1．Pairwise TS events

Significant differential transcript usage (DTU), which refers to the abundance of a transcript changes significantly under two conditions, was detected using SUPPA2 (version 2.3)[1]. A TS event is called if the expression of a pair DTU transcripts change oppositely. Figure S1 displays a pairwise TS event intuitively, C1 and C2 represent two different conditions, respectively. The y-axis (**Ratio**) in Figure S1 represents relative abundance of a transcript in the specific condition (e.g. the expression of transcript A divided by the sum of all transcript expression in a gene).

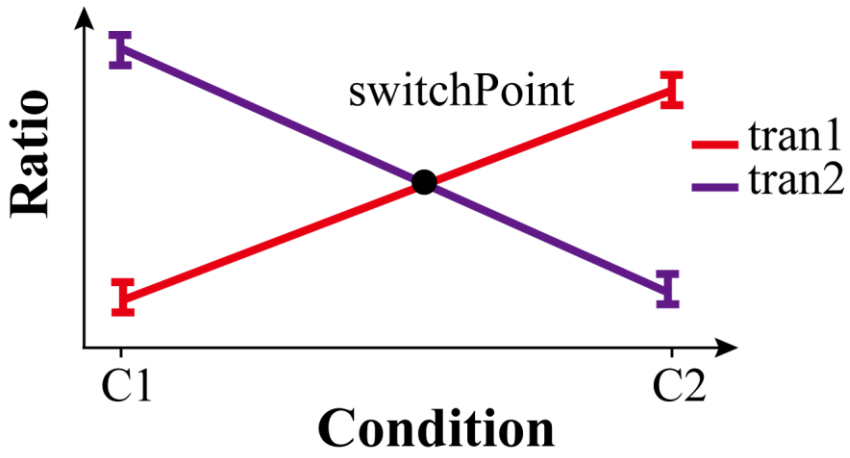

Figure S1 The schematic of a pairwise TS event

To help users to select TS events with specific patterns, deepTS provided five parameters which are described as below:

meanRa represents the average ratio of a transcript across N_C biological replicates under condition C,

$$meanRa = \frac{sum(Ratio_{tran}|C)}{N_c}$$

Where **Ratio$_{tran}$C** represents ratios of N biological replicates under condition C, N_C is the number of biological replicates under condition C.

meanExp is similar to **meanRa**, which represents the average expression abundance of a transcript across N_C biological replicates under condition C, the formula is as follows:

$$meanExp = \frac{sum(Exp_{tran}|C)}{N_c}$$

Here, **Exp$_{tran}$|C** is the expression abundance of the transcript "tran" in N_C biological replicates under condition C. TS events can be further filtered using the following four parameters.

Filtered parameters

1) **switchPoint:** a logic value indicating whether there is a cross point between the paired ratio curves in a TS event (default: TRUE).

$$sign(\ meanRatio_{\text{tran1}}|C1 - meanRatio_{\text{tran2}}|C1)$$

$$\neq \text{sign}(\ meanRatio_{\text{tran1}}|C2 - meanRatio_{\text{tran2}}|C2)$$

2) **max_Exp:** a numerical vector describing the maximum **meanExp** of two transcripts in a TS events (default: (1, 1)).

$$(\max(\ meanExp_{\text{tran1}}|C1, meanExp_{\text{tran1}}|C2), \max(\ meanExp_{\text{tran2}}|C1, meanExp_{\text{tran2}}|C2))$$

3) **max_FC:** a numerical parameter presenting the log2 of maximum fold change of transcripts under two conditions (default: 2).

$$max(\log_2 \frac{meanExp_{\text{tran1}}|C2}{meanExp_{\text{tran1}}|C1}, \log_2 \frac{meanExp_{\text{tran2}}|C2}{meanExp_{\text{tran2}}|C1})$$

4) **min_Ratiodiff:** a numerical parameter specifying the minimum **Ratio** difference of two transcripts under two conditions (default: 0.15).

$$min(|meanRa_{\text{tran1}}|C1 - meanRatio_{\text{tran2}}|C1|, |meanRa_{\text{tran1}}|C2 - meanRa_{\text{tran2}}|C2|)$$

2. Time-series TS events

For the time-series transcriptome analysis, TS events are identified using R package TSIS (version 0.2.0) [2]. The first step is searching switch points. It calculates the average expression values of the replicates for each time point of each transcript, following by searching the cross points. Then TS events are defined and filtered with a series of criterions described as below chart.

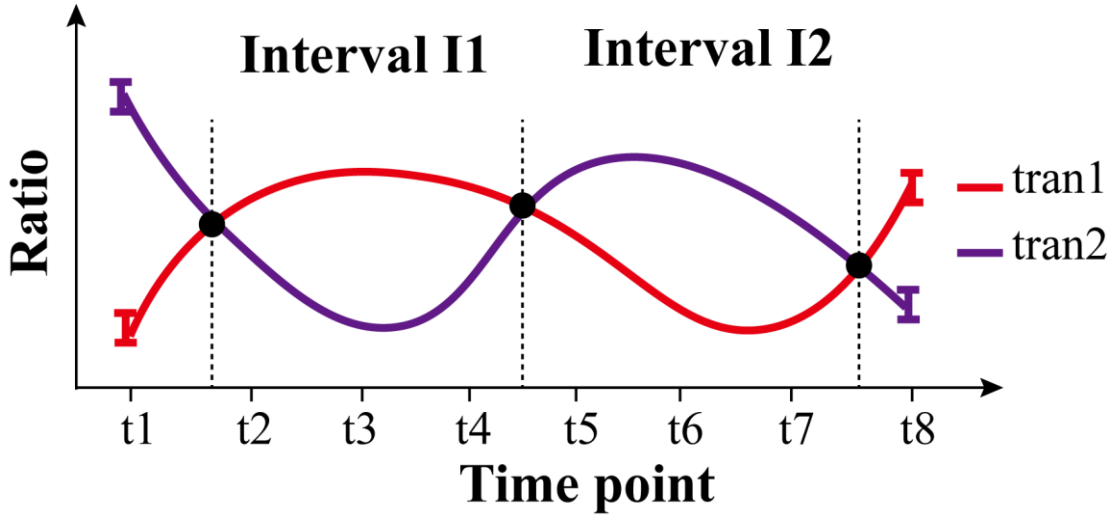

Figure S2. The schematic of a time-series TS event

Figure S2 shows a time-series TS event between transcript “trans1”and “trans2”, where **Ratio** represents relative abundance for a transcript at a time point. The time series with eight time points are divided into four intervals by the intersection points of average expression. For each switch point, a series of scores are calculated and used to construct filtered metrics. For example, I_1 and I_2 are before and after time interval of a switch point, each contains three consecutive time points.

$$I1:\{t2,t3,t3\} \qquad I2:\{t5,t6,t7\}$$

meanRa represents the average Ratio of a transcript at time interval I,

$$meanRa = \frac{sum(Ratio\_m_{\text{tran}}|I)}{N_I}$$

Where **Ratio_m$_{tran}$|I** represents the average ratio of biological replicates at each time point in interval I, **N$_I$** is the number of time points at time interval I. Similarly, **meanExp** represents the average expression abundance of a transcript at time interval I,

$$meanExp = \frac{sum(Exp\_m_{tran}|I)}{N_I}$$

Where **Exp_m$_{tran}$|I** represents the average expression abundance of biological replicates at each time point in interval I TS events can be further filtered using the following eight parameters:

Filtered parameters

1) ***num***: a numerical vector describes the least number of consecutive time points in interval I_1 and I_2 (default: (1,1));

$$(N_{I1}, N_{I2})$$

2) ***cor***: the Pearson's correlation coefficient between the expression values of the paired transcripts across time courses in interval I_1 and I_2 (default: -0.2);

$$correlation(Ratio\_m_{tran1}|(I1+I2), Ratio\_m_{tran2}|(I1+I2))$$

3) ***freq***: a numerical value in the range of 0–1, reflecting that the frequencies of samples of one transcript is greater than that of the other in interval I_1 and is less in interval I_2 (default: 0.5);

$$|P\left(Ratio_{m_{tran1}} > Ratio_{m_{tran2}}|I1\right) + P(Ratio\_M_{tran1} < Ratio\_m_{tran2}|I2) - 1|$$

4) ***diff_ra***: a numerical vector describes the mean difference between two transcripts in terms of relative abundance in intervals I_1 and I_2 (default: (0.3, 0.3));

$$((meanRa_{tran1} - meanRa_{tran2})|I1, (meanRa_{tran1} - meanRa_{tran2})|I2))$$

5) ***diff_pvalue***: a numerical vector describes the significance level of the difference between the relative abundances of two transcripts in intervals I_1 and I_2 (default: (0.05, 0.05));

$$(t.test\left(Ratio_{m_{tran1}}|I1, Ratio_{m_{tran2}}|I1\right), t.test\_(Ratio\_m_{tran1}|I2, Ratio\_m_{tran2}|I2))$$

6) ***change_ra***: a numerical vector describes the difference in relative abundance of each transcript between interval I_1 and I_2 (default: (0.2,0.2));

$$((meanRa_{tran1}|I1 - meanRa_{tran1}|I2), (meanRa_{tran2}|I1 - meanRa_{tran2}|I2))$$

7) ***diff_exp***: a numerical vector describes the mean difference between two transcripts in terms of expression values in intervals I_1 and I_2 (default: (3, 3));

$$((meanExp_{tran1} - meanExp_{tran2})|I1, (meanExp_{tran1} - meanExp_{tran2})|I2)$$

8) ***change_exp***: a numerical vector describes the difference in average expression value of each transcript between interval I_1 and I_2 (default: (3,3)).

$$(\ (meanExp_{tran1}|I1 - meanEXP_{tran1}|I2), (meanExp_{tran2}|I1 - meanExp_{tran2}|I2) \ )$$

3. Population TS events

TS identification from population transcriptome data has recently been introduced and validated by [3]. In this study, we developed popTS to enable uses identify TS events from population transcriptome data by integrating existing tools (GAPIT2 and PLINK) and in-house scripts. popTS focuses on genes with multiple transcripts, each transcript should express (e.g., TPM > 1) in at least *N* (e.g., 18) individuals in the population. The ratio of each transcript in these genes is used as phenotype for genome-wide association analysis to identify sQTLs using GAPIT2 [4] with mixed linear model. The Bonferroni-corrected *P*-values are used to detect significant associations. For genes with multiple sQTLs, unique sQTLs are identified when associated single nucleotide polymorphisms (SNPs) are not in linkage disequilibrium (LD) (e.g., $r^2 < 0.1$) with any other associated SNPs on the same chromosome for the target genes. LD analysis is performed using PLINK (v1.9) [5]. Unique sQTLs are further eliminated if their associated transcripts exhibit less than 0.05 difference in relative abundance in two genotypes. TS events are defined using a pair of transcripts associated with the same unique sQTL, which can be further filtered according to the **Ratio** (described as below) difference between a pair of transcripts in two genotypes described in Figure S3.

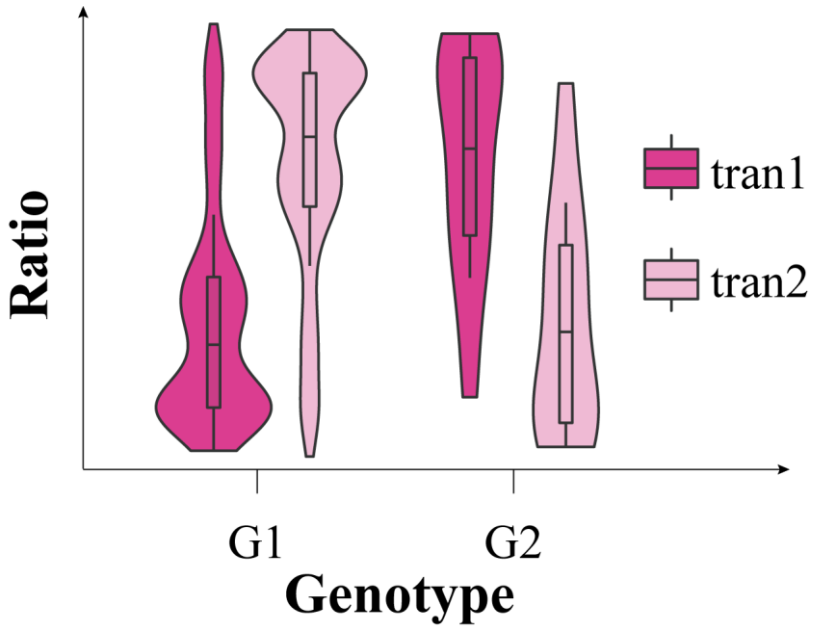

Figure S3. The schematic of a popultaion TS event

Figure S3 shows a sQTL associated TS event, **Ratio** represents the relative abundance of a transcript in a genotype, G1 and G2 are two different genotypes. Several parameters are defined to filter population transcriptome derived TS events. **meanRa** represents the average **Ratio** of transcript "tran" with genotype G.,

$$meanRa = \frac{sum(Ratio_{tran}|G)}{N_G} \qquad G:\{G1, G2$$

Where $\mathbf{Ratio_{tran}|G}$ is the **Ratio** of transcript "tran" in population samples with genotype G; $\mathbf{N_G}$ is the number of population samples with genotype G. TS events can be further filtered using parameter **diff_Ratio,** which is the relative difference of two transcripts between two genotypes.

$$(meanRa_{tran1} - meanRa_{tran2})|G1 - (meanRa_{tran1} - meanRa_{tran2})|G2$$