Supplementary material

Contents

Supplementary Tables

Table S1: Results of conventional PCR and sequencing pre-screening on the *Y. pestis* specific *pla* fragment (133 bp)

Individual	Tooth sample 1	Tooth sample 2
AE96	-	-
AE97	-	-
AE127	-	-
AE128	-	-
AE349	-	-
AE350	-	-
AE468	-	-
AE469	-	-
AE887	-	-
AE888	-	-
AE1004	-	-
AE1005	-	-
AE1154	-	-
AE1155	-	-
AE1175	-	+
AE1176	+	-

AE1184	-	-
AE1185	-	-
AE1223	-	-
AE1241	-	-

Table S2: Carbon-dating

Individual	Sample	C14 date	+/-	Cal 1 sigma	Cal 2 sigma	C:N	%C	percent collagen
AE1175	metacarpal	1541	19	Cal AD 434-556	Cal AD 428-571	3.3[a]	37.8[a]	13.8[a]
AE1176	metacarpal	1563	20	Cal AD 430-538	Cal AD 426-545	3.3[a]	36.1[a]	3.4[a]

[a] The C:N relation, percent C and percent collagen values are in the normal range and show a good collagen quality.

Table S3: Copy number estimation of plasmids

	Chromosome	pCD1	pMT1
Reference sequence (CO92)	NC_003143.1	NC_003131.1	NC_003134.1
Average read length	67.41 bp	68.7 bp	61 bp
Selected region of full mappability (positions in reference)	2306929 - 2311929	24206-29205	2000-6999
"G+C" content in region of full mappability	48.04 %	47.98 %	47.96 %
Average coverage in region of full mappability	21.2 X	60.8 X	54 X
Estimated copy number[a]	-	2.87	2.55

a The copy number of the plasmids was estimated on the average coverage in the region of full mappability divided by that of the chromosome, and is likely to be affected by ascertainment bias in the array capture.

Table S4: Genomes used for SNP calling and phylogeny

Strain ID	SNP Calls	Coverage (fold)	Coverage (percent)	accession
0.ANT1a_42013	162	63.19	93.93	ADPG00000000 47685
0.ANT1b_CMCC49003	172	66.88	94.59	ADQX00000000 47685
0.ANT1c_945	185	50.49	92.05	ADPV00000000 47685
0.ANT1d_164	179	47.76	93.37	ADOW00000000 47685
0.ANT1e_CMCC8211	188	55.41	94.27	ADRD00000000 47685
0.ANT1f_42095	185	47.24	93.98	ADPJ00000000 47685
0.ANT1g_CMCC42007	185	53.09	93.43	ADQV00000000 47685

0.ANT1h_CMCC43032	182	38.54	93.05	ADQW00000000 47685
0.ANT2_B42003004	90	94.52	95.25	NZ_AAYU00000000
0.ANT2a_2330	90	66.53	92.44	ADOY00000000 47685
0.ANT3a_CMCC38001	93	67.2	93.74	ADQU00000000 47685
0.ANT3b_A1956001	91	66.62	93.98	ADPX00000000 47685
0.ANT3c_42082	92	27.29	92.06	ADPH00000000 47685
0.ANT3d_CMCC21106	99	42.78	91.74	ADQP00000000 47685
0.ANT3e_42091b	98	85.13	93.89	ADPI00000000 47685
0.PE2_PEST-F	507	92.06	93.07	NC_009381
0.PE2b_G8786	567	51.87	91.33	ADSG00000000 47685
0.PE3_Angola	889	89.73	91.04	NC_010159
0.PE4_Microtus91001	351	93.27	94.25	NC_005810
0.PE4Aa_12	267	49.15	92.27	ADOV00000000 47685
0.PE4Ab_9	264	46.84	93.04	ADPT00000000 47685
0.PE4Ba_PestoidesA	315	93.44	94.42	NZ_ACNT00000000
0.PE4Ca_CMCCN010025	325	61.42	92.9	ADRT00000000 47685
0.PE4Cb_M0000002	316	49.02	93.05	ADST00000000 47685
0.PE4Cc_CMCC18019	322	35.08	93.45	ADQO00000000 47685
0.PE4Cd_CMCC93014	331	52.98	94.06	ADRM00000000 47685
0.PE4Ce_CMCC91090	333	87.7	93.18	ADRJ00000000 47685
0.PE7b_620024	444	38.79	92.68	ADPM00000000 47685
1.ANT1_Antiqua	156	93.95	94.9	NC_008150
1.ANT1_UG05-0454	163	91.51	92.34	NZ_AAYR00000000
1.IN1a_CMCC11001	48	52.46	93.59	ADQK00000000 47685
1.IN1b_780441	51	54.96	94.66	ADPS00000000 47685
1.IN1c_K21985002	62	60.58	92.21	ADSS00000000 47685
1.IN2a_CMCC640047	43	45.14	92.33	ADRA00000000 47685
1.IN2b_30017	46	89.81	94.69	ADPC00000000 47685
1.IN2c_CMCC31004	46	56.78	92.84	ADQR00000000 47685
1.IN2d_C1975003	40	49.75	92.45	ADPZ00000000 47685
1.IN2e_C1989001	50	47.25	95.07	ADQB00000000 47685
1.IN2f_710317	49	42.58	94.17	ADPP00000000 47685
1.IN2g_CMCC05013	50	24.84	93.45	ADQF00000000 47685
1.IN2h_5	49	36.98	93.36	ADPK00000000 47685
1.IN2i_CMCC10012	53	59.62	93.98	ADQG00000000 47685
1.IN2j_CMCC27002	54	46.36	92.45	ADQQ00000000 47685
1.IN2k_970754	55	61.24	93.04	ADPW00000000 47685
1.IN2l_D1991004	52	38.21	94.56	ADRX00000000 47685
1.IN2m_D1964002b	53	76.63	94.7	ADRV00000000 47685
1.IN2n_CMCC02041	56	62.95	94.48	ADQC00000000 47685
1.IN2o_CMCC03001	54	41.46	92.42	ADQD00000000 47685

1.IN2p_D1982001	50	47.49	93.11	ADRW00000000 47685
1.IN2q_D1964001	52	33.69	92.63	ADRU00000000 47685
1.IN3a_F1954001	48	60.11	95.31	ADSC00000000 47685
1.IN3b_E1979001	44	40.72	94.92	NZ_AAYV00000000
1.IN3c_CMCC84038b	45	70.76	94.72	ADRF00000000 47685
1.IN3d_YN1683	46	32.63	93.19	ADTD00000000 47685
1.IN3e_YN472	44	57.37	95.34	ADTH00000000 47685
1.IN3f_YN1065	45	60.74	94.36	ADTC00000000 47685
1.IN3g_E1977001	45	50.42	93.47	ADRY00000000 47685
1.IN3h_CMCC84033	44	38.97	94	ADRE00000000 47685
1.IN3i_CMCC84046	48	66.26	95.35	ADRG00000000 47685
1.ORI1_CA88	13	94.97	95.69	NZ_ABCD00000000
1.ORI1_CO92	0	95.06	95.74	NC_003143
1.ORI1a_CMCC114001	26	44.58	92.37	ADQL00000000 47685
1.ORI1b_India195	28	92.74	93.47	NZ_ACNR00000000
1.ORI1c_F1946001	34	42.44	94.74	ADSB00000000 47685
1.ORI2_F1991016	40	94.5	95.2	NZ_ABAT00000000
1.ORI2a_YN2179	41	40.78	92.33	ADTE00000000 47685
1.ORI2c_YN2551b	42	44.84	94.5	ADTF00000000 47685
1.ORI2d_YN2588	40	48.06	91.99	ADTG00000000 47685
1.ORI2f_CMCC87001	38	59.25	94.71	ADRH00000000 47685
1.ORI2g_F1984001	40	42.89	92.63	ADSD00000000 47685
1.ORI2h_YN663	38	56.2	92.37	ADTI00000000 47685
1.ORI2i_CMCCK100001a	38	70.87	95.36	ADRR00000000 47685
1.ORI2i_CMCCK110001b	39	50.54	92.56	ADRS00000000 47685
1.ORI3_IP275	42	94.47	95.21	NZ_AAOS00000000
1.ORI3_MG05-1020	34	94.73	95.36	NZ_AAYS00000000
1.ORI3a_EV76	30	50.95	91.01	ADSA00000000 47685
2.ANT1_Nepal516	138	91.87	92.7	NZ_ACNQ00000000
2.ANT1a_34008	122	61.73	92.57	ADPD00000000 47685
2.ANT1b_34202	122	53.84	93.97	ADPE00000000 47685
2.ANT2a_2	117	52.78	93.05	ADOX00000000 47685
2.ANT2b_351001	121	40.92	92.98	ADPF00000000 47685
2.ANT2c_CMCC347001	119	32.57	93.87	ADQS00000000 47685
2.ANT2d_G1996006	117	56.48	94.12	ADSE00000000 47685
2.ANT2e_G1996010	121	68.03	94.21	ADSF00000000 47685
2.ANT2f_CMCC348002	120	57.17	94.3	ADQT00000000 47685
2.ANT3a_CMCC92010	128	60.52	91.48	ADRL00000000 47685
2.ANT3b_CMCC95001	126	49.74	92.38	ADRN00000000 47685
2.ANT3c_CMCC96001	127	74.21	94.22	ADRO00000000 47685
2.ANT3d_CMCC96007	129	69.28	92.53	ADRP00000000 47685
2.ANT3e_CMCC67001	137	62.64	91.29	ADRB00000000 47685

2.ANT3f_CMCC104003	126	71.42	93.99	ADQH00000000 47685
2.ANT3g_CMCC51020	127	50.32	93.12	ADQY00000000 47685
2.ANT3h_CMCC106002	126	68.1	93.94	ADQI00000000 47685
2.ANT3i_CMCC64001	133	56.49	91.43	ADQZ00000000 47685
2.ANT3j_H1959004	124	51.89	93.69	ADSI00000000 47685
2.ANT3k_5761	131	52.31	91.38	ADPL00000000 47685
2.ANT3l_735	135	49.37	92.53	ADPR00000000 47685
2.MED1b_2506	158	70.57	93.99	ADPA00000000 47685
2.MED1c_2654	157	29	93.57	ADPB00000000 47685
2.MED1d_2504	158	37.87	92.65	ADOZ00000000 47685
2.MED2_KIM10	171	93.81	94.65	NC_004088
2.MED2b_91	131	77.47	91.46	ADPU00000000 47685
2.MED2c_K11973002	129	59.49	93.09	NZ_AAYT00000000
2.MED2d_A1973001	129	31.97	91.78	ADPY00000000 47685
2.MED2e_7338	132	62.53	90.23	ADPQ00000000 47685
2.MED3a_J1963002	134	51.58	93.19	ADSP00000000 47685
2.MED3b_CMCC125002b	134	49.53	93.86	ADQN00000000 47685
2.MED3c_I1969003	133	73.87	93.97	ADSK00000000 47685
2.MED3d_J1978002	122	60.65	94.71	ADSQ00000000 47685
2.MED3f_I1970005	139	46.96	93.47	ADSL00000000 47685
2.MED3g_CMCC99103	136	41.71	93.66	ADRQ00000000 47685
2.MED3h_CMCC90027	136	50.58	91.2	ADRI00000000 47685
2.MED3i_CMCC92004	140	42.18	91.35	ADRK00000000 47685
2.MED3j_I2001001	137	78.49	94.82	ADSO00000000 47685
2.MED3k_CMCC12003	138	51.11	91.09	ADQM00000000 47685
2.MED3l_I1994006	141	62.9	93.9	ADSN00000000 47685
2.MED3m_SHAN11	142	51.92	92.72	ADTA00000000 47685
2.MED3n_SHAN12	142	42.92	93.19	ADTB00000000 47685
2.MED3o_I1991001	140	48.81	91.82	ADSM00000000 47685
2.MED3p_CMCC107004	141	100.25	94.78	ADQJ00000000 47685
3.ANT1a_7b	96	65.01	94.49	ADPN00000000 47685
3.ANT1b_CMCC71001	95	42	92.33	ADRC00000000 47685
3.ANT1c_C1976001	92	59.96	95.27	ADQA00000000 47685
3.ANT1d_71021	94	44.74	94.2	ADPO00000000 47685
3.ANT2a_MGJZ6	112	54.85	91.3	ADSX00000000 47685
3.ANT2b_MGJZ7	107	40.09	91.68	ADSY00000000 47685
3.ANT2c_MGJZ9	111	56.22	93.67	ADSZ00000000 47685
3.ANT2d_MGJZ11	113	53.19	94.25	ADSU00000000 47685
3.ANT2e_MGJZ3	111	63.11	93.09	ADSW00000000 47685
4.ANT1a_MGJZ12	94	55.06	93.32	ADSV00000000 47685
BD 8124_8291_11972	65	16.7	88.07	SRR341963, SRR341961,

				SRR341962
Altenerding	157	17.9	91.5	PRJEB14851
Aschheim	120	3.94	30.24	SRP033879
Y. pseudotuberculosis (outgroup)	12389	88.08	91.16	NC_006155

Table S5: 157 chromosomal SNPs called for the Altenerding genome compared to the CO92 reference (5X coverage and SNP allele frequency of at least 90%)

Position	CO92	Altenerding	Coverage in Altenerding	Ancestral/Derived	SNP type
74539	C	T	31	Anc	non-synonymous
86824*	A	G	8	der	intergenic
130643	G	A	24	Anc	non-synonymous
155747*	A	G	20	Anc	synonymous
189227*	C	T	19	Anc	non-synonymous
189912*	A	G	18	der	intergenic
228268	T	G	31	Anc	synonymous
260148*	C	T	15	der	non-synonymous
271114*	C	A	19	der	non-synonymous
286528*	T	A	17	Anc	synonymous
325836	T	C	24	Anc	synonymous
341720	A	G	19	Anc	non-synonymous
399533	C	A	17	Anc	non-synonymous
420208*	G	T	7	Anc	intergenic
485976*	C	T	15	der	synonymous
545488	T	C	25	Anc	stop lost
547131	T	G	18	Anc	synonymous

549767*	T	C	10	Anc	intergenic
557841*	C	T	14	der	non-synonymous
699494	A	G	22	Anc	synonymous
699647	T	C	22	Anc	synonymous
727741	G	A	14	der	non-synonymous
779365*	C	T	17	der	synonymous
877258	T	C	26	Anc	non-synonymous
898980	A	T	11	der	intergenic
918790	C	T	13	Anc	non-synonymous
1017647	T	C	16	Anc	synonymous
1025278	T	G	20	Anc	non-synonymous
1051913	A	G	26	Anc	non-synonymous
1067966	C	A	20	der	non-synonymous
1098675*	A	C	15	Anc	non-synonymous
1102174	A	G	14	Anc	non-synonymous
1178178	T	C	9	Anc	intergenic
1178459	T	C	28	Anc	synonymous
1211729*	A	C	18	der	synonymous
1251046	T	C	10	Anc	non-synonymous
1263337	G	A	5	Anc	intergenic
1272559	T	C	16	Anc	non-synonymous
1296743*	C	T	27	der	non-synonymous
1306718	T	C	17	Anc	intergenic

1385780	T	C	23	Anc	synonymous
1387701*	C	T	13	der	intergenic
1387756*	A	G	16	der	intergenic
1413031*	C	A	8	der	intergenic
1434752*	C	A	16	der	non-synonymous
1489055	C	T	31	der	synonymous
1512930	A	G	17	Anc	non-synonymous
1530658	C	A	12	der	non-synonymous
1540754	A	G	17	Anc	synonymous
1609461*	T	C	14	der	non-synonymous
1705810	A	C	14	Anc	non-synonymous
1735263	A	C	26	Anc	synonymous
1749443	T	C	15	Anc	synonymous
1754708	C	T	22	der	non-synonymous
1804559	C	T	12	Anc	synonymous
1808946	T	C	19	Anc	intergenic
1859946*	T	C	15	Anc	non-synonymous
1868678	G	T	11	der	intergenic
1871476	G	A	10	Anc	intergenic
1956162	T	C	9	der	intergenic
2012524	T	G	19	Anc	non-synonymous
2022335	A	C	16	Anc	intergenic
2092152*	C	T	21	der	synonymous

2097520	G	T	22	der	synonymous
2098628	T	C	16	Anc	non-synonymous
2262577	T	G	31	Anc	non-synonymous
2277583	G	A	8	Anc	intergenic
2278317	A	G	20	Anc	non-synonymous
2300659	T	G	23	Anc	non-synonymous
2352174*	T	G	17	der	non-synonymous
2356003	T	A	20	Anc	non-synonymous
2419529*	G	A	25	der	synonymous
2495165*	C	A	8	der	intergenic
2508389	T	C	22	Anc	non-synonymous
2655012	C	T	13	Anc	intergenic
2656129*	T	G	20	Anc	synonymous
2684793	A	G	18	Anc	intergenic
2721828	C	A	16	Anc	synonymous
2725715	C	T	11	der	intergenic
2739149	C	A	13	Anc	synonymous
2744933	A	G	9	Anc	non-synonymous
2753572*	A	T	14	der	synonymous
2773647	A	G	18	Anc	non-synonymous
2797988*	A	G	13	Anc	synonymous
2812384	G	T	18	Anc	non-synonymous
2829833	A	G	32	Anc	non-synonymous

2903882	T	G	18	Anc	non-synonymous
2934972	C	G	22	Anc	synonymous
2936268	G	A	18	Anc	non-synonymous
2950954	G	A	11	Anc	non-synonymous
2958327	C	T	23	Anc	intergenic
2977542*	C	A	26	der	non-synonymous
2995771	A	G	8	Anc	intergenic
3078807*	C	A	21	der	non-synonymous
3085079	A	G	16	Anc	non-synonymous
3096319	G	A	17	Anc	non-synonymous
3145523	A	C	22	Anc	non-synonymous
3179828*	C	A	30	der	synonymous
3190399	A	G	35	Anc	non-synonymous
3210101	A	G	18	Anc	synonymous
3223359*	C	A	5	Anc	intergenic
3244204	A	G	26	Anc	non-synonymous
3267118*	A	G	16	Anc	non-synonymous
3274298*	A	T	8	der	synonymous
3324959	A	G	18	Anc	intergenic
3360963*	A	C	14	der	non-synonymous
3360984*	C	T	6	der	non-synonymous
3362591	A	G	18	Anc	non-synonymous
3397040*	A	G	16	Anc	synonymous

3398153	G	A	12	der	synonymous
3409414	T	C	5	der	intergenic
3421335	A	G	10	Anc	non-synonymous
3426560*	A	G	20	Anc	synonymous
3442617*	A	T	15	Anc	non-synonymous
3500922	T	G	20	der	non-synonymous
3535148*	G	T	11	der	non-synonymous
3560088	G	A	31	der	non-synonymous
3564026	C	T	24	Anc	non-synonymous
3568597	C	T	23	der	non-synonymous
3571531	A	G	30	Anc	synonymous
3616733	A	G	17	Anc	non-synonymous
3645151*	C	G	20	Anc	non-synonymous
3658233*	T	G	12	Anc	non-synonymous
3667806	A	G	13	Anc	non-synonymous
3726726	A	G	18	Anc	stop lost
3750736	G	A	16	der	intergenic
3755861	C	T	7	der	intergenic
3767613	C	T	11	Anc	intergenic
3806677	C	T	12	Anc	non-synonymous
3843195	C	A	5	der	synonymous
3892488*	C	T	21	Anc	non-synonymous
3973746	C	T	27	Anc	non-synonymous

4066494*	C	T	22	der	non-synonymous
4080579	T	C	19	Anc	non-synonymous
4081612	T	C	17	Anc	intergenic
4082562*	T	C	21	Anc	synonymous
4083536	A	G	19	Anc	intergenic
4087224	T	C	28	Anc	intergenic
4173149	A	C	6	Anc	intergenic
4194600	G	A	21	Anc	non-synonymous
4243823	A	T	9	Anc	non-synonymous
4307755	G	A	25	der	non-synonymous
4339366	T	G	21	Anc	non-synonymous
4371886*	A	G	19	Anc	synonymous
4399470	A	G	29	Anc	non-synonymous
4412624*	A	G	12	der	intergenic
4421633	T	C	36	Anc	non-synonymous
4421689	A	G	32	Anc	non-synonymous
4423366*	G	A	38	der	synonymous
4460688	C	T	18	der	non-synonymous
4465967	C	A	24	der	synonymous
4518401	G	A	18	Anc	synonymous
4527483	A	G	14	Anc	intergenic
4579183	A	G	20	Anc	non-synonymous
4628496	C	A	13	der	synonymous

4629169	G	A	33	der	intergenic
4634287	A	G	13	Anc	non-synonymous

* Chromosomal SNPs detected in the Altenerding genome that were not called in the Wagner et al. Aschheim genome.

Table S6: 11 SNPs called for plasmids pCD1 and pMT1 (5X coverage and SNP frequency of 90% minimum).

Plasmid	Position	CO92	Altenerding	Coverage
pCD1 (NC_003131.1)	23564	T	G	67
	29959	A	G	40
	50462	T	C	33
	54237	T	G	44
	55839	G	A	53
	66608	C	T	83
pMT1 (NC_003134.1)	12976*	A	C	70
	32569	T	G	82
	47365	C	T	79
	62994	T	C	90
	82435	C	T	99

* pMT1 SNP detected in the Altenerding genome and not called in the Wagner et al. Aschheim genome.

Table S7: SNPs published by Wagner et al. for the Aschheim genome that were not called in the Altenerding genome

Position in CO92	CO92	Altenerding	Coverage in Altenerding	Aschheim genome published in Wagner et al.	Coverage in the Aschheim genome published in Wagner et al.	Re-analyzed Aschheim genome	Coverage in the re-analyzed Aschheim genome	Variant frequency in the re-analyzed Aschheim genome (%)	Reason SNP was not called in the Altenerding genome
105187	A	C	17	C	19	C	14	100	Excluded non-core region
107738	A	G	14	G	13	G	10	100	Excluded non-

									core region
221811	A	A	24	G	63	G	7	70	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
225902	A	A	16	T	6	N/A	0	0	Potential false positive in Wagner et al.: position not covered in the re-analysis of Wagner et al.
333342	A	A	22	G	46	G	5	63	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
417323	G	A	3	A	30	A	18	100	Excluded non-core region
442439	T	C	19	C	46	C	33	100	Excluded non-core region
497800	T	T	17	A	122	A	13	72	False positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
567757	C	A	2	A	6	A	6	100	Excluded non-core region
698477	C	A	19	A	21	A	15	100	Excluded non-core region
754287	T	C	13	C	18	C	13	100	Excluded non-core region
773110	T	C	17	C	24	C	13	100	Excluded non-core region
809132	A	G	15	G	13	G	9	100	Excluded non-core region
1044488	A	N/A	0	G	23	G	17	100	Less than 5X coverage in Altenerding
1137603	G	T	13	T	6	T	2	100	Excluded non-core region

1138676	G	T	22	T	6	T	5	83	Excluded non-core region
1237756	C	N/A	0	T	8	T	3	100	Less than 5X coverage in Altenerding
1371020	C	C	17	A	8	C	1	100	Potential false positive in Wagner et al.: SNP does not exist in the re-analysis of Wagner et al.
1371025	C	C	18	T	24	T	6	86	False positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
1440494	A	C	29	C	12	C	11	100	Excluded non-core region
1444672	A	G	2	G	9	G	7	88	Less than 5X coverage in Altenerding
1796044	T	C	14	C	16	C	13	93	Excluded non-core region
1864793	A	A	16	G	162	G	7	54	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
1895361	C	A	22	A	14	A	10	100	Excluded non-core region
1914093	T	C	19	C	14	C	8	100	Excluded non-core region
1982740	A	C	11	C	9	C	8	89	Excluded non-core region
2072914	G	G	35	A	13	A	4	80	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
2117516	G	A	12	A	22	A	15	100	Excluded non-core region

2119347	T	T	45	A	7	N/A	0	0	Potential false positive in Wagner et al.: position not covered in the re-analysis of Wagner et al.
2141322	T	C	27	C	5	C	3	60	Excluded non-core region
2141910	C	A	18	A	10	A	6	100	Excluded non-core region
2218046	G	T	17	T	5	T	1	100	Excluded non-core region
2235109	T	C	1	C	12	C	11	100	Less than 5X coverage in Altenerding
2281856	A	N/A	0	C	10	C	7	100	Less than 5X coverage in Altenerding
2304950	A	G	11	G	27	G	14	100	Excluded non-core region
2317730	A	C	10	C	18	C	9	100	Excluded non-core region
2453454	A	G	14	G	11	G	7	100	Excluded non-core region
2548551	G	T	20	T	19	T	12	100	Excluded non-core region
2575152	G	A	4	A	33	A	14	100	Less than 5X coverage in Altenerding
2577686	A	G	28	G	24	G	12	100	Excluded non-core region
2607034	C	T	23	T	18	T	15	100	Excluded non-core region
2619611	T	G	15	G	9	G	5	100	Excluded non-core region
2787770	T	G	25	G	13	G	2	100	Excluded non-core region
2865494	A	A	30	C	9	C	3	100	Potential false positive in Wagner et al.: less than 5X coverage in the re-analysis of Wagner et al.
2894703	T	N/A	0	C	16	C	10	100	Excluded non-core region

2896636	A	G	10	G	41	G	21	88	Excluded non-core region
3143800	G	G	21	T	29	G	1	100	Potential false positive in Wagner et al.: SNP does not exist in the re-analysis of Wagner et al.
3155055	G	G	26	C	54	C	5	71	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
3248223	T	C	9	C	10	C	7	100	Excluded non-core region
3358603	G	T	9	T	30	T	23	100	Excluded non-core region
3392897	A	A	22	G	79	G	11	85	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
3403167	G	T	6	T	16	T	10	100	Excluded non-core region
3472427	G	A	17	A	18	A	16	100	Excluded non-core region
3725545	T	C	14	C	19	C	12	100	Excluded non-core region
3732919	A	G	15	G	20	G	13	100	Excluded non-core region
3737968	G	A	13	A	19	A	11	100	Excluded non-core region
3739401	C	A	18	A	9	A	6	100	Excluded non-core region
3813424	C	C	10	A	23	A	4	100	Potential false positive in Wagner et al.: less than 5X coverage in the re-analysis of Wagner et al.; abnormal cover

									peak in region
3909258	T	C	8	C	10	C	10	100	Excluded non-core region
4170791	A	A	25	G	87	G	20	80	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
4199187	A	A	47	G	70	A	3	75	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
4199190	T	T	27	C	123	C	3	38	Potential false positive in Wagner et al.: heterogeneous position with abnormal cover peak in region
4203596	G	G	22	T	51	G	3	100	Potential false positive in Wagner et al.: SNP does not exist in the re-analysis of Wagner et al.
4210011	T	T	34	C	262	T	8	100	Potential false positive in Wagner et al.: SNP does not exist in the re-analysis of Wagner et al.
4232217	C	N/A	0	T	5	T	5	100	Less than 5X coverage in Altenerding
4542642	A	G	8	G	9	G	7	100	Excluded non-core region

Table S8: 19 potential false positive SNPs called by Wagner et al.

Position in CO92	SNP type defined in Wagner et al.	Gene
221811	Derived, non-synonymous	*rpsJ*
225902	Ancestral	
333342	Derived, synonymous	*uvrA*
497800	Derived, synonymous	*dnaK*
1371020	Ancestral	
1371025	Derived, synonymous	*gyrA*
1864793	Derived, synonymous	*mnmA*
2072914	Derived, synonymous	*fliI*
2119347	Ancestral	
2865494	Ancestral	
3143800	Derived, non-synonymous	*ynbD*
3155055	Derived, synonymous	*purI*
3392897	Derived, synonymous	*napA*
3813424	Derived, synonymous	*acnB*
4170791	Derived, synonymous	*aceA*
4199187	Derived, synonymous	*rpoC*
4199190	Derived, synonymous	*rpoC*
4203596	Derived, synonymous	*rpoB*
4210011	Ancestral	

Table S9: 63 Unique SNPs in the Altenerding genome cross-referenced against all *Y. pestis* genomes in the data set (excluding the Aschheim genome)

Position in CO92	SNP type	Ancestral AA	Derived AA	Gene ID	Gene name
Chromosom					
86824*	intergenic	N/A	N/A		
189912*	intergenic	N/A	N/A		
260148*	non-synonymous	P	S	YPO0257	
271114*	non-synonymous	L	I	YPO0270	
485976*	synonymous	A	A	YPO0460	*thrB*

557841*	non-synonymous	R	H	YPO0517	*hepA*
727741	non-synonymous	E	K	YPO0668	*parE*
779365*	synonymous	S	S	YPO0717	*fliI*
898980	intergenic	N/A	N/A		
1067966	non-synonymous	G	C	YPO0966	
1211729*	synonymous	V	V	YPO1068	*proS*
1296743*	non-synonymous	V	M	YPO1150	*bioA*
1387701*	intergenic	N/A	N/A		
1387756*	intergenic	N/A	N/A		
1413031*	intergenic	N/A	N/A		
1434752*	non-synonymous	D	Y	YPO1275	*spr*
1489055	synonymous	Q	Q	YPO1322	*deoR*
1530658	non-synonymous	R	I	YPO1363	
1609461*	non-synonymous	T	A	YPO1417	
1754708	non-synonymous	P	L	YPO1539	*galU*
1868678	intergenic	N/A	N/A		
1956162	intergenic	N/A	N/A		
2092152*	synonymous	G	G	YPO1847	*yecS*
2097520	synonymous	A	A	YPO1851	*putA*
2352174*	non-synonymous	V	G	YPO2071	
2419529*	synonymous	L	L	YPO2150	
2495165*	intergenic	N/A	N/A		
2725715	intergenic	N/A	N/A		
2753572*	synonymous	P	P	YPO2455	
2977542*	non-synonymous	S	I	YPO2649	*nrdE*
3078807*	non-synonymous	R	S	YPO2747	*fadJ*
3179828*	synonymous	S	S	YPO2847	*yegM*
3274298*	synonymous	A	A	YPO2930	*pdxJ*
3360963*	non-synonymous	T	P	YPO3008	
3360984*	non-synonymous	H	Y	YPO3008	
3398153	synonymous	L	L	YPO3043	

3409414	intergenic	N/A	N/A		
3500922	non-synonymous	V	G	YPO3141	*tesB*
3535148*	non-synonymous	A	S	YPO3171	*apbA*
3560088	non-synonymous	P	S	YPO3199	
3568597	non-synonymous	G	E	YPO3205	*phoB*
3750736	intergenic	N/A	N/A		
3755861	intergenic	N/A	N/A		
3843195*	synonymous	V	V	YPO3438	*intB*
3892488*	non-synonymous	A	T	YPO3483	
4066494*	non-synonymous	V	I	YPO3646	*pcp*
4307755	non-synonymous	A	V	YPO3839	
4412624*	intergenic	N/A	N/A		
4423366*	synonymous	A	A	YPO3938	*glgP*
4460688	non-synonymous	R	Q	YPO3963	
4465967	synonymous	S	S	YPO3966	
4628496	synonymous	A	A	YPO4107	*yieG*
4629169	intergenic	N/A	N/A		
pCD1 plasmid					
23564	synonymous	A	A	YPCD1.33c	*lcrR*
29959	non-synonymous	N	S	YPCD1.41	*yscO*
50462	non-synonymous	K	E	YPCD1.71c	*yopJ*
54237	intergenic	N/A	N/A		
55839	intergenic	N/A	N/A		
66608	non-synonymous	L	F	YPCD1.92	
pMT1 plasmid					
32569	synonymous	T	T	YPMT1.30	
47365	synonymous	R	R	YPMT1.44	
62994	intergenic	N/A	N/A		
82435	intergenic	N/A	N/A		

*30 unique SNPs detected in the Altenerding genome that were not called in *Wagner et al.*

Table S10: Non-synonymous SNPs compared to CO92

Position in CO92	CO92	Altenerding	Ancestral /Derived	Codon Change	Amino Acid Change	Gene ID	Gene name	Gene function
Chromosome								
74539	C	T	Anc	GCC to ACC	A to T	YPO0063		
130643	G	A	Anc	ACC to ATC	T to I	YPO0122	*glpE*	thiosulfate sulfurtransferase
189227*	C	T	Anc	CGT to TGT	R to C	YPO0169	*pabA*	para-aminobenzoate synthase component II
260148*	C	T	der	CCG to TCG	P to S	YPO0257		type III secretion protein
271114*	C	A	der	CTT to ATT	L to I	YPO0270		type III secretion system protein (iron-sulfur binding protein)
341720	A	G	Anc	CAC to CGC	H to R	YPO0332	*rhaS*	transcriptional activator
399533	C	A	Anc	TTC to TTA	F to L	YPO0383	*aidB*	isovaleryl CoA dehydrogenase
557841*	C	T	der	CGT to CAT	R to H	YPO0517	*hepA (RapA)*	ATP-dependent helicase (transcription regulator)
727741	G	A	der	GAA to AAA	E to K	YPO0668	*parE*	DNA to poisomerase IV subunit B
877258	T	C	Anc	TTT to CTT	F to L	YPO0797	*lysR*	LysR family transcriptional regulator
918790	C	T	Anc	CCA to CTA	P to L	YPO0839	*kduD2*	2-deoxy-D-gluconate 3-dehydrogenase
1025278	T	G	Anc	TCT to GCT	S to A	YPO0932		
1051913	A	G	Anc	AGG to GGG	R to G	YPO0953		
1067966	C	A	der	GGC to TGC	G to C	YPO0966		kinase
1098675*	A	C	Anc	AGC to CGC	S to R	YPO0989	*iucA*	pseudo gene
1102174	A	G	Anc	ACA to GCA	T to A	YPO0993	*iucD*	siderophore biosynthesis protein
1251046	T	C	Anc	ATC to ACC	I to T	YPO1107	*GrpE*	heat shock protein
1272559	T	C	Anc	GTT to GCT	V to A	YPO1126		l-pal system protein YbgF
1296743*	C	T	der	GTG to ATG	V to M	YPO1150	*bioA*	adenosylmethionine-8-amino-7-oxononanoate

								aminotransferase (part of the Biotin operon)
1434752*	C	A	der	GAT to TAT	D to Y	YPO1275	*spr*	outer membrane lipoprotein (Murein hydrolase)
1512930	A	G	Anc	ACG to GCG	T to A	YPO1348		
1530658	C	A	der	AGA to ATA	R to I	YPO1363		virulence factor
1609461*	T	C	der	ACA to GCA	T to A	YPO1417		iron-sulfur binding protein
1705810	A	C	Anc	GTG to GGG	V to G	YPO1502		alcohol dehydrogenase
1754708	C	T	der	CCA to CTA	P to L	YPO1539	*galU*	UTP-glucose-1-phosphate uridylyltransferase
1859946*	T	C	Anc	AGT to GGT	S to G	YPO1634	*phoP*	DNA-binding transcriptional regulator
2012524	T	G	Anc	TTG to GTG	L to V	YPO1767	*hpaI (hpcH)*	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase
2098628	T	C	Anc	AAT to AGT	N to S	YPO1851	*putA*	trifunctional transcriptional regulator/proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase
2262577	T	G	Anc	CTG to CGG	L to R	YPO1990		
2278317	A	G	Anc	GTT to GCT	V to A	YPO2005		
2300659	T	G	Anc	GAC to GCC	D to A	YPO2029		
2352174*	T	G	der	GTG to GGG	V to G	YPO2071		DEAD box family helicase
2356003	T	A	Anc	AAT to AAA	N to K	YPO2074	*fadD*	long chain fatty acid CoA ligase
2508389	T	C	Anc	ACA to GCA	T to A	YPO2234	*cstA*	carbon starvation protein A
2744933	A	G	Anc	ATT to GTT	I to V	YPO2446		2-deoxyglucose-6-phosphatase
2773647	A	G	Anc	GTC to GCC	V to A	YPO2472		
2812384	G	T	Anc	CCG to CAG	P to Q	YPO2502	*gutB*	zinc-binding dehydrogenase
2829833	A	G	Anc	TGC to CGC	C to R	YPO2519		SAM-dependent methyltransferase
2903882	T	G	Anc	TGT to GGT	C to G	YPO2582		sugar transport ATP-binding protein
2936268	G	A	Anc	CTT to TTT	L to F	YPO2614	*gltJ*	glutamate/Faspartate transport system permease
2950954	G	A	Anc	GCG to GTG	A to V	YPO2625	*nagC*	N-acetylglucosamine regulatory protein
2977542*	C	A	der	AGC to ATC	S to I	YPO2649	*nrdE*	ribonucleotide-diphosphate reductase subunit alpha
3078807*	C	A	der	CGT to AGT	R to S	YPO2747	*fadJ (faoA)*	multifunctional fatty acid oxidation complex subunit

								alpha
3085079	A	G	Anc	ACT to GCT	T to A	YPO2752	*mepA*	penicillin-insensitive murein endopeptidase
3096319	G	A	Anc	GGA to GAA	G to E	YPO2762		AraC family transcriptional regulator
3145523	A	C	Anc	CTC to CGC	L to R	YPO2814	*ynbD*	
3190399	A	G	Anc	CAG to CGG	Q to R	YPO2853	*baeR*	DNA-binding transcriptional regulator
3244204	A	G	Anc	GTC to GCC	V to A	YPO2901		
3267118*	A	G	Anc	CTG to CCG	L to P	YPO2921	*purL*	phosphoribosylformylglycinamidine synthase
3360963*	A	C	der	ACC to CCC	T to P	YPO3008		two-component sensor histidine kinase (TCSs)
3360984*	C	T	der	CAT to TAT	H to Y	YPO3008		two-component sensor histidine kinase (TCSs)
3362591	A	G	Anc	AGC to GGC	S to G	YPO3009		two-component response regulator
3421335	A	G	Anc	ATG to GTG	M to V	YPO3064	*bcp*	thioredoxin-dependent thiol peroxidase
3442617*	A	T	Anc	AGA to AGT	R to S	YPO3086	*copA*	copper exporting ATPase
3500922	T	G	der	GTG to GGG	V to G	YPO3141	*tesB*	acyl-CoA thioesterase
3535148*	G	T	der	GCT to TCT	A to S	YPO3171	*apbA (panE)*	2-dehydropantoate 2-reductase (vitamin B5 biosynthesis)
3560088	G	A	der	CCA to TCA	P to S	YPO3199		short chain dehydrogenase
3564026	C	T	Anc	TGT to TAT	C to Y	YPO3201	*proY*	permease
3568597	C	T	der	GGA to GAA	G to E	YPO3205	*phoB*	phosphate regulon transcriptional regulator
3616733	A	G	Anc	CTA to CCA	L to P	YPO3247	*hmwA*	adhesin
3645151*	C	G	Anc	GCC to GGC	A to G	YPO3272	*yfiQ*	acetyltransferase
3658233*	T	G	Anc	AGA to AGC	R to S	YPO3277	*rluD*	23S rRNA pseudouridine synthase D
3667806	A	G	Anc	AAA to GAA	K to E	YPO3287	*yehT*	two-component response-regulatory protein
3806677	C	T	Anc	AGG to AAG	R to K	YPO3408	*hpt*	hypoxanthine phosphoribosyltransferase
3892488*	C	T	Anc	GCT to ACT	A to T	YPO3483		multidrug efflux protein
3973746	C	T	Anc	ACT to ATT	T to I	YPO3559		
4066494*	C	T	der	GTT to ATT	V to I	YPO3646	*pcp (pcpY,*	outer membrane lipoprotein

							slyB)	
4080579	T	C	Anc	CAC to CGC	H to R	YPO3659	*accB*	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit
4194600	G	A	Anc	GTT to ATT	V to I	YPO3742	*thiG*	thiazole synthase
4243823	A	T	Anc	TTT to TAT	F to Y	YPO3781	*ubiE*	ubiquinone,menaquinone biosynthesis methyltransferase
4307755	G	A	der	GCG to GTG	A to V	YPO3839		
4339366	T	G	Anc	AGT to CGT	S to R	YPO3865	*wzzE*	lipopolysaccharide biosynthesis protein
4399470	A	G	Anc	AGC to GGC	S to G	YPO3917		dihydrolipoamide dehydrogenase
4421633	T	C	Anc	GTG to GCG	V to A	YPO3937	*glpD*	glycerol-3-phosphate dehydrogenase
4421689	A	G	Anc	ACA to GCA	T to A	YPO3937	*glpD*	glycerol-3-phosphate dehydrogenase
4460688	C	T	der	CGG to CAG	R to Q	YPO3963		sugar transport system permease
4579183	A	G	Anc	AGC to GGC	S to G	YPO4060	*fdhD*	formate dehydrogenase accessory protein
4634287	A	G	Anc	TCG to CCG	S to P	YPO4113	*phoU*	transcriptional regulator
pCD1								
29959	A	G	der	AAC to AGC	N to S	YPCD1.41	*yscO*	type III secretion apparatus component
50462	T	C	der	AAA to GAA	K to E	YPCD1.71 c	*yopJ*	targeted effector protein
66608	C	T	der	CTT to TTT	L to F	YPCD1.92		
pMT1								
12976*	A	C	anc	TAT to GAT	Y to D	YPMT1.09 c		minor tail fiber protein L

*SNPs detected in the Altenerding genome that were not called in the *Wagner et al.* Aschheim genome.

Table S11: Regions missing in CO92 but present in the Altenerding genome. Positions and annotated genes refer to the *Yersinia pseudotuberculosis* reference genome (GenBank accession NC_006155)

Start Position	End Position	Region Length (bp)	Mean Coverage	ID of genes in region	Name of genes in region	Function
97811	98932	1122	17.37	YPTB0085	*glpX*	type II fructose 1,6-bisphosphatase (part of the glpFKX operon that enables aerobic glycerol fermentation).
				YPTB0086	*glpK*	glycerol kinase (part of the glpFKX operon that enables aerobic glycerol fermentation).
263822	264118	297	7.34	YPTB0221	*ftsY*	cell division protein
369602	369881	280	12.98	YPTB0305	*ytaP*	
408757	409016	260	3.2	YPTB0343		
				YPTB0344		coproporphyrinogen III oxidase
799654	799932	279	5.24	YPTB0668	*setA (yadM)*	major facilitator superfamily transporter sugar efflux pump
807313	807587	275	5.88	Intergenic		
994244	994536	293	2.26	Intergenic		
1433136 (DFR4)	1448749	15614	20.85	YPTB1202	*xapB*	major facilitator superfamily xanthosine permease
				YPTB1203	*zraP*	zinc resistance protein
				YPTB1204		two component Histidine kinase sensor
				YPTB1205	*hydG*	transcriptional regulator
				YPTB1206	*morB*	morphinone reductase
				YPTB1207		LysR family transcriptional regulator
				YPTB1208		
				YPTB1209		multidrug ABC transporter permease
				YPTB1210		multidrug ABC transporter permease
				YPTB1211		ABC transporter ATPase
				YPTB1212		
				YPTB1213		DNA binding transcriptional regulator
				YPTB1214	*rhlE*	ATP dependent RNA helicase
1753403	1753691	289	12.94	YPTB1458	*helD*	DNA helicase IV

2222016	2222305	290	6.05	YPTB1880		
2603581	2604597	1017	13.44	YPTB2210	*tauB*	taurine/sulfonate transporter (part of the tauABC operon)
				YPTB2211	*amn*	AMP nucleosidase
3877157	3879821	2665	18.66	YPTB3285		Va autotransporter
				YPTB3286		Va autotransporter
3975571	3975841	271	10.76	YPTB3344		
				YPTB3345	*flil*	flagellum specific ATP synthase
4373203	4373468	266	12.02	YPTB3659		transferase
4490444	4490698	255	15.12	YPTB3782	*glpD*	glycerol 3-phosphate dehydrogenase
4510659	4510920	262	22.16	YPTB3789		Ig-like domain containing protein

Supplementary figures

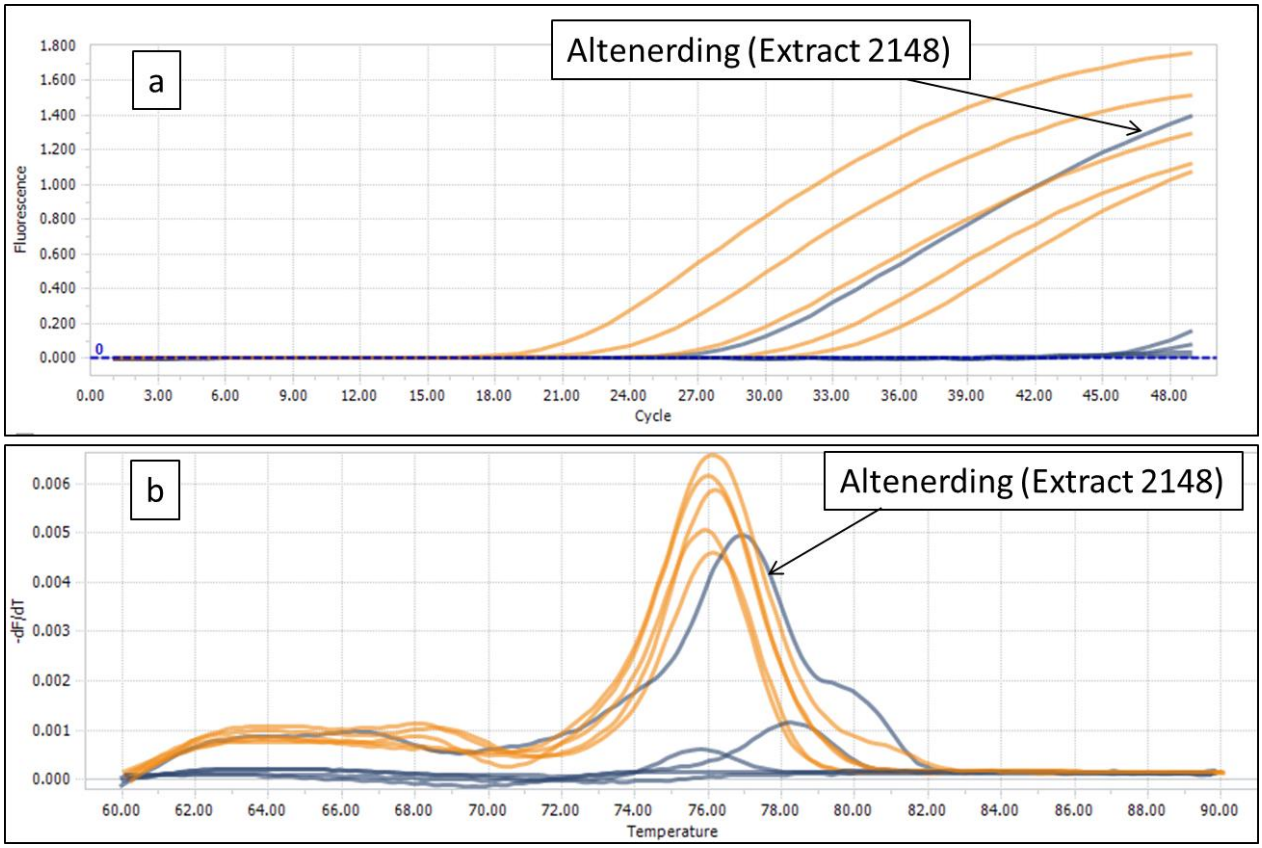


Fig.S1: *pla* gene qPCR amplification and melting curves used for sample screening. The standards of known copy number (22,300, 2230, 223, 22.3 and 2.23 copies/uL) are in duplicates, shown in yellow and grey. (a) Amplification curves. (b) Melting peaks.

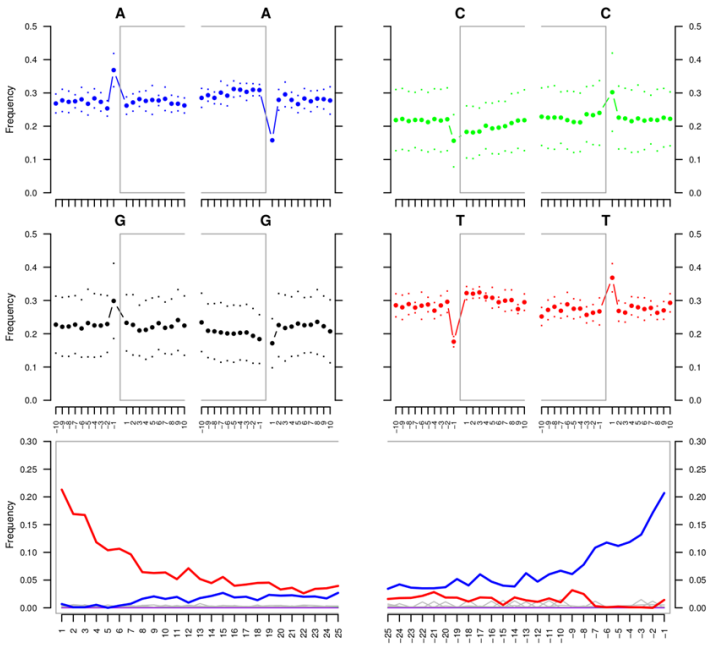


Fig.S2: mapDamage fragment misincorporation plot based on the non-UDG treated Altenerding library mapped to the CO92 chromosome reference (5510 fragments).

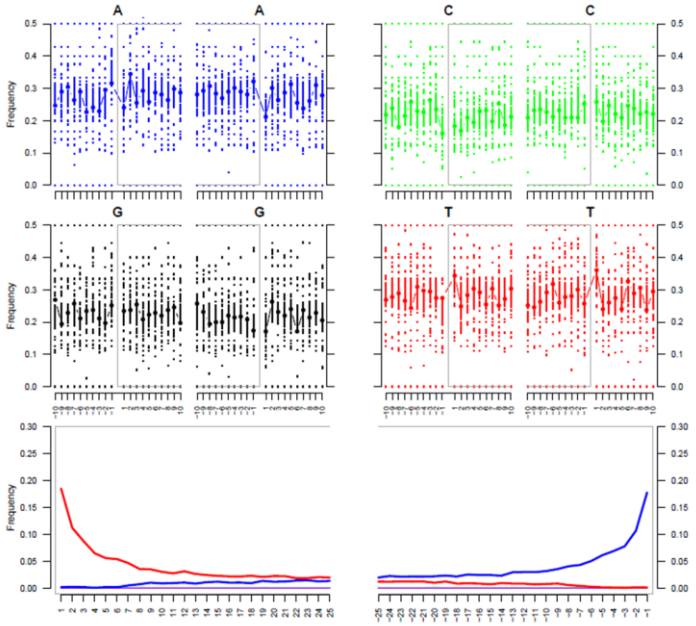


Fig.S3: mapDamage fragment misincorporation plot based on the non-UDG treated Altenerding library mapped to the human hg19 reference (45,359 fragments).

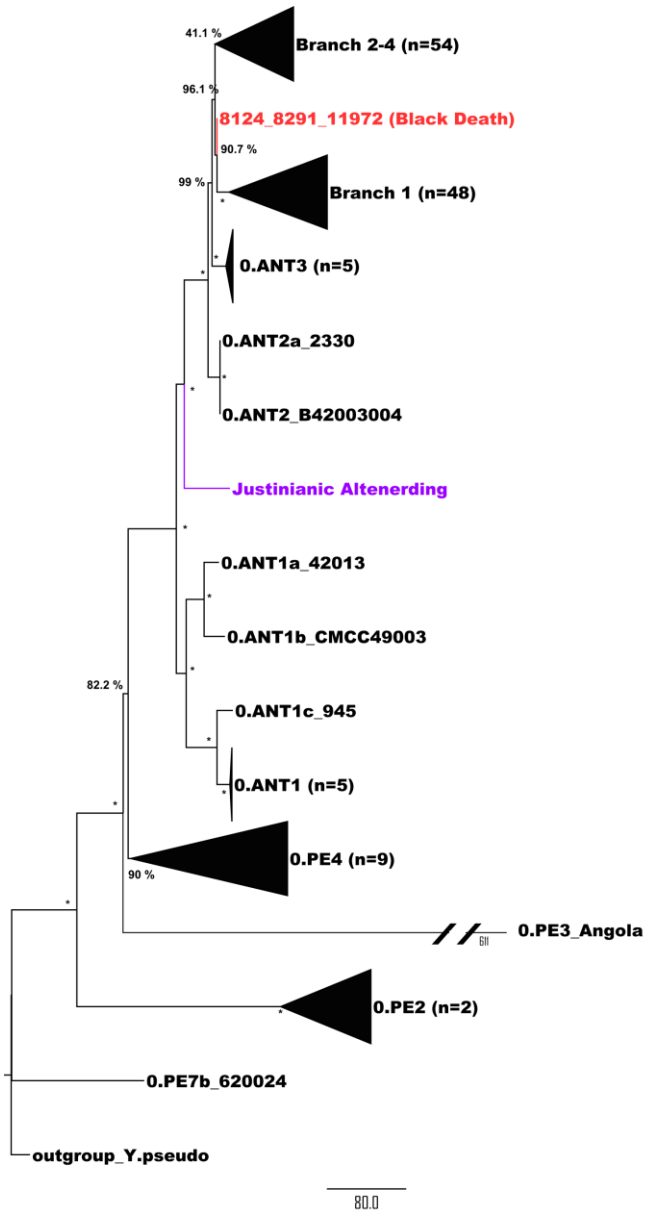


Fig.S4: Maximum Parsimony tree excluding the Aschheim genome. Maximum Parsimony analysis of 2603 nucleotide positions from genomes of 132 *Y. pestis* strains (the Aschheim genome was excluded from this analysis). All positions containing missing data were eliminated. Bootstrap values are next to nodes and bootstrap values of 100 % are indicated by an asterisk. The tree is rooted using the genome of *Y. pseudotuberculosis* (strain IP32953). Branches leading to isolates from the historical pandemics are colored red and purple representing the second and first pandemics respectively. Number of isolates in a collapsed node is indicated in brackets.

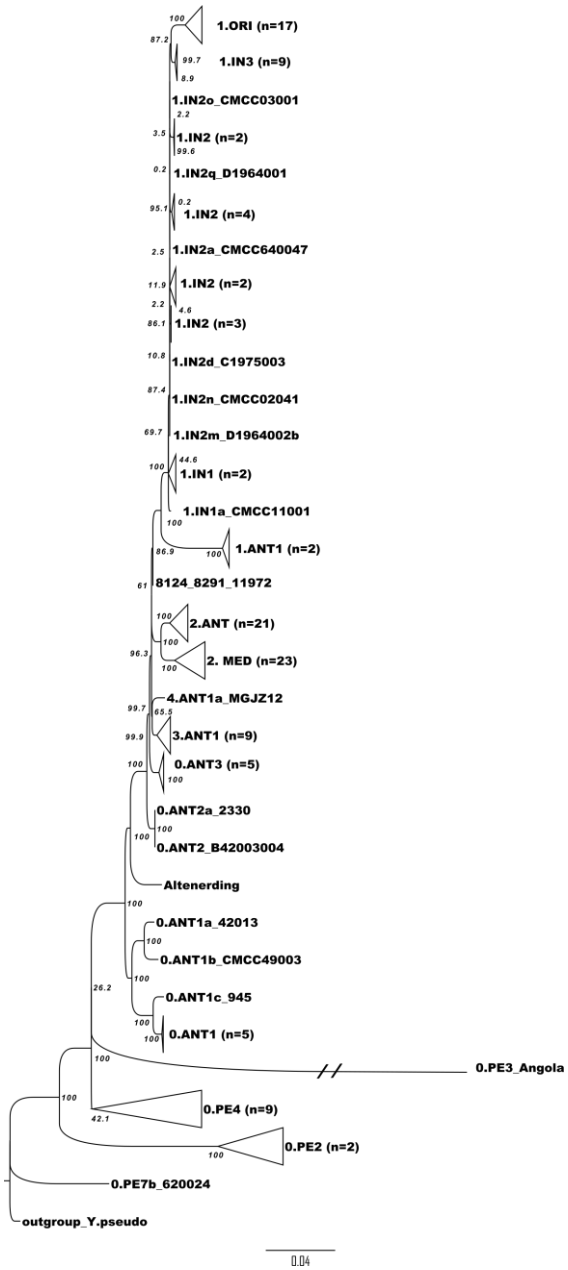


Fig.S5: Maximum Likelihood tree excluding the Aschheim genome. Maximum Likelihood analysis of 2603 nucleotide positions from genomes of 132 *Y. pestis* strains (the Aschheim genome was excluded from this analysis). All positions containing gaps and missing data were eliminated. Bootstrap values in italics. The tree is rooted using the genome of *Y. pseudotuberculosis* (strain IP32953). Number of isolates in a collapsed node is indicated in brackets.

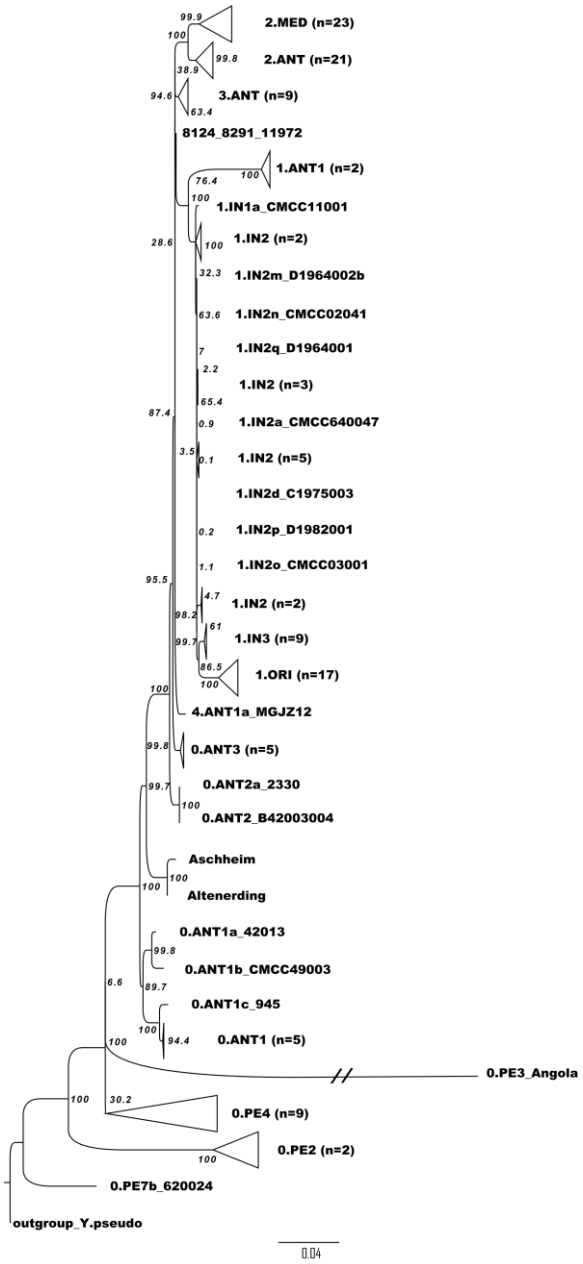
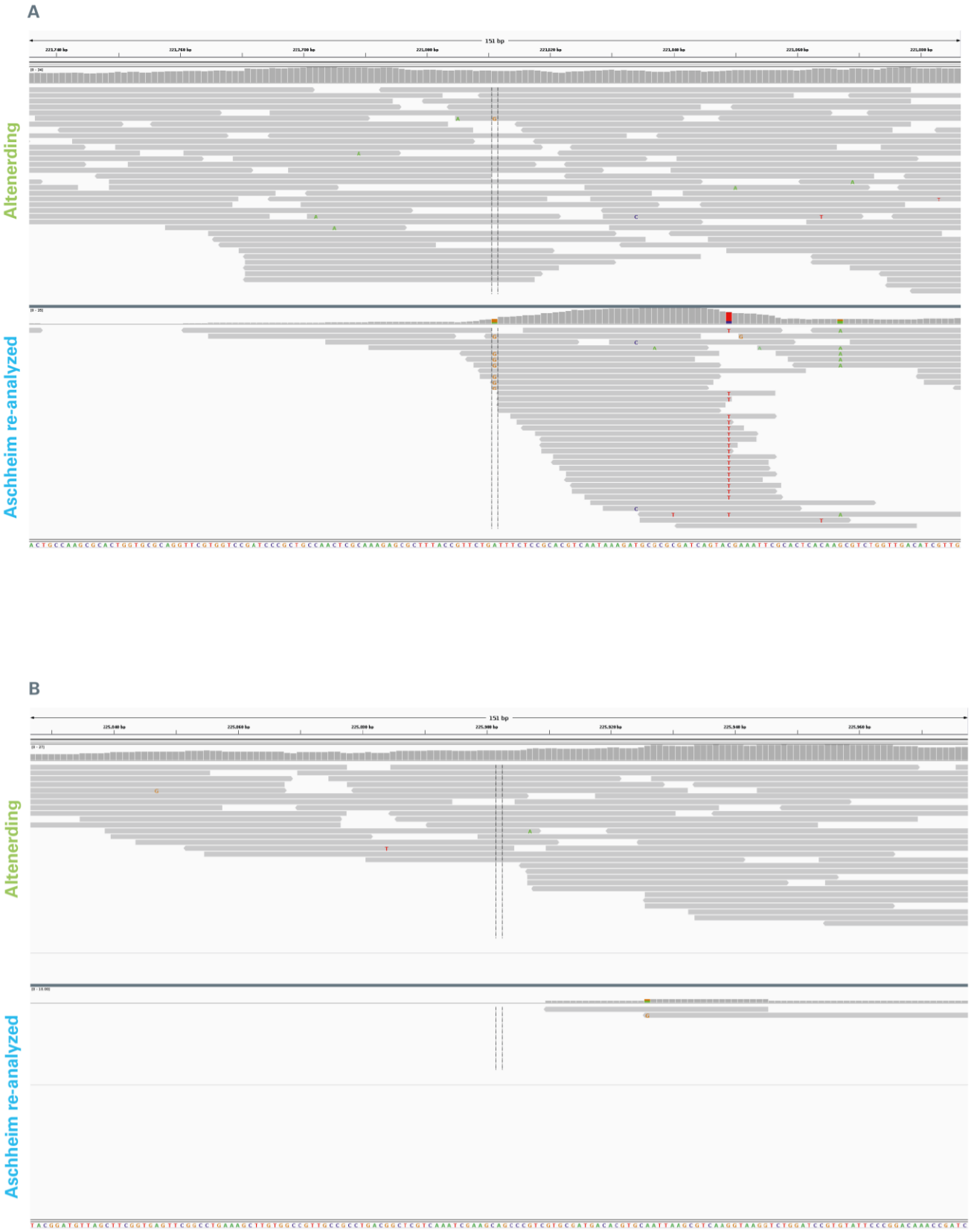
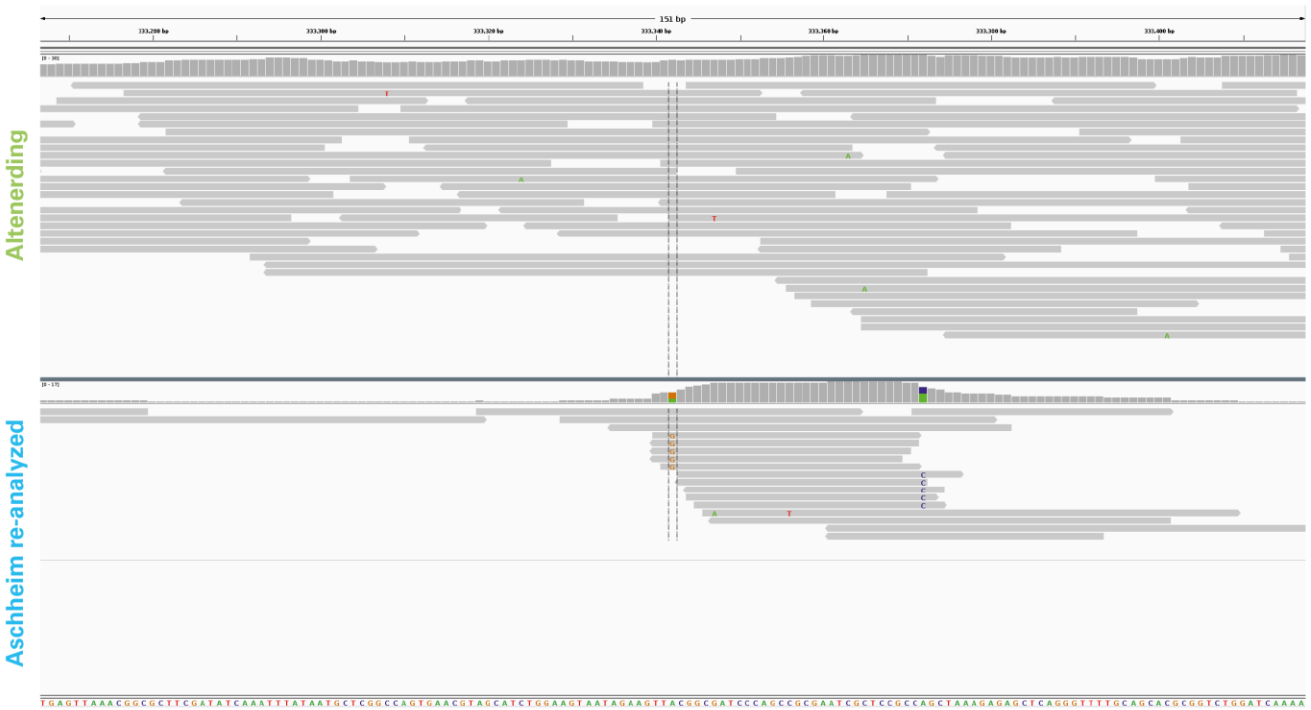


Fig.S6: Maximum Likelihood tree. Maximum Likelihood analysis of 1418 nucleotide positions from genomes of 133 *Y. pestis* strains. All positions containing gaps and missing data were eliminated. Bootstrap values in italics. The tree is rooted using the genome of *Y. pseudotuberculosis* (strain IP32953). Number of isolates in a collapsed node is indicated in brackets.


A
151 bp
Altenerding
Aschheim re-analyzed
B
151 bp
Altenerding
Aschheim re-analyzed

C


151 bp
Altenerding
Aschheim re-analyzed

D


151 bp
Altenerding
Aschheim re-analyzed

E


151 bp
Altenerding
Aschheim re-analyzed

F


231 bp
Altenerding
Aschheim re-analyzed

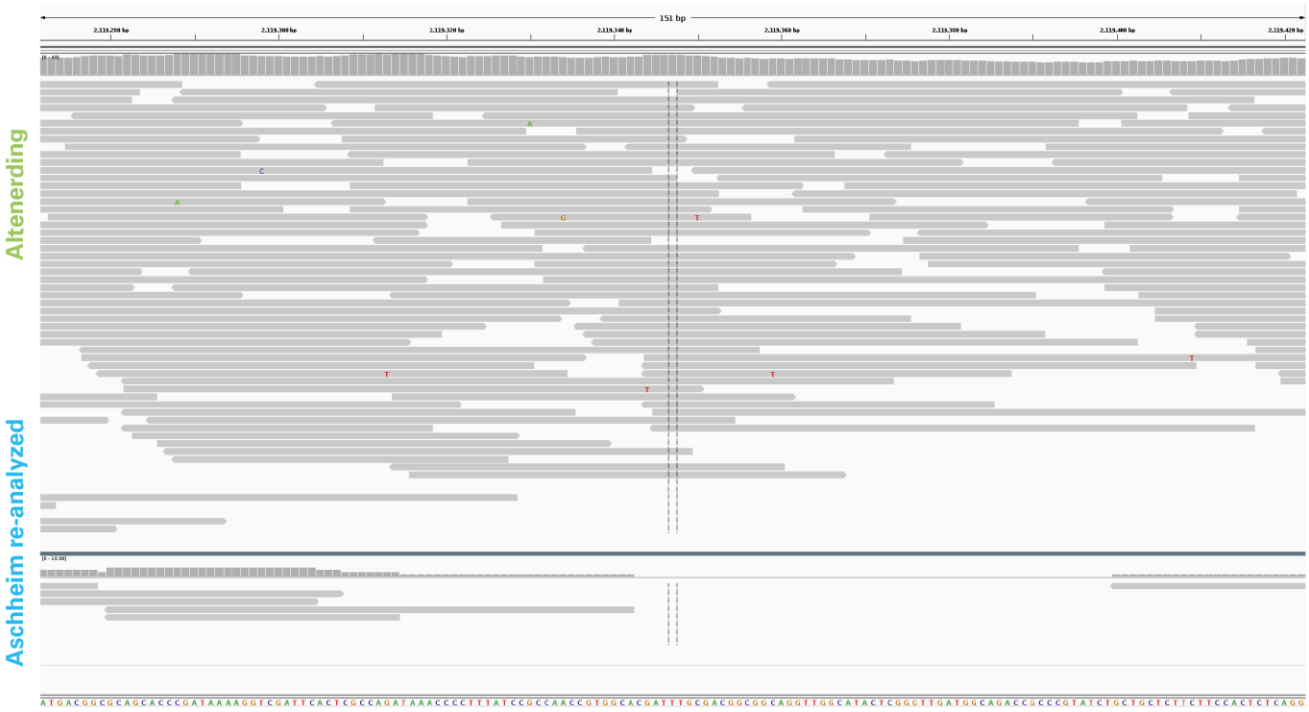
G


Altenerding
Aschheim re-analyzed

H


Altenerding
Aschheim re-analyzed

I


151 bp
Altenerding
Aschheim re-analyzed

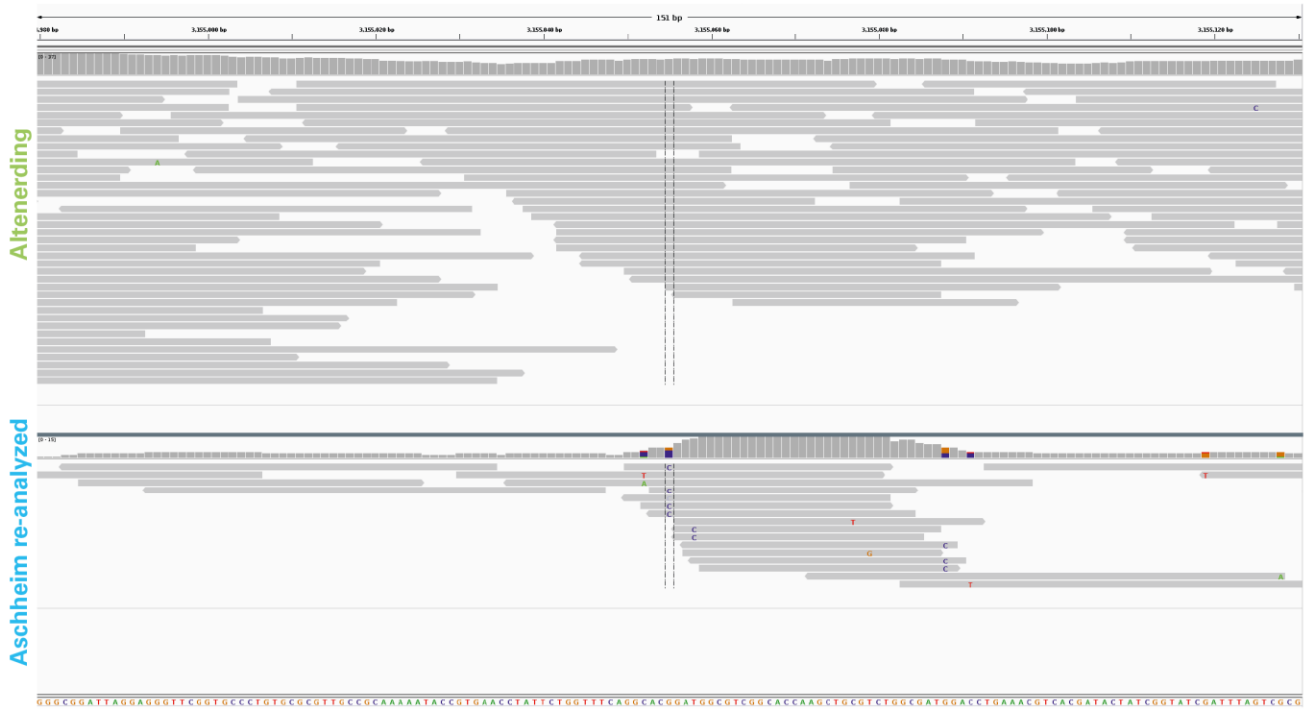
J


151 bp
Altenerding
Aschheim re-analyzed

K


151 bp
Altenerding
Aschheim re-analyzed

L


151 bp
Altenerding
Aschheim re-analyzed

M


151 bp
Altenerding
Aschheim re-analyzed

N


151 bp
Altenerding
Aschheim re-analyzed

O


Altenerding
Aschheim re-analyzed

P


Altenerding
Aschheim re-analyzed

Q


151 bp
Altenerding
Aschheim re-analyzed

R

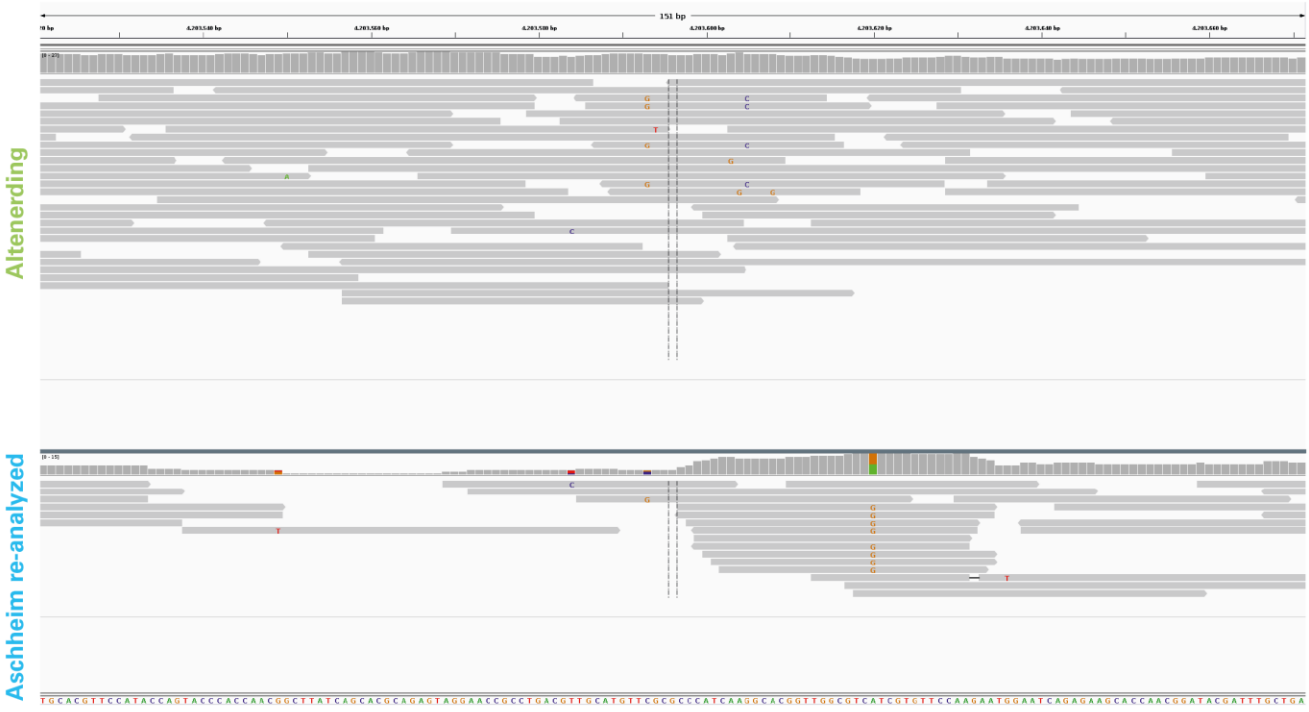

151 bp
Altenerding
Aschheim re-analyzed

Fig.S7 (A-S): Visualization of positions containing potential false positive SNPs called by *Wagner et al.* 2014. Reads were mapped to the CO92 reference with sensitivity of 0.1 and minimum mapping quality of 30 and visualized on IGV gene browser. Upper bend shows coverage plot for the region corresponding to the genome beneath. Upper scale shows position (bp) in reference sequence. Bottom sequence shows the 150 bp in the CO92 reference. Dotted line marks the potential false positive SNP.

(A) Position 221811: Re-analysis of the Aschheim raw data shows 70 % variant frequency for the “A” to “G” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The “G” variants are located at end of reads in a region with an abnormal cover peak. The Altenerding mapping shows even 24 fold coverage in the region and a 96 % variant frequency that supports the “A” variant (identical to reference).

(B) Position 225902: Re-analysis of the Aschheim raw data shows no coverage in position 225902 called as an “A” to “T” SNP by Wagner et al., in contrast to a 6 fold coverage in the original analysis (Wagner et al. 2014). The Altenerding mapping shows even 16 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference).

(C) Position 333342: Re-analysis of the Aschheim raw data shows 63 % variant frequency for the “A” to “G” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The “G” variants are located at end of reads in a region with an abnormal peak in coverage. The Altenerding mapping shows even 22 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference).

(D) Position 497800: Re-analysis of the Aschheim raw data shows 72 % variant frequency for the “T” to “A” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The “A” variants are located at end of reads in a region with an abnormal peak in coverage. The Altenerding mapping shows even 17 fold coverage in the region and a 100 % variant frequency that supports the “T” variant (identical to reference).

(E) Position 137020: Re-analysis of the Aschheim raw data shows 1 fold coverage in position 137020 called as a “C” to “A” SNP by Wagner et al., in contrast with a 8 fold coverage in the original analysis (Wagner et al. 2014). The one read covering the position shows the “C” variant (identical to reference). The Altenerding mapping shows even 17 fold coverage in the region and a 100 % variant frequency that supports the “C” variant (identical to reference).

(F) Position 137025: Re-analysis of the Aschheim raw data shows 86 % variant frequency for the “C” to “T” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The “T” variants are located at end of reads in a region with an abnormal peak in coverage. The Altenerding mapping shows even 18 fold coverage in the region and a 100 % variant frequency that supports the “C” variant (identical to reference).

(G) Position 1864793: Re-analysis of the Aschheim raw data shows 54 % variant frequency for the “A” to “G” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The reads containing the “G” variants are located in a region with an abnormal peak in coverage. The Altenerding mapping shows even 16 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference).

(H) Position 2072914: Re-analysis of the Aschheim raw data shows 80 % variant frequency for the “G” to “A” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The “A” variants are located at end of reads in a region with an abnormal peak in coverage. The Altenerding mapping shows 37 fold coverage in the region and a 95% variant frequency that supports the “G” variant (identical to reference).

(I) Position 2119347: Re-analysis of the Aschheim raw data shows no coverage in position 2119347 called as a “T” to “A” SNP by Wagner et al., in contrast with a 7 fold coverage in the original analysis (Wagner et al. 2014). The Altenerding mapping shows even 45 fold coverage in the region and a 100 % variant frequency that supports the “T” variant (identical to reference).

(J) Position 2865494: Re-analysis of the Aschheim raw data shows 3 fold coverage in position 2865494 called as an “A” to “C” SNP by Wagner et al., in contrast with a 9 fold coverage in the original analysis (Wagner et al. 2014). Coverage is lower than the 5 fold minimum set by Wagner et al. The Altenerding mapping shows even 30 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference).

(K) Position 3143800: Re-analysis of the Aschheim raw data shows 1 fold coverage in position 3143800 called as a “G” to “T” SNP by Wagner et al., in contrast with a 29 fold coverage in the original analysis (Wagner et al. 2014). The one read covering the position shows the “G” variant (identical to reference). The Altenerding mapping shows even 21 fold coverage in the region and a 100 % variant frequency that supports the “G” variant (identical to reference).

(L) Position 3155055: Re-analysis of the Aschheim raw data shows 71 % variant frequency for the “G” to “C” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The “C” variants are located at end of reads in a region with an abnormal peak in coverage. The Altenerding mapping shows even 27 fold coverage in the region and a 100 % variant frequency that supports the “G” variant (identical to reference).

(M) Position 3392897: Re-analysis of the Aschheim raw data shows 85 % variant frequency for the A to G variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The reads containing the “G” variants are located

in a region with an abnormal peak in coverage. The Altenerding mapping shows even 22 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference).

(N) Position 3813424: Re-analysis of the Aschheim raw data shows 4 fold coverage in position 3813424 called as a “C” to “A” SNP by Wagner et al., in contrast with a 23 fold coverage in the original analysis (Wagner et al. 2014). Coverage is lower than the 5 fold minimum set by Wagner et al. The A variants are located at end of reads in a region with an abnormal peak in coverage. The Altenerding mapping shows 10 fold coverage in the region and a 100 % variant frequency that supports the “C” variant (identical to reference).

(O) Position 4170791: Re-analysis of the Aschheim raw data shows 80 % variant frequency for the “A” to “G” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set in Wagner et al. The reads containing the “G” variants are located in a region with an abnormal peak in coverage. The Altenerding mapping shows even 25 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference).

(P) Position 4199187: Re-analysis of the Aschheim raw data shows 75 % variant frequency and 3 fold coverage for the “A” to “G” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % and coverage is lower than the 5 fold minimum set by Wagner et al. The only “G” variant is located at the end of a read in a region with an abnormal peak in coverage. The Altenerding mapping shows 47 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference).

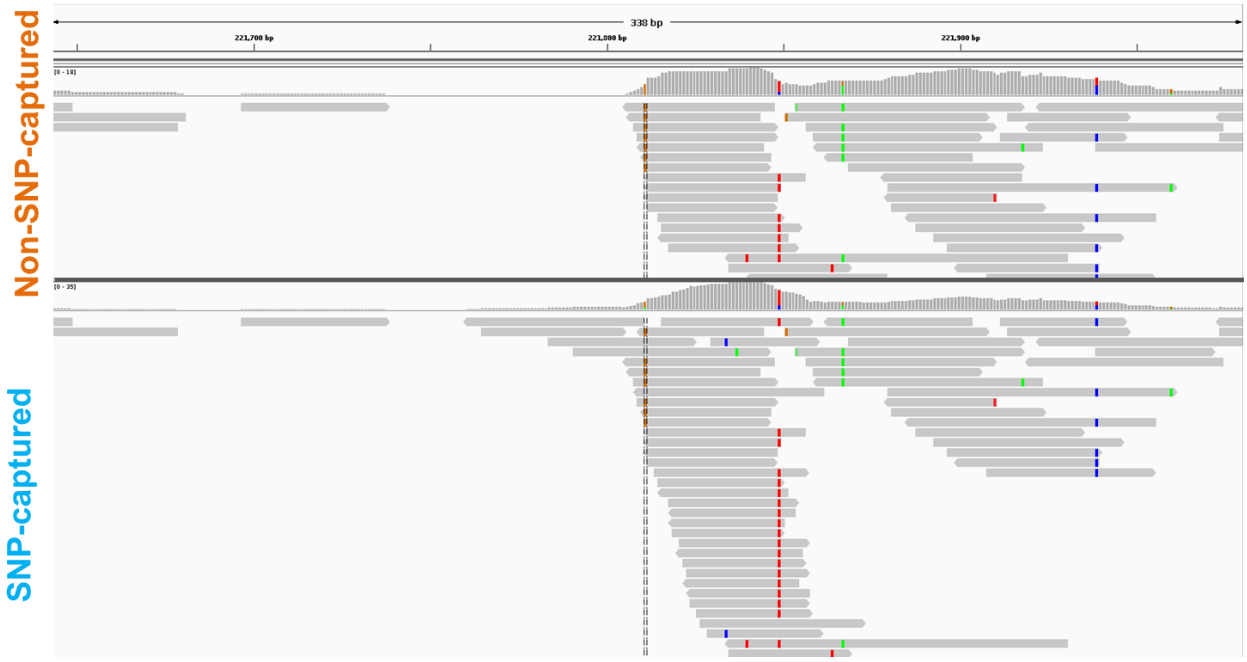
(Q) Position 4199190: Re-analysis of the Aschheim raw data shows 38 % variant frequency for the “T” to “C” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The “C” variants are located at end of reads in a region with an abnormal peak in coverage. The Altenerding mapping also shows a cover peak in this region with 50 fold coverage and a 54% variant frequency of the “T” variant (identical to reference).

(R) Position 4203596: Re-analysis of the Aschheim raw data shows 3 fold coverage in position 4203596 called as a “G” to “T” SNP by Wagner et al., in contrast with a 51 fold coverage in the

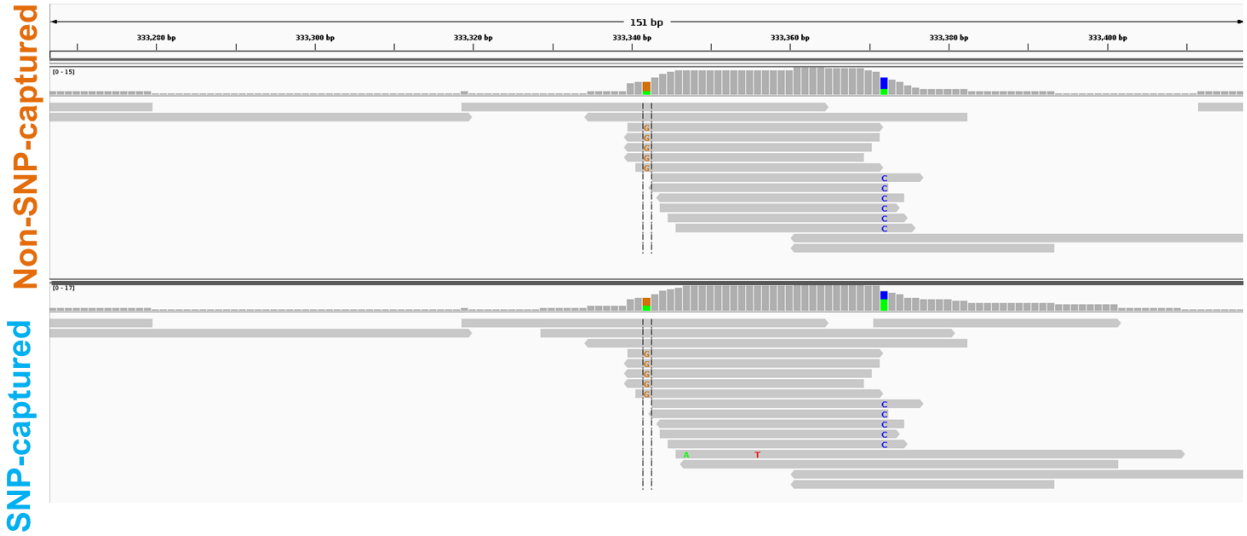
original analysis (Wagner et al. 2014). All 3 reads contain the “G” variant (identical to reference). The Altenerding mapping shows even 22 fold coverage in the region and a 100 % variant frequency that supports the “G” variant (identical to reference).

(S) Position 4210011: Re-analysis of the Aschheim raw data shows 8 fold coverage in position 4210011 called as a “T” to “C” SNP by Wagner et al., in contrast with a 262 fold coverage in the original analysis (Wagner et al. 2014). All 8 reads contain the “T” variant (identical to reference). The Altenerding mapping shows 40 fold coverage in the position and an 85 % variant frequency of the “T” variant (identical to reference).

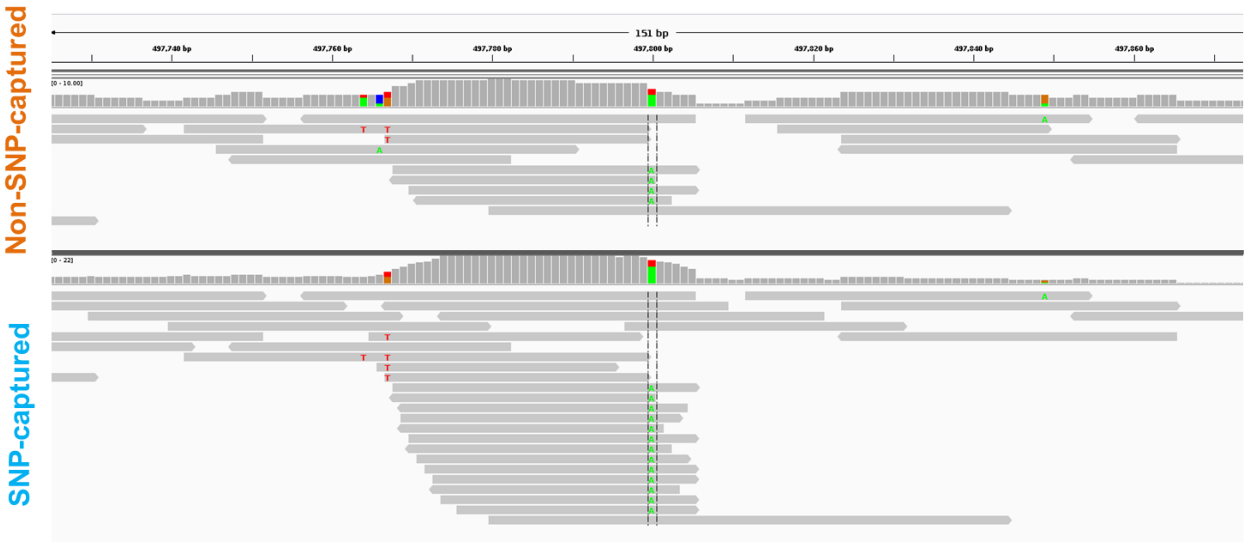
A


Non-SNP-captured
SNP-captured
338 bp
221,700 bp
221,800 bp
221,900 bp

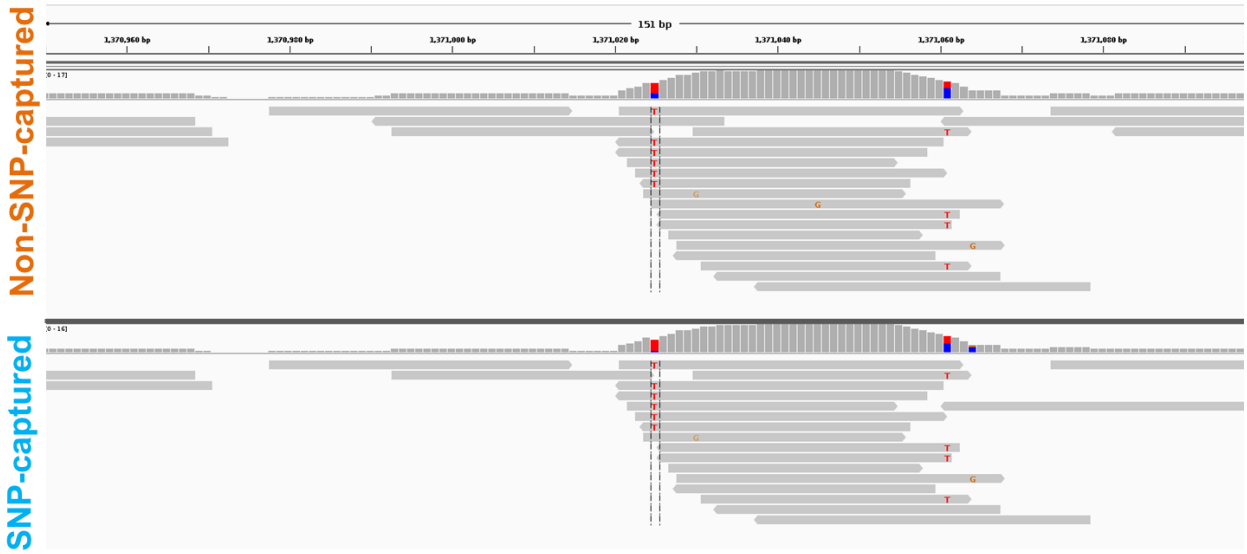
B


Non-SNP-captured
SNP-captured
151 bp
333,280 bp
333,300 bp
333,320 bp
333,340 bp
333,360 bp
333,380 bp
333,400 bp

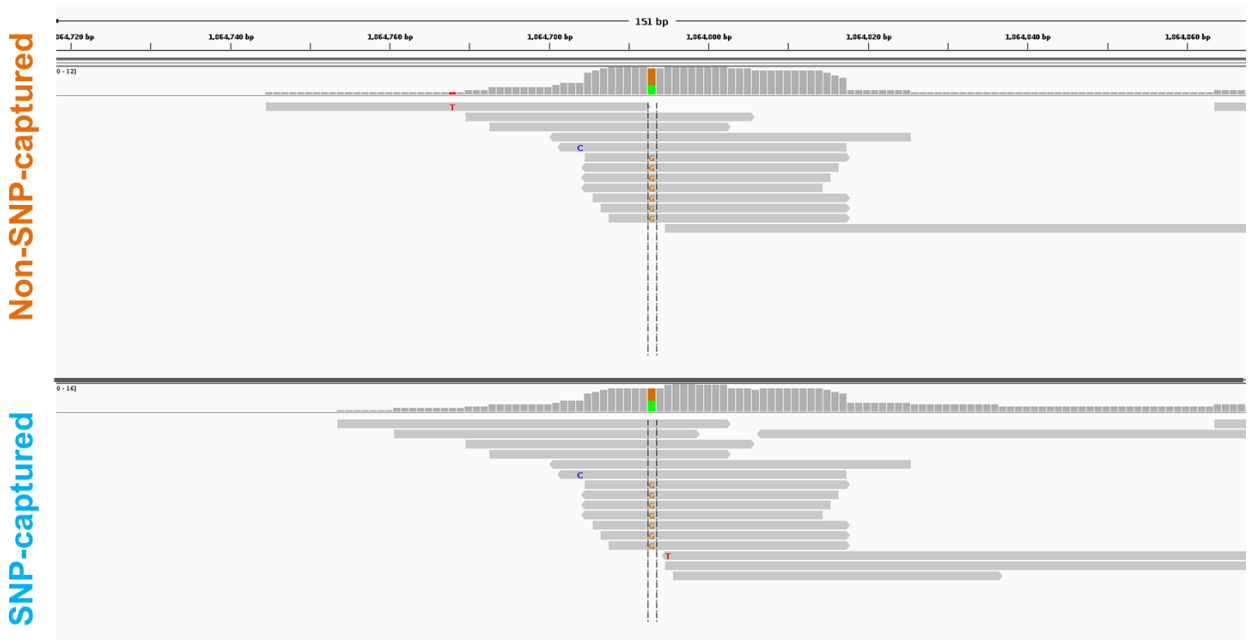
C


Non-SNP-captured
SNP-captured
151 bp
457,740 bp
457,760 bp
457,780 bp
457,800 bp
457,820 bp
457,840 bp
457,860 bp

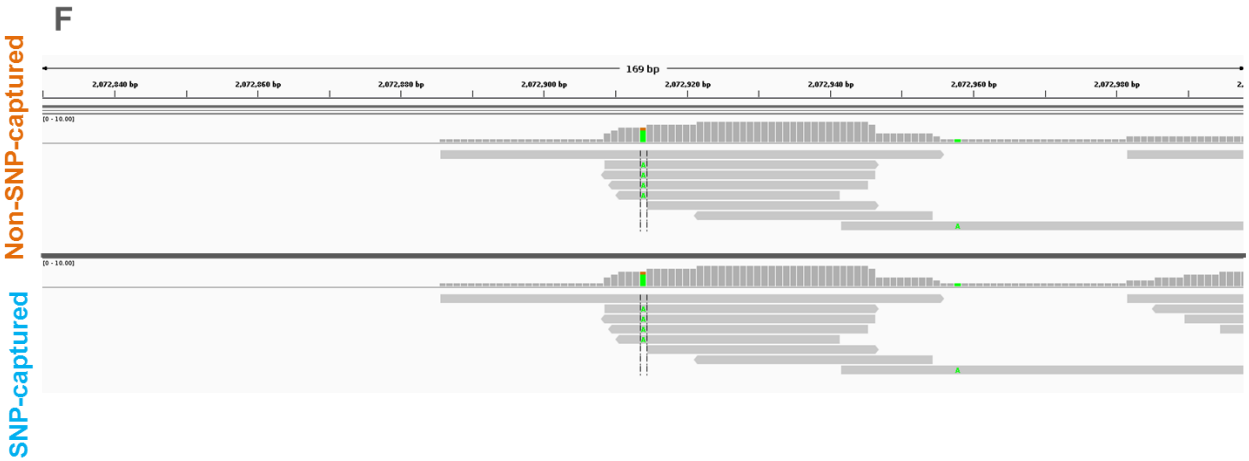
D


Non-SNP-captured
SNP-captured
151 bp
1,370,960 bp
1,370,980 bp
1,371,000 bp
1,371,020 bp
1,371,040 bp
1,371,060 bp
1,371,080 bp

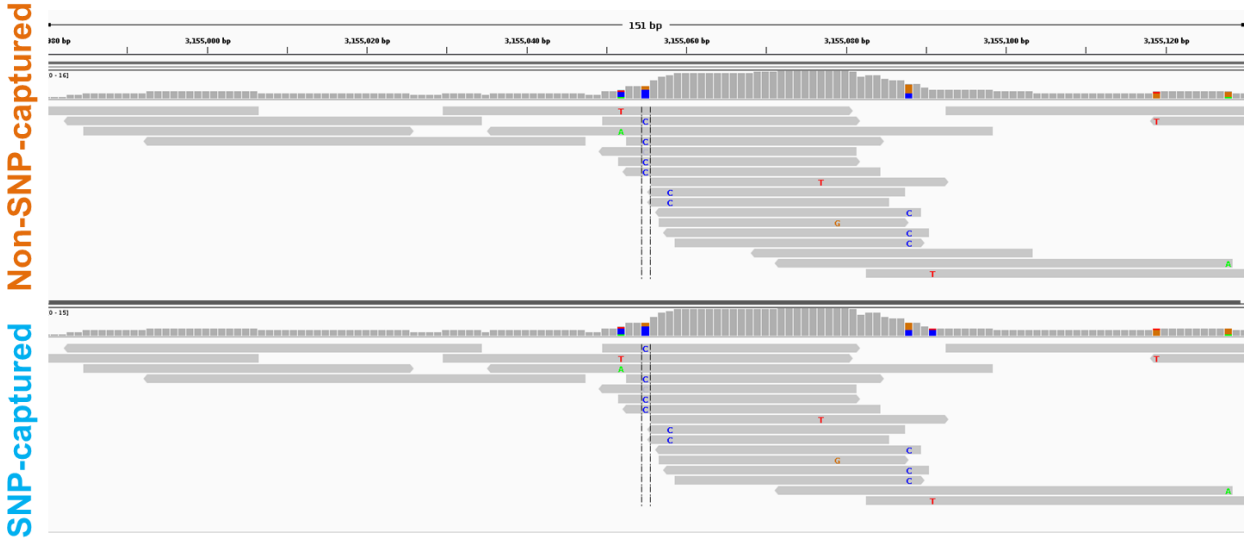
E


Non-SNP-captured
SNP-captured
151 bp

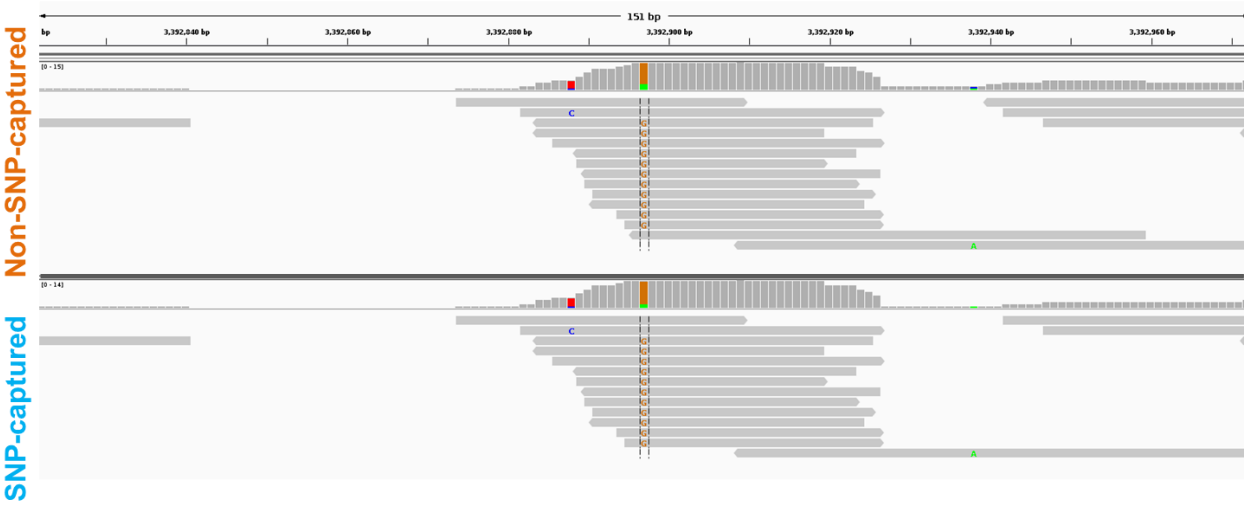
F


Non-SNP-captured
SNP-captured
169 bp

G


Non-SNP-captured
SNP-captured
151 bp
3,155,000 bp
3,155,020 bp
3,155,040 bp
3,155,060 bp
3,155,080 bp
3,155,100 bp
3,155,120 bp

H


Non-SNP-captured
SNP-captured
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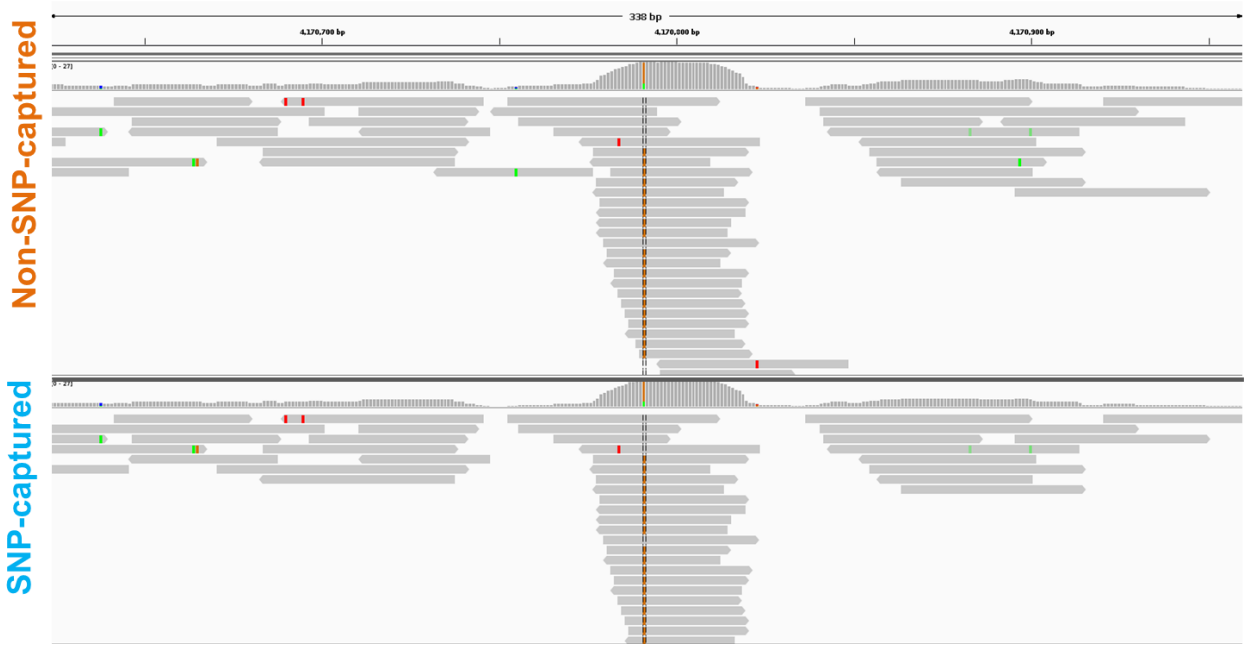
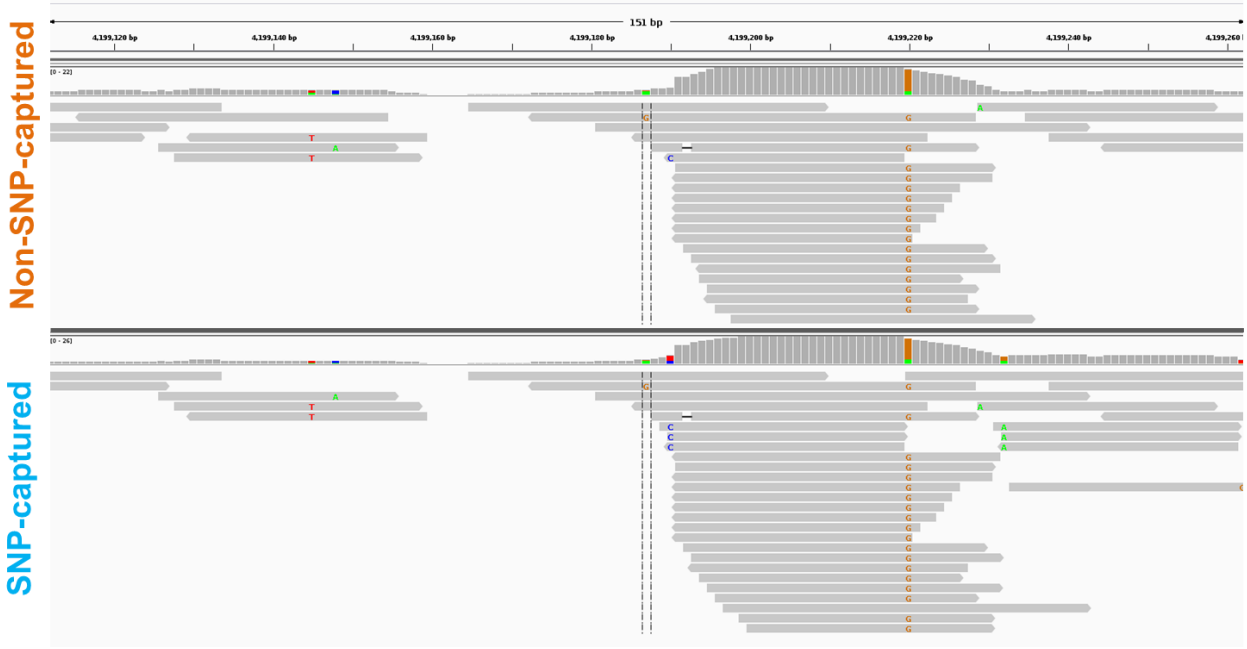
3,392,040 bp
3,392,060 bp
3,392,080 bp
3,392,900 bp
3,392,920 bp
3,392,940 bp
3,392,960 bp

I


Non-SNP-captured
SNP-captured
338 bp
4,170,700 bp
4,170,000 bp
4,170,900 bp

J


Non-SNP-captured
SNP-captured
151 bp
4,199,120 bp
4,199,140 bp
4,199,160 bp
4,199,180 bp
4,199,200 bp
4,199,220 bp
4,199,240 bp
4,199,260

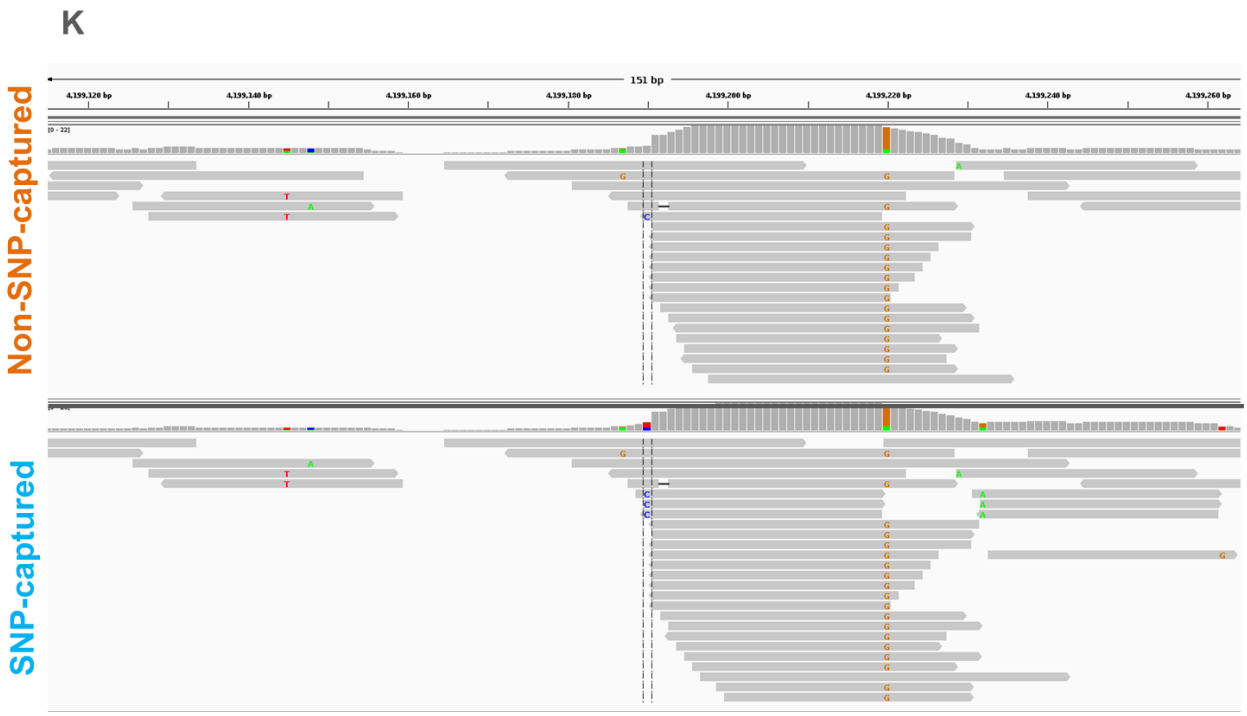
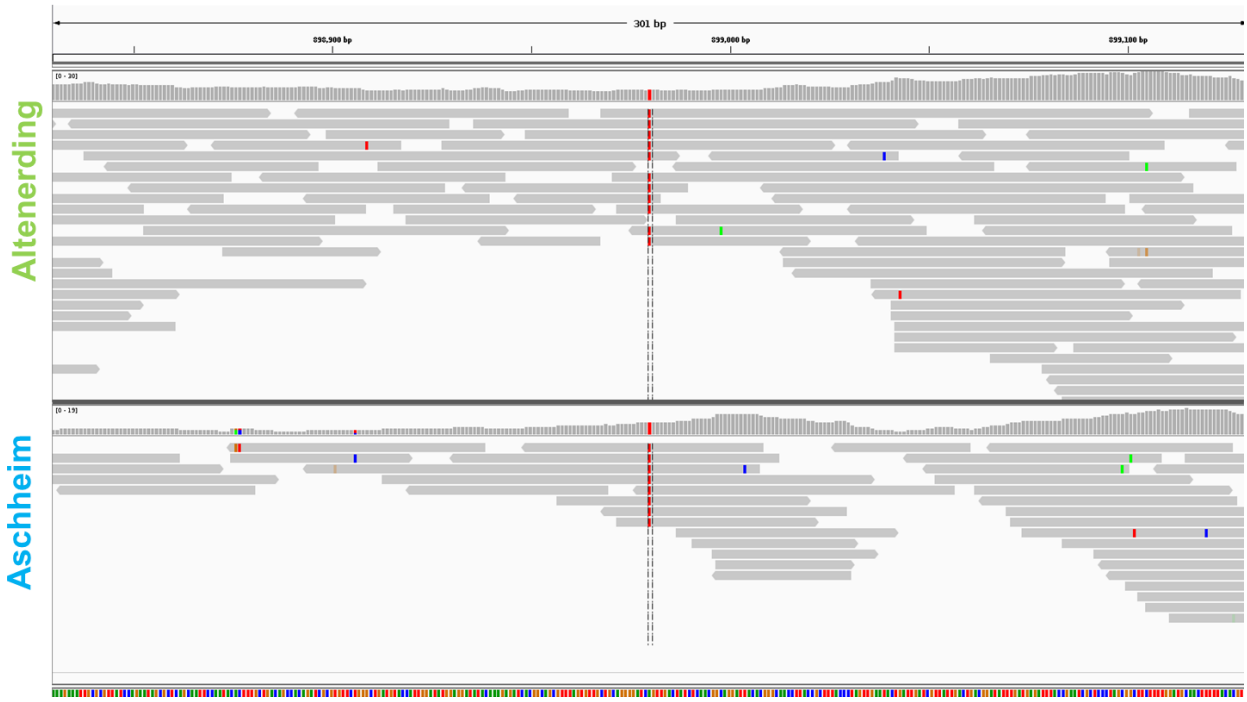
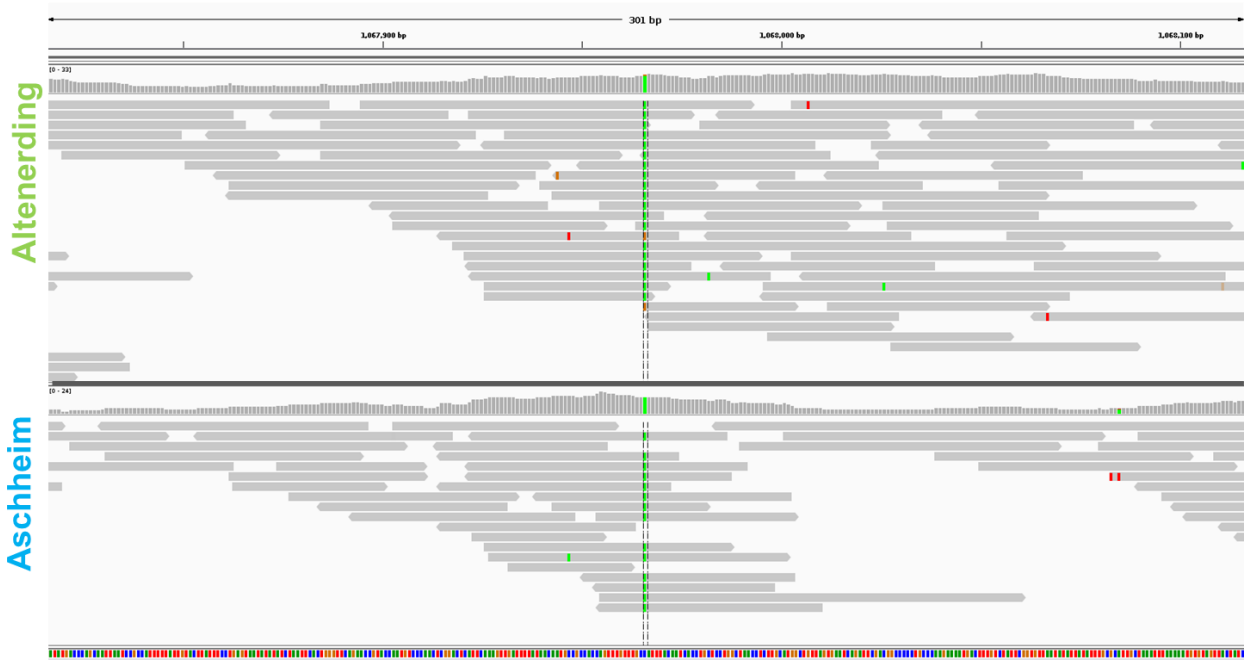


Fig. S8 (A-K): Visualization of the abnormal coverage peaks in the re-analyzed Aschheim SNP enriched data and non-SNP enriched data, containing potential false positive SNPs called by *Wagner et al.* 2014. Reads were mapped to the CO92 reference with sensitivity of 0.1 and minimum mapping quality of 30 and visualized on IGV gene browser. Upper bend shows coverage plot for the region corresponding to the genome beneath. Upper scale shows position (bp) in reference sequence. Dotted line marks the SNP.

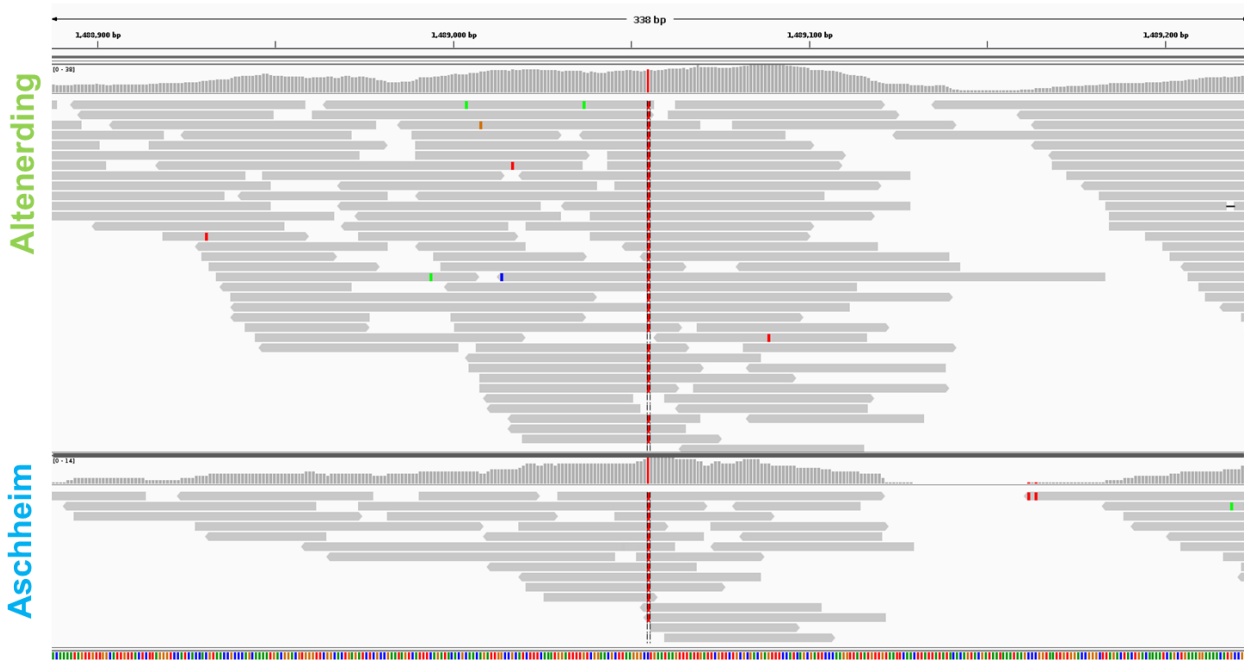
A


301 bp
898,900 bp
899,000 bp
899,100 bp
Altenerding
Aschheim

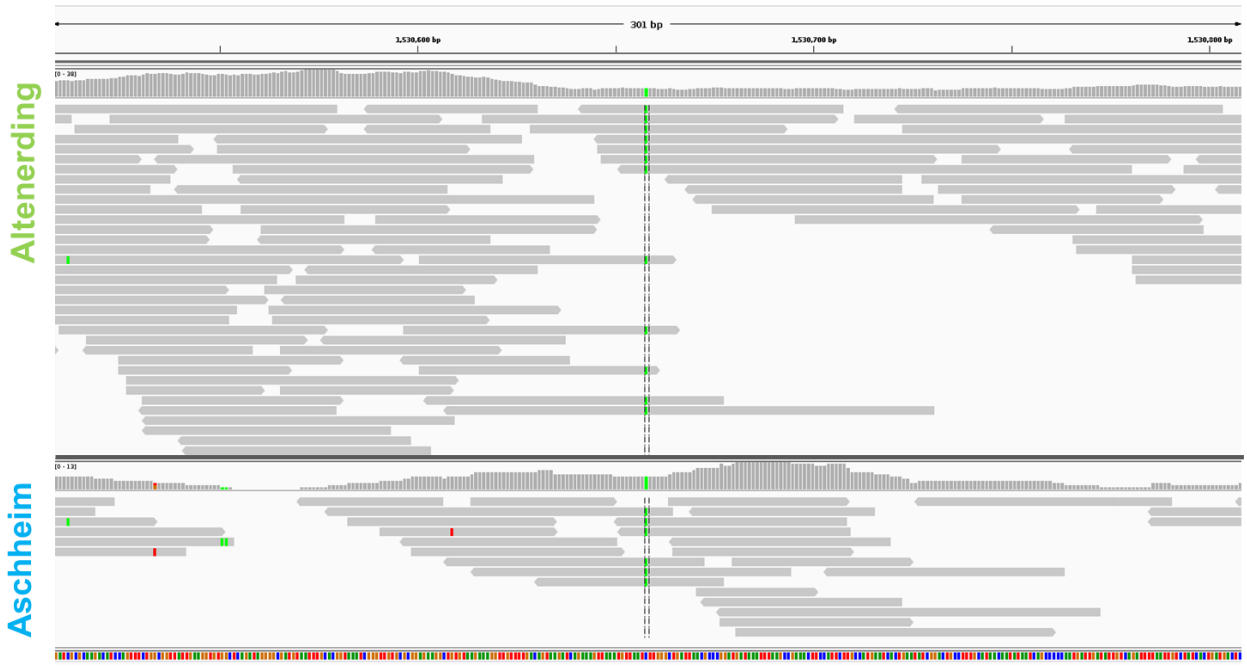
B


301 bp
1,067,900 bp
1,068,000 bp
1,068,100 bp
Altenerding
Aschheim

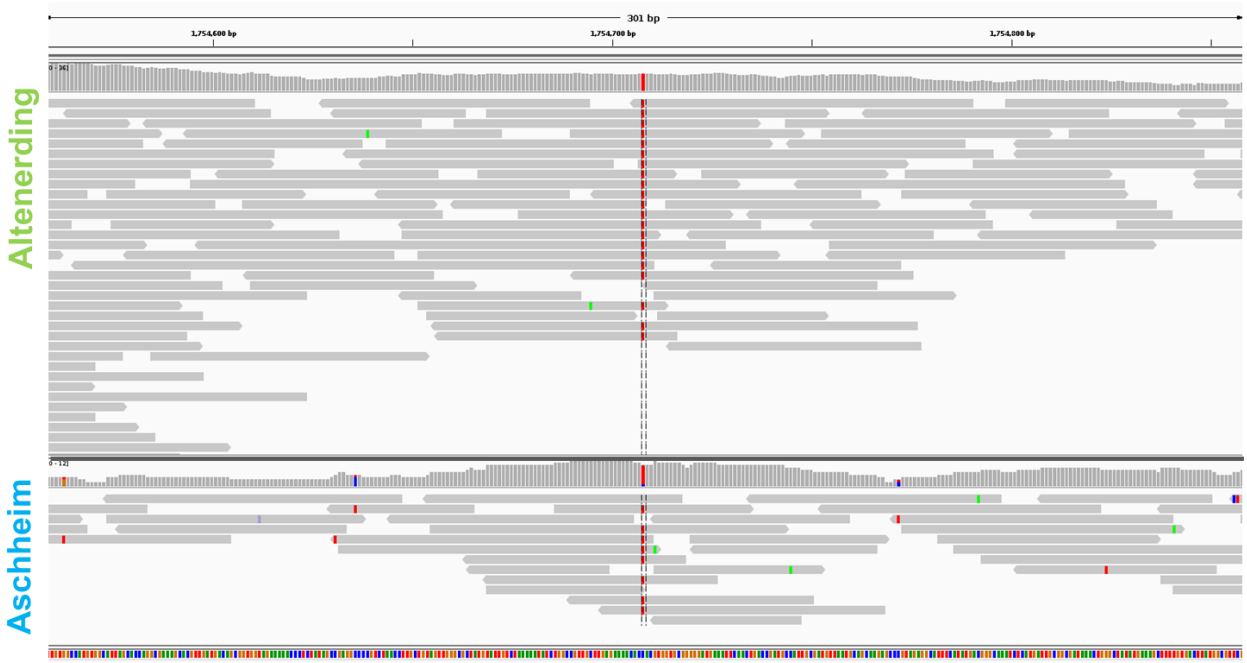
C


338 bp
1,409,000 bp
1,409,100 bp
1,409,200 bp
Altenerding
Aschheim

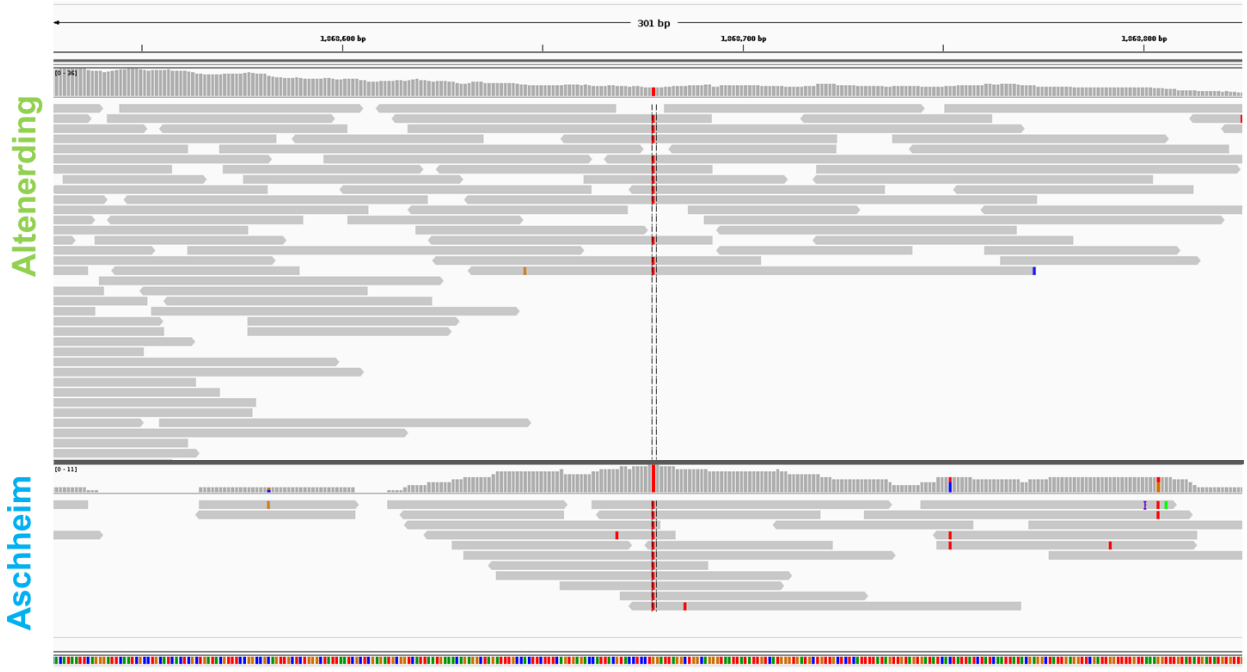
D


301 bp
1,530,600 bp
1,530,700 bp
1,530,800 bp
Altenerding
Aschheim

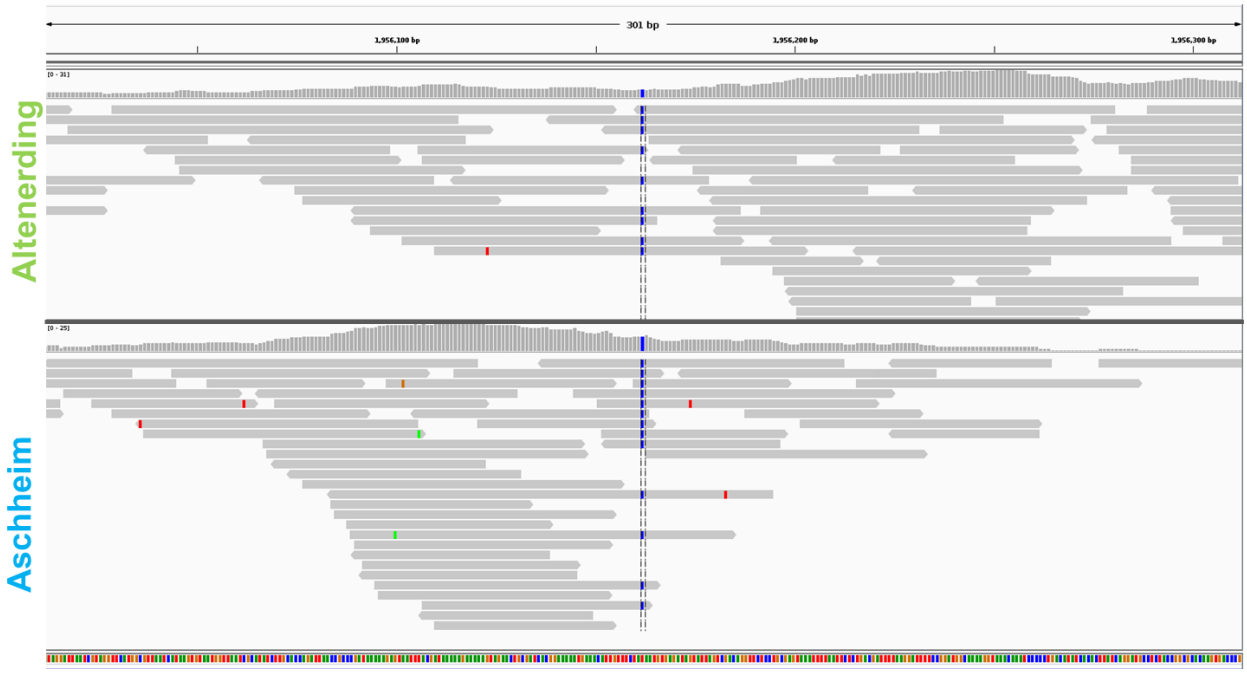
E


301 bp
1,754,600 bp
1,754,700 bp
1,754,800 bp
Altenerding
Aschheim

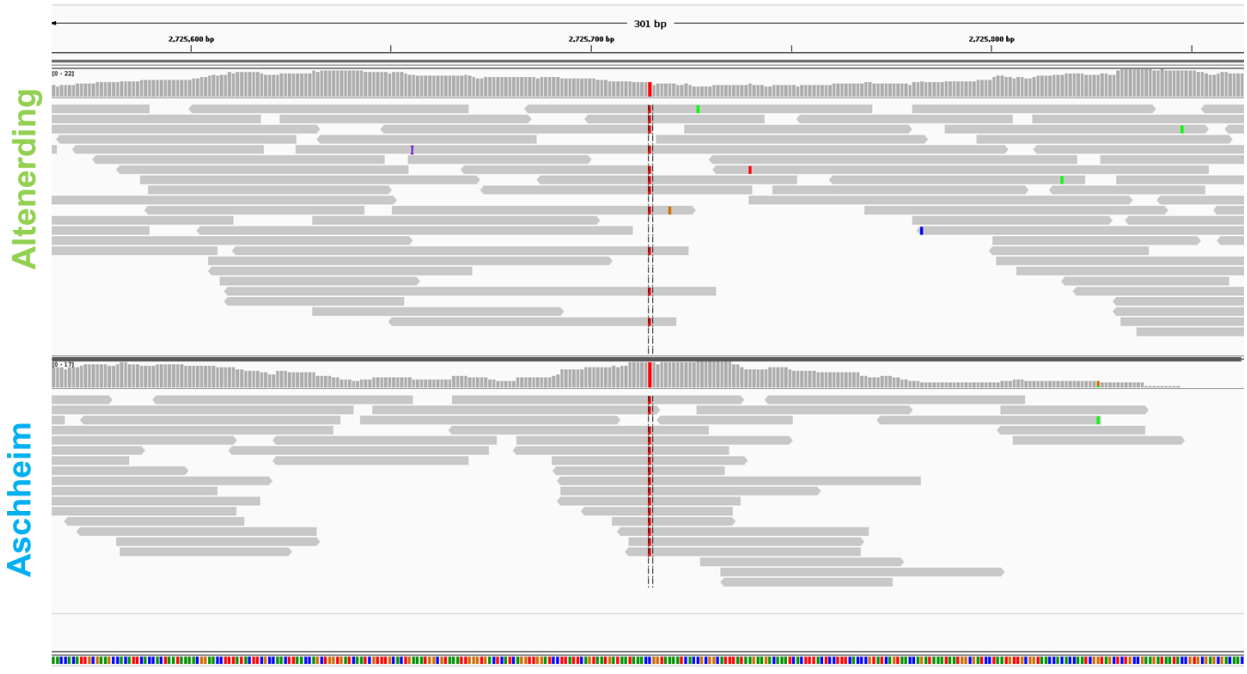
F


301 bp
1,060,600 bp
1,060,700 bp
1,060,800 bp
Altenerding
Aschheim

G


301 bp
1,956,100 bp
1,956,200 bp
1,956,300 bp
Altenerding
Aschheim

H


301 bp
2,725,600 bp
2,725,700 bp
2,725,800 bp
Altenerding
Aschheim

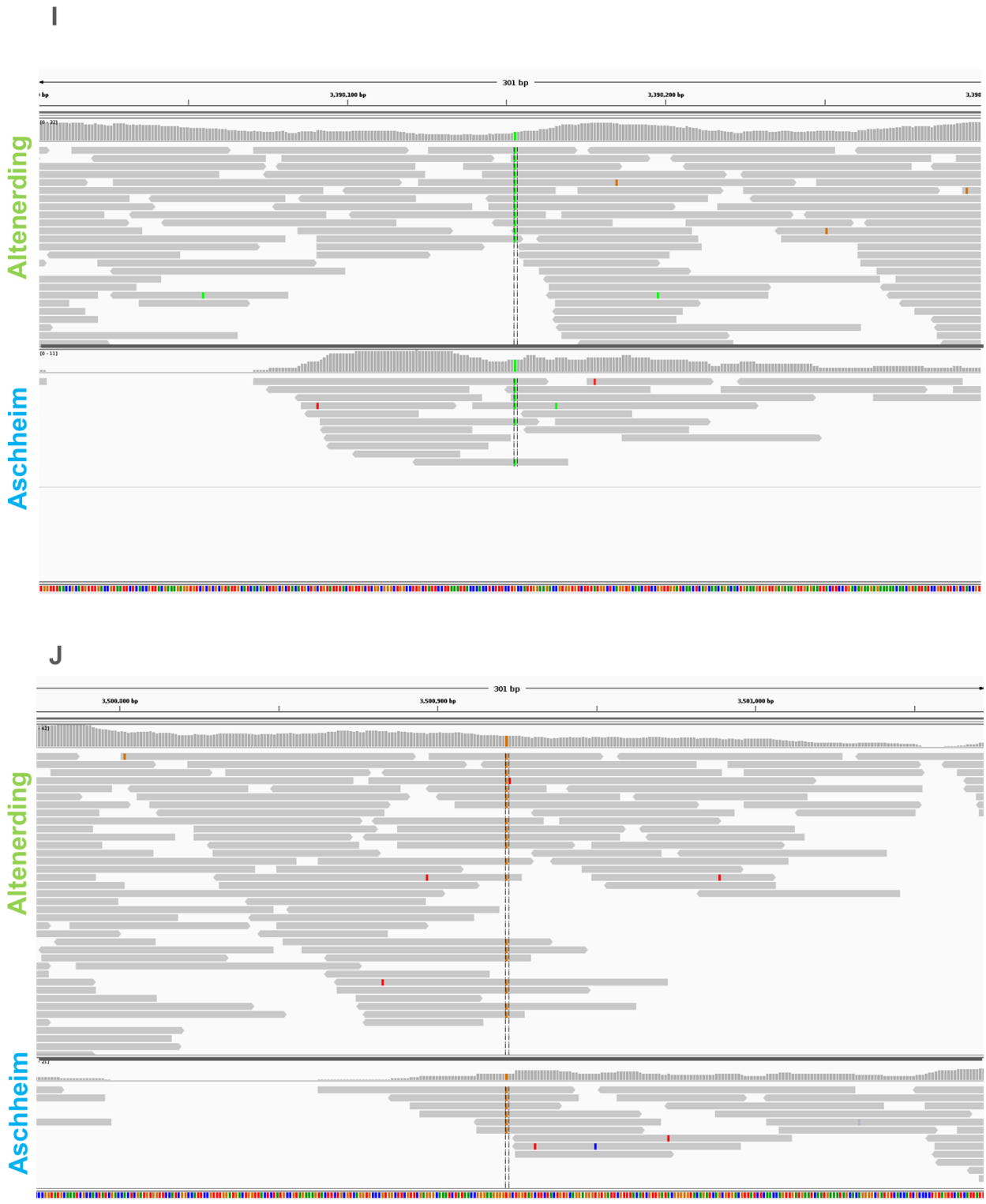
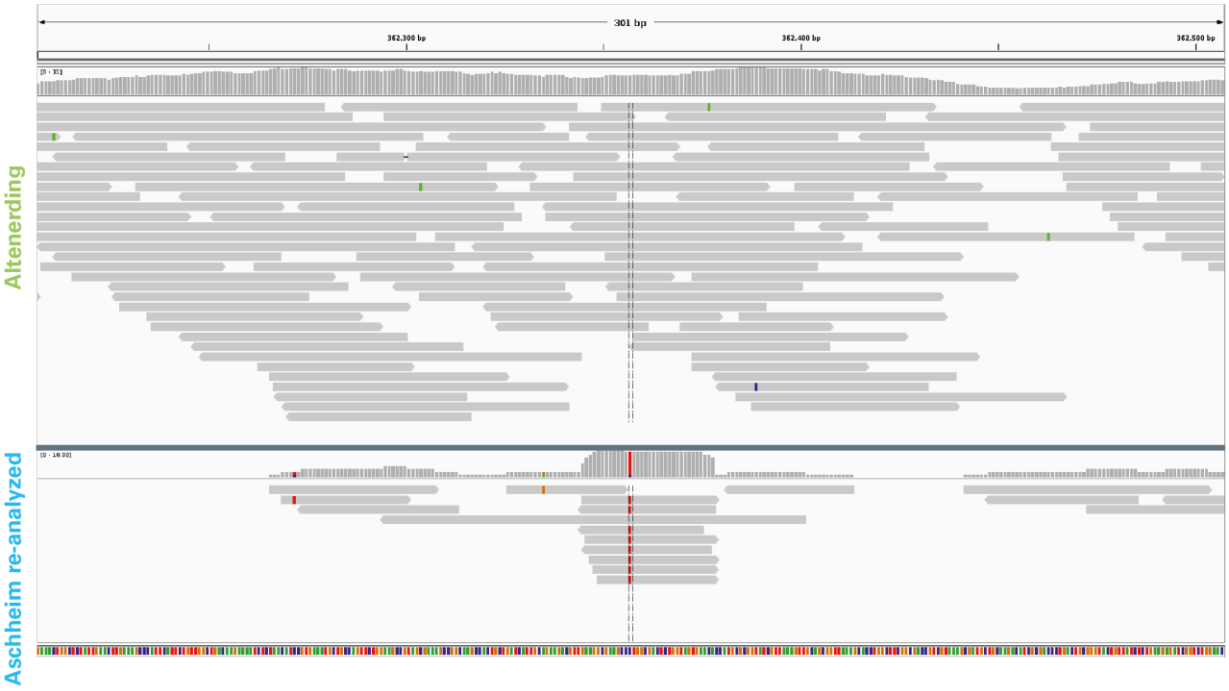


Fig. S9 (A-J): Visualization of 10 positions containing true SNPs called for the re-analyzed Aschheim genome as well as for the Altenerding genome. The positions were randomly picked to represent visual patterns consistent with the set criteria for SNP calling and with a

relative even coverage, in contrast with the pattern of the abnormal coverage peaks shown in figures S4 and S5. Reads were mapped to the CO92 reference with sensitivity of 0.1 and minimum mapping quality of 30 and visualized on IGV gene browser. Upper bend shows coverage plot for the region corresponding to the genome beneath. Upper scale shows position (bp) in reference sequence. Bottom sequence shows the 150 bp in the CO92 reference. Dotted line marks the SNP. **A:** position 898980 (A to T) **B:** position 1067966 (C to A) **C:** position 1489055 (C to T) **D:** position 1530658 (C to A) **E:** position 1754708 (C to T) **F:** position 1868678 (G to T) **G:** position 1956162 (T to C) **H:** position 2725715 (C to T) **I:** position 3398153 (G to A) **J:** position 3500922 (T to G).

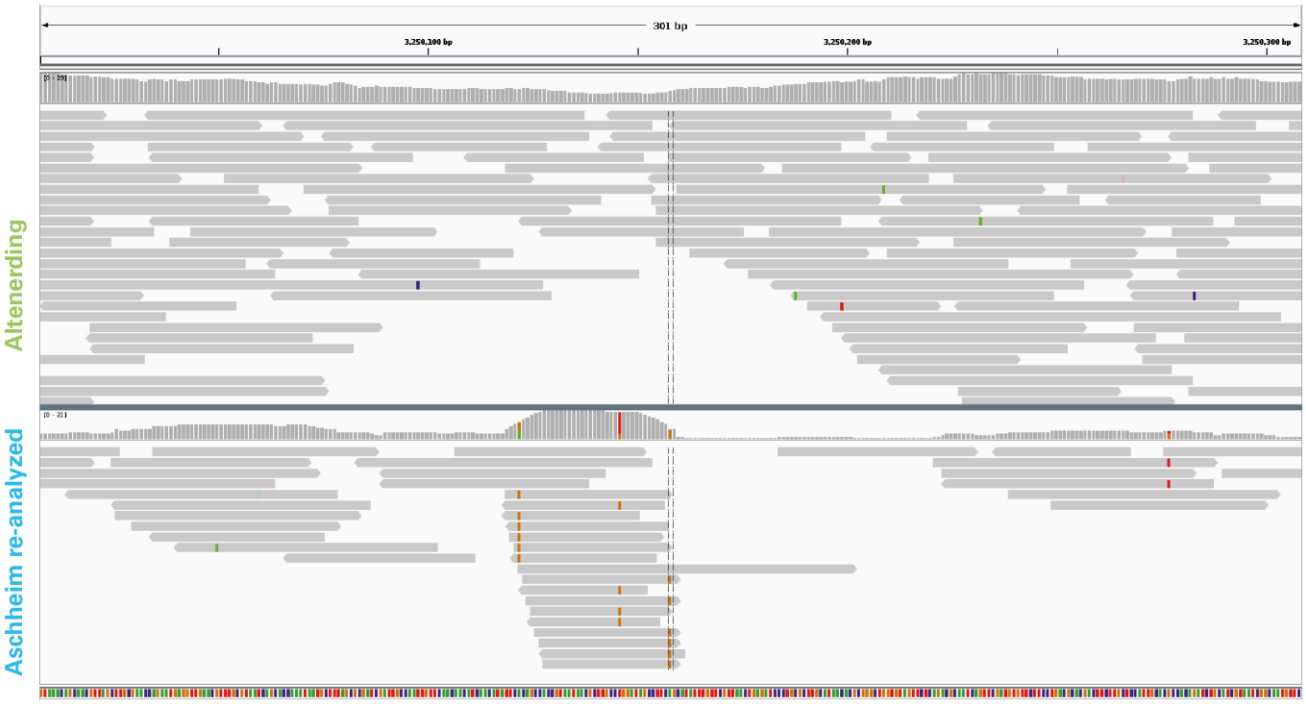
A


301 bp
362,300 bp
362,400 bp
362,500 bp
Altenerding
Aschheim re-analyzed

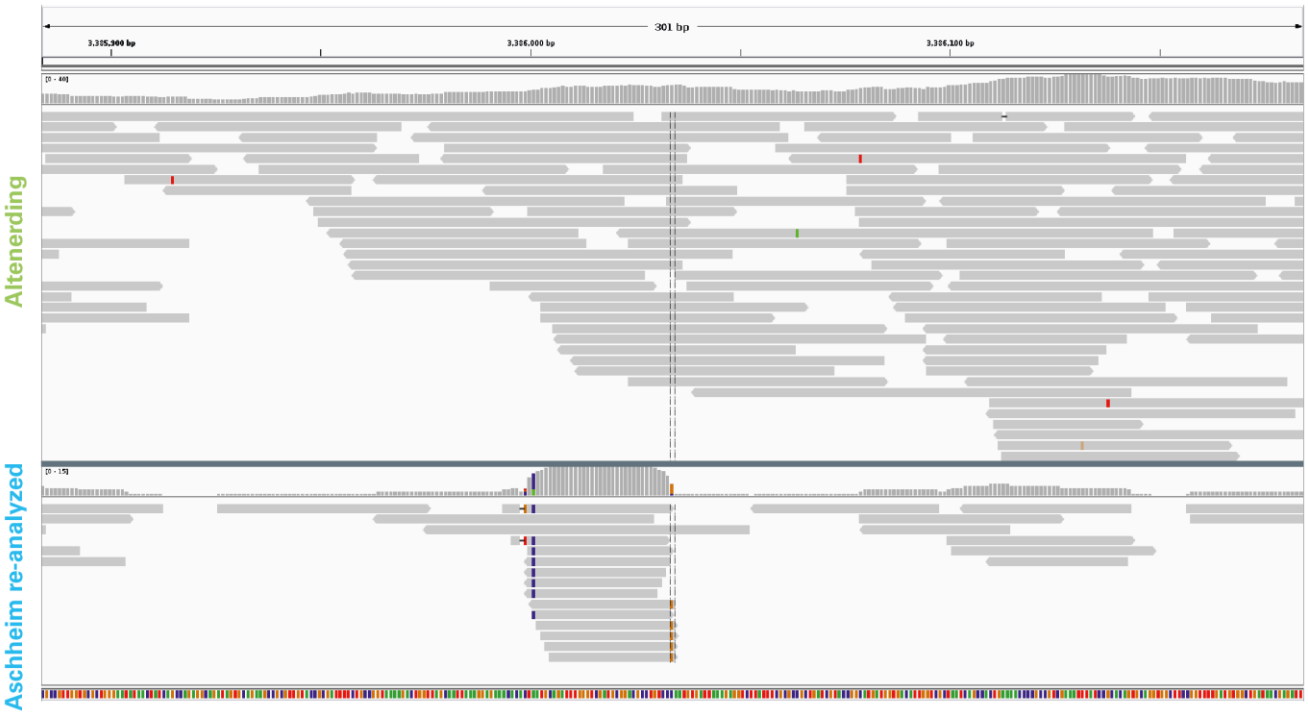
B

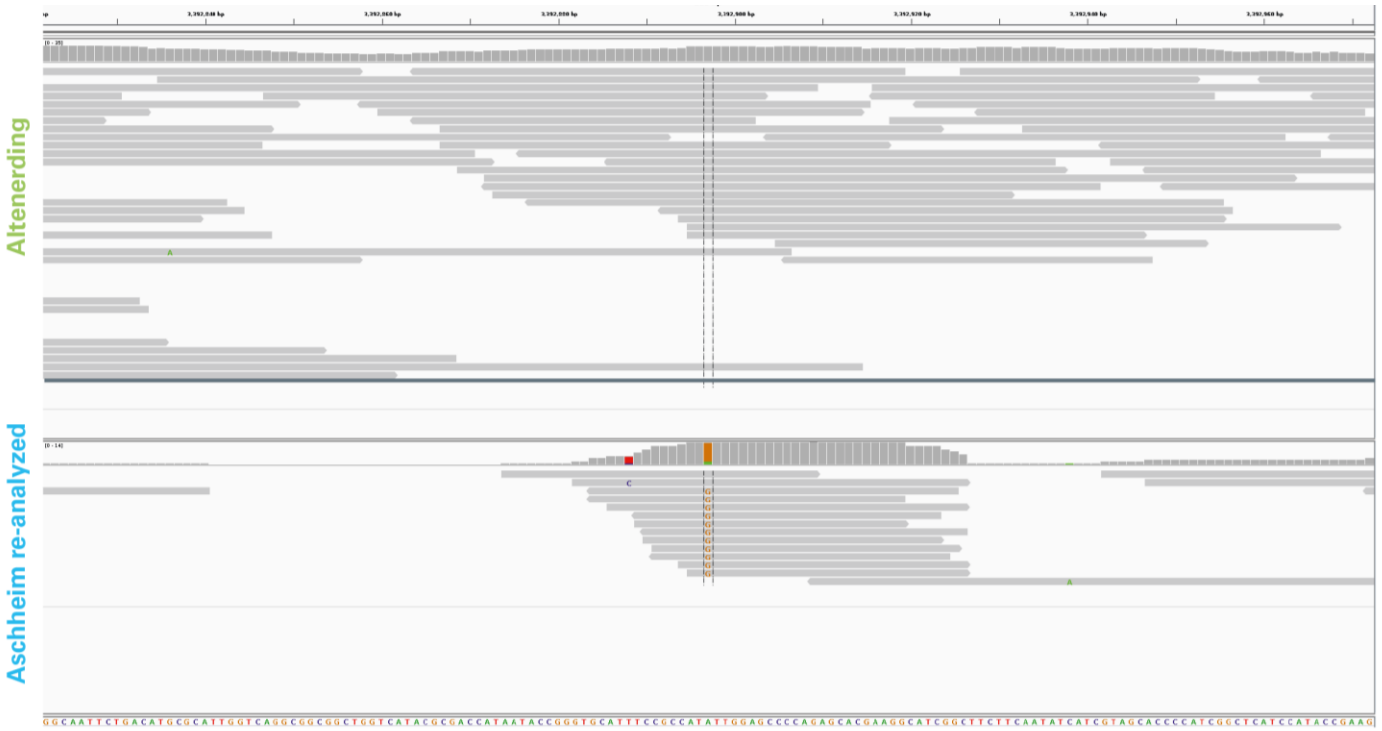

231 bp
Altenerding
Aschheim re-analyzed

C

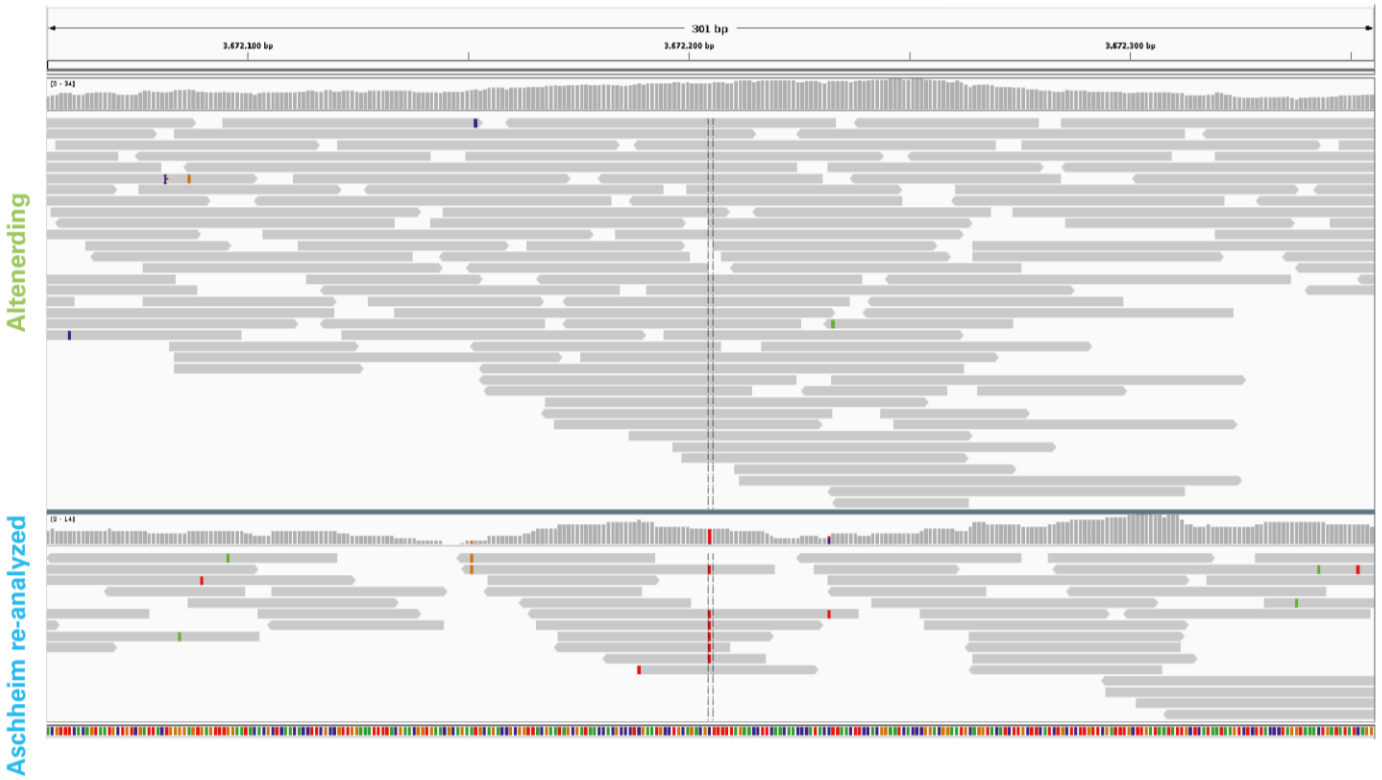

301 bp
3,250,300 bp
3,250,200 bp
3,250,300 bp
Altenerding
Aschheim re-analyzed

D

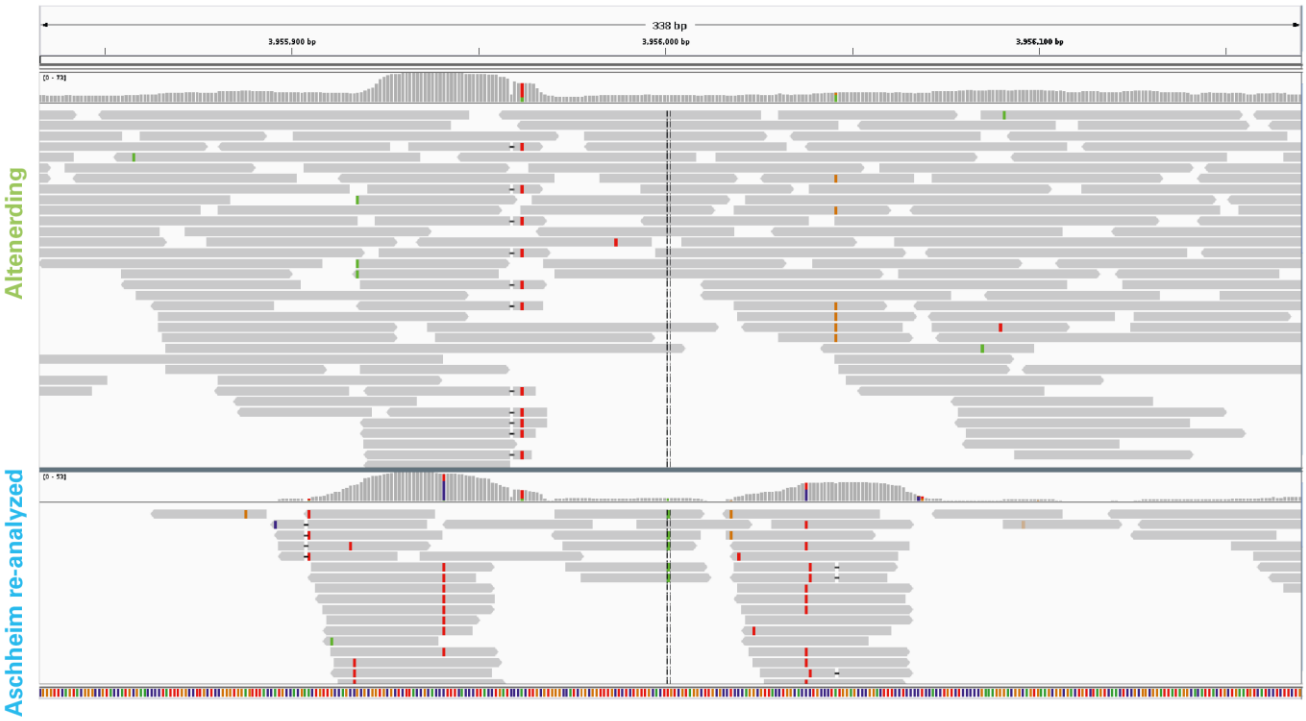

301 bp
3,305,900 bp
3,306,000 bp
3,306,100 bp
Altenerding
Aschheim re-analyzed


Altenerding
Aschheim re-analyzed

F


301 bp
3,672,100 bp
3,672,200 bp
3,672,300 bp
Altenerding
Aschheim re-analyzed

G


338 bp
3,955,900 bp
3,956,000 bp
3,956,100 bp
Altenerding
Aschheim re-analyzed

H

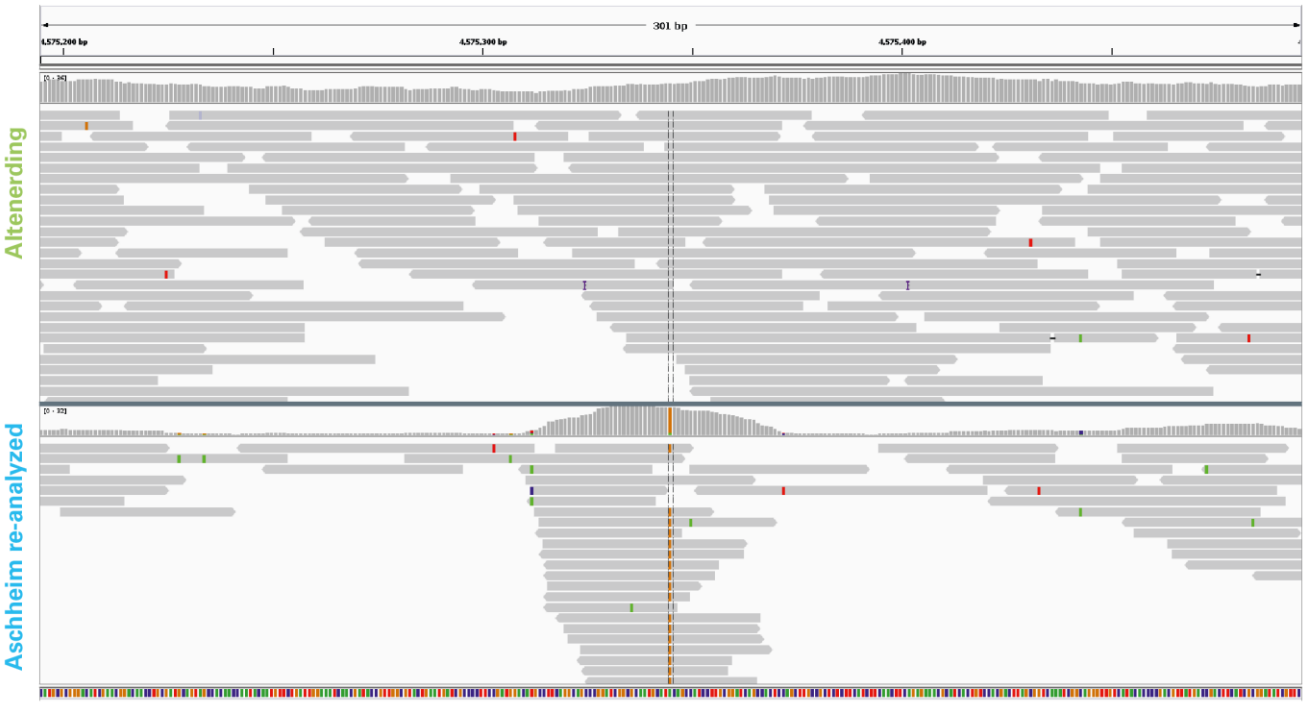

301 bp
4,575,200 bp
4,575,300 bp
4,575,400 bp
Altenerding
Aschheim re-analyzed

Fig.S10 (A-H): Visualization of positions containing potential false positive SNPs specifically derived in the re-analyzed Aschheim. Reads were mapped to the CO92 reference with sensitivity of 0.1 and minimum mapping quality of 30 and visualized on IGV gene browser. Upper bend shows coverage plot for the region corresponding to the genome beneath. Upper scale shows position (bp) in reference sequence. Bottom sequence shows the 150 bp in the CO92 reference. Dotted line marks the false positive SNP.

(A) Position 362357: Re-analysis of the Aschheim raw data shows 89 % variant frequency for the “C” to “T” variant at the position. The “T” variants are located in a region with an abnormal cover peak. The Altenerding mapping shows even 21 fold coverage in the region and a 100 % variant frequency that supports the “C” variant (identical to reference). This SNP was removed from final analysis by *Wagner et al.*, 2014 following a visual inspection.

(B) Position 1371025: Re-analysis of the Aschheim raw data shows 86 % variant frequency for the “C” to “T” variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The “T” variants are located at end of reads in a region with an abnormal peak in coverage. The Altenerding mapping shows even 18 fold coverage in the region and a 100 % variant frequency that supports the “C” variant (identical to reference).

(C) Position 3250158: Re-analysis of the Aschheim raw data shows 86 % variant frequency for the “A” to “G” variant at the position. The “G” variants are located at end of reads in a region with an abnormal cover peak. The Altenerding mapping shows even 11 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference). This SNP was not called by *Wagner et al.*, 2014.

(D) Position 3386034: Re-analysis of the Aschheim raw data shows 83 % variant frequency for the “C” to “G” variant at the position. The “G” variants are located at end of reads in a region with an abnormal cover peak. The Altenerding mapping shows even 24 fold coverage in the region and a 100 % variant frequency that supports the “C” variant (identical to reference). This SNP was not called by *Wagner et al.*, 2014.

(E) Position 3392897: Re-analysis of the Aschheim raw data shows 85 % variant frequency for the A to G variant called as SNP by Wagner et al. Variant frequency is lower than the minimum variant frequency of 95 % set by Wagner et al. The reads containing the “G” variants are located in a region with an abnormal peak in coverage. The Altenerding mapping shows even 22 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference).

(F) Position 3672205: Re-analysis of the Aschheim raw data shows 86 % variant frequency for the “C” to “T” variant at the position. The “T” variants are located in a region with an abnormal cover peak. The Altenerding mapping shows even 28 fold coverage in the region and a 100 % variant frequency that supports the “C” variant (identical to reference). This SNP was not called by *Wagner et al.*, 2014.

(G) Position 3956001: Re-analysis of the Aschheim raw data shows 83 % variant frequency for the “T” to “A” variant at the position. The “A” variants are located between two abnormal cover peaks, in a region with high variability. The Altenerding mapping shows even 18 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference). This SNP was removed from final analysis by Wagner et al., 2014 following a visual inspection.

(H) Position 4575345: Re-analysis of the Aschheim raw data shows 90 % variant frequency for the “A” to “G” variant at the position. The “G” variants are located in a region with an abnormal cover peak. The Altenerding mapping shows even 23 fold coverage in the region and a 100 % variant frequency that supports the “A” variant (identical to reference). This SNP was removed from final analysis by Wagner et al., 2014 following a visual inspection.

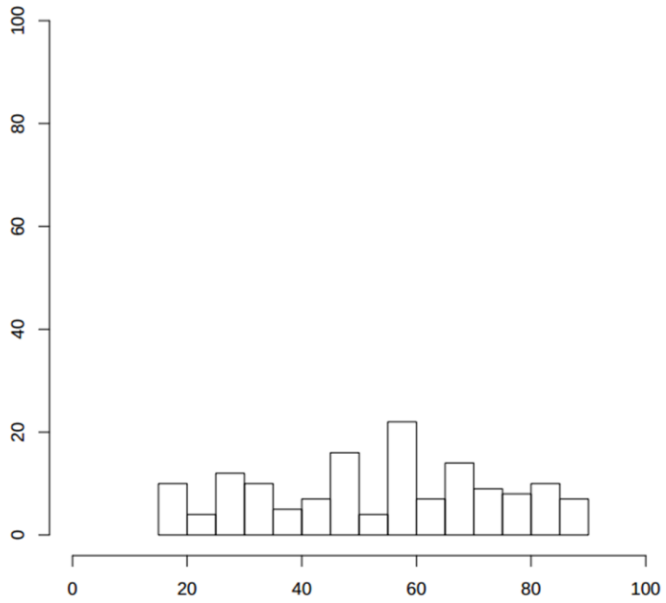


Fig.S11: Genome-wide SNP allele frequency plot of the re-analyzed Aschheim draft genome. The x axis indicates the frequency of reads covering a SNP position in which the SNP was detected in the re-analyzed Aschheim draft genome. The y axis indicates the number of SNP calls with the respective frequency. The observed frequencies are not showing any bimodal pattern or any other pattern that could indicate an infection with multiple strains.

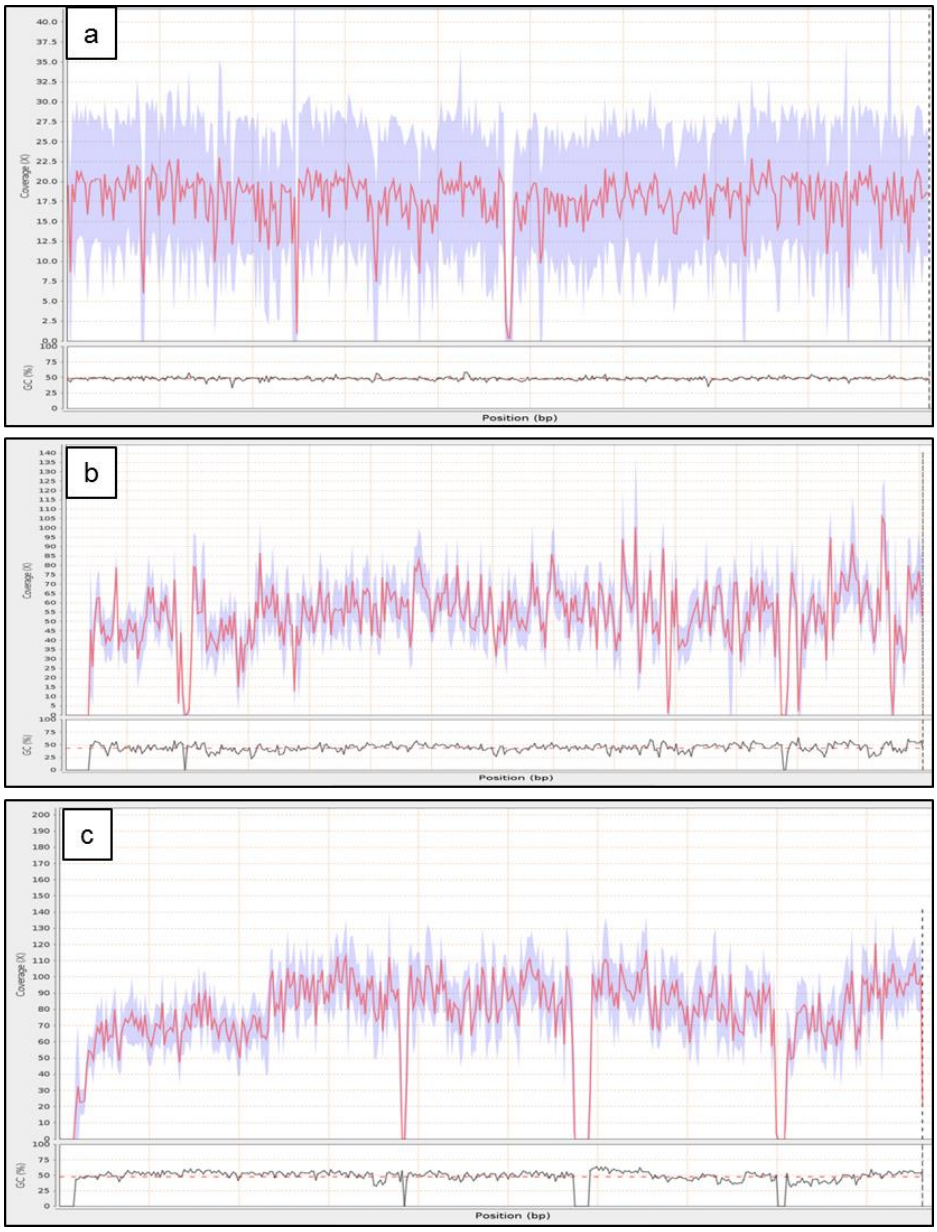


Fig.S12: Coverage plots across the CO92 reference for the Altenerding genome. Coverage across the reference was plotted using QualiMap version 2.1. (a) Coverage across the CO92 chromosome (NC_003143.1). (b) Coverage across the pCD1 plasmid (NC_003131.1). (c) Coverage across the pMT1 plasmid (NC_003134.1). Coverage in red, percent GC content in grey and mean GC content in dashed line.

Supplementary archaeological and historical information

The early medieval cemetery from Altenerding (also called Altenerding/Klettham) is located near Munich in southern Germany. It contains around 1450 inhumations and is therefore one of the largest early medieval cemeteries in Central Europe. Altenerding was excavated from 1966 to 1973 (Sage 1984) and was used from the second half of the fifth century until the seventh century AD (Losert and Pleterski 2003).

Within the cemetery 16 double burials and no multiple burials could be identified. Ten double burials were chosen randomly for screening of *Y. pestis* presence (Table S9). All of them contained grave furnishings, mostly a set of brooches and other dress ornaments for women and a combination of weapons for men, as is typical to this time period (Sage 1984). This is also true for the plague-positive individuals in the double-burial 1175/1176. Here, a 25- to 30-year old woman was laid to rest together with a 20 to 25 year old male. The dead woman (1175) was buried with a variety of clothes and jewels (Fig. 1C) typical of the middle of the 6th century, including an iron arm ring; a belt with bronze belt buckle; a chatelaine with antler pendant; Roman brooches; iron keys; chained links and scales; a knife; a fragment of La Tène glass arm ring; a necklace of glass and amber beads and fragments of a blue Roman glass vessel. She wore a pair of garnet disk fibulas whose typology has been dated between ~530 and ~570 (Vielitz 2003) and among other ornaments she was equipped with a so-called Hercules- or Donar club amulet, which probably expresses a special hope for growth and fertility (Losert and Pleterski 2003). Due to her young age only minor expressions of degenerative lesions in the great joints and the spine are visible on the bones. However, the orbital roofs show porotic lesions on the bone surface (*cribra orbitalia*). These kinds of lesions are rather unspecific and can occur in a variety of diseases (Walker et al. 2009).

The young man was buried without weapons, but a bag hanging on a belt could be reconstructed as containing an iron knife, a lighter and nails (Fig. 1D). Despite the young age of the individual some degenerative lesions are visible in the spine including Schmorl's nodes. The right orbital roof shows porotic lesions on the bone surface (the left orbital roof is missing). Both tibiae exhibit an extensive inflammation of the bone

surface (periostitis). Both symptoms are rather unspecific and can be connected to different kinds of infectious diseases or anemia.

Wooden traces indicate the existence of two coffins or wooden planking in the grave. This is a further sign that the dead were carefully arranged. Furthermore, both individuals were buried with rather expensive clothes and jewels. This indicates that the victims had been dressed and prepared carefully for their funeral. Burial rites, which probably also included washing and public laying out of the body seem to have been conducted also for these plague victims. The same has been noted in the neighboring Aschheim cemetery (Gutsmiedl-Schümann et al. 2010).

No historical record has yet been adduced that mentions the impact of the Justinianic Pandemic in this region. In fact, an 8th-century historian who used some reliable early sources, with respect to the wave dated ~565-571 states explicitly that this outbreak went as far as this region, but stayed within “Italy”: “In his [Narses] time, the greatest plague emerged, particularly in the province of Liguria.... And what is more, these evils occurred only within Italy up to the region of the Alamannian and Bavarian peoples, to the Romans alone."(Bethmann and Waitz 1878)

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