SUPPLEMENTARY DATA LEGENDS

Suppl. Figure 1. TR50 and Segmentation profiles for 12 of the ENCODE regions.

Suppl. Figure 2. Mid-Scores are elevated when two alleles of a gene replicate asynchronously in the same cell.

The Mid-Score for a time point is the percentage of cells that have replicated at least one, but not all alleles for the given probe. Thus it is the percentage of cells where G1 dots/cell < actual dots/cell < G2 dots/cell. For a locus with two alleles, G1 cells will have 2 dots/cell while G2 cells will have 4 dots/cell. Thus, the mid-score for a given time point is the percentage of cells with 3 dots/cell.

For an early replicating locus with synchronous alleles (i.e. Early-SA), the two alleles replicate together in an early time point. The mid-score is always 5%, since 5% of the cells (background) have 3 dots/cell in all of the time points.

For a Pan-S replicating locus with synchronous alleles (i.e. Pan-S-SA), the two alleles replicate together in a given cell, but <u>intercellular asynchrony</u> results in some cells replicating their two alleles in each time point. Since the alleles replicate synchronously in a given cell, this locus also gives a 5% mid-score (background) for all time points.

Contrast this with a Pan-S replicating locus with asynchronous alleles (Pan-S-AA). In this case the two alleles replicate asynchronously (<u>interallelic asynchrony</u>), one replicating earlier than the other. Here the majority of the cells replicate their first allele at an early time point (say 0-2 hr) and then the majority of the cells replicate their second allele in a later time point (say 6-8 hr). The Mid-Score starts at 5% (background) but then jumps to 85% at the 2 hr time point, because 85% of the cells have replicated exactly one allele. The mid-score stays at 85% until the 6 hr time point when the second allele replicates and the mid-score drops to 5% again at the 8 hr time point.

Suppl. Figure 3. Comparison of two methods of analyzing replication data for ENm001. The upper panel shows UCSC genome browser generated image representing the two sample based analyses of replication timing for ENm001 used in (Jeon et al., 2005). Each track shows the significant enrichment of replication signal in indicated 2 hr interval relative to the 0-10 hr interval. The bottom track labeled pan-S indicates the chromosomal regions that appeared to have replication signal in more than one time interval of S-phase. The lower panel shows the new probe-by-probe method of analyzing replication signal for ENm001.

Suppl. Figure 4. Replication timing of chromosome X. **(*A*)** Panel 1: UCSC genome browser displaying replication profile of a 1.2 Mb region of chromosome X (ENm006). Panel 2: This browser picture details the genes (track I) and replication time (track II) of a 62kb pan-S replicating region from ENm006. **(*B*)** UCSC genome browser displaying replication profile of a 500kb region of chromosome X (ENr324).

Suppl. Table 1. Examples of the TR50 calculation and temporal specificity classification.

Probe 1 is classified as temporally specific because the 0-2 hour time point contains all of the signal. Hence, probe 1 passes either criterion for temporal specificity. For example, the 0-2 hour time point contains at least twice the signal of any of the other four time points. So probe 1 clearly passes the first criterion. Probe 3 is classified as temporally specific due to the second criterion. That is, the sum of the 2-4 and 4-6 hour time points is 200, which is at least three times any of the other three time points. Probe 3 does not pass the first criterion, but it only needs to pass one of the criteria to be called temporally specific. Probes 2 and 4 are classified as temporally non-specific because they do not pass either of the criteria for specificity.

Suppl. Table 2. Interphase FISH probes from ENCODE regions are listed alongside FISH results of replication time in HeLa cells released from thymidine/aphidicolin block or nocodozole block or in MCF10A cells released from thymidine/aphidicolin block. The classification of the same regions on the microarrays as early, mid or late-S-phase or pan-S-phase is indicated. Blank cells: not determined

Suppl. Table 3. Primer details for chromatin immunoprecipitation assay.

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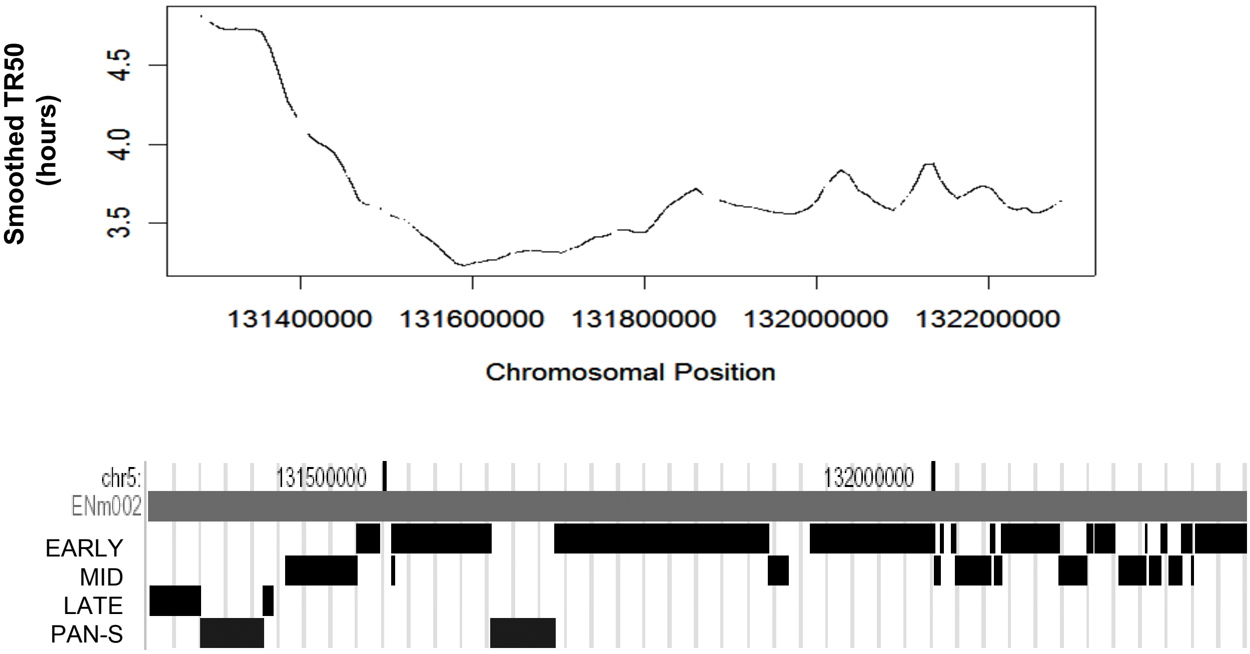
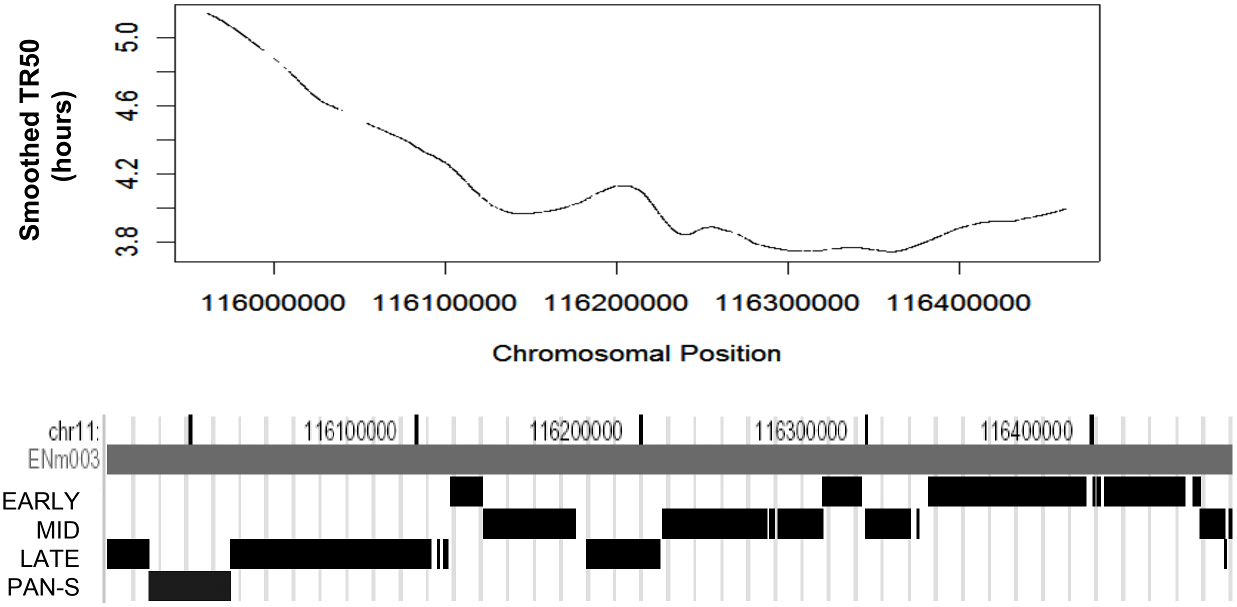
chr5:131284314-132284313 (ENm002: Interleukin _cluster)



chr11:115962316-116462315 (ENm003: Apo _cluster)



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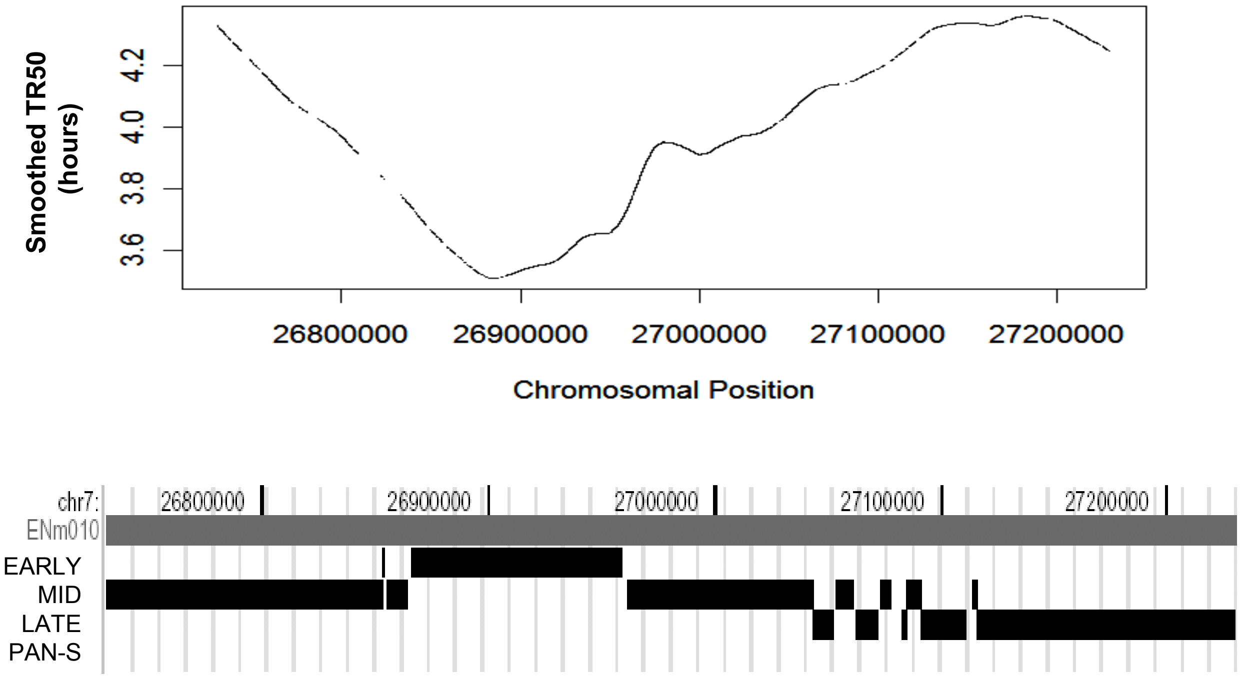
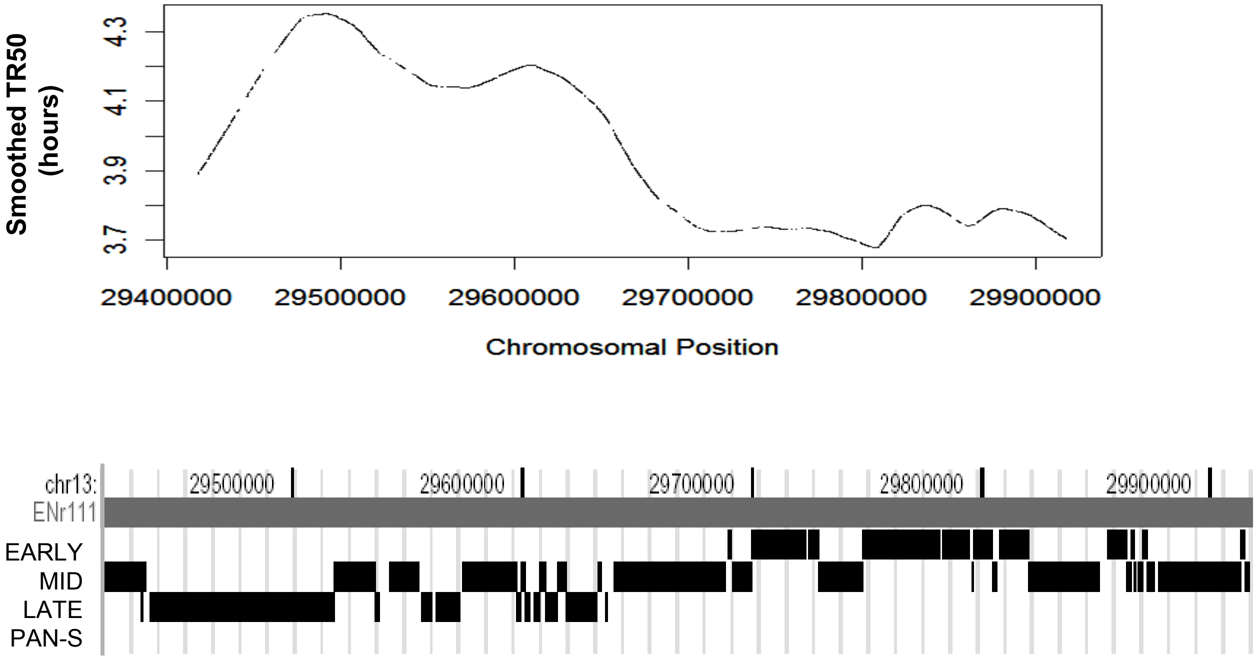
chr7:26730761-27230760 (ENm010: HOXA _cluster)



chr13:29418016-29918015 (ENr111)



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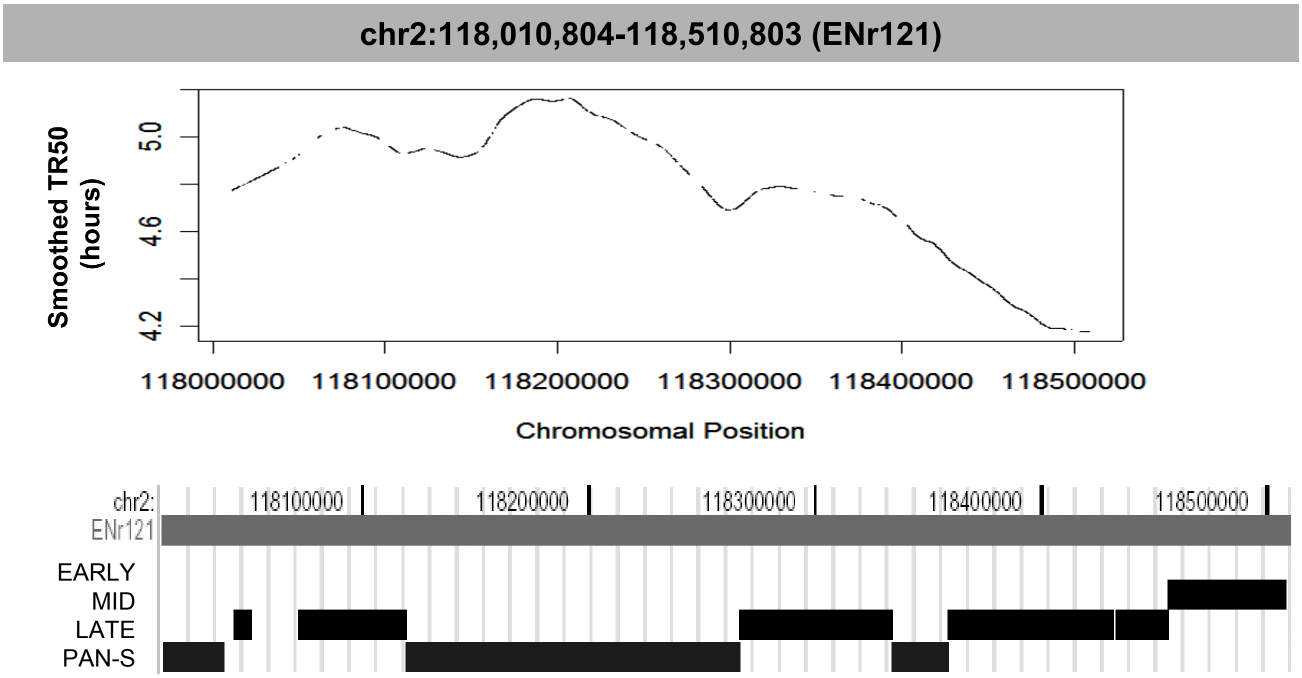
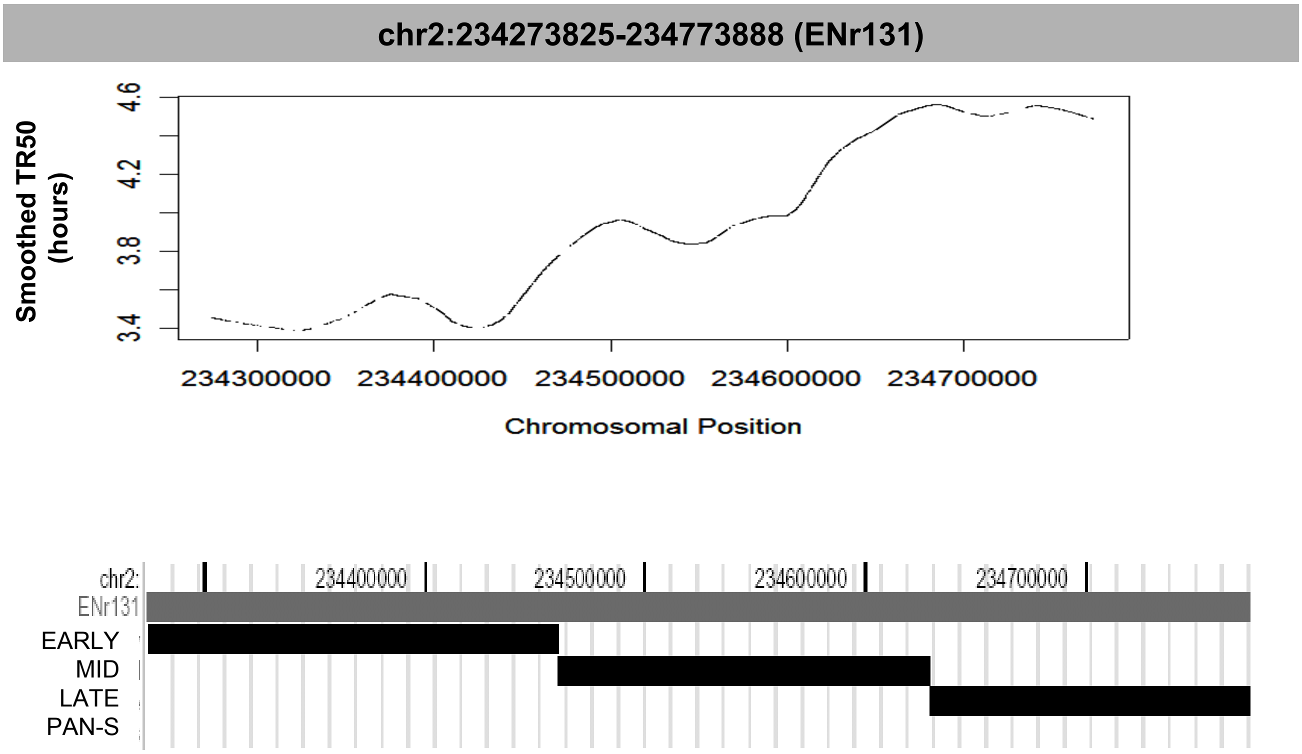

chr2:118,010,804-118,510,803 (ENr121)


chr2:234273825-234773888 (ENr131)

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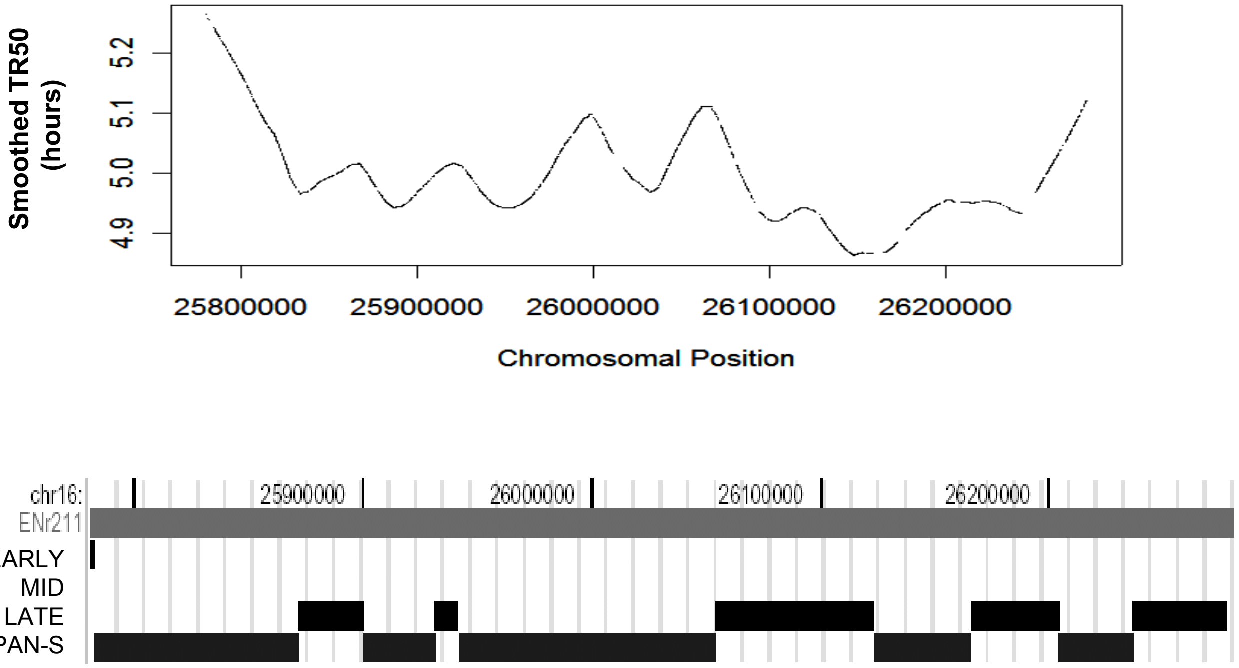
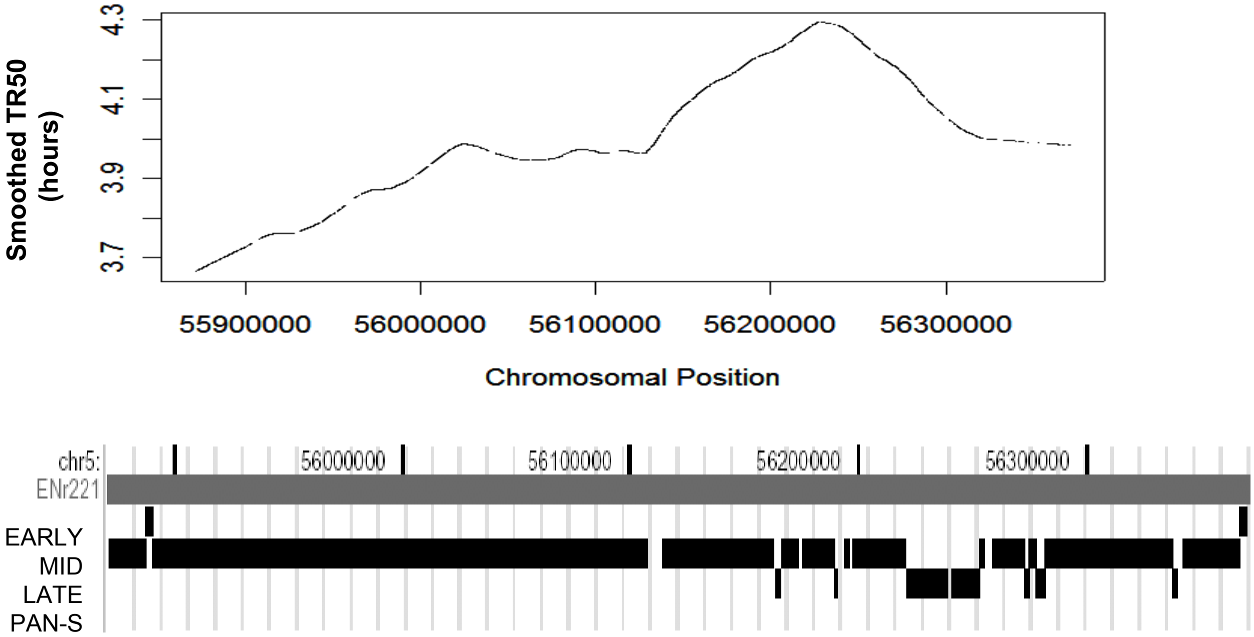
chr16:25780428-26280428 (ENr211)



chr5:55871007-56371006 (ENr221)



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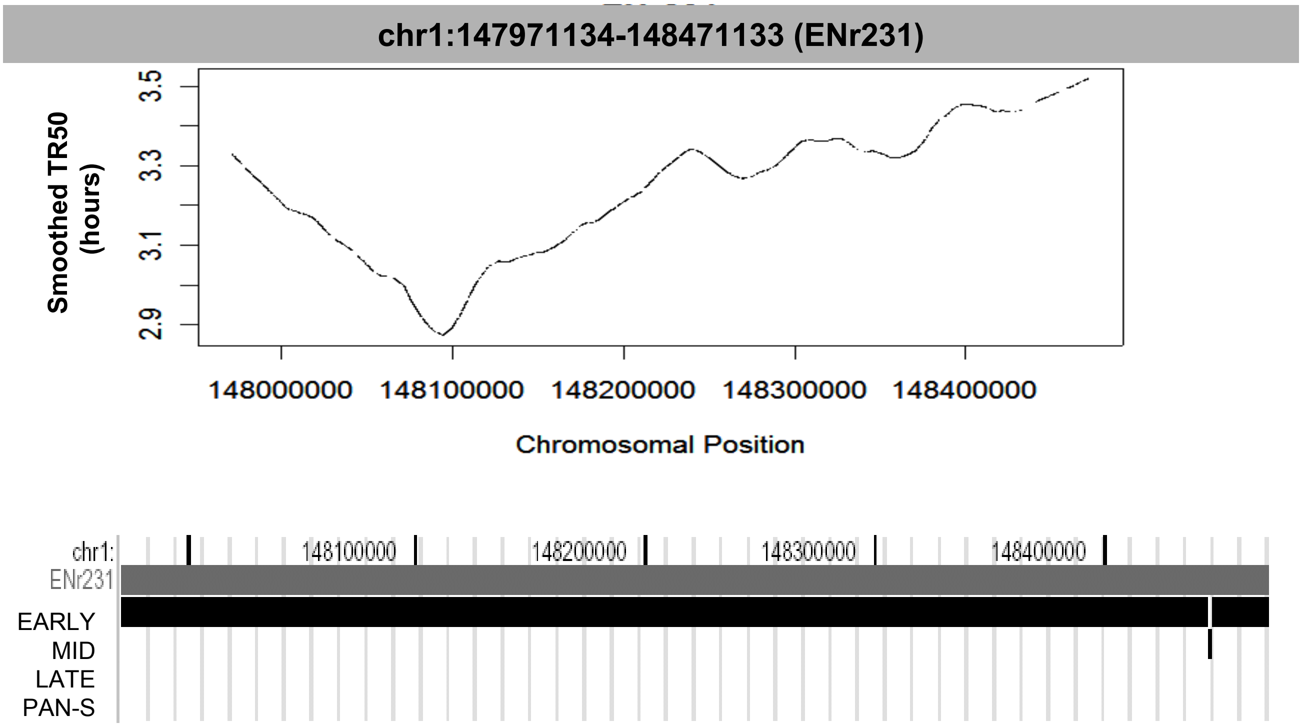

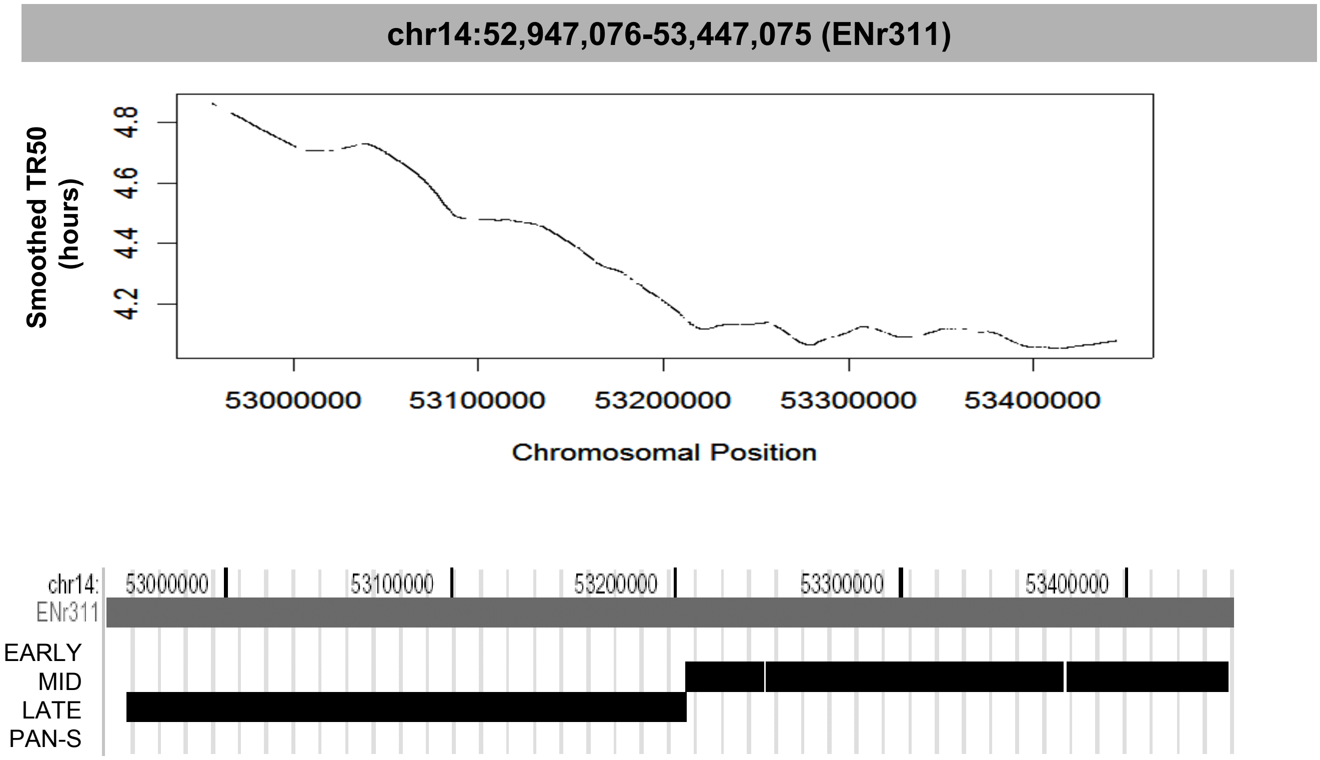

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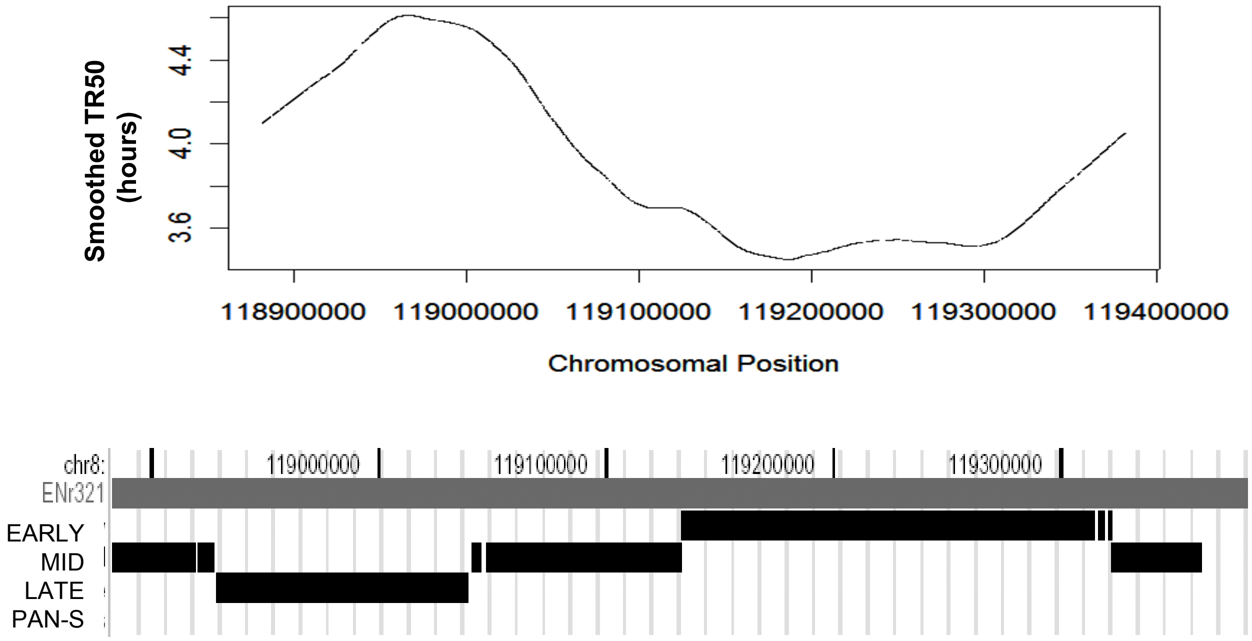
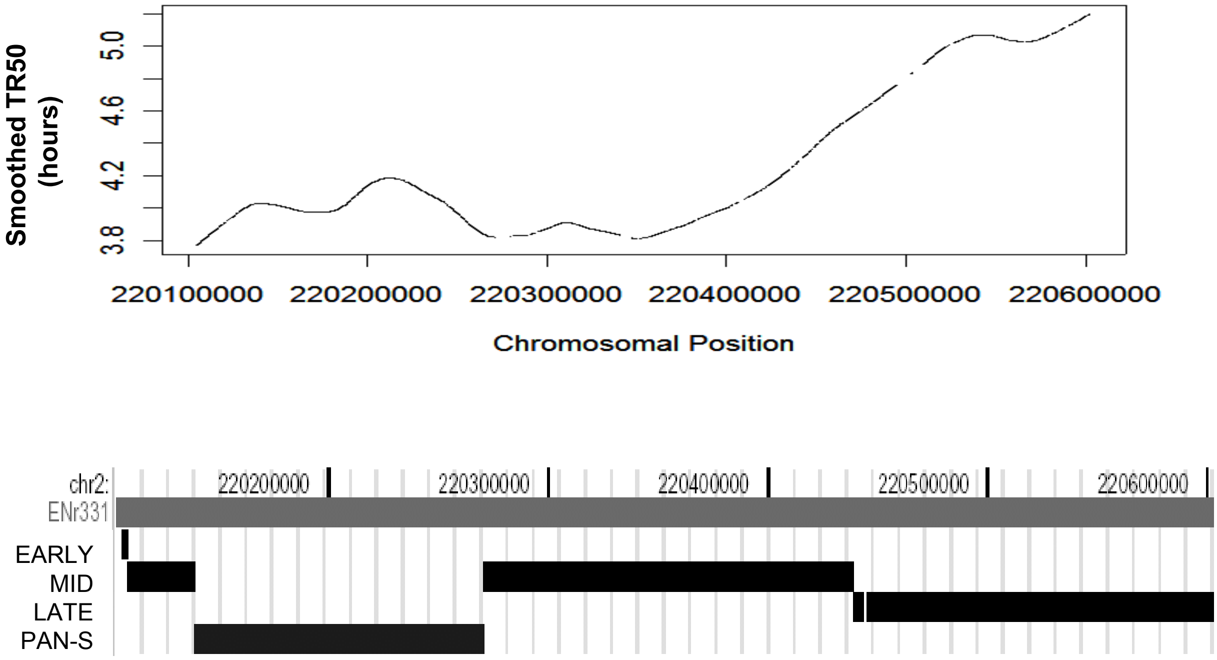
chr8:118882221-119382220 (ENr321)



chr2:220102851-220602850 (ENr331)



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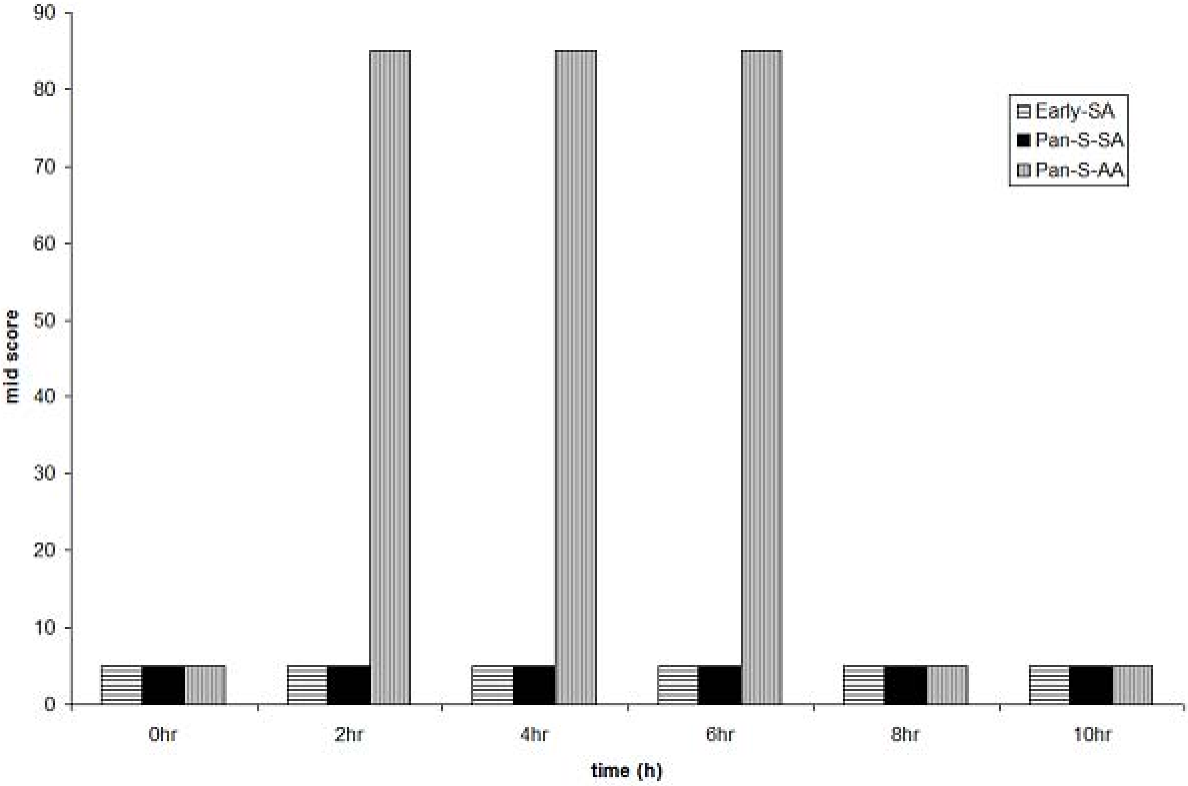

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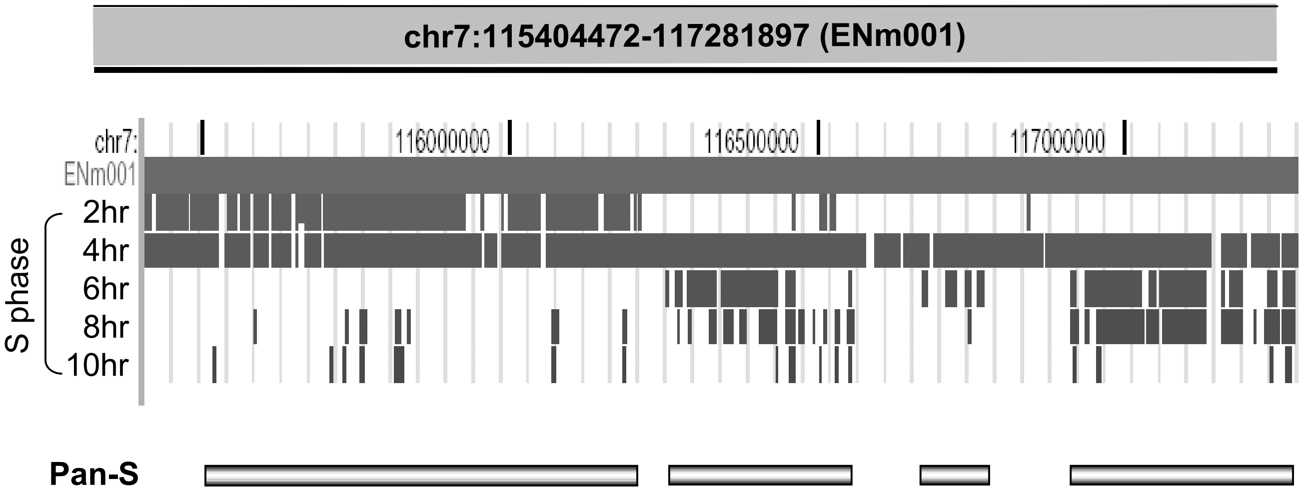
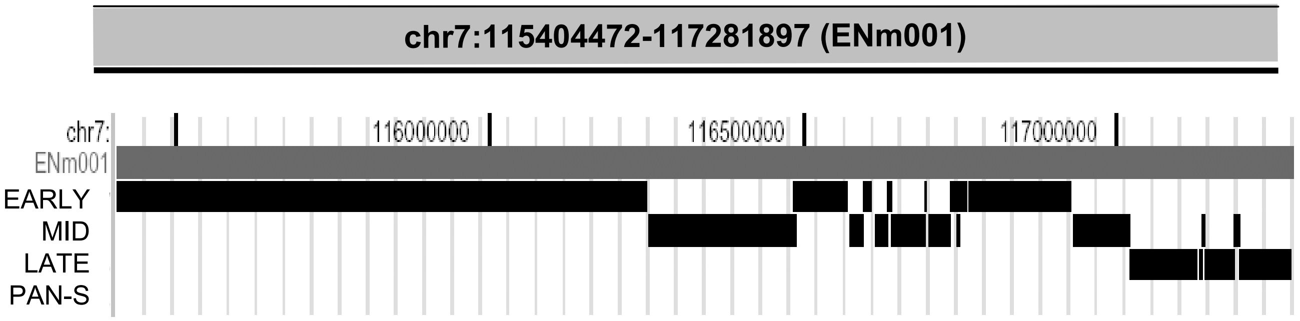
GTRANS based analysis (old method)



Segregation based analysis (new method)



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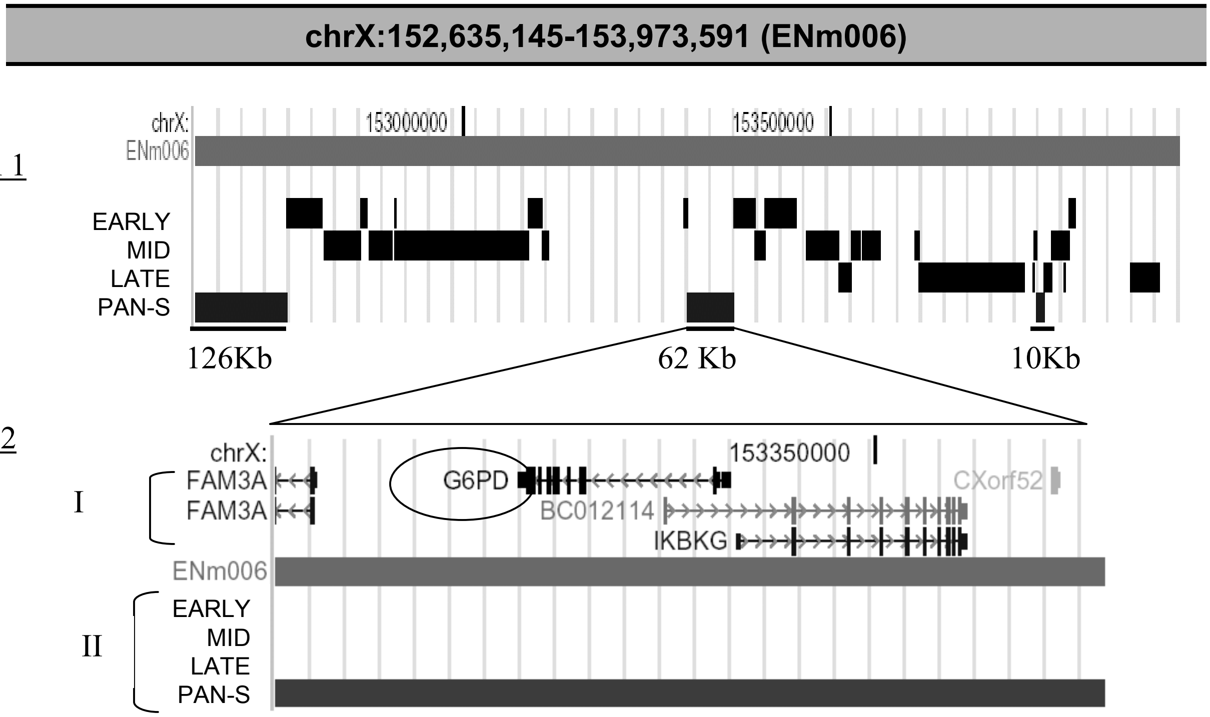
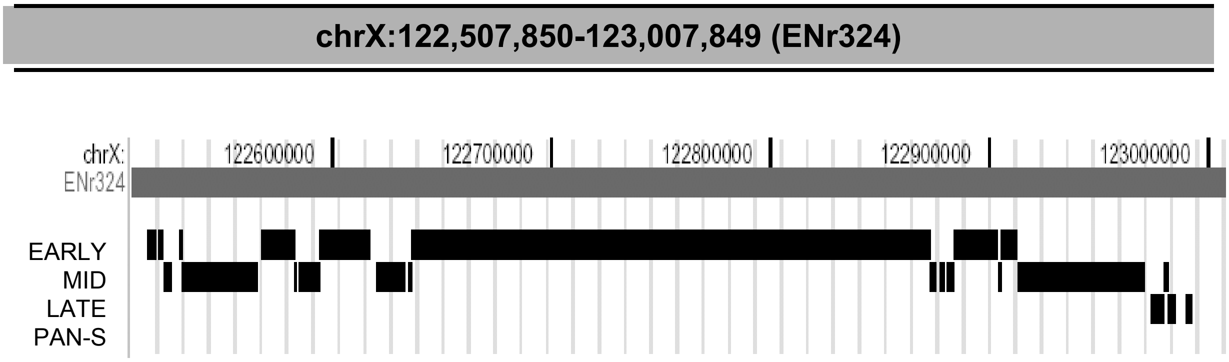
A.



B.



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Probe	**PM-MM Replication Score**						**TR50**	**Temporal Specificity**
	0-2hr	***2-4hr***	***4-6hr***	***6-8hr***	***8-10hr***	***Total***		
Probe 1	200	0	0	0	0	200	1.00hr	Specific
Probe 2	100	100	0	100	0	300	3.00hr	Non-Specific
Probe 3	0	100	100	50	0	250	4.50hr	Specific
Probe 4	80	60	0	0	100	240	3.33hr	Non-Specific

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ENCODE Region	FISH Clone	Chromosome No.	Chromosome Position	FISH RESULT			ARRAY RESULT
				HELA (APHI/THY)	HELA (NOC)	MCF10A (APHI/THY)	HELA (APHI/THY)
ENm001	RP11-51M22	7	115435013 - 115597337	EARLY	EARLY	EARLY	EARLY
ENm005	RP11-54F16	21	32694022 - 32849422	EARLY + MID			EARLY+MID
ENr331	RP11-316O14	2	220032424 - 220216048	EARLY	EARLY	LATE	EARLY + PANS
ENr112	RP11-18019	2	51942654 - 52109389	LATE			LATE
ENm005	RP11-79D9	21	33204657 - 33369197	LATE			MOSTLY LATE
ENr313	RP11-3I14	16	61098254 - 61253550	LATE	LATE	LATE	LATE
ENr111	RP11 90M5	13	29310416 - 29471953	LATE			LATE
ENm004	RP1-127L4	22	30829975 - 30877786	PAN S			PANS AND LATE
ENr132	RP11-88E10	13	112208159 - 112395315	PAN S	PANS	PAN S	MOSTLY PANS + SOME LATE
ENm008	RP11-243K18	16	266994 - 431803	PANS	PAN S	EARLY	MOSTLY PANS, SOME EARLY

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	Primer	Chromosome position	Amplicon size	Sequence (5’→3’)
1	005HM1F	chr21:32686521-32686813	293bp	GTATGAGTGTTGGGGGATGG
2	005HM1R			TGAGGTTCAGGACTGCTGTG
3	005HM2F	chr21:33022187-33022292	106bp	GTCACTTCCGCTCCAGCA
4	005HM2R			GTGGTCTCCCTCCTGATGC
5	005HM3F	chr21:33066160-33066267	108bp	GAGAGAGAGAGAGCCCCACA
6	005HM3R			TGACCTGTCAAACAACACCAG
7	005HM4F	chr21:33319521-33319737	217bp	CCACGTGTTGATGAAACCAG
8	005HM4R			AATACGGCCACCACAGAGTC
9	005HM5F	chr21:33363802-33364027	226bp	TTAATGGGTGGAGCTTCAGG
10	005HM5R			CCTTCCTCCAGCAACTGTCT
11	005HM6F	chr21:33618899-33619020	122bp	CATAGGCCGGAAAGAGTGAG
12	005HM6R			TAGCTAGCACCCCTTCTCCA
13	005HM7F	chr21:33836675-33836809	135bp	CATCCGGCCTATTGCTAAAA
14	005HM7R			GATATGCATCCGGGAGAGAA
15	005HM8F	chr21:33936279-33936457	179bp	TAGTGTCTCTCACGGCAACG
16	005HM8R			CAGAGTGACAAGCCGAATGA
17	005HM9F	chr21:34210077-34210183	107bp	CGGGCTGGGAGTTGTAGTT
18	005HM9R			CGCTTGGCCAGTAACAATG
19	132HM1F	chr13:112390183-112390371	189bp	GCAGCAGGATTTTGGTGAAT
20	132HM1R			AATGCTCCTTTTTCCCTGGT
21	132HM2F	chr13:112395710-112395881	172bp	GGGTGATGTATGTGCTGTCG
22	132HM2R			CAACACATACGCCAGTCCAC
23	132HM3F	chr13:112647902-112648128	227bp	GCCTTTTTGACCTTGAGCAG
24	132HM3R			TGTCAGTGCCAACCCAATTA