

Supplementary material

No	Sample ID	Concentration (ng/ μ l)	Volume (μ l)	Quantity (μ g)	Purity	
					RIN	28s/18s
1	E12.5_incisor_Set1	25.42	12	0.305	9.4	1.4
2	E12.5_incisor_Set2	101.00	14	1.414	9.8	1.6
3	E12.5_incisor_Set3	118.00	14	1.652	9.3	1.3
4	E12.5_diastema_Set1	125.00	8	1.000	9.7	1.6
5	E12.5_diastema_Set2	45.00	14	0.630	9.9	1.6
6	E12.5_diastema_Set3	75.00	14	1.050	9.8	1.6
7	E12.5_molar_Set1	68.33	15	1.025	9.5	1.3
8	E12.5_molar_Set2	74.00	14	1.036	10.0	1.6
9	E12.5_molar_Set3	134.00	14	1.876	9.7	1.4
10	E15.5_diastema_Set1	250.00	16	4.000	9.8	1.7
11	E15.5_diastema_Set2	241.00	14	3.374	9.4	1.5
12	E15.5_diastema_Set3	234.00	13	3.042	9.8	1.9
13	E15.5_molar_Set1	285.00	16	4.560	10.0	1.7
14	E15.5_molar_Set2	279.00	14	3.906	9.7	1.6
15	E15.5_molar_Set3	235.00	15	3.525	9.8	1.7
16	E17.5_diastema_Set1	168.38	8	1.347	8.9	1.6
17	E17.5_diastema_Set2	217.00	14	3.038	9.0	1.6
18	E17.5_diastema_Set3	195.00	16	3.120	9.4	1.5
19	E17.5_molar_Set1	164.00	8	1.312	9.6	1.7
20	E17.5_molar_Set2	327.00	14	4.578	9.7	1.8
21	E17.5_molar_Set3	180.00	15	2.700	9.8	1.7

Supplementary Table 1 | QC data of RNA preparation

Gene		Forward (5'-3')	Reverse (5'-3')	Product size
RT-qPCR	Zfp618	GTCGAACCAGTCGGGAAAA	TCTGCTGCTGCTGCCTTG	141
	Gfra2	ACGAAACCCTCCGCTCTTTG	AGCGGCAGTCATACAATGGG	243
	Klk4	GTGTCAGCAGCCGGATCATA	CCAAGACTCCCGAGCAGAAA	105
	Cxcl14	CTGAGTCACCGAGTGGTTCTG	CGGCTTGGTTCTCGGTTTCA	228
	Cdh2	CGCAGTCTTACCGAAGGATGT	TCTCACAGCATACACCGTGC	148
<i>In situ</i> hybridization	Zfp618	TCGGAGTCGGTATTCAGGGA	TCGGAACGTCTGAGTTTGGG	667
	Gfra2	TTAGACGAAACCCTCCGCTC	ACAGTTGGCGTGGAAGTCTG	789
	Klk4	CTGCTCGGGAGTCTTGGTG	CTGAGGTGGTACACAGGGTC	402
	Cxcl14	GAGTCACCGAGTGGTTCTGCAT	CTTCGTAGACCCTGCGCTTC	582
	Cdh2	ATGTGCCGGATAGCGGGAG	AGTTGATTGGCGGGATGACC	502

Supplementary Table 2 | Primer sequences for RT-qPCR and *in situ* hybridization

No	Sample ID	Raw (Adapter trimmed)	Filtered	Mapped	Uniquely* Mapped	READ 1** / READ 2	Strand(+)** / Strand(-)	Splice****
1	E12.5_incisor_Set1	48,715,568	46,449,704 95.3%	40,705,265 87.6%	38,878,314 83.7%	21,038,358 17,839,956	19,398,692 19,479,622	15,550,402 33.5%
2	E12.5_incisor_Set2	47,362,550	45,972,810 97.1%	40,053,846 87.1%	38,337,609 83.4%	20,924,805 17,412,804	19,130,703 19,206,906	15,768,685 34.3%
3	E12.5_incisor_Set3	67,657,256	65,783,410 97.2%	56,441,728 85.8%	53,708,152 81.6%	29,802,157 23,905,995	26,783,768 26,924,384	22,692,644 34.5%
4	E12.5_diastema_Set1	57,962,590	55,705,366 96.1%	49,454,256 88.8%	47,761,329 85.7%	25,554,348 22,206,981	23,858,589 23,902,740	20,286,257 36.4%
5	E12.5_diastema_Set2	40,265,360	38,914,678 96.6%	33,813,188 86.9%	32,360,700 83.2%	17,641,966 14,718,734	16,153,004 16,207,696	13,157,577 33.8%
6	E12.5_diastema_Set3	70,319,504	68,340,666 97.2%	57,387,493 84.0%	54,779,372 80.2%	31,063,031 23,716,341	27,316,225 27,463,147	22,074,764 32.3%
7	E12.5_molar_Set1	56,314,710	53,430,272 94.9%	46,438,486 86.9%	44,346,422 83.0%	24,104,280 20,242,142	22,112,809 22,233,613	17,287,676 32.4%
8	E12.5_molar_Set2	41,484,366	40,165,268 96.8%	34,541,334 86.0%	33,037,716 82.3%	18,201,981 14,835,735	16,475,137 16,562,579	13,329,552 33.2%
9	E12.5_molar_Set3	74,032,748	72,230,840 97.6%	62,549,392 86.6%	59,830,291 82.8%	32,964,214 26,866,077	29,857,510 29,972,781	24,985,344 34.6%
10	E15.5_diastema_Set1	68,201,930	65,578,424 96.2%	58,127,043 88.6%	56,110,249 85.6%	30,071,632 26,038,617	28,022,154 28,088,095	23,535,501 35.9%
11	E15.5_diastema_Set2	45,951,920	44,552,930 97.0%	39,043,651 87.6%	37,273,622 83.7%	20,250,115 17,023,507	18,627,610 18,646,012	16,244,147 36.5%
12	E15.5_diastema_Set3	47,605,924	46,449,162 97.6%	40,104,295 86.3%	38,411,415 82.7%	21,041,237 17,370,178	19,159,787 19,251,628	15,426,257 33.2%
13	E15.5_molar_Set1	72,807,624	69,933,160 96.1%	61,992,858 88.6%	59,914,986 85.7%	32,093,881 27,821,105	29,932,211 29,982,775	25,042,822 35.8%
14	E15.5_molar_Set2	40,305,860	39,182,120 97.2%	34,555,595 88.2%	33,216,574 84.8%	17,934,265 15,282,309	16,593,997 16,622,577	14,032,009 35.8%
15	E15.5_molar_Set3	57,631,506	56,401,312 97.9%	48,252,320 85.6%	46,314,813 82.1%	25,339,023 20,975,790	23,070,976 23,243,837	18,716,158 33.2%

continued

16	E17.5_diastema_Set1	67,759,816	64,452,330 95.1%	56,786,196 88.1%	54,481,654 84.5%	29,361,607 25,120,047	27,219,514 27,262,140	23,484,004 36.4%
17	E17.5_diastema_Set2	37,430,834	36,154,878 96.6%	31,314,258 86.6%	29,935,593 82.8%	16,268,121 13,667,472	14,963,718 14,971,875	12,545,996 34.7%
18	E17.5_diastema_Set3	53,086,272	51,944,572 97.8%	45,225,300 87.1%	43,162,099 83.1%	23,502,047 19,660,052	21,580,320 21,581,779	18,302,097 35.2%
19	E17.5_molar_Set1	49,579,086	47,172,774 95.1%	41,316,457 87.6%	39,778,971 84.3%	21,519,035 18,259,936	19,872,971 19,906,000	17,265,023 36.6%
20	E17.5_molar_Set2	50,722,064	49,153,708 96.9%	41,862,482 85.2%	40,327,864 82.0%	22,309,490 18,018,374	20,128,714 20,199,150	16,565,283 33.7%
21	E17.5_molar_Set3	53,950,508	52,679,148 97.6%	45,403,135 86.2%	43,663,884 82.9%	23,947,002 19,716,882	21,778,928 21,884,956	18,426,622 35.0%
	Total	1,149,147,996	1,110,647,532 96.65%	965,368,578 84.01%	925,631,629 80.55%			384,718,820 33.48%

Supplementary Table 3 | Summary statistics of RNA-seq data

*	Uniquely Mapped	reads mapped to exactly one location within the reference genome
**	READ1/READ2	reads on Forward / reads on Reverse
***	Strand(+)/Strand(-)	DNA sense / DNA antisense
****	Splice	mapped reads on splicing site

E12.5_incisor_Set1

Category	Genome		Genes	
	Reads Number	%	Reads Number	%
Total Reads	46,449,704	100.00	46,449,704	100.00
Mapped Reads	40,705,265	87.63	40,734,204	87.70
Properly paired*	34,101,544	73.42	39,845,992	85.78

E12.5_incisor_Set2

Genome		Genes	
Reads Number	%	Reads Number	%
45,972,810	100.00	45,972,810	100.00
40,053,846	87.13	40,972,748	89.12
33,328,074	72.50	40,161,072	87.36

E12.5_incisor_Set3

Genome		Genes	
Reads Number	%	Reads Number	%
65,783,410	100.00	65,783,410	100.00
56,441,728	85.80	58,730,667	89.28
45,829,610	69.67	57,608,208	87.57

E12.5_diastema_Set1

Total Reads	55,705,366	100.00	55,705,366	100.00
Mapped Reads	49,454,256	88.78	49,960,602	89.69
Properly paired	42,175,588	75.71	49,007,730	87.98

E12.5_diastema_Set2

38,914,678	100.00	38,914,678	100.00
33,813,188	86.89	34,564,591	88.82
28,202,780	72.47	33,907,244	87.13

E12.5_diastema_Set3

68,340,666	100.00	68,340,666	100.00
57,387,493	83.97	61,358,817	89.78
45,473,744	66.54	60,229,754	88.13

E12.5_molar_Set1

Total Reads	53,430,272	100.00	53,430,272	100.00
Mapped Reads	46,438,486	86.91	46,367,353	86.78
Properly paired	38,619,580	72.28	45,307,556	84.80

E12.5_molar_Set2

40,165,268	100.00	40,165,268	100.00
34,541,334	86.00	35,746,168	89.00
28,368,764	70.63	35,029,168	87.21

E12.5_molar_Set3

72,230,840	100.00	72,230,840	100.00
62,549,392	86.60	64,619,353	89.46
51,544,286	71.36	63,363,120	87.72

E15.5_diastema_Set1

Total Reads	65,578,424	100.00	65,578,424	100.00
Mapped Reads	58,127,043	88.64	58,451,766	89.13
Properly paired	49,246,130	75.10	57,187,746	87.21

E15.5_diastema_Set2

44,552,930	100.00	44,552,930	100.00
39,043,651	87.63	40,443,846	90.78
32,416,126	72.76	39,822,998	89.38

E15.5_diastema_Set3

46,449,162	100.00	46,449,162	100.00
40,104,295	86.34	40,757,878	87.75
33,257,798	71.60	40,015,284	86.15

Total Reads	69,933,160	100.00	69,933,160	100.00
Mapped Reads	61,992,858	88.65	62,289,602	89.07
Properly paired	52,789,374	75.49	60,948,288	87.15

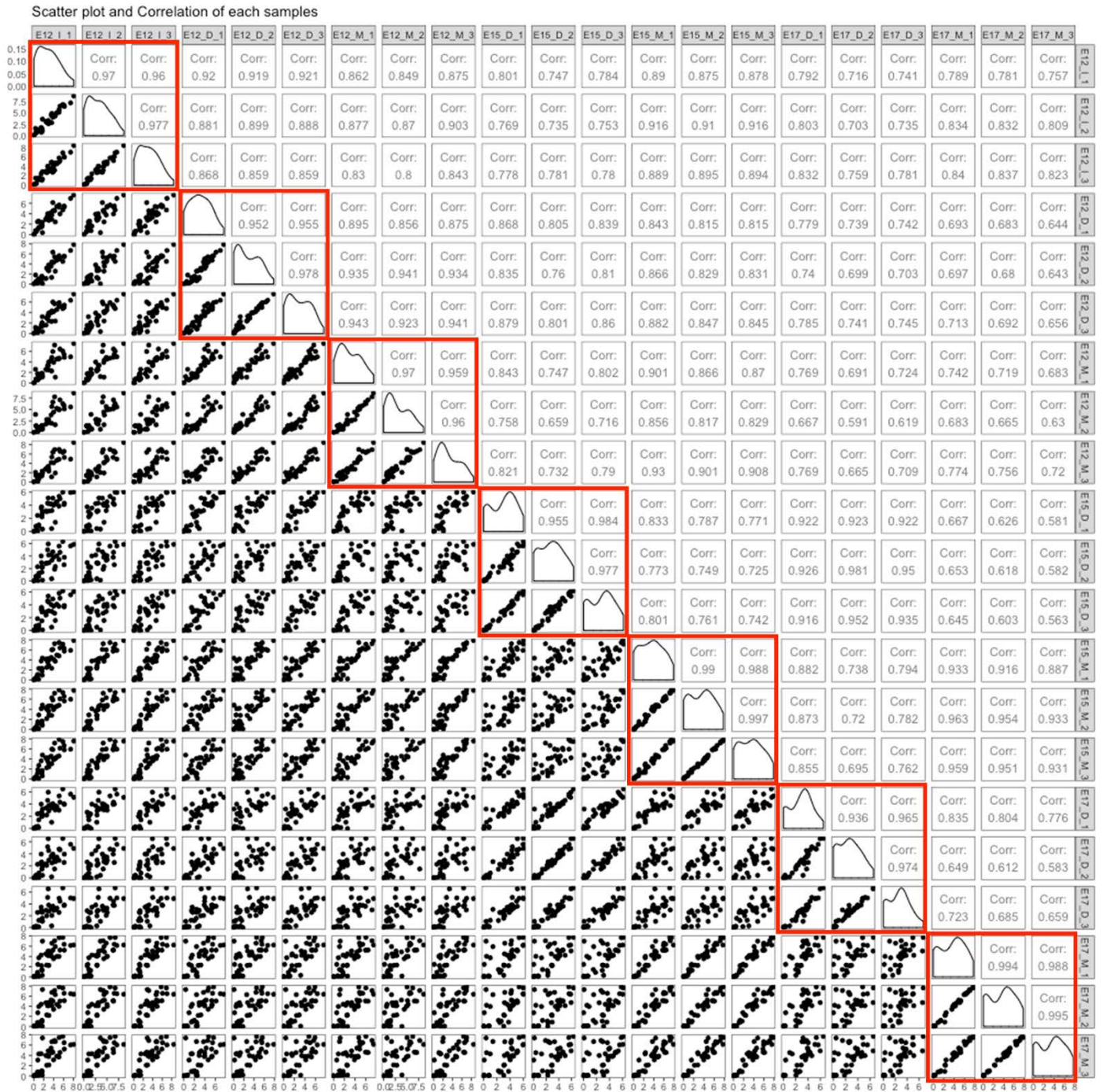
39,182,120	100.00	39,182,120	100.00
34,555,595	88.19	35,267,282	90.01
29,188,058	74.49	34,604,804	88.32

56,401,312	100.00	56,401,312	100.00
48,252,320	85.55	48,415,238	85.84
40,142,360	71.17	47,529,236	84.27

continued

E17.5_diastema_Set1					E17.5_diastema_Set2				E17.5_diastema_Set3			
Total Reads	64,452,330	100.00	64,452,330	100.00	36,154,878	100.00	36,154,878	100.00	51,944,572	100.00	51,944,572	100.00
Mapped Reads	56,786,196	88.11	58,080,749	90.11	31,314,258	86.61	32,552,421	90.04	45,225,300	87.06	46,205,389	88.95
Properly paired	47,450,652	73.62	56,882,798	88.26	26,043,910	72.03	32,005,344	88.52	37,737,786	72.65	45,415,206	87.43
E17.5_molar_Set1					E17.5_molar_Set2				E17.5_molar_Set3			
Total Reads	47,172,774	100.00	47,172,774	100.00	49,153,708	100.00	49,153,708	100.00	52,679,148	100.00	52,679,148	100.00
Mapped Reads	41,316,457	87.59	42,559,211	90.22	41,862,482	85.17	43,352,755	88.20	45,403,135	86.19	46,542,900	88.35
Properly paired	34,666,286	73.49	41,779,360	88.57	34,348,512	69.88	42,551,446	86.57	37,898,544	71.94	45,753,282	86.85

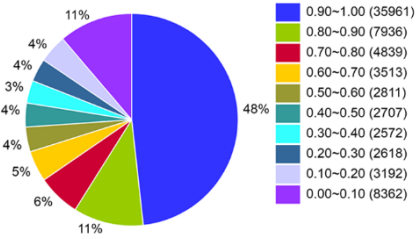
Supplementary Table 4 | Statistics of mapping to genome and genes



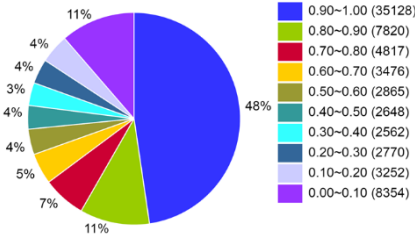
Supplementary Figure 1 | Scatter plot and correlation of each sample

Pearson's correlation values in each experimental group are higher than 0.95 (red boxes); only the correlation value of E17.5 diastema set1 and set 2 was found to be 0.936.

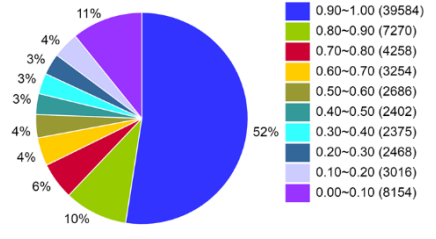
E12.5 incisor Set1



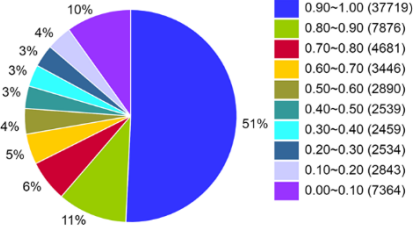
E12.5 incisor Set2



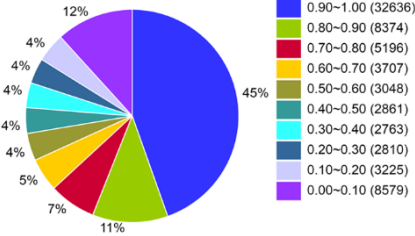
E12.5 incisor Set3



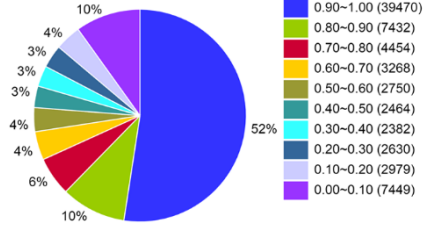
E12.5 diastema Set1



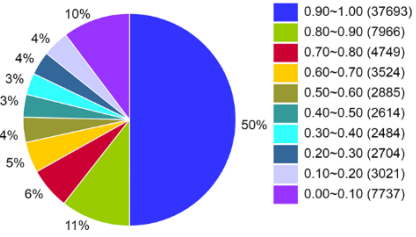
E12.5 diastema Set2



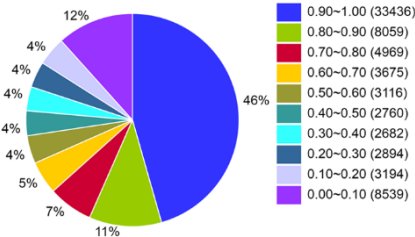
E12.5 diastema Set3



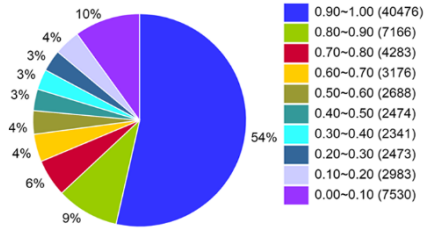
E12.5 molar Set1



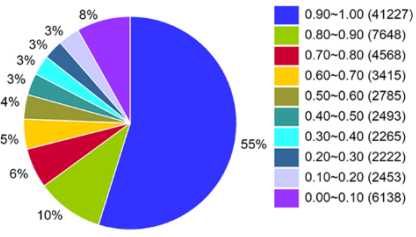
E12.5 molar Set2



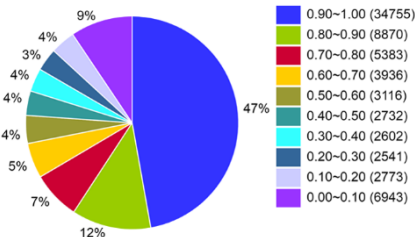
E12.5 molar Set3



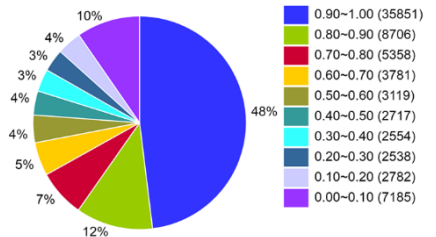
E15.5 diastema Set1



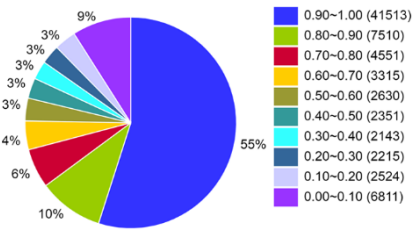
E15.5 diastema Set2



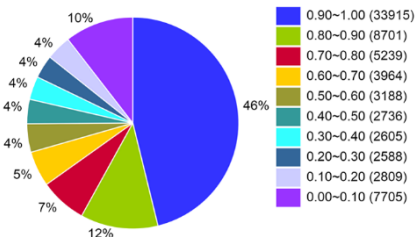
E15.5 diastema Set3



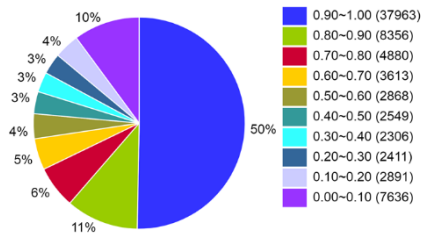
E15.5 molar Set1



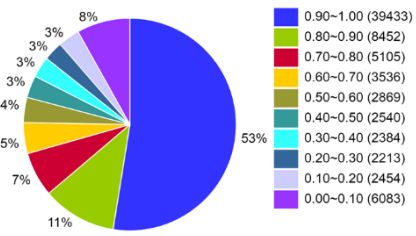
E15.5 molar Set2



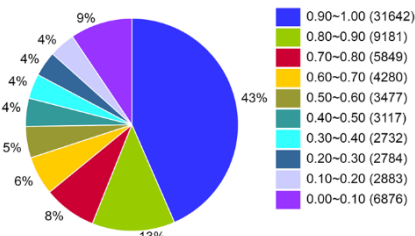
E15.5 molar Set3



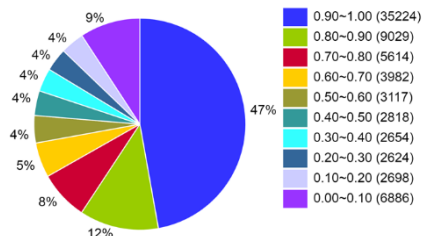
E17.5 diastema Set1



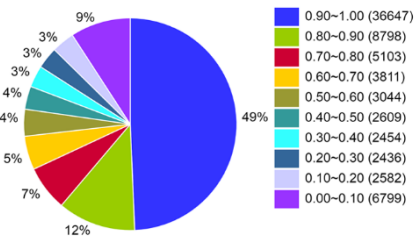
E17.5 diastema Set2



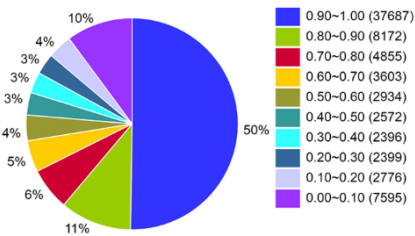
E17.5 diastema Set3



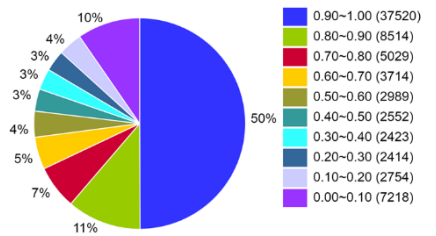
E17.5 molar Set1



E17.5 molar Set2

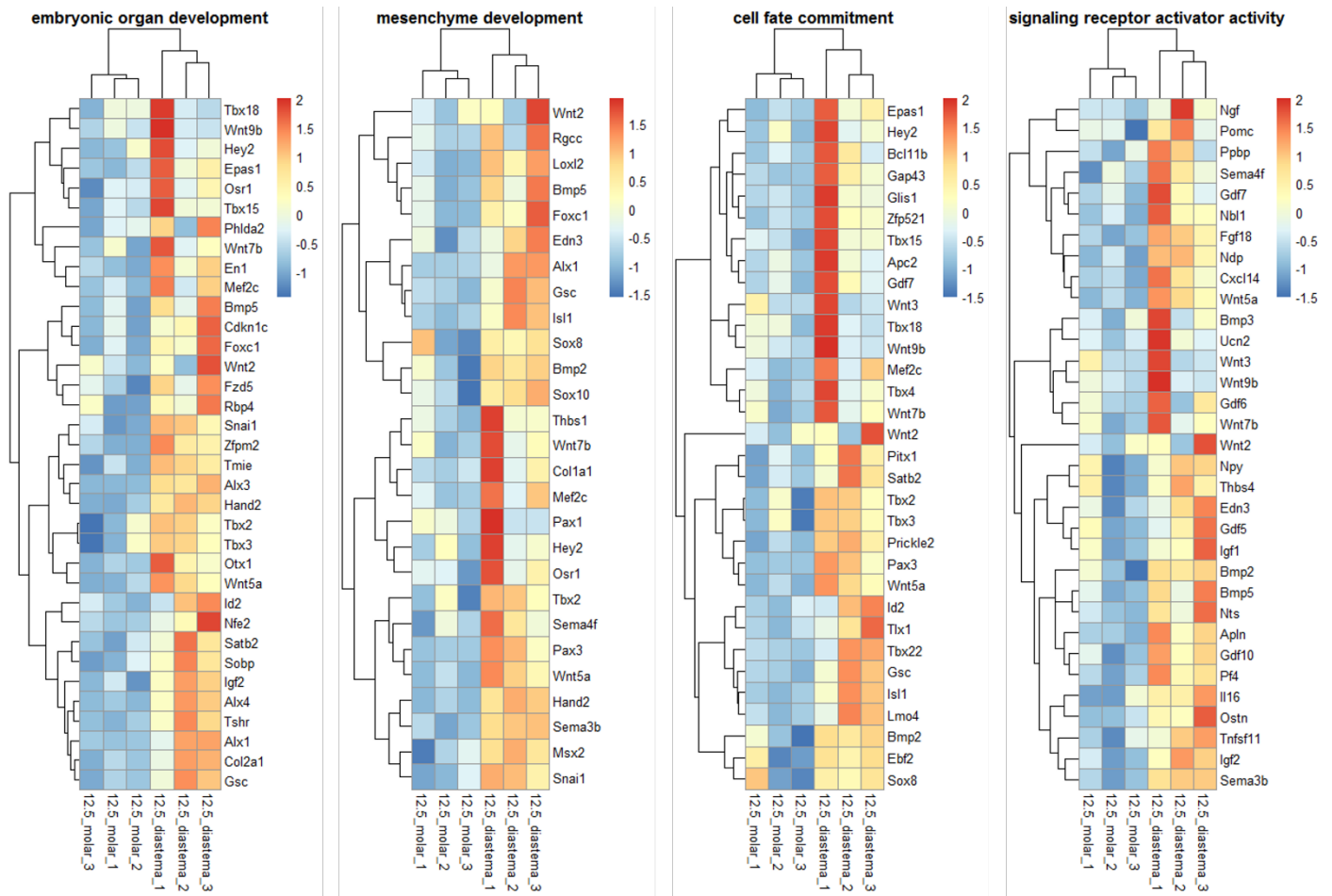


E17.5 molar Set3



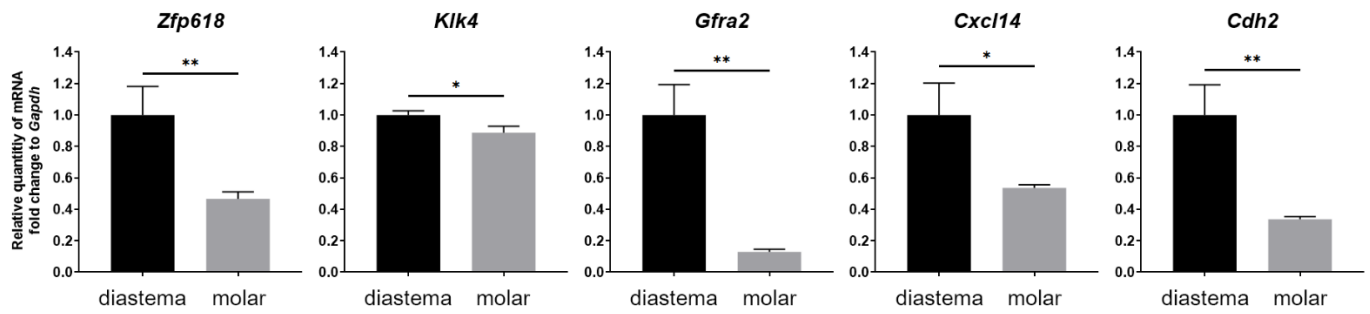
Supplementary Figure 2 | Average coverage of the reads aligned to mRNA

The coverage range was divided by 0.1 and from 0 to 1. The number of reads for the range covering the mRNA sequence references is presented in the legends of each sample. Reads with a coverage range of 0.9 to 1.0 accounted for 45%–55% in almost all samples.



Supplementary Figure 3 | E12.5 mRNA expression by GO term in molar and diastema region

Among the GO terms ranked higher in E12.5 diastema and molar, only genes belonging to terms related to organogenesis and tooth development were expressed as heatmaps.



Supplementary Figure 4 | RT-qPCR results of E12.5 non-odontogenic and odontogenic regions

Related quantities of mRNA of selected genes were analyzed by RT-qPCR. * $p < 0.05$, ** $p < 0.01$.