Supplementary Table S1

SAG assembly and annotation data

cell	D10	C07	J10	L09	P20	L15	B11	M09	N17	C06
Lake	Mendota	Mendota	Mendota	Sparkling	Damariscotta	Mendota	Damariscotta	Damariscotta	Sparkling	Damariscotta
Collected (2009)	May 19	May 19	May 19	May 28	Aug 19	May 19	Aug 19	Aug 19	May 28	Aug 19
IMG name	alpha proteobacterium SCGC AAA028-D10	alpha proteobacterium SCGC AAA028-C07	alpha proteobacterium SCGC AAA027-J10	alpha proteobacterium SCGC AAA023-L09	alpha proteobacterium SCGC AAA280-P20	alpha proteobacterium SCGC AAA027-L15	alpha proteobacterium SCGC AAA280-B11	alpha proteobacterium SCGC AAA487-M09	alpha proteobacterium SCGC AAA024-N17	alpha proteobacterium SCGC AAA027-C06
Assembly size bp	925,141	846,566	792,980	774,923	720,523	719,587	674,250	627,365	328,144	775,384
Scaffolds	57	32	82	76	65	56	47	97	45	90
Genes	1091	974	952	921	838	840	815	800	397	936
Proteins	1057	955	921	892	814	821	797	776	383	916
rRNA genes[a]	5,16,23S	5S	5,16,23S	5S, 23S, 23S, 23S	5,16,23S	-	5,16,23S	-	5S,5S, 16S,23S	16S, 23S,23S
Largest scaffolds bp (GC)	176,148 (0.29) 145,420 (0.29) 126,387 (0.29) 103,388 (0.31)	203,146 (0.29) 138,806 (0.30)	96,767 (0.30) 91,089 (0.31) 69,426 (0.29) 52,972 (0.31)	59,964 (0.29) 54,879 (0.32)	90,870 (0.30) 48,602 (0.31) 48,167 (0.28)	245,217 (0.30) 126,188(0.28) 88,810 (0.29) 79,072 (0.30)	54,526 (0.28) 53,946 (0.31)	54,427 (0.28) 54,274 (0.29)	35,260 (0.28) 29,946 (0.30)	70,157 (0.30) 65,619 (0.29) 64,320 (0.29) 52,346 (0.29)
microcluster	A		B	A	C	B		C	A	B
Patchwork contruction (patchwork start-end):scaffold:start-end:orientation	(1-42082)sc5:21559-63640:1 (693535-695310)sc6:17015-18790:-1 (944309-963781)sc4:72945-92417:-1 (967411-1035006)sc4:1309-68904:-1	(120287-138346)sc2:68210-86269:1 (470765-525902)sc1:45082-100219:1 (555014-569229)sc1:134439-148654:1	(223342-227654)sc1:62601-66913:1 (705898-723218)sc12:3330-20650:1 (827988-862604)sc6:5222-39838:1 (879880-944308)sc2:16613-81041:-1 (963782-967410)sc18:4512-8140:-1	(862605-879879)sc7:17273-34547:1	(785837-827987)sc1:15416-57566:1	(42083-120286)sc4:237-78440:1 (138347-223341)sc3:2869-87863:1 (227655-470764)sc1:85-243194:1 (525903-555013)sc5:10515-39625:-1 (569230-693534)sc2:1153-125457:1 (695311-	(1035007-1054073)sc5:16867-35933:-1	(753541-760945)sc1:14137-21541:-1		

						705897)sc8:671-11257:-1 (723219-753540)sc6:10247-40568:-1 (760946-785836)sc7:1714-26604:-1				

[a] Multiple annotations indicate partial genes located on different contigs

Supplementary Table S2

Sequences included in the analysis with ClonalFrame

Block	Position in patchwork genome[a]	Gene name
1	108973..110196	coaBC
2-1	110196..110636	dut
2-2	110643..111395	moeB
3	111392..111871	485[b]
4-1	111943..112449	fabA
4-2	112459..113673	fabB
5	113684..114463	fabI
6 & 7-1	c(114460..116535)	pnp
7-2	c(116542..116811)	rpsO
8	c(116815..117693)	truB
9-1	c(117686..118063)	rbfA
9-2	c(118070..120286)	infB
10	143515..144288	dapB
11	144318..144956	nth
12	c(145907..146539)	11939[b]
13 & 14	150239..152770	leuS
15	152760..153224	23820[b]
16-1	153230..154252	[b]
16-2	c(154211..155077)	parB
17-1	c(155078..155878)	parA
17-2	c(155887..156516)	gidB
18	c(156522..158390)	gidA
19	c(158438..159763)	trmE
20-1	c(309116..310042)	acuC
20-2	c(310039..311199)	dapE
21	347456..348757	11936[b]
22	348771..349685	OG11893[b]
23	c(350465..351775)	OG11892[b]
24-1	503872..504540	23783[b]
24-2 & 25	504544..505776	OG11235[b]
26	507641..509677	ligA

[a] Numbers listed give full-length gene position in the patchwork genome, from which gaps were removed by Gblocks as described in Methods. To obtain position in SAGs find these positions in patchwork construction from SAGs (Supplementary Table S5), for example coaBC matches scaffold 4 from cell L15.

[b] Genes without a gene name were not used for the sequence divergence test.

Supplementary Table S3

Number of SAGs for each of the 57 proteins used in the concatenated protein alignment.

aspS	6
ftsQ	0
gidA	9
glyS	7
hypA	6
hypB	8
hypC	6
hypD	6
hypE	9
hypF	6
hypG	5
hypH	6
ksgA	7
ligA	9
nusA	9
nusB	6
obgE	5
pcnB	9
pheT	6
pnp	9
pth	8
recR	6
rnc	4
rplA	4
rplI	6
rplJ	4
rplK	4
rplL	3
rplM	4
rplO	4
rplQ	7
rplR	4
rplS	7
rplU	5
rplY	8
rpmA	4
rpmE	6
rpmI	6

rpoA	7
rpoB	5
rpsB	8
rpsD	4
rpsE	4
rpsH	5
rpsI	4
rpsK	4
rpsN	5
rpsO	9
rpsP	7
rpsT	8
rrf	8
secY	6
serS	7
smpB	4
thdF	9
tig	5
trmD	7
tsf	8

Supplementary Table S4

Protein clusters putatively gained in gene flux analyses with penalties for gain ranging from 2 to 5.

Cluster	G 2	A 3	I 4	N 5	Gene	Product
LDA01479	1	0	0	0	NA	hypothetical:19, rubrerythrin:9, transmembrane:1
LDA01850	1	0	0	0	livG:3	ABC:16, branched-chain:5, ABC-type:2, amino:1, branched:1, high-affinity:1
LDA01890	1	0	0	0	livG:1	ABC:17, branched-chain:5, inner-membrane:1, branched:1, glutelin:1, high-affinity:1
LDA02108	1	0	0	0	NA	hypothetical:11, transporter,:2, inner:2, conserved:1, transmembrane:1
LDA02119	1	0	0	0	mntH:4	manganese:20, Mn2+/Fe2+:2, manganese/divalent:1
LDA02133	1	0	0	0	NA	ABC:8, nitrate/sulfonate/bicarbonate:4, NMT1/THI5-like:3, extracellular:2, sulfonate/nitrate:1, amino:1, putative:1, substrate-binding:1, taurine-binding:1
LDA02178	1	0	0	0	ansA:2	L-asparaginase:12, hypothetical:7
LDA02194	1	0	0	0	NA	UDP-glucose/GDP-mannose:12, UDP-glucose:2, udpglucosegdpmannose:1, putative:1, nucleotide:1, UDP-N-acetyl-D-mannosaminuronic:1, UDP-N-acetyl-D-mannosaminuronate:1, nucleotide-sugar:1, udpglucose:1
LDA02200	1	0	0	0	NA	ABC:18, amino:1, ABC-transport:1, taurine:1
LDA02201	1	0	0	0	NA	ABC:10, binding-protein-dependent:7, binding-protein:2, amino:1, taurine:1
LDA02298	1	0	0	0	dppA:3, dppAch1:1, dppA1:1	extracellular:7, periplasmic:4, dipeptide:3, peptide:3, 4-phytase:2, ABC:1, hypothetical:1, peptide/opine/nickel:1
LDA02381	1	0	0	0	gatA1:1, gatA:1	amidase:17, amidotransferase:1, glutamyl-tRNA(Gln):1
LDA02391	1	0	0	0	NA	hypothetical:10, membrane-associated:2, sulfatase:2, phosphatidylethanolamine:Kdo2-lipid:1
LDA02440	1	0	0	0	rffE:4	UDP-N-acetylglucosamine:18, lipopolysaccharide:1, putative:1, UDP-GlcNAc-2-epimerase.:1, udp-n-acetylglucosamine:1
LDA02514	1	0	0	0	dctQ:3	tripartite:9, TRAP:4, TRAP-type:1
LDA02517	1	0	0	0	NA	hypothetical:12, signal:1, MtN3:1
LDA02611	1	0	0	0	NA	branched-chain:4, extracellular:3, ABC:2, twin-arginine:2, ABC-type:2, amino:1, Leu/Ile/Val-binding:1, branched:1, leucine-specific:1
LDA02738	1	0	0	0	NA	hypothetical:12
LDA02740	1	0	0	0	hsdR:7	type:7, Type:6, HsdR:4, putative:2, DEAD/DEAH:1
LDA02798	1	0	0	0	NA	hypothetical:12, lipoprotein:2, signal:1
LDA02823	1	0	0	0	gltJ:4, gltJ1:1, gltJ2:1	amino:8, glutamate/aspartate:4, polar:3, ABC:2
LDA02925	1	0	0	0	NA	oxidoreductase:6, Gfo/Idh/MocA:4, oxidoreductase-like:3, NAD-binding:1, hypothetical:1, trans-1,2-dihydrobenzene-1,2-diol:1
LDA03005	1	0	0	0	NA	hypothetical:14, DMT:3
LDA03006	1	0	0	0	NA	hypothetical:5, branched-chain:3, branched:2, putative:1, leucine-,:1, Fis:1

LDA03008	1	0	0	0	NA	inner-membrane:12, branched:2, ABC:1, branched-chain:1, transmembrane:1
LDA03124	1	0	0	0	gltK:2, gltK2:1, gtpC:1, gltK1:1	polar:5, glutamate/aspartate:4, ABC:3, amino:3
LDA03154	1	0	0	0	NA	hypothetical:14, signal:1
LDA03268	1	0	0	0	NA	hypothetical:13, TRAP-T:1, transmembrane:1
LDA03313	1	0	0	0	NA	molybdopterin:6, sulfite:4, twin-arginine:2, oxidase:1, oxidoreductase,:1
LDA03330	1	0	0	0	NA	hypothetical:9, transmembrane:1
LDA03331	1	0	0	0	fer2:1	ferredoxin,:4, ferredoxin-like:2, (2Fe-2S):2, ferredoxin:2
LDA03501	1	0	0	0	NA	glycosyl:11, glycosyltransferase:3, dolichol-p-glucose:1
LDA03547	1	0	0	0	NA	hypothetical:12, integral:1
LDA03637	1	0	0	0	adh:1	alcohol:9, zinc-containing:1, zinc-type:1
LDA03718	1	1	0	0	pldA:1	phospholipase:5, hypothetical:4, cardiolipin:2, phosphatidylserine/phosphatidylglycerophosphate/:1, nuclease-related:1
LDA03823	1	0	0	0	rci:1	phage:5, integrase:2, DNA:2, Phage:1, integrase/recombinase:1, site-specific:1, shufflon-specific:1
LDA03889	1	0	0	0	NA	glycosyl:4, group:4, glycosyltransferase:2, putative:1
LDA03891	1	0	0	0	NA	spore:5, hypothetical:2, late-developmental:1, protein:1
LDA03906	1	1	0	0	bcpB:1	thiol-specific:2, BcpB:2, redoxin:1, thioredoxin:1
LDA04129	1	0	0	0	gltL:4, gltL1:1, gltL2:1	glutamate/aspartate:4, ABC:3, glutamate:2, amino:1
LDA04183	1	0	0	0	lpeA:1	methyltransferase:4, hypothetical:4, putative:2, lipolysaccharide:1, SAM-dependent:1
LDA04204	1	1	0	0	NA	hypothetical:11
LDA04259	1	0	0	0	NA	hypothetical:7, membrane:1
LDA04351	1	0	0	0	traF:4	hypothetical:6, F:2, conjugative:2, TraF-like:1
LDA04391	1	1	0	0	braC:1, braC1:1, livJ:1	extracellular:3, ABC:1, amino:1, solute-binding:1, putative:1, leucine-,:1, Leu/Ile/Val-binding:1, branched-chain:1
LDA04406	1	0	0	0	fimD:1	hypothetical:4, P:3, outer:1, fimbrial:1
LDA04536	1	0	0	0	chrR:1	hypothetical:3, transcription:2, anti-sigm:1, allophanate:1, anti-ECFsigma:1
LDA04774	1	0	0	0	NA	major:8
LDA04805	1	1	0	0	NA	hypothetical:3
LDA04976	1	1	0	0	NA	methyltransferase:4, hypothetical:3, putative:2
LDA05211	1	1	1	0	NA	hypothetical:8, acetamidase/formamidase:1
LDA05213	1	0	0	0	traH:2	pilus:3, F:2, hypothetical:2, TraH:2
LDA05222	1	0	0	0	NA	6-phosphofructokinase:5, pyrophosphate-dependent:1
LDA05230	1	0	0	0	NA	group:4, glycosyl:2, glycosyltransferase-like:1, hypothetical:1, glycosyltransferase:1
LDA05245	1	0	0	0	corAf:1	magnesium:2, metal:2, metal-transport:2, transmembrane:1, CorA-like:1, magnesium/cobalt:1
LDA05607	1	1	0	0	NA	hypothetical:2, putative:1, putataive:1, Pilus:1, chaperone:1
LDA05671	1	1	1	1	NA	NA
LDA05745	1	0	0	0	NA	hypothetical:6
LDA05815	1	0	0	0	lpsB1:1	sugar:3, putative:1, galactosyltransferase:1, hypothetical:1, glycosyltransferase:1

LDA06129	1	0	0	0	NA	hypothetical:4, TPR:2, serine/threonine:1
LDA06319	1	1	1	1	NA	NA
LDA06338	1	1	0	0	NA	glycosyltransferase:3, glycosyl:1, group:1, alpha-glycosyltransferase:1
LDA06377	1	1	1	1	NA	NA
LDA06381	1	1	1	1	NA	NA
LDA06386	1	1	1	1	NA	NA
LDA06387	1	1	1	0	NA	hypothetical:4
LDA06397	1	1	1	0	NA	epimerase:2, dTDP-glucose:1, NAD-dependent:1, hypothetical:1, epimerase/dehydratase:1, NDP-glucose:1
LDA06398	1	1	0	0	NA	hypothetical:1
LDA06704	1	0	0	0	NA	thioredoxin:4, thioredoxin-related:1, hypothetical:1, thioredoxin-like:1
LDA06830	1	0	0	0	NA	acetaldehyde:6
LDA06989	1	0	0	0	NA	cephalosporin:3, hypothetical:3
LDA07069	1	0	0	0	NA	hypothetical:6
LDA07073	1	0	0	0	NA	acid:3, nonspecific:1, Acid:1, PA-phosphatase-like:1
LDA07079	1	0	0	0	NA	hypothetical:5
LDA07083	1	1	1	1	NA	NA
LDA07085	1	1	0	0	NA	extracellular:3, ABC:2, twin-arginine:1
LDA07086	1	1	1	1	NA	NA
LDA07088	1	1	1	1	NA	NA
LDA07119	1	1	0	0	NA	group:4, hypothetical:1, glycosyltransferase:1
LDA07273	1	1	1	1	NA	NA
LDA07274	1	1	1	1	NA	NA
LDA07275	1	1	1	1	NA	NA
LDA07279	1	1	1	1	NA	NA
LDA07280	1	1	1	1	NA	NA
LDA07283	1	1	1	1	NA	NA
LDA07477	1	1	1	1	NA	NA
LDA07478	1	1	1	0	NA	hypothetical:6
LDA07527	1	1	0	0	NA	hypothetical:6
LDA07627	1	1	0	0	NA	hypothetical:3, dienelactone:1, dipeptidyl:1
LDA07689	1	0	0	0	NA	hypothetical:3, tetratricopeptide:2
LDA07691	1	0	0	0	NA	4-hydroxy-2-ketovalerate:4, pyruvate:1
LDA07958	1	0	0	0	NA	FkbM:3, methyltransferase:1
LDA08012	1	1	1	1	NA	NA
LDA08016	1	1	1	0	NA	hypothetical:2
LDA08020	1	1	1	1	NA	NA
LDA08107	1	1	0	0	NA	hypothetical:5
LDA08179	1	1	1	1	NA	NA
LDA08180	1	1	1	1	NA	NA
LDA08183	1	1	0	0	NA	S-adenosyl-L-methionine-dependent:1, hypothetical:1, 2-polyprenyl-3-methyl-5-hydroxy-6-metoxy-1,4-:1, Generic:1
LDA08184	1	1	1	0	NA	putative:2, signal:1
LDA08185	1	1	1	1	NA	NA
LDA08186	1	1	1	1	NA	NA
LDA08189	1	1	1	1	NA	NA
LDA08191	1	1	1	1	NA	NA
LDA08375	1	1	1	1	NA	hypothetical:5
LDA08421	1	0	0	0	NA	sialic:1, polysaccharide:1, N-acylneuraminate-9-phosphate:1
LDA08484	1	1	1	1	NA	NA
LDA08537	1	1	1	1	NA	hypothetical:5
LDA08588	1	1	0	0	NA	DNA:3, hypothetical:1, adenine-specific:1
LDA08738	1	1	1	1	NA	NA

LDA09008	1	1	1	0	NA	ABC:3, binding-protein-dependent:1
LDA09104	1	1	1	0	NA	TRAP:4
LDA09128	1	1	1	1	NA	NA
LDA09129	1	1	1	1	NA	NA
LDA09130	1	1	1	1	NA	NA
LDA09133	1	1	1	1	NA	NA
LDA09134	1	1	1	1	NA	NA
LDA09135	1	1	0	0	NA	YceI:3
LDA09270	1	1	1	1	NA	NA
LDA09273	1	1	1	1	NA	NA
LDA09274	1	1	1	1	NA	NA
LDA09275	1	1	1	1	NA	NA
LDA09276	1	1	1	1	NA	NA
LDA09277	1	1	1	1	NA	NA
LDA09278	1	1	1	1	NA	NA
LDA09280	1	1	1	1	NA	NA
LDA09318	1	0	0	0	NA	SAM-dependent:2, type:2
LDA09577	1	1	1	1	NA	NA
LDA09691	1	1	1	1	NA	NA
LDA09965	1	1	1	0	NA	hypothetical:3
LDA09996	1	0	0	0	NA	NAD-dependent:4
LDA10041	1	1	0	0	NA	hypothetical:2, O-methyltransferase:1
LDA10190	1	1	1	1	NA	hypothetical:2, capsule:1
LDA10552	1	1	1	1	NA	NA
LDA10557	1	1	1	1	NA	NA
LDA10562	1	1	1	1	NA	NA
LDA10583	1	1	1	1	NA	NA
LDA10610	1	0	0	0	NA	NAD-dependent:1
LDA10694	1	1	1	1	NA	NA
LDA10696	1	1	1	1	NA	NA
LDA10714	1	1	1	1	NA	hypothetical:2, FkbM:1
LDA11154	1	1	1	1	NA	NA
LDA11155	1	1	1	1	NA	NA
LDA11157	1	1	1	1	NA	hemolysin:1
LDA11159	1	1	1	1	NA	NA
LDA11483	1	1	1	0	NA	abortive:2
LDA11795	1	1	1	1	NA	NA
LDA12080	1	1	1	1	NA	NA
LDA12227	1	1	1	1	NA	hypothetical:1
LDA12497	1	1	0	0	NA	glycosyltransferase:1
LDA12538	1	1	1	1	NA	NA
LDA12541	1	1	1	1	NA	NA
LDA12542	1	1	1	0	NA	cytochrome:1
LDA12547	1	1	1	1	NA	NA
LDA12549	1	1	1	1	NA	MOSC:1, hypothetical:1
LDA12559	1	1	1	0	NA	formate:2
LDA12573	1	1	1	0	NA	lipopolysaccharide:1
LDA12594	1	1	1	1	NA	NA
LDA12695	1	1	1	1	NA	NA
LDA12696	1	1	1	1	NA	NA
LDA12697	1	1	1	1	NA	NA
LDA12698	1	1	1	1	NA	NA
LDA13213	1	1	0	0	NA	hypothetical:2

LDA13215	1	1	1	1	NA	NA
LDA13216	1	1	1	1	NA	NA
LDA13241	1	1	1	1	NA	regulatory:1, nitrogen:1
LDA13406	1	1	1	1	NA	short-chain:2
LDA13536	1	1	1	1	NA	NA
LDA13652	1	1	1	1	NA	NA
LDA13653	1	1	1	0	NA	TRAP:1
LDA13654	1	1	0	0	NA	hypothetical:1, glycosyl/methyltransferase:1
LDA13656	1	1	1	1	NA	NA
LDA13859	1	1	1	1	NA	hypothetical:1
LDA13860	1	1	1	1	NA	hypothetical:1
LDA13861	1	1	1	1	NA	hypothetical:1
LDA13862	1	1	1	1	NA	serine:1
LDA14385	1	1	1	1	NA	NA
LDA14386	1	1	1	1	NA	NA
LDA14387	1	1	1	1	NA	NA
LDA14388	1	1	1	1	NA	NA
LDA14389	1	1	1	1	NA	NA
LDA14390	1	1	1	1	NA	NA
LDA14391	1	1	0	0	NA	putative:1, secreted:1
LDA15221	1	1	1	1	NA	NA
LDA15349	1	1	1	1	NA	NA
LDA15785	1	1	1	1	NA	NA
LDA16072	1	1	1	1	NA	NA
LDA16073	1	1	1	1	NA	NA
LDA16074	1	1	1	1	NA	NA
LDA16076	1	1	1	1	NA	NA
LDA16078	1	1	1	1	NA	NA
LDA16080	1	1	1	1	NA	NA
LDA16082	1	1	1	1	NA	NA
LDA16085	1	1	1	1	NA	NA
LDA16089	1	1	1	1	NA	NA
LDA16090	1	1	1	1	NA	NA
LDA16202	1	1	1	1	NA	methyltransferase:1
LDA16205	1	1	1	1	NA	oxidoreductase:1
LDA16206	1	1	1	1	NA	NA
LDA16207	1	1	1	1	NA	NA
LDA16208	1	1	1	1	NA	NA
LDA16220	1	1	1	1	NA	hypothetical:1
LDA16337	1	1	1	0	NA	cyclase:1
LDA16612	1	1	1	1	NA	NA
LDA16613	1	1	1	1	NA	NA
LDA16614	1	1	1	1	NA	NA
LDA16615	1	1	1	1	NA	NA
LDA16616	1	1	1	1	NA	NA
LDA16617	1	1	1	1	NA	NA
LDA16618	1	1	1	1	NA	NA
LDA16619	1	1	1	1	NA	glycosyl:1
LDA16620	1	1	1	1	NA	NA
LDA16621	1	1	1	1	NA	NA
LDA16622	1	1	1	1	NA	NA
LDA16623	1	1	1	1	NA	NA
LDA16625	1	1	1	1	NA	NA

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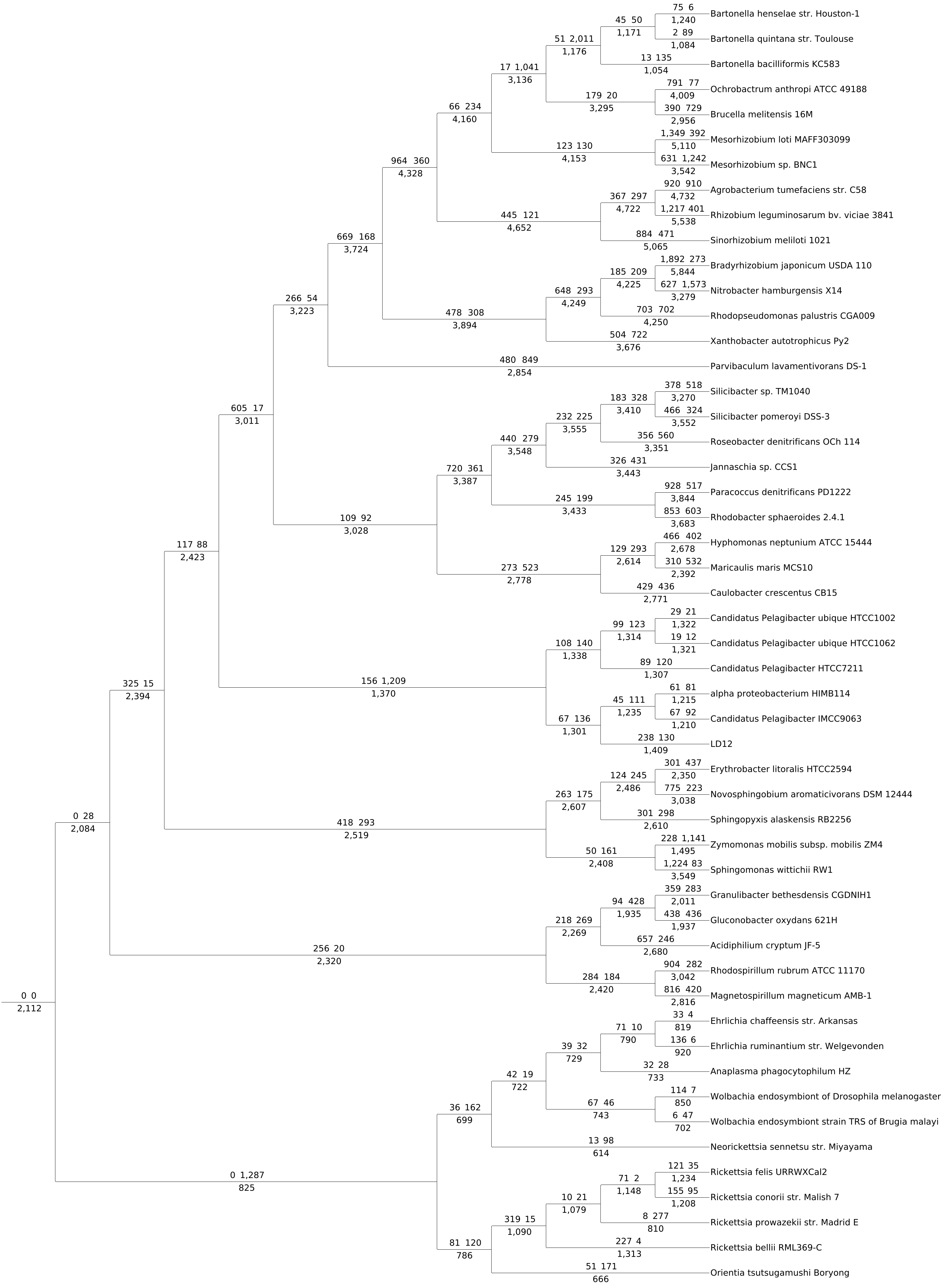

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Bartonella bacilliformis KC583
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Ochrobactrum anthropi ATCC 49188
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Brucella melitensis 16M
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Agrobacterium tumefaciens str. C58
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Sinorhizobium meliloti 1021
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Bradyrhizobium japonicum USDA 110
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Wolbachia endosymbiont strain TRS of Brugia malayi
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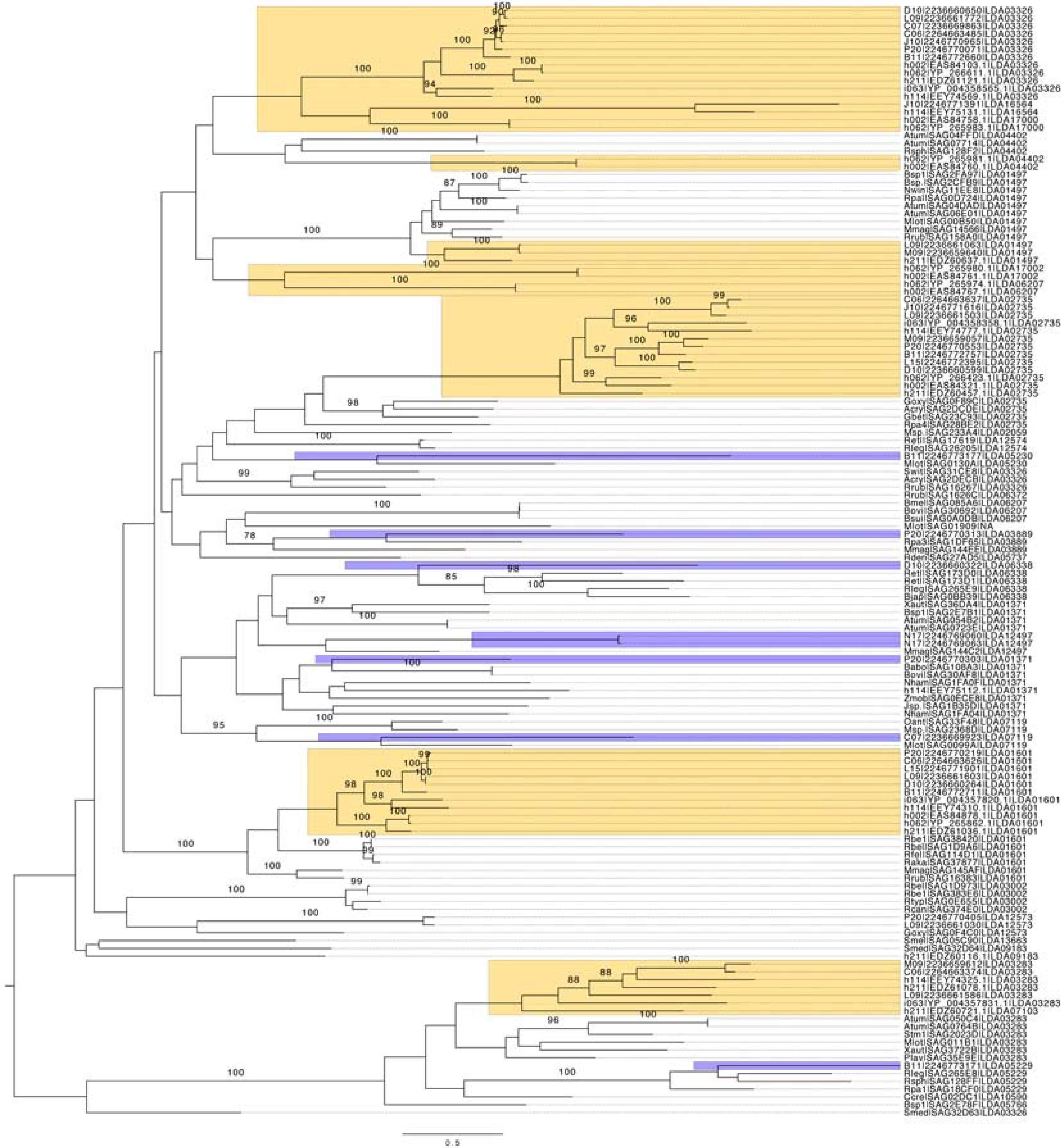
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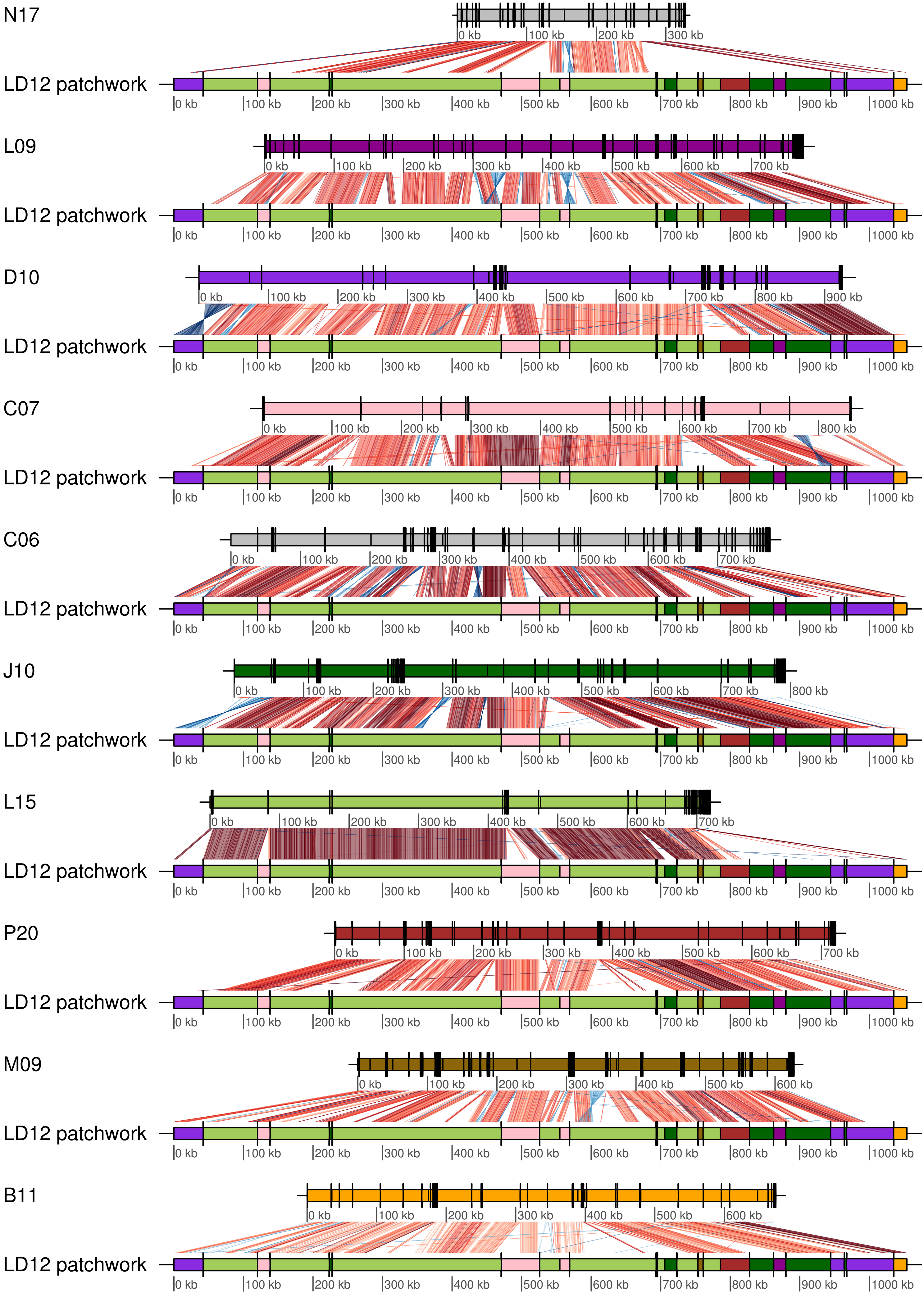
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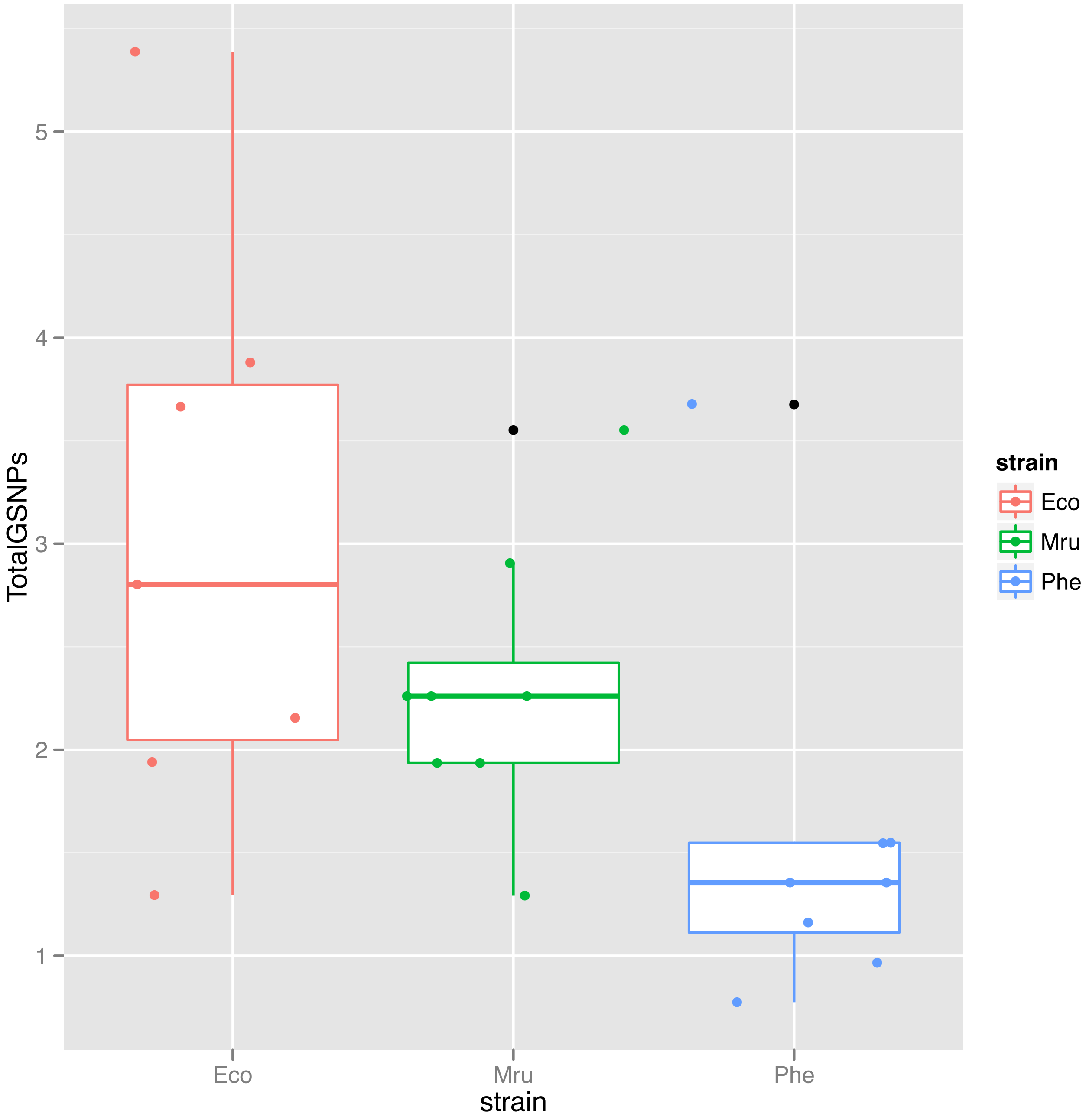
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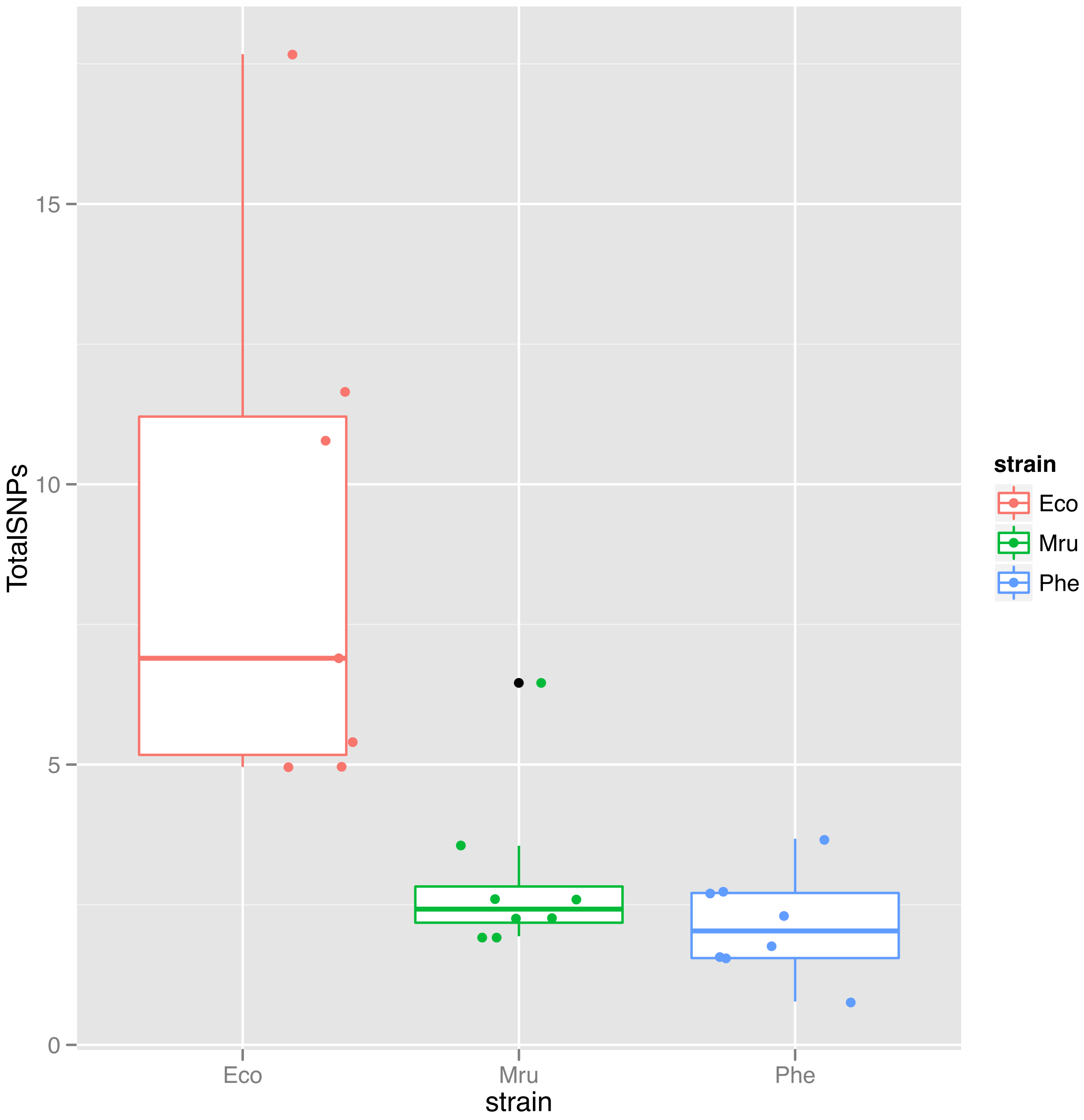
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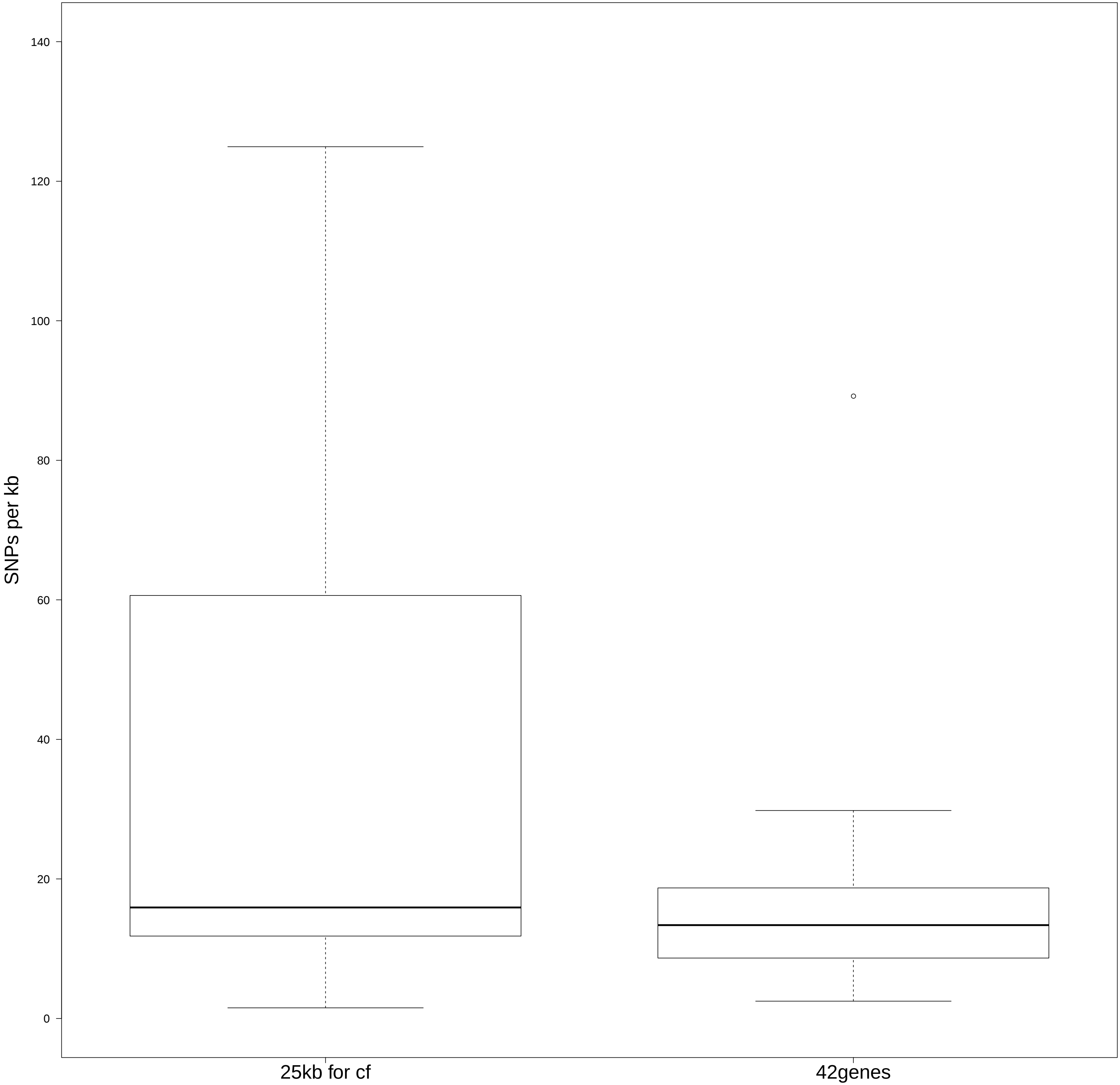
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