Materials List for:

Genome-wide Purification of Extrachromosomal Circular DNA from Eukaryotic Cells

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Materials

Name	Company	Catalog Number	Comments
Bacto peptone	BD Difco	211677	Alternative product can be used.
Brilliant III SYBR Green PCR Master Mix	Agilent Technologies	600882	For qPCR analysis. Alternative product can be used.
Dextrose (D-glucose)	Carl Roth	HN06.4	Alternative product can be used.
Disruptor Beads, 0.5 mm	Scientific Industries, Inc.	SI-BG05	Glass beads to disrupt plasma cell membranes. Alternative product can be used.
Ethidium bromide	Carl Roth	2218.2	Agarose gel stain for detecting DNA/RNA.
GeneJet plasmid miniprep kit	Thermo Fisher	K0502	Plasmid purifcation from bacteria. Alternative product can be used.
NotI, FastDigest	Life Technologies - Thermo Fisher Scientific, USA	FD0594	Endonuclease. Alternative product can be used.
Plasmid Mini AX kit	A&A Biotechnology, Poland	010-50	Plasmid purifcation kit used to purify eccDNA.
Plasmid-Safe ATP-dependent DNase kit	Epicentre, USA	E3105K	ATP-dependent exonuclease kit. Alternative product can be used.
Propidium iodide	Sigma-Aldrich, USA	81845	Alternative product can be used.
pUG6 plasmid	EUROSCARF, Germany	P30114	Marker gene: loxP-PAgTEF1-kanMX-TAgTEF1-loxP.
QIAGEN genomic-tip 100/G	Qiagen, USA	13343	Genomic DNA purifcation from yeast. Alternative product can be used.
REPLI-g Mini Kit protocol	Qiagen, USA	150023	Amplification of eccDNA by the phi29 polymerase.
Yeast extract	BD Difco	210929	Alternative product can be used.
Zymolyase 100T (Lyticase, Yeast Lytic Enzyme)	Nordic BioSite, Sweden	Z1004-3	Alternative product can be used.
Data access to sequence files	European Nucleotide Archive		EccDNA dataset from *Saccharomyces cerevisiae* CEN.PK113-7D. Study accession number PRJEB9684. 2nd accession number is ERP010820. Locus tag prefix is BN2032.
Name	**Company**	**Catalog Number**	**Comments**
Strains			

Saccharomyces cerevisiae CEN.PK113-7D			Genotype MATa MAL2-8c SUC2
Saccharomyces cerevisiae yeast deletion library pool	EUROSCARF, Germany		S288c BY4741 pool of 4400 viable single-gene deletion mutants disrubted by KanMX module. Genotypes *MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 genexxx::KanMX*.
Name	**Company**	**Catalog Number**	**Comments**
Equipments			
DNA Spectrophotometer	NanoDrop 1000 Spectrophotometer, Thermo Fisher		Measuring DNA concentration. Alternative product can be used.
Fluorescence microscopy	Nikon Optronics Magnafire. Red excitation fluorescence filter, 663-738 nm.		Alternative product can be used.
Robotic library-build system	Apollo 324, IntegenX Inc.		DNA library preparation. Alternative product can be used.
Sequencing platform	Illumina HiSeq 2000 platform, Illumina Inc.		DNA sequencing. Alternative product can be used.
Ultrasonicator	Covaris LE220, microTUBE AFA Fiber tubes		Alternative product can be used.
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Methods			
2% YPD media			Mix 10 g Dextrose, 10 g Yeast extract, 20 g Bacto peptone and add H2O to a total volume of 1000 ml and autoclave.
Circle-Seq test on genomic DNA			Genomic DNA was purified (Qiagen) from a pool of the yeast deletion library (Euroscarf). The DNA concentration was measured by nanodrop and 30 µg genomic DNA was pipetted into two micro centrifuge tubes. One micro centrifuge tube was supplemented with 100 nanogram plasmid (pUG6). The DNA samples were purified by Circle-Seq, omitting the protocol steps 1.1-1.3 and 1.5-1.7. The eluted DNA concentrations were measured by nanodrop and the entire DNA yield from sample GD and GD+P was treated with exonuclease for a period of 29 hours. A 10% fraction was collected for phi29-amplification and PCR analysis, while the remaining DNA was subjected to 72 hour exonuclease treatment. The samples were analyzed for linear DNA content by PCR, using the *ACT1* gene as chromosomal marker. A 5% fraction of each of the exonuclease treated samples was amplified by the phi29 DNA polymerase for 16 hours (Qiagen). The presence of DNA in each sample was examined by loading an equal amount (7 µl) in wells on an 0.5 µg/ml ethidium-bromide 0.9% agarose gel after running gel-electrophoresis.

Mapping software	Bowtie2 aligner, John Hopkins University		Ultrafast short read alignment. Reference: 29.
Propidium iodide stain			Images of propidium iodine stained DNA were captured by fluorescence microscopy at 100x magnification (100x/1.30 oil, Nikon) in the RFP channel (red excitation fluorescence filter, 663-738 nm) using identical exposition time (5 seconds).
Workflow bioinformatic system	Galaxy, Open source.		A free web-based platform for data intensive biomedical research. References: 27-28.