Supplementary material

Fig. S1: A scheme of workflow to generate variants from Oxford Nanopore technologies (ONT) and Illumina reads

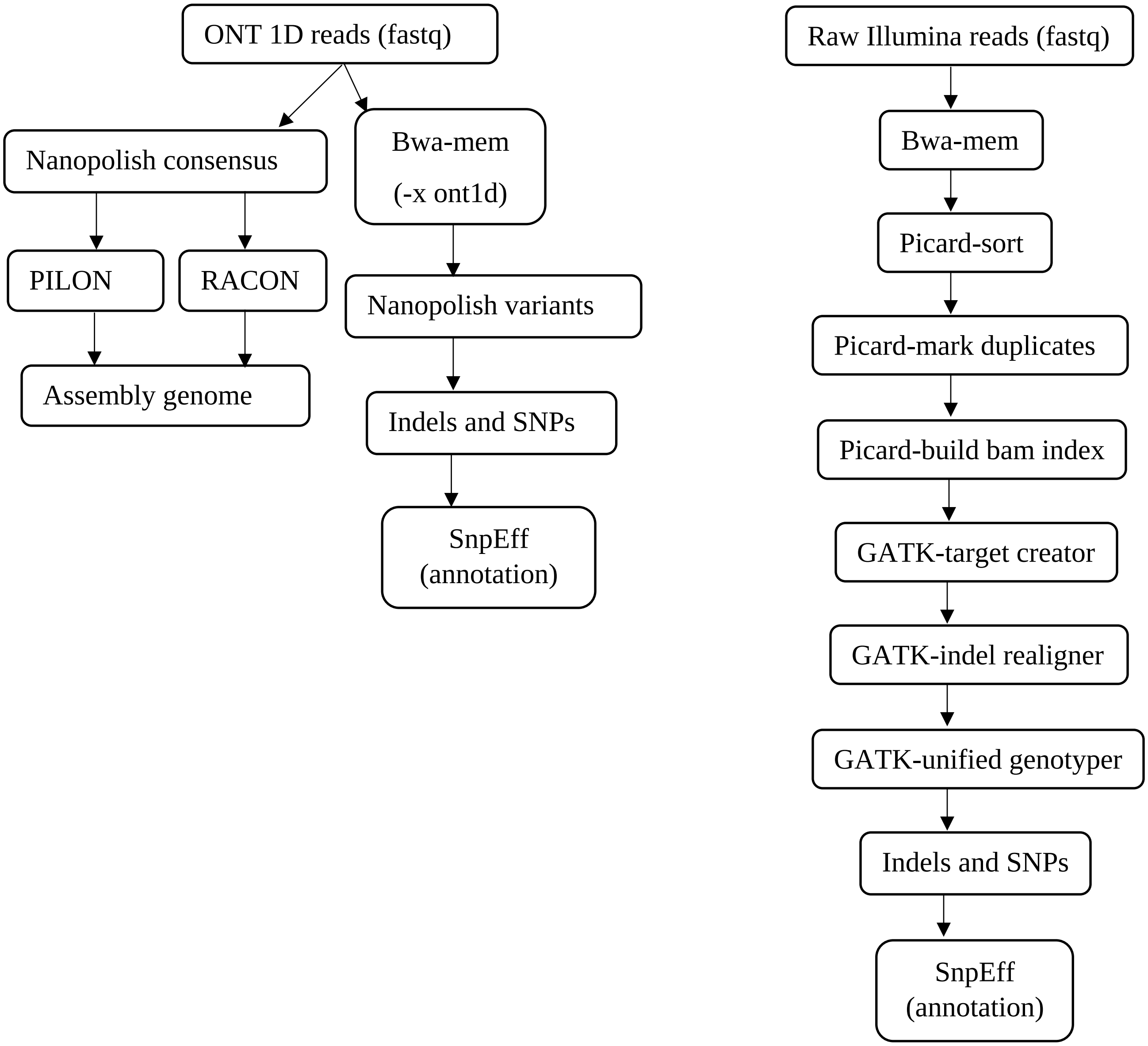

Fig. S2: Dot plot of sequence accuracy between the draft WP-XDR genome and the reference genome H37Rv which revealed 99.7% Average Nucleotide Identity (ANI) score. The red dots show a forward orientation while blue dots show reverse orientation

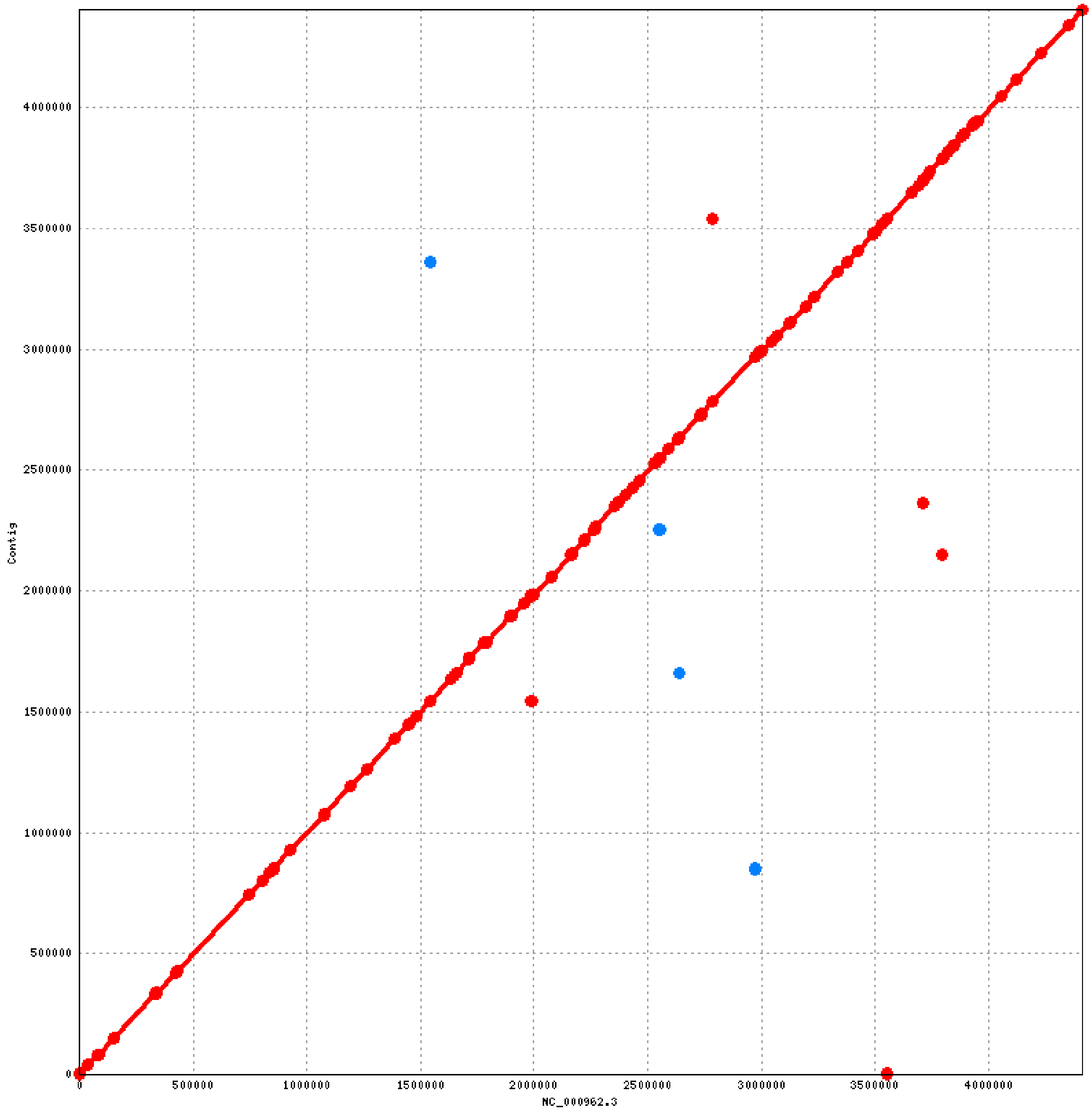

Fig. S3: Plot of sequence similarity of 168 PE/PPE family genes identified from the assembly and their sequence and depth coverage from Oxford minion® reads. The marks on the x-axis represent the different PE/PPE genes while y-axis respects their percentage sequence similarity from blastn (red), percentage breadth of coverage (black), and read depth (blue). Sample was sequenced to an average depth of 238X

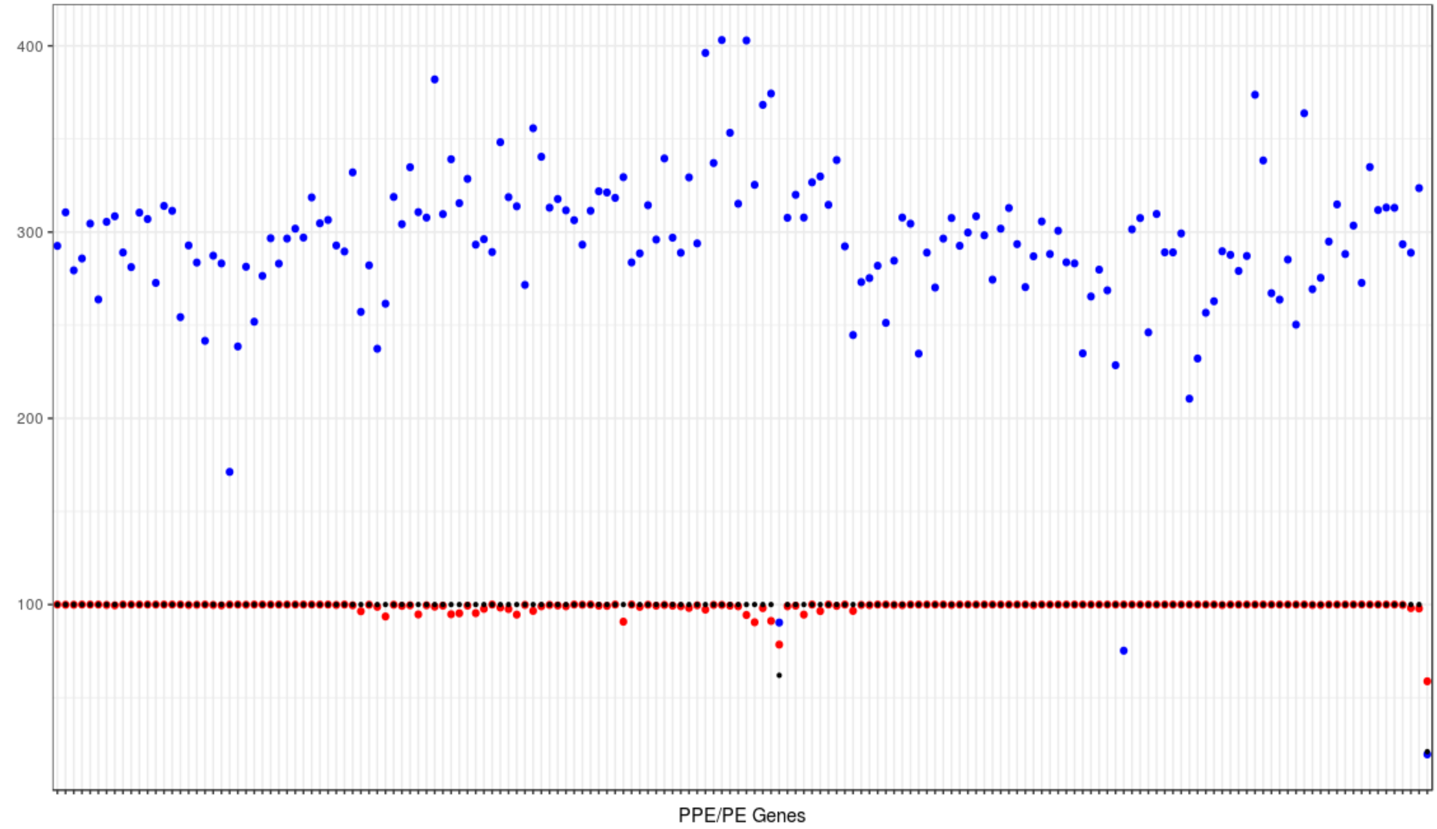

Fig. S4: Plot of sequence and depth coverage for the assembly of 168 PE/PPE family genes using Illumina reads. The marks on the x-axis represent the different 168 PE/PPE family genes while y-axis respective their percentage breadth of coverage (red), and read depth (blue). Sample was sequenced to average 46.3x depth

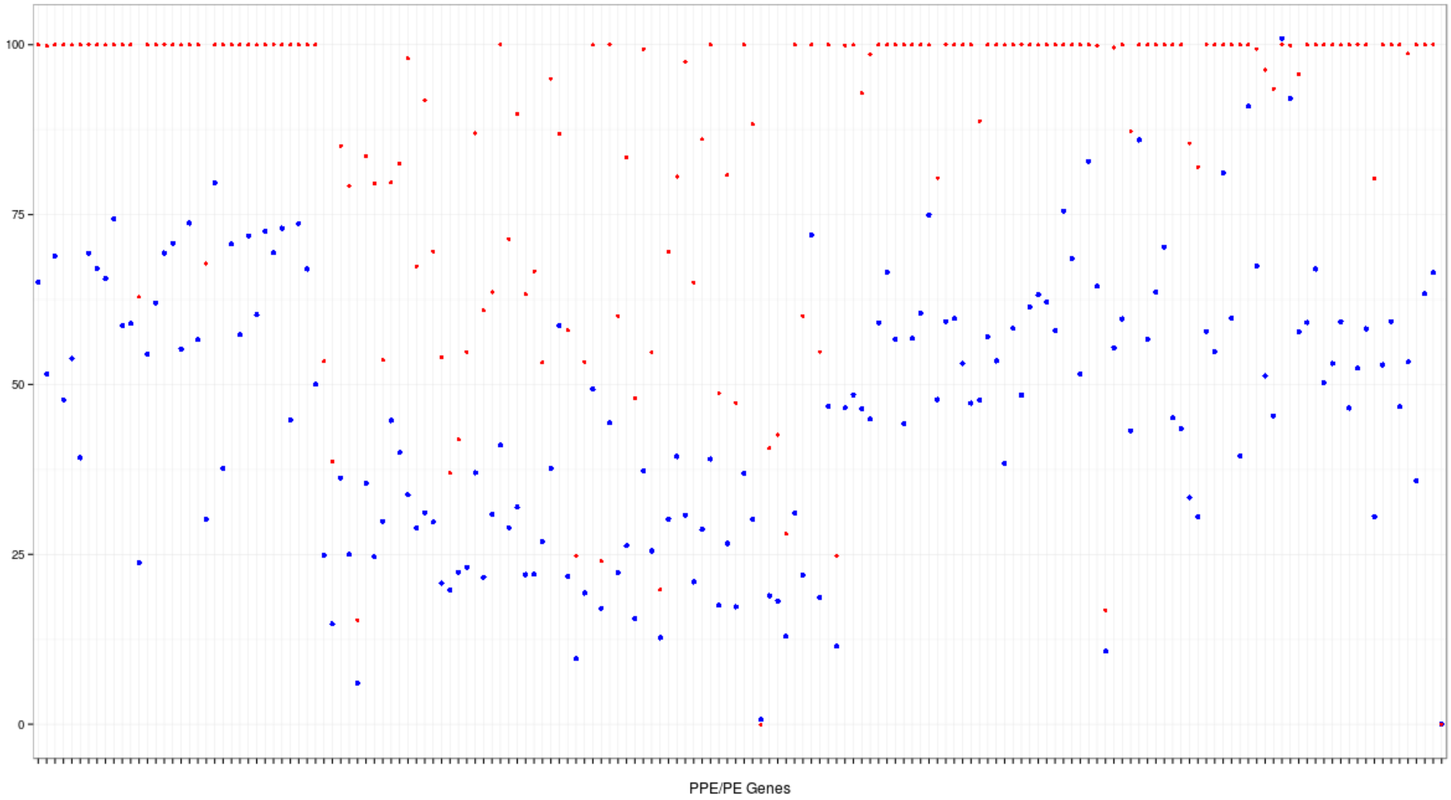

Fig. S5: Protein structure of the PPE family protein predicted using Phyre2 for a 654bp gene sequence with an end 323bp insertion identified within the assembled genome but absent in the reference genome H37Rv.

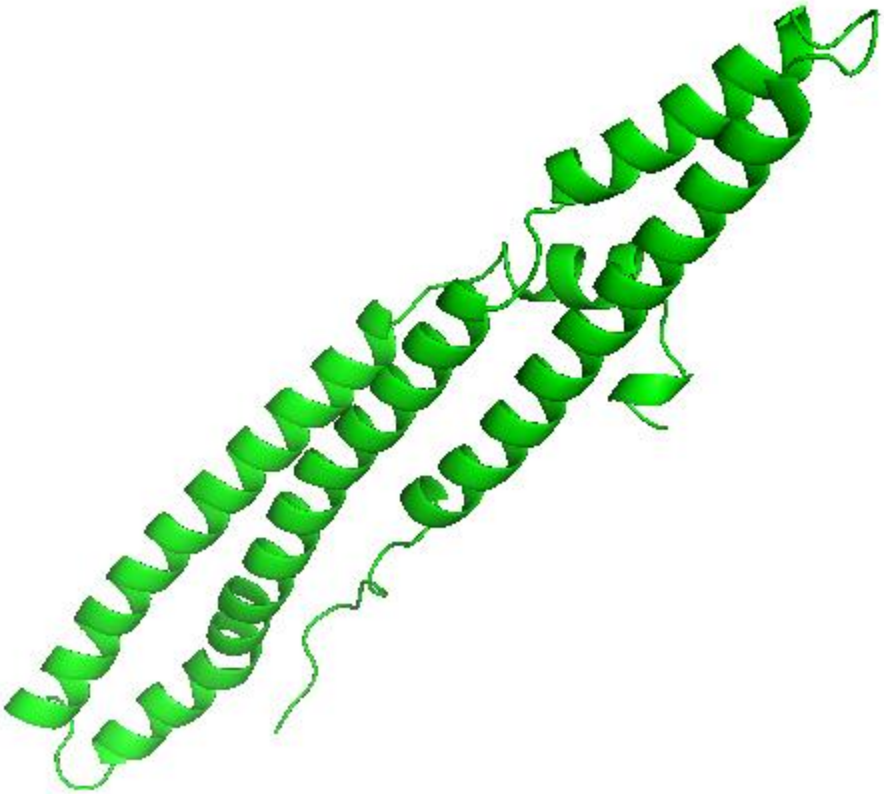

Table S1: List of PE/PPE family genes and their genomic position in H37Rv (NC_000962.3)

Locus Number	Gene	Start	End
Rv0096	PPE1	105324	106715
Rv0109	PE_PGRS1	131382	132872
Rv0124	PE_PGRS2	149533	150996
Rv0151c	PE1	177543	179309
Rv0152c	PE2	179319	180896
Rv0159c	PE3	187433	188849
Rv0160c	PE4	188931	190451
Rv0256c	PPE2	307877	309547
Rv0278c	PE_PGRS3	333437	336310
Rv0279c	PE_PGRS4	336560	339073
Rv0280	PPE3	339364	340974
Rv0285	PE5	349624	349932
Rv0286	PPE4	349935	351476
Rv0297	PE_PGRS5	361334	363109
Rv0304c	PPE5	366150	372764
Rv0305c	PPE6	372820	375711
Rv0335c	PE6	399535	400050
Rv0354c	PPE7	424269	424694
Rv0355c	PPE8	424777	434679
Rv0388c	PPE9	467459	468001
Rv0442c	PPE10	530751	532214
Rv0453	PPE11	543174	544730
Rv0532	PE_PGRS6	622793	624577
Rv0578c	PE_PGRS7	671996	675916
Rv0742	PE_PGRS8	832981	833508
Rv0746	PE_PGRS9	835701	838052
Rv0747	PE_PGRS10	838451	840856
Rv0754	PE_PGRS11	846159	847913
Rv0755c	PPE12	848103	850040
Rv0832	PE_PGRS12	924951	925364
Rv0833	PE_PGRS13	925361	927610
Rv0834c	PE_PGRS14	927837	930485
Rv0872c	PE_PGRS15	968424	970244
Rv0878c	PPE13	976872	978203
Rv0915c	PPE14	1020058	1021329
Rv0916c	PE7	1021344	1021643
Rv0977	PE_PGRS16	1090373	1093144
Rv0978c	PE_PGRS17	1093361	1094356

Rv0980c	PE_PGRS18	1095078	1096451
Rv1039c	PPE15	1161297	1162472
Rv1040c	PE8	1162549	1163376
Rv1067c	PE_PGRS19	1188421	1190424
Rv1068c	PE_PGRS20	1190757	1192148
Rv1087	PE_PGRS21	1211560	1213863
Rv1088	PE9	1214513	1214947
Rv1089	PE10	1214769	1215131
Rv1091	PE_PGRS22	1216469	1219030
Rv1135c	PPE16	1262272	1264128
Rv1168c	PPE17	1298764	1299804
Rv1169c	lipX	1299822	1300124
Rv1172c	PE12	1301755	1302681
Rv1195	PE13	1339003	1339302
Rv1196	PPE18	1339349	1340524
Rv1214c	PE14	1357293	1357625
Rv1243c	PE_PGRS23	1384989	1386677
Rv1325c	PE_PGRS24	1488154	1489965
Rv1361c	PPE19	1532443	1533633
Rv1386	PE15	1561464	1561772
Rv1387	PPE20	1561769	1563388
Rv1396c	PE_PGRS25	1572127	1573857
Rv1430	PE16	1606386	1607972
Rv1441c	PE_PGRS26	1618209	1619684
Rv1450c	PE_PGRS27	1630638	1634627
Rv1452c	PE_PGRS28	1636004	1638229
Rv1468c	PE_PGRS29	1655609	1656721
Rv1548c	PPE21	1751297	1753333
Rv1646	PE17	1855764	1856696
Rv1651c	PE_PGRS30	1862347	1865382
Rv1705c	PPE22	1931497	1932654
Rv1706c	PPE23	1932694	1933878
Rv1753c	PPE24	1981614	1984775
Rv1759c	wag22	1989833	1992577
Rv1768	PE_PGRS31	2000614	2002470
Rv1787	PPE25	2025301	2026398
Rv1788	PE18	2026477	2026776
Rv1789	PPE26	2026790	2027971
Rv1790	PPE27	2028425	2029477
Rv1791	PE19	2029904	2030203
Rv1800	PPE28	2039453	2041420
Rv1801	PPE29	2042001	2043272
Rv1802	PPE30	2043384	2044775

Rv1803c	PE_PGRS32	2044923	2046842
Rv1806	PE20	2048072	2048371
Rv1807	PPE31	2048398	2049597
Rv1808	PPE32	2049921	2051150
Rv1809	PPE33	2051282	2052688
Rv1818c	PE_PGRS33	2061178	2062674
Rv1840c	PE_PGRS34	2087971	2089518
Rv1917c	PPE34	2162932	2167311
Rv1918c	PPE35	2167649	2170612
Rv1983	PE_PGRS35	2226244	2227920
Rv2098c	PE_PGRS36	2356729	2358033
Rv2099c	PE21	2358033	2358206
Rv2107	PE22	2367359	2367655
Rv2108	PPE36	2367711	2368442
Rv2123	PPE37	2381071	2382492
Rv2126c	PE_PGRS37	2387202	2387972
Rv2162c	PE_PGRS38	2423240	2424838
Rv2328	PE23	2600731	2601879
Rv2340c	PE_PGRS39	2617667	2618908
Rv2352c	PPE38	2632923	2634098
Rv2353c	PPE39	2634528	2635592
Rv2356c	PPE40	2637688	2639535
Rv2371	PE_PGRS40	2651753	2651938
Rv2396	PE_PGRS41	2692799	2693884
Rv2408	PE24	2706017	2706736
Rv2430c	PPE41	2727336	2727920
Rv2431c	PE25	2727967	2728266
Rv2487c	PE_PGRS42	2795301	2797385
Rv2490c	PE_PGRS43	2801254	2806236
Rv2519	PE26	2835785	2837263
Rv2591	PE_PGRS44	2921551	2923182
Rv2608	PPE42	2935046	2936788
Rv2615c	PE_PGRS45	2943600	2944985
Rv2634c	PE_PGRS46	2960105	2962441
Rv2741	PE_PGRS47	3053914	3055491
Rv2768c	PPE43	3076894	3078078
Rv2769c	PE27	3078158	3078985
Rv2770c	PPE44	3079309	3080457
Rv2853	PE_PGRS48	3162268	3164115
Rv2892c	PPE45	3200794	3202020
Rv3018c	PPE46	3376939	3378243
Rv3018A	PE27A	3378329	3378415
Rv3021c	PPE47	3379376	3380452

Rv3022c	PPE48	3380440	3380682
Rv3022A	PE29	3380679	3380993
Rv3097c	lipY	3465778	3467091
Rv3125c	PPE49	3490476	3491651
Rv3135	PPE50	3501334	3501732
Rv3136	PPE51	3501794	3502936
Rv3144c	PPE52	3510088	3511317
Rv3159c	PPE53	3527391	3529163
Rv3343c	PPE54	3729364	3736935
Rv3344c	PE_PGRS49	3736984	3738438
Rv3345c	PE_PGRS50	3738158	3742774
Rv3347c	PPE55	3743711	3753184
Rv3350c	PPE56	3755952	3767102
Rv3367	PE_PGRS51	3778568	3780334
Rv3388	PE_PGRS52	3801653	3803848
Rv3425	PPE57	3842239	3842769
Rv3426	PPE58	3843036	3843734
Rv3429	PPE59	3847165	3847701
Rv3477	PE31	3894093	3894389
Rv3478	PPE60	3894426	3895607
Rv3507	PE_PGRS53	3926569	3930714
Rv3508	PE_PGRS54	3931005	3936710
Rv3511	PE_PGRS55	3939617	3941761
Rv3512	PE_PGRS56	3941724	3944963
Rv3514	PE_PGRS57	3945794	3950263
Rv3532	PPE61	3969343	3970563
Rv3533c	PPE62	3970705	3972453
Rv3539	PPE63	3978059	3979498
Rv3558	PPE64	3997980	3999638
Rv3590c	PE_PGRS58	4031404	4033158
Rv3595c	PE_PGRS59	4036731	4038050
Rv3621c	PPE65	4060648	4061889
Rv3622c	PE32	4061899	4062198
Rv3650	PE33	4091233	4091517
Rv3652	PE_PGRS60	4093632	4093946
Rv3653	PE_PGRS61	4093940	4094527
Rv3738c	PPE66	4189285	4190232
Rv3739c	PPE67	4190284	4190517
Rv3746c	PE34	4196171	4196506
Rv3812	PE_PGRS62	4276571	4278085
Rv3872	PE35	4350745	4351044
Rv3873	PPE68	4351075	4352181
Rv3892c	PPE69	4374484	4375683

Rv3893c	PE36	4375762	4375995

Table S2: Non-consensus SNPs identified from ONT reads mapped to reference H37Rv (159/1254 SNPs)

SNP	COUNT	Proportion (%)
A->C	6	0.48
A->G	15	1.20
T->C	18	1.44
T-G	13	1.04
C->A	9	0.72
C->T	19	1.52
C->G	23	1.83
G->A	22	1.75
G->T	20	1.59
G->C	14	1.12

Table S3: Number of SNPs and average base coverage of PE/PPE family genes identified from ONT and Illumina reads

PE/PPE genes	ONT	Illumina	Overlap SNPs	Illumina average base coverage	ONT average base coverage
PPE1	1	1	1	46	273
PE_PGRS1	1	0	0	24	332
PE1	1	1	1	65	279
PE3	1	1	1	79	281
PPE2	2	2	2	47	307
PPE3	1	2	2	61	305
PPE6	2	2	2	59	269
PPE47	0	14	0	19	232
PPE8	4	4	4	53	288
PE_PGRS6	3	0	0	12	307
PE_PGRS7	4	2	2	18	338
PPE9	0	9	0	35	323

PE_PGRS9	2	0	0	11	244
PE_PGRS10	6	4	4	14	257
PE_PGRS13	3	0	0	6	261
PE_PGRS14	1	0	0	35	318
PE_PGRS15	1	0	0	24	304
PE_PGRS17	3	1	1	44	310
PE_PGRS18	4	0	0	39	307
PPE16	0	3	0	44	234
PE8	1	1	1	66	292
PE_PGRS20	4	0	0	31	339
PE_PGRS44	0	2	0	37	339
PE_PGRS21	2	0	0	29	315
PE_PGRS22	5	2	2	20	328
PPE17	1	1	1	56	288
PPE18	1	0	0	60	270
PE14	1	1	1	53	305
PPE19	2	0	0	74	296
PPE20	1	1	1	59	292
PPE31	0	1	0	62	313
PE_PGRS25	1	1	1	23	289
PE16	1	1	1	69	289
PE_PGRS26	1	1	1	37	348
PE_PGRS27	2	0	0	21	318
PE_PGRS28	6	3	3	30	313
PPE21	1	0	0	59	299
PPE22	1	1	1	53	308
PPE24	2	0	0	47	274
PPE30	1	0	0	63	288
PE_PGRS32	3	1	1	22	317
PPE32	2	0	0	57	283
PPE33	2	2	2	75	283
PE_PGRS33	1	0	0	26	311
PPE34	2	3	2	68	234
PPE35	2	1	1	51	265
PPE37	1	0	0	64	268
PE_PGRS37	1	0	0	9	321
PE23	2	2	2	62	292
PE24	1	1	1	69	274
PE_PGRS42	1	0	0	26	314
PE_PGRS43	8	0	0	15	295
PE26	1	1	1	55	287
PE_PGRS47	4	0	0	30	329
PE27	2	2	2	73	283

PPE44	1	1	1	70	289
PE_PGRS48	3	1	1	39	293
lipY	2	1	1	66	310
PPE51	1	0	0	59	279
PPE52	1	0	0	39	287
PPE53	1	0	0	90	373
PPE54	11	9	9	67	338
PE_PGRS50	1	1	1	28	403
PPE55	7	17	7	51	267
PPE56	3	3	3	45	263
PPE60	2	2	2	66	275
PE_PGRS53	1	0	0	26	402
PE_PGRS54	7	4	4	17	325
PE_PGRS55	1	1	1	36	368
PE_PGRS56	3	6	3	30	374
PE_PGRS58	2	1	1	18	307
PE_PGRS59	1	0	0	18	320
PPE66	1	1	1	58	334
PE35	1	0	0	60	296
PPE69	1	1	1	59	313
Total	**158**	**124**	**81**		

Table S4: Mutations identified in genes that encode for potential virulence proteins in theWP-XDR strain

Gene	**Nucleotide change**	**Position**	**Amino acid**
mak	T->C	154283	p.Ser18Pro
mce1A	T->G	199470	p.Ser313Ala
mce1D	T->C	203038	p.Ile188Thr
mce1F	T->C	206339	p.Leu370Pro
mce2A	T->C	686972	p.Phe51Ser
mce2F	A->G	694531	p.Asn432Ser
Rv0634c	A->G	731015	p.Tyr7His
mazF3	G->A	1230778	p.Thr65Ile
ephB	G->T	2191498	p.Gly158Trp
mce3A	G->A	2209465	p.Ala47Thr
mce3F	C->A	2216443	p.Ala396Glu
cstA	C->A	3428917	p.Arg559Ser
mce4C	G->T	3916386	pArg191Ser
proV	T->C	4204168	p.Asn84Asp
proX	A->G	4205120	p.Leu85Pro
mycP1	T->C	4364046	Thr238Ala

Table S5: Mutations in genes involved in cell wall biosynthesis identified in the WP-XDR strain

Gene	**Position**	**Mutation**
mmpL5	776182	p.Asp767Asn
mmpL12	1715165	p.Ile816Met
mmpL10	1322741	p.Thr408Ala
fadD6	1349733	p.Glu134Asp
fadE9	844023	p.Asp131Gly
fadD28	3284640	p.Thr436Ala
fadD30	484596	p.Pro207Leu
fadD34	37305	p.Ser16Trp
fadD34	37334	p.Thr26Ser
fadD36	1336164	p.Pro124Leu
fadD31	2177366	p.Arg94Cys
fadE17	2185674	p.Ile172Thr
fadE20	3036826	p.Val156Met
fadD28	3284640	p.Thr436Ala
fadE24	3505648	p.Arg96Gly
fadE26	3922552	p.Leu218Met
fadD19	3952308	p.Asp55Tyr|
fadE35	4252066	p.Ser328Pro
fadD32	4262388	p.Gly227Ser
fadD23	4301075	p.Glu422Gln
pks5	1722228	p.Leu2061Arg
pks7	1876976	p.Thr558Lys
pks8	1885385	p.Leu1228Val
pks11	1891388	p.Pro55Ala
pks12	2295685	p.Val3768Leu
pks12	2302033	p.Arg1652Cys
pks13	4256210	p.Ala1646Asp

Table S6: Blast search results of the gene sequence with insertion confirming the uniqueness of insertion sequence among lineage 2 genomes

Query Number	Reference genomes	Identification (%)	Identified (bp)	Query coverage (%)	start	end	E-value	Max score
117001	CP007027.1	100	332	50	3500716	3501047	1.11E-175	614
117001	CP009480.1	100	332	50	3494479	3494810	1.11E-175	614
117001	CP003248.2	100	332	50	3501331	3501662	1.11E-175	614
117001	AL123456.3	100	332	50	3501334	3501665	1.11E-175	614
159769	CP022014.1	100	654	100	3502186	3502839	0.00	654
159769	AP018036.1	100	654	100	3489334	3489987	0.00	654
159769	AP018035.1	100	654	100	3498189	3498842	0.00	654
159769	AP018034.1	100	654	100	3486855	3487508	0.00	654
159769	CP017920.1	100	654	100	4292533	4291880	0.00	654
159769	CP013475.1	100	654	100	3481453	3482106	0.00	654
159769	CP011510.1	100	654	100	4254434	4253781	0.00	654
159769	CP016888.1	100	654	100	3496444	3497097	0.00	654
159769	CP012506.2	100	654	100	3493237	3493890	0.00	654
159769	CP016794.1	100	654	100	3493988	3494641	0.00	654
159769	CP010968.1	100	654	100	3981473	3982126	0.00	654
159769	CP010339.1	100	654	100	3499781	3500434	0.00	654
159769	CP010335.1	100	654	100	3493892	3494545	0.00	654
159769	CP012090.1	100	654	100	917081	916428	0.00	654
159769	CP010895.1	100	654	100	3955706	3956359	0.00	654
159769	CP007299.1	100	654	100	3490224	3490877	0.00	654
159769	HG813240.1	100	654	100	3491900	3492553	0.00	654
159769	CP009426.1	100	654	100	3470402	3471055	0.00	654
159769	CP008983.1	100	654	100	518032	517379	0.00	654
159769	CP008981.1	100	654	100	518033	517380	0.00	654
159769	CP008980.1	100	654	100	518033	517380	0.00	654
159769	CP008979.1	100	654	100	518032	517379	0.00	654
159769	CP008978.1	100	654	100	518033	517380	0.00	654
159769	CP008977.1	100	654	100	518032	517379	0.00	654
159769	CP008976.1	100	654	100	518032	517379	0.00	654
159769	CP008975.1	100	654	100	518032	517379	0.00	654
159769	CP008974.1	100	654	100	518032	517379	0.00	654
159769	CP008973.1	100	654	100	518033	517380	0.00	654
159769	CP008972.1	100	654	100	518033	517380	0.00	654
159769	CP008970.1	100	654	100	518032	517379	0.00	654

159769	CP008969.1	100	654	100	518033	517380	0.00	654
159769	CP008968.1	100	654	100	518032	517379	0.00	654
159769	CP008966.1	100	654	100	518032	517379	0.00	654
159769	CP008964.1	100	654	100	518032	517379	0.00	654
159769	CP008962.1	100	654	100	518033	517380	0.00	654
159769	CP008961.1	100	654	100	518032	517379	0.00	654
159769	CP008960.1	100	654	100	518033	517380	0.00	654
159769	CP008959.1	100	654	100	518033	517380	0.00	654
159769	CP007809.1	100	654	100	3488153	3488806	0.00	654
159769	CP007803.1	100	654	100	3478240	3478893	0.00	654
159769	CP002885.1	100	654	100	3491717	3492370	0.00	654
159769	CP002883.1	100	654	100	3486486	3487139	0.00	654
159769	CP002882.1	100	654	100	3480758	3481411	0.00	654
159769	CP002871.1	100	654	100	3490732	3491385	0.00	654
159769	CP002884.1	100	654	100	3492943	3493596	0.00	654
159769	CP001642.1	100	654	100	3486529	3487182	0.00	654
159769	CP001641.1	100	654	100	3478627	3479280	0.00	654
159769	CP017598.1	100	654	100	3495720	3496373	0.00	654
159769	CP017597.1	100	654	100	3522988	3523641	0.00	654
159769	CP017596.1	100	654	100	3493170	3493823	0.00	654
159769	CP017595.1	100	654	100	3508711	3509364	0.00	654
159769	CP017594.1	100	654	100	3519190	3519843	0.00	654
159769	CP017593.1	100	654	100	3505356	3506009	0.00	654
159769	CP010873.1	100	654	100	3488175	3488828	0.00	654
159769	CP008963.1	100	654	100	518033	517379	0.00	654