Genomics Data 4 (2015) 12–16

Contents lists available at ScienceDirect

Genomics Data

journal homepage: http://www.journals.elsevier.com/genomics-data/

Data in Brief

Data for chromosome contacts and matched transcription profiles at three cell cycle phases in the fission yeast

Ralph S. Grand [a,b], Justin M. O'Sullivan [a,*]

[a] Liggins institute, University of Auckland, Grafton Auckland 1032, New Zealand
[b] Institute of Natural and Mathematical Sciences, Massey University, Albany, Auckland 0745, New Zealand

ARTICLE INFO

Article history:
Received 22 December 2014
Accepted 13 January 2015
Available online 20 January 2015

ABSTRACT

The data described in this article pertains to Grand et al. (2014), "Chromosome conformation maps in fission yeast reveal cell cycle dependent sub nuclear structure" [1]. Temperature sensitive *Schizosaccharomyces pombe* cell division cycle (cdc) mutants, which are induced by a shift in temperature to 36 °C, were chosen for the analysis of genome structure in the G1 phase, G2 phase and mitotic anaphase of the cell cycle. Chromatin and total RNA were isolated from the same cell culture following synchronization. Two biological replicates were analyzed for each condition. The global, three-dimensional organization of the chromosomes was captured at high resolution using Genome Conformation Capture (GCC). GCC libraries and RNA samples were sequenced using an Illumina Hi-Seq 2000 platform (Beijing Genomics Institute (China)). DNA sequences were processed using the Topography suite v1.19 [2] to obtain chromosome contact frequency matrices. RNA sequences were processed using the Cufflinks pipeline [3] to measure gene transcript levels and how these varied between the conditions. All sequence data, processed GCC and transcriptome files are available under the Gene Expression Omnibus (GEO) accession number GSE52287 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52287).

Specifications	
Subject area	*Biology.*
More specific subject area	*Chromosome contacts and transcriptome datasets from fission yeast cells synchronized at three cell cycle phases.*
Type of data	*Fastq sequencing files, table (Excel spreadsheet), and text files.*
How data was acquired	*High throughput DNA sequencing using the Illumina Hi-Seq 2000.*
Data format	*Raw and analyzed.*
Experimental factors	*Fission yeast cultures were grown, synchronized by raising the temperature, and split. 4/5ths of the culture was cross-linked and chromosome contacts captured according to the Genome Conformation Capture (GCC) protocol. Total RNA was extracted from the remaining 1/5th of the culture and prepared for mRNA-Seq analysis.*
Experimental features	*Chromatin and RNA were isolated from fission yeast cells synchronized at three cell cycle phases (strains: G1 phase, cdc10-129; G2 phase, cdc25-220; and* mitotic anaphase, *nuc2-663).*
Data source location	*The University of Auckland, Auckland, New Zealand.*
Data accessibility	*All sequencing data and processed GCC and transcriptome files are available from Gene Expression Omnibus (GEO) accession number GSE52287.*

Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52287.

Value of the data

- Provides high resolution information about the three-dimensional organization of chromosomes in Fission yeast cells synchronized in G1 phase, G2 phase and mitotic anaphase of the cell cycle.
- Medium depth mRNA-Seq data from Fission yeast cells synchronized in G1, G2 and mitotic anaphase of the cell cycle.
- Presents an unique opportunity to investigate how variations in genome organization correlate with changes in genome function (*i.e.* transcription).

Experimental design, materials and methods

Objective of experiment

Our objective was to investigate how the three-dimensional organization of genomes changes through the cell cycle and whether the

* Corresponding author. Tel.: +64 9 923 9868.
E-mail address: justin.osullivan@auckland.ac.nz (J.M. O'Sullivan).

http://dx.doi.org/10.1016/j.gdata.2015.01.005

phase specific organization is related to the regulation of gene transcription. We set out to capture the three-dimensional organization of the fission yeast (*Schizosaccharomyces pombe*) genome and the transcriptome from cells synchronized at the G1, G2 and mitotic anaphase of the cell cycle. The selection of a consistent method to obtain highly synchronized cell populations at these different cell cycle phases was important because it minimized experimental variation due to environmental differences.

Choice of synchronization method

We considered a number of methods (*e.g.* centrifugal elutriation, lactose gradient, nitrogen deprivation, chemical treatment, and temperature sensitive cell division cycle mutants [4–8]) for obtaining populations of *S. pombe* cells synchronized at G1, G2, and mitotic anaphase of the cell cycle. Centrifugal elutriation and lactose gradient methods separate cells based on their size, allowing for the isolation of cell size fractions. In the case of *S. pombe* the smallest cells are in the G2 phase [5]. These G2 phase cells can be isolated and grown further to obtain subsequent cell cycle phases. Both centrifugal elutriation and lactose gradient methods minimally perturb the cells and produce reasonably high levels of G2 phase synchronized cells. However, the level of synchronization diminishes rapidly upon continued culturing.

The addition of chemicals (*e.g.* Thymidine treatment), or removal of nitrogen from the growth medium, can be used to produce high levels of synchronized *S. pombe* cells [4,8]. However, the varied effects of these treatments on the cell may confound the results and subsequent interpretation. Furthermore, as for the cell size selection methods, the cells must be released and cultured further to obtain cells synchronized in subsequent phases of the cell cycle. Alternatively, combinations of treatments could be used to isolate different cell cycle phases; however, the varied effects of the treatments would complicate the resulting data and interpretation.

Temperature sensitive *S. pombe* mutant cells become synchronized at specific stages of the cell cycle when they are shifted from a permissive to restrictive temperature for a defined period of time. These temperature sensitive mutants have been shown to have little influence on normal cell growth and produce cell populations with a high proportion of synchronized cells. To reduce the confounding effects of different synchronization methods/temperatures, we selected three mutant strains (*S. pombe* My291, MY284, and MY286) that are sensitive to the same temperature shift (Table 1) [6,7,9,10].

Strains, growth conditions and synchronization

S. pombe strains MY291 (h- lue1 cdc10-129), MY284 (h- lue1 cdc25-220) and MY286 (h- lue1 nuc2-663) (Table 1) were stored at −80 °C and recovered on YES [11] (2% agar) plates (26 °C, 4 days). YES medium (12 ml) starter cultures were inoculated and incubated (26 °C, 200 rpm) until the OD_{595} measured ~0.8 (after ~24 h). Synchronization cultures (125 ml EMM2 [11], in baffled flasks) were inoculated with starter culture to an OD_{595} = ~0.05 and incubated (26 °C, 120 rpm). Cultures were grown for four generations (OD_{595} ~ 0.8) before synchronization was induced by the addition of pre-warmed EMM2 medium (125 ml, 46 °C), instantly raising the temperature of the culture to the restrictive temperature (36 °C). Cultures were incubated in a hot water bath (36 °C, 140 rpm, for 4 h) to complete synchronization.

Table 1
Schizosaccharomyces pombe strains used in this study.

Strain Name	Genotype	Reference
MY291	h- lue1 cdc10-129	[10]
MY284	h- lue1 cdc25-22	[12]
MY286	h- lue1 nuc2-663	[6]

All strains were obtained from the National BioResource Project — Yeast (http://yeast.lab.nig.ac.jp/nig/index_en.html). Reprinted from Grand et al. 2014 [1].

Synchronization efficiency

Synchronization efficiency was checked using cell samples taken from cultures before induction and following synchronization. Cells were harvested by centrifugation (1 ml, 4000 rpm, 2 min) before being snap frozen (dry ice/ethanol (100%) bath) and stored at −20 °C until use. Cells were thawed, washed once with ice-cold 1% PBS (500 μl, 4000 rpm, 2 min) and suspended in PBS (100 μl). Cells were stained with calcofluor white (1 g/l with 10% potassium hydroxide) and DAPI (25 mg/ml) and photographs were taken of each sample using a fluorescence microscope (ZEISS, HBO 100 Axiostart plus). The level of cell cycle phase synchronization was calculated for the G1 and G2 phases by calculating the proportion of cells that had a septum, in >200 cells, in the synchronized cell populations and comparing it to the pre-synchronized populations (Fig. 1 and Table 2). The estimation of >80% synchronization for mitotic anaphase cells was based on the observation of characteristic traits described for cultures undergoing a *nuc2* arrest; increased septation index (from ~16% to ~50%), highly condensed chromosomes, and the presence of enucleate cells, following DAPI staining [6].

Chromatin isolation for genome conformation capture (GCC)

Chromatin isolation and GCC were performed as previously described [2,13], with the following modifications. Synchronized cultures (200 ml) were cross-linked with 1% formaldehyde (with shaking, 10 min, room temperature), quenched with glycine (125 mM; with shaking, 10 min, room temperature), washed twice and suspended in FA-lysis buffer (50 mM HEPES–KOH (pH 7.5), 140 mM NaCl, 1 mM EDTA (pH 8.0), 1% Triton-X100, and 0.1% SDS). To determine the number of cells in each sample, dilutions were counted with a hemocytometer. Aliquots of ~9.5 * 10 [8] cells were made up to a final volume of 330 μl with FA-lysis buffer in a 2 ml microfuge tube. Cell walls were digested with T20 Zymolyase (70 μl at 75 mg/ml; 35 °C, 40 min with periodic inversion) before the zymolyase was heat inactivated (60 °C, 5 min). Acid washed glass beads (425–600 μm, Sigma; 500 μl) were added to each sample and the cells were lysed in a Geno/Grinder (−20 °C; 1750 rpm, 2 × 30 s on 60 s off; SPEX sample prep 2010). The glass beads were removed by piercing the bottom of the tube with a 271/2 gauge needle and centrifuging the chromatin through the hole into a clean microfuge tube (2000 rpm, 1 min). Chromatin was pelleted (13,000 rpm, 15 min, 4 °C), washed with FA-lysis buffer, suspended in chromatin digestion buffer (500 μl; 10 mM Tris–HCl (pH 8.0), 5 mM $MgCl_2$, and 0.1% Triton-X100) and stored (−80 °C).

Each chromatin sample was treated with SDS (0.1% final concentration, 37 °C, 10 min) and quenched with TritonX-100 (1% final concentration). Chromatin samples were divided into ten sets of 9.5 *10 [7] cells and digested with AseI (100 U, New England Biolabs, 37 °C, 2 h) in a total volume of 100 μl. An external ligation control (sees below and Table 3) was added to the AseI digested chromatin, samples were diluted (~20-fold) and ligated with T4 DNA ligase (20 U, Invitrogen, 16 °C, 4 h). Following ligation, cross-links and protein were removed by incubation at 65 °C (16 h) in the presence of high salt and Proteinase K (5 mM EDTA (pH 8.0), 30 mM NaCl, 0.6 mM Tris–HCl (pH 7.5) and 10 μg Proteinase K) before the removal of RNA by treatment with RNaseA (20 μg, 37 °C, 15 min). pUC19 plasmid (27.4 pg/2 ml) was added as a sequence library preparation ligation control before the GCC libraries were purified by three extractions with phenol:chloroform (1:1) and a final purification using a DNA clean and concentrator kit (Zymo Research, according to the manufacturer's instructions). 3 μg of each GCC library was sent for paired-end sequencing (50 bp, Illumina Hi-Seq platform, BGI China).

Production of external ligation controls for GCC library preparation

External ligation controls containing an AseI restriction enzyme site at one end were PCR amplified from the *Escherichia coli* genome,