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Data in Brief

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

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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

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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,

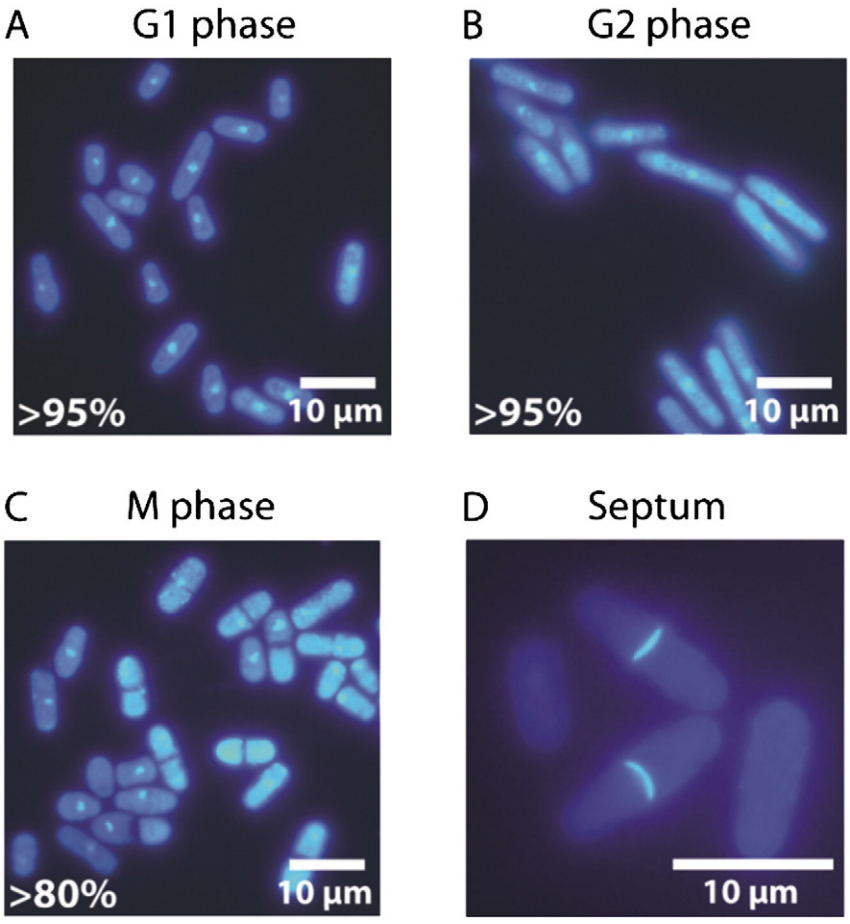

Fig. 1. Representative images of synchronized cells. Micrographs (400×) of calcofluor stained *S. pombe* cells were taken pre- and post-synchronization. Representative images of: (A) G1 phase; (B) G2 phase; and (C) mitotic anaphase synchronized cells. The numbers of visible septa (D, 1000×) were counted in >200 cells (total) from 10 fields of view. Cell cycle phase synchronization was calculated for the G1 and G2 phases by comparing the proportion of cells that had a visible septum in the pre-synchronized and synchronized cell populations. Percentages indicate the minimal level of synchronization obtained for both biological replicates at each cell cycle phase.
Adapted from Grand et al. 2014 [1].

Lambda phage genome and pRS426 plasmid (Table 3) [13]. PCR amplicons were digested with Ase1, purified (DNA clean and concentrator kit; Zymo Research), and shown to be ligation proficient. The purified ligation proficient PCR products ($9.5 * 10$ [7] copies, *i.e.* one fragment per genome copy used to make the GCC library) were introduced into the GCC samples (*i.e. E. coli*: G1 phase, pRS426: G2 phase, Lambda: M phase) prior to the ligation step of the GCC protocol. The addition of these controls enables the estimate of random inter-molecular ligation events during the GCC library preparation. Following sequencing, only one ligation event was detected between the pRS426 ligation control and an AseI fragment in one of the G2 phase biological replicate.

The pUC19 that was added to the purified GCC libraries prior to sending them for sequencing, controls for inter-molecular ligation events that occur during the addition of the sequencing adaptors. A number of ligation events were detected between the *S. pombe* genome and the pUC19 control (G1 phase: 14, G2 phase: 7, and M phase: 2), indicating that intermolecular ligation events occurred during preparation for sequencing at the BGI.

Table 2
The synchronization efficiency for each of the G1 and G2 cell cycle phase biological replicates was calculated by comparing the proportion of cells with a septum before and after synchronization.

G2 phase (cdc25-22) biological replicate #1		
	Before synchronization	After synchronization
Total cells counted	225	204
Number with a visible septum	48	2
Percentage	21.33	0.98
Synchronization efficiency	$100 - ((0.98 / 21.33) \times 100) = 95.41\%$	

An example calculation of the cell culture synchronization efficiency for one of the G2 phase biological replicates is shown. Reprinted from Grand et al. 2014 [1].

Table 3
PCR primers for external ligation controls used in this study.

Primer name	Sequence	Length of product (bp)
E.coli191bp3′AseIF	TAGGCAGGATAAGGCGTTCA	191
E.coli191bp3′AseIR	GTGATTAATGCGGTCTGATGAGTCGTTTC	
pRS426_185bp3′AseIF	TTGGTCTGACAGTTACCAATGC	185
pRS426_185bp3′AseIR	GTGATTAATGATAAATCTGGAGCCGGTGA	
Lambda187bp3′AseIF	TTTACAGCGTGATGGAGCAG	187
Lambda187bp3′AseIR	GTGATTAATACCAATCCAGCCGGTCAG	

Three short DNA sequences were amplified from the *E. coli* genome, pRS426 plasmid, and Lambda phage DNA for use as external ligation controls. An AseI site (red) was introduced into each amplicon within the reverse (AseIR) primer sequence. PCR amplicons were purified, digested with AseI and introduced into the GCC samples (at a 1:1 ratio with genome/cell number) before ligation to control for random inter-molecular ligation events. Reprinted from Grand et al. 2014 [1].

Table 4
The number of chromosomal loci that had the highest (top 5%) and lowest (bottom 5%) transcript levels at each cell cycle phase.

Cell cycle phases	G1 phase	G2 phase	M phase
Number of chromosome loci that contained genes with high transcript levels	178	182	179
Number of chromosome loci that contained genes with low transcript levels	181	189	186

Following analysis by Cufflinks, the genes that had the highest (top 5%) and lowest (bottom 5%, excluding genes that were not expressed) transcript levels were determined for each phase of the cell cycle. Reprinted from Grand et al. 2014 [1].

Network assembly

GCC networks were constructed from 50 bp paired-end Illumina Genome Analyser sequence reads using the Topography suite v1.19 [2]. Topography uses the SOAP algorithm [14] to position paired-end sequence tags and single ends which contain an AseI restriction enzyme site onto the reference genome. In this instance, the reference genome consisted of the *S. pombe* genome sequence (ASM294v2), and the *E. coli*, pRS426, Lambda phage and pUC19 (SYNPUC19CV) ligation control sequences. No mismatches or unassigned bases (N) were allowed during positioning. The sequences and analyses are available at (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52283).

Unless explicitly stated, all bioinformatics analysis was performed on significant (≥FDR cut-off; see below), uniquely positioned, non-adjacent (only interactions between restriction fragments that were not adjacent to each other in the linear sequence) interactions data using in house Perl and Python scripts. Except where indicated, statistical analyses were performed using R and Venn-diagrams were drawn with the Vennerable package [15].

FDR cut-off calculations

Random ligation events can occur during the two ligation steps in the GCC protocol: 1) the ligation of the cross-linked fragments; and 2) linker addition during preparation for sequencing. We employed two methods for the identification of significant interactions: 1) a statistical method that calculates a false detection rate (FDR) cut-off as in [2]; and 2) the external ligation controls during the GCC library preparation allowed us to measure the rates of intermolecular ligation events. Only one intermolecular ligation event was detected and it occurred at a frequency below the calculated FDR cut-off (≥3). Therefore, we determined our significance cut-off to be ≥3 using the statistical method described in [2].

RNA extraction

For RNA extraction, cells were harvested from 12 ml of each synchronized cell culture prior to cross-linking (4000 rpm, 2 min, RT), washed with 5 ml of AE buffer (50 mM sodium acetate, 10 mM EDTA, pH 5.3), and suspended in AE buffer (400 μl). Cell suspensions were transferred to tubes containing an equal volume of phenol/chloroform/isoamyl alcohol (24/24/1 Ambion) and 400 μl of acid washed glass beads (Invitrogen). Tubes were transferred to a chilled block (−20 °C) in a Geno/Grinder (SPEX sample prep 2010) and the cells were lysed (1750 rpm, 8 × 30 s on 60 s off). Lysis was completed by a freeze thaw (−80 °C, ~15 min) before centrifugation (14,500 rpm, 5 min, 4 °C). The aqueous phase was extracted three times with an equal volume of phenol/chloroform/isoamyl alcohol. RNA was isolated by precipitation with 1/10th volume ammonium acetate (5 M, pH 5.3) and two volumes of 100% ethanol at −80 °C (>30 min/over-night) before centrifugation (14,500 rpm, 10 min, 4 °C). RNA was washed with 70% ethanol (350 μl; 14,500 rpm, 5 min, 4 °C) and air dried (37 °C, ~15 min). RNA pellets were suspended in RNASecure (80 μl; Ambion) and dissolved by heating (60 °C, 10 min). The RNA concentration was determined by Nano-Drop (ACTGene ASP-3700) and 2 μg of each sample visually inspected following electrophoresis through a 1% (w/v) agarose gel. RNA was stored at −80 °C before RNA sequencing (BGI China, 90 bp paired-end RNA sequencing analysis). The RNA integrity numbers (RINs) for the RNA samples were: G1-rep1, 9.6; G1-rep2, 9.6; G2-rep1, 9.4; G2-rep2, 8.8; M-rep1, 9.6; M-rep2, 9.5.

Transcriptome analysis

RNA sequences (90 bp) were quality assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). To maximize the quality of the sequence reads, 10 bp was trimmed off either end of the sequences using fastx_trimmer (http://hannonlab.cshl.edu/fastx_toolkit/index.html) resulting in final sequence lengths of 70 bp.

The identification of differentially expressed genes was performed using cufflinks [3] to analyze the trimmed RNA sequences as a time course (G1 → G2 → M → G1). This enabled the isolation of effects due to the temperature shift, thus maximizing the chances of identifying genes that are differentially regulated during each cell cycle transition.

Briefly, trimmed RNA-seq reads were aligned to the *S. pombe* reference genome (ASM294v2) using Tophat version 2.0.7 (http://tophat.cbcb.umd.edu/) without providing the *S. pombe* GTF file. This allowed for novel transcript discovery. Aligned reads were assembled for differential expression analysis using cufflinks version 2.0.2 (http://cufflinks.cbcb.umd.edu/) and merged using cuffmerge (http://cufflinks.cbcb.umd.edu/manual.html#cuffmerge) with an "assemblies" file containing the transcripts.gtf output files from cufflinks for the two biological replicates of each cell cycle phase in the order G1–G2–M–G1. Finally, differential expression analysis was performed using the merged.gtf output

Table 5
Numbers of *S. pombe* genes that were significantly differentially regulated during each cell cycle transition.

Cell cycle phase transitions	G1 → G2 phase	G2 → M phase	M → G1 phase
Total number of genes differentially expressed	198	346	239
Number of significantly upregulated genes	102	138	150
(percentage of total)	(51.51%)	(39.88%)	(62.76%)
Number of significantly downregulated genes	96	208	89
(Percentage of total)	(48.49%)	(60.12%)	(37.24%)
Genes with a fold change in transcript level ≥2	91	142	70
Number of genes upregulated (cut-off ≥2)	77	46	26
(Percentage of total)	(84.62%)	(32.39%)	(37.14%)
Number of genes downregulated (cut-off ≥ −2)	14	96	44
(Percentage of total)	(15.38%)	(67.61%)	(62.86%)

RNA-seq data was analyzed using Cufflinks [3,16] to identify genes that were significantly up- and downregulated during each *S. pombe* cell cycle transition: G1 → G2 phase, G2 → M phase, and M → G1 phase. The total number of genes, and those that had a ≥2-fold change in transcript level, are displayed. Reprinted from Grand et al. 2014 [1].

file from cuffmerge, the –T operator, and the accepted_hits.bam output files from tophat in the time series order G1–G2–M–G1.

The raw transcript levels for genes in individual biological replicates were highly correlated ($R2 > 0.91$). For downstream analyses, transcription data sets were divided into: 1) genomic regions that were in the top and bottom 5% of transcript levels in each cell cycle phase (Table 4 and http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52284); and 2) genomic regions whose transcript levels were differentially regulated during the three cell cycle transitions (G1 → G2, G2 → M, and M → G1) (Table 5 and http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52284).

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References

[1] R.S. Grand, et al., Chromosome conformation maps in fission yeast reveal cell cycle dependent sub nuclear structure. Nucleic Acids Res. (2014) 1–15. http://dx.doi.org/10.1093/nar/gku965 ("Breakthrough artical").
[2] C.D.M. Rodley, F. Bertels, B. Jones, J.M. O'Sullivan, Global identification of yeast chromosome interactions using Genome conformation capture. Fungal Genet. Biol. 46 (2009) 879–886.
[3] C. Trapnell, et al., Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 7 (2012) 562–578.
[4] D.B.F. Faraday, et al., Synchronization studies on *Schizosaccharomyces pombe*. *Inst. Chem. Eng. Symp. Ser*, 1994, pp. 154–156.
[5] G.M. Walker, Synchronization of yeast cell populations. Methods Cell Sci. 21 (1999) 87–93.
[6] T. Hirano, Y. Hiraoka, M. Yanagida, A temperature-sensitive mutation of the *Schizosaccharomyces pombe* gene nuc2+ that encodes a nuclear scaffold-like protein blocks spindle elongation in mitotic anaphase. J. Cell Biol. 106 (1988) 1171–1183.
[7] P.A. Fantes, P. Nurse, Control of the timing of cell division in the fission yeast. 115 (1978) 317–329.
[8] E.B. Gómez, S.L. Forsburg, Analysis of the fission yeast *Schizosaccharomyces pombe* cell cycle. Methods Mol. Biol. 241 (2004) 93–111.
[9] P. Nurse, Genetic control of cell size at cell division in yeast. Nature 256 (1975) 547–551.
[10] S.J. Aves, B.W. Durkacz, A. Carr, P. Nurse, Cloning, sequencing and transcriptional control of the Schizosaccharomyces pombe cdc10 "start" gene. EMBO J. 4 (1985) 457–463.
[11] S.A. Sabatinosa, S.L. Forsburga, Methods Enzymol, 2010, pp. 759–795.
[12] P. Nurse, P. Thuriaux, K. Nasmyth, Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. 146 (1976) 167–178.
[13] C. Cagliero, R.S. Grand, M.B. Jones, D.J. Jin, J.M. O'Sullivan, Genome conformation capture reveals that the *Escherichia coli* chromosome is organized by replication and transcription. Nucleic Acids Res. 41 (2013) 6058–6071.
[14] R. Li, Y. Li, K. Kristiansen, J. Wang, SOAP: short oligonucleotide alignment program. Bioinforma. Appl. Note 24 (2008) 713–714.
[15] R Development Core Team, R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2008, ISBN 3-900051-07-0. (URL http//www.R-project.org).
[16] C. Trapnell, et al., Differential analysis of gene regulation at transcript resolution with RNA-seq. Nat. Biotechnol. 31 (2012) 46–53.