Supporting Information

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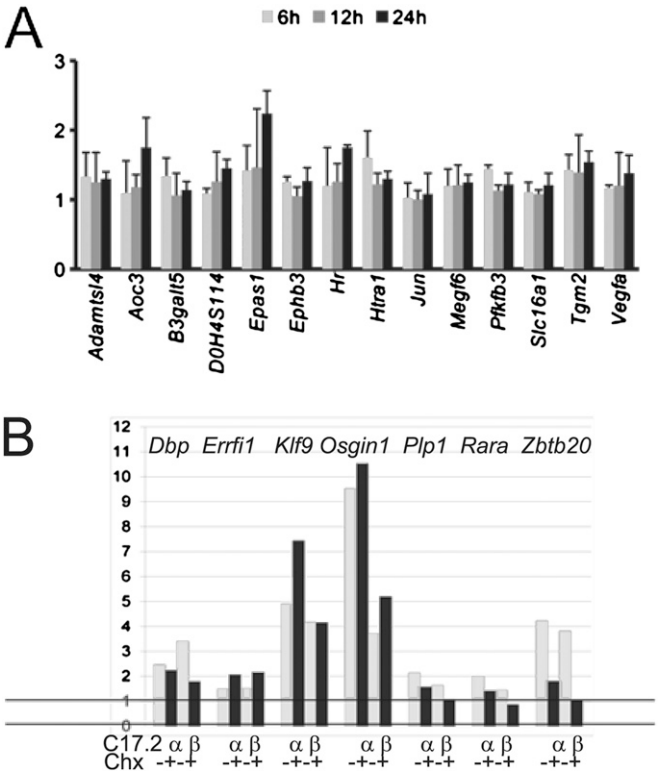

Fig. S1. Absence of response without exogenous TR and effect of cycloheximide. (*A*) Time-course analysis [0, 6, 12, and 24 h after 3,3′,5-triiodo-L-thyronine (T3) treatment] of gene expression in C17.2. Quantitative (q)PCR indicates minimal effect of T3, compared with that in Fig. 3. (*B*) Induction by T3 (6 h) in the presence of cycloheximide, a translation inhibitor. The drug has no effect if genes are directly regulated by liganded TR. The observed effect for *Zbtb20* is an indication that T3-mediated induction of this gene is a secondary event and that this gene is not a direct TR target.

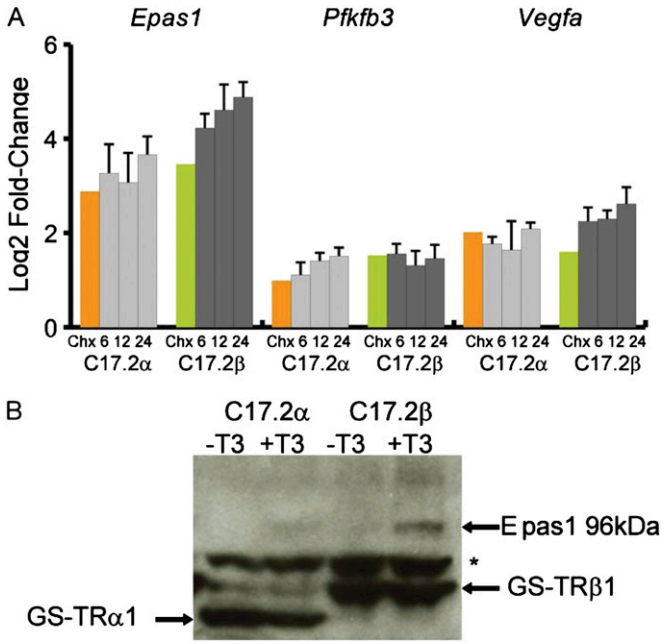

Fig. S2. Activation by T3 of hypoxia pathway genes in C17.2α and C17.2β cells. (*A*) Quantitative (Q)-RT-PCR analysis of *Epas1*, *Pfkfb3*, and *Vegfa* expression after 6, 12, or 24 h of T3 stimulation (light grey: C17.2α, dark grey: C17.2β). The presence of cycloheximide has no significant effect on the response at 6 h (orange: C17.2α, green: C17.2β), suggesting that the three genes can be directly regulated by T3. *Pfkfb3* and *Vegfa* are, however, thought to be EPAS1 target genes and to provide a link between EPAS1, glucose metabolism, and vasculogenesis. (*B*) Western blot analysis of EPAS1 in C17.2α and C17.2β cells after 24 h of T3 stimulation. The antibody identifies the EPAS1 protein in T3-stimulated cells only. Due to cross-reactivity with the GS-derived tag, the antibody also recognizes GS-TRα1 and GS- TRβ1. Asterisk indicates a nonspecific band.

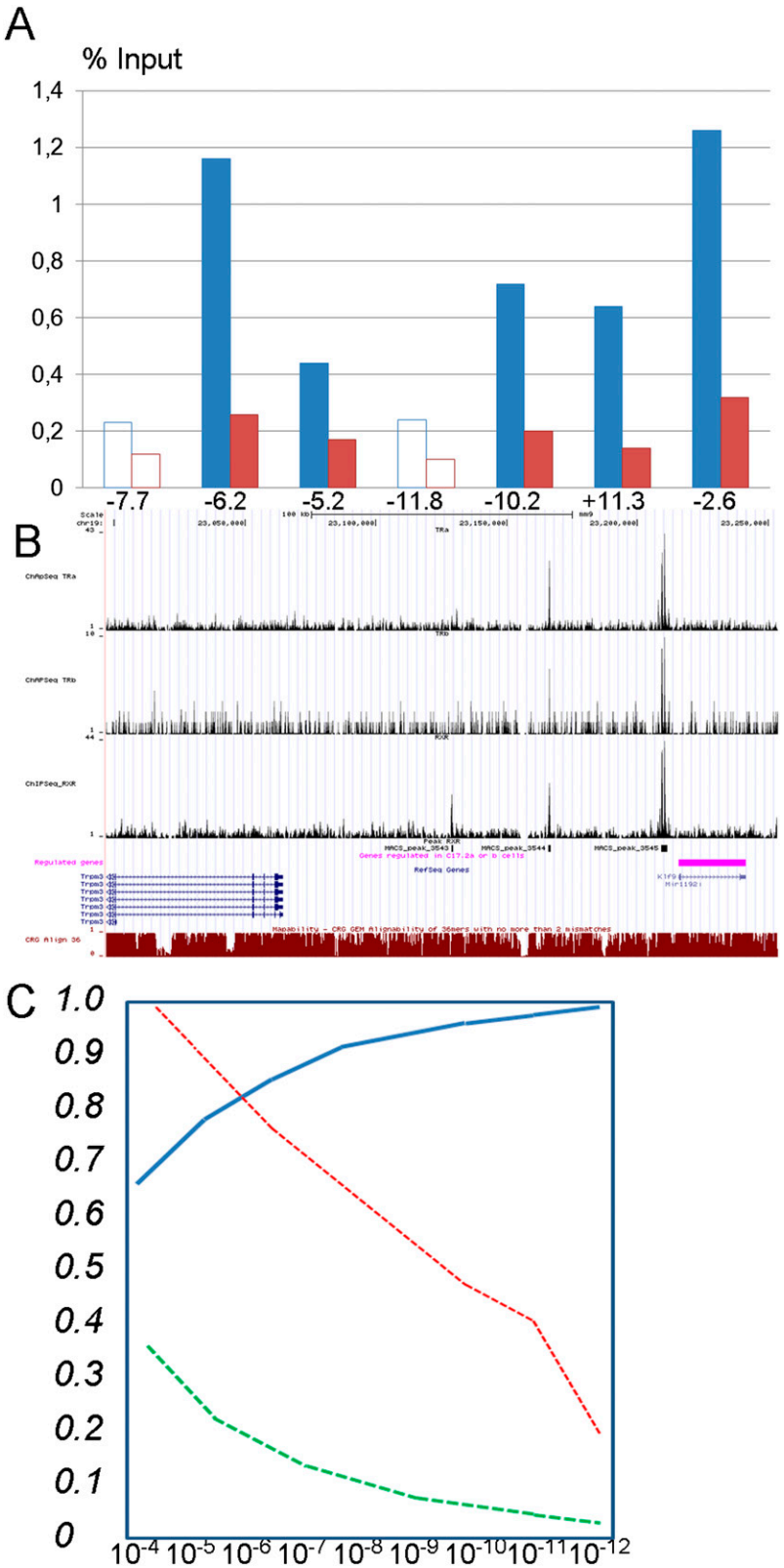

Fig. S3. Retinoid X receptor (RXR) chromatin immunoprecipitation (ChIP)-Seq in C17.2α cells. (*A*) Chromatin immunoprecipitation in C17.2α cells with a RXR antibody. Cross-linked chromatin was incubated first with an anti-RXR antibody and then with IgA-coupled beads. Blue columns represents the percentage of input observed after chromatin immunoprecipitation with a RXR antibody. A small but significant enrichment (red columns) was also observed at the TR/RXR binding site when the RXR antibody is omitted, due to the cross-reaction between the IgA-coupled beads and the protein-G–derived tag present on TR. Open columns are control regions without TR binding, used to evaluate background contamination. (*B*) Example of ChIP-Seq result. Some binding sites are different from the one obtained after TR chromatin affinity purification (ChAP)-Seq and thus correspond to complexes containing RXR but not TR. (*C*) Influence of P-value threshold. *x* axis is the P value calculated by the MACS algorithm for peak calling. The *y* axis represent various ratios. The blue line represents the ratio

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of TRBS detected in the TRα1 ChAP-Seq experiment that overlap with the RXR binding sites detected by ChIP-Seq. As explained in the text, IgG/protein-A interaction introduces a contamination of the RXR ChIP-Seq by GS-TRα1–containing complexes, and 100% of overlap is thus expected. On the basis of this assumption we calculated the false discovery rate for TRα1 binding sites (green dashed line). The discovery rate (red dashed line) was calculated for the peaks that overlap between the TRα1 ChAP-Seq dataset and the RXR ChIP-Seq dataset, with a P value $<10^{-4}$ in each experiment. Comparison with Fig. 5 confirms the existence of TRβ1-specific peaks.

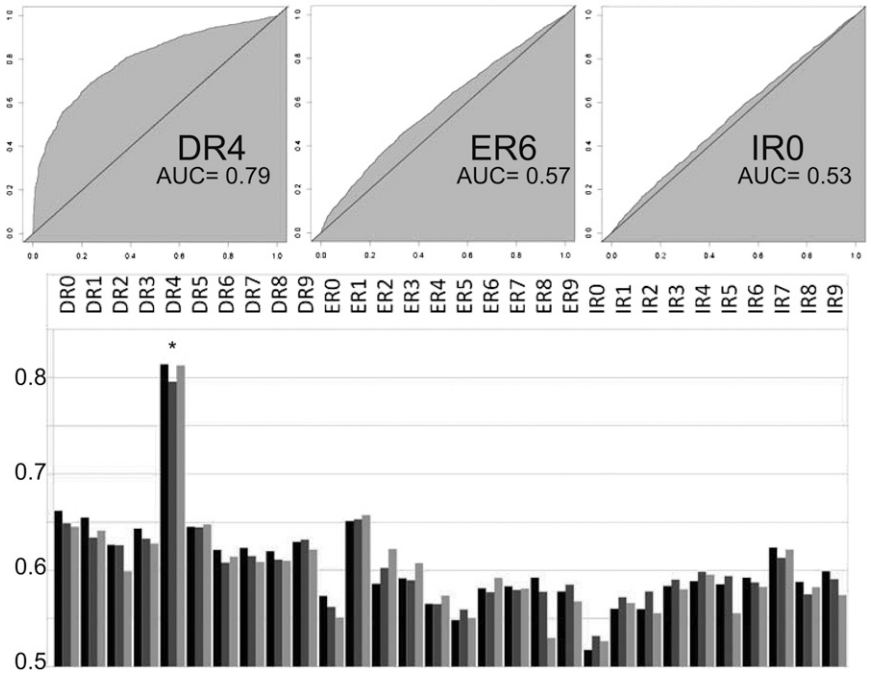

Fig. S4. ROC curves analysis. Enrichment in the various types of response elements was assessed by comparing the frequency of discovery of the consensus in the TRBS (*y* axis) and in a set of randomly generated sequences (same size, same GC content, *x* axis) for a changing score threshold. In absence of enrichment, the area under the curve (AUC) is close to 0.5. (*Upper*) The ROC curve for DR4, ER6, and IR0 elements is presented for all TRBS, confirming that only DR4 elements are overrepresented in TRBS. (*Lower*) The histogram provides all AUCs obtained when the analysis is performed for three groups of TRBS: all TRBS (solid bars),TRα-specific TRBS (bars with dark shading), and TRBS proximal to T3-regulated genes (bars with light shading). As only 240 TRβ1-specific TRBS were detected, this small set of TRBS was not included in the analysis. DR4 is the only arrangement for which the AUC clearly stands out. The enrichment values are not different for the three categories of TRBS that are considered.

Table S1. Digital gene expression libraries

Sample	Total beads, $\times 10^7$	Total counted, $\times 10^7$	Total match unique, $\times 10^7$	Total match multiple, $\times 10^6$
C17.2α, 6 h, −T3	2.06	1.29	1.09	2.07
C17.2α, 6 h, +T3	1.53	1.01	0.81	1.88
C17.2α, 12 h, −T3	1.65	1.13	0.89	2.35
C17.2α, 12 h, +T3	2.18	1.41	1.13	2.81
C17.2α, 24 h, −T3	1.69	0.98	0.92	0.59
C17.2α, 24 h, +T3	1.43	0.83	0.78	0.52
C17.2β, 6 h, −T3	2.89	1.91	1.54	3.66
C17.2β, 6 h, +T3	1.39	0.94	0.75	1.85
C17.2β, 12 h, −T3	1.67	1.12	0.90	2.09
C17.2β, 12 h, +T3	1.72	1.13	0.93	1.97
C17.2β, 24 h, −T3	1.27	0.73	0.67	0.62
C17.2β, 24 h, +T3	1.24	0.71	0.65	0.59

Total beads, number of beads with a single tag; total counted, number of sequenced tags matching a RefSeq transcript with at most one mismatch; unique/multiple indicates the number of tags allocated to a unique transcript or to several transcripts.

Table S2. Chromatin affinity/immunoprecipitation high-throughput sequencing libraries

Sample	Total quality filtered reads, $\times 10^7$	Total reads aligned, $\times 10^7$	Total unique match, $\times 10^7$
ChAP C17.2α	3.14	2.03	1.98
ChAP C17.2β	2.85	1.98	1.66
ChIP RXR	3.08	1.66	1.60

Total quality filtered reads, number of reads that passed the sequencing quality controls; total reads aligned, number of reads aligned on the mm9 mouse genome; total unique match, number of reads that match only one genomic location (no repeat sequence).

Table S3. Transactivation properties of individual TRBS

Binding site	Chr	Summit position	DR4-like sequence (similarity)	Gene (distance to TSS, kb)	T3 induction rate of gene, 12 h		Transactivation in pGL2-TRBS-luc by	
					C17.2α	C17.2β	TRα1	TRβ1
A-TRBS-5973	7	52,963,226	CCGGGTCAGCCGAGGACAC (0.97)	*Dbp* (+2.6)	1.3	2.1	1.42	4.78
A-TRBS-3567	19	23,209,439	CGGGGTTATGCGAGGTAAC (0.89)	*Klf9* (−6.2)	2.0	3.9	1.24	1.47
A-TRBS-3568	19	23,210,491	CAGGTTCATTTGAGGACAG (0.92)	*Klf9* (−5.2)	2.0	3.9		
B-TRBS-6508	4	94,719,071	No DR4	*Jun* (−0.1)	0.4	0.4	0.19	0.53
A-TRBS-4394	3	95,480,909	ATTGGTCATCTGAGGACAG (1.0)	*Adamtsl4* (−10.9)	0.8	6.0	0.95	1.87
A-TRBS-6181	7	138,112,268	No DR4	*Htra1* (+32.5)	1.9	3.0	8.02	9.72
A-TRBS-6182	7	138,121,594	TAAGGTCACCTGAGGCCGG (0.85)	*Htra1* (+41.9)	1.9	3.0	0.36	1.05
A-TRBS-3280	17	87,268,845	CGGGGTGACCTGGGGCCAC (0.98)	*Epas1* (+115.6)	21.4	25.9	0.19	0.42
pGL2-DR4-Luc	NA	NA	AGGTCANNNNAGGTCA (1.0)	NA	NA	NA	104	85
pGL2-Luc	NA	NA	NA	NA	NA	NA	0.26	0.37

Data on T3 induction are from Table 2. The last two columns report assays performed in HEK293 cells after insertion of TRBS-containing fragments in the pGL2-Luciferase reporter vector. For *Klf9*, the two TRBS were inserted together. Underlined bases indicate the TRBS consensus half-sites. The indicated consensus DR4 was used as a positive control. NA: not applicable.

Table S4. Genes with a proximal TRBS overlapping a CTCF binding site

Gene symbol	Chr	Cluster	Binding site(s) name(s)
Abca1	Chr4	3	A_MACS_peak_4697
Adamtsl4	Chr3	6	A_MACS_peak_4394
Apol9b	Chr15	5	A_MACS_peak_2573
Asb8	Chr15	6	A_MACS_peak_2660
C1qtnf1	Chr11	3	A_MACS_peak_1465
Cebpb	Chr2	6	A_MACS_peak_4126
Cuedc1	Chr11	6	A_MACS_peak_1303
Ddr1	Chr17	10	A_MACS_peak_3121
Fasn	Chr11	4	A_MACS_peak_1475
Foxred2	Chr15	6	A_MACS_peak_2575
Fxyd3	Chr7	0	B_MACS_peak_8015
Gpr146	Chr5	6	A_MACS_peak_5442, A_MACS_peak_5444
Grhpr	Chr4	1	A_MACS_peak_4671
H1fx	Chr6	10	A_MACS_peak_5696
Hadh	Chr3	2	A_MACS_peak_4508
Hibch	Chr1	2	A_MACS_peak_129
Hk1	Chr10	6	A_MACS_peak_733
Hr	Chr14	3	A_MACS_peak_2260
Inpp1	Chr1	2	A_MACS_peak_129
Itgb3	Chr11	6	A_MACS_peak_1392
Jun	Chr4	9	B_MACS_peak_6508
Ldlrap1	Chr4	6	A_MACS_peak_4979
Lgi4	Chr7	4	B_MACS_peak_8015
Lpin3	Chr2	3	A_MACS_peak_4098
Mapkapk3	Chr9	4	A_MACS_peak_6854
Mcam	Chr9	7	A_MACS_peak_6672
Mtap7d1	Chr4	6	A_MACS_peak_4942, A_MACS_peak_4943
Ncoa4	Chr14	2	A_MACS_peak_2163
Nfix	Chr8	6	A_MACS_peak_6428
Nr1d1	Chr11	2	A_MACS_peak_1350
Nrp1	Chr8	2	A_MACS_peak_6562
Nudt18	Chr14	6	A_MACS_peak_2260
Pcdh7	Chr5	5	B_MACS_peak_7008
Plxnd1	Chr6	6	A_MACS_peak_5773
Rfng	Chr11	6	A_MACS_peak_1475
Slc25a33	Chr4	5	A_MACS_peak_5050
Usp2	Chr9	5	A_MACS_peak_6672
Zfp809	Chr9	5	A_MACS_peak_6611
Zhx3	Chr2	1	A_MACS_peak_4098
Zmiz1	Chr14	1	B_MACS_peak_2761

Data from the ENCODE/LICR adult mouse cerebellum CTCF ChIP-Seq track of the University of California, Santa Cruz (UCSC) mouse genome browser were compared with our cistrome analysis to identify genes where CTCF and TR binding might coincide in cerebellum neurons.

Table S5. Primers used for gene expression

Gene	Forward primer	Reverse primer
Adamtsl4	TCCAAGCCCCGATGCCAGAGTA	GGCAGCTGATGAGAAGGTCCAAACA
Aoc3	TCACTTGGCTGTGACCCAGAGGA	AAAAAGCCAGCCGTCACCCAGG
B3galt5	CGAACGTGAGCTCTTACTCGGCA	TCACCAGCAGCACCAGGAAAGG
D0H4S114	TGAACAGGGAGCGGGGCTTTTG	AGCGCCAGTCTCCTCCATCTTCT
Dbp	GAAGGCAAGGAAAGTCCAGGTGCC	GCTCCTGCCGCACAGCCA
Epas1	TGGACATCCCCCTGGACAGCAA	GGTCATGTTCTCCGAATCCAGGGCA
Ephb3	TCTACTGCAATGGCGACGGGGA	CCGAGTCTGAGTCTGCACGGTAGAA
Errfi1	AAGTCCTGCTGGGGCAGTCACA	TCACGCTGTCCCGAGCTTTCAC
Hprt	CAGCGTCGTGATTAGCGATG	CGAGCAAGTCTTTCAGTCCTGTCC
Hr	GCCTTGCTTCCTATGATTGTCTCC	AGAGGTCCAAGGAGCATCAAGG
Htra1	ACGCCAAGACCTACACCAACCTGT	ACGCAAACTGTTGGGATCTTCCTGC
Jun	AGAACGTGACCGACGAGCAGGA	CCGGGTTGAAGTTGCTGAGGTTGG
Klf9	CACGCCTCCGAAAAGAGGCACAA	CTTTTCCCCAGTGTGGGTCCGGTA
Megf6	ACGTGTGTACATGCGGGCAAGG	AGCATGACACAGGCCCCCATTC
Osgin1	TCCAGACAACCCAGGCTCACCA	TGGACGGCTCCTGGTTTCACGTA
Pfkfb3	CAGCTTTGAGGAGCGTGTGGCT	GGCACAAGGCAGGCTGTGGAAA
Plp1	GGCGACTACAAGACCACCATCTGC	ACACCAGGAGCCATACAACAGTCAGG
Rara	TCAGCGCCTGTGAGGGCTGTAA	TCAGGCGTCAGCGTGTAGCTCT
Slc16a1	TCGTGTAGGTGCAGCAGCCAAG	TGTATCCCACTGGCCCTCCAATCG
Tgm2	AGAAACTGGTGCTGCGTCGTGG	TGCAGAGCTGTAGCGAGAGGACA
Thra (TRα1)	TGCCTTTAACCTGGATGACAC	TCGACTTTCATGTGGAGGAAG
Thrb	AGCCAGAACCCACGGATGAGGA	TGCCACCTTCTGGGGCATTCAC
Vegfa	CCAGGCTGCACCCACGACAG	CGCATCAGCGGCACACAGGA
Zbtb20	CCAGCCCTCATCCACTCGACACAT	GCGAACCGTCACGTCACAGAAGT

Table S6. Primers used for chromatin immuno- or affinity precipitation analysis

Binding site	Forward primer	Reverse primer
A-TRBS-5973	TCGGCTGACCCGGGCTTATTTG	TTTGTTCCCACTGCTGTGTCCGAG
A-TRBS-3567	ACATCGGGGAGAATGGGTGGGA	TTGTCCAACGAGCGCCAGACAC
A-TRBS-3568	TGCACGAGTTTGGGGCGGATTC	TGGGCCTGGCATCGCCCTTTTA
B-TRBS-6508	TGTCTCTGCCACACTCAGTGCAAC	CCCTGAGAACGACGCAAGCCAA
A-TRBS-4394	GCGGCAGCAGGTAGTTGTGTAGTAG	GGGGATGGAGTTGGACTATGGGCA
A-TRBS-6181	TCCCATGATTTGGCCCTGCCTG	ACGGGAAGAGGACAGCATCCAGA
A-TRBS-6182	AGCTTGCTGCGTGCCATTGGA	TGCTTGGCCAGTGGGTCAGCTA
A-TRBS-3280	GTCACCCCGGGGCTCAAGGA	CACCAGCCGAATGTGACAGGCA
A-TRBS-3281	CGGCTGGGGGAGGCCTACAG	GCCAGGGCAAGGGTGTGACC
A-TRBS-6445	AGAGGCCTAGTTAGCTCATCCGTGG	TGCAGTAGCAGGGGGAGGAGG
B-TRBS-6937	CCTGGTGGCCGCTCCAACATC	TCGCTCAATCTCCCCTCCGTCA
A-TRBS-3729	ACGCAGACCTGCTTCTAGGCCA	ATCCTCTACTGTATCCGCGCCCA
A-TRBS-4531	GCCCAAACAACCACTCGGTTCCA	TCGAGAAGCGAAAGCACCCAACC