Description

This track displays replication timing and was produced as part of the ENCODE Project. Replication timing refers to the order in which DNA is duplicated during the synthesis phase of the cell cycle and is correlated with the expression of genes and the structure of chromosomes. This track shows genome-wide assessment of DNA replication timing in cell lines using NimbleGen tiling CGH microarrays. Each experiment represents the relative enrichment of early versus late S-phase nascent strands in a given cell line, with data represented as a loess-smoothed function of individual timing values at probes spaced at even intervals across the genome. Regions with high values indicate domains of early replication where initiation occurs earlier in S-phase.

Display Conventions and Configuration

The graph displays a wavelet-smoothed signal of mean early/late S-phase ratios. Metadata for a particular subtrack can be found by clicking the down arrow in the list of subtracks.

Methods

Experimental Procedures

Cells were grown according to the approved ENCODE cell culture protocols. Methods for replication timing profile creation and analysis are described in detail in Hiratani *et al*. (2008) and Ryba *et al*. (June 2011). Methods for individual stages of extraction, hybridization, scanning and processing are summarized below.

For the extraction protocol, replication timing data were obtained by hybridizing early and late replication intermediates to NimbleGen oligonucleotide arrays. Replication intermediates were prepared from cells that were first pulse-labeled with 5'-bromo-2'-deoxyuridine (BrdU) and then sorted into early and late stages of S-phase by flow cytometry, followed by anti-BrdU immunoprecipitation of the BrdU-substituted (nascent) replication intermediates newly synthesized either early or late during S-phase. Samples were labeled after unbiased amplification of recovered DNA by whole-genome amplification (WGA; Sigma, GenomePlex).

The hybridization set used the NimbleGen standard hybridization protocol. Cy3- and Cy5-labeled DNA samples (6 µg each) were co-hybridized to Nimblegen CGH arrays containing evenly-spaced oligonucleotide probes across the human genome, with a median probe spacing of 1.1-5.8 kb. No differences in smoothed data have been detected with probe densities from 100 bp to 5.8 kb. The NimbleGen MS 200 2 µm resolution scanner and GenePix software were used per NimbleGen's standard scanning protocol.

Data Processing

NimbleScan software was used to obtain .pair raw data per manufacturer's instructions. Raw early/late data (i.e. from .pair files) from two independent biological replicates, in which early- and late-replicating DNA were labeled reciprocally, were loess-normalized to remove signal intensity-dependent bias. The data were then scaled to a reference data set to have the same median absolute

deviation and then averaged (limma package, R/Bioconductor). The mean early/late ratios were used to generate a final smoothed profile (i.e. processed data) using local polynomial smoothing (loess, 300 kb span) for each chromosome using basic functions in the statistical language R.

Verification

Technical data quality was assessed by verifying high autocorrelation between neighboring timing values. Biological identity was confirmed by verifying consistent early or late replication by PCR at individual loci, as well as uniformity in replication profiles between replicate experiments.

Release Notes

This is Release 2 (July 2012) of this track. It adds 6 more data sets including additional replicates for H1-hESC and H7-hESC and all new data for the iPS skin fibroblast bio samples.

Credits

These data were generated by the Florida State University ENCODE group.

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References

Hiratani I, Ryba T, Itoh M, Rathjen J, Kulik M, Papp B, Fussner E, Bazett-Jones DP, Plath K, Dalton S *et al*. Genome-wide dynamics of replication timing revealed by in vitro models of mouse embryogenesis. *Genome Res*. 2010 Feb;20(2):155-69.

Hiratani I, Ryba T, Itoh M, Yokochi T, Schwaiger M, Chang CW, Lyou Y, Townes TM, Schübeler D, Gilbert DM. Global reorganization of replication domains during embryonic stem cell differentiation. *PLoS Biol*. 2008 Oct 7;6(10):e245.

Pope BD, Tsumagari K, Battaglia D, Ryba T, Hiratani I, Ehrlich M, Gilbert DM. DNA replication timing is maintained genome-wide in primary human myoblasts independent of D4Z4 contraction in FSH muscular dystrophy. *PLoS One*. 2011;6(11):e27413.

Ryba T, Battaglia D, Pope BD, Hiratani I, Gilbert DM. Genome-scale analysis of replication timing: from bench to bioinformatics. *Nat Protoc*. 2011 Jun;6(6):870-95.

Ryba T, Hiratani I, Lu J, Itoh M, Kulik M, Zhang J, Schulz TC, Robins AJ, Dalton S, Gilbert DM. Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types.*Genome Res.* 2010 Jun;20(6):761-70.

Ryba T, Hiratani I, Sasaki T, Battaglia D, Kulik M, Zhang J, Dalton S, Gilbert DM. Replication timing: a fingerprint for cell identity and pluripotency. *PLoS Comput Biol*. 2011 Oct;7(10):e1002225.

Data Release Policy

Data users may freely use ENCODE data, but may not, without prior consent, submit publications that use an unpublished ENCODE dataset until nine months following the release of the dataset. This date is listed in the *Restricted Until*column, above. The full data release policy for ENCODE is available here.