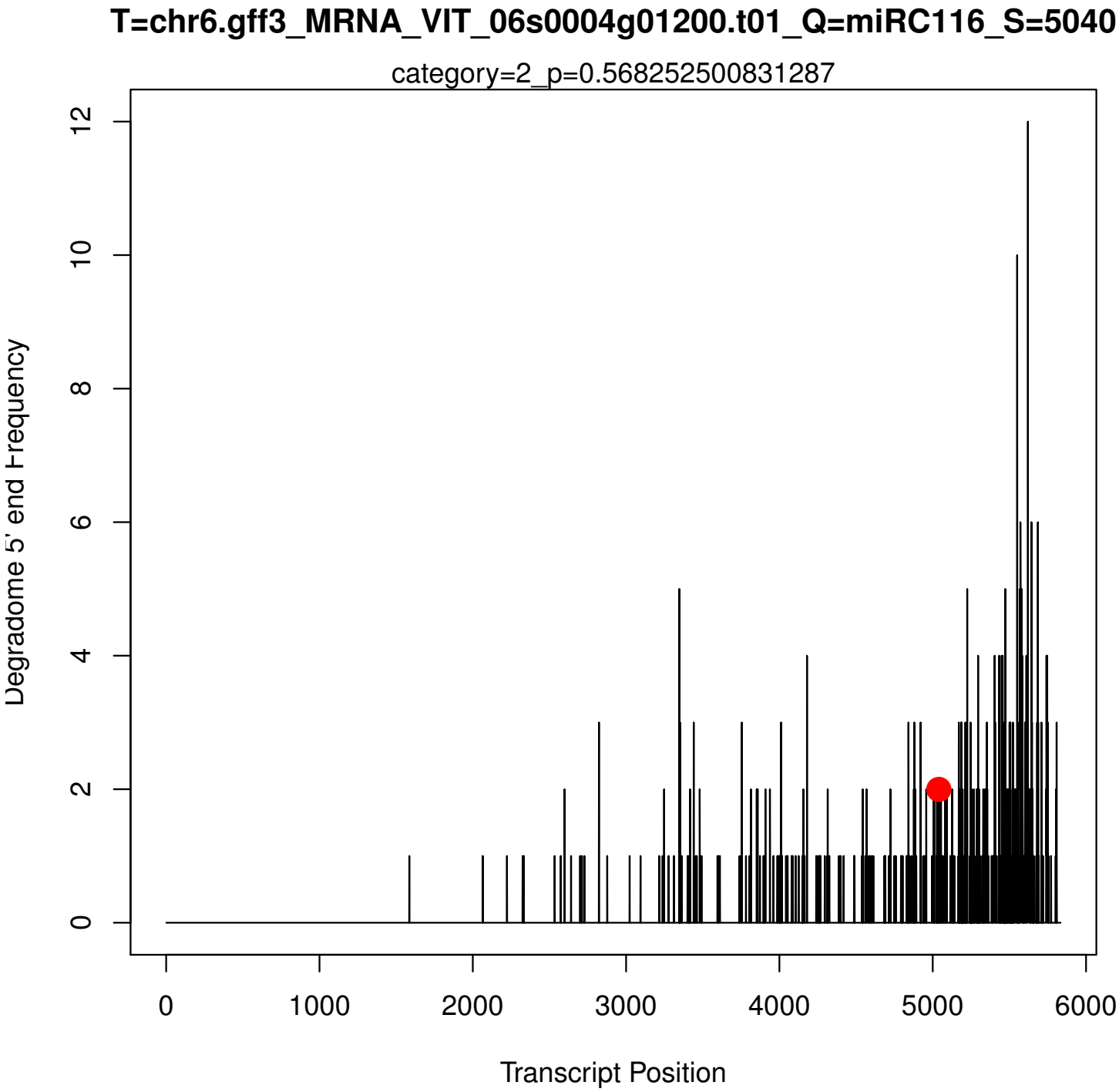
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0004g01200.t01_Q=miRC116_S=5040
category=2_p=0.568252500831287
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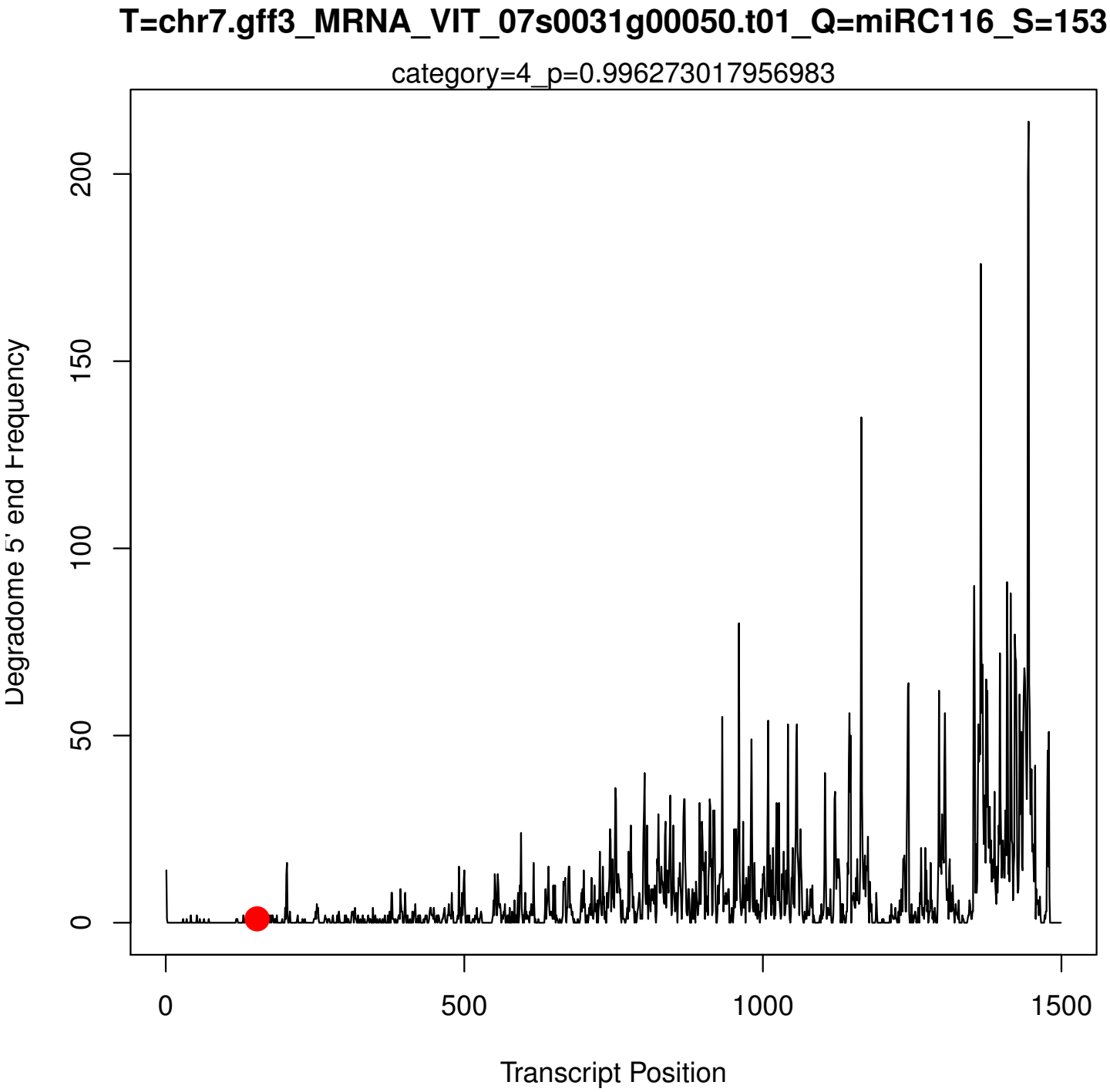
Transcript Position


T=chr7.gff3_MRNA_VIT_07s0031g00050.t01_Q=miRC116_S=153
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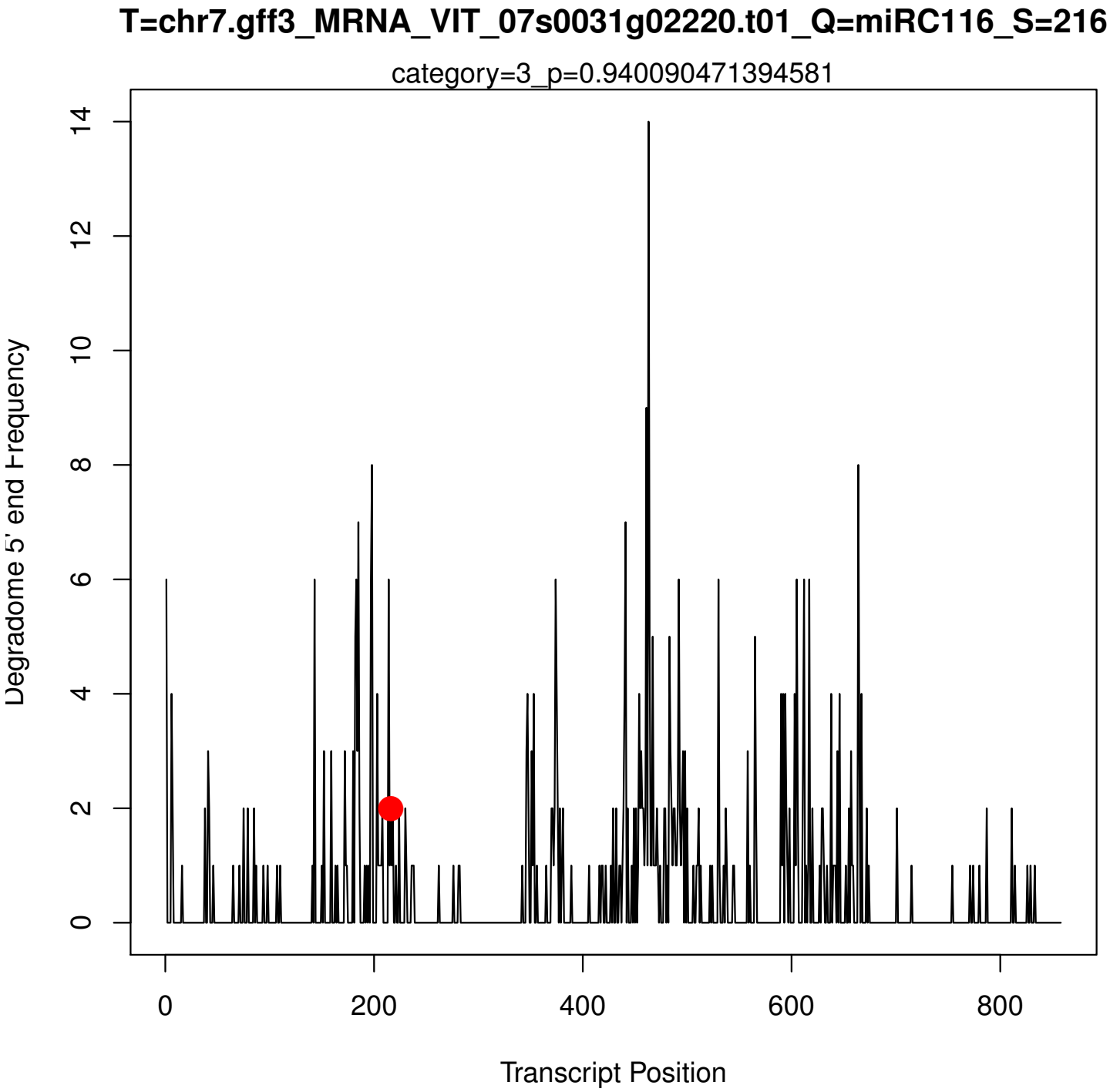
Transcript Position


T=chr7.gff3_MRNA_VIT_07s0031g02220.t01_Q=miRC116_S=216
category=3_p=0.940090471394581
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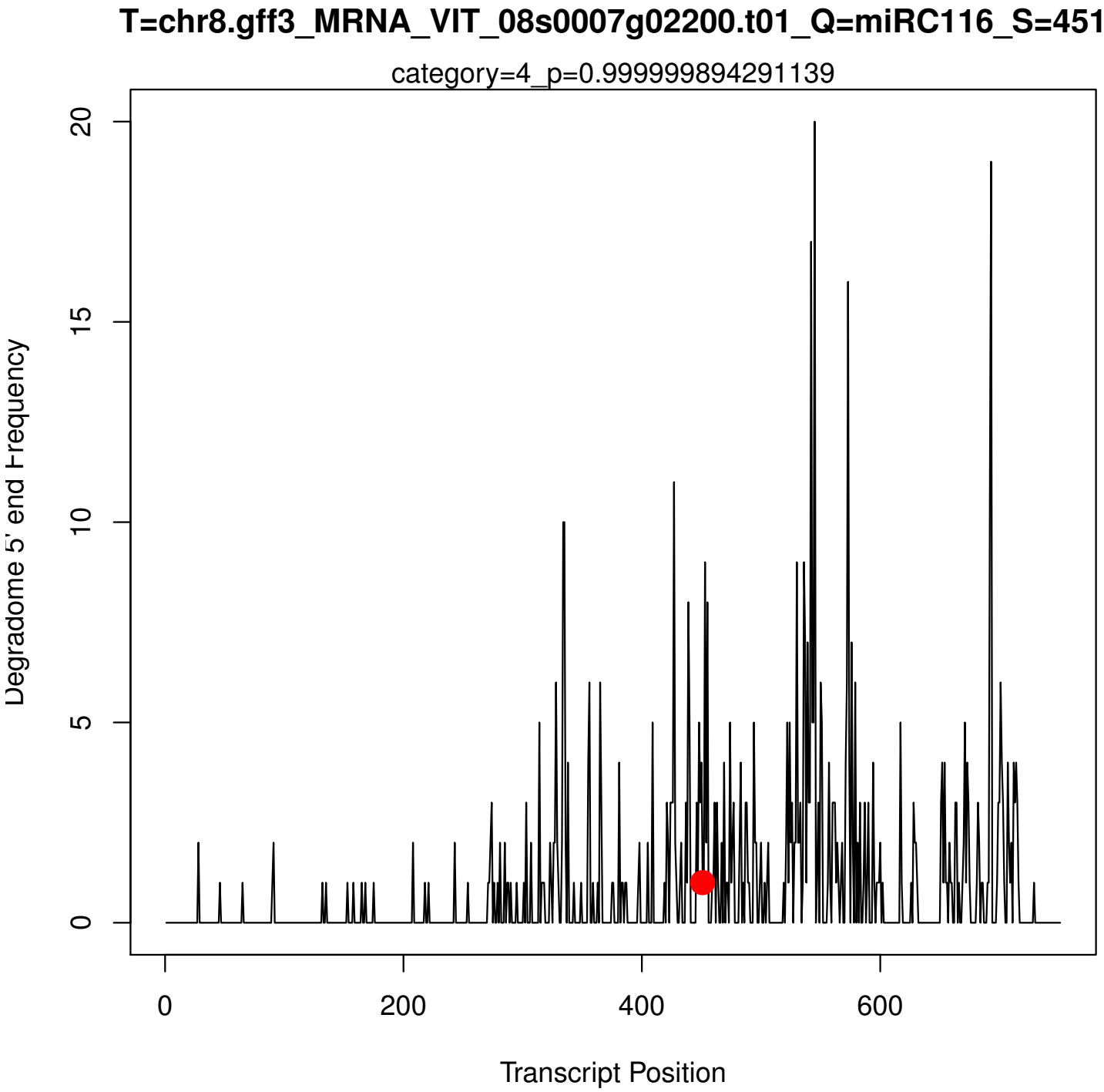
Transcript Position


T=chr8.gff3_MRNA_VIT_08s0007g02200.t01_Q=miRC116_S=451
category=4_p=0.999999894291139
Degradome 5' end Frequency
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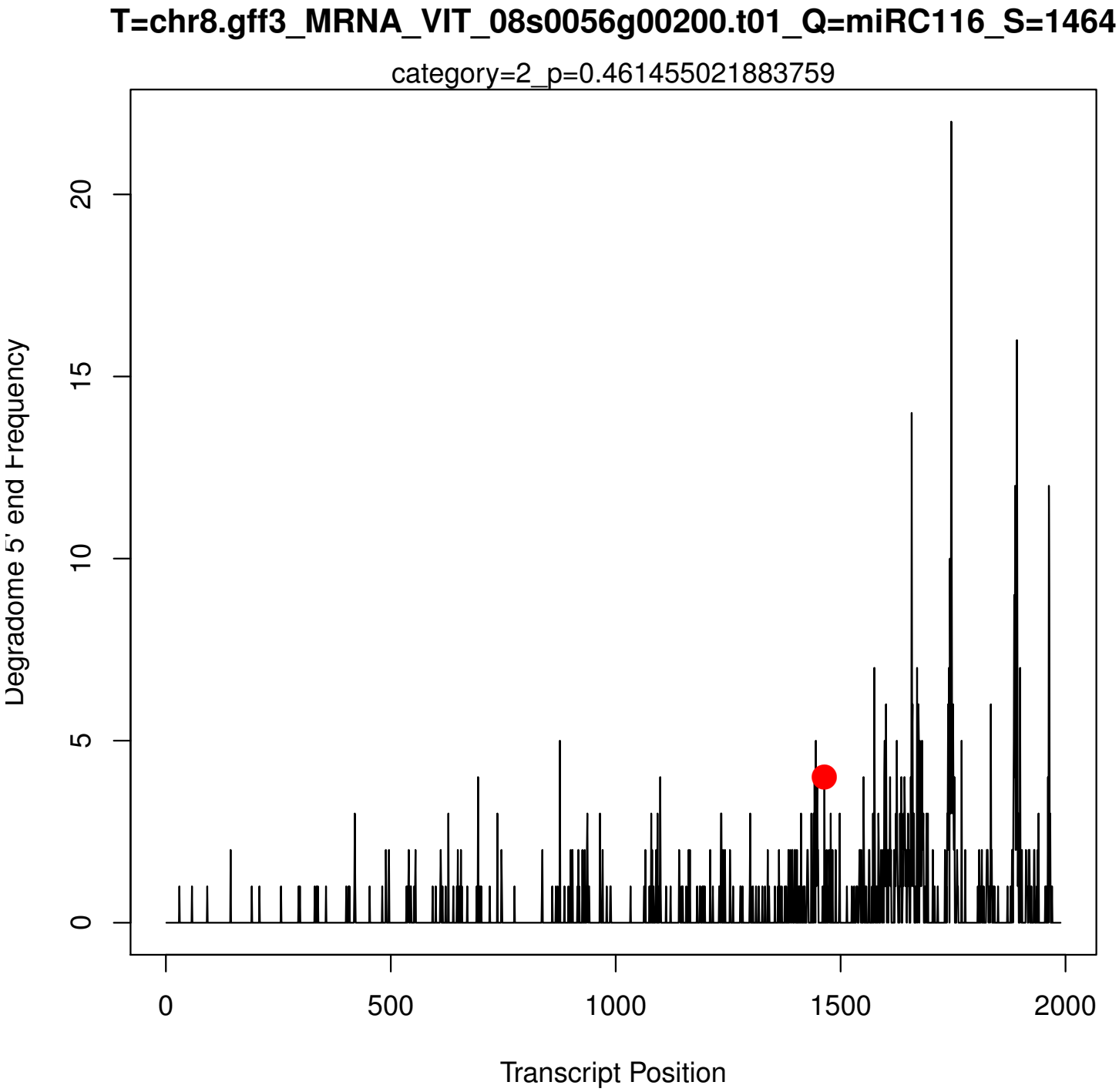
15
20
0
200
400
600
Transcript Position


T=chr8.gff3_MRNA_VIT_08s0056g00200.t01_Q=miRC116_S=1464
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Degradome 5' end Frequency
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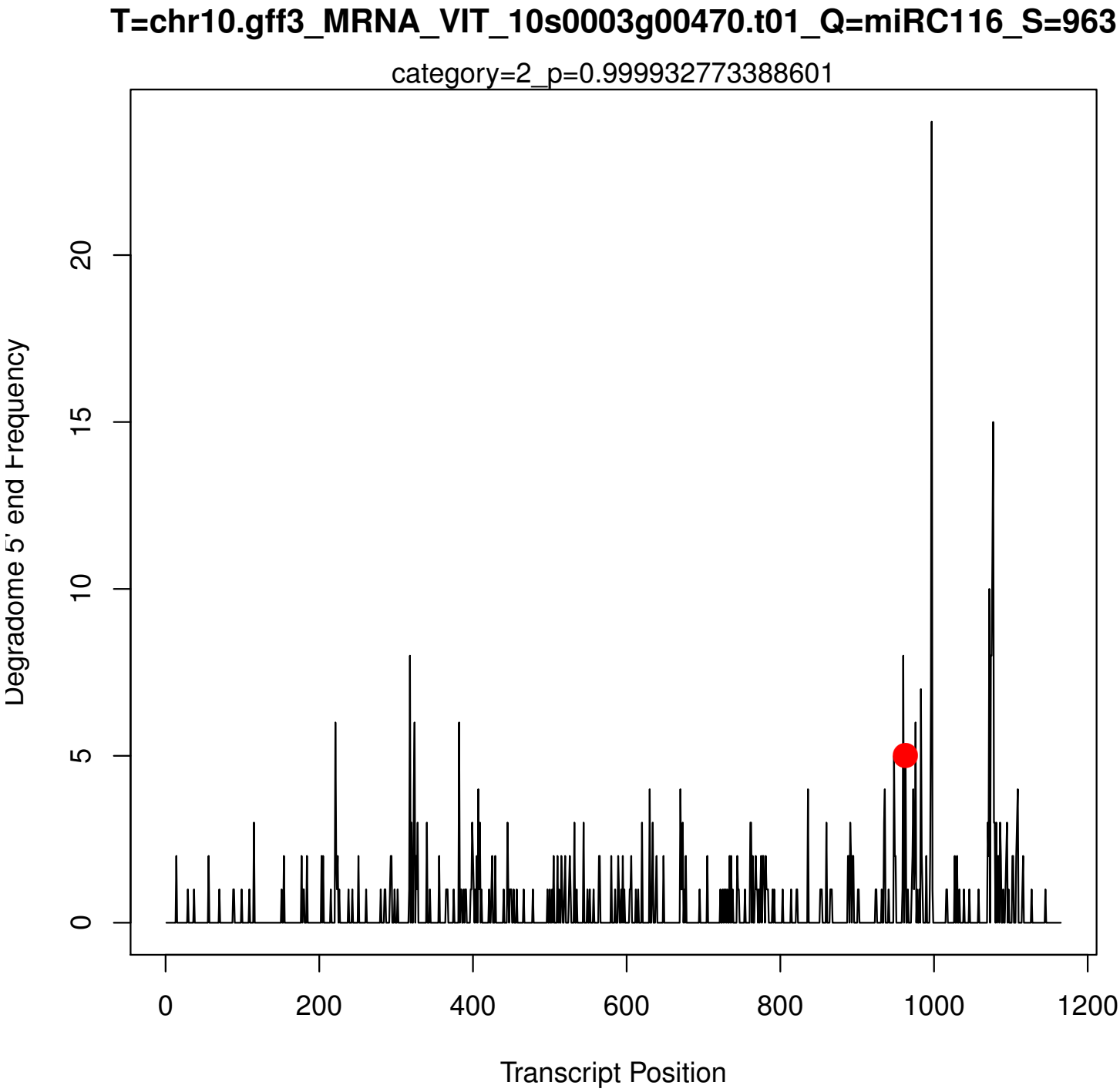
Transcript Position


T=chr10.gff3_MRNA_VIT_10s0003g00470.t01_Q=miRC116_S=963
category=2_p=0.999932773388601
Degradome 5' end Frequency
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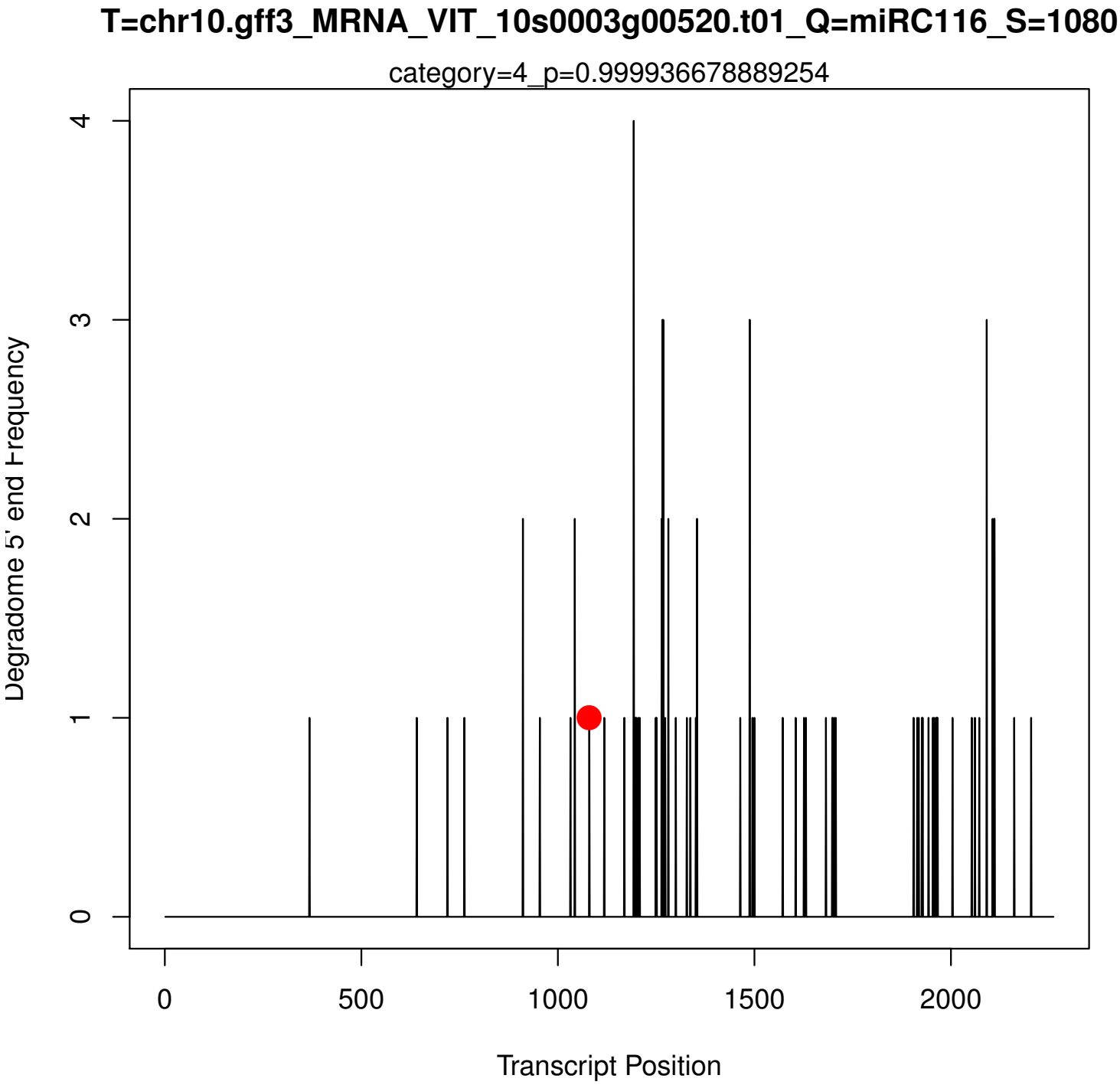
Transcript Position


T=chr10.gff3_MRNA_VIT_10s0003g00520.t01_Q=miRC116_S=1080
category=4_p=0.999936678889254
Degradome 5' end Frequency
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3
4
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1000
1500
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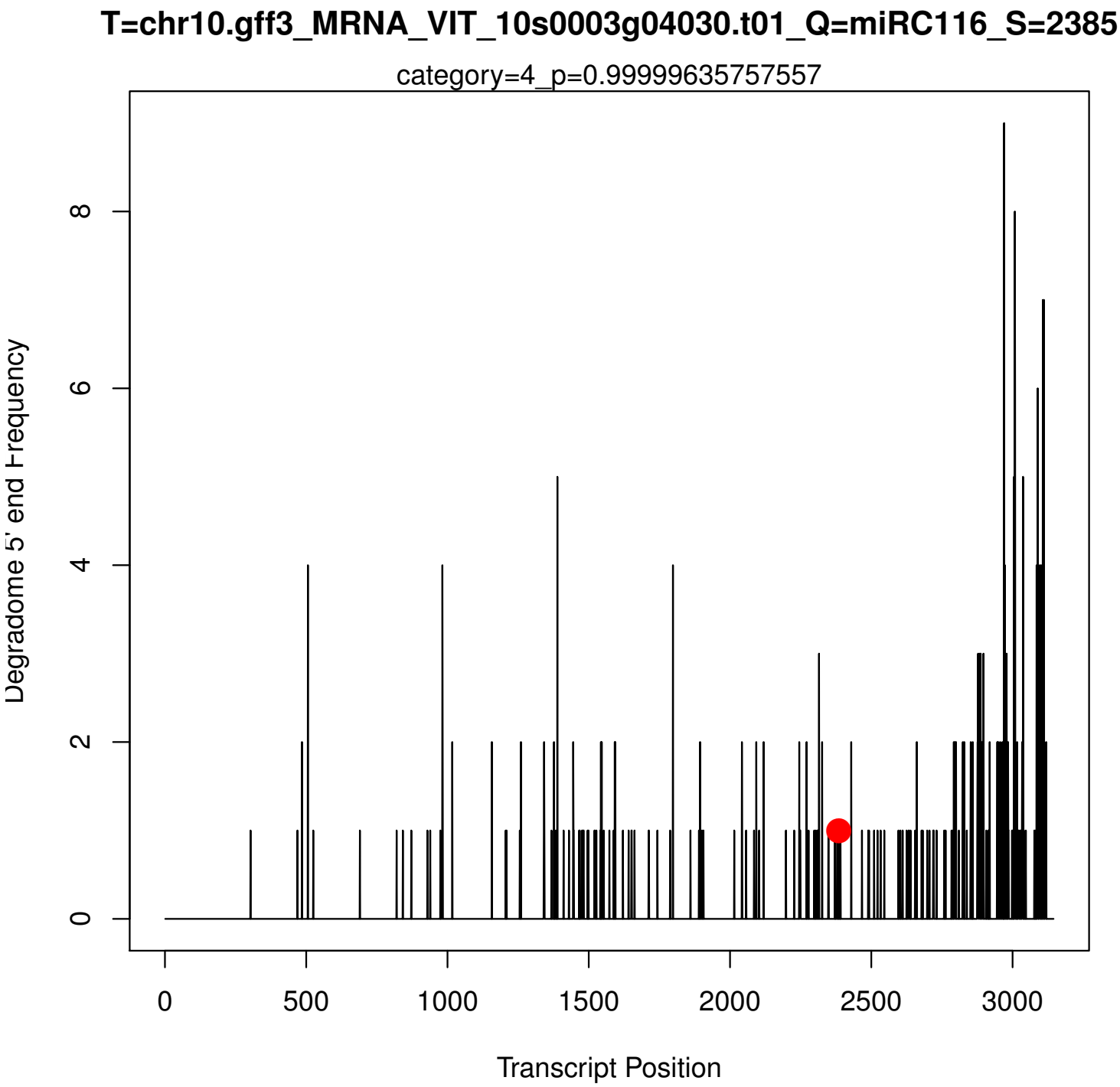
Transcript Position


T=chr10.gff3_MRNA_VIT_10s0003g04030.t01_Q=miRC116_S=2385
category=4_p=0.99999635757557
Degradome 5' end Frequency
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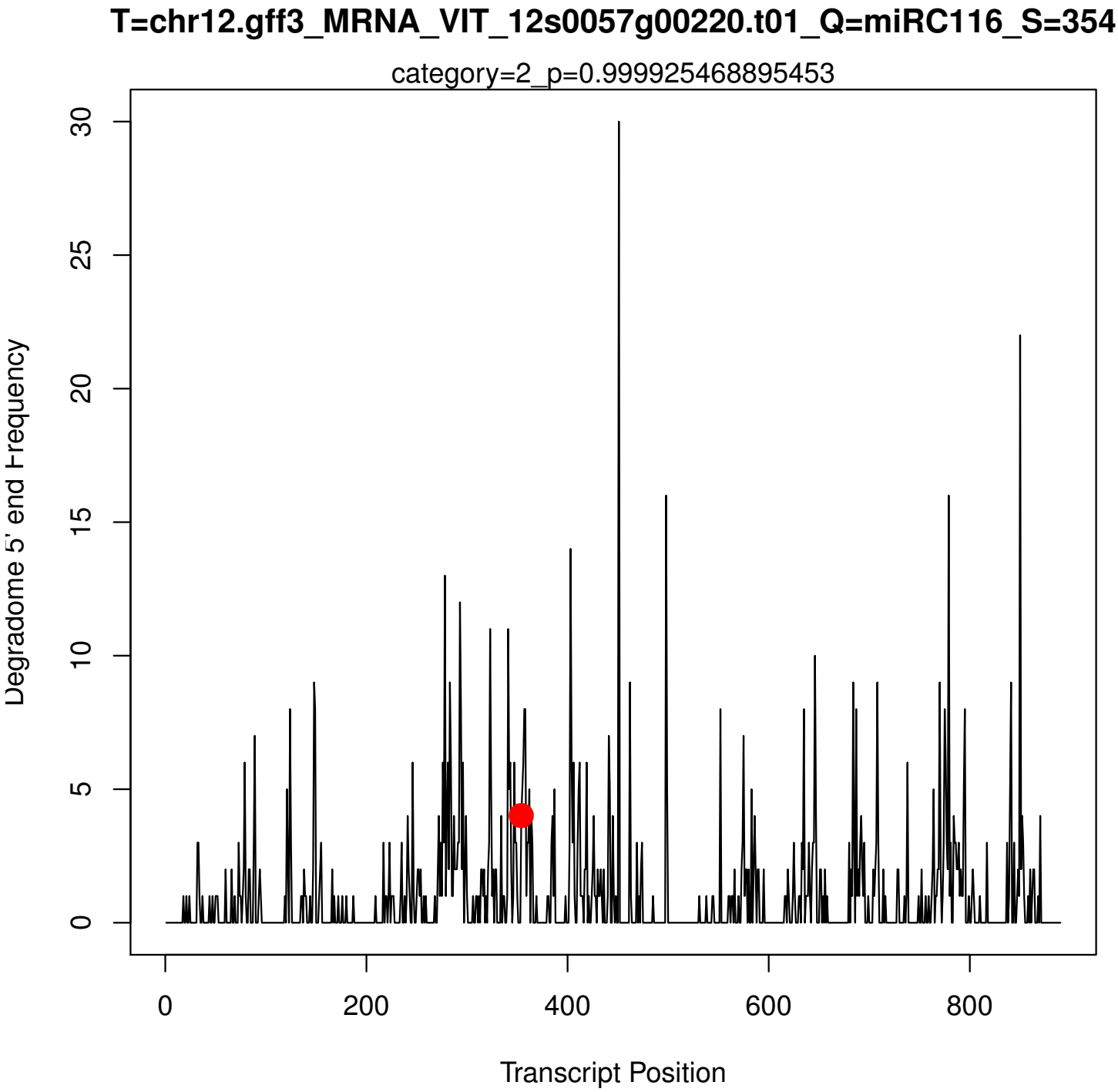
Transcript Position


T=chr12.gff3_MRNA_VIT_12s0057g00220.t01_Q=miRC116_S=354
category=2_p=0.999925468895453
Degradome 5' end Frequency
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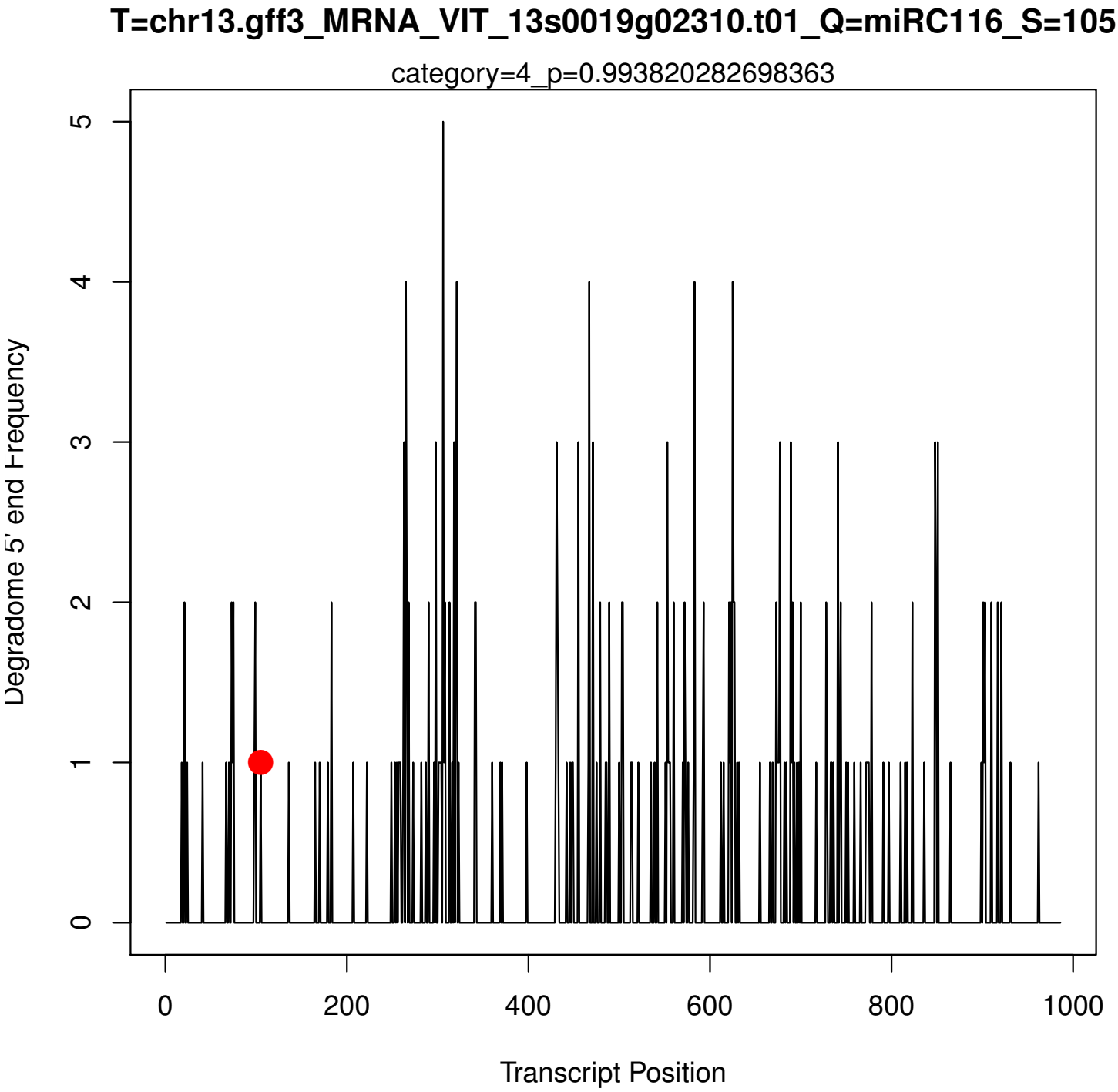
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0019g02310.t01_Q=miRC116_S=105
category=4_p=0.993820282698363
Degradome 5' end Frequency
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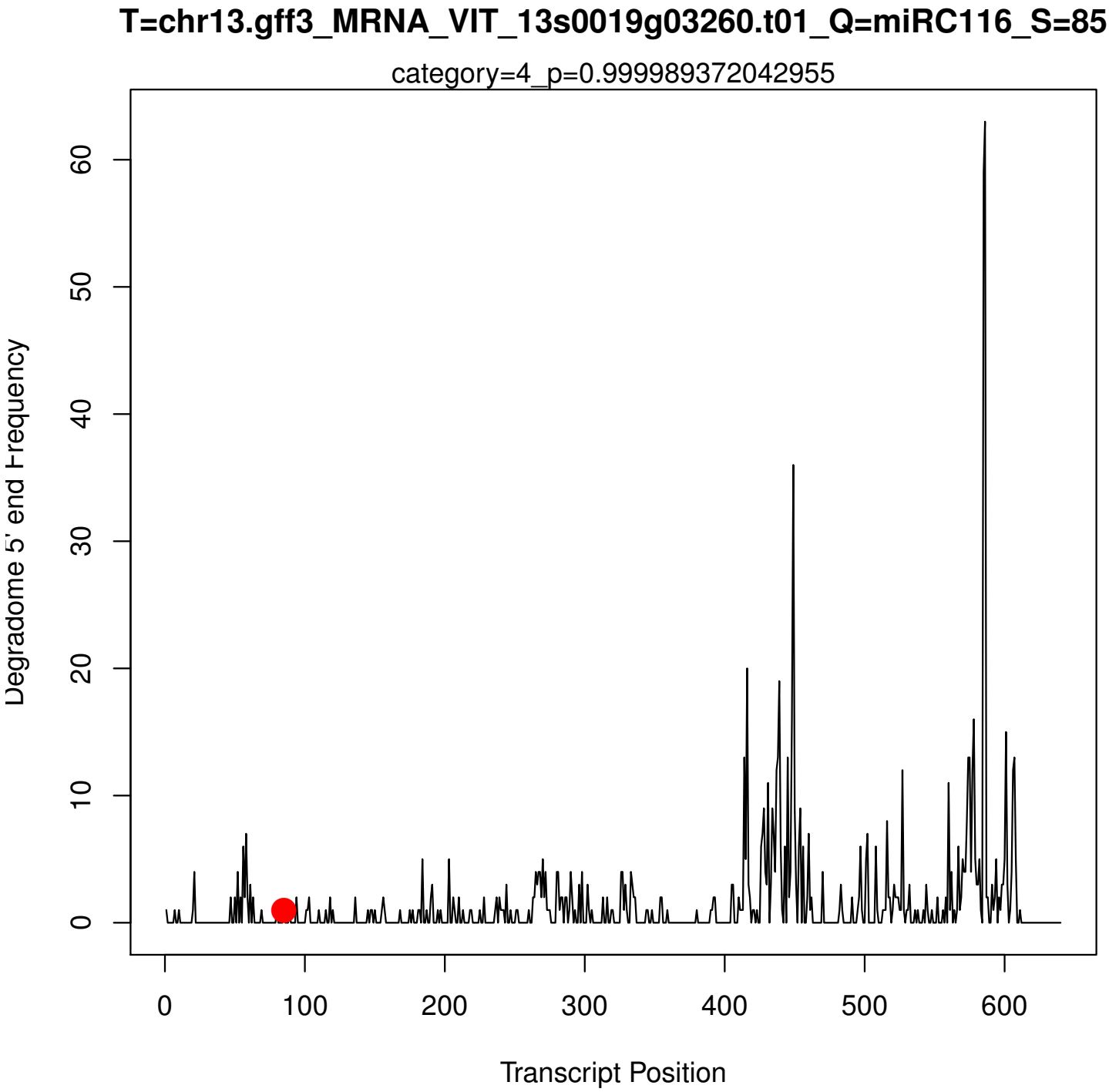
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0019g03260.t01_Q=miRC116_S=85
category=4_p=0.999989372042955
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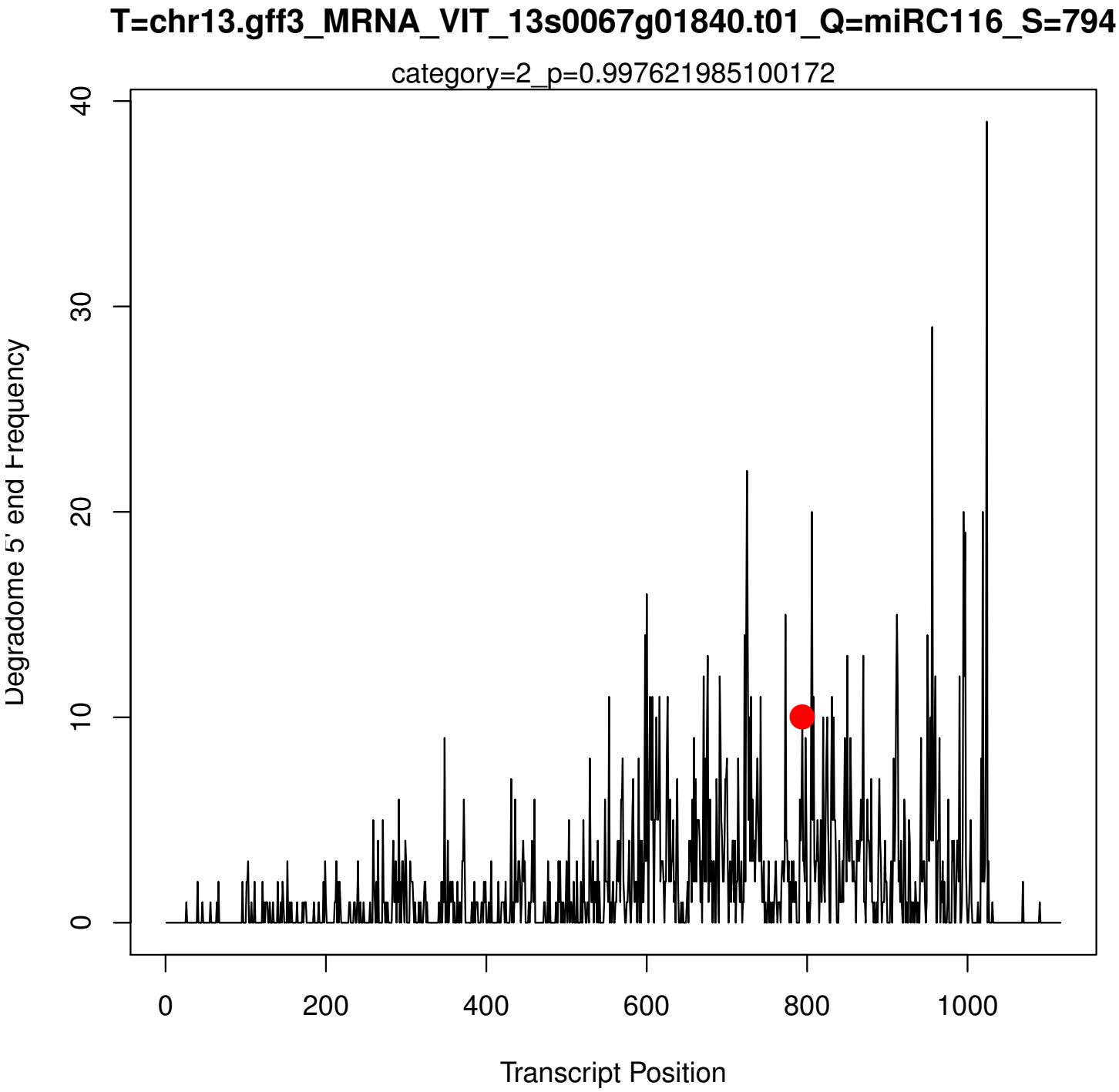
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0067g01840.t01_Q=miRC116_S=794
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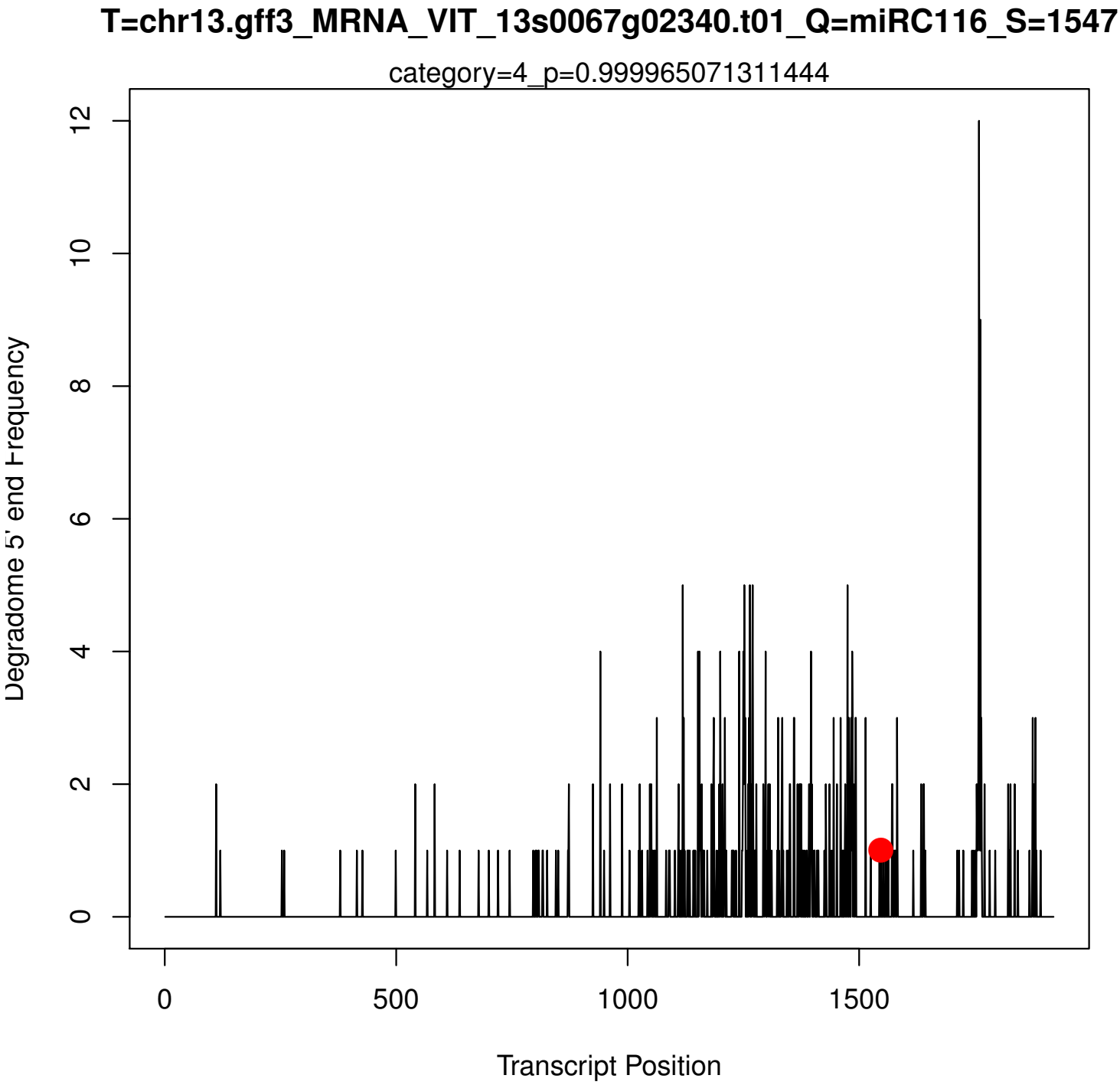
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0067g02340.t01_Q=miRC116_S=1547
category=4_p=0.999965071311444
Degradome 5' end Frequency
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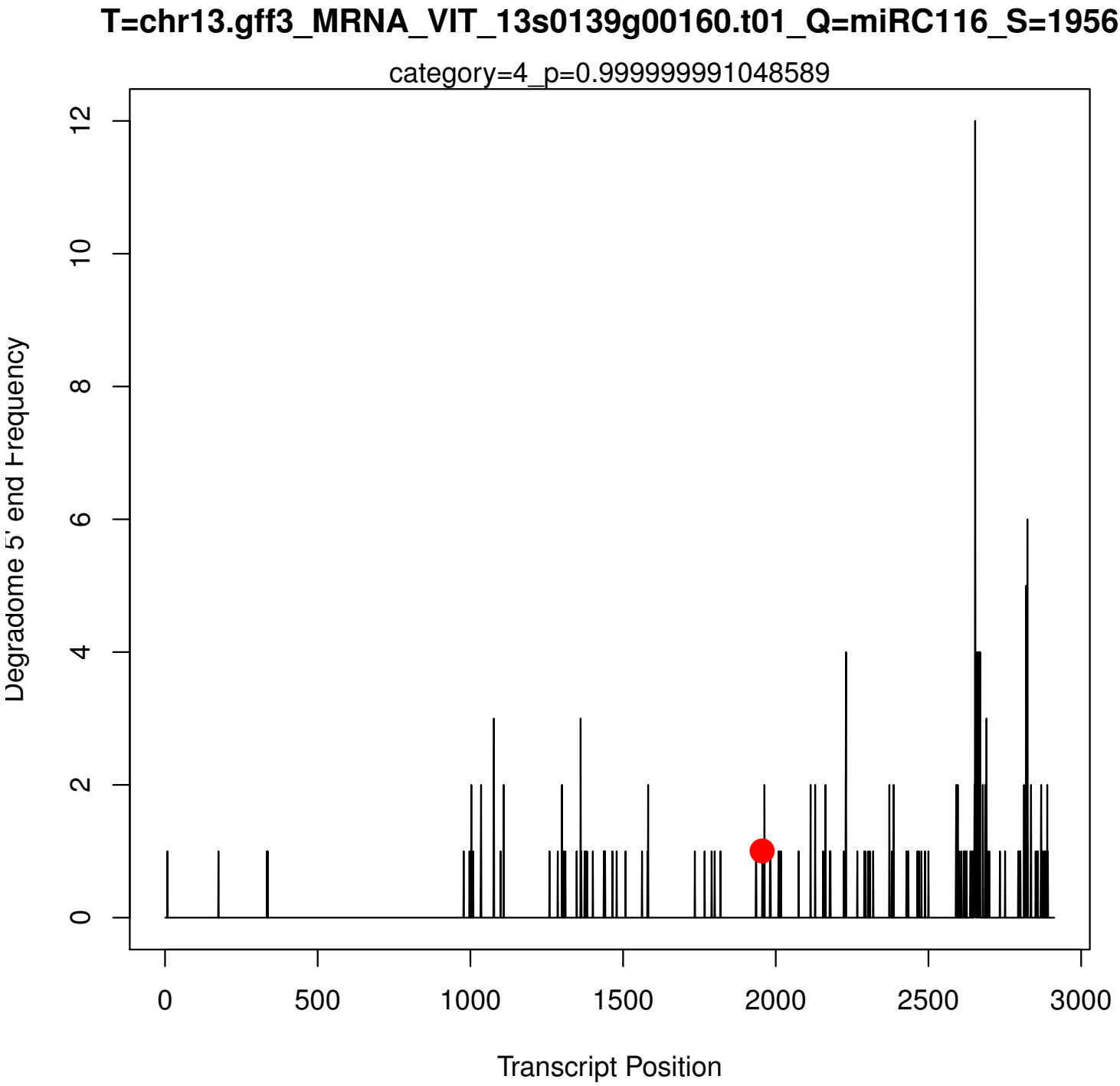
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0139g00160.t01_Q=miRC116_S=1956
category=4_p=0.999999991048589
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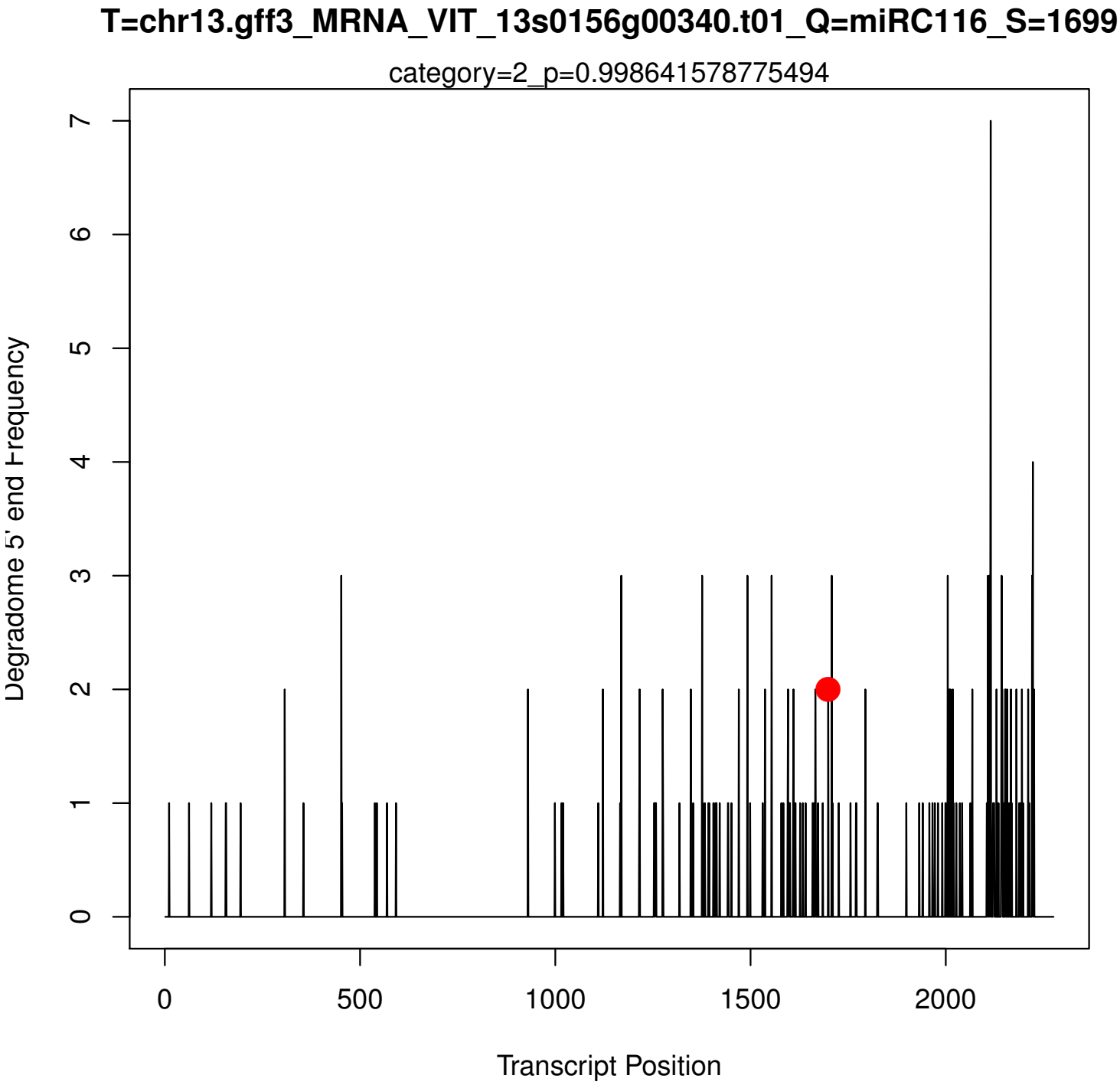
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0156g00340.t01_Q=miRC116_S=1699
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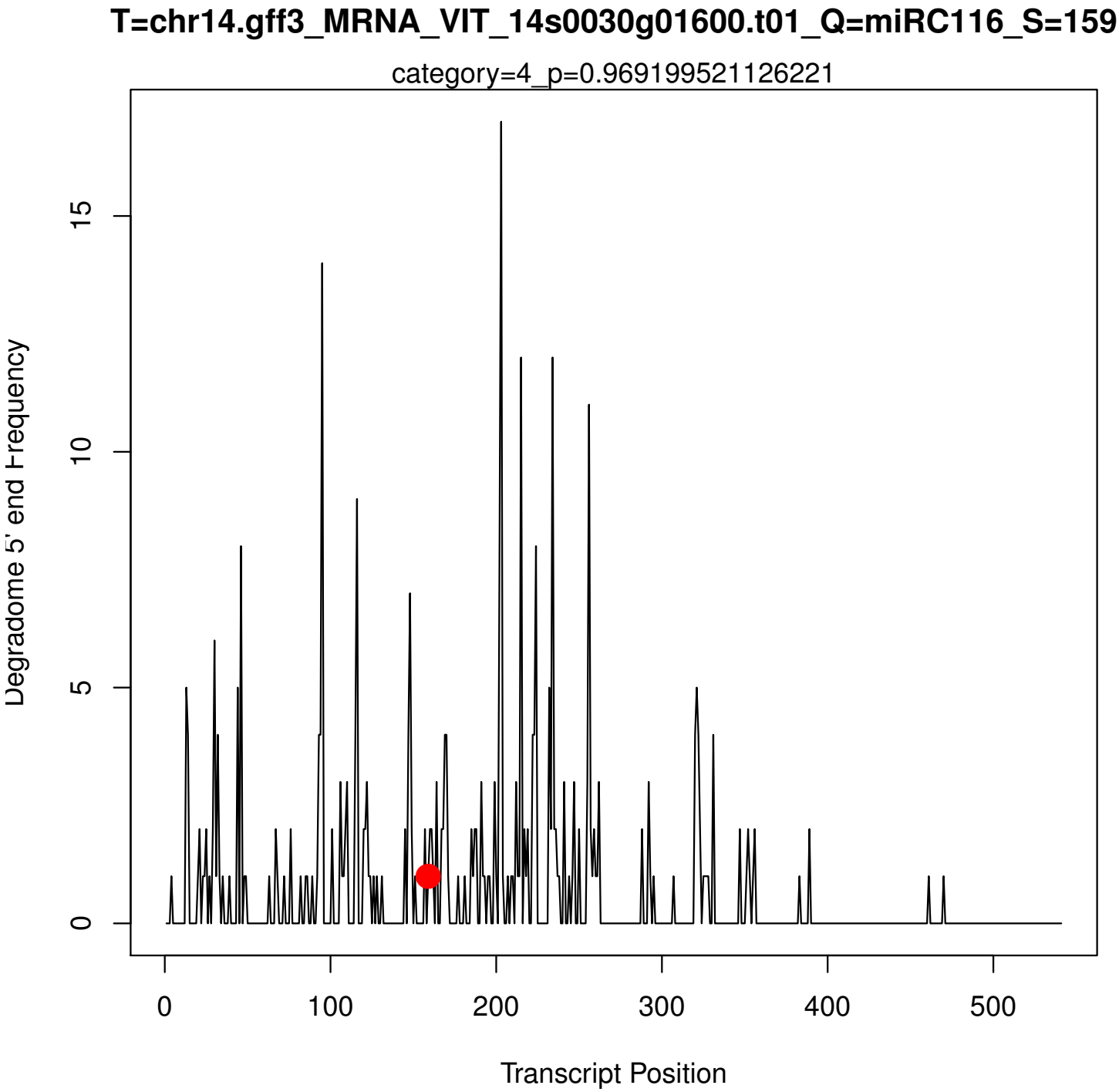
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0030g01600.t01_Q=miRC116_S=159
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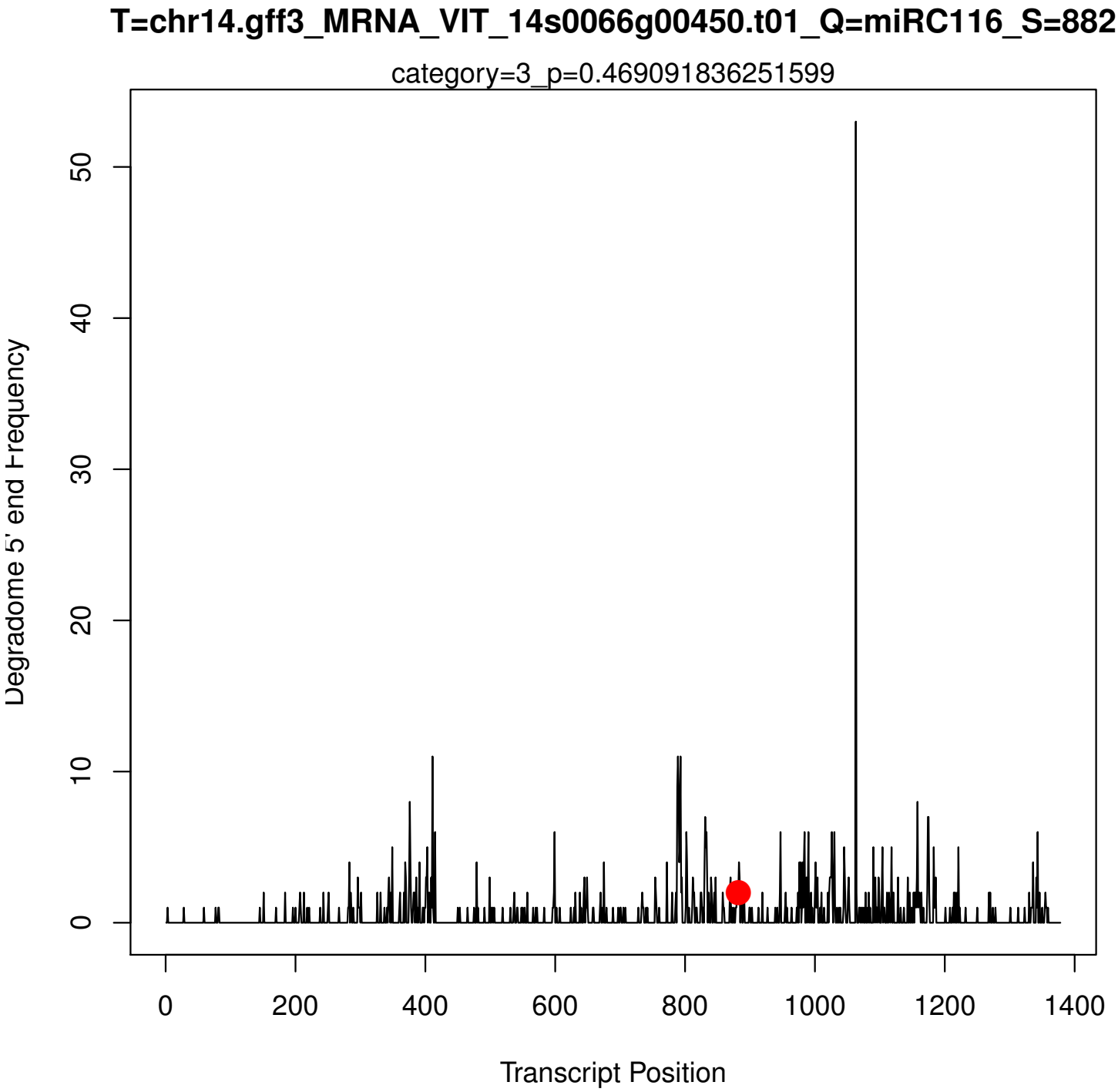
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0066g00450.t01_Q=miRC116_S=882
category=3_p=0.469091836251599
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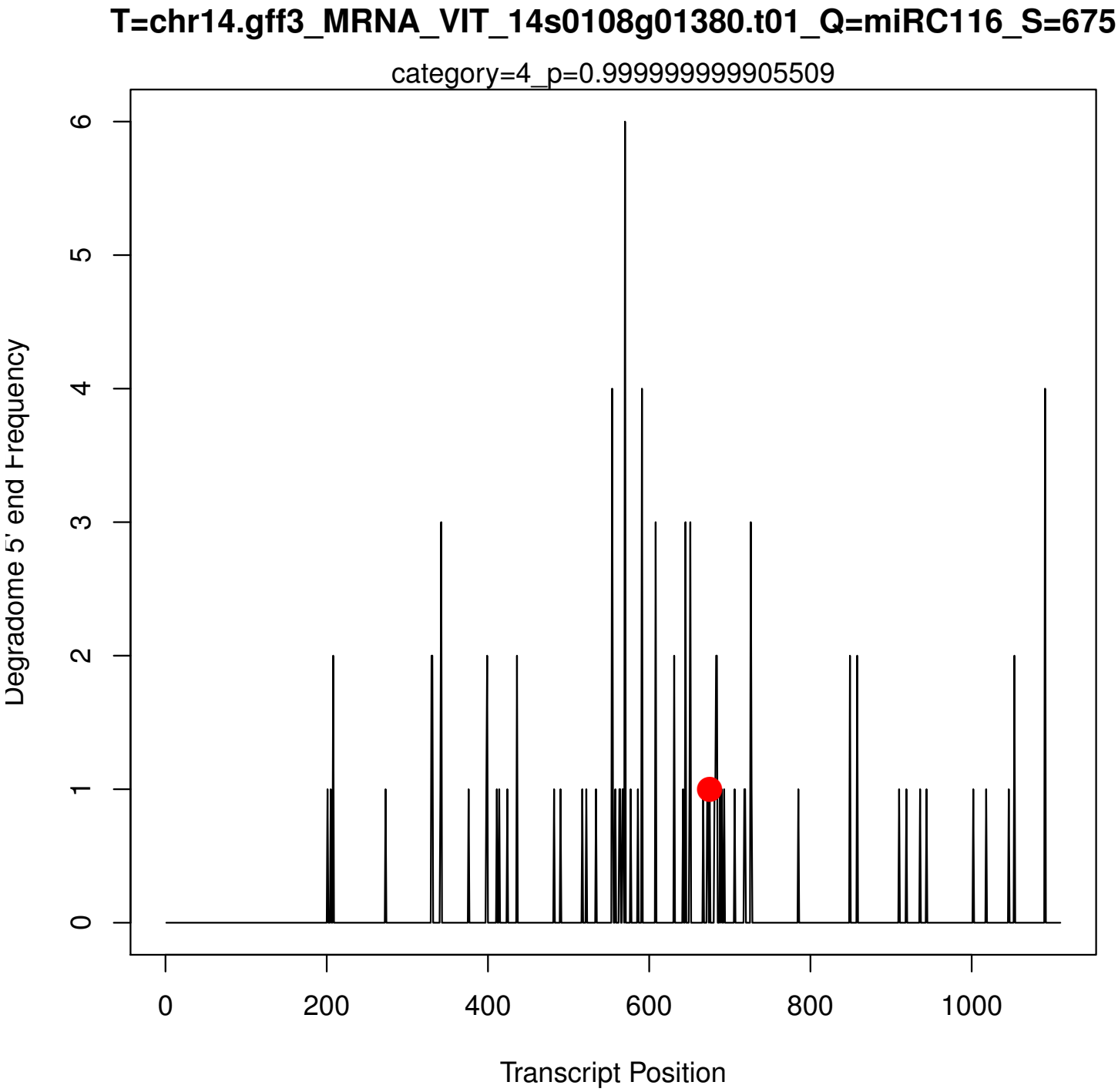
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0108g01380.t01_Q=miRC116_S=675
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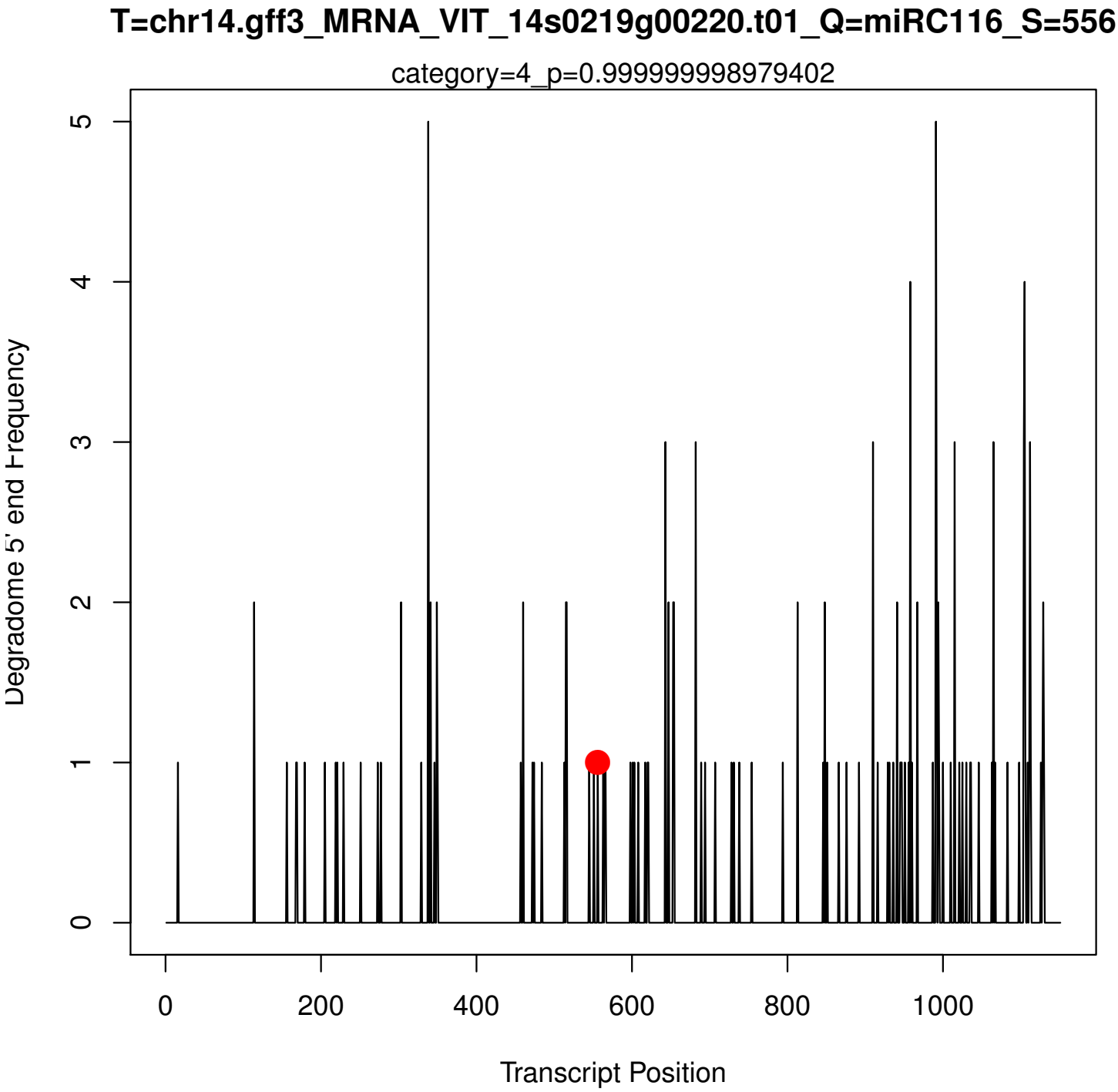
1000
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0219g00220.t01_Q=miRC116_S=556
category=4_p=0.999999998979402
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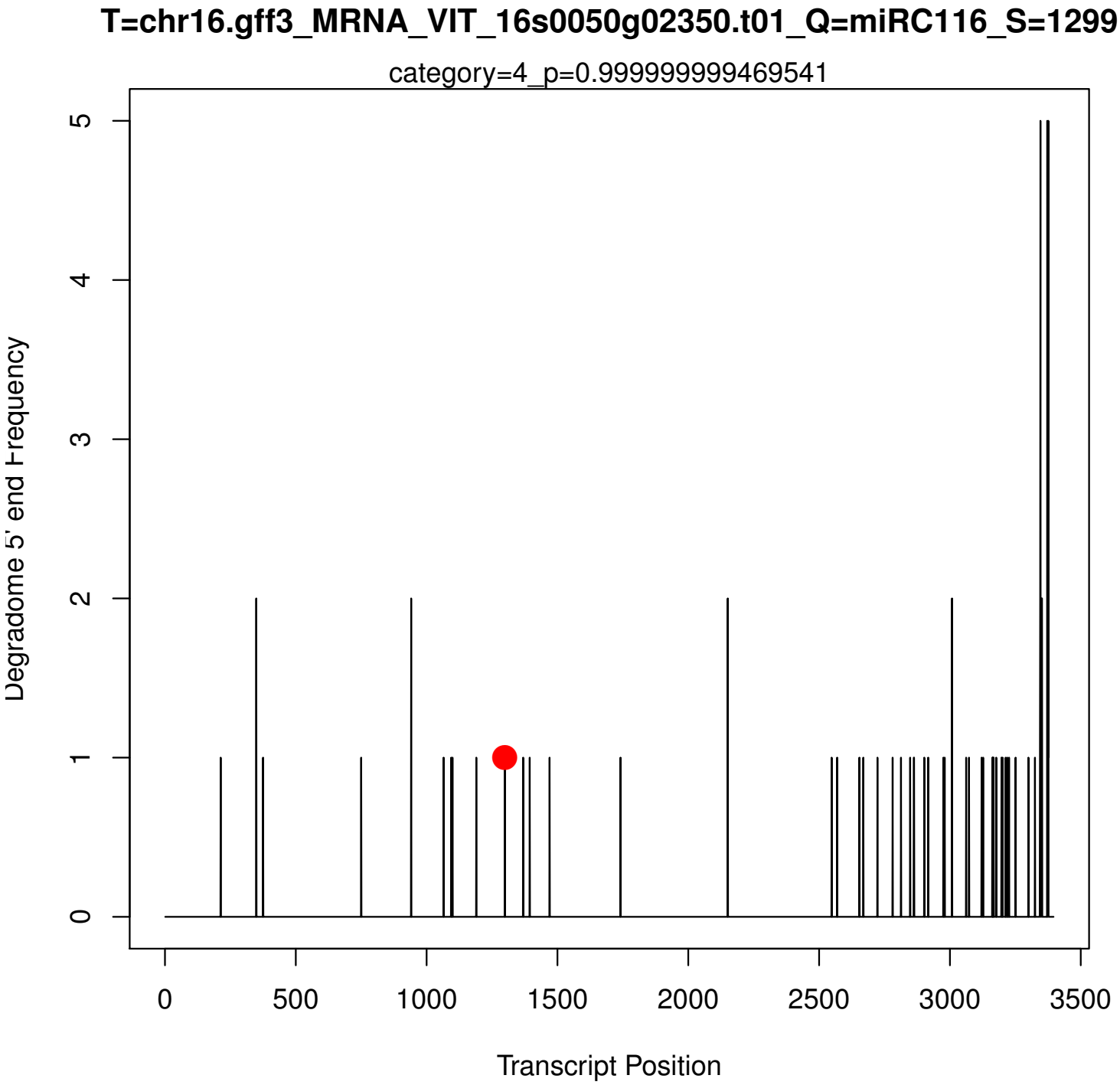
Transcript Position


T=chr16.gff3_MRNA_VIT_16s0050g02350.t01_Q=miRC116_S=1299
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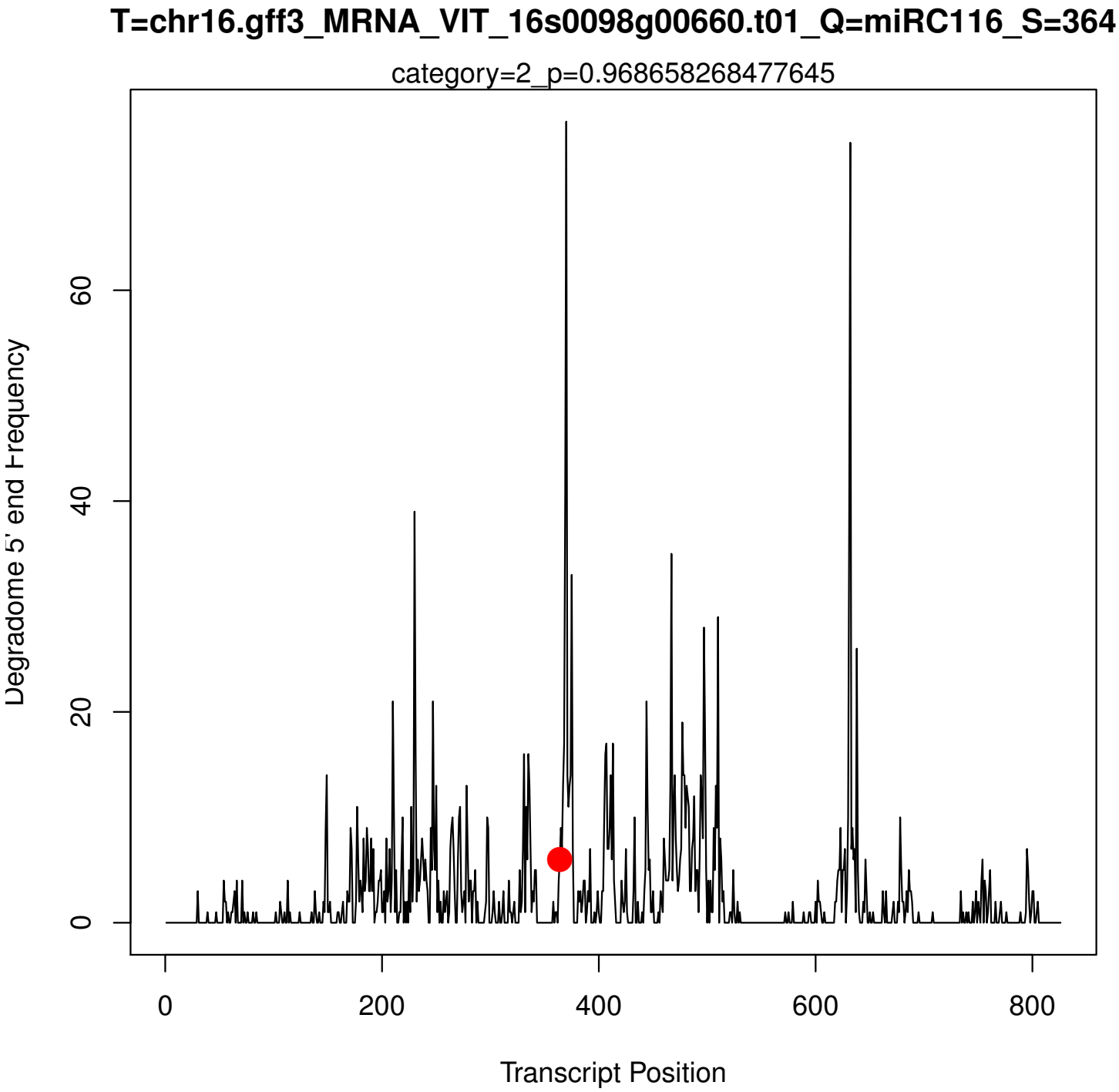
3500
Transcript Position


T=chr16.gff3_MRNA_VIT_16s0098g00660.t01_Q=miRC116_S=364
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600
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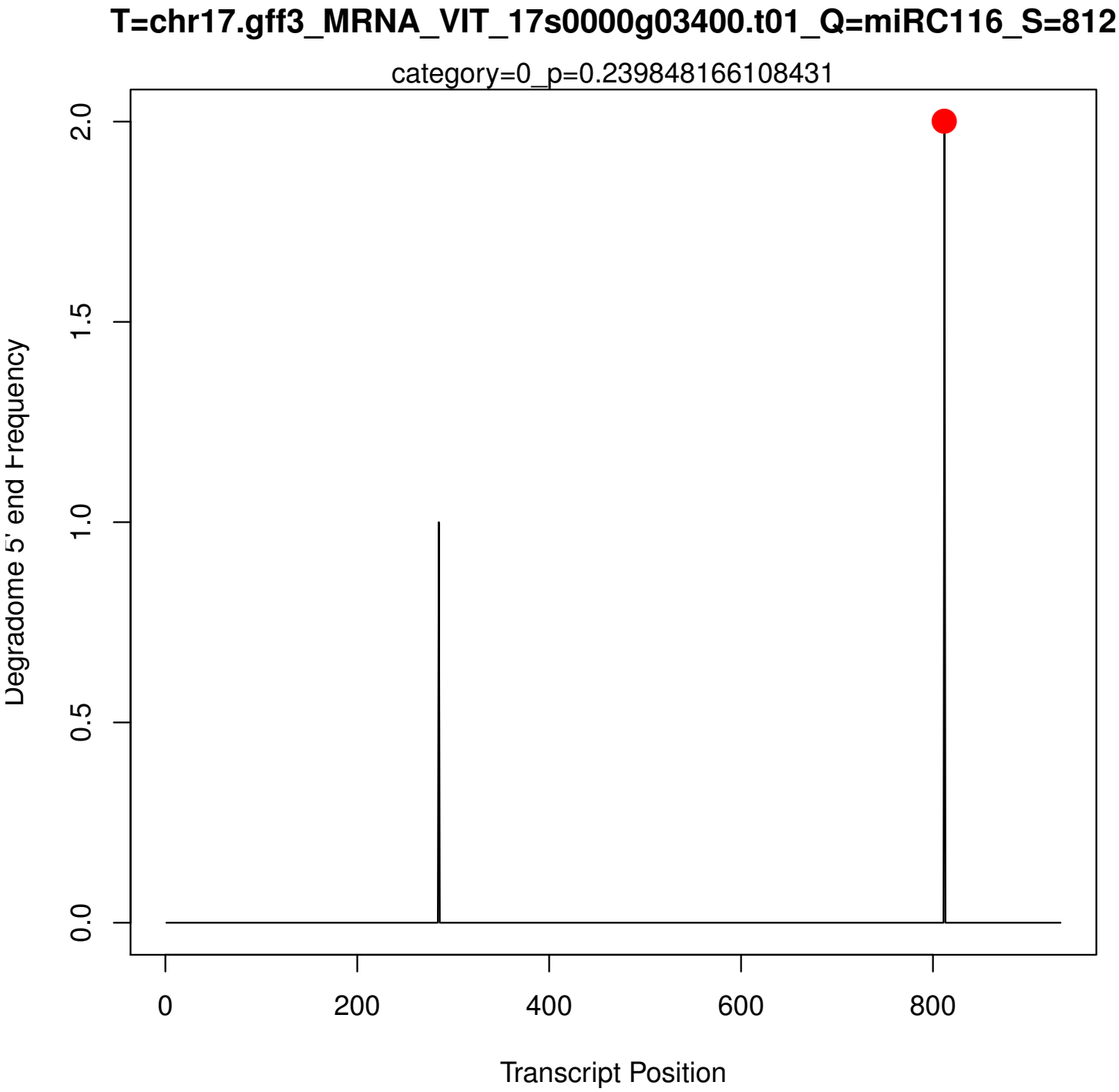
Transcript Position


T=chr17.gff3_MRNA_VIT_17s0000g03400.t01_Q=miRC116_S=812
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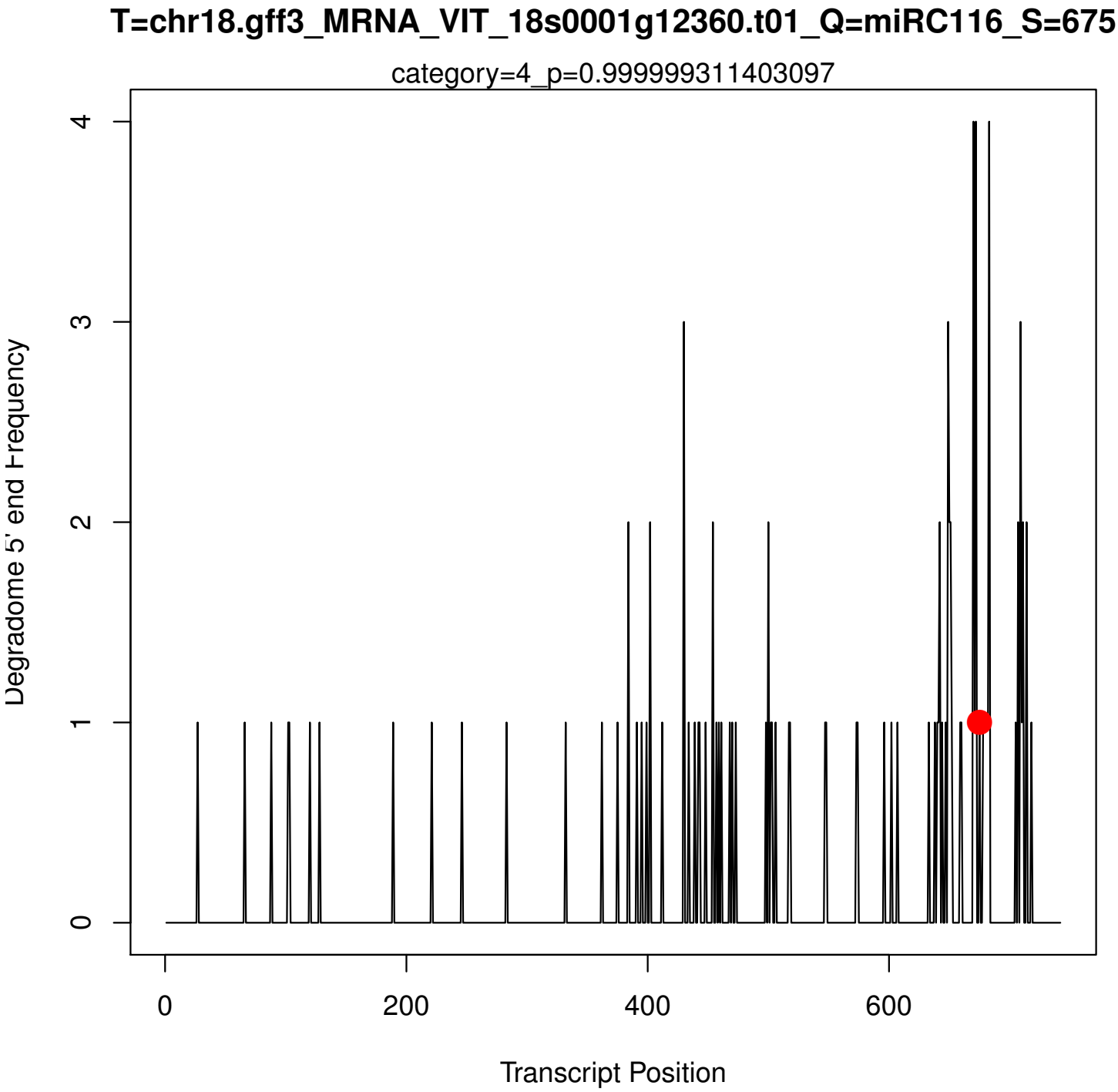
Transcript Position


T=chr18.gff3_MRNA_VIT_18s0001g12360.t01_Q=miRC116_S=675
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Degradome 5' end Frequency
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3
4
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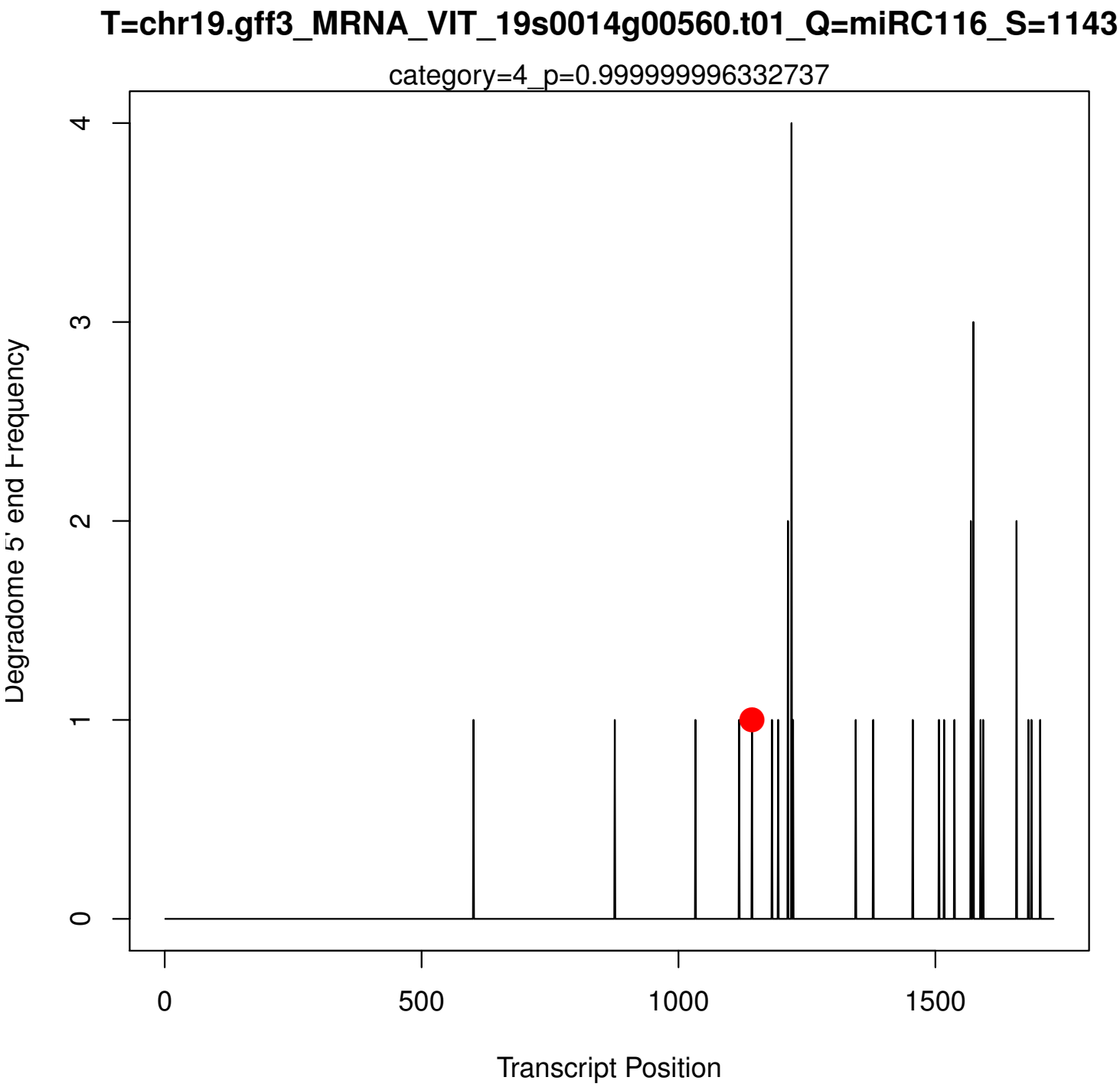
400
600
Transcript Position


T=chr19.gff3_MRNA_VIT_19s0014g00560.t01_Q=miRC116_S=1143
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3
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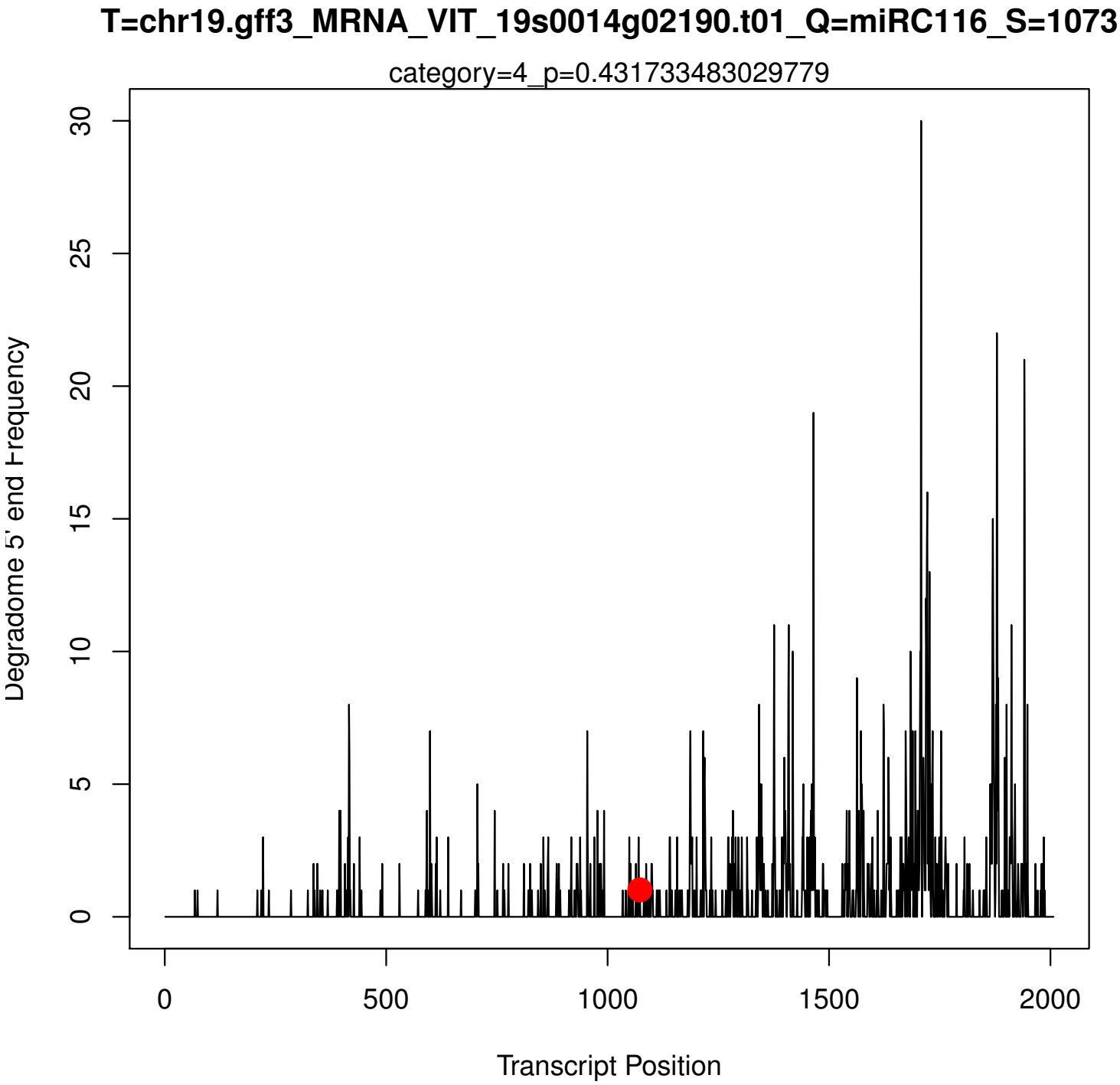
Transcript Position


T=chr19.gff3_MRNA_VIT_19s0014g02190.t01_Q=miRC116_S=1073
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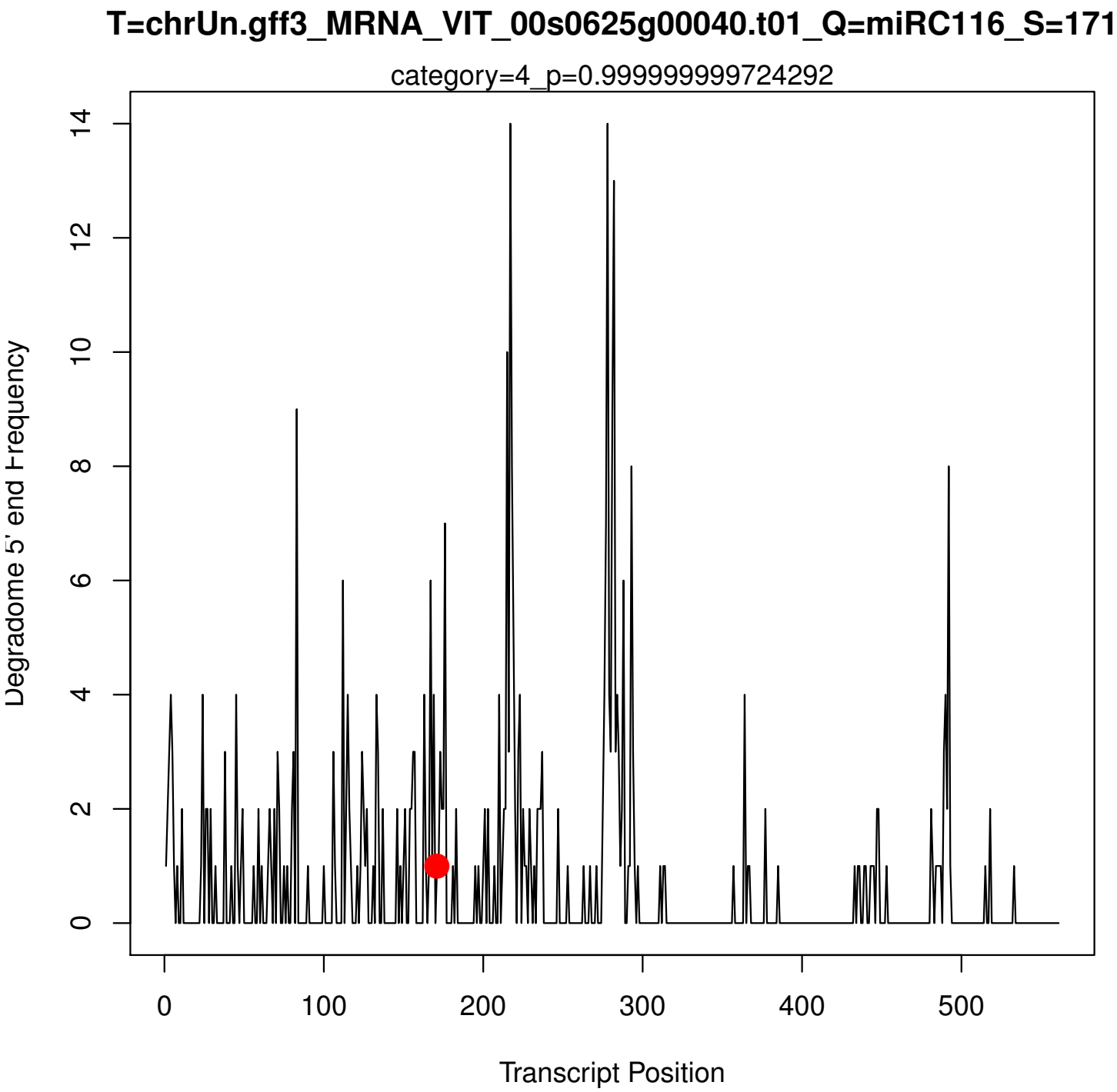
Transcript Position


T=chrUn.gff3_MRNA_VIT_00s0625g00040.t01_Q=miRC116_S=171
category=4_p=0.999999999724292
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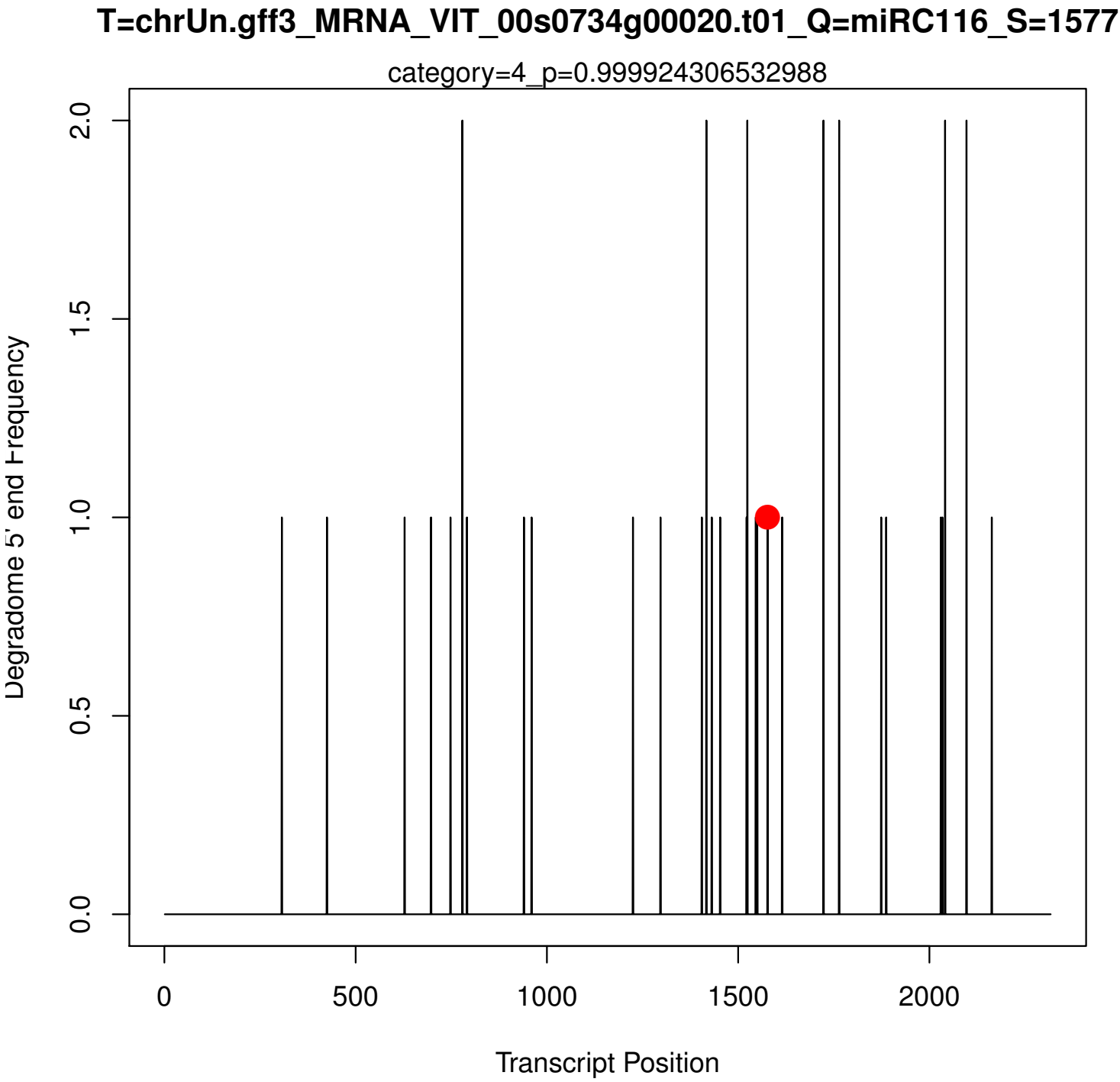
500
Transcript Position


T=chrUn.gff3_MRNA_VIT_00s0734g00020.t01_Q=miRC116_S=1577
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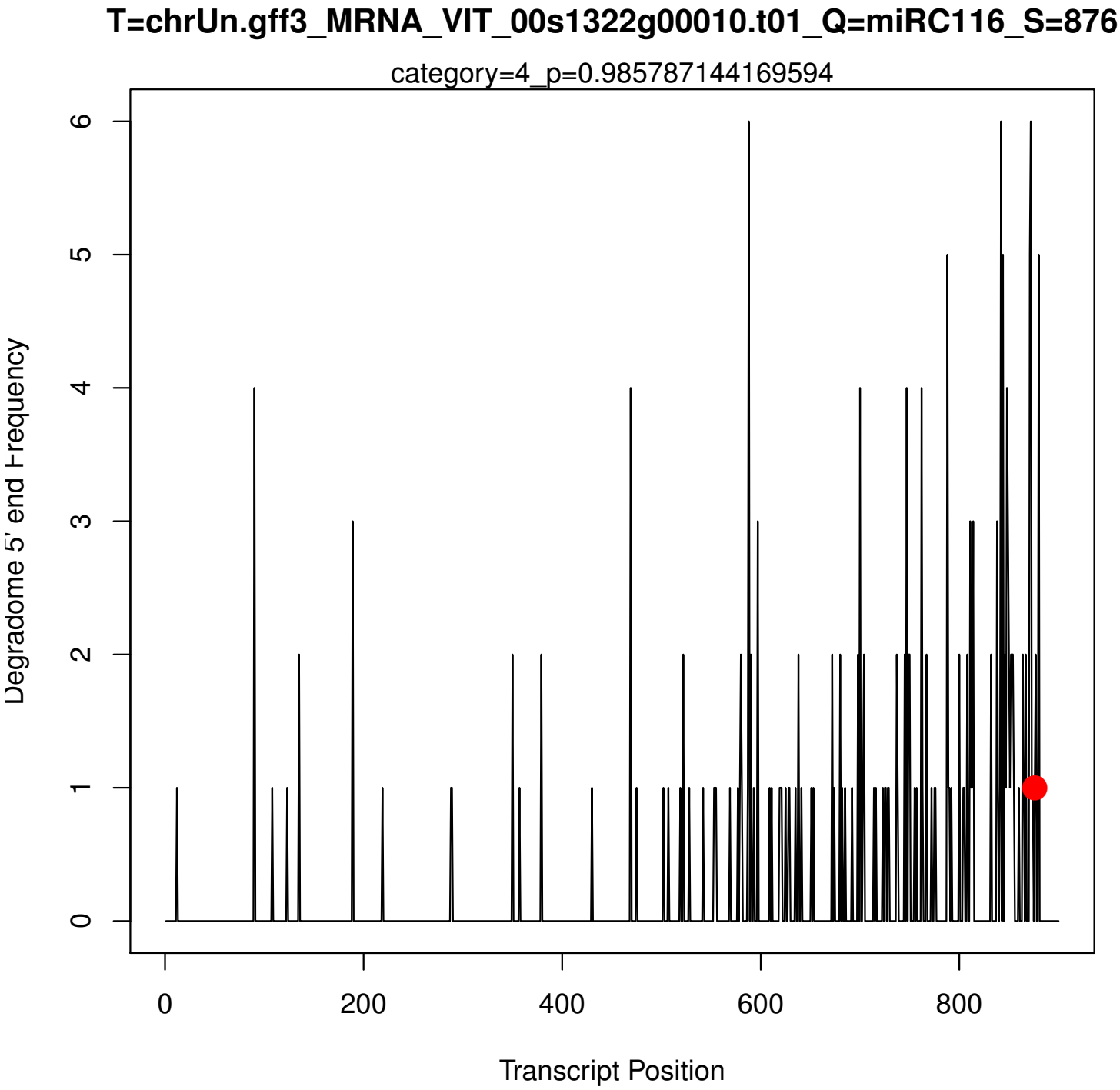
2000
Transcript Position


T=chrUn.gff3_MRNA_VIT_00s1322g00010.t01_Q=miRC116_S=876
category=4_p=0.985787144169594
Degradome 5' end Frequency
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2
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5
6
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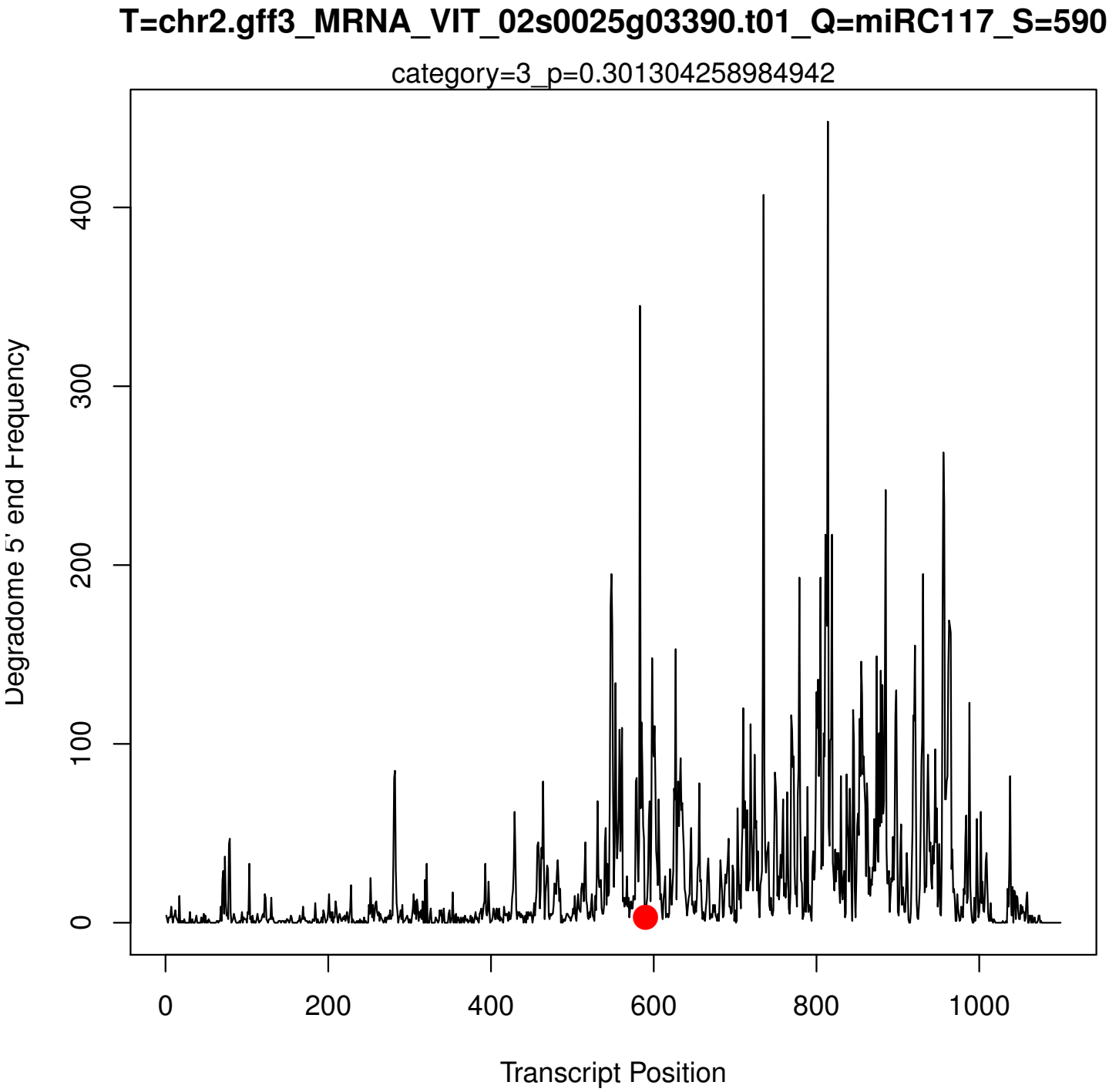
600
800
Transcript Position


T=chr2.gff3_MRNA_VIT_02s0025g03390.t01_Q=miRC117_S=590
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Degradome 5' end Frequency
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400
0
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400
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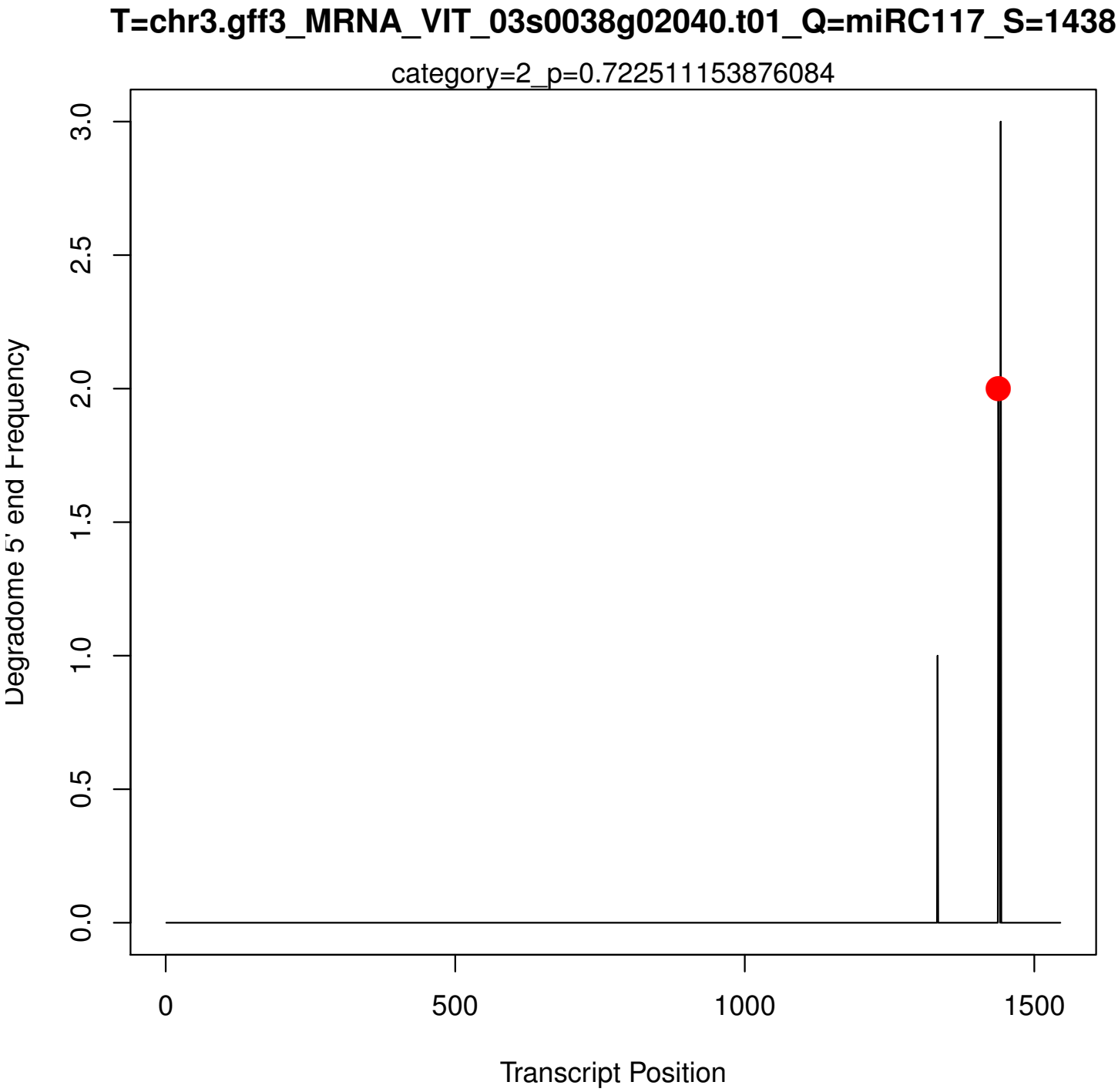
800
1000
Transcript Position


T=chr3.gff3_MRNA_VIT_03s0038g02040.t01_Q=miRC117_S=1438
category=2_p=0.722511153876084
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1000
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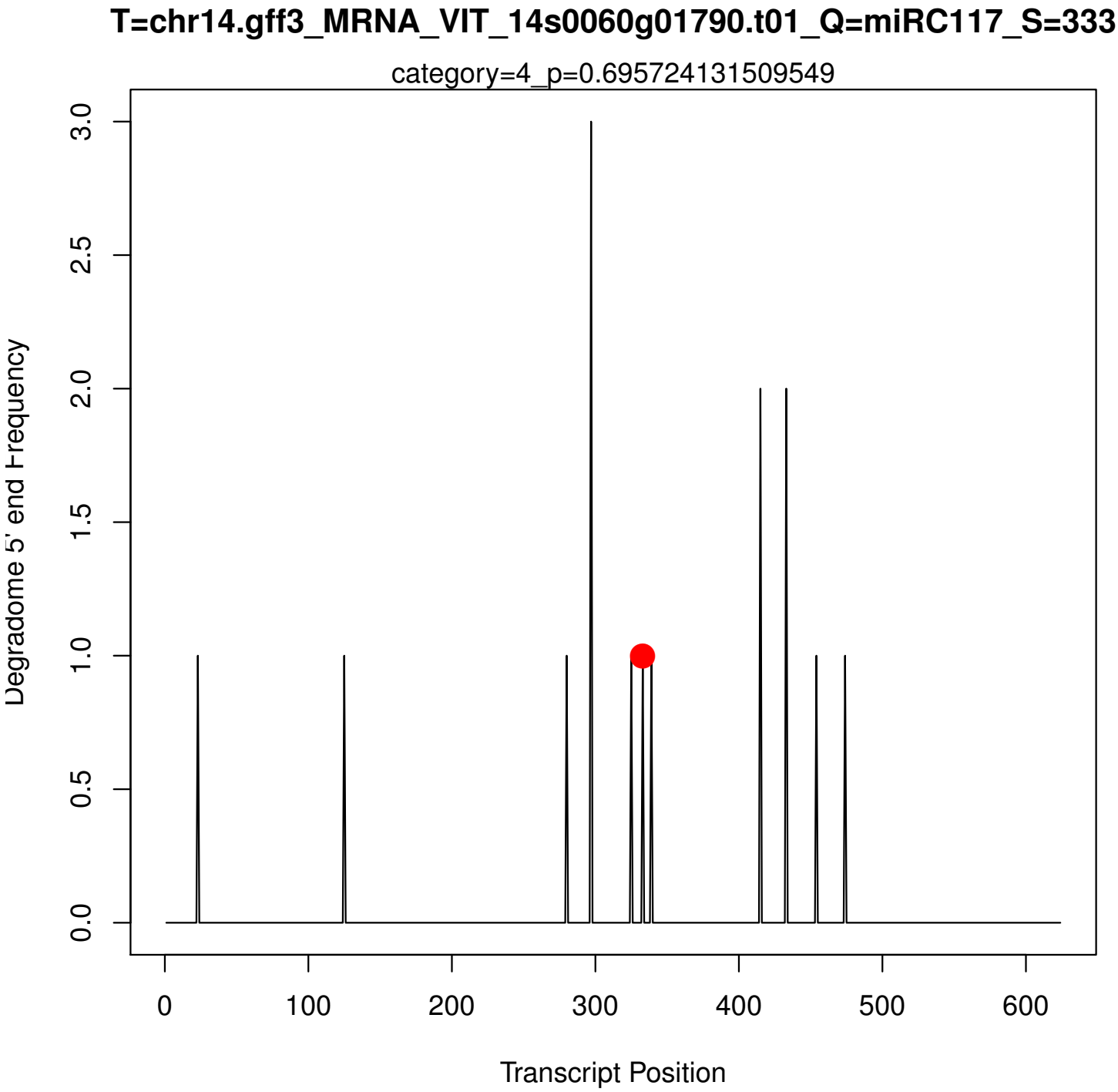
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0060g01790.t01_Q=miRC117_S=333
category=4_p=0.695724131509549
Degradome 5' end Frequency
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3.0
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100
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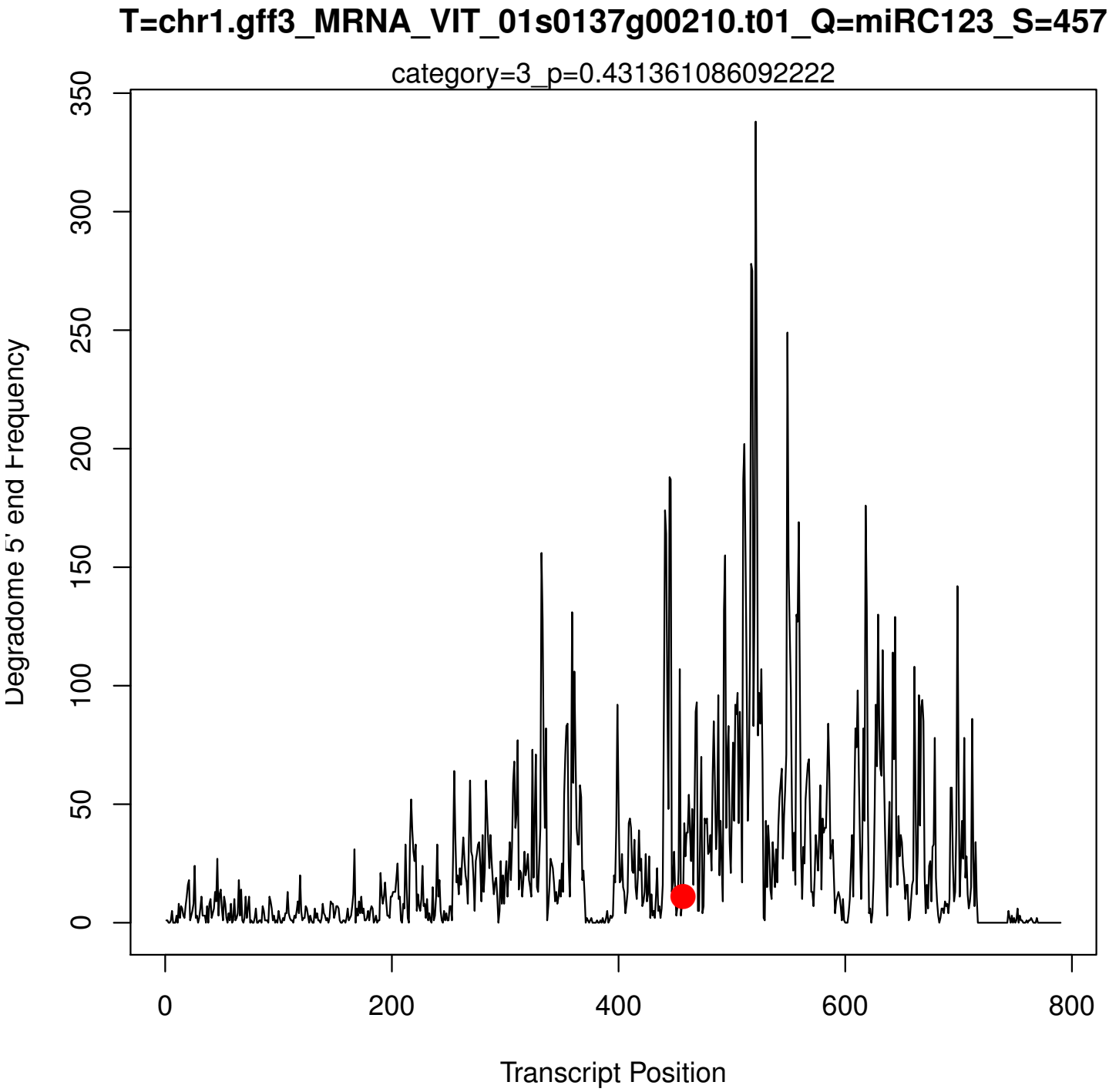
400
500
600
Transcript Position


T=chr1.gff3_MRNA_VIT_01s0137g00210.t01_Q=miRC123_S=457
category=3_p=0.431361086092222
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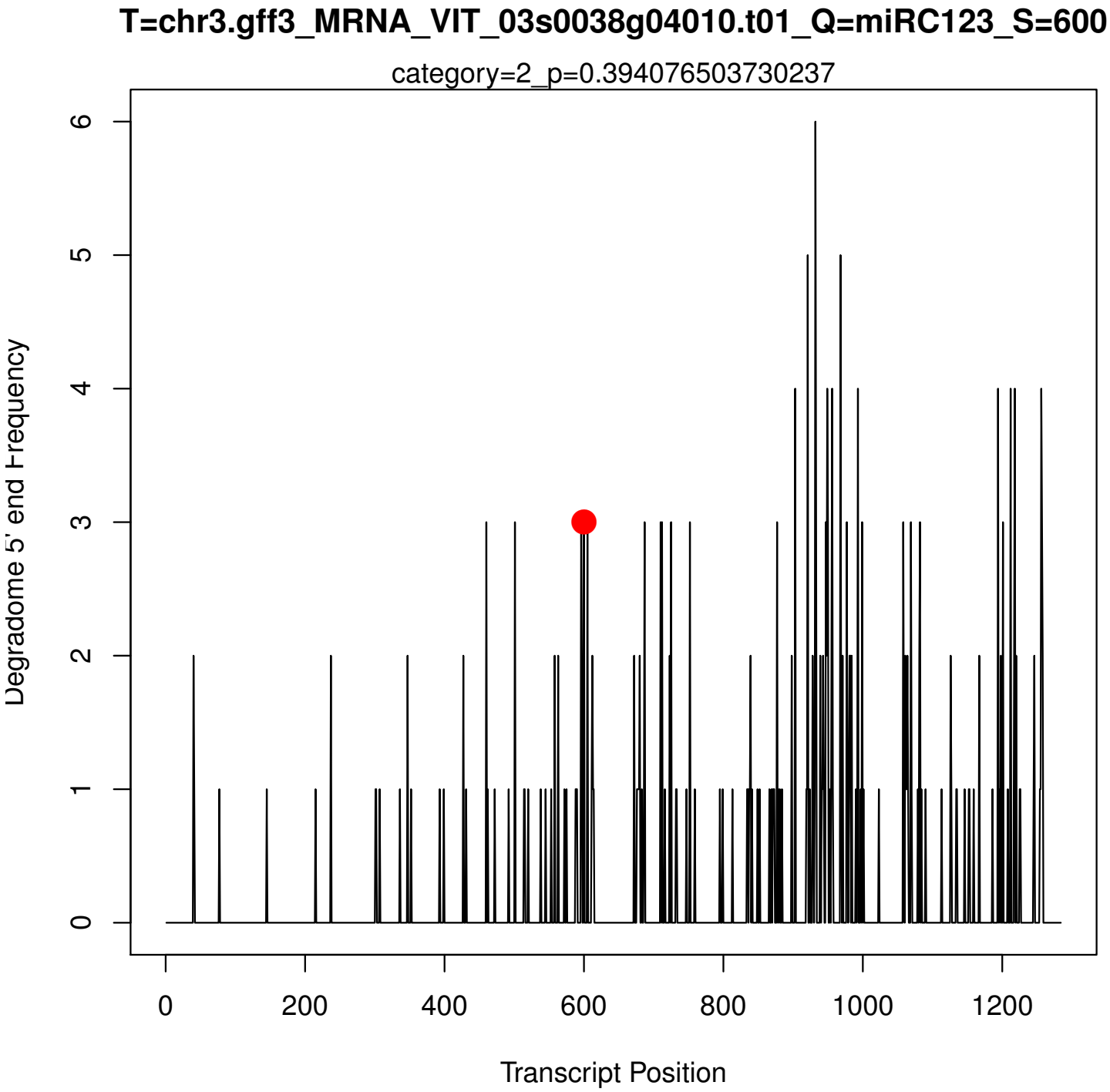
600
800
Transcript Position


T=chr3.gff3_MRNA_VIT_03s0038g04010.t01_Q=miRC123_S=600
category=2_p=0.394076503730237
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1
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3
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5
6
0
200
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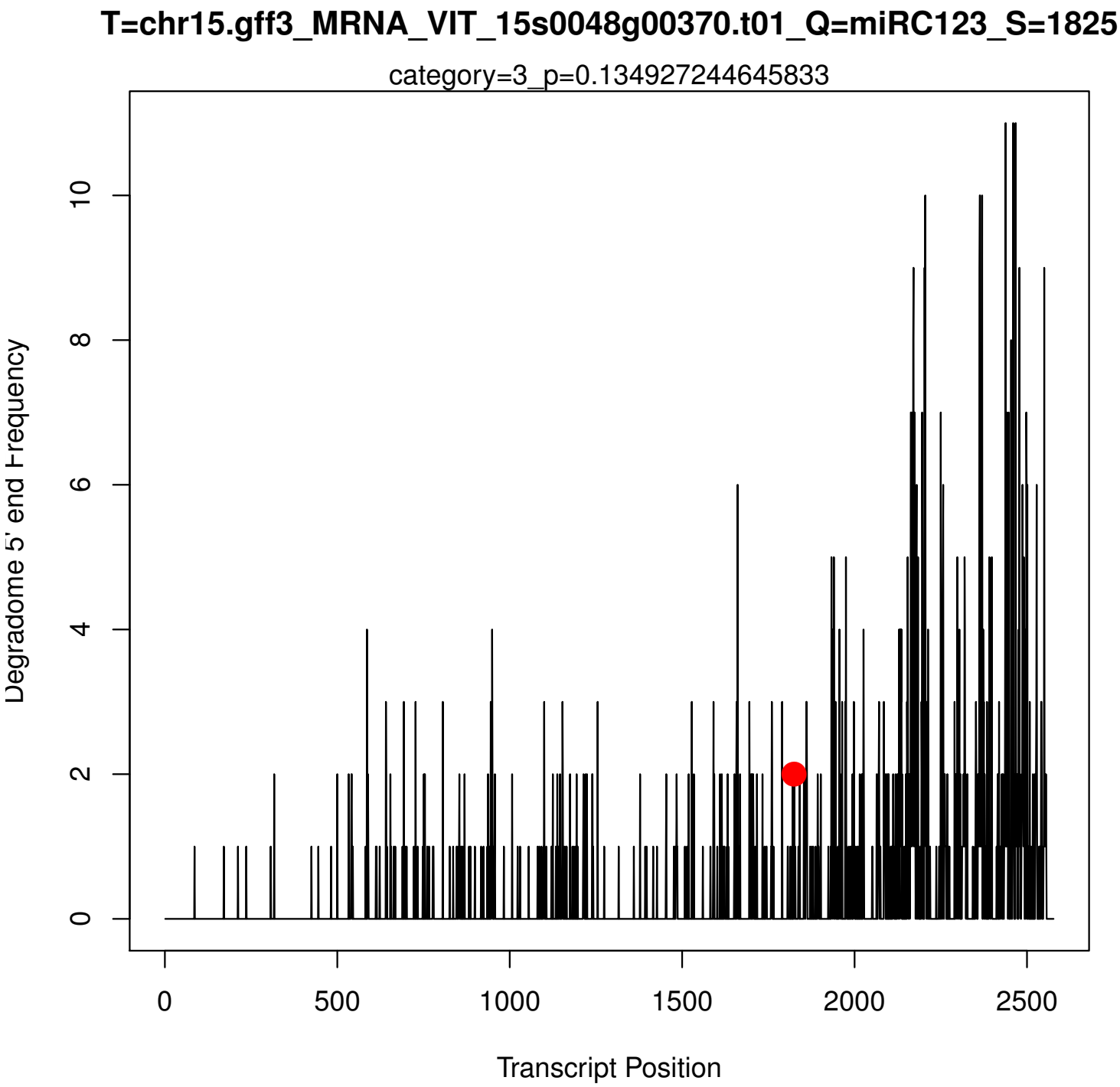
600
800
1000
1200
Transcript Position


T=chr15.gff3_MRNA_VIT_15s0048g00370.t01_Q=miRC123_S=1825
category=3_p=0.134927244645833
Degradome 5' end Frequency
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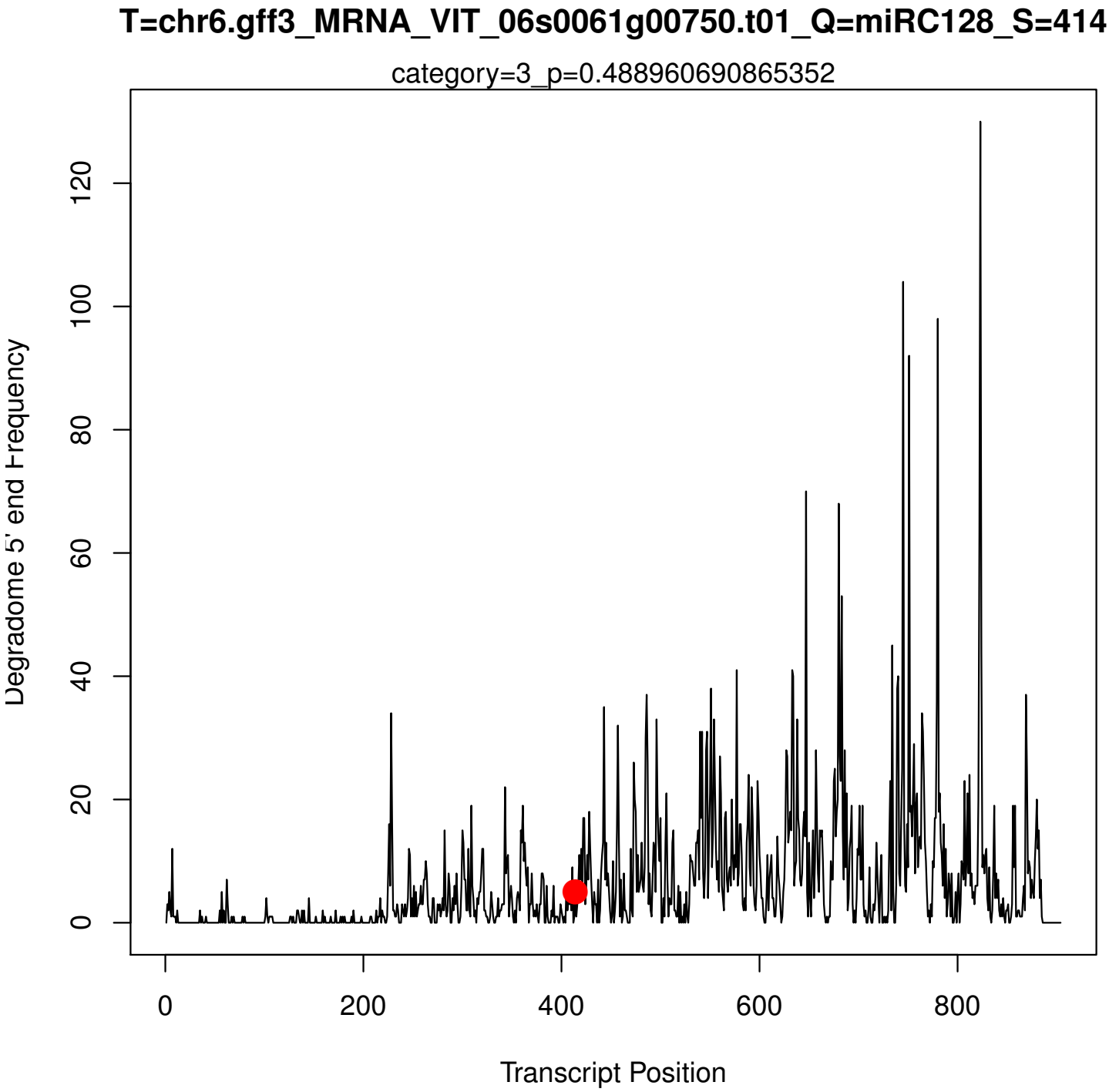
2
4
6
8
10
0
500
1000
1500
2000
2500
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0061g00750.t01_Q=miRC128_S=414
category=3_p=0.488960690865352
Degradome 5' end Frequency
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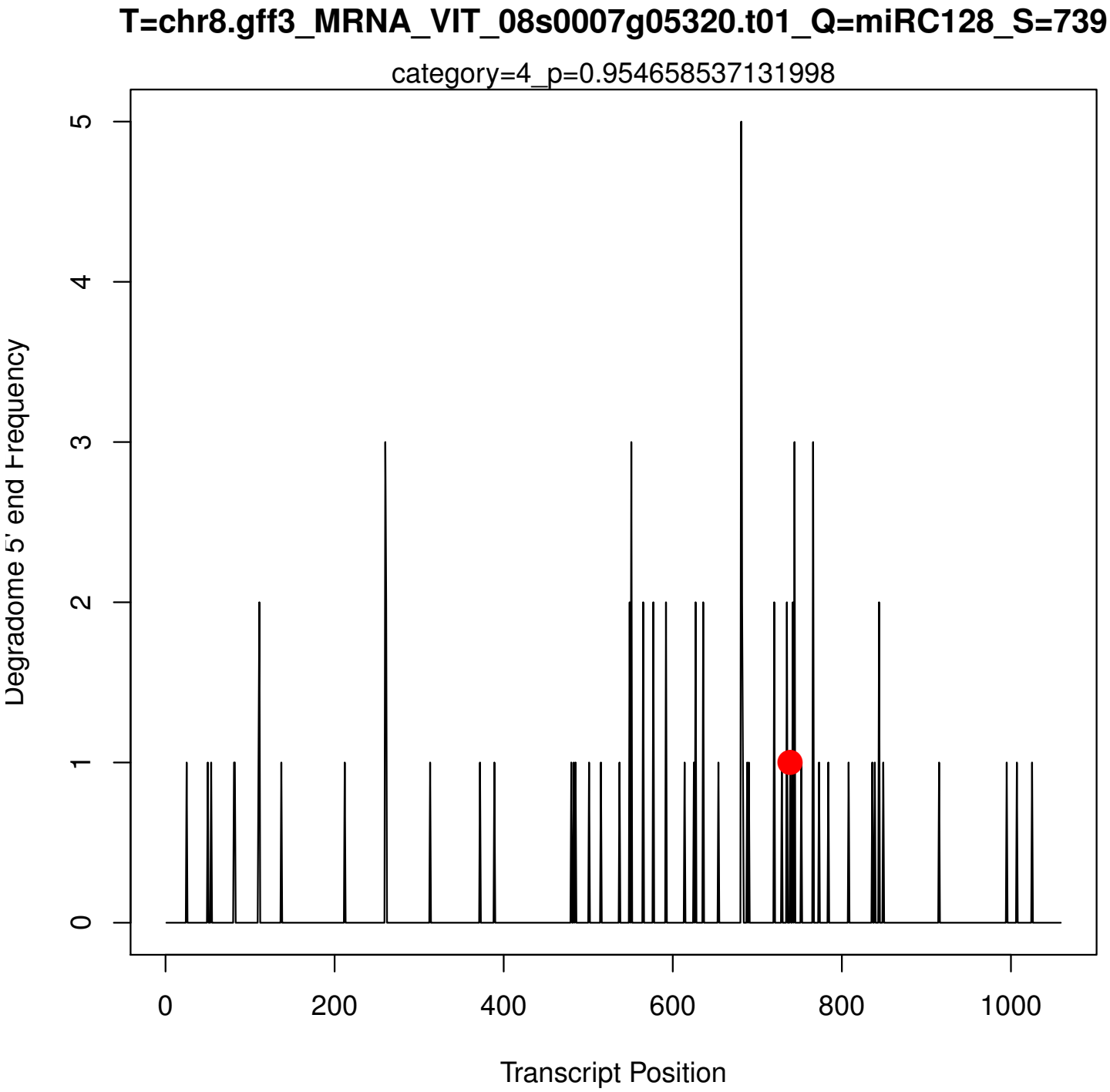
100
120
0
200
400
600
800
Transcript Position


T=chr8.gff3_MRNA_VIT_08s0007g05320.t01_Q=miRC128_S=739
category=4_p=0.954658537131998
Degradome 5' end Frequency
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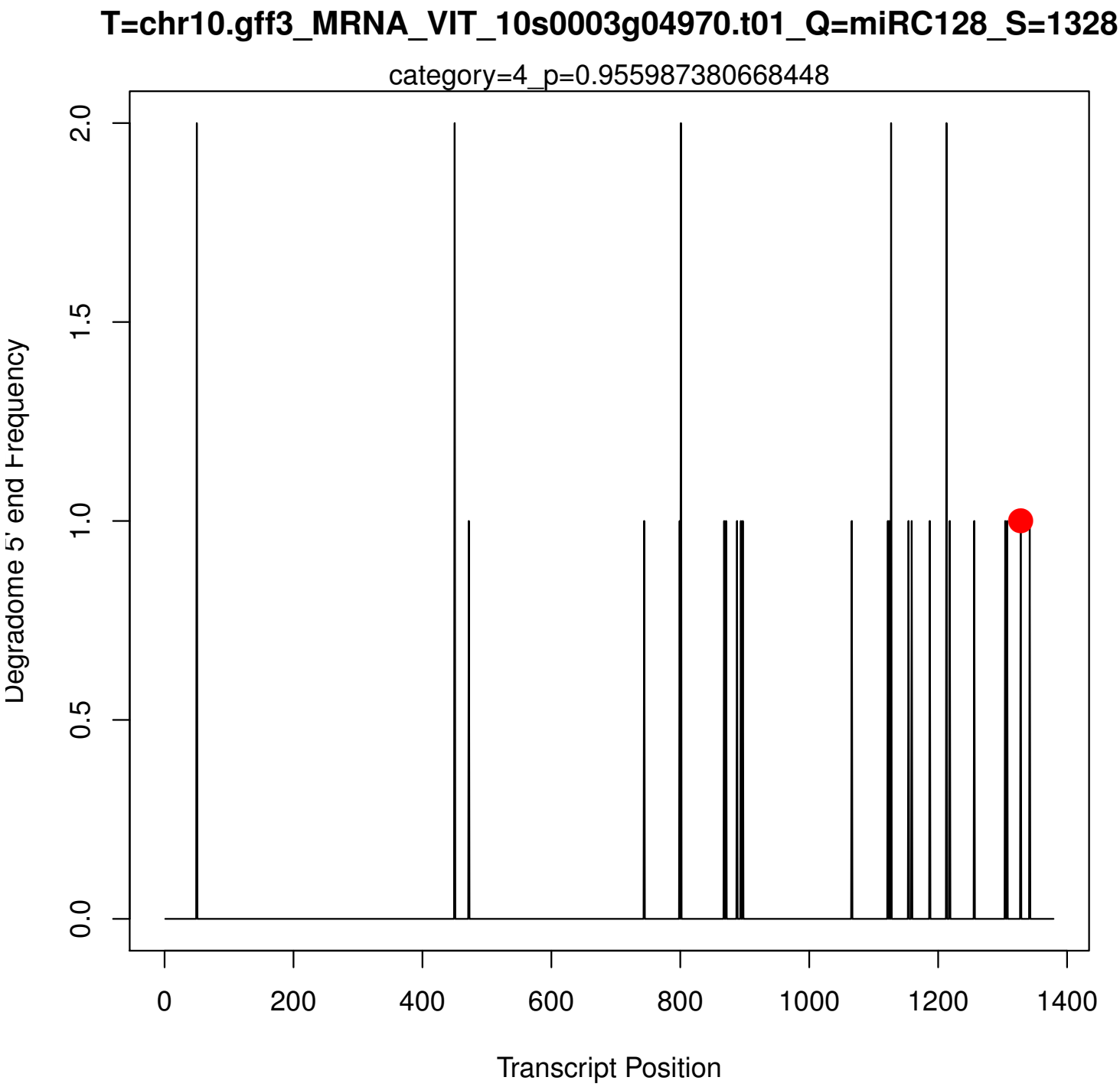
1
2
3
4
5
0
200
400
600
800
1000
Transcript Position


T=chr10.gff3_MRNA_VIT_10s0003g04970.t01_Q=miRC128_S=1328
category=4_p=0.955987380668448
Degradome 5' end Frequency
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1.0
1.5
2.0
0
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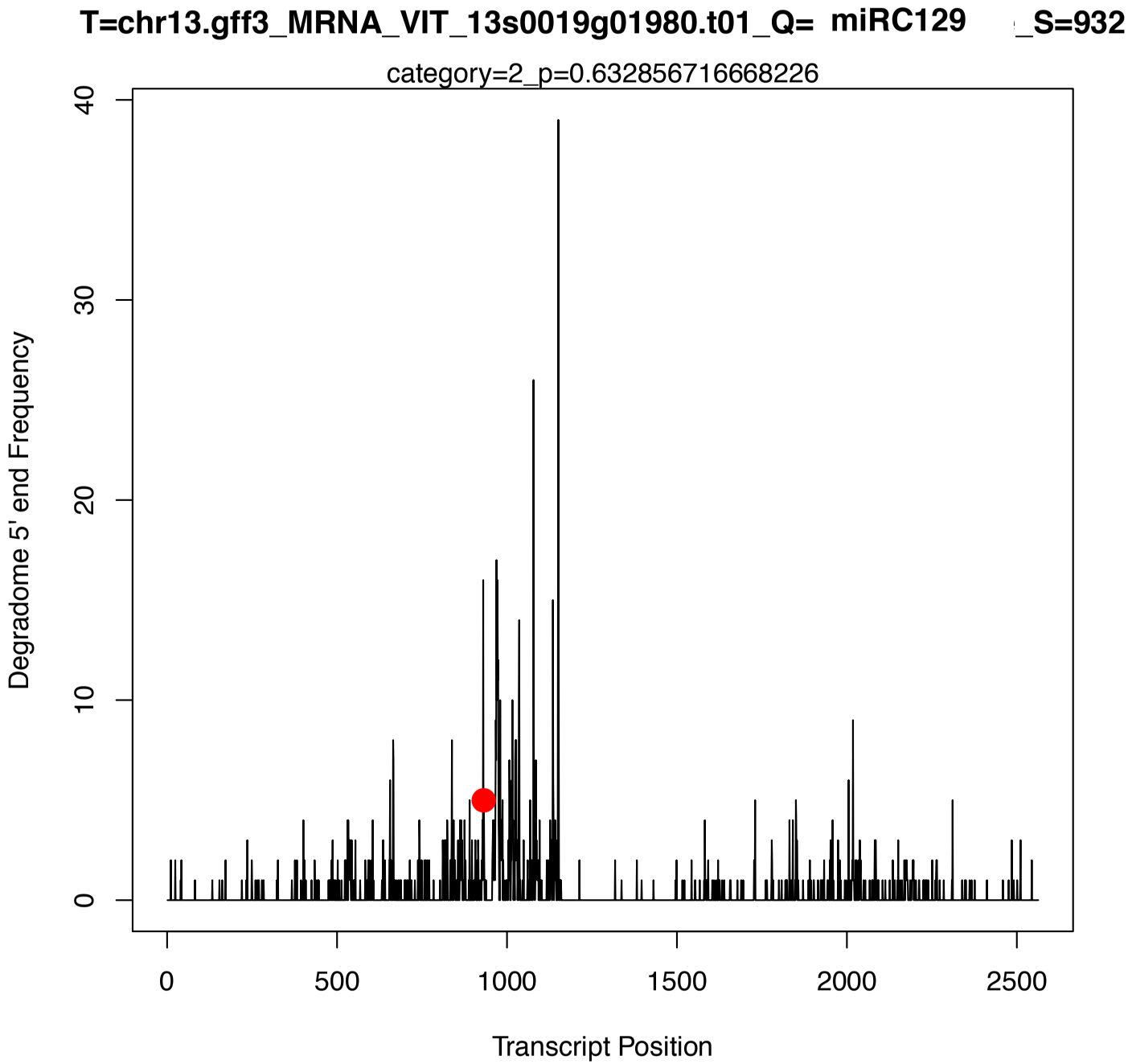
400
600
800
1000
1200
1400
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0019g01980.t01_Q= miRC129 _S=932
category=2_p=0.632856716668226
Degradome 5' end Frequency
0
10
20
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0
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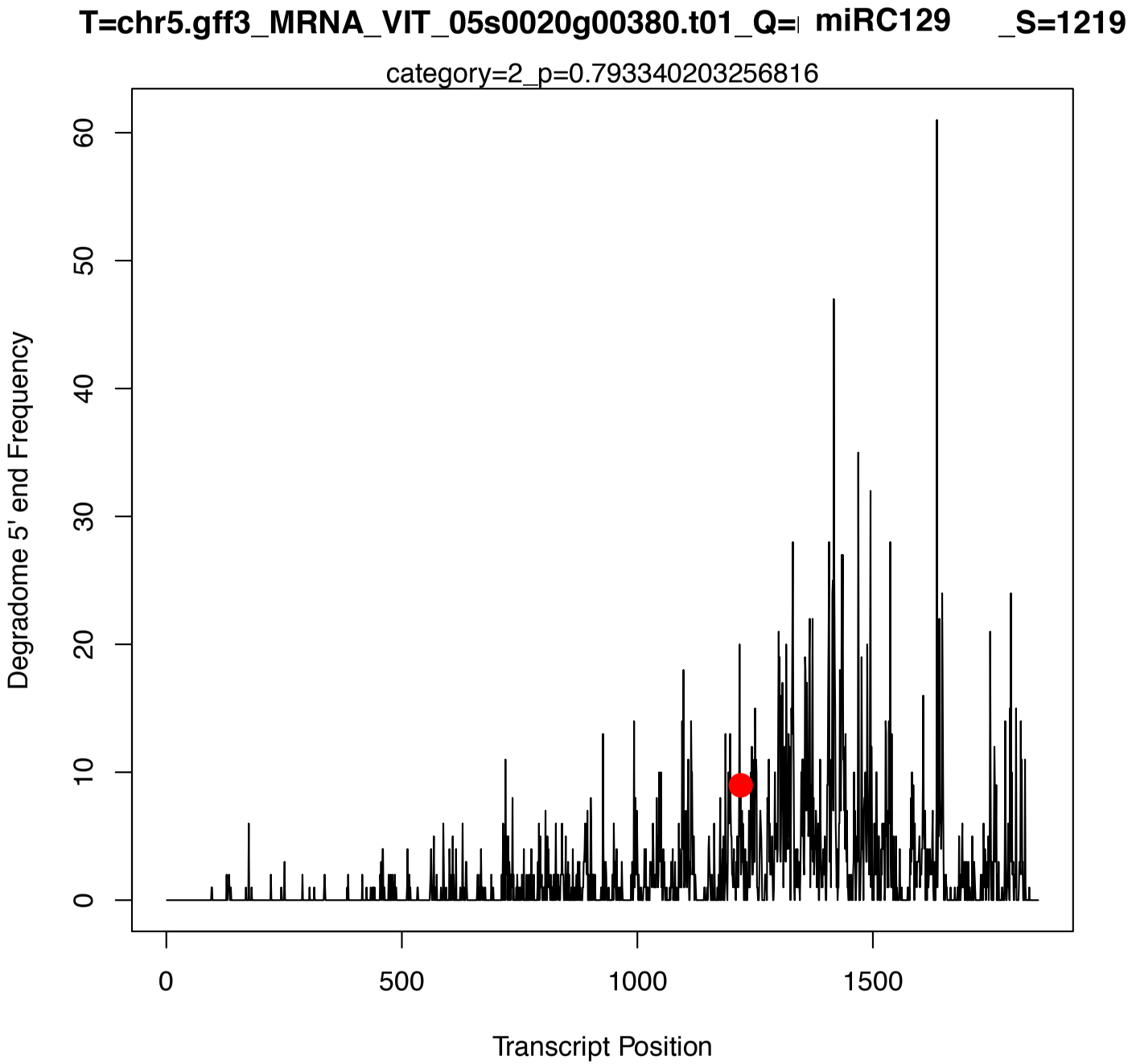
1000
1500
2000
2500
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0020g00380.t01_Q=I miRC129 _S=1219
category=2_p=0.793340203256816
Degradome 5' end Frequency
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10
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50
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0
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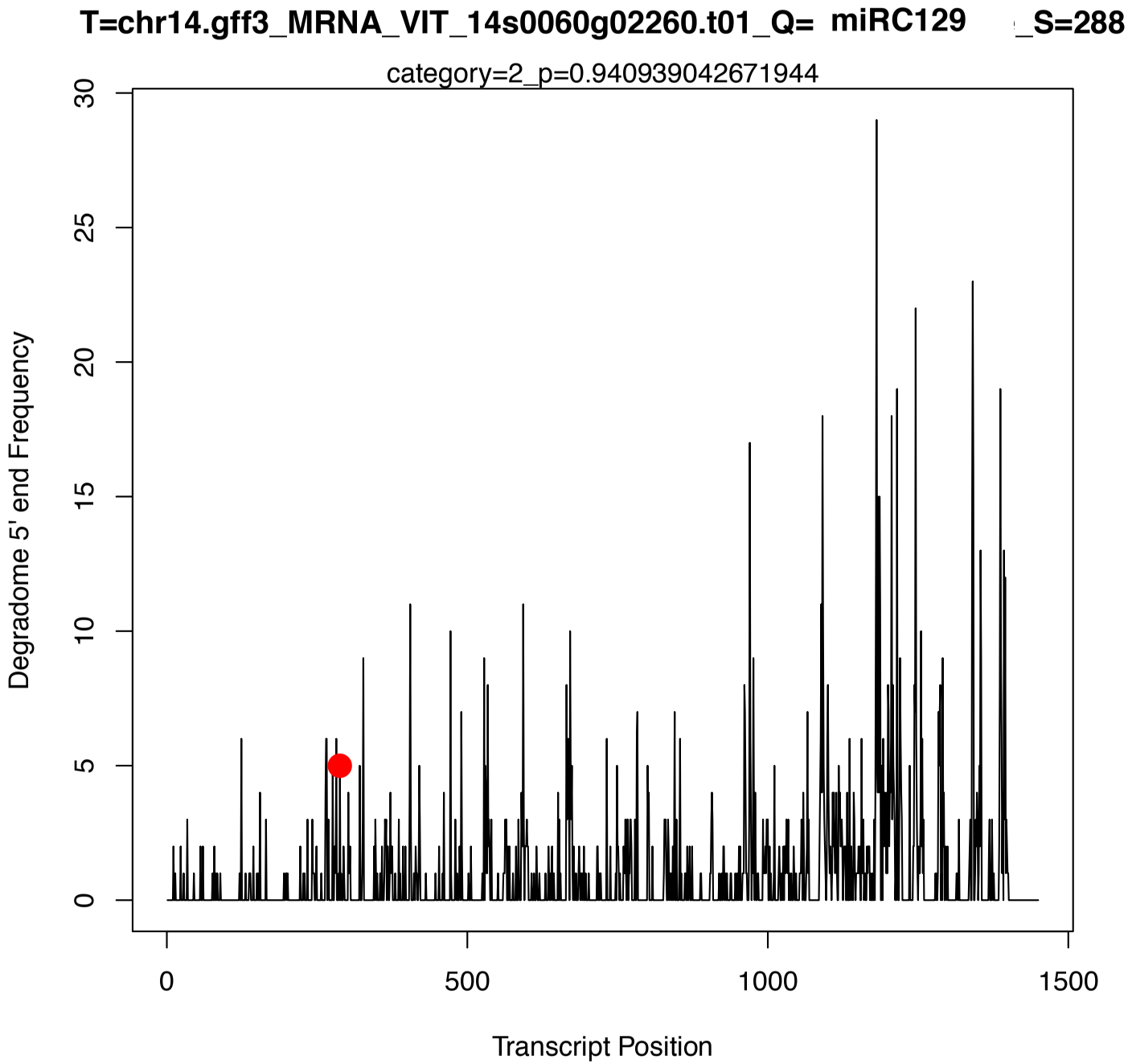
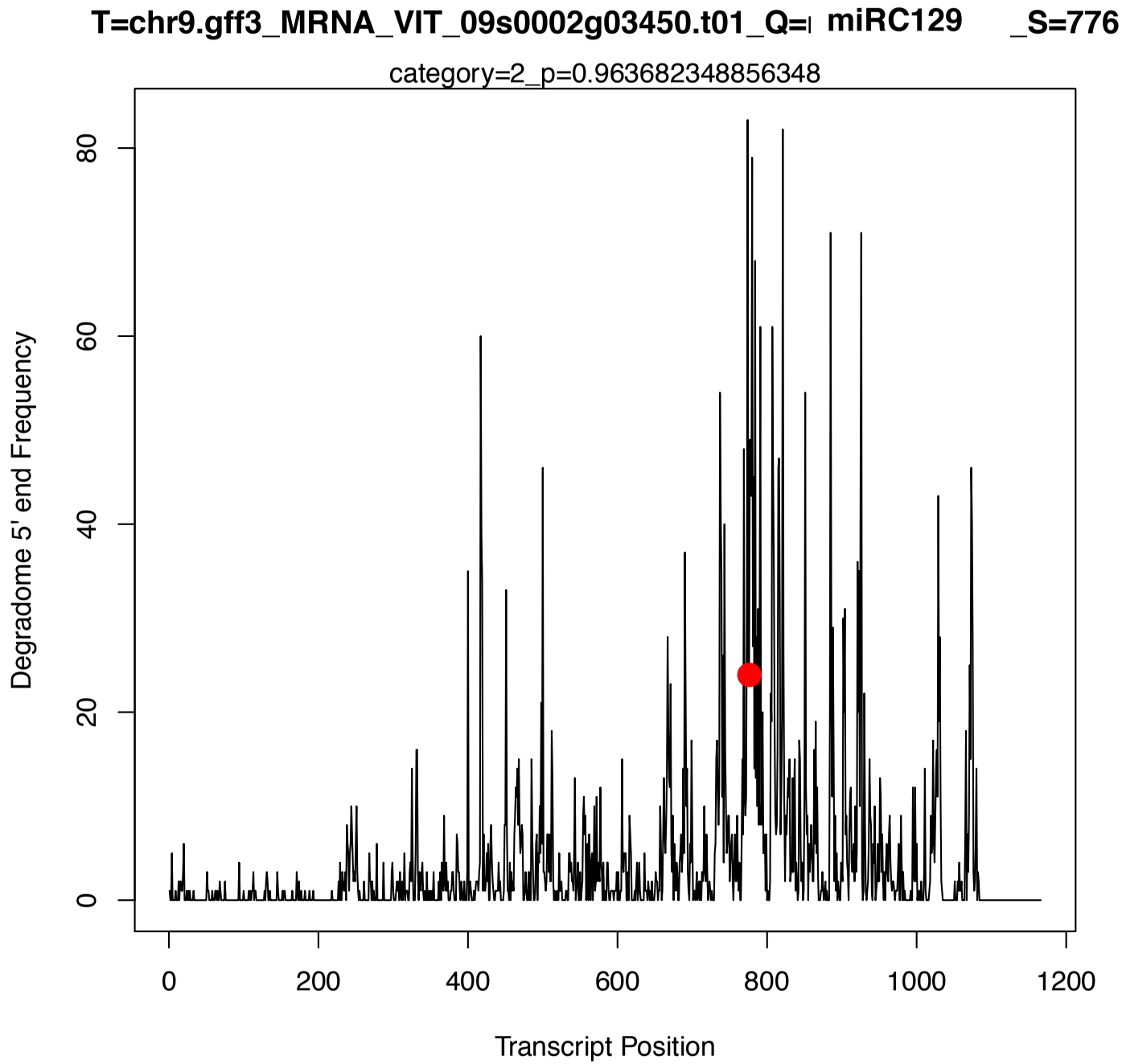
1000
1500
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0060g02260.t01_Q= miRC129 _S=288
category=2_p=0.940939042671944
Degradome 5' end Frequency
0
5
10
15
20
25
30
0
500
1000
1500
Transcript Position


T=chr9.gff3_MRNA_VIT_09s0002g03450.t01_Q=I miRC129 _S=776
category=2_p=0.963682348856348
Degradome 5' end Frequency
0
20
40
60
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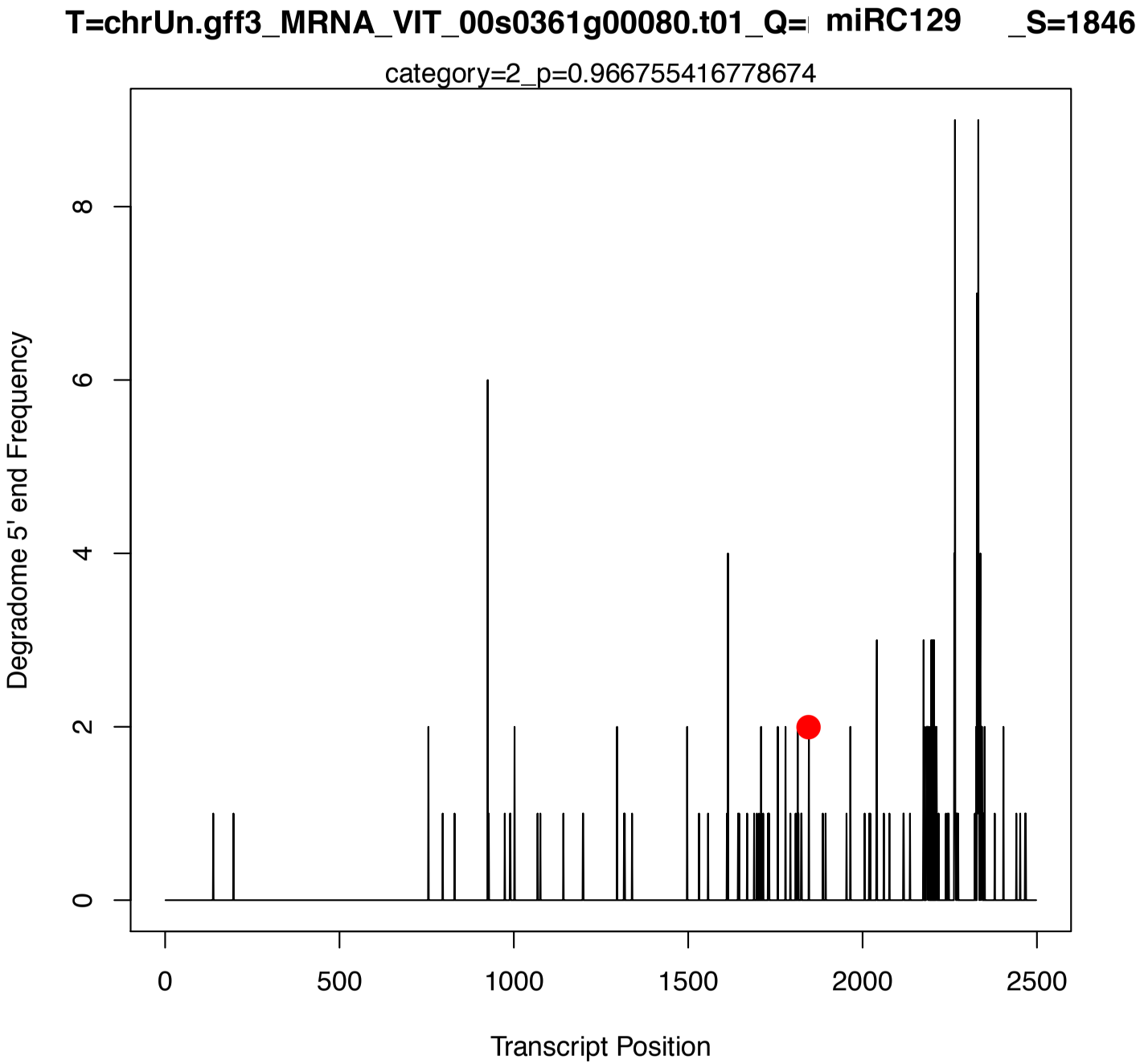
200
400
600
800
1000
1200
Transcript Position


T=chrUn.gff3_MRNA_VIT_00s0361g00080.t01_Q= miRC129 _S=1846
category=2_p=0.966755416778674
Degradome 5' end Frequency
0
2
4
6
8
0
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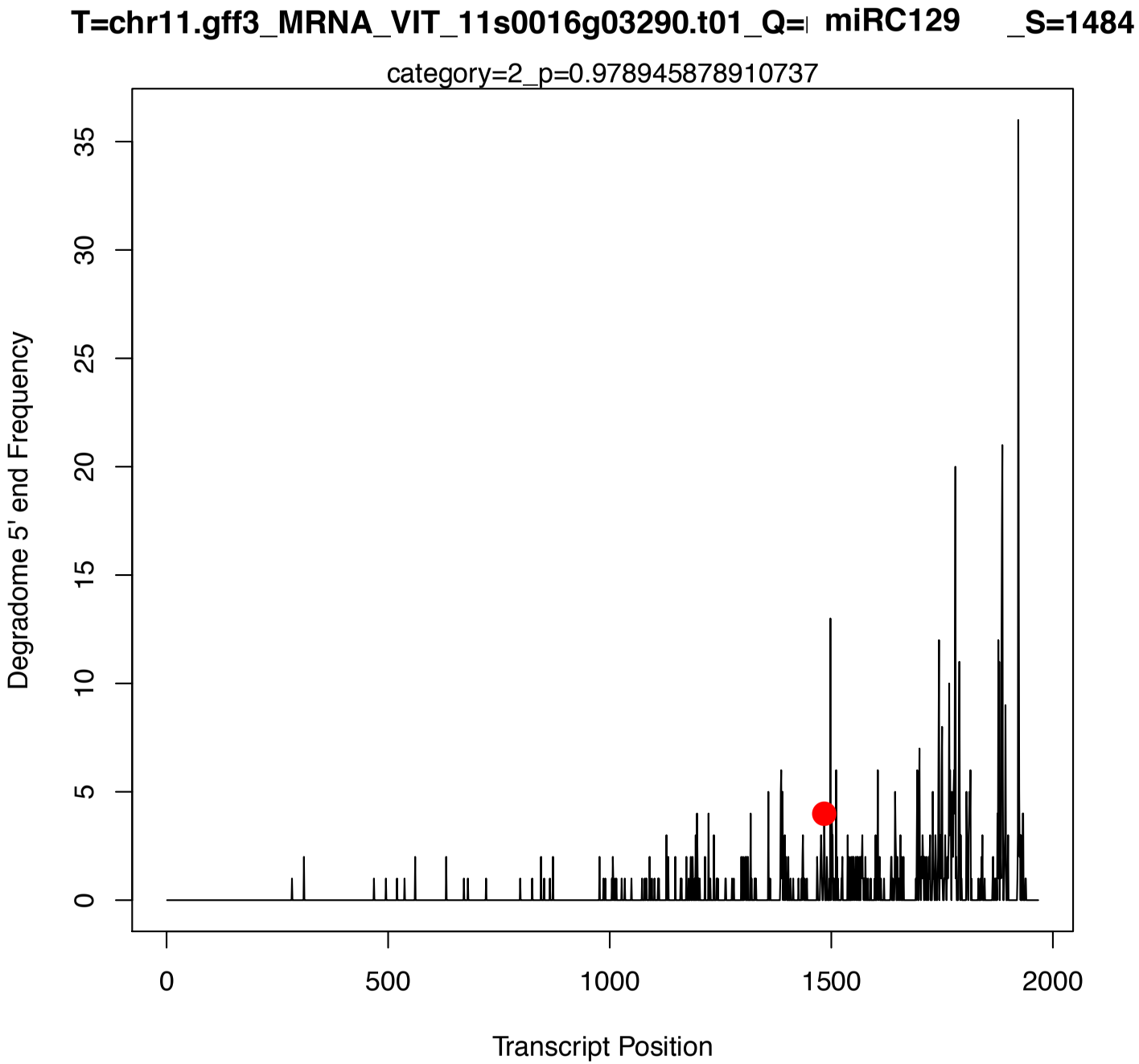
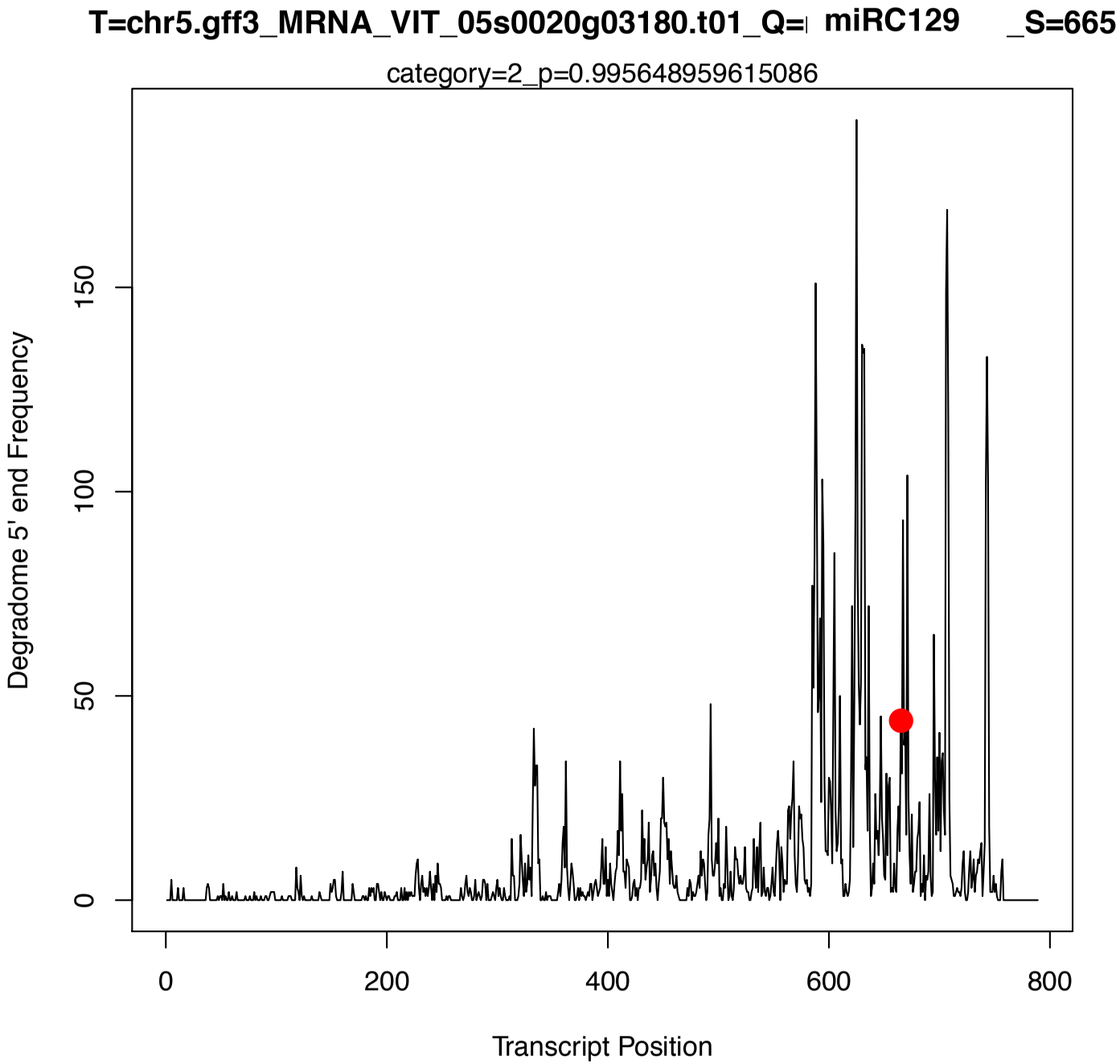
1000
1500
2000
2500
Transcript Position


T=chr11.gff3_MRNA_VIT_11s0016g03290.t01_Q=
miRC129
_S=1484
category=2_p=0.978945878910737
Degradome 5' end Frequency
0
5
10
15
20
25
30
35
0
500
1000
1500
2000
Transcript Position


T=chr5.gff3_MRNA_VIT_05s0020g03180.t01_Q=
miRC129
_S=665
category=2_p=0.995648959615086
Degradome 5' end Frequency
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150
0
200
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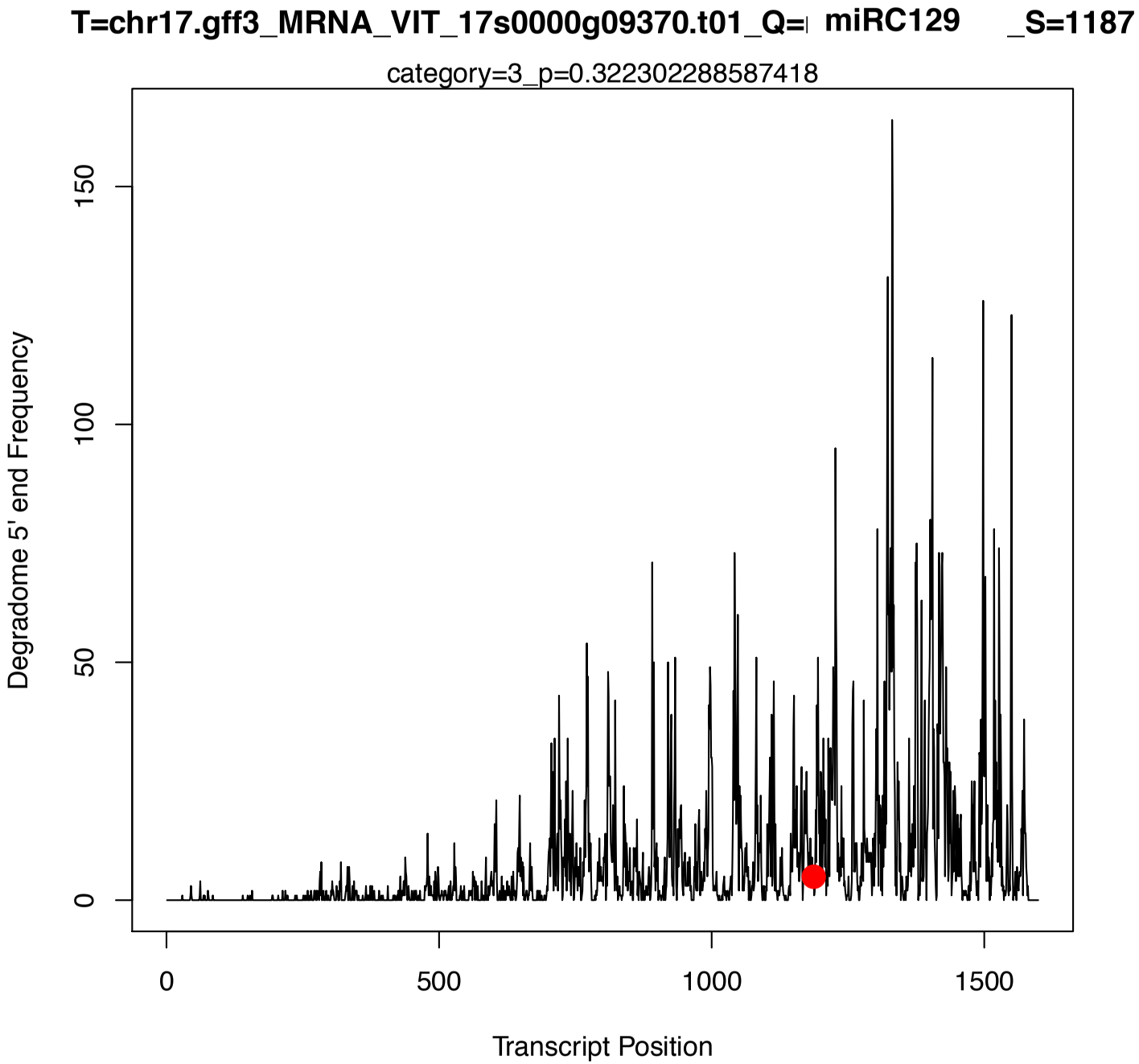
600
800
Transcript Position


T=chr17.gff3_MRNA_VIT_17s0000g09370.t01_Q=
miRC129
_S=1187
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Degradome 5' end Frequency
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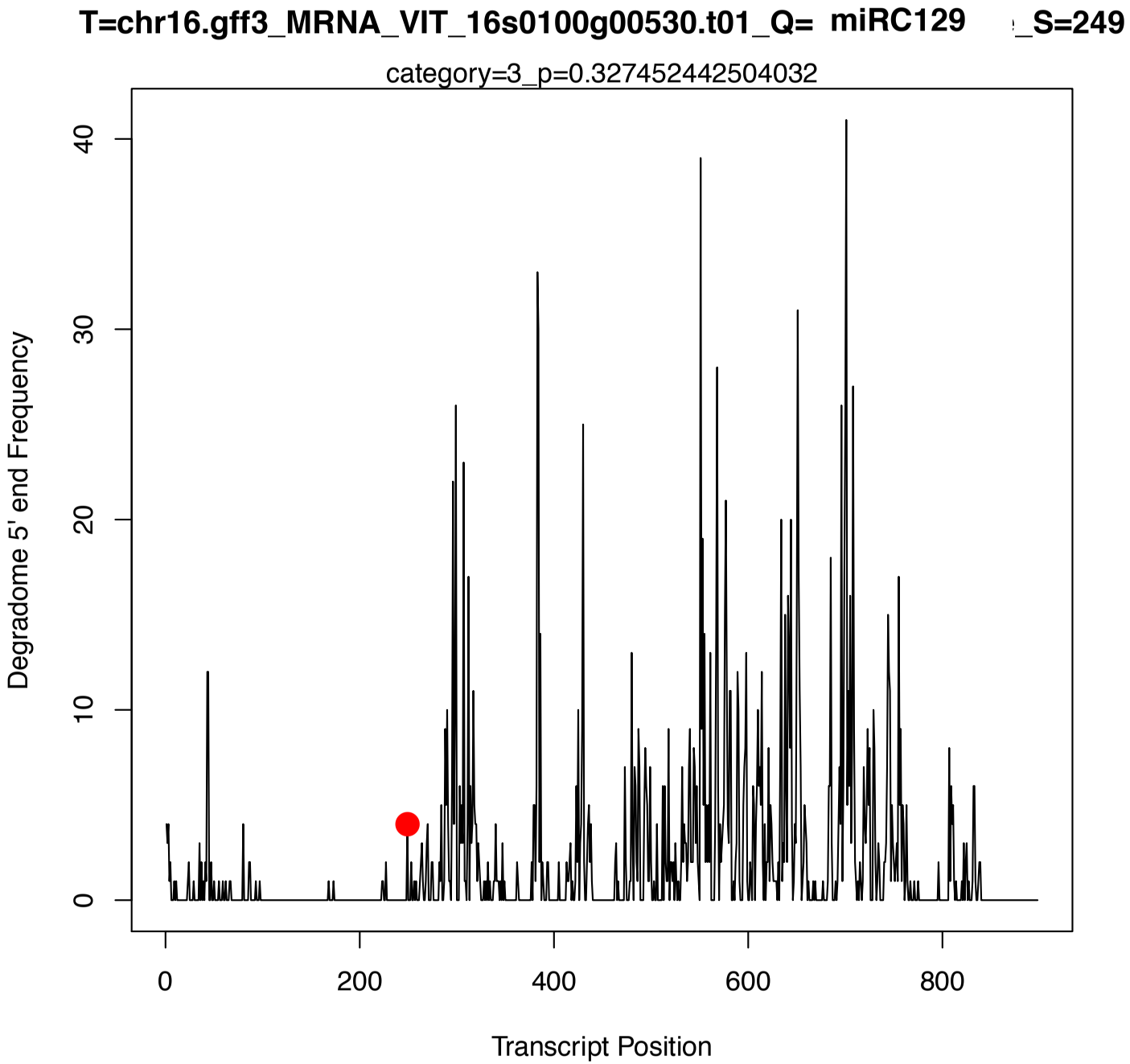
100
150
0
500
1000
1500
Transcript Position


T=chr16.gff3_MRNA_VIT_16s0100g00530.t01_Q= miRC129 _S=249
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Degradome 5' end Frequency
0
10
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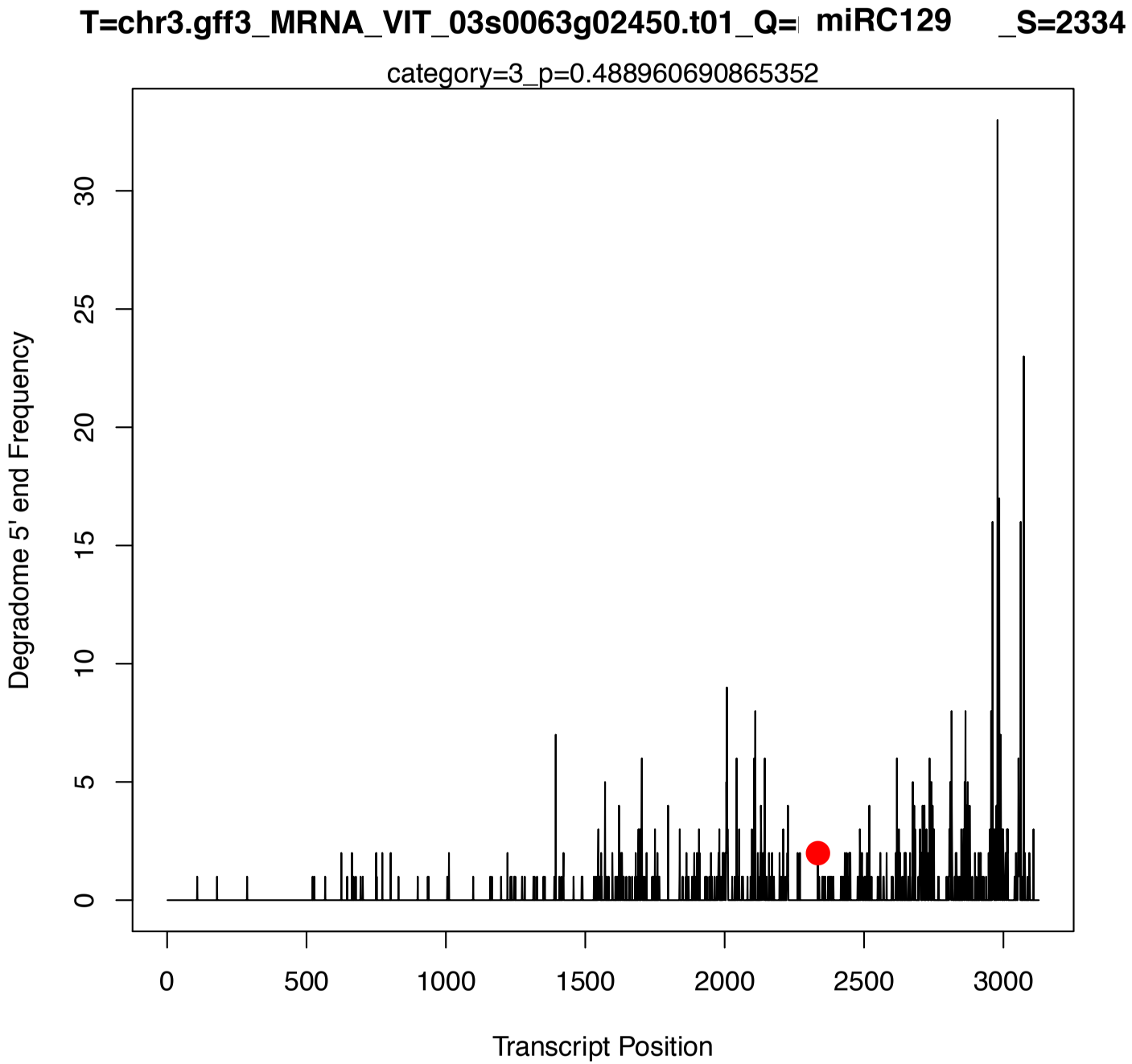
30
40
0
200
400
600
800
Transcript Position


T=chr3.gff3_MRNA_VIT_03s0063g02450.t01_Q= miRC129 _S=2334
category=3_p=0.488960690865352
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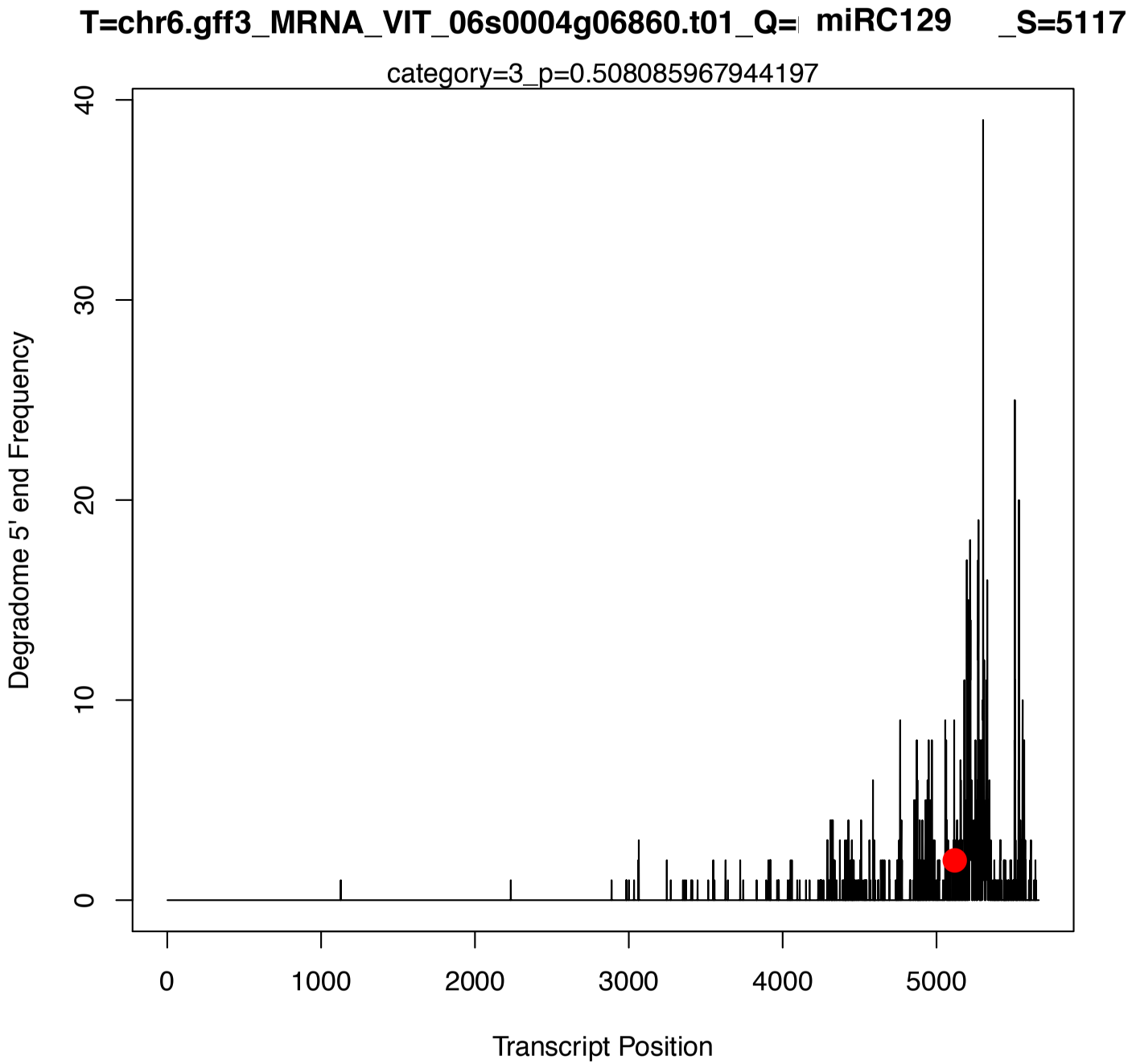
2500
3000
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0004g06860.t01_Q= miRC129 _S=5117
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0
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2000
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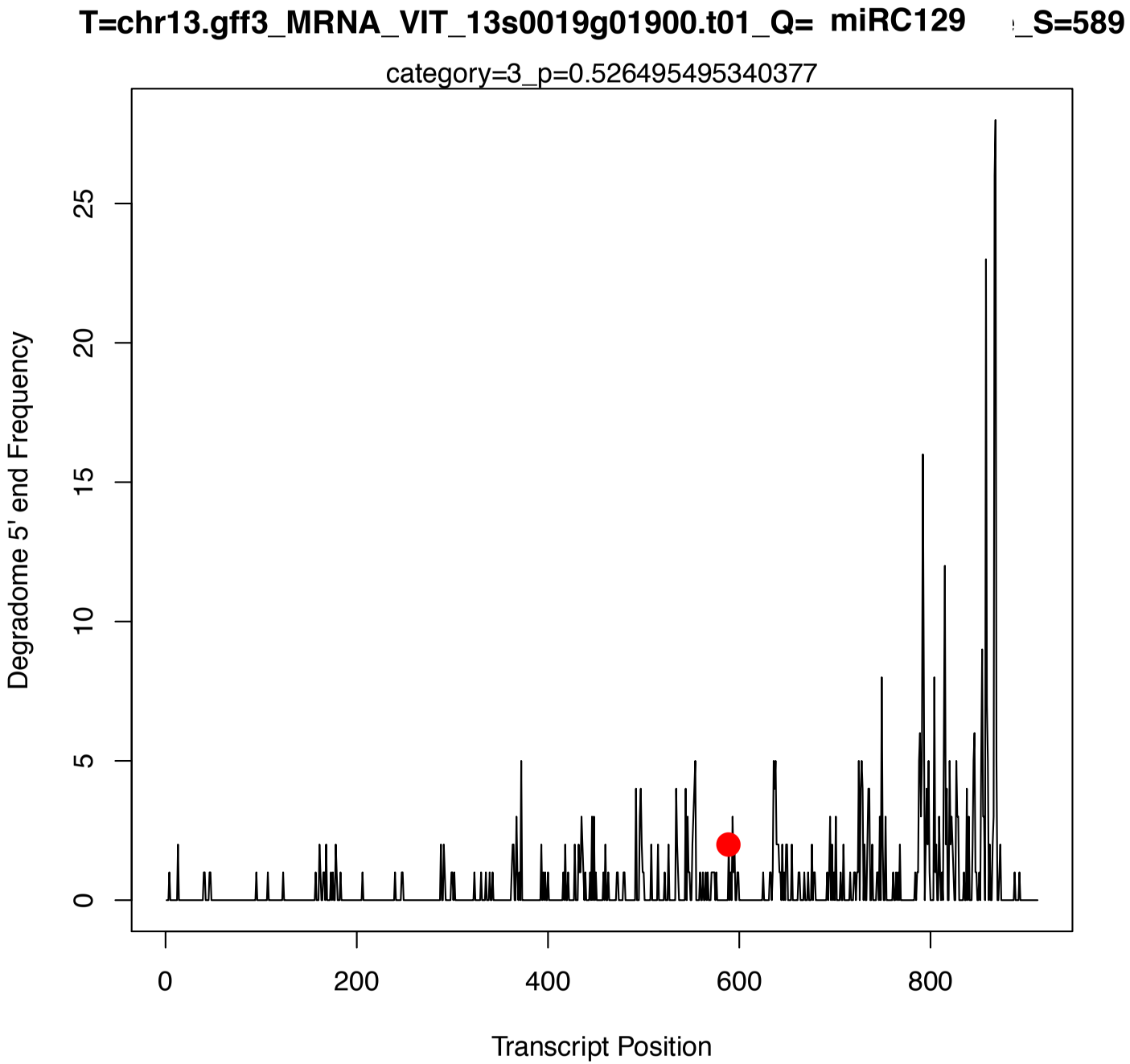
4000
5000
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0019g01900.t01_Q= miRC129 _S=589
category=3_p=0.526495495340377
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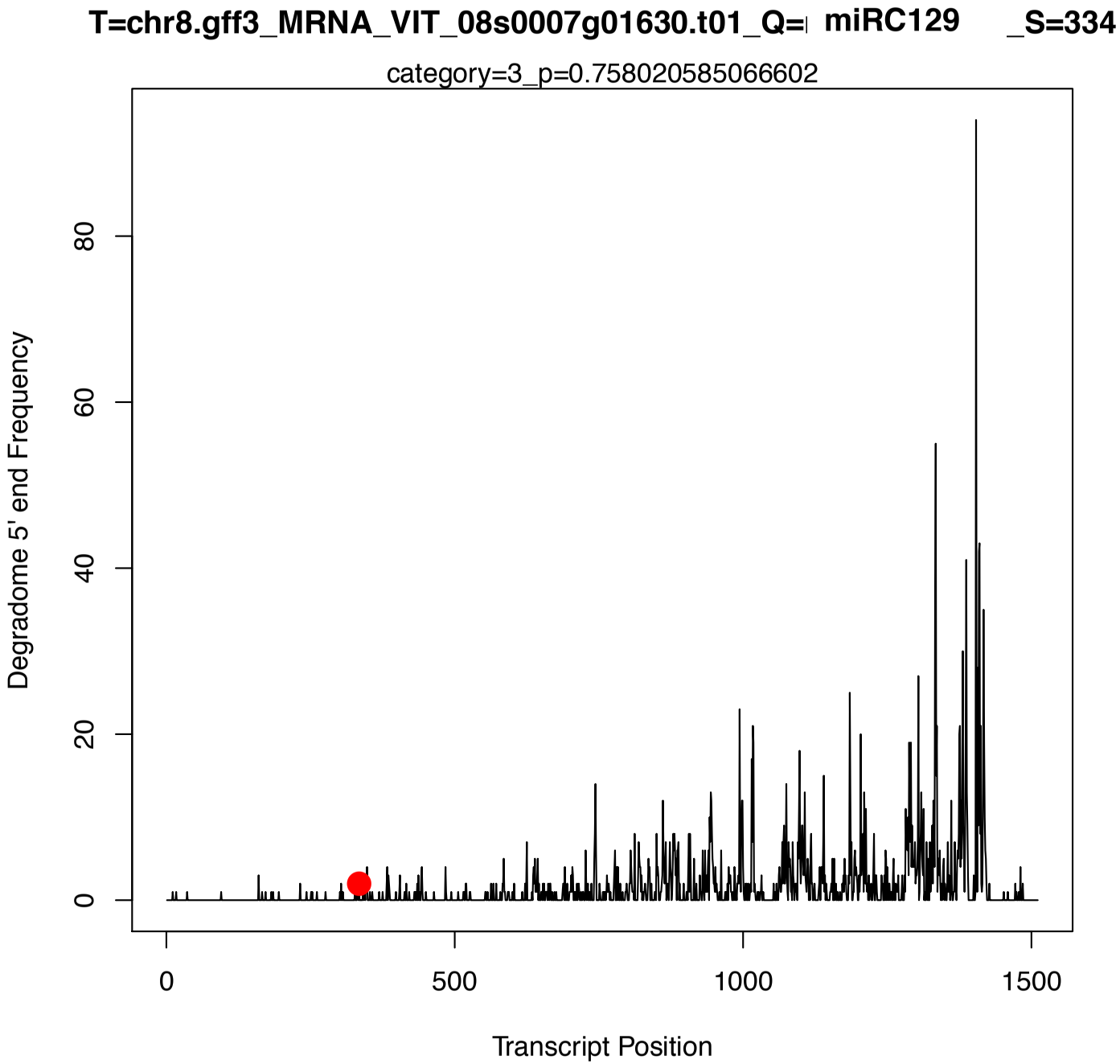
600
800
Transcript Position


T=chr8.gff3_MRNA_VIT_08s0007g01630.t01_Q=I miRC129 _S=334
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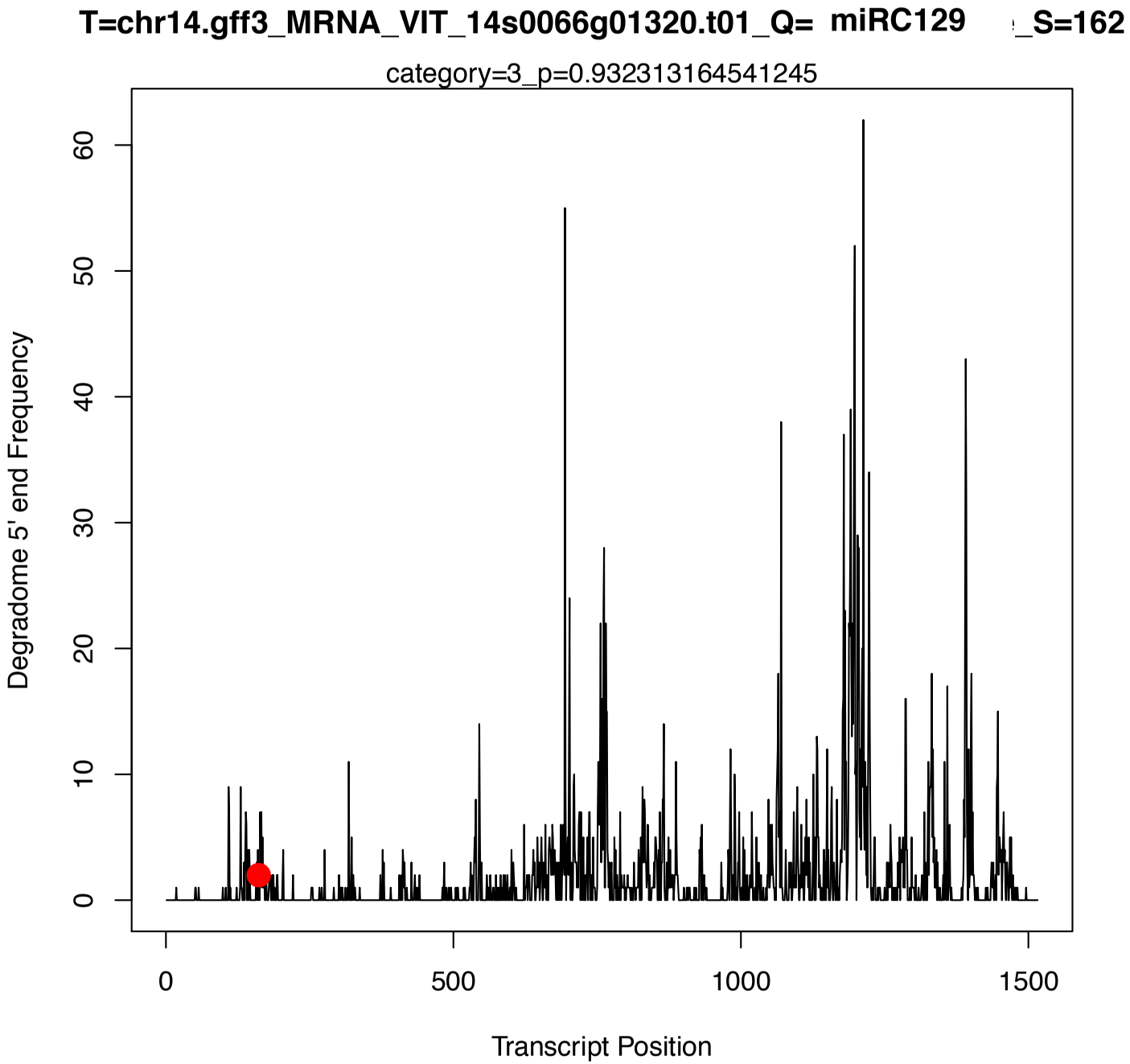
1000
1500
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0066g01320.t01_Q= miRC129 _S=162
category=3_p=0.932313164541245
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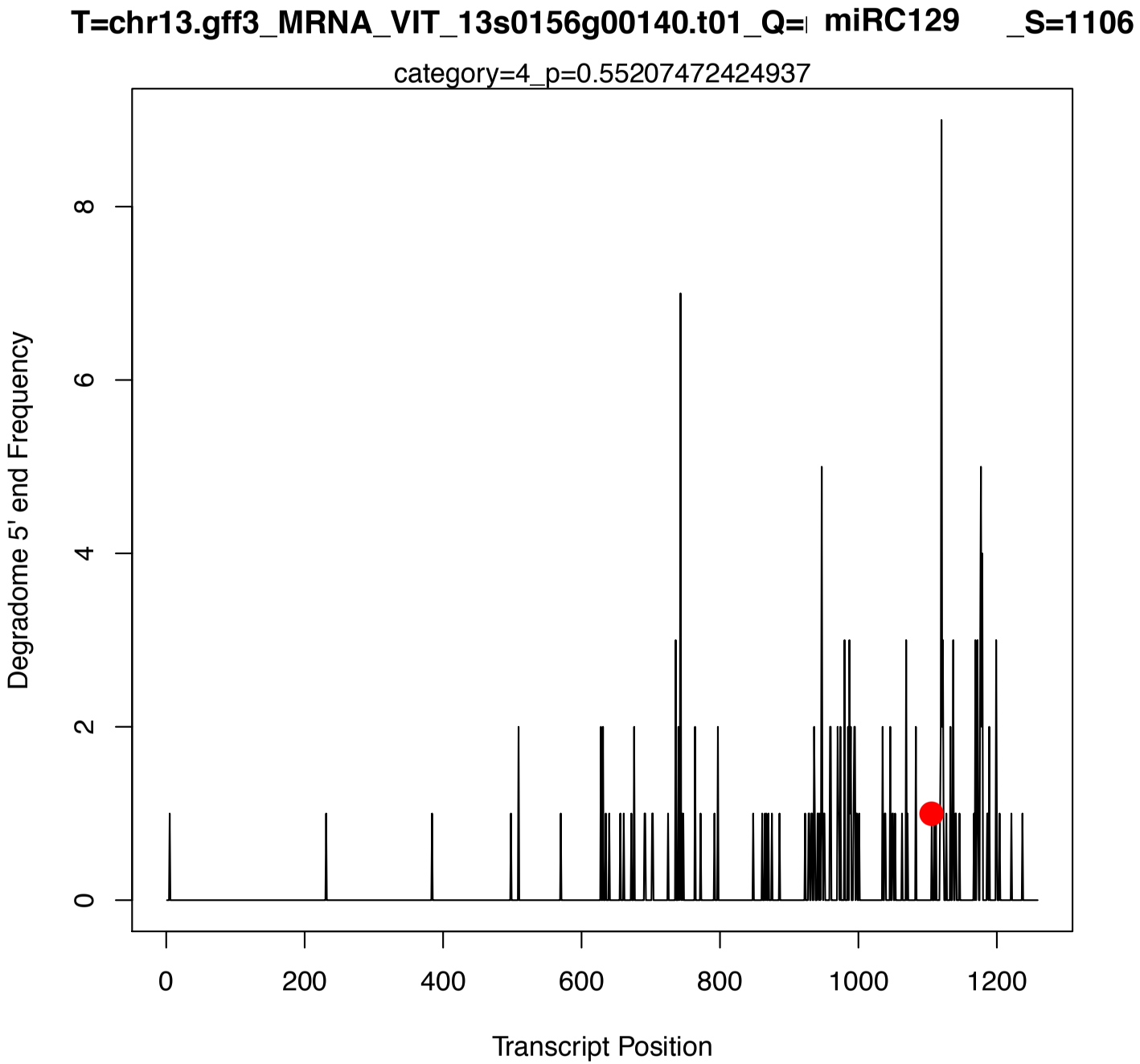
1000
1500
Transcript Position


T=chr13.gff3_MRNA_VIT_13s0156g00140.t01_Q=
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_S=1106
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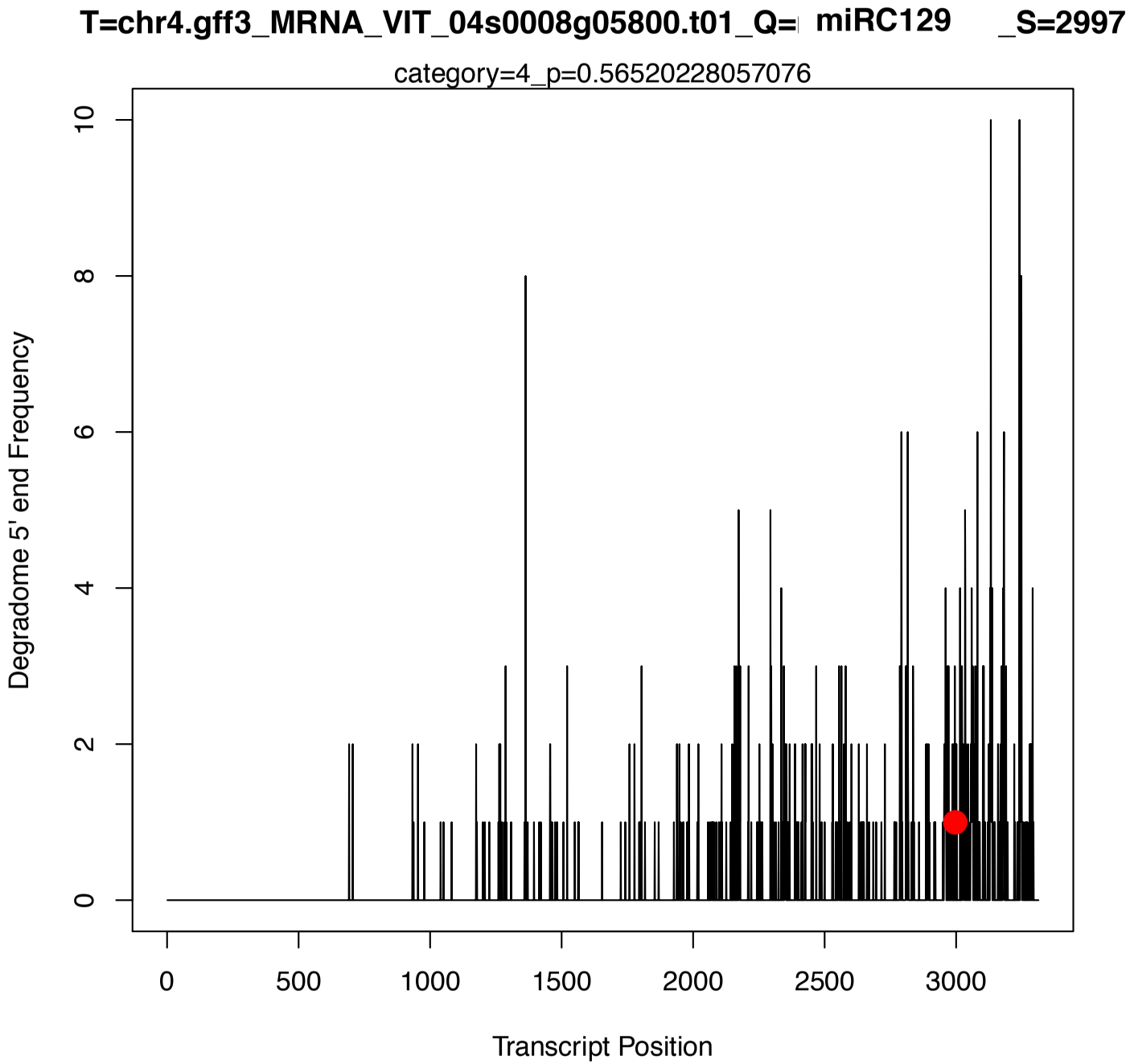
1000
1200
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0008g05800.t01_Q= miRC129 _S=2997
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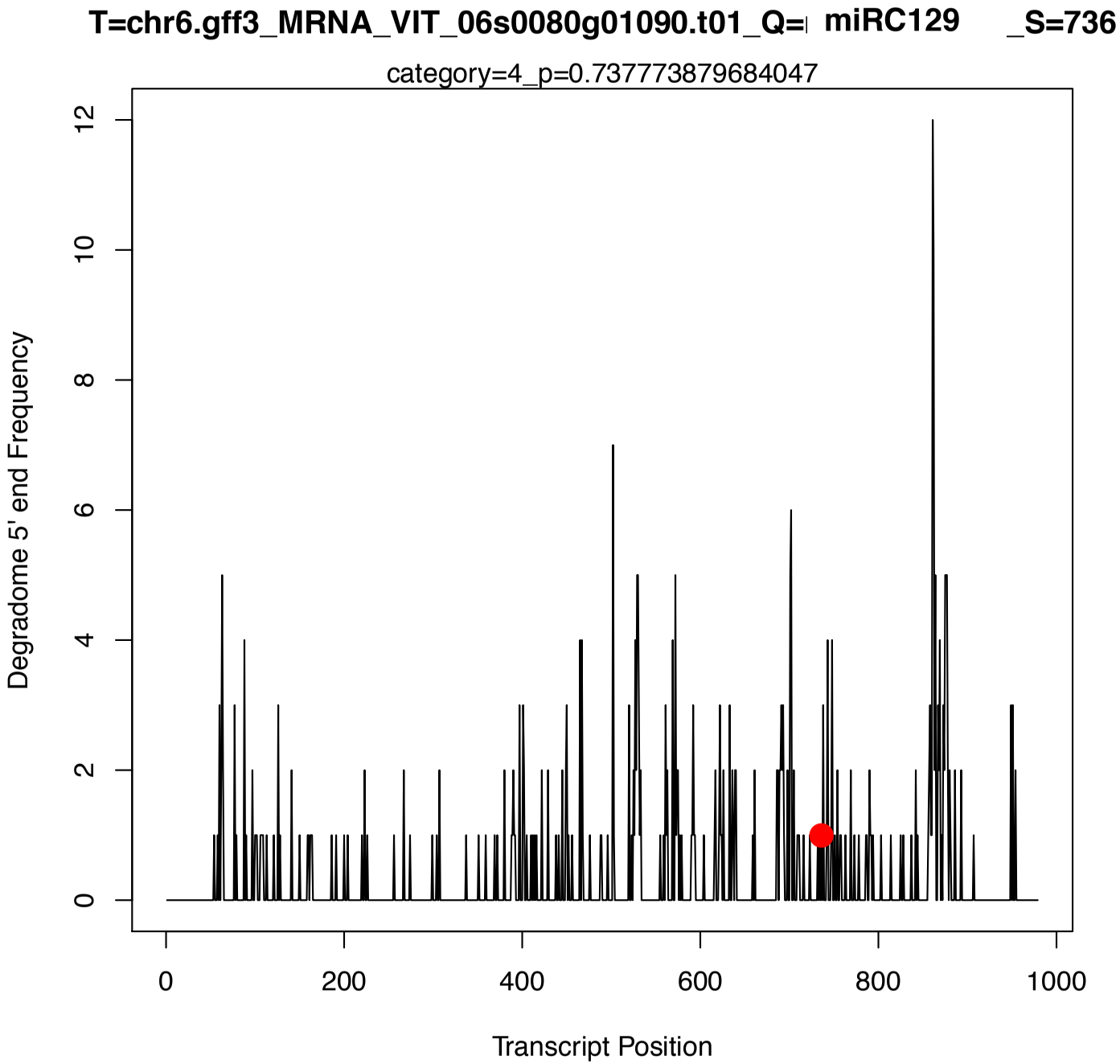
Transcript Position


T=chr6.gff3_MRNA_VIT_06s0080g01090.t01_Q=
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_S=736
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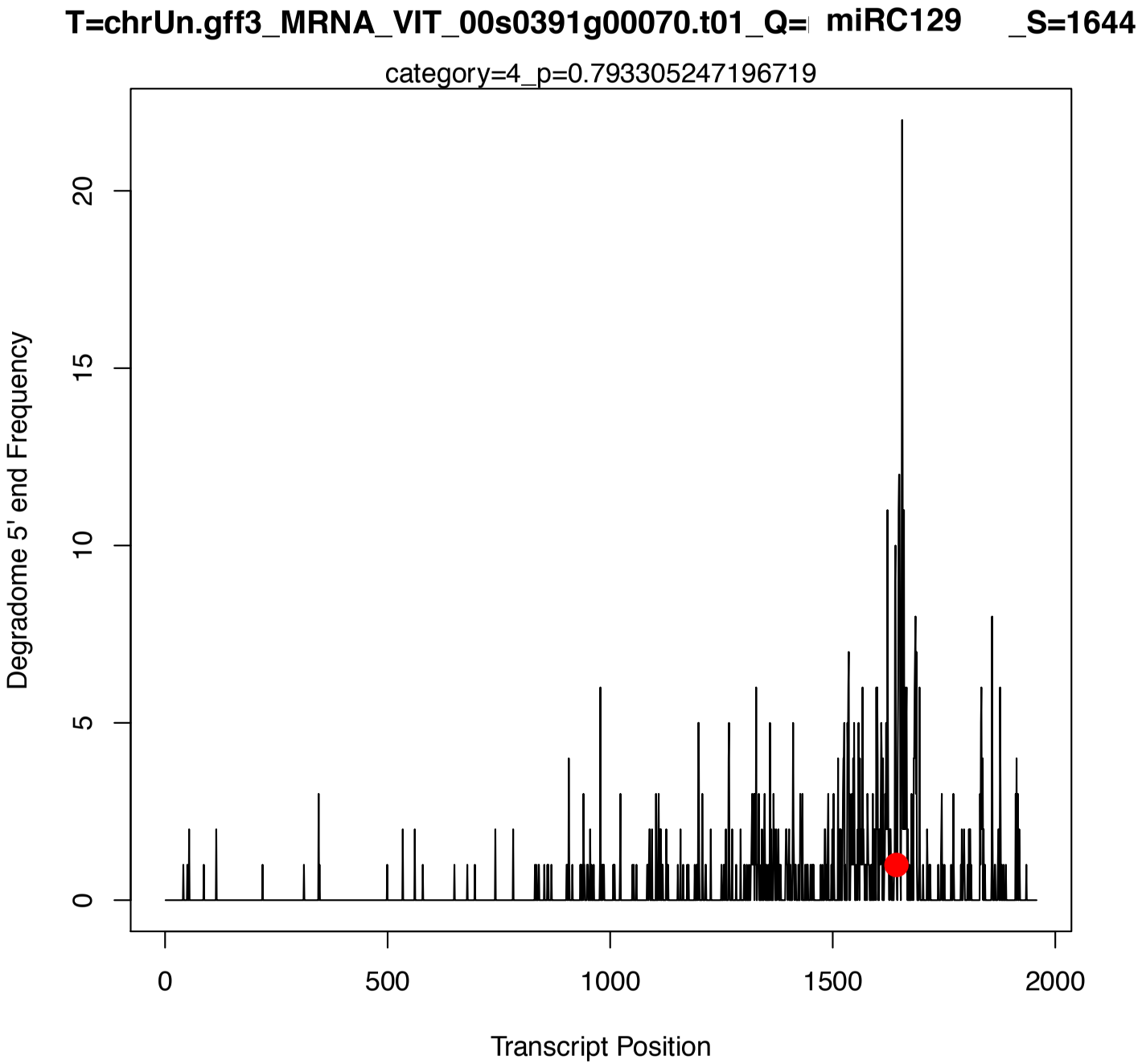
1000
Transcript Position


T=chrUn.gff3_MRNA_VIT_00s0391g00070.t01_Q= miRC129 _S=1644
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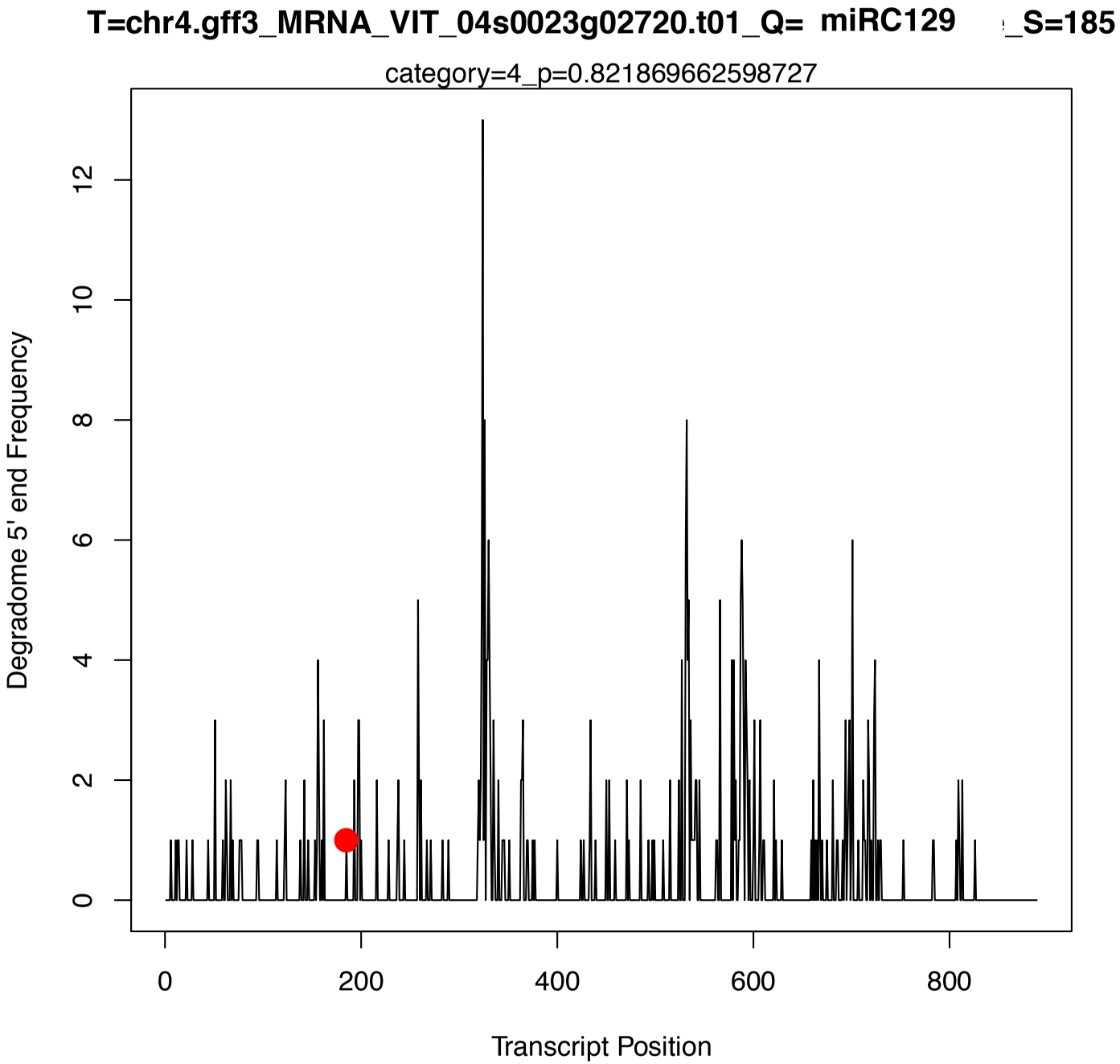
Transcript Position


T=chr4.gff3_MRNA_VIT_04s0023g02720.t01_Q= miRC129 _S=185
category=4_p=0.821869662598727
Degradome 5' end Frequency
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12
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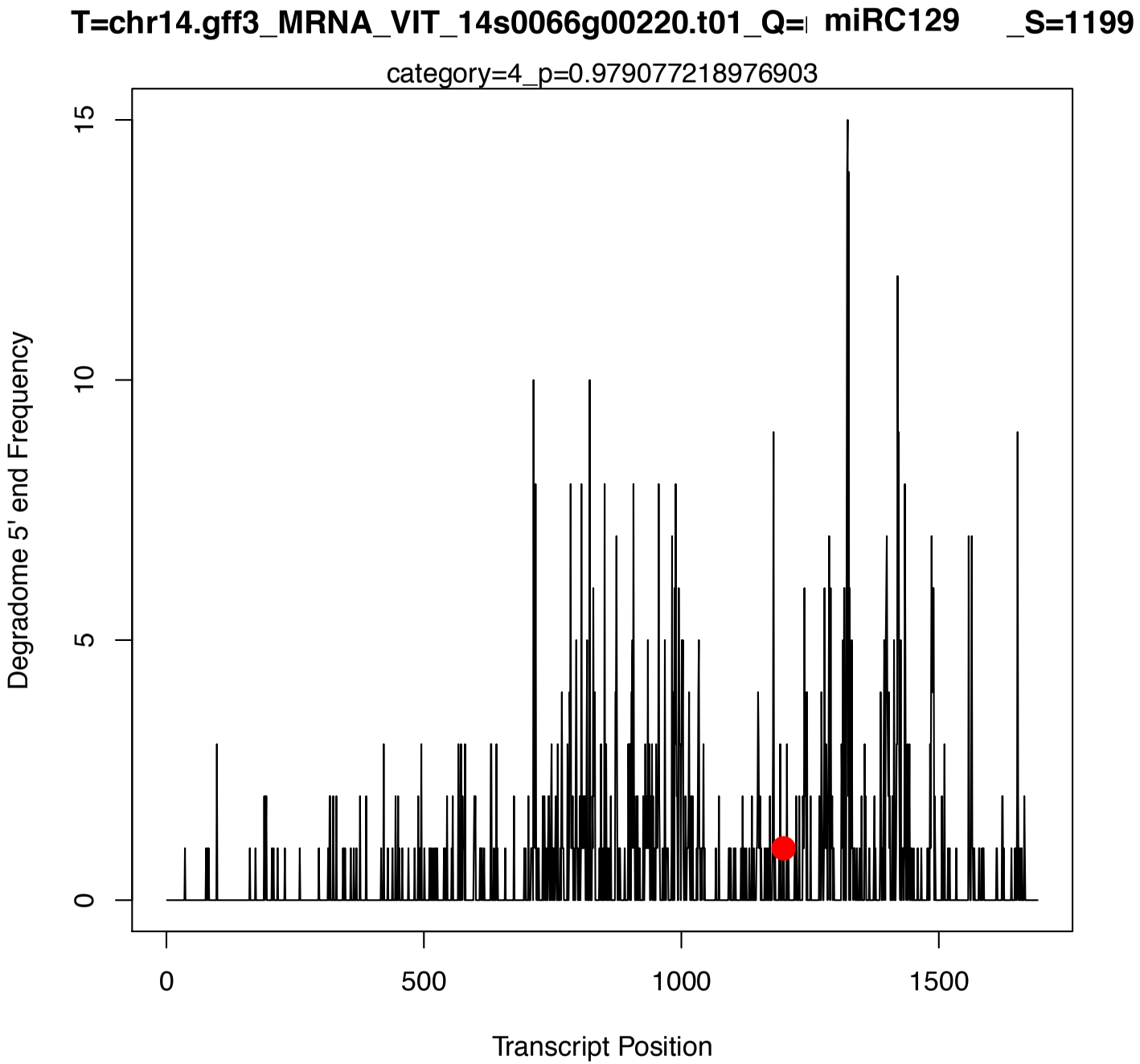
600
800
Transcript Position


T=chr14.gff3_MRNA_VIT_14s0066g00220.t01_Q=
miRC129
_S=1199
category=4_p=0.979077218976903
Degradome 5' end Frequency
0
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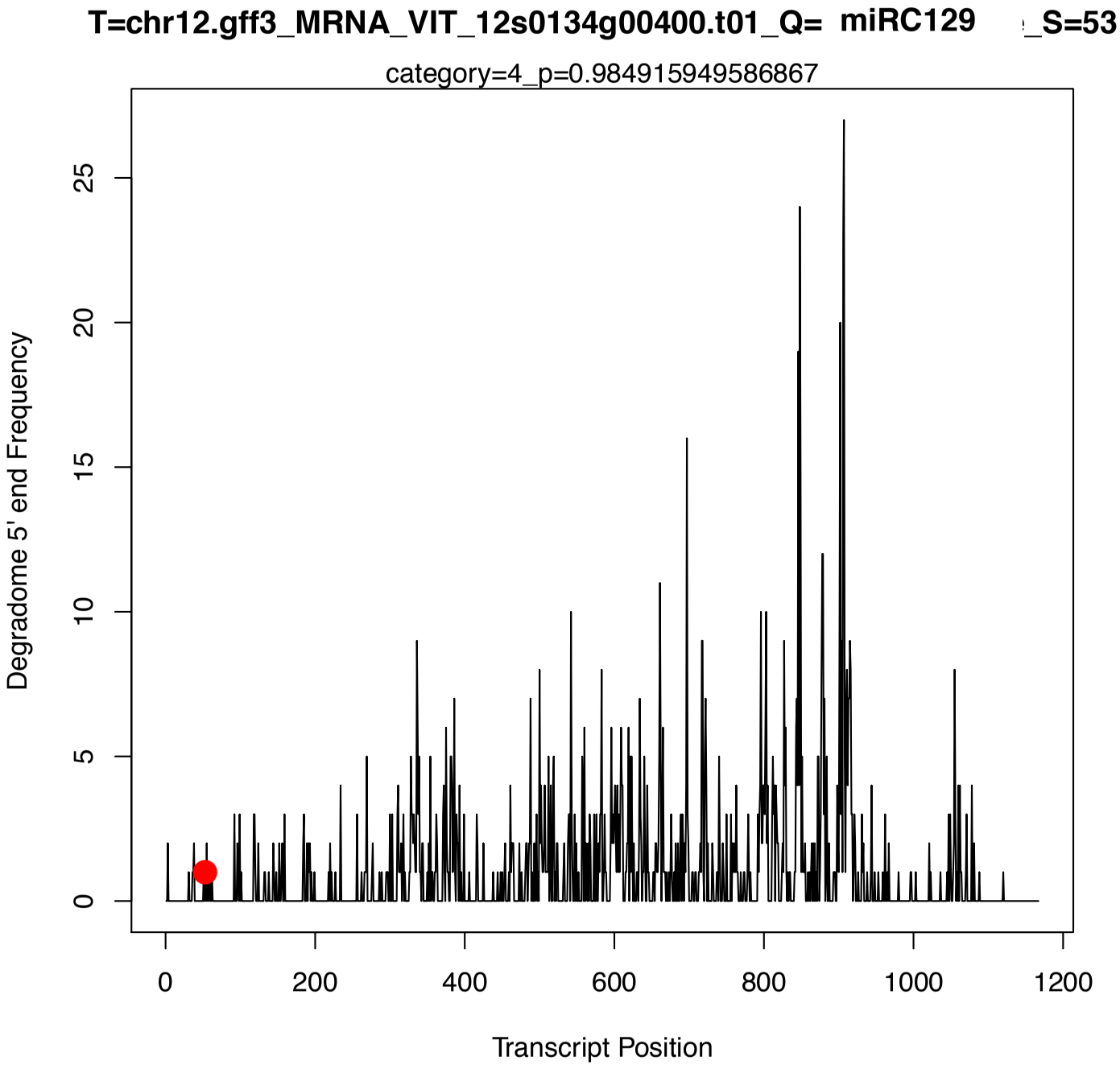
10
15
0
500
1000
1500
Transcript Position


T=chr12.gff3_MRNA_VIT_12s0134g00400.t01_Q= miRC129 _S=53
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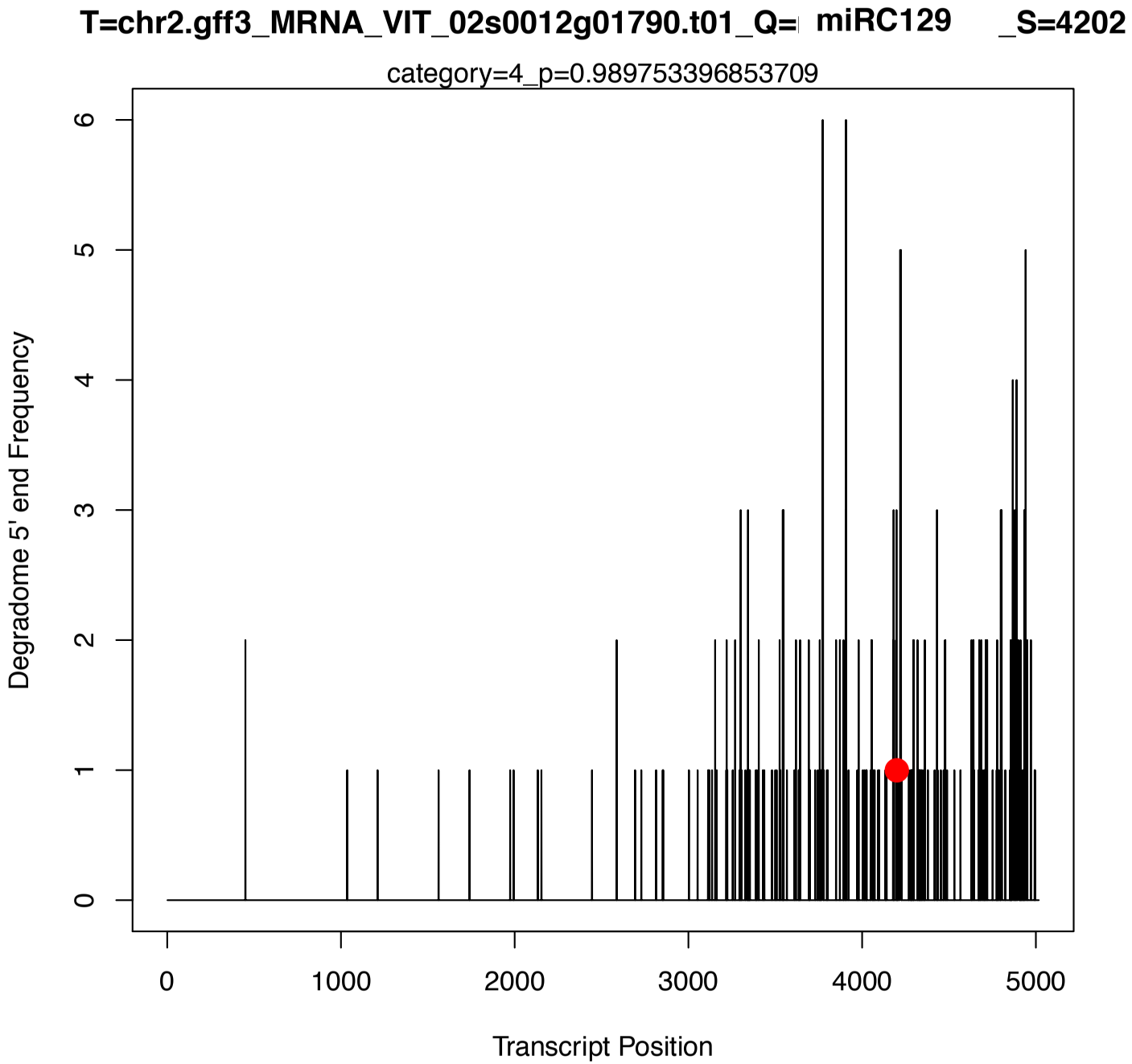
1000
1200
Transcript Position


T=chr2.gff3_MRNA_VIT_02s0012g01790.t01_Q= miRC129 _S=4202
category=4_p=0.989753396853709
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0
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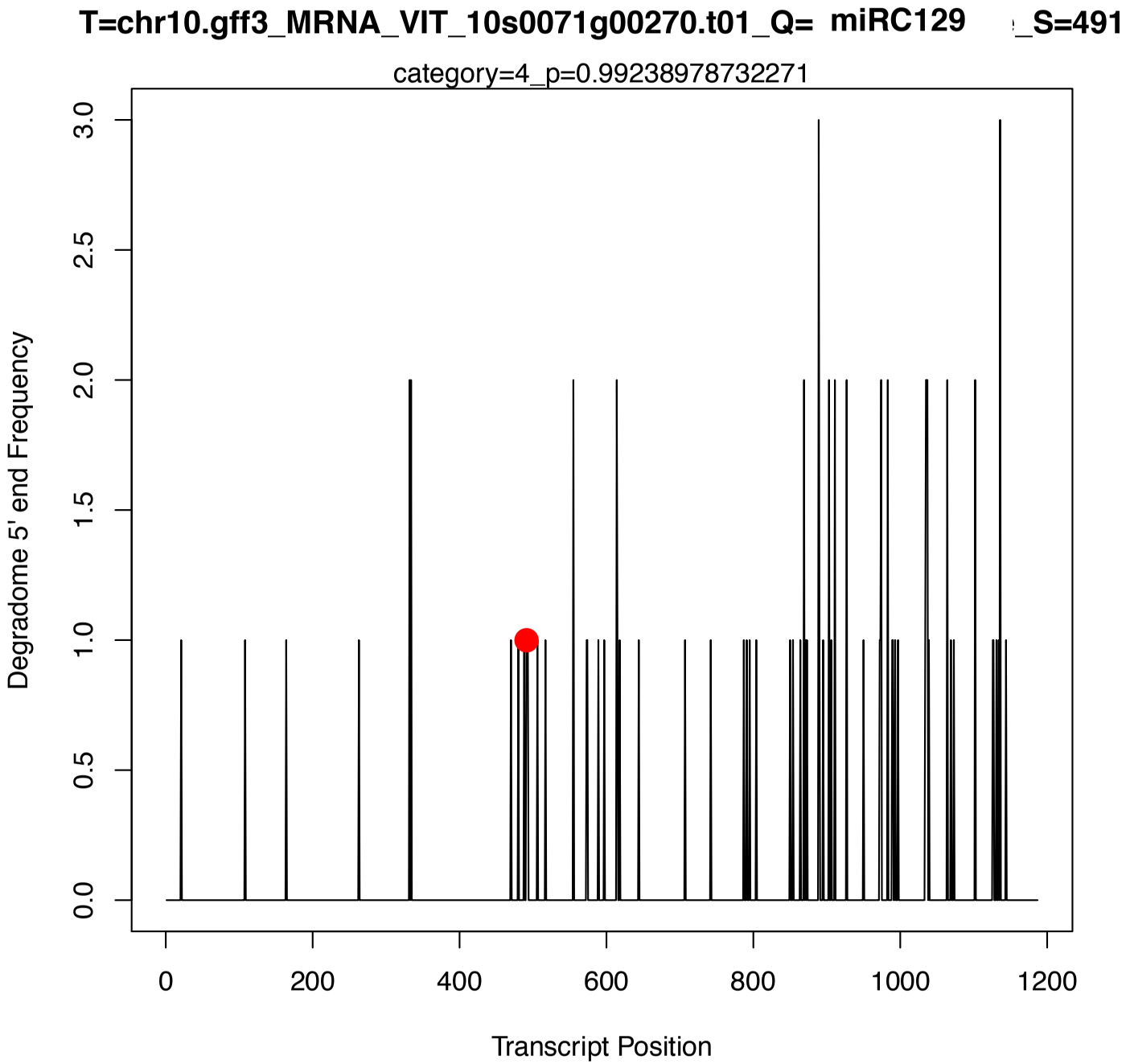
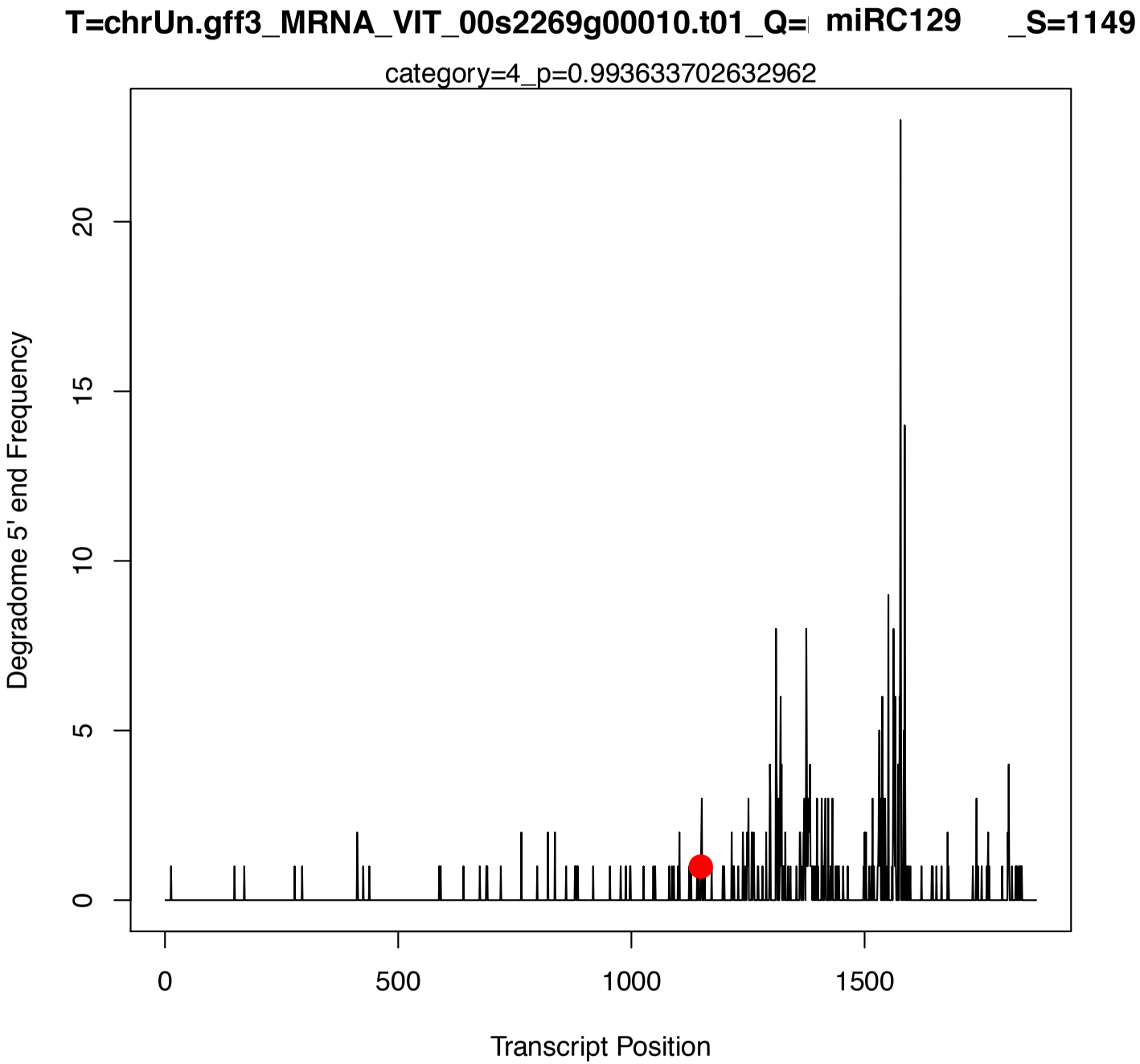
2000
3000
4000
5000
Transcript Position


T=chr10.gff3_MRNA_VIT_10s0071g00270.t01_Q= miRC129 _S=491
category=4_p=0.99238978732271
Degradome 5' end Frequency
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
200
400
600
800
1000
1200
Transcript Position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5
10
15
20
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1000
1500
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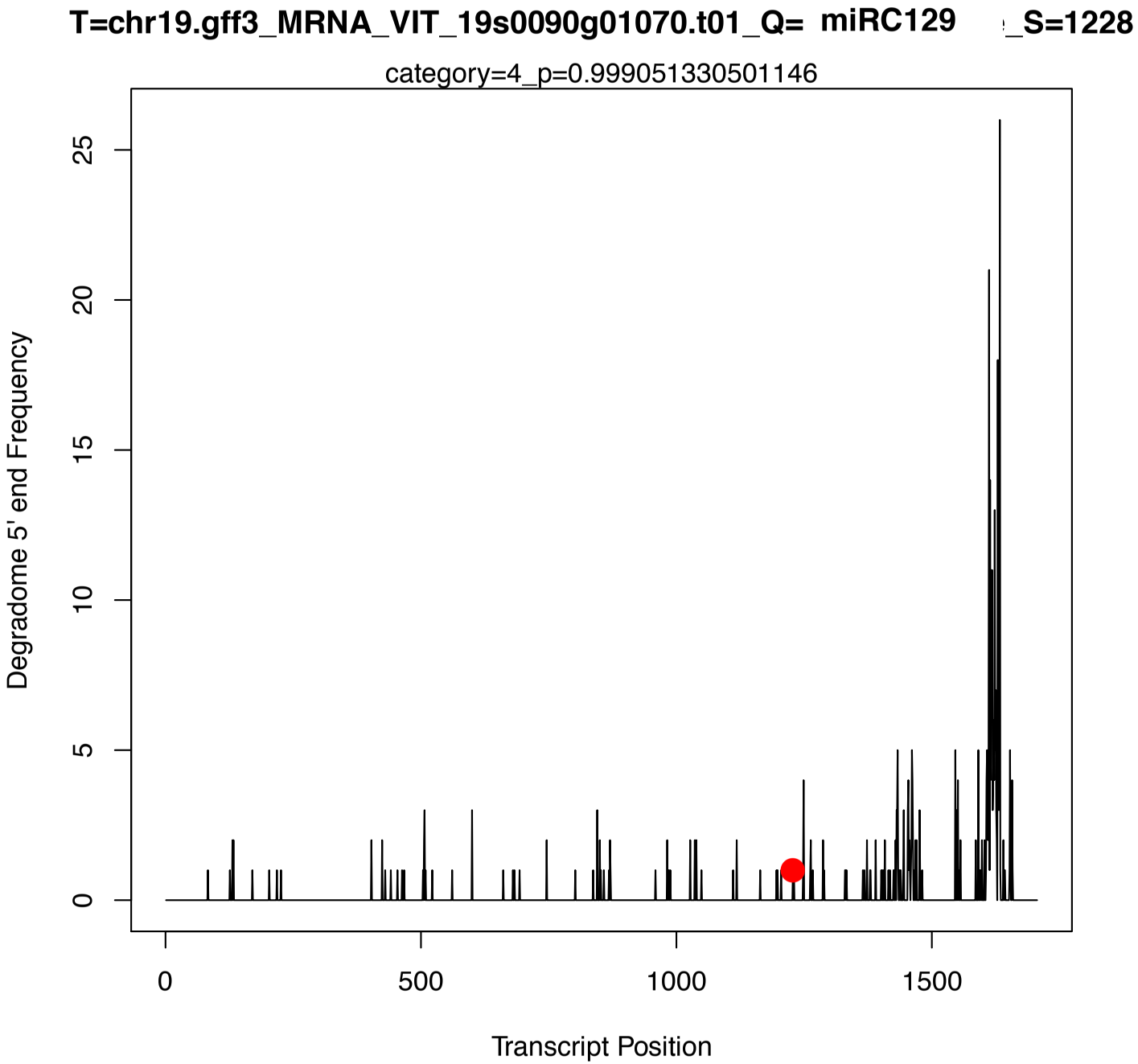
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20
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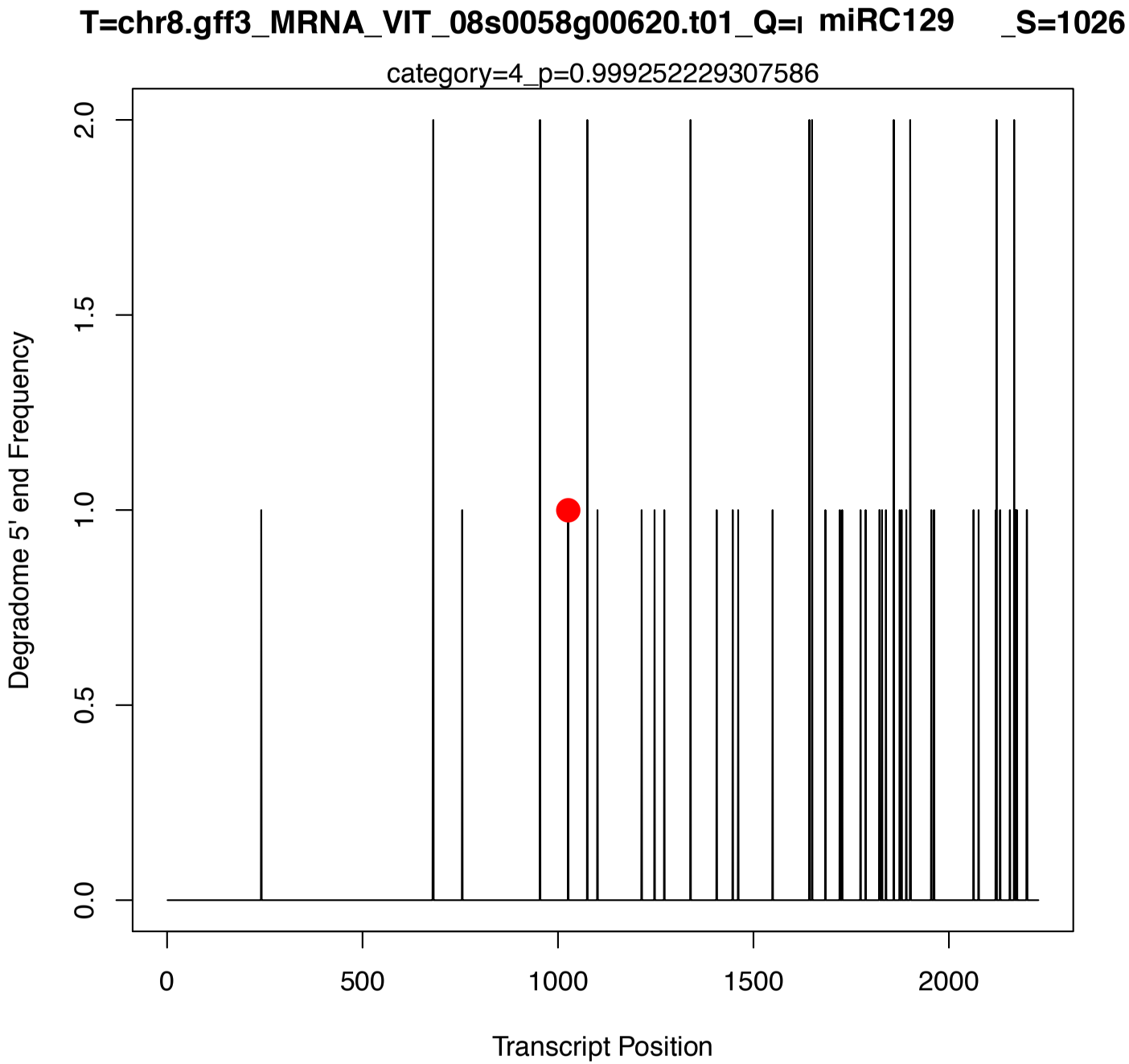
1000
1500
Transcript Position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1.5
2.0
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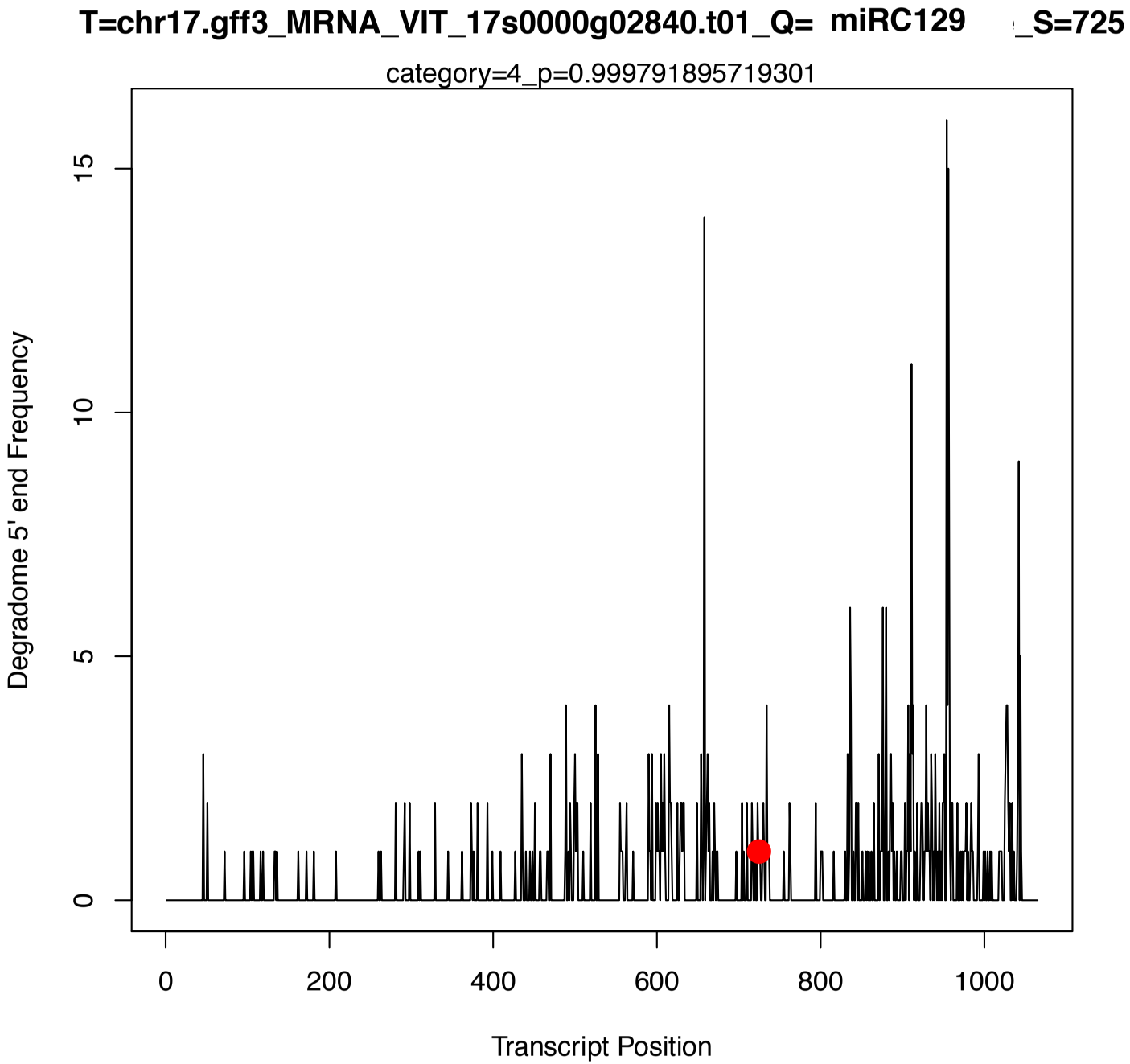
Transcript Position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15
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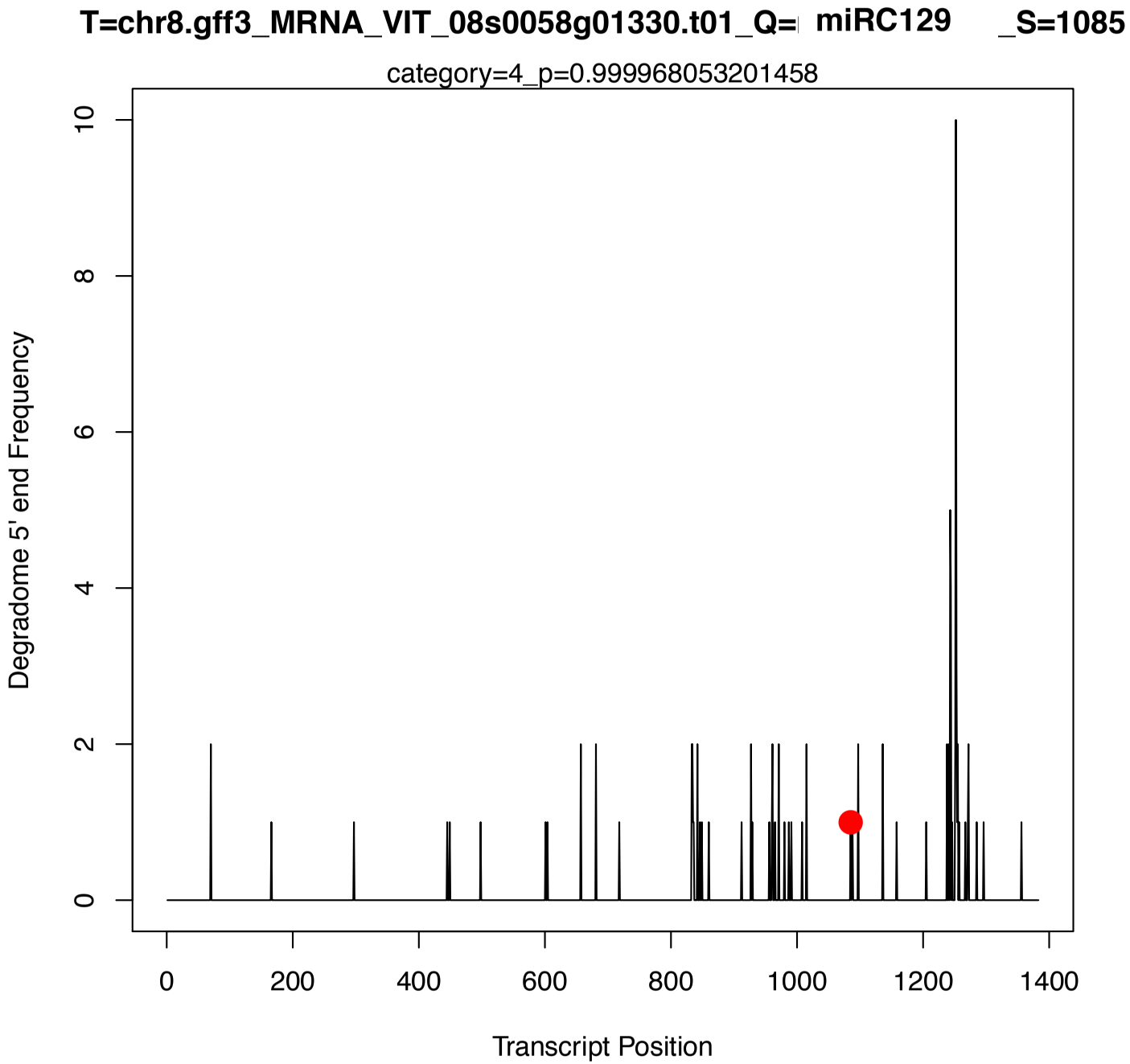
Transcript Position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6
8
10
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400
600
800
1000
1200
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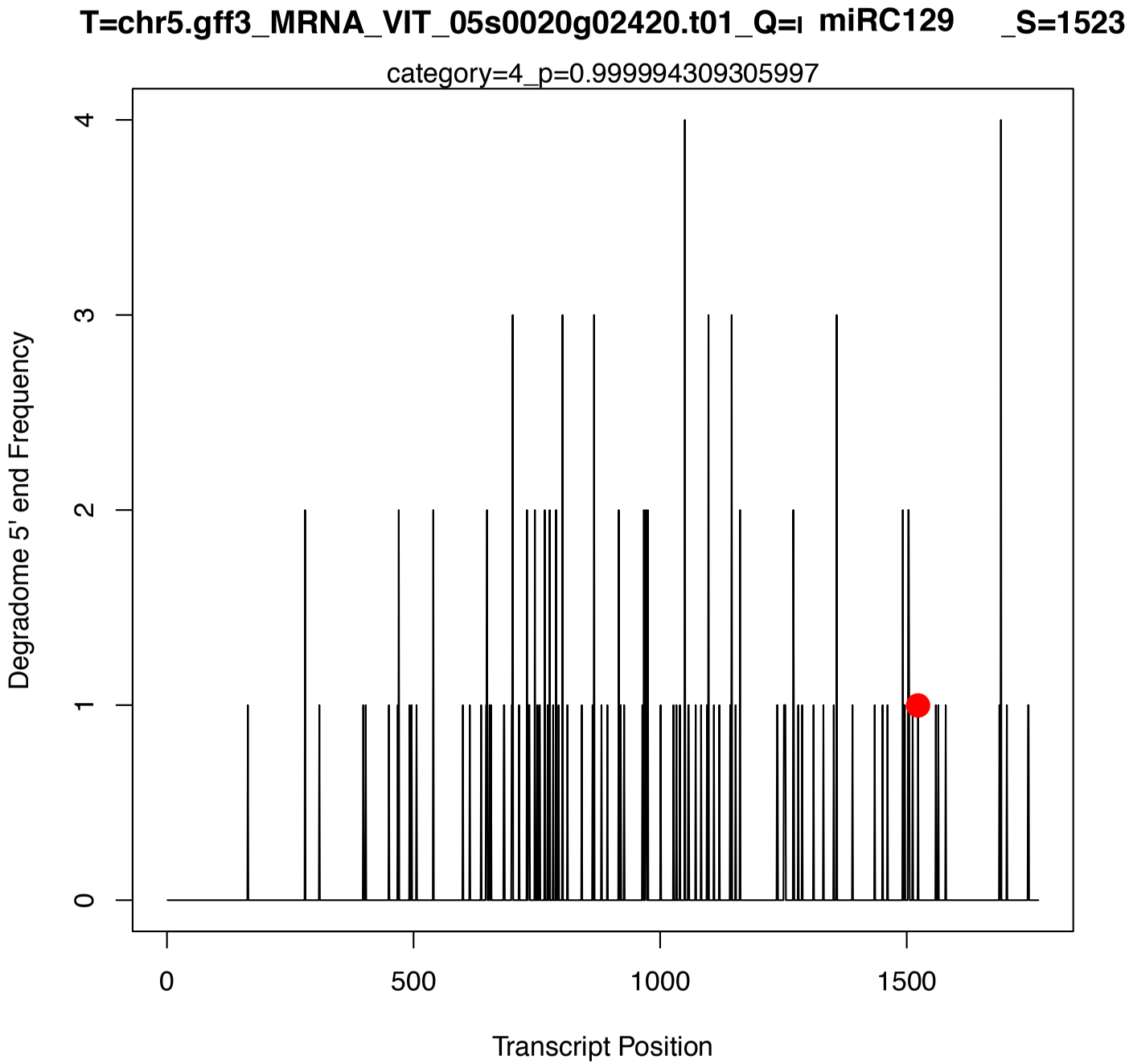
Transcript Position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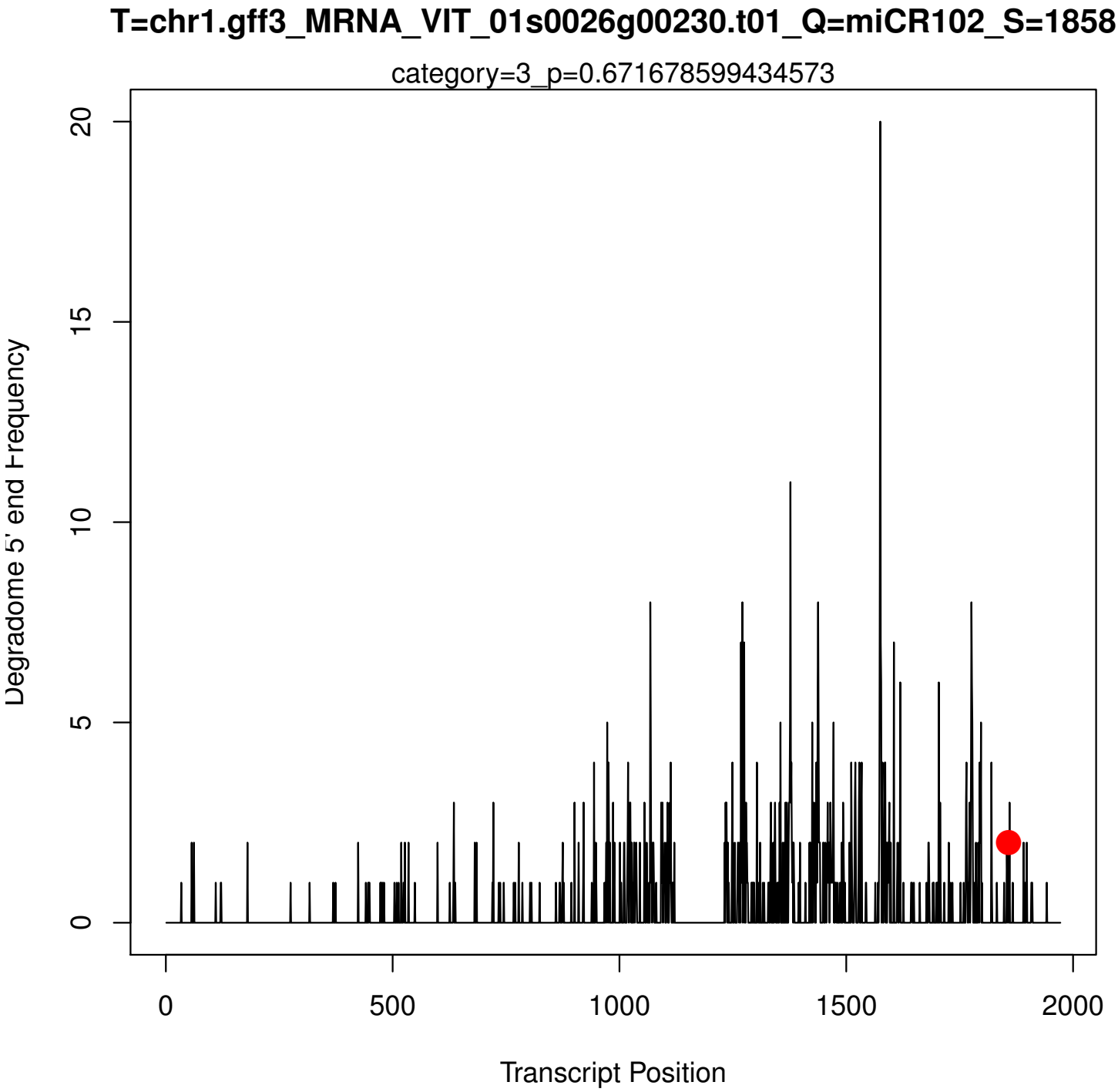
2
3
4
0
500
1000
1500
Transcript Position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Degradome 5' end Frequency
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15
20
0
500
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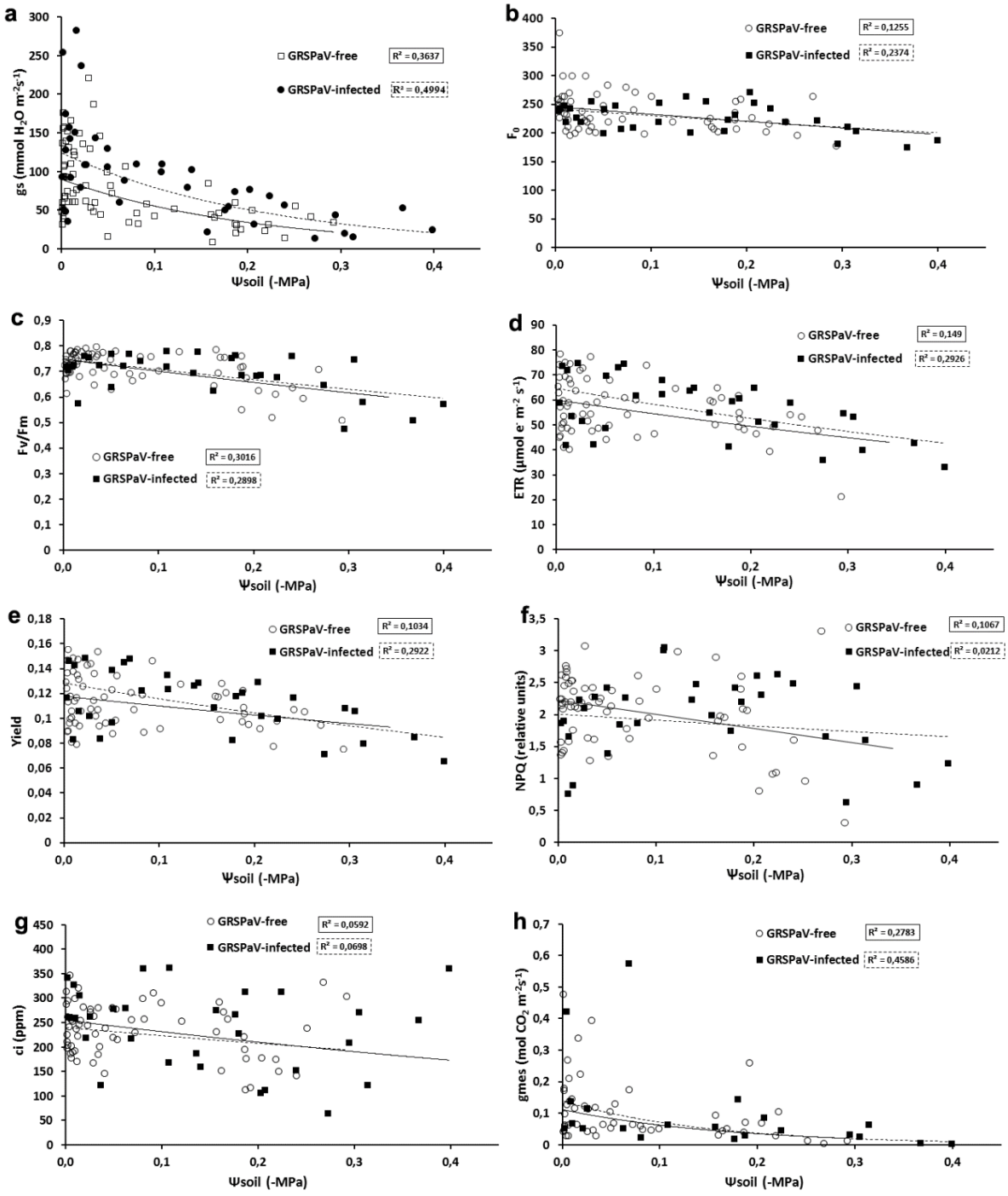
2000
Transcript Position



Supplementary Figure S10. Relationship between soil water potential (Ψsoil) and: a) estimated stomatal conductance (g_s), b) maximum fluorescence in dark adapted leaves (F0), c) maximum quantum efficiency of PSII (Fv/Fm), d) electron transport rate (ETR), e) quantum efficiency of PSII (Yield), f) non-photochemical quenching (NPQ), g) substomatal internal carbon concentration (ci) and h) mesophyll conductance to CO_2 (gmes) in *Grapevine rupestris stem pitting-associated* (GRSPaV)-infected (filled squares, dashed trend line) and GRSPaV-free (open circles, continuous trend line) plants.