

Supplemental Material

Characterization and molecular analysis of the viable but nonculturable state of a yeast *Candida* sp. strain LN1 induced by high phenol concentrations

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Table S1 Information and quality of Illumina whole-genome sequencing of the strain LN1.

Sample	R1				R2			
	R1_reads	R1_bases	Q20	Q30	R2_reads	R2_bases	Q20	Q30
LN1	5350439	794571467	0.9867	0.9529	5350439	792751210	0.9800	0.9346

Table S2 Genome assembly and annotation statistics of LN1 and the most closely related strain MYA-3404.

	LN1	MYA-3404
Assembly statistics		
Assembly length (≥ 1000 bp)	14463479	14630139
Contigs (≥ 1000 bp)	660	24
Scaffold N50 (bp)	61951	1654078
Scaffold L50	72	4
Scaffold N75 (bp)	29321	1255791
Scaffold L75	158	6
GC content of genome (%)	33.06	33.15
N's per 100 kbp	0	372.79
Gene statistics		
Number of genes	6504	6441
Number of exons	7034	6475
Number of introns	530	34
Number of CDS	6504	6254
Total gene length	9369400	9122989
Gene length (bp, average)	1441	1416
CDS length (bp, average)	1428	1455
Exon Length (bp, average)	1320	1408
Intron length (bp, average)	161	201
% of genome covered by genes	64	62.4
% of genome covered by CDS	63.4	62.2
Functional annotations		
Genes with KEGG annotation	3448	3322
Genes with Pfam domain	4403	4296
Genes with SignalP peptide	417	429

Table S3 Raman bands observed in spectra of LN1 cells and tentative assignment.

Wavelength (cm^{-1})	Assignment	Reference
1125	(COC) Symmetric glycosidic link (C–N) and n (C–C)	(1, 2)
1338	AMP, GMP, as well as to the aromatic amino acids tyrosine and tryptophan	(3)
1445	CH ₂ bending mode of proteins and lipids	(4, 5)
1575	DNA bases guanine and adenine	(1, 5)
1665	Amide I	(5, 6)
2935	stretching vibrations of CH ₂ and CH ₃	(5)

Table S4 An overview of the Illumina high throughput RNA-sequencing statistics.

R1							
sample	sequence	base	min_len	max_len	avg_len	Q20	Q30
CG1	17616833	2617696439	36	150	148.59	0.9855	0.95
CG2	18486034	2745932896	36	150	148.54	0.9854	0.9498
CG3	22795240	3386672538	36	150	148.57	0.9856	0.9501
TG1	18235369	2713360377	36	150	148.8	0.986	0.9502
TG2	17632919	2618253145	36	150	148.49	0.9849	0.9473
TG3	18412994	2735302269	36	150	148.55	0.985	0.9473
R2							
sample	sequence	base	min_len	max_len	avg_len	Q20	Q30
CG1	17616833	2624025923	36	150	148.95	0.9845	0.9433
CG2	18486034	2753681348	36	150	148.96	0.9848	0.9443
CG3	22795240	3393169959	36	150	148.85	0.9834	0.9402
TG1	18235369	2703368861	36	150	148.25	0.9756	0.92
TG2	17632919	2612522548	36	150	148.16	0.9775	0.9249
TG3	18412994	2725237698	36	150	148.01	0.9752	0.9192

Table S5 98 up-regulated genes with expression ration of $|\log_2 \text{TG/CG}| > 3.0$.

Gene ID	TG_base Mean	CG_base Mean	log₂ ratio	Regula tion	Annotation
LN1_1979.t	798.75	2.20	8.56	Up	Spore wall maturation protein DIT1
LN1_1980.t	473.11	1.82	7.95	Up	Cytochrome P450
LN1_1648.t	39.96	0.34	6.82	Up	Unknown
LN1_5013.t	39.96	0.34	6.82	Up	Unknown
LN1_2044.t	564.04	6.18	6.52	Up	MFS antiporter QDR1
LN1_4912.t	385.05	4.86	6.29	Up	Mitogen-activated protein kinase
LN1_2090.t	1406.42	18.98	6.21	Up	Putative aryl-alcohol dehydrogenase
LN1_2615.t	2538.57	38.06	6.06	Up	Unknown
LN1_5210.t	344.72	6.71	5.68	Up	Unknown
LN1_1174.t	33.39	0.80	5.55	Up	Unknown
LN1_2355.t	65.81	1.42	5.54	Up	Unknown
LN1_2756.t	16612.34	408.69	5.35	Up	Catabolic L-serine/threonine dehydratase
LN1_0877.t	750.57	19.33	5.28	Up	Unknown
LN1_4976.t	1141.84	31.09	5.20	Up	Unknown
LN1_5864.t	8932.33	281.09	4.99	Up	Unknown
LN1_3339.t	1230.73	40.16	4.94	Up	Multidrug resistance protein 1
LN1_3902.t	103.11	3.81	4.77	Up	Unknown
LN1_2724.t	947.72	36.00	4.72	Up	Putative NADPH-dependent methylglyoxal reductase GRP2
LN1_5742.t	74.72	3.02	4.62	Up	Iron transport multicopper oxidase FET3
LN1_4934.t	31846.92	1307.95	4.61	Up	Transaminated amino acid decarboxylase
LN1_5442.t	450.64	19.01	4.56	Up	Unknown
LN1_1843.t	56.16	2.65	4.40	Up	Vitamin H transporter 1
LN1_4585.t	91.10	4.69	4.33	Up	Unknown
LN1_2664.t	67.86	3.48	4.30	Up	Lipase 8
LN1_4596.t	7594.43	401.32	4.24	Up	Probable quinate permease
LN1_0275.t	36.17	2.01	4.16	Up	Transcriptional regulator EFH1
LN1_4493.t	1059.11	59.81	4.15	Up	DNA polymerase alpha catalytic subunit A
LN1_1981.t	3839.00	223.09	4.10	Up	Dityrosine transporter 1
LN1_1286.t	139.00	8.11	4.10	Up	Unknown
LN1_4412.t	183.22	11.28	4.03	Up	Azole resistance protein 1
LN1_3646.t	2098.44	129.48	4.02	Up	Ribonucleoside-diphosphate reductase large chain 1
LN1_2483.t	250.57	15.81	3.99	Up	Unknown
LN1_0574.t	428.04	27.99	3.94	Up	Structural maintenance of chromosomes protein 3
LN1_1056.t	326.39	21.53	3.92	Up	DNA primase large subunit
LN1_6310.t	331.30	22.28	3.90	Up	Serine/threonine-protein kinase RAD53

LN1_6464.t	44.68	3.00	3.84	Up	Unknown
LN1_3058.t	265.39	19.23	3.79	Up	Unknown
LN1_1326.t	1046.26	78.64	3.73	Up	Proliferating cell nuclear antigen
LN1_0905.t	418.30	31.77	3.72	Up	DNA polymerase alpha subunit B
LN1_4399.t	451.45	34.68	3.70	Up	Unknown
LN1_5172.t	4999.62	386.22	3.70	Up	Unknown
LN1_1473.t	161.70	12.98	3.66	Up	Uracil-DNA glycosylase
LN1_3139.t	12705.67	1043.78	3.61	Up	Glycine dehydrogenase
LN1_3140.t	12706.01	1044.22	3.60	Up	Glycine dehydrogenase
LN1_5184.t	457.79	38.00	3.59	Up	Threonylcarbamoyl-AMP synthase
LN1_1827.t	114.54	9.66	3.59	Up	Unknown
LN1_1230.t	429.59	35.94	3.58	Up	Vacuolar basic amino acid transporter 5
LN1_5009.t	20373.34	1727.90	3.56	Up	Cell wall acid trehalase ATC1
LN1_2751.t	225.46	19.14	3.56	Up	Unknown
LN1_3407.t	114.28	9.70	3.55	Up	Unknown
LN1_3065.t	89.34	7.74	3.52	Up	Unknown
LN1_6363.t	939.35	81.82	3.52	Up	Deoxyuridine 5'-triphosphate nucleotidohydrolase
LN1_5867.t	388.78	34.01	3.51	Up	Pirin-like protein
LN1_2614.t	182.93	16.02	3.51	Up	Agglutinin-like protein 3
LN1_6316.t	117.84	10.74	3.48	Up	Oligopeptide transporter 2
LN1_3717.t	713.63	64.42	3.47	Up	Mediator of replication checkpoint protein 1
LN1_3722.t	1323.06	120.14	3.46	Up	Vacuolar basic amino acid transporter 5
LN1_2212.t	162.74	14.88	3.45	Up	Unknown
LN1_1168.t	49.65	4.71	3.44	Up	Unknown
LN1_2504.t	614.68	56.84	3.44	Up	Histone chaperone ASF1
LN1_1129.t	236.67	22.25	3.41	Up	Unknown
LN1_0988.t	966.02	90.68	3.41	Up	DNA topoisomerase 2
LN1_1130.t	236.34	22.25	3.41	Up	Unknown
LN1_2211.t	162.73	15.57	3.39	Up	Unknown
LN1_1532.t	1171.17	112.19	3.38	Up	Probable metabolite transport protein GIT1
LN1_0210.t	37.75	3.57	3.36	Up	Cell wall protein RHD3
LN1_5188.t	460.49	45.14	3.35	Up	Unknown
LN1_0725.t	82.72	8.33	3.33	Up	Unknown
LN1_4865.t	73.32	7.54	3.30	Up	Protein ATC1
LN1_2072.t	911.51	93.10	3.29	Up	Replication factor A protein 2
LN1_4282.t	49.59	5.14	3.28	Up	Probable ferric reductase transmembrane component
LN1_5107.t	206.91	21.30	3.28	Up	Unknown
LN1_2776.t	31.18	3.27	3.27	Up	Unknown
LN1_4457.t	375.46	39.14	3.26	Up	DNA replication licensing factor MCM6
LN1_1717.t	115.13	12.25	3.23	Up	Unknown

LN1_0672.t	309.89	33.45	3.21	Up	Unknown
LN1_1558.t	1138.26	125.62	3.18	Up	Putative aryl-alcohol dehydrogenase
LN1_4939.t	103.49	11.42	3.18	Up	Pheromone alpha factor receptor
LN1_5738.t	374.93	41.77	3.17	Up	Unknown
LN1_5040.t	955.92	107.43	3.15	Up	Unknown
LN1_4553.t	521.04	59.01	3.14	Up	Unknown
LN1_2314.t	5782.71	663.68	3.12	Up	Probable pyridoxal 5'-phosphate synthase subunit SNO3
LN1_1684.t	181.45	20.79	3.12	Up	Unknown
LN1_5136.t	95.42	11.01	3.11	Up	Unknown
LN1_6246.t	1116.05	130.02	3.10	Up	Unknown
LN1_0591.t	508.80	59.35	3.10	Up	ATP-dependent DNA helicase PIF1
LN1_1425.t	204.58	24.23	3.08	Up	Unknown
LN1_0291.t	260.31	31.00	3.07	Up	Beta-glucosidase 2
LN1_1123.t	424.61	51.10	3.05	Up	Kinesin-like protein CIN8
LN1_1018.t	97.52	12.00	3.04	Up	Unknown
LN1_4483.t	10797.54	1318.34	3.03	Up	Low-specificity L-threonine aldolase
LN1_6456.t	122.16	14.93	3.02	Up	Unknown
LN1_4311.t	31.66	3.97	3.02	Up	Unknown
LN1_5319.t	862.58	107.06	3.01	Up	Flap endonuclease 1
LN1_3881.t	39.33	4.87	3.01	Up	Unknown
LN1_4842.t	166.16	20.62	3.00	Up	Unknown
LN1_6172.t	698.84	88.44	2.98	Up	specific cyclin CCN1
LN1_5858.t	122.97	15.78	2.97	Up	Unknown

Table S6 50 down-regulated genes with expression ration of $|\log_2 \text{TG/CG}| > 3.0$.

Gene ID	TG_bas eMean	CG_base Mean	log2 ratio	Regula tion	Annotation
LN1_2214.t	23.93	1103.92	-5.53	Down	Proline-specific permease
LN1_2804.t	6.82	275.67	-5.34	Down	Unknown
LN1_2803.t	7.15	276.02	-5.27	Down	Unknown
LN1_0298.t	302.89	10989.41	-5.18	Down	Unknown
LN1_6026.t	37.21	1302.91	-5.13	Down	Unknown
LN1_6149.t	53.29	1710.98	-5.01	Down	Unknown
LN1_0836.t	32.12	1007.85	-4.97	Down	High-affinity nicotinic acid transporter
LN1_0835.t	32.45	1008.19	-4.96	Down	High-affinity nicotinic acid transporter
LN1_2988.t	328.37	10164.95	-4.95	Down	Opaque-phase-specific protein OP4
LN1_3779.t	159.77	4857.62	-4.93	Down	Unknown
LN1_2345.t	103.00	2657.92	-4.69	Down	Probable family 17 glucosidase SCW4
LN1_1885.t	53.49	1343.34	-4.65	Down	Unknown
LN1_0426.t	188.09	3612.13	-4.26	Down	Unknown
LN1_0198.t	97.90	1799.42	-4.20	Down	Maltose permease MAL61
LN1_0297.t	680.81	12003.93	-4.14	Down	Unknown
LN1_3601.t	49.75	727.47	-3.87	Down	Allantoin permease OS
LN1_0462.t	62.61	841.53	-3.75	Down	Phenol 2-monooxygenase
LN1_2684.t	227.13	3000.72	-3.72	Down	Unknown
LN1_3074.t	326.86	4071.23	-3.64	Down	Unknown
LN1_0061.t	2.91	35.91	-3.63	Down	Unknown
LN1_1413.t	1122.46	13396.64	-3.58	Down	Unknown
LN1_2055.t	537.24	6314.34	-3.56	Down	Spermidine synthase
LN1_1933.t	2.30	26.74	-3.55	Down	Unknown
LN1_0757.t	1239.24	14147.21	-3.51	Down	Unknown
LN1_0327.t	6365.88	72420.19	-3.51	Down	Unknown
LN1_1614.t	99.83	1107.08	-3.47	Down	Sulfate adenylyltransferase
LN1_3475.t	117.50	1269.26	-3.43	Down	Glutathione S-transferase 1
LN1_6301.t	16.48	177.07	-3.43	Down	Accumulation of dyads protein 2
LN1_0066.t	9306.64	99175.81	-3.41	Down	Acetyl-coenzyme A synthetase 1
LN1_4337.t	461.18	4873.49	-3.40	Down	Unknown
LN1_3077.t	1942.72	20062.90	-3.37	Down	Sodium transport ATPase 1
LN1_2059.t	207.86	2045.15	-3.30	Down	Unknown
LN1_0809.t	588.98	5694.88	-3.27	Down	Maltose permease MAL61
LN1_1600.t	20.24	194.00	-3.27	Down	Threonine--tRNA ligase, cytoplasmic
LN1_3627.t	7578.64	72410.89	-3.26	Down	Aldehyde dehydrogenase 5

LN1_1599.t	20.54	194.00	-3.25	Down	Threonine--tRNA ligase, cytoplasmic
LN1_5919.t	3198.97	29636.22	-3.21	Down	Unknown
LN1_6044.t	562.62	5044.86	-3.16	Down	N amino acid transport system protein
LN1_6110.t	2822.54	25307.70	-3.16	Down	Uncharacterized membrane protein
LN1_0660.t	346.32	3004.62	-3.12	Down	Unknown
LN1_3587.t	1414.77	12274.57	-3.12	Down	Sorbose reductase homolog SOU2
LN1_2825.t	205.53	1735.58	-3.08	Down	URIC_CYBJA Uricase
LN1_4966.t	1908.85	15979.20	-3.07	Down	Probable methylmalonate-semialdehyde dehydrogenase
LN1_2575.t	411.90	3420.99	-3.05	Down	Cell wall protein RBR3
LN1_2021.t	1081.23	8822.90	-3.03	Down	Unknown
LN1_5288.t	10.05	81.39	-3.03	Down	Agglutinin-like protein 1
LN1_5462.t	61.65	484.69	-2.98	Down	Unknown
LN1_3494.t	47.22	368.34	-2.96	Down	Formate dehydrogenase 1
LN1_0659.t	329.36	2563.00	-2.96	Down	Unknown
LN1_5485.t	4.82	37.45	-2.96	Down	Unknown

Table S7 KEGG pathway enrichment of down-regulated genes for xenobiotics degradation, energy and carbohydrate metabolism.

KEGG category	Definition	Down-regulated genes in category	Number of DEGs in category	Ratio
ko01220	Degradation of aromatic compounds	13	13	1
ko00362	Benzoate degradation	6	6	1
ko00791	Atrazine degradation	2	2	1
ko00624	Polycyclic aromatic hydrocarbon degradation	2	2	1
ko00010	Glycolysis	47	48	0.98
ko04146	Peroxisome	68	70	0.97
ko00020	Citrate cycle (TCA cycle)	27	28	0.96
ko02010	ABC transporters	22	23	0.96
ko00511	Other glycan degradation	25	27	0.93
ko00500	Starch and sucrose metabolism	46	51	0.90

Table S8 The DEGs involved in amino acid metabolism.

KEGG category	Definition	Number of DEGs in the category	Up-regulated genes in category	Down-regulated genes in category
ko00250	Alanine, aspartate and glutamate metabolism	34	4	30
ko00260	Glycine, serine and threonine metabolism	38	9	29
ko00270	Cysteine and methionine metabolism	39	3	36
ko00280	Valine, leucine and isoleucine degradation	21	1	20
ko00290	Valine, leucine and isoleucine biosynthesis	13	1	12
ko00300	Lysine biosynthesis	13	0	13
ko00310	Lysine degradation	23	3	20
ko00330	Arginine and proline metabolism	44	7	37
ko00340	Histidine metabolism	13	1	12
ko00350	Tyrosine metabolism	25	1	24
ko00360	Phenylalanine metabolism	20	3	17
ko00380	Tryptophan metabolism	27	3	24
ko00400	Phenylalanine, tyrosine and tryptophan biosynthesis	19	0	19

Table S9 The DEGs with expression ratios of at least 8-fold associated with six KEGG categories of amino acid metabolism.

KEGG category	Definition	Up-regulated genes in category	Number of DEGs in category	Ratio
ko00250	Alanine, aspartate and glutamate metabolism	3	6	0.5
ko00260	Glycine, serine and threonine metabolism	7	14	0.5
ko00220	Arginine biosynthesis	1	2	0.5
ko00350	Tyrosine metabolism	1	2	0.5
ko00360	Phenylalanine metabolism	3	3	1
ko00460	Cyanoamino acid metabolism	3	3	1

Table S10 The DEGs involved in the category of DNA replication and repair.

KEGG category	Definition	Number of DEGs in the category	Up-regulated genes in category	Down-regulated genes in category
ko03030	DNA replication	36	13	23
ko03410	Base excision repair	22	5	17
ko03420	Nucleotide excision repair	42	8	34
ko03430	Mismatch repair	22	9	13
ko03440	Homologous recombination	22	8	14
ko03450	Non-homologous end-joining	7	3	4

Table S11 The DGEs with expression ratios of at least 8-fold involved in DNA replication and repair.

KEGG category	Definition	Up-regulated genes in category	Number of DEGs in category	Ratio
ko03030	DNA replication	13	13	1
ko03410	Base excision repair	5	5	1
ko03430	Mismatch repair	9	9	1
ko03440	Homologous recombination	8	8	1
ko03450	Non-homologous end-joining	2	2	1

Table S12 Reverse transcription quantitative PCR validation of the results of Illumina high throughput RNA-sequencing.

Gene ID	KEGG	Function	Regulation	Expression fold change of TG/CG	
				RNA-seq	RT-qPCR
LN1_5322.t	superoxide dismutase	response to superoxide	Up	2.37	2.62
LN1_4997.t	catalase	response to oxidative stress	Down	-2.24	-3.20
LN1_0659.t	glutamate decarboxylase	catalytic activity	Down	-2.96	-2.36
LN1_4655.t	bloom syndrome protein	regulation of DNA recombination	Up	1.95	2.10
LN1_4623.t	DNA repair and recombination protein RAD54	telomere maintenance	Up	1.39	1.27
LN1_4135.t	ATP-dependent DNA helicase 2 subunit 1	telomere maintenance	Up	1.85	1.87
LN1_5319.t	flap endonuclease-1	telomere maintenance	Up	3.01	3.78
LN1_1509.t	NAD(P)H dehydrogenase	extracellular region	Down	-2.74	-3.43
LN1_5075.t	NADH dehydrogenase alpha	organic substance biosynthetic process	Down	-1.86	-2.93
LN1_2055.t	spermidine synthase	catalytic activity	Down	-3.56	-5.17
LN1_0066.t	acetyl-CoA synthetase	catalytic activity	Down	-3.43	-6.19
LN1_3077.t	Na ⁺ -exporting ATPase	catalytic activity	Down	-3.37	-5.10
LN1_1979.t	Spore wall maturation protein DIT1	cell wall biogenesis	Up	8.56	9.00
LN1_3339.t	multidrug resistance protein	transporter activity	Up	4.94	4.23
LN1_2724.t	NADPH-dependent methylglyoxal reductase	carbonyl reductase (NADPH) activity	Up	4.71	4.20
LN1_4493.t	DNA polymerase alpha subunit A	DNA replication checkpoint	Up	4.17	3.71
LN1_4412.t	Azole resistance protein 1	secondary metabolic process	Up	4.03	3.41
LN1_0574.t	structural maintenance of chromosome 3	condensed nuclear chromosome	Up	3.94	4.23
LN1_0905.t	DNA polymerase alpha subunit B	alpha DNA polymerase:primase	Up	3.72	2.99
LN1_2072.t	replication factor A2	telomere maintenance	Up	3.29	3.39

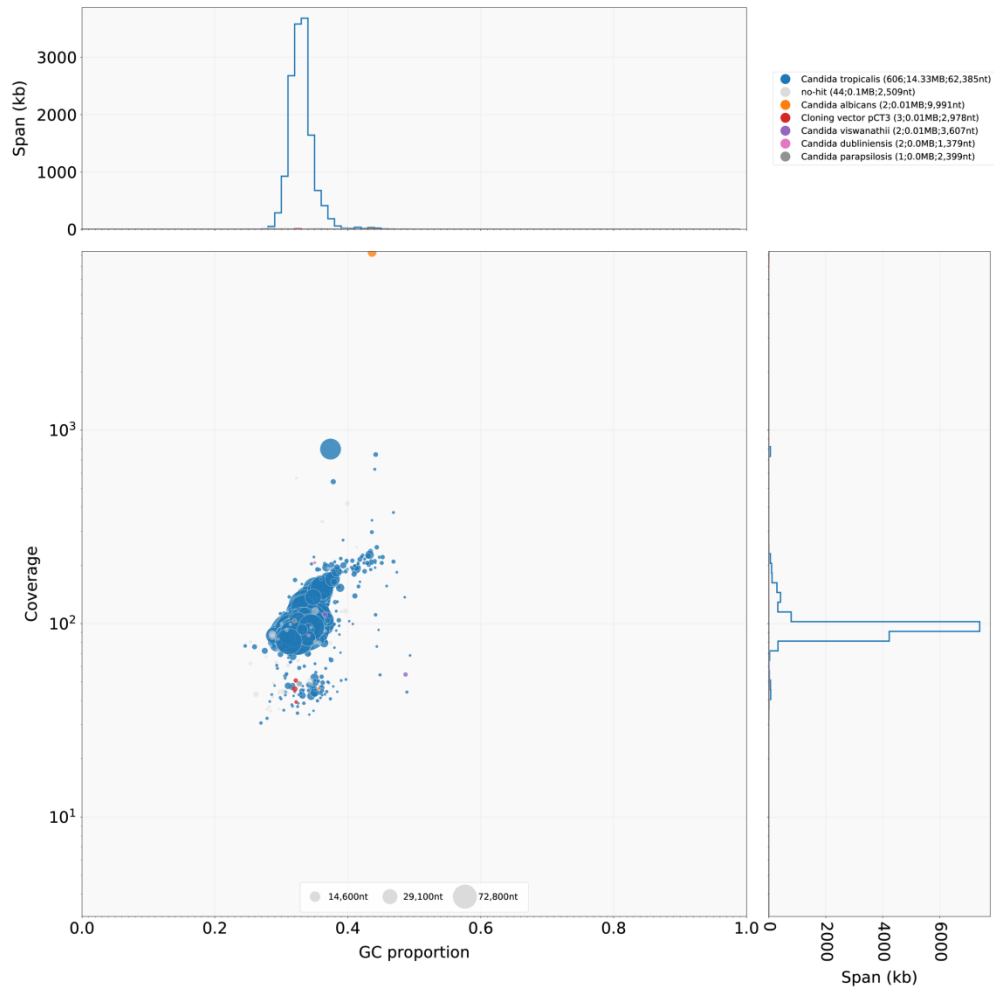


Fig. S1. Visualization of the assembly of sequencing library. Sequences in the assembly are depicted as circles, with diameter scaled proportional to sequence length and taxonomic annotation with different color.

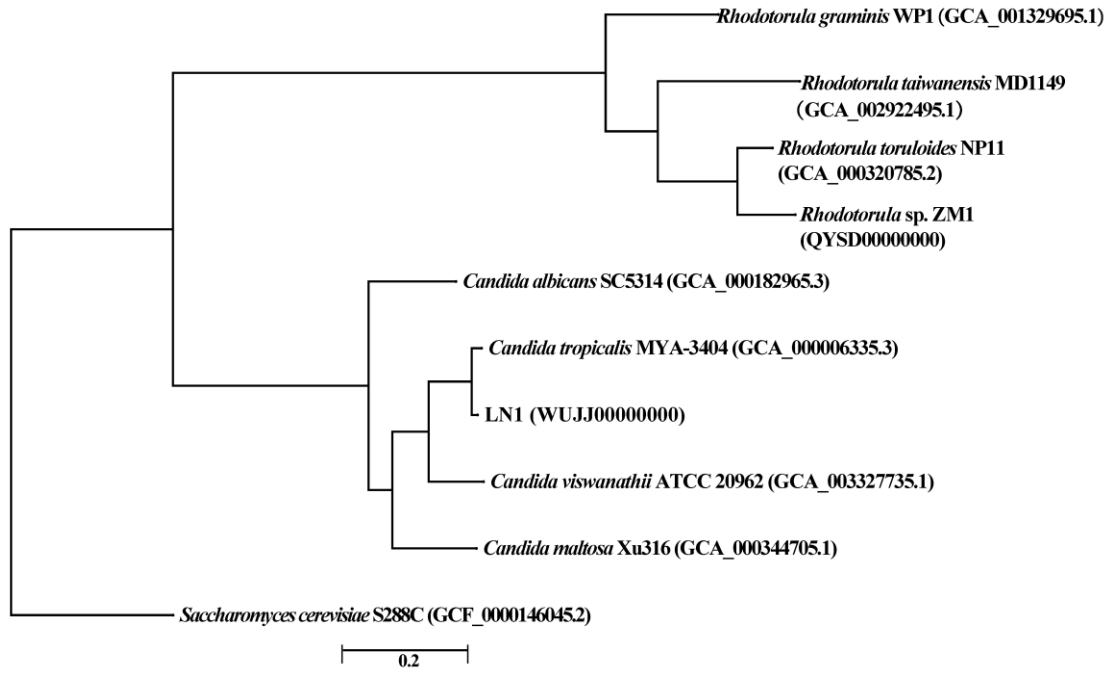


Fig. S2. Maximum-likelihood species tree based on genome sequences showing the relationship of the strain LN1 and eight of their related GenBank genome sequences. Bootstrap values of above 50% are shown at the branch points. *Saccharomyces cerevisiae* S288C was used as an outgroup.

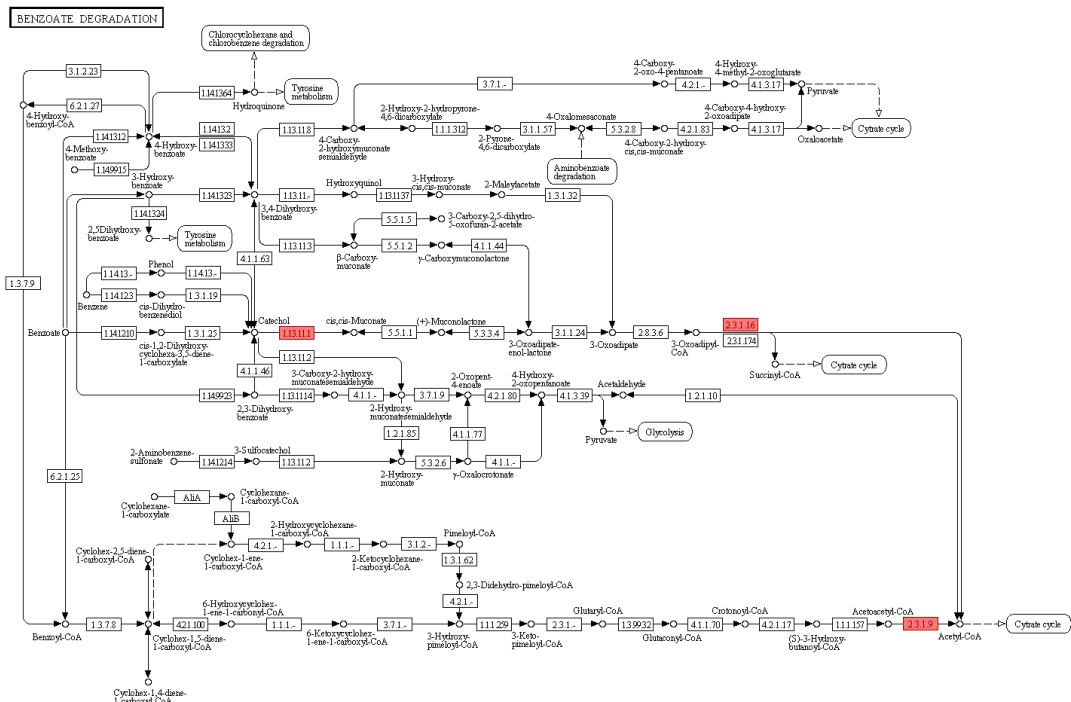


Fig. S3. Genes involved in benzoate degradation pathway. The red box means the enzymes encoded by the detected genes in the strain LN1.

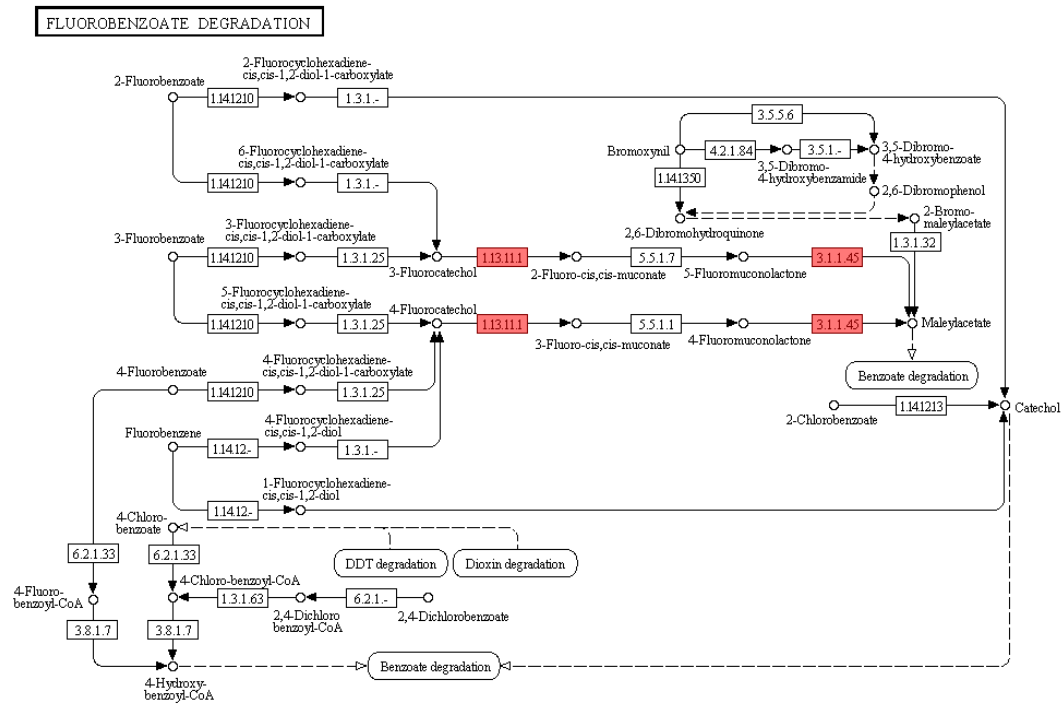


Fig. S4. Genes involved in fluorobenzoate degradation pathway. The red box means the enzymes encoded by the detected genes in the strain LN1.

TOLUENE DEGRADATION

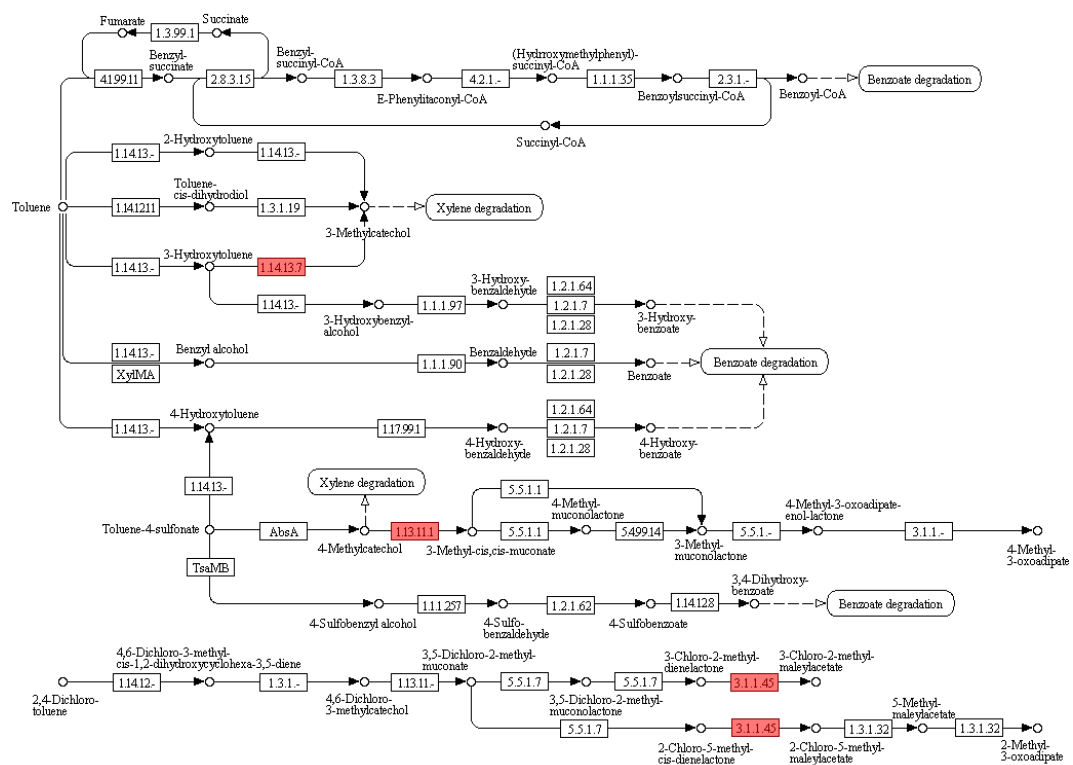


Fig. S5. Genes involved in toluene degradation pathway. The red box means the enzymes encoded by the detected genes in the strain LN1.

CHLOROCYCLOHEXANE AND CHLOROBENZENE DEGRADATION

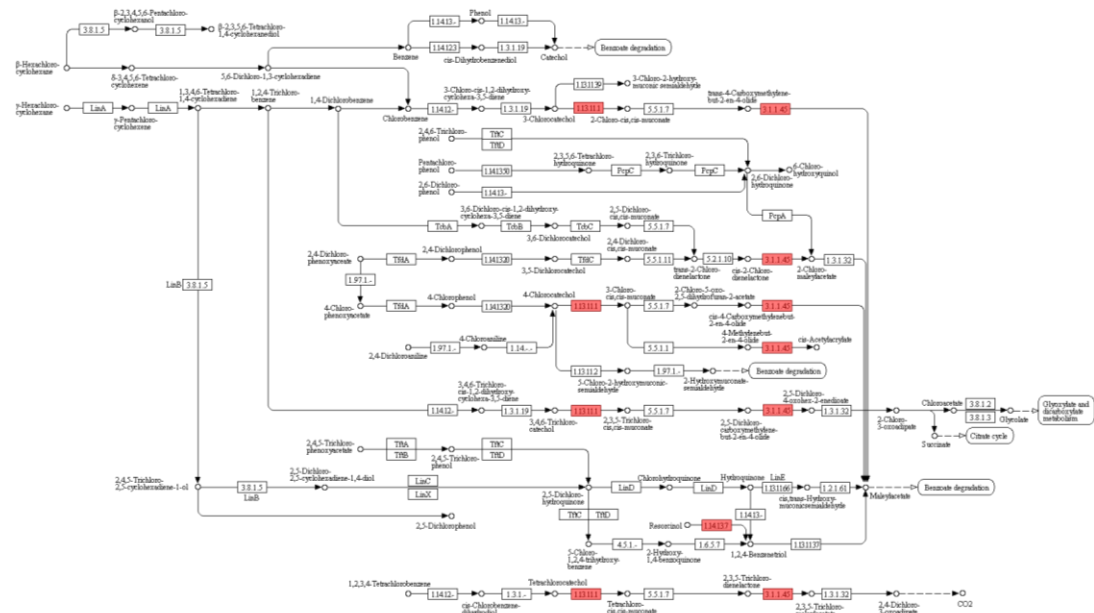


Fig. S6. Genes involved in chlorocyclohexane and chlorobenzene degradation pathway. The red box means the enzymes encoded by the detected genes in the strain LN1.

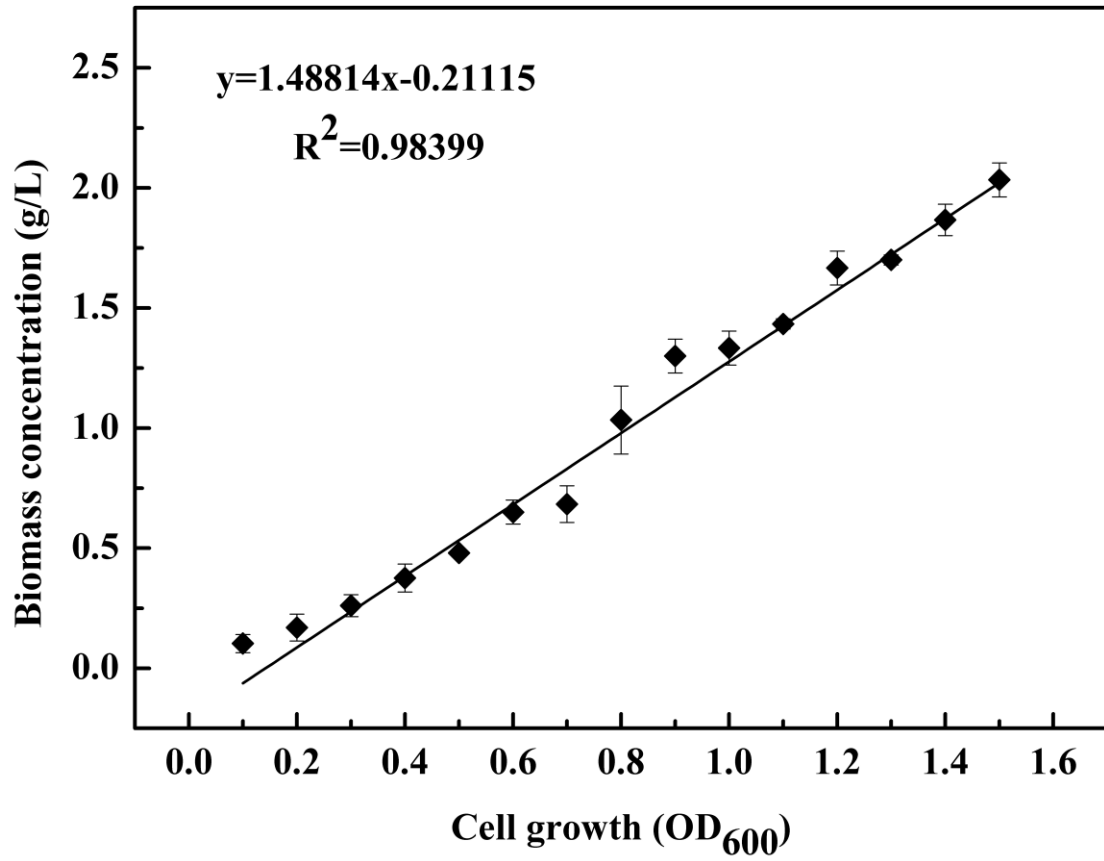


Fig. S7. The relationships of cell growth (OD₆₀₀) with biomass concentration.

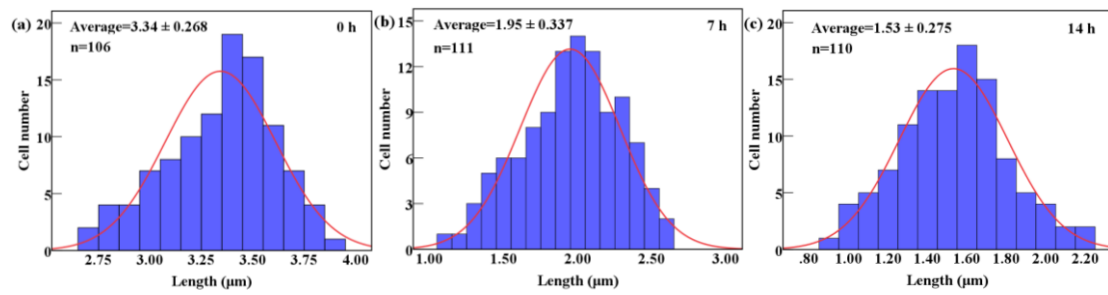


Fig. S8. Lengths of *Candida* sp. LN1 observed under a scanning electron microscope. Strain LN1 was incubated at MSM with 6000 mg/L phenol, and the length changes were observed at 0 h (a), 7 h (b) and 14 h (c).

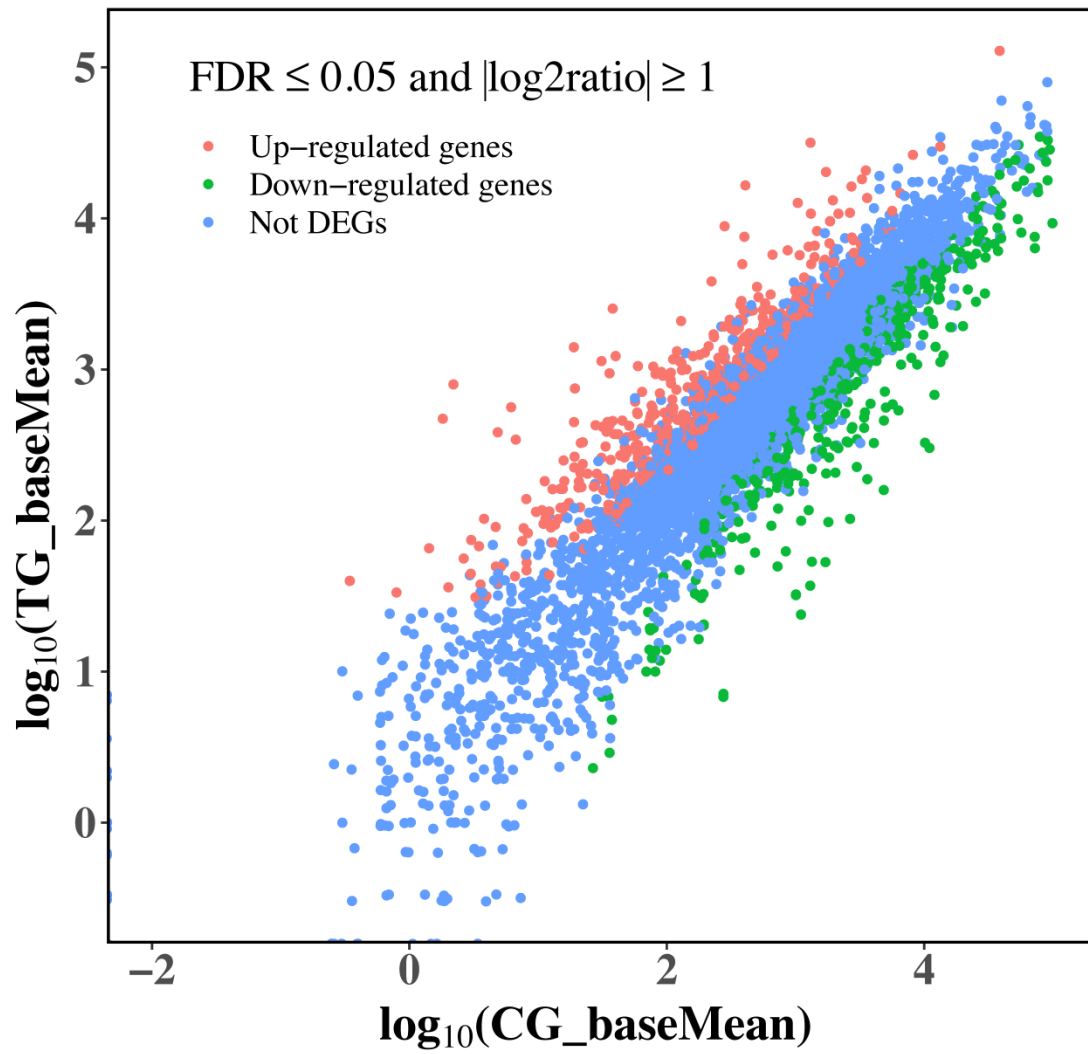


Fig. S9. Differentially expressed genes in the VBNC cells (TGs) compared to those in normal cells (CGs).

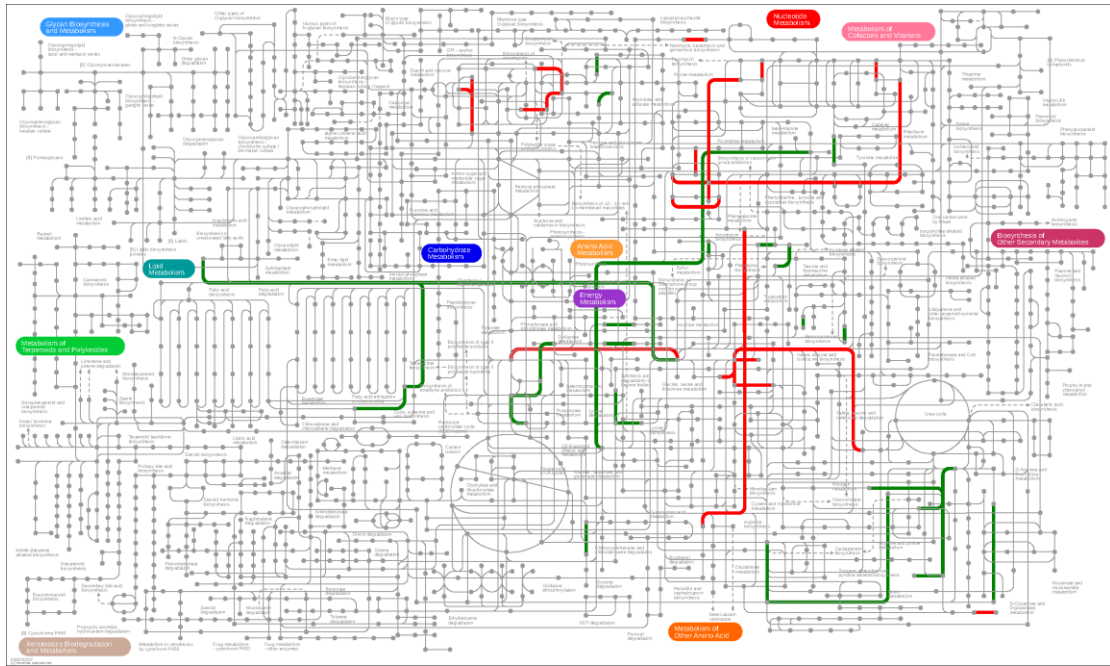


Fig. S10. The mapping KEGG pathway of the 98 up-and 50-down-regulated (red and green lines) genes.

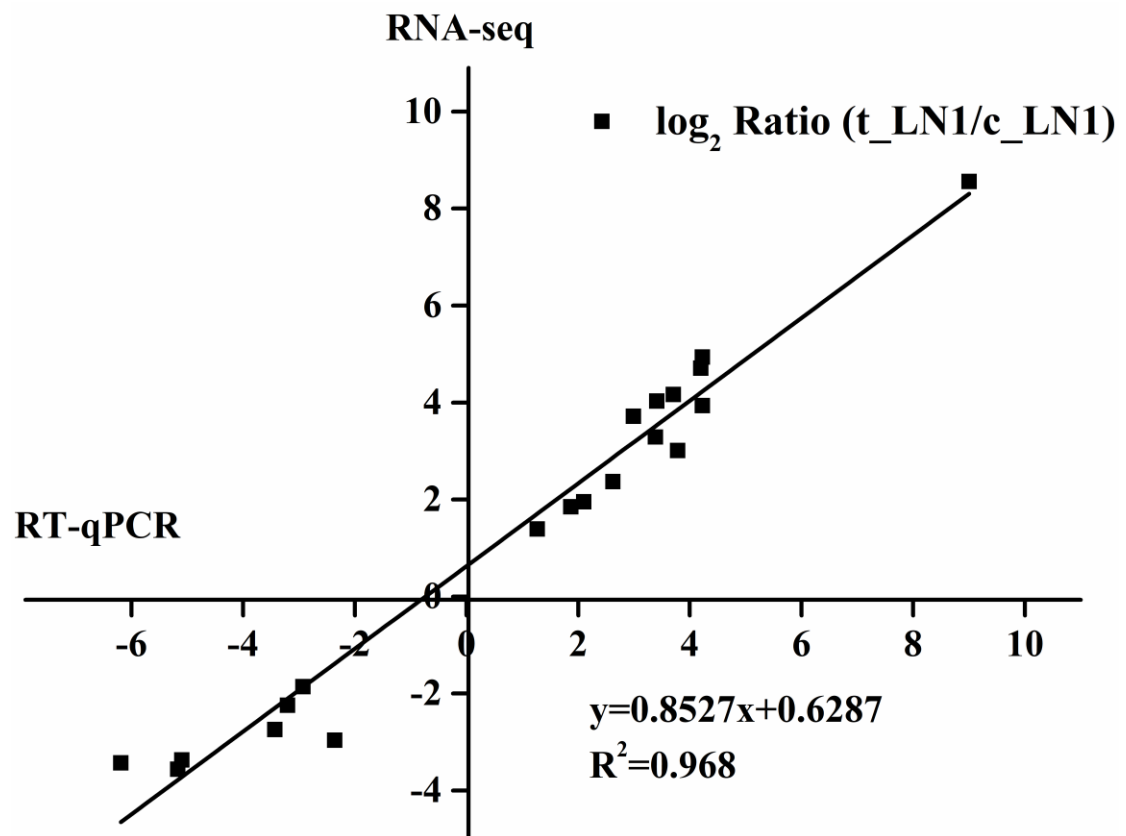


Fig. S11. Reverse transcription quantitative PCR (RT-qPCR) validation analysis of RNA-seq results with selected twenty genes.

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