Canola Agronomic Research Program (CARP)
FINAL REPORT

The Final Report should fully describe the work completed for the year, noting the personnel involved and any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. A final financial statement summary of expenses must also be submitted. In the event of major changes within the budget, supporting notes are required. The final report is a summary of activity for the final year and an overview of the entire project.

Project Title: ***From field to the genome. Application of 3rd generation sequencing to direct genotyping of canola pathogens***

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Hossein Borhan	AAFC Saskatoon	Principal Investigator
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Stephen Strelkov	University of Alberta	Co-Investigator
Tim Dumonceaux	AAFC Saskatoon	Co-Investigator

Project Start Date: April 1, 2019 **Project Completion Date:** March 31, 2024

Reporting Period: April 1, 2023 **to** March 31, 2024

CARP Project Number: **2019.28**

Instructions: This Final Project Report shall be completed and submitted on or about February 15th of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition to the Final Research Report, a *Final Research Abstract/Extension Report* is due upon completion of the project, maximum 2-3 pages, to be used for publication on the Funders' websites and in the *Canola Digest*. Content will be used in extension material, for consumers and/or industry. Include an Executive Summary, brief project description, key findings and conclusions (with a summary graph/table or supporting image for the project), translation of key findings into best management practices and/or relevance to the canola sector and future research, and funding acknowledgment as determined in the grant award letter. The Final Extension Report

is intended to support messaging to all audiences. Information needs to be clear, concise and in "grower-friendly" language.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of completion & status of activity (please check one)

Date of completion: March 31, 2024

☐ **Ahead of Schedule** ☐ **On Schedule** ☐ **Behind Schedule** ☐ **Completed**

Comments: Completed

2. Summary - Maximum of one page. This must include project objectives, results, and conclusions.

As described in the previous annual project reports, we re-designed target capture arrays by designing baits from a subset of 126 predicted clubroot effectors identified from our nearly chromosome level assembled genome of *Plasmodiophora brassicae* (Pb) pathotype 3H (Pb3H). Unlike the first array that was consist of 652 predicted Pb3H effectors, in the second capture array, only 126 effectors that their gene structure was supported by transcriptome (RNA-seq) data were used. Additionally, we improved the target capture sensitivity by modifying the PCR amplification steps. In this array we included targets against all the cloned *Leptosphaeria maculans*, probes to differentiate Leptosphaeria species and probes against several common fungi present in the soil.

Here we report on the application of second target capture array to DNA samples prepared from spore mixture of Pb3H and Pb6, DNA from potting soil spiked with an equal mixture of Pb3H and Pb6 spores at 10^1, 10^3, 10^5, 10^7 spore/gr of soil and DNA from field samples of canola stem canker. DNA extracted from potting soil with no spore was used as control. We also applied second target capture array to clubroot infected soils from two canola fields in Alberta. All samples, except for the Alberta field samples, were multiplexed, and run on our inhouse llumine MiSeq platform. We were able to identify SNP mutation that differentiated Pb3 and Pb6 effector alleles for sequence reads of the DNA prepared from potting soil with the mixture of both pathotypes' spores at 10^5 and 10^7 spore/gr of soil, but not at the lower than 10^5 concentrations. The average number of reads for various effectors was between 300 to 700 for 10^5 soil samples spiked with Pb and 4,000 to 9,000 for 10^7 spiked soils as well as pure spore samples. Pb3H and Pb6 genotypes were detected in sequence reads generated from a sample of mixed spores from both pathotypes. By applying target enrishment sequencing technique we were able to determine the genotype of clubroot pathogen in gall and soil samples collected from three locations in Alberta.

Target sequencing of *AvrLm-AvrLep* effectors from cankered tissues of canola stem generated up to 20,000 sequence reads per target genes allowing accurate genotyping of several effector at once and detection of *L. biglobosa* as a mix in one of samples. Clubroot as well as blackleg field soil samples provided a picture of other prevalent fungi.

1- To expand the existing genome sequence database by sequencing recently discovered Canadian clubroot pathotypes.
2- To apply and compare direct sequencing and targeted sequencing as diagnostic and genotyping method.
3- To compare laboratory detection method with direct in-field detection method.

3. Introduction – Brief project background, rationale, and objectives.

Proteins and small molecules known as effectors are virulent factors secreted by pathogen to overcome plant defense (1). Under selection pressure from the host, new virulent races/pathotypes, enriched with mutation within the effector genes, emerge. Due to their high sequence variability, effector gene sequence are the best targets to develop PCR based markers for race determination (2). However, existing sequence information is needed to design PCR markers for each known effector gene. Moreover, with PCR markers, undefined mutations in a given effector will remain undetected and PCR markers could become unfunctional due to new mutations within primers/probes target sequences. As an alternative method here we investigated the effector targeted re-sequencing to enrich the DNA mixture extracted from clubroot infested soil or plant tissues for the target effector genes. As we reported previously effector genes were predicted from the high quality draft genome of *Plasmodiophora brassicae* (Pb) pathotype 3H (Pb3H) (Borhan et al., unpublished). The first set of effector capture arrays that we developed consisted of 652 predicted effector genes. To improve the efficiency of target capture we designed a new set of capture array with 126 effectors. These effectors were selected based on being expressed during the infection and their gene structure being supported by RNA-sequencing data. In the same target capture array, we included probes to target all known *AvrLm* effectors as well as sequences that differentiate *Leptosphaeria* species as well as under other commonly occurring fungal pathogens.

4. Methods – Include approaches, experimental design, methodology, materials, sites, etc. Major changes from original plan should be cited and the reason(s) for the change should be specified.

Soil Preparation:

Cocomix and Sunshine #3 were mixed into 1:1 ratio (Final weight : 1000 g). 400 ml tap water was added to bring up the soil humidity (weight : 1370 grams).

1390 ul of ssPb3 inoculum ($7.2X10^8$ RS/ml) was added to a zip lock bag containing 100 g soil and mixed thoroughly to have 10^7 ssPb3 per gram of soil. One gram of the 10^7 ssPB3 soil was added to a bag containing 99g of soil to get 10^5 ssPb3 soil. This was repeated twice to make sequential 10^3 and 10^1 concentrations of soil. Soil was stored at -80.

Other samples included clubroot contaminated soil received from Bruce Gossen from a Manitoba farm in 2017 as well as the same soil spiked with 6.95 ul of the original ssPb3 RS suspension to add an extra 10^7 spores into the soil.

Soil DNA extraction:

Illum1802 and 1803:

For experiments, Illum1802 & Illum1803, frozen soil was ground in liquid N2 with pre-cooled mortar and pestle. DNA was extracted using the Fast DNA spin kit for Soil from MP Biomed.

Illum 2101:

For experiment Illum2101, the DNA samples prepped in 2018 were used again but treated with RNAse A followed by cleaning and concentrating using the DCC column from Zymo Research.

Illum2102:

For Illum2102, previously prepped soil was dried out in a petri dish at 37 degrees for 2-3 days and then ground at room temperature with mortar and pestle. 250mg of soil was homogenized in the Fast Prep homogenizer (for 3 x 1' at speed 6 with 1' rests on ice in between) and DNA was extracted using the Qiagen Powersoil Kit.

Clubroot Spore & gall DNA extraction:

For experiment Illum 2103, P2, P5, P6 and P8 galls were ground in liquid N2 using pre-cooled mortars and pestle followed by DNA extraction using the Qiagen DNeasy Max

DNA from P3 and P3A was prepped in 2021 by spinning down 2-3mL of clubroot resting spore (10^8), resuspending to 200uL, homogenizing with the Fast Prep homogenizer (4 x 40" at speed 6 with 2' on ice in

between, followed by 30' rest at room temperature). DNA extractions was done using the DNA Spin kit (MPBiomed), followed by RNAse A treatment and cleaning and concentration using the Zymo DCC column. DNA quality and quantity were checked on the Nanodrop and Qubit.

Conducting DNA target capture

Library preparation was done using the Nextera DNA Flex kit from Illumina and IDT for Illumina UD Indexes (Set A). Libraries were quantified using the Qubit dsDNA HS (High Sensitivity) kit and the quality of the final library was checked on the Tapestation from Agilent using D1000 tape.
Target Capture was done using MyBaits kit and custom designed probe sets from Daciel/Arbor Biosciences. Depending on the experiment, hybridization temperatures ranged from 65 down to 60 degrees for 48 hours at a time and both single and higher sensitivity dual capture protocols were followed to try and minimize background noise. PCR was done following each capture and the number of cycles used post capture ranged from 8-20 in an effort to produce post-capture libraries of a suitable concentration for sequencing. Post-capture libraries were quantified using qPCR and the Kapa Universal Library Quantification Kit.

Performing Illumina sequencing

Sequencing was done on the MiSeq sequencer from Illumina. Libraries were denatured and loaded onto MiSeq Reagent Kit v3 (600-cycle) at a concentration of 12pM and included a 30% PhiX control. Paired end sequencing (2x150bp or 2x 300bp) was performed.

Oxford Nano-Technology Adaptive Sampling

Oxford Nano-Technology sequencing was conducted in-house on a MiniION-MK1C device. The clubroot Pb3 genome sequence was supplied to the MinKNOW software as the reference for the selection and enrichment of target sequence. Figure 1 shows a comparison between adaptive sampling and target enrichment by capture array.

Detection of sequence variation

Generated data was first checked by FastQC for quality control. Reads were trimmed and adaptors were removed using CLC software. Trimmed read sequenced were mapped to the designed baits using CLC Genomics Workbench. All variants were called and visualized by the Variant -detection option implemented in the CLC Genomics Workbench software (Qiagen).

5. Results – Present and discuss project results, including data, graphs, models, maps, design, and technology development.

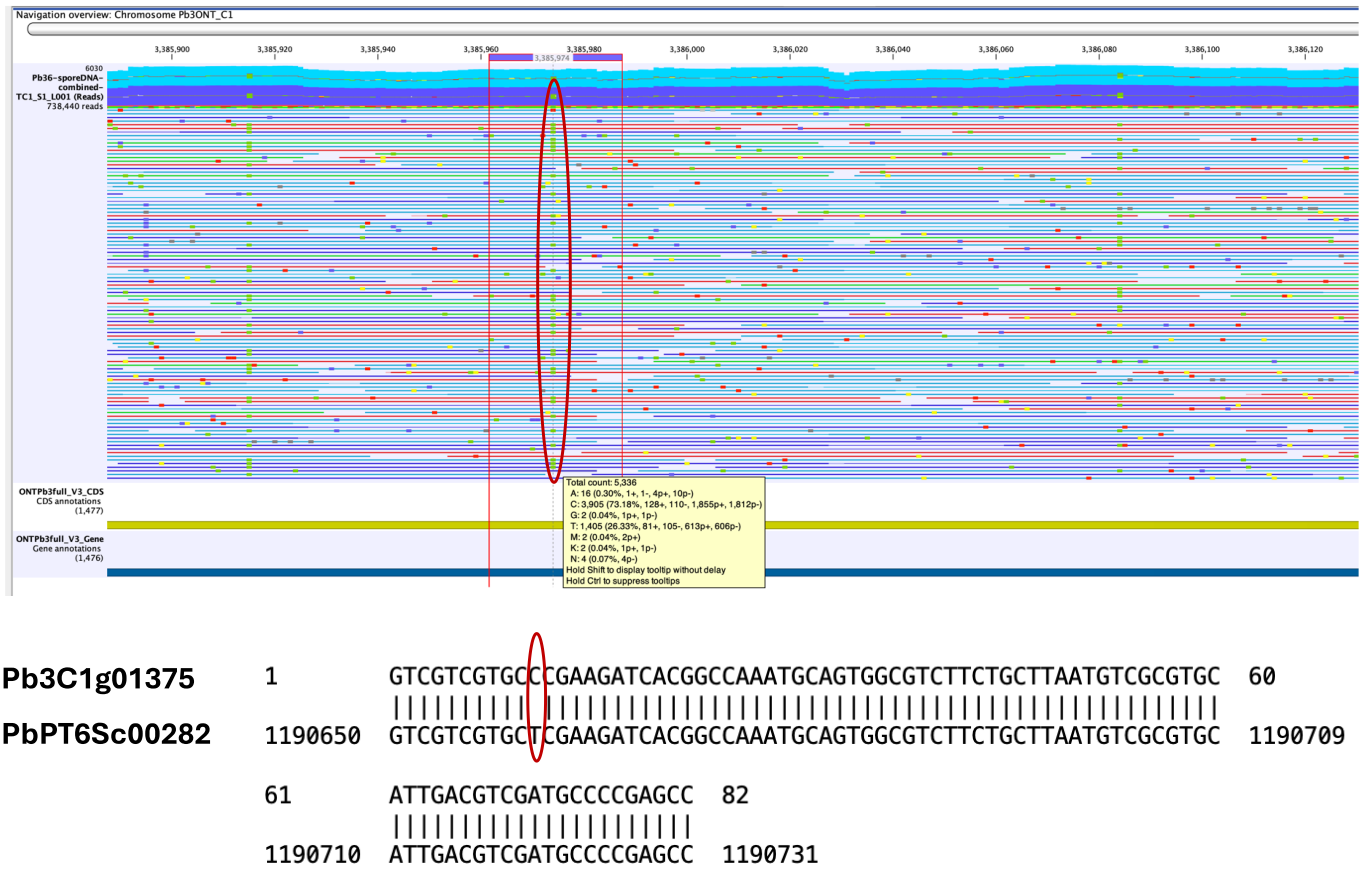
1- **To expand the existing genome sequence database by sequencing recently discovered Canadian clubroot pathotypes.**

As we reported previously chromosome level reference genome for the *P. brassicae* pathoype 3H (Pb3H) was generated and 10,348 genes were predicted from which 724 were annotated as potential effectors genes. We also re-sequenced the recently discovered virulent Pb3A using short read Illumina sequencing. A permit to import single spore purified pathotype from Dr. Strelkov lab was recently issued allowing us to acquire these pathotypes in future. Dr. Strelkov lab, independently from this project, has completed whole genome sequencing of several Pb pathotypes including Pb3A and is in the process of generating genome sequence for several other pathotypes identified so far.

2- **To apply and compare direct sequencing and targeted sequencing as diagnostic and genotyping method.**

2.1- Targeted sequencing of Pb spores.

Our sequencing of several Pb pathotypes (3) showed that Pb3 (renamed as Pb3H) and Pb6 had the most sequence diversity. Therefore, DNA from spores of Pb3 and Pb6 were mixed in equal concentration (250 ng of each sample) and used for target capture and sequencing. Sequencing was conducted at AAFC using Illumina MiSeq. The spore sample was sequenced in a multiplex with six other samples (from soil spiked with Pb spores). Over 6 million reads were produced for the spore samples alone, and 86.9% of the reads were mapped to the genome. Appendix table S1 shows the statistic of sequence reads mapped to the Pb3 reference genome. Sequence coverage for the target genes was between 3,000 to 7,000 sequence reads, while off-target sequence coverage was on average around 10 sequence reads indicating target gene specific sequence enrichment. This depth of sequence coverage ensures reliability of SNP calling. Figure one shows specificity and reliability of target capture to detect SNP in one of the effector genes.




```
Pb3C1g01375     1         GTCGTCGTGCCCGAAGATCACGGCCAAATGCAGTGGCGTCTTCTGCTTAATGTCGCGTGC  60
                          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
PbPT6Sc00282    1190650   GTCGTCGTGCTCGAAGATCACGGCCAAATGCAGTGGCGTCTTCTGCTTAATGTCGCGTGC  1190709

                61        ATTGACGTCGATGCCCCGAGCC  82
                          |||||||||||||||||||||||
                1190710   ATTGACGTCGATGCCCCGAGCC  1190731
```

Figure 1: An example of SNP detected by targeted re-sequencing of 126 *P. brassica* effectors. Top panel shows one of the SNPs in the effector Pb3C1g01375 vs its Pb6 orthologue. Targeted sequencing of DNA from Pb3H and Pb6 spores mixed with potting soil was mapped to the Pb3H reference genome. One of the SNPs marked by the red oval in top panel shows a C to T mutation indicating presence of two alleles one (C nucleotide) being the reference allele and the other (T nucleotide) is the orthologous sequence on the scaffold 282 (PbPT6Sc00282) of Pb6 genome. Lower panel shows alignment of the Pb3 and Pb6 alleles confirming the C to T change.

2.2 Targeted sequencing of galls from farms in Alberta.

Galls from clubroot infected canola from farms in Lacombe, Red Deer and municipal district (MD) Lesser

Slave River in Alberta were processed for sequencing. Targeted sequencing was conducted on DNA extracted from gall tissues enriched for spore by filtering ground tissues through cheesecloth. Galls were small and decomposed as shown in the supplementary figure S1. Despite the poor tissue quality, targeted sequencing resulted in deep coverage of all 126 effector genes with up to 10,000 sequence reads mapped to some of the genes. Sequence reads generated from Lacombe gall samples were closely examined for sequence variation. As shown in table 1 only 9 out of 126 Pb effectors showed sequence variation from the reference alleles. Comparison of the sequence variations with the data of blast search using the target effector genes as queries against the Pb3H genome, showed that these variant alleles were paralogues of the target gene as shown in figure 2 and identified the pathotype of Lacombe gall as Pb3H.

We generated over 4,000,000 sequence reads from the MD Lesser Slave River gall sample. Approximately 56% of the reads were mapped to the target genes. Sequence polymorphism profile of target effectors was similar to the Lacombe gall sample indicating the same Pb genotype (i.e. Pb3H) was present in the gall samples from both locations. We compared the sequence variation with the Pb3A genome reported recently (4). However, within the limits of our comparison (polymorphic sequences from 126 effector genes) we did not find any variation that could differentiate Pb3H from Pb3A.

Similar analysis was conducted on the sequence reads from Red Deer gall samples. Unlike the samples from Lacombe and Lesser Salve locations, the Red Deer galls showed polymorphism in some of the effector genes and the presence of alleles different from Pb3H and Pb3A (figure 3), indicating presence of a different isolate or co-infection of two Pb pathotypes with one being Pb3. To determine the genotype of the Pb pathotype of Red Deer gall sample a comprehensive genome sequence database of the Canadian clubroot isolates will be required.

Table S2 and table S3 show sequence read mapping statistics and sequence variations in target effector genes respectively.

2.3 Targeted sequencing of soil samples from Alberta

DNA was extracted from soil samples from Lacombe, MD Lesser Salve River, and Red Deer. Targeted sequencing generated 5.5, 4.9 and 1.1 million reads for Lacombe, Lesser Salve and Red Deer soil samples respectively, however only less than 1% of the reads were mapped to the genome indicating either spore concentration of lower than 10^5 spore per gram of soil or low quality of soil DNA. It should be noted that our first attempt in making the sequencing library failed, despite having recommended DNA concentration by the sequencing kit manufacturer. The second library prepared for these samples had poor quality for the Red Deer soil and lower than recommended for the other two soil samples. The lowest read coverage for target effectors on average was about 10 and the highest was about 400 sequence reads. Sequence comparison of effector genes with 100 or more reads did not show sequence variation from the reference Pb3H genome sequence. It is noteworthy that in the potting soil samples spiked with Pb spores, we could only generate a good sequence coverage for soil with 10^5 or higher spore per gram of soil. Applying the same method that we used for this report we were able to genotype clubroot in soil samples from Leduc Alberta as described in the 2022-2023 fiscal year report.

Table 1: Effector genes, from Lacombe gall sample, with sequence variation from the target alleles.

Gene nname	Region	Type	Reference	Allele	Count	Coverage	Frequency
Pb3C12g08016	230	SNV	A	G	1911	3200	59.71875
Pb3C12g08016	232	SNV	G	A	1880	3215	58.47589
Pb3C12g08016	232	SNV	G	G	1326	3215	41.24417
Pb3C5g04527	575	SNV	C	G	2014	2025	99.45679
Pb3C1g00089	261	SNV	A	G	1953	4038	48.36553
Pb3C1g00089	261	SNV	A	A	2082	4038	51.56018
Pb3C10g07394	1090	SNV	A	G	2188	4770	45.87002
Pb3C10g07394	1090	SNV	A	A	2577	4770	54.02516
Pb3C4g03984	1096^1097	Insertion	-	T	1655	2543	65.08061
Pb3C4g03984	1100	SNV	T	G	1655	2555	64.77495
Pb3C8g06091	267	Deletion	T	-	2593	4444	58.34833
Pb3C8g06091	267	SNV	T	T	1851	4444	41.65167
Pb3C5g04803	480	SNV	G	A	3926	10050	39.06468
Pb3C5g04803	480	SNV	G	G	6108	10050	60.77612
Pb3C5g04803	483	SNV	A	T	3918	9994	39.20352
Pb3C5g04803	483	SNV	A	A	6052	9994	60.55633
Pb3C5g04803	536	SNV	C	T	4717	11221	42.03725
Pb3C5g04803	536	SNV	C	C	6486	11221	57.80233
Pb3C5g04803	573	SNV	T	C	4466	11298	39.52912
Pb3C5g04803	573	SNV	T	T	6798	11298	60.16994
Pb3C5g04803	748..749	MNV	GC	AG	3284	7489	43.85098
Pb3C5g04803	748..749	MNV	GC	GC	4166	7489	55.62825
Pb3C5g04803	821	SNV	T	C	3334	7255	45.95451
Pb3C5g04803	821	SNV	T	T	3899	7255	53.74225
Pb3C5g04803	827	SNV	T	C	3347	7117	47.02824
Pb3C5g04803	827	SNV	T	T	3758	7117	52.80315
Pb3C5g04803	845	SNV	A	G	3366	6930	48.57143
Pb3C5g04803	845	SNV	A	A	3552	6930	51.25541
Pb3C5g04803	933	SNV	C	T	1519	3374	45.02075
Pb3C5g04803	933	SNV	C	C	1850	3374	54.83106
Pb3C5g04803	975	SNV	A	T	870	1986	43.80665
Pb3C5g04803	975	SNV	A	A	1115	1986	56.143
Pb3C6g05176	107^108	Insertion	-	G	203	384	52.86458
Pb3C6g05176	107^108	Insertion	-	-	180	384	46.875
Pb3C4g03832	106..107	Replaceme	TT	G	724	1407	51.457
Pb3C4g03832	106..107	MNV	TT	TT	679	1407	48.25871

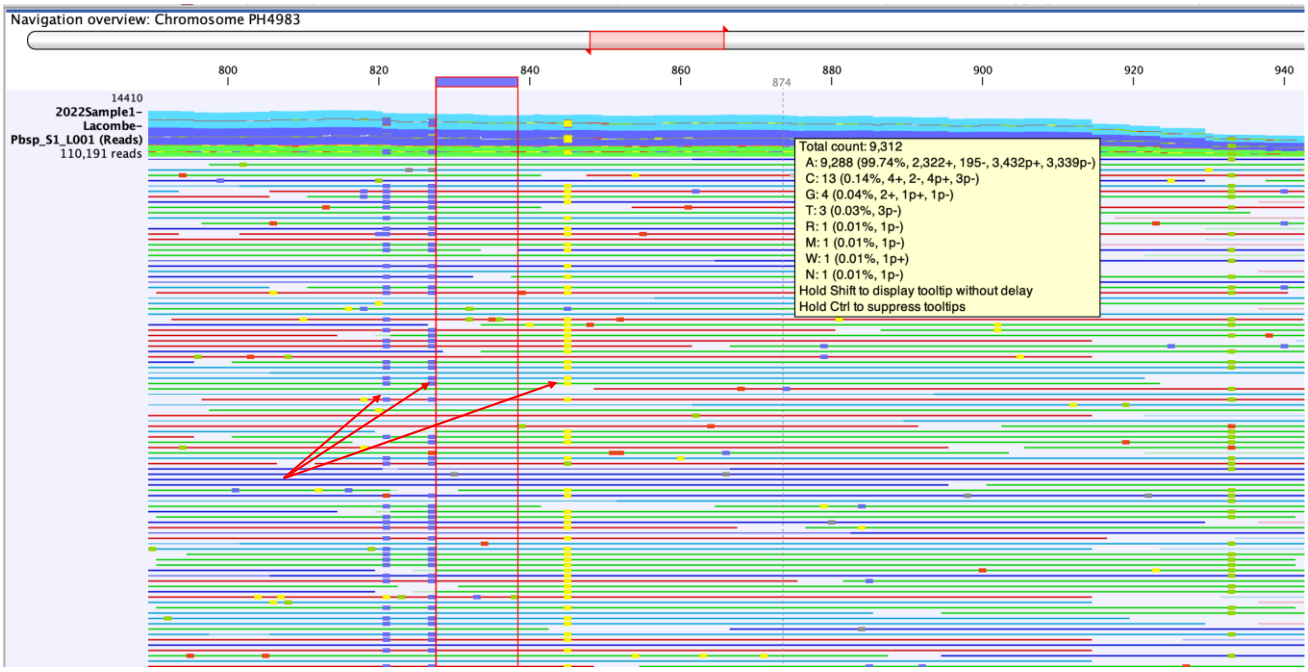


Figure 2: Sequence variations (SNPs; shown by red arrows) in effector gene Pb3C5g04803 sequence reads generated from Lacombe gall sample. SNPs are due to hybridization of probes to a paralogue allele. The read coverage as noted in the yellow inserted box, was over 9,000 reads.

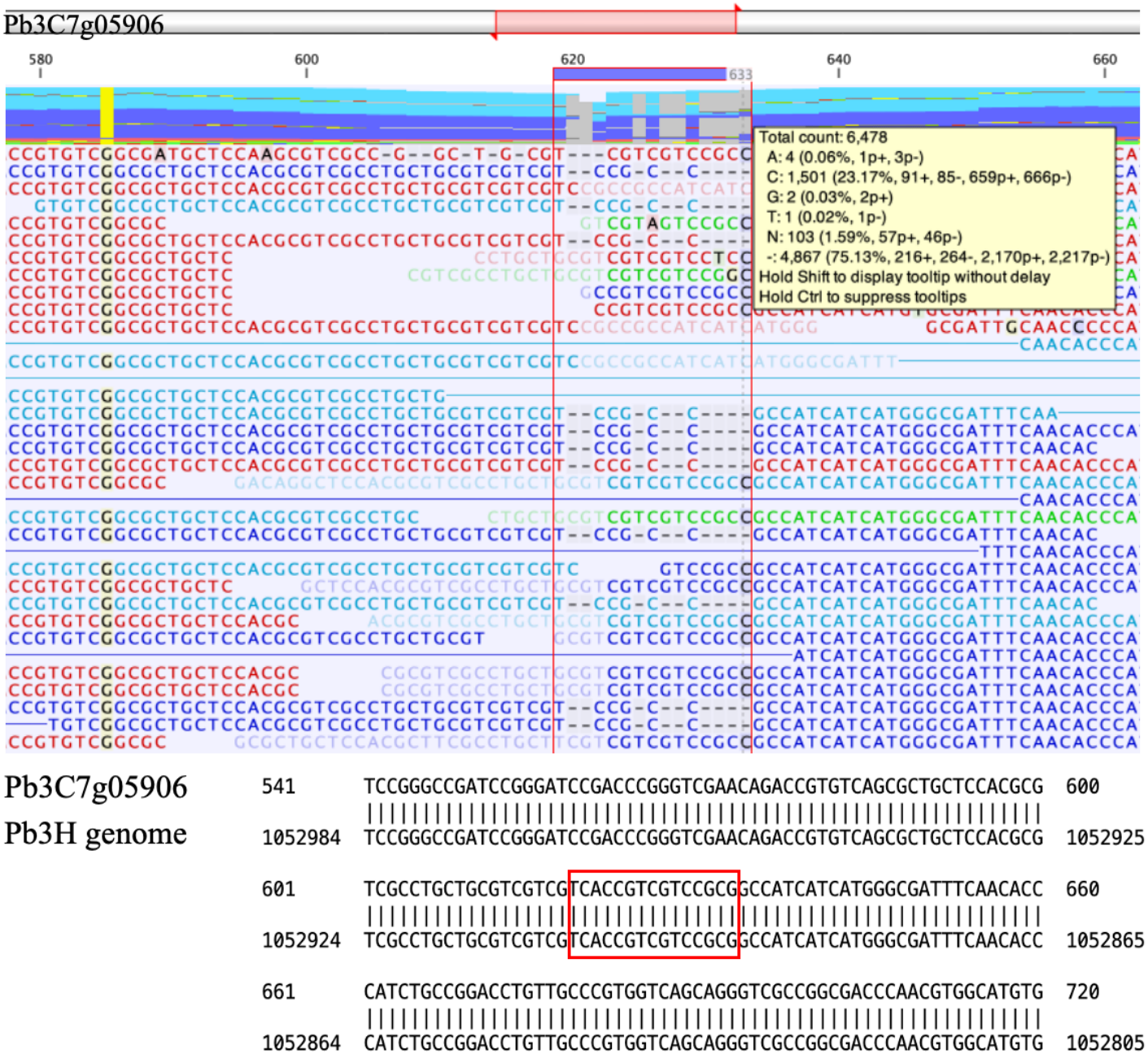


Figure 3: The top panel shows several indels (delimited by the red lines) with the sequence reads generated for the Pb3C7g05906 effector gene from the Red Deer gall samples. These sequence variations from the Pb3H reference do not correspond to the recently published Pb3A or Pb6 the only other two Canadian pathotypes that we have access to their genome sequences. The lower panel is the blast-generated alignment of Pb3C7g05906 effector with the corresponding interval on Pb3H genome sequence. The red box marks the sequence fragment corresponding to the sequence fragment within the red lines in the top panel.

2.4 Targeted sequencing of stem canker of blackleg infected canola plants from field.

To test the efficiency of genotyping by targeted sequencing on blackleg samples from canola fields, we prepared DNA from 3 stem samples infected with blackleg. Samples were old canola stem from our historic collection (SK field samples). Small cross section of cankered stems was cut, surface sterilized and placed in liquid culture media to grow the pathogen. DNA was extracted from mycelia collected from the liquid cultures and used for targeted

sequencing. Mapping the sequence reads to target *AvrLm/AvrLep* genes generated an extremely high coverage (close to 20,000 reads). The three samples had the same Avr gene profile and genotype determined to be *avrLm1, AvrLm2, AvrLm3, avrLm4, AvrLm7, avrLm9, AvrLm11 and AvrLms-Lep2*. Genotypes with lower case (avr) represent virulence and uppercase names (Avr) are avirulence genes.

Stem Sample number two had a mixture of both *L. macul*ans and *L. biglobosa* canadensis. In the sample one, about 300 reads matching to *L. maculans thlaspii* were detected indicating the presence of this subspecies on the canola field sample. *Lm.lepidii* was detected on sample two. Sample three had noticeable reads from other fungi namely the endophyte *Trichoderma gamsii* and *Fusarium redolens* a root pathogen causing root rot and wilting.

6. Conclusions and Recommendations – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development, and communication needs, if applicable.

We conducted targeted sequencing on potting soils mixed with spores of Pb3H and Pb6 together and were able to reliably differentiate (genotype) the two clubroot pathotypes. Genotyping by targeted sequencing of potting soil samples with added mixture of Pb3H and Pb6 spores were achieved for 107 and 105 spore/gr of soil but not for 10^3 and lower spore concentration. It should be noted that based on the current studies (5)10^3 spore per gram of soil is likely insufficient to cause infection. We conducted sequencing on galls collected from three locations in Alberta. Although gall tissues were of low quality and severely decomposed, we were able to generate a high sequencing coverage and to determine the Pb genotype for Lacombe and MD Lesser Slave to be Pb3H and for Red Deer an unknown pathotypes (within the limited genome data that currently exist for the Canadian clubroot isolates) or a mix of Pb3H and another pathotype. Soil samples from the same locations did not produce high sequence coverage. Coverage of 100 or more sequence reads was generated for a limited number of effector gene for Lacombe and DM Lesser Salve but not for Red Deer soil sample. This level of coverage was enough to determine the genotype of clubroot in these samples as Pb3H albeit with lower confidence. In our previous report (2022-2023) we were able to genotype clubroot from infected canola fields in Leduc. Sequencing soil DNA is more challenging due to presence of other microorganisms and organic materials which could affect quality of DNA and cause overestimation of the target DNA quantity.

We also sequenced DNA from stem lesion (canker) of blackleg infected canola samples. Cross sections of stem were placed in liquid media to allow fungal growth. Sequencing of the culturally grown samples produced high coverage and allowed us to not only genotype the blackleg isolates within the stem but also identify other Leptosphaeria species as well as other pathogenic and non-pathogenic fungi.

In conclusion target sequencing of pathogen effectors and other highly variable genes form pathogens, not only allows to detect the known genotypes but also to identify new variation in target genes that could indicate shift in the pathogen population. This method allows to genotype many genes at once. We used the Illumina MiSeq platform which is a low output sequencer, generating about 25 million reads on average. Despite this we were able to sequence up to 7 samples at once and generate 4 to 5 million reads per sample allowing us to genotype with high accuracy. It is possible to sequence 100s and thousands of samples on higher output Illumina sequencers to increase throughput and lower cost per sample.

7. Extension and communication activities: (e.g. extension meetings, extension publications, peer-reviewed publications, conference presentations, photos, etc).

A draft manuscript was prepared in the 2nd year based on the data obtained from clubroot spiked soil samples and DNA from individually cultured blackleg isolates. However we decided to postpone publishing the manuscript and add the field data to prove the applicability of this method under field condition. We intend to publish the manuscript by the end of 2024.

8. Acknowledgements – Include actions taken to acknowledge support by the Funders.

Funders will be acknowledged in a manuscript that is in preparation.

9. Literature Cited

1- Toruño, T. Y., Stergiopoulos, I., & Coaker, G. (2016). Plant-Pathogen Effectors: Cellular Probes Interfering with Plant Defenses in Spatial and Temporal Manners. Annual review of phytopathology, 54, 419–441. https://doi.org/10.1146/annurev-phyto-080615-100204.

2- Thilliez, G. J. A., Armstrong, M. R., Lim, T. Y., Baker, K., Jouet, A., Ward, B., van Oosterhout, C., Jones, J. D. G., Huitema, E., Birch, P. R. J., & Hein, I. (2019). Pathogen enrichment sequencing (PenSeq) enables population genomic studies in oomycetes. The New phytologist, 221(3), 1634–1648. https://doi.org/10.1111/nph.15441.

3- Rolfe, S.A., Strelkov, S.E., Links, M.G. et al. The compact genome of the plant pathogen Plasmodiophora brassicae is adapted to intracellular interactions with host Brassica spp . BMC Genomics 17, 272 (2016). https://doi.org/10.1186/s12864-016-2597-2.

4- Muhammad Asim Javed, Soham Mukhopadhyay, Eric Normandeau, Anne-Sophie Brochu, Edel Pérez-López (2024) Telomere-to-telomere genome assembly of the clubroot pathogen Plasmodiophora brassicae. bioRxiv; doi: https://doi.org/10.1101/2024.04.03.587992.

5- HwangS. F., AhmedH. U., StrelkovS. E., GossenB. D., TurnbullG. D., PengG., and HowardR. J. (2011) Seedling age and inoculum density affect clubroot severity and seed yield in canola. Canadian Journal of Plant Science. 91(1): 183-190. https://doi.org/10.4141/cjps10066.

10. Other Administrative Aspects: HQP personnel (PhD and/or MSc students) trained and involved; equipment bought; project materials developed

N/A

11. Appendices - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.

Supplementary data:

Table S1. Statistics of sequence reads for spores and potting soils mixed with spores.

Sample		Count	Percentage c	Average length	Number of bases	Percentage of bases (%
Pb3H reference genome		19 contigs	-	1,337,690.58	25,416,121	-
Pb3-6 pure spore mix						
	Mapped reads	6,235,179	86.93	138.3	862,353,000	87.48
	Total reads	7,172,838	100	137.43	985,742,788	100
Pb3-6, Soil 101 spore/g of soil						
	Mapped reads	3,060	0.04	74.24	227,173	0.02
	Total reads	7,947,976	100	138.43	1,100,227,546	100
Pb3-6, Soil 103 spore/g of soil						
	Mapped reads	3,060	0.04	74.24	227,173	0.02
	Total reads	7,947,976	100	138.43	1,100,227,546	100
Pb3-6, Soil 105 spore/g of soil						
	Mapped reads	3,060	0.04	74.24	227,173	0.02
	Total reads	7,947,976	100	138.43	1,100,227,546	100
Pb3-6, Soil 107 spore/g of soil						
	Mapped reads	8,437,358	81.42	138.8	1,171,124,727	81.74
	Total reads	10,363,136	100	138.26	1,432,760,535	100
soil without spore						
	Mapped reads	10,934	0.12	112.79	1,233,299	0.1
	Total reads	9,080,806	100	138.22	1,255,176,722	100

Figure S1: Clubroot gall samples collected from canola fields in Alberta (Dr. Strelkov). (A) Red Deer county; (B) MD Lesser Salve River; (C) Lacombe county.

Table S2: Sequence read statistic fro field gall samples from three locations in Alberta.

	Count	**Percentage of reads (%)**	**Average length**	**Number of bases**	**Percentage of bases (%)**
Gall sampes-Lacombe					
Mapped reads	5292897	60.7459119	150.098388		60.8299709
Not mapped reads	3420277	39.2540881	149.569994	511570809	39.1700291

Reads in pairs	4326188	49.6511145	338.884536	649251468	49.7119821
Broken paired reads	966709	11.0947974	150.204288	145203837	11.1179888
Total reads	11433284	100	149.662129	1711129621	100
Gall sampes- MD Lesser Slave River					
Mapped reads	2795994	56.5371526	150.173952	419885468	56.6616804
Not mapped reads	2149416	43.4628474	149.414582	321154094	43.3383196
Reads in pairs	2219348	44.8769263	361.500196	333263708	44.9724583
Broken paired reads	576646	11.6602264	150.216528	86621760	11.6892221
Total reads	4945410	100	149.843908	741039562	100
Gall sampes- Red Deer					
Mapped reads	6758922	59.1161909	150.025572	1014011136	59.25975
Not mapped reads	4674362	40.8838091	149.136606	697118485	40.74025
Reads in pairs	5434676	47.5338144	336.46428	815081210	47.6341009
Broken paired reads	1324246	11.5823765	150.221278	198929926	11.6256491
Total reads	11433284	100	149.662129	1711129621	100

Table S3: Profile of polymorphic sequences in gall samples from Alberta canola fields.

Chromosome	**Region**	**Type**	**Reference**	**Allele**	**Reference allele**	**Count**	**Coverage**	**Frequency**
Lacombe gall samples								
PH8325	230	SNV	A	G	No	1911	3200	59.71875
PH8325	232	SNV	G	A	No	1880	3215	58.475894
PH8325	232	SNV	G	G	Yes	1326	3215	41.244168
PH4694	575	SNV	C	G	No	2014	2025	99.45679
PH94	261	SNV	A	G	No	1953	4038	48.365527
PH94	261	SNV	A	A	Yes	2082	4038	51.560178
PH7676	1090	SNV	A	G	No	2188	4770	45.870021
PH7676	1090	SNV	A	A	Yes	2577	4770	54.025157
PH4134	1096^1097	Insertion	-	T	No	1655	2543	65.080613
PH4134	1100	SNV	T	G	No	1655	2555	64.774951
PH6320	267	Deletion	T	-	No	2593	4444	58.348335
PH6320	267	SNV	T	T	Yes	1851	4444	41.651665
PH4983	480	SNV	G	A	No	3926	10050	39.064677
PH4983	480	SNV	G	G	Yes	6108	10050	60.776119
PH4983	483	SNV	A	T	No	3918	9994	39.203522
PH4983	483	SNV	A	A	Yes	6052	9994	60.556334
PH4983	536	SNV	C	T	No	4717	11221	42.037252
PH4983	536	SNV	C	C	Yes	6486	11221	57.802335
PH4983	573	SNV	T	C	No	4466	11298	39.52912
PH4983	573	SNV	T	T	Yes	6798	11298	60.169942

PH4983	748..749	MNV	GC	AG	No	3284	7489	43.850981
PH4983	748..749	MNV	GC	GC	Yes	4166	7489	55.628255
PH4983	821	SNV	T	C	No	3334	7255	45.954514
PH4983	821	SNV	T	T	Yes	3899	7255	53.742247
PH4983	827	SNV	T	C	No	3347	7117	47.028242
PH4983	827	SNV	T	T	Yes	3758	7117	52.803147
PH4983	845	SNV	A	G	No	3366	6930	48.571429
PH4983	845	SNV	A	A	Yes	3552	6930	51.255411
PH4983	933	SNV	C	T	No	1519	3374	45.020747
PH4983	933	SNV	C	C	Yes	1850	3374	54.831061
PH4983	975	SNV	A	T	No	870	1986	43.806647
PH4983	975	SNV	A	A	Yes	1115	1986	56.143001
PH5360	107^108	Insertion	-	G	No	203	384	52.864583
PH5360	107^108	Insertion	-	-	Yes	180	384	46.875
PH3972	106..107	Replacement	TT	G	No	724	1407	51.457001
PH3972	106..107	MNV	TT	TT	Yes	679	1407	48.258706
Lesser Salve River gall samples								
PH8325	230	SNV	A	G	No	969	1575	61.52381
PH8325	230	SNV	A	A	Yes	604	1575	38.349206
PH8325	232	SNV	G	A	No	945	1574	60.038119
PH8325	232	SNV	G	G	Yes	623	1574	39.580686
PH1617	191	SNV	T	G	No	712	1582	45.006321
PH1617	191	SNV	T	T	Yes	869	1582	54.930468
PH4694	575	SNV	C	G	No	1135	1142	99.38704
PH94	261	SNV	A	G	No	1043	2139	48.761103
PH94	261	SNV	A	A	Yes	1095	2139	51.192146
PH7676	1090	SNV	A	G	No	1200	2613	45.924225
PH7676	1090	SNV	A	A	Yes	1408	2613	53.884424
PH4134	1096^1097	Insertion	-	T	No	840	1297	64.764842
PH4134	1096^1097	Insertion	-	-	Yes	457	1297	35.235158
PH4134	1100	SNV	T	G	No	835	1303	64.082886
PH4134	1100	SNV	T	T	Yes	467	1303	35.840368
PH6320	267	Deletion	T	-	No	1294	2203	58.738084
PH6320	267	SNV	T	T	Yes	909	2203	41.261916
PH4983	480	SNV	G	A	No	1954	5300	36.867925
PH4983	480	SNV	G	G	Yes	3334	5300	62.90566
PH4983	483	SNV	A	T	No	1952	5251	37.173872
PH4983	483	SNV	A	A	Yes	3287	5251	62.5976
PH4983	536	SNV	C	T	No	2360	5967	39.550863
PH4983	536	SNV	C	C	Yes	3587	5967	60.11396
PH4983	573	SNV	T	C	No	2265	6005	37.718568
PH4983	573	SNV	T	T	Yes	3726	6005	62.048293

PH4983	748..749	MNV	GC	AG	No	1535	3598	42.66259
PH4983	748..749	MNV	GC	GC	Yes	2034	3598	56.531406
PH4983	821	SNV	T	C	No	1573	3529	44.573534
PH4983	821	SNV	T	T	Yes	1944	3529	55.086427
PH4983	827	SNV	T	C	No	1580	3453	45.757312
PH4983	827	SNV	T	T	Yes	1861	3453	53.895164
PH4983	845	SNV	A	G	No	1607	3382	47.516263
PH4983	845	SNV	A	A	Yes	1770	3382	52.335896
PH5360	107^108	Insertion	-	G	No	68	139	48.920863
PH5360	107^108	Insertion	-	-	Yes	71	139	51.079137
PH3972	106..107	Replacement	TT	G	No	279	571	48.861646
PH3972	106..107	MNV	TT	TT	Yes	289	571	50.61296
Red Deer gall samples								
PH101	260	SNV	C	A	No	1444	1444	100
PH101	334..335	MNV	CG	AA	No	4208	4223	99.644802
PH101	423	SNV	C	T	No	4629	4634	99.892102
PH8061	496	SNV	T	C	No	5048	5054	99.881282
PH9260	934	SNV	G	A	No	5168	5196	99.461124
PH9260	973	SNV	T	C	No	5362	5377	99.721034
PH8325	56	SNV	G	A	No	2440	2450	99.591837
PH8325	119	SNV	T	C	No	3930	3940	99.746193
PH8325	230	SNV	A	G	No	2563	4117	62.254068
PH8325	230	SNV	A	A	Yes	1551	4117	37.673063
PH8325	232	SNV	G	A	No	2465	4103	60.077992
PH8325	232	SNV	G	G	Yes	1627	4103	39.653912
PH8325	345	SNV	C	T	No	4029	4043	99.653722
PH8325	400..401	MNV	TT	AG	No	1676	1683	99.584076
PH8325	579	SNV	T	G	No	2918	2922	99.863107
PH8325	1062	SNV	G	A	No	7871	7885	99.822448
PH8325	1123	SNV	G	C	No	7391	7398	99.90538
PH8325	1188	SNV	C	T	No	7495	7519	99.680809
PH8325	1711	SNV	G	A	No	1617	1620	99.814815
PH1617	1173	SNV	G	T	No	1640	1650	99.393939
PH131	801	SNV	G	A	No	6488	6501	99.800031
PH131	1049	SNV	G	A	No	5066	5072	99.881703
PH131	1088	SNV	G	A	No	5035	5051	99.683231
PH131	1136	SNV	T	C	No	3522	3527	99.858236
PH131	1187	SNV	C	T	No	2596	2596	100
PH131	1209	SNV	G	A	No	1965	1972	99.64503
PH9819	4	SNV	A	G	No	601	602	99.833887
PH9819	42..45	Replacement	ACTA	G	No	928	933	99.464094
PH9819	50	SNV	C	T	No	1085	1089	99.632691
PH9819	319	SNV	A	G	No	1921	1923	99.895996
PH9819	340..342	MNV	CGG	GAC	No	1618	1619	99.938233

PH9819	375	SNV	T	A	No	773	774	99.870801
PH9819	381..382	MNV	CT	TG	No	608	608	100
PH6286	107	SNV	T	A	No	2854	2864	99.650838
PH6286	117	SNV	C	G	No	2850	2854	99.859846
PH6286	121	SNV	G	A	No	3391	3397	99.823374
PH6286	1001..1002	MNV	AA	GG	No	1656	1663	99.579074
PH4694	575	SNV	C	G	No	2644	2663	99.286519
PH94	19	SNV	A	C	No	484	509	95.088409
PH94	30	SNV	T	A	No	560	585	95.726496
PH94	58^59	Insertion	-	CGT	No	871	900	96.777778
PH94	126	SNV	A	G	No	1960	1966	99.694812
PH94	187	SNV	T	C	No	1507	1510	99.801325
PH94	206..207	MNV	AT	GC	No	1354	1363	99.339692
PH94	230	SNV	G	A	No	855	856	99.883178
PH94	232	SNV	G	A	No	853	856	99.649533
PH94	261	SNV	A	G	No	1299	1301	99.846272
PH94	305	SNV	C	T	No	3386	3391	99.852551
PH94	317	SNV	T	C	No	3619	3622	99.917173
PH94	390	SNV	C	T	No	5479	5487	99.854201
PH94	417	SNV	G	C	No	5261	5271	99.810283
PH94	590	SNV	C	T	No	6063	6073	99.835337
PH94	670	SNV	G	A	No	5524	5534	99.819299
PH94	723	SNV	G	A	No	5252	5261	99.82893
PH94	768	SNV	C	G	No	5022	5027	99.900537
PH94	846..847	Replacement	CA	GTGGT	No	888	903	98.33887
PH94	853..854	MNV	AA	GG	No	902	903	99.889258
PH94	860..861	MNV	GT	CA	No	902	903	99.889258
PH94	869	SNV	T	A	No	903	903	100
PH94	876	SNV	C	T	No	903	903	100
PH94	878	SNV	A	G	No	903	903	100
PH94	885	SNV	C	G	No	799	801	99.750312
PH94	908	SNV	T	A	No	387	387	100
PH2705	92	SNV	T	C	No	5281	5290	99.829868
PH2705	99	SNV	A	C	No	5212	5229	99.67489
PH2705	101..102	MNV	CG	TC	No	5201	5231	99.426496
PH2705	106	SNV	G	A	No	5613	5631	99.680341
PH2705	144	SNV	G	A	No	4641	4676	99.251497
PH2705	210	SNV	G	C	No	5295	5298	99.943375
PH2705	424	SNV	G	A	No	3765	3770	99.867374
PH2705	458	SNV	C	A	No	4051	4061	99.753755
PH2705	524	SNV	T	G	No	2676	2677	99.962645
PH2705	528	SNV	C	A	No	2622	2622	100
PH5219	312..313	MNV	AA	GG	No	4868	4896	99.428105
PH5219	319	SNV	A	C	No	5033	5048	99.702853

PH5219	381	SNV	G	A	No	5687	5696	99.841994
PH5219	426	SNV	T	G	No	6482	6492	99.845964
PH5219	619	SNV	A	G	No	4521	4527	99.867462
PH5219	642	SNV	G	T	No	4321	4339	99.585158
PH5219	834	SNV	A	G	No	1942	1943	99.948533
PH5219	863	SNV	C	T	No	970	973	99.691675
PH5219	885	SNV	A	T	No	633	634	99.842271
PH5367	52	SNV	G	C	No	1457	1459	99.86292
PH5367	182	SNV	G	C	No	3803	3817	99.63322
PH5367	225	SNV	G	C	No	3546	3559	99.634729
PH5367	244	SNV	C	T	No	3412	3416	99.882904
PH5367	261	SNV	G	T	No	3467	3479	99.655073
PH5367	263	SNV	G	A	No	3463	3477	99.597354
PH5367	369	SNV	G	A	No	4781	4787	99.874661
PH5367	375	SNV	C	A	No	4557	4569	99.73736
PH5367	403	SNV	A	T	No	4220	4234	99.669343
PH7676	1090	SNV	A	G	No	3081	6635	46.435569
PH7676	1090	SNV	A	A	Yes	3551	6635	53.519216
PH7854	763	SNV	C	T	No	7761	7793	99.589375
PH7854	867	SNV	T	C	No	7989	8002	99.837541
PH7854	873	SNV	A	C	No	7983	7993	99.874891
PH7854	1231	SNV	C	G	No	7117	7134	99.761705
PH7854	1262	SNV	A	G	No	6243	6264	99.664751
PH8045	44	SNV	T	C	No	725	728	99.587912
PH8045	72	SNV	C	G	No	794	795	99.874214
PH8045	192^193	Insertion	-	G	No	995	1001	99.400599
PH8045	193^194	Insertion	-	GC	No	994	1001	99.300699
PH8045	195	SNV	G	T	No	992	1001	99.100899
PH8045	227	SNV	C	T	No	768	769	99.869961
PH8045	241	SNV	C	A	No	665	671	99.105812
PH8045	281..283	Deletion	CGC	-	No	106	106	100
PH8045	311	SNV	A	G	No	641	641	100
PH8045	344	SNV	C	T	No	1202	1207	99.58575
PH8045	357	SNV	A	G	No	1425	1427	99.859846
PH8045	466	SNV	T	C	No	1606	1609	99.813549
PH8045	498	SNV	T	C	No	1079	1080	99.907407
PH8045	500	SNV	T	C	No	1079	1081	99.814986
PH8045	533	SNV	G	A	No	574	578	99.307958
PH8045	537^538	Insertion	-	CCTC	No	142	142	100
PH8045	538^539	Insertion	-	GC	No	142	142	100
PH10351	464	SNV	A	C	No	5405	5416	99.796898
PH10351	511	SNV	A	G	No	4756	4765	99.811123
PH10351	516	SNV	T	C	No	4737	4754	99.642406
PH10351	623	SNV	T	G	No	2843	2860	99.405594
PH10351	675	SNV	A	C	No	2582	2618	98.624905
PH6184	43	SNV	A	G	No	2469	2472	99.878641

PH6184	166	SNV	G	A	No	4591	4604	99.717637
PH6184	169	SNV	G	A	No	4575	4603	99.391701
PH6184	253	SNV	G	T	No	6310	6331	99.668299
PH6184	870	SNV	C	G	No	6743	6759	99.763279
PH6184	1038	SNV	T	G	No	3071	3078	99.77258
PH6184	1064	SNV	T	C	No	2748	2751	99.890949
PH10557	62	SNV	T	G	No	2853	2855	99.929947
PH10557	608	SNV	A	T	No	6970	6981	99.842429
PH10557	676	SNV	C	T	No	5633	5642	99.840482
PH10557	683	SNV	A	G	No	4689	4694	99.893481
PH10557	705	SNV	G	C	No	4501	4510	99.800443
PH10557	738	SNV	T	C	No	4513	4517	99.911446
PH10557	741	SNV	T	C	No	4497	4519	99.513167
PH10557	809	SNV	C	T	No	6891	6900	99.869565
PH10557	859..860	MNV	CA	AG	No	6012	6048	99.404762
PH10557	939	SNV	G	C	No	7330	7344	99.809368
PH10557	992	SNV	C	T	No	6962	6971	99.870894
PH10557	1048	SNV	G	A	No	7236	7250	99.806897
PH10557	1052	SNV	T	C	No	7320	7326	99.9181
PH10557	1364	SNV	G	A	No	3427	3429	99.941674
PH621	40	SNV	A	T	No	931	934	99.678801
PH621	258	SNV	G	T	No	4856	4884	99.426699
PH621	324	SNV	A	G	No	5211	5223	99.770247
PH621	330	SNV	A	G	No	5227	5235	99.847182
PH621	614	SNV	G	C	No	4426	4429	99.932265
PH621	694	SNV	G	A	No	2271	2275	99.824176
PH9095	304	SNV	T	A	No	3666	3673	99.80942
PH9095	1190	SNV	T	A	No	2873	2876	99.895688
PH7790	345	SNV	G	A	No	6696	6725	99.568773
PH7790	424	SNV	G	C	No	4805	4830	99.482402
PH7790	426^427	Insertion	-	G	No	4715	4822	97.781004
PH7790	431	Deletion	C	-	No	4667	4827	96.685312
PH7790	466	SNV	A	G	No	5268	5284	99.697199
PH7790	567	SNV	C	T	No	4850	4856	99.876442
PH7790	594	SNV	G	A	No	4885	4896	99.775327
PH7790	670	SNV	T	G	No	3617	3623	99.834391
PH7790	693	SNV	T	C	No	3264	3270	99.816514
PH7790	744	SNV	G	A	No	2042	2044	99.902153
PH6122	16	SNV	G	T	No	3156	3160	99.873418
PH6122	94	SNV	G	C	No	3567	3573	99.832074
PH6122	180	SNV	G	C	No	5576	5589	99.7674
PH6122	183	SNV	C	G	No	5584	5589	99.910539
PH6122	215	SNV	G	A	No	6294	6317	99.635903
PH6122	342	SNV	G	C	No	6723	6736	99.807007
PH6122	386	SNV	C	T	No	6928	6941	99.812707
PH6122	392	SNV	T	C	No	6657	6663	99.90995

PH6122	395	SNV	T	A	No	6645	6664	99.714886
PH6122	402	SNV	T	G	No	6795	6803	99.882405
PH6122	423	SNV	C	T	No	6459	6477	99.722094
PH6122	504	SNV	A	G	No	7846	7885	99.50539
PH6122	585	SNV	A	G	No	6811	6822	99.838757
PH6122	620..621	Deletion	CA	-	No	4521	5479	82.515057
PH6122	625	Deletion	T	-	No	4437	5606	79.147342
PH6122	627..628	Deletion	GT	-	No	4422	5630	78.543517
PH6122	630..633	Deletion	CGCG	-	No	4367	5719	76.359503
PH6122	708	SNV	A	G	No	7093	7112	99.732846
PH6122	717	SNV	T	G	No	7247	7254	99.903502
PH6122	834	SNV	C	T	No	5449	5467	99.670752
PH6122	841	SNV	A	G	No	5197	5203	99.884682
PH6122	935	SNV	G	T	No	3701	3715	99.623149
PH6122	951	SNV	G	C	No	3359	3361	99.940494
PH4981	21	SNV	A	T	No	1087	1090	99.724771
PH4981	118	SNV	A	G	No	1068	1069	99.906455
PH4981	141	SNV	A	G	No	1455	1456	99.931319
PH4134	1096^1097	Insertion	-	T	No	2086	3247	64.243917
PH4134	1100	SNV	T	G	No	2075	3260	63.650307
PH3645	13	SNV	T	C	No	975	976	99.897541
PH3645	75	Deletion	G	-	No	1467	1472	99.660326
PH3645	126	SNV	T	C	No	1555	1555	100
PH3645	322	SNV	C	G	No	2778	2781	99.892125
PH3645	375	SNV	C	T	No	3340	3353	99.612288
PH3645	384	SNV	G	A	No	3190	3198	99.749844
PH3645	393	SNV	C	T	No	3146	3149	99.904732
PH3645	480	SNV	A	G	No	4133	4137	99.903312
PH3645	552	SNV	C	G	No	4776	4784	99.832776
PH3645	918	SNV	G	A	No	3758	3771	99.655264
PH3645	1152	SNV	A	G	No	3032	3044	99.605782
PH3645	1195	SNV	G	A	No	3474	3477	99.913719
PH10579	15	SNV	A	G	No	2447	2450	99.877551
PH10579	348	SNV	A	C	No	4656	4661	99.892727
PH10579	528	SNV	T	C	No	3137	3143	99.8091
PH10579	546	SNV	T	A	No	2345	2352	99.702381
PH10579	555	SNV	G	A	No	1905	1909	99.790466
PH10579	567	SNV	C	T	No	1750	1754	99.77195
PH10579	597	SNV	G	A	No	678	679	99.852725
PH10579	610	SNV	G	A	No	250	253	98.814229
PH10579	702	SNV	T	C	No	586	588	99.659864
PH10579	724	SNV	G	C	No	999	1002	99.700599
PH10579	740..741	MNV	CG	GA	No	1180	1188	99.326599
PH10579	743	SNV	G	A	No	1186	1188	99.83165
PH10579	751	SNV	A	G	No	1384	1387	99.783706

PH10579	864	SNV	T	G	No	1568	1575	99.555556
PH10579	891	SNV	G	A	No	1163	1165	99.828326
PH10579	894	SNV	C	T	No	1164	1165	99.914163
PH10579	909	SNV	G	A	No	905	916	98.799127
PH10579	939	SNV	C	T	No	385	386	99.740933
PH10579	978	SNV	C	A	No	203	203	100
PH10579	991	SNV	G	T	No	295	298	98.993289
PH10579	1035	SNV	G	A	No	809	812	99.630542
PH10579	1041	SNV	G	A	No	887	889	99.775028
PH10579	1080	SNV	A	G	No	1061	1063	99.811853
PH10579	1199..1200	MNV	GT	AA	No	683	684	99.853801
PH10579	1208	SNV	G	T	No	668	671	99.552906
PH10579	1217	SNV	C	A	No	626	626	100
PH10579	1230	SNV	C	T	No	528	528	100
PH9963	166	SNV	A	G	No	2889	2890	99.965398
PH9963	333	SNV	T	C	No	4370	4383	99.703399
PH9963	354	SNV	C	T	No	4422	4433	99.751861
PH9963	365	SNV	C	T	No	4453	4460	99.843049
PH9963	377..378	MNV	CT	TC	No	4313	4336	99.469557
PH9963	426	SNV	A	T	No	4343	4354	99.747359
PH9963	485	SNV	A	G	No	5107	5110	99.941292
PH9963	497	SNV	T	C	No	4656	4676	99.572284
PH9963	500	SNV	G	A	No	4667	4678	99.764857
PH9963	579	SNV	A	G	No	4752	4764	99.748111
PH9963	737	SNV	C	A	No	2739	2745	99.781421
PH9963	761	SNV	C	T	No	1668	1668	100
PH9963	764	SNV	A	C	No	1665	1668	99.820144
PH9963	797	SNV	T	A	No	841	844	99.64455
PH9963	824..825	MNV	GG	CC	No	212	212	100
PH9963	828	SNV	A	G	No	211	212	99.528302
PH9963	844	SNV	A	G	No	621	623	99.678973
PH9963	879^880	Insertion	-	CCACCA	No	856	944	90.677966
PH9963	906	SNV	T	G	No	2083	2089	99.712781
PH9963	1012	SNV	A	G	No	2553	2560	99.726563
PH9963	1047	SNV	G	A	No	2432	2439	99.712997
PH9963	1053	SNV	T	G	No	2458	2464	99.756494
PH9963	1113	SNV	C	T	No	2549	2559	99.609222
PH967	24	SNV	C	G	No	3427	3430	99.912536
PH967	84	SNV	T	C	No	4655	4665	99.785638
PH967	138	SNV	G	A	No	5339	5347	99.850383
PH967	162	SNV	T	A	No	5592	5601	99.839314
PH9553	129	SNV	T	G	No	3447	3454	99.797336
PH9553	139..141	Deletion	GGC	-	No	2548	2984	85.38874
PH9553	292	SNV	C	G	No	4860	4872	99.753695

PH9553	296	SNV	G	A	No	5047	5068	99.585635
PH9553	336	SNV	G	C	No	5257	5267	99.810139
PH9553	512	SNV	C	G	No	7951	7972	99.736578
PH9553	600	SNV	T	C	No	6122	6138	99.739329
PH9553	795	SNV	C	T	No	7135	7146	99.846068
PH9553	1122	SNV	G	C	No	6053	6084	99.490467
PH9553	1194	SNV	G	A	No	3564	3568	99.887892
PH9553	1205	SNV	T	A	No	2687	2690	99.888476
PH9136	30	SNV	G	T	No	4394	4398	99.90905
PH9136	129	SNV	T	C	No	7763	7783	99.74303
PH9136	147	SNV	G	C	No	6636	6654	99.729486
PH9136	207	SNV	G	C	No	7708	7719	99.857494
PH9136	213	SNV	C	G	No	7850	7864	99.821974
PH9136	222	SNV	T	C	No	8259	8277	99.78253
PH9136	471	SNV	C	G	No	9412	9428	99.830293
PH9136	577	SNV	G	C	No	9014	9028	99.844927
PH9136	590	SNV	C	T	No	9041	9070	99.680265
PH9136	672	SNV	C	T	No	7982	7999	99.787473
PH9136	690	SNV	T	A	No	7586	7602	99.789529
PH9136	697	SNV	A	G	No	7450	7482	99.572307
PH6366	93	SNV	C	G	No	3154	3159	99.841722
PH6366	348	SNV	G	C	No	5448	5465	99.68893
PH6366	396	SNV	G	A	No	4733	4765	99.328437
PH6366	411	SNV	C	G	No	4627	4632	99.892055
PH6366	669	SNV	T	G	No	4857	4861	99.917712
PH6366	726	SNV	C	G	No	4818	4821	99.937772
PH6366	840	SNV	G	C	No	5186	5197	99.788339
PH6366	957	SNV	A	G	No	3957	3964	99.823411
PH6366	991	SNV	A	T	No	3018	3032	99.538259
PH6366	997	SNV	A	T	No	2812	2819	99.751685
PH6320	267	Deletion	T	-	No	3478	6026	57.716562
PH6320	267	SNV	T	T	Yes	2548	6026	42.283438
PH4996	37^38	Insertion	-	C	No	1877	1889	99.364743
PH4996	289	SNV	T	C	No	6444	6450	99.906977
PH4996	359	SNV	G	A	No	7131	7150	99.734266
PH4996	375	SNV	C	A	No	7147	7181	99.526528
PH4996	531	SNV	G	A	No	5447	5459	99.78018
PH4996	546	SNV	C	T	No	5391	5405	99.740981
PH4996	561	SNV	C	G	No	5410	5416	99.889217
PH4996	588	SNV	C	T	No	6119	6136	99.722947
PH4983	80	SNV	G	A	No	3704	3720	99.569892
PH4983	136	SNV	G	A	No	5430	5445	99.724518
PH4983	480	SNV	G	A	No	5315	13906	38.220912
PH4983	480	SNV	G	G	Yes	8567	13906	61.606501
PH4983	483	SNV	A	T	No	5306	13790	38.477157
PH4983	483	SNV	A	A	Yes	8465	13790	61.385062

PH4983	536	SNV	C	T	No	6441	15521	41.498615
PH4983	536	SNV	C	C	Yes	9058	15521	58.359642
PH4983	573	SNV	T	C	No	6129	15759	38.892062
PH4983	573	SNV	T	T	Yes	9595	15759	60.885843
PH4983	716	SNV	C	A	No	5845	11886	49.175501
PH4983	716	SNV	C	C	Yes	6027	11886	50.706714
PH4983	748	SNV	G	A	No	4702	9140	51.444201
PH4983	748..749	MNV	GC	AG	No	4394	9346	47.014766
PH4983	805	SNV	G	A	No	4633	9293	49.854729
PH4983	805	SNV	G	G	Yes	4654	9293	50.080706
PH4983	821	SNV	T	C	No	4401	9004	48.878276
PH4983	821	SNV	T	T	Yes	4586	9004	50.932919
PH4983	827	SNV	T	C	No	4419	8918	49.551469
PH4983	827	SNV	T	T	Yes	4477	8918	50.201839
PH4983	845	SNV	A	G	No	4422	8718	50.722643
PH4983	845	SNV	A	A	Yes	4291	8718	49.220005
PH4983	862	SNV	A	C	No	4103	8488	48.338831
PH4983	862	SNV	A	A	Yes	4377	8488	51.566918
PH4983	933	SNV	C	T	No	3467	3472	99.855991
PH4983	944	SNV	G	T	No	1441	3319	43.416692
PH4983	944	SNV	G	G	Yes	1878	3319	56.583308
PH4983	975	SNV	A	G	No	779	1887	41.282459
PH4983	975	SNV	A	T	No	1107	1887	58.664547
PH4983	2007	SNV	A	C	No	2810	2812	99.928876
PH4983	2037	SNV	A	G	No	1916	1923	99.635985
PH4983	2045	SNV	G	A	No	1806	1807	99.94466
PH3740	7	SNV	C	T	No	1605	1607	99.875544
PH3740	81	SNV	G	T	No	3074	3074	100
PH3740	176	SNV	A	G	No	4048	4052	99.901283
PH3740	560	SNV	C	T	No	5297	5309	99.773969
PH3740	652	SNV	G	A	No	2526	2530	99.841897
PH3740	669	SNV	C	A	No	2076	2077	99.951854
PH3543	182	SNV	A	G	No	4244	4254	99.764927
PH3543	384	SNV	T	C	No	3624	3631	99.807216
PH3543	437	SNV	G	A	No	2561	2565	99.844055
PH3543	460	SNV	G	A	No	2285	2285	100
PH2890	38	SNV	T	C	No	3142	3145	99.90461
PH2890	165	SNV	G	C	No	5562	5570	99.856373
PH2890	239	SNV	A	T	No	6184	6202	99.709771
PH2890	255	SNV	G	C	No	6350	6356	99.905601
PH2890	366	SNV	G	T	No	6418	6433	99.766827
PH2324	9	SNV	G	C	No	1846	1849	99.83775
PH2324	140..141	MNV	CT	TC	No	2603	2618	99.427044
PH2324	184	SNV	C	T	No	3096	3106	99.678042
PH2324	393	SNV	C	T	No	2387	2392	99.79097
PH1544	416	SNV	C	G	No	7141	7162	99.706786

PH109	45	SNV	G	A	No	2742	2755	99.528131
PH109	117	SNV	C	G	No	3938	3946	99.797263
PH109	136	SNV	A	G	No	4059	4062	99.926145
PH109	138	SNV	T	C	No	4049	4062	99.679961
PH109	234	SNV	T	C	No	5525	5532	99.873463
PH109	575	SNV	C	T	No	7327	7362	99.524586
PH109	583	SNV	G	A	No	7653	7670	99.778357
PH109	597	SNV	T	C	No	7462	7474	99.839443
PH9428	74	SNV	C	T	No	2296	2300	99.826087
PH9428	96	SNV	G	C	No	2201	2214	99.412827
PH9428	151..152	MNV	AA	TG	No	2771	2776	99.819885
PH9428	198	SNV	T	G	No	3567	3570	99.915966
PH9428	273	SNV	A	T	No	4136	4159	99.446982
PH9428	334	SNV	C	A	No	3954	3963	99.772899
PH9428	669	SNV	C	G	No	1252	1254	99.84051
PH8682	66	SNV	T	C	No	3491	3497	99.828424
PH8682	198	SNV	C	G	No	5721	5731	99.82551
PH8682	468	SNV	C	A	No	4821	4825	99.917098
PH7779	71	SNV	G	A	No	2430	2431	99.958865
PH7779	79	SNV	C	T	No	2440	2440	100
PH7779	95	SNV	G	A	No	2537	2550	99.490196
PH7779	101	SNV	C	T	No	2526	2526	100
PH7779	153	SNV	A	C	No	2525	2530	99.802372
PH7779	183	SNV	C	A	No	2797	2800	99.892857
PH7779	274	SNV	A	T	No	3124	3130	99.808307
PH7779	330	SNV	C	T	No	2402	2405	99.87526
PH7779	375	SNV	G	C	No	1142	1142	100
PH7779	396	SNV	G	T	No	682	682	100
PH7779	403	SNV	T	G	No	571	578	98.788927
PH7779	435	SNV	C	T	No	116	116	100
PH7770	466	SNV	C	T	No	5273	5289	99.697485
PH7770	473	SNV	G	A	No	5290	5313	99.5671
PH7770	482	SNV	C	G	No	4998	5013	99.700778
PH7770	488	SNV	T	C	No	4939	4946	99.858471
PH7770	514	SNV	A	G	No	3627	3628	99.972437
PH7770	527	SNV	C	T	No	3534	3541	99.802316
PH7770	604	SNV	C	G	No	5525	5531	99.891521
PH7770	642	SNV	A	G	No	4199	4200	99.97619
PH7770	645	SNV	C	A	No	4193	4201	99.809569
PH7770	733	SNV	C	A	No	2670	2679	99.664054
PH7363	621	SNV	G	C	No	495	495	100
PH7363	623	SNV	G	A	No	493	495	99.59596
PH7363	638	SNV	A	G	No	801	801	100
PH7363	688	SNV	C	A	No	837	841	99.524376
PH7363	717	SNV	C	T	No	806	807	99.876084
PH7363	726	SNV	G	A	No	744	748	99.465241

PH7363	745	SNV	A	G	No	654	658	99.392097
PH7363	747	SNV	G	A	No	655	657	99.695586
PH7363	750	SNV	T	C	No	655	657	99.695586
PH7363	765^766	Insertion	-	A	No	861	999	86.186186
PH7363	777	SNV	C	G	No	1001	1001	100
PH7363	817	SNV	C	A	No	1503	1503	100
PH7363	823	SNV	T	A	No	1598	1601	99.812617
PH7363	885	SNV	G	C	No	2298	2300	99.913043
PH7363	999	SNV	G	A	No	1818	1822	99.780461
PH7363	1003	SNV	T	C	No	1672	1674	99.880526
PH7363	1040	SNV	A	G	No	1202	1205	99.751037
PH7363	1076	SNV	C	T	No	143	143	100
PH7363	1177	SNV	G	A	No	308	310	99.354839
PH7363	1213	SNV	G	T	No	951	969	98.142415
PH7363	1218	SNV	C	T	No	1025	1029	99.611273
PH7363	1276..1277	MNV	GA	CG	No	2744	2766	99.204628
PH7363	1469	SNV	T	C	No	4892	4915	99.532045
PH7363	1476	SNV	T	A	No	5048	5067	99.625025
PH7363	1683	SNV	G	T	No	3009	3013	99.867242
PH7363	1725	SNV	G	A	No	2109	2109	100
PH7080	86	SNV	G	A	No	2416	2444	98.854337
PH7080	151	SNV	A	G	No	2389	2393	99.832846
PH7080	189	SNV	T	C	No	3624	3632	99.779736
PH7080	277	SNV	A	G	No	4323	4325	99.953757
PH7080	282	SNV	G	C	No	4371	4376	99.88574
PH7080	523	SNV	G	A	No	2479	2480	99.959677
PH7080	571	SNV	A	G	No	1124	1126	99.82238
PH7080	573..574	MNV	GG	AA	No	1123	1126	99.73357
PH7080	576	SNV	A	G	No	1125	1126	99.91119
PH7080	607	SNV	A	G	No	477	481	99.168399
PH7080	609..610	MNV	GG	AA	No	477	480	99.375
PH7080	612	SNV	A	G	No	480	480	100
PH7080	621	SNV	C	G	No	450	454	99.118943
PH7080	633	SNV	A	G	No	224	236	94.915254
PH7080	825	SNV	T	C	No	249	249	100
PH7080	847	SNV	A	G	No	309	310	99.677419
PH7080	865^866	Insertion	-	ACG	No	354	399	88.721805
PH7080	953	SNV	T	G	No	460	460	100
PH7080	969	SNV	A	G	No	347	347	100
PH7080	999	SNV	T	C	No	222	222	100
PH6608	438	SNV	A	G	No	4473	4475	99.955307
PH6608	528	SNV	C	T	No	1815	1815	100
PH630	482	SNV	A	C	No	5097	5102	99.901999
PH630	828	SNV	G	A	No	2121	2122	99.952875
PH5360	78	SNV	C	T	No	800	800	100

PH5360	107^108	Insertion	-	G	No	228	500	45.6
PH5360	107^108	Insertion	-	-	Yes	272	500	54.4
PH5263	75	SNV	G	A	No	1931	1938	99.638803
PH5263	91	SNV	C	T	No	1773	1776	99.831081
PH5263	95	SNV	G	C	No	1685	1691	99.64518
PH5263	97	SNV	G	T	No	1684	1691	99.586044
PH5263	115..116	MNV	AA	CG	No	1502	1507	99.668215
PH5263	130	SNV	A	C	No	1945	1947	99.897278
PH5263	148	SNV	A	G	No	1730	1732	99.884527
PH5263	152	SNV	T	G	No	1939	1942	99.84552
PH5263	171	SNV	A	C	No	1933	1935	99.896641
PH5263	176	SNV	G	A	No	2188	2190	99.908676
PH5263	218	SNV	A	G	No	2890	2898	99.723948
PH5263	257..258	MNV	TT	GA	No	3151	3175	99.244094
PH5263	334	SNV	C	G	No	3232	3238	99.8147
PH5263	339	SNV	G	A	No	3239	3243	99.876657
PH5263	400	SNV	C	G	No	3044	3049	99.836012
PH5261	208	SNV	A	G	No	4227	4235	99.811098
PH5261	231	SNV	T	G	No	5206	5220	99.731801
PH3972	106..107	Replacement	TT	G	No	943	1792	52.622768
PH3972	106..107	MNV	TT	TT	Yes	843	1792	47.042411
PH3938	270	SNV	C	T	No	6346	6355	99.858379
PH3938	284	SNV	G	A	No	5502	5511	99.83669
PH3938	414	SNV	T	C	No	6059	6066	99.884603
PH3938	495	SNV	G	C	No	4499	4505	99.866815
PH3938	536	SNV	A	C	No	3993	4001	99.80005
PH3938	564..566	MNV	AGC	CAA	No	3837	3893	98.561521
PH3938	603	SNV	C	A	No	4679	4690	99.765458
PH1620	621	SNV	T	C	No	6947	6960	99.813218
PH1620	624	SNV	G	C	No	6901	6960	99.152299
PH1620	642	SNV	T	C	No	6700	6709	99.865852
PH1620	722	SNV	T	A	No	6114	6123	99.853013
PH1620	730	SNV	A	G	No	5633	5640	99.875887
PH1620	774	SNV	C	G	No	6399	6407	99.875137
PH1620	777	SNV	G	T	No	6388	6407	99.703449
PH1620	807	SNV	G	C	No	6165	6176	99.821891
PH1620	883	SNV	G	A	No	7025	7040	99.786932
PH1620	911	SNV	G	A	No	6577	6587	99.848186
PH1620	1011	SNV	T	C	No	3817	3821	99.895315
PH1620	1015	SNV	G	A	No	2537	2551	99.451196
PH1620	1018	SNV	G	A	No	2535	2551	99.372795
PH1620	1021	SNV	T	C	No	2543	2551	99.686397
PH1620	1023	SNV	C	T	No	2545	2551	99.764798
PH1620	1032	SNV	G	A	No	2242	2250	99.644444
PH1620	1048	SNV	T	C	No	1170	1170	100

PH1620	1050	SNV	G	A	No	1169	1170	99.91453
PH1620	1067	SNV	C	A	No	531	539	98.51577
PH1620	1069	SNV	T	G	No	534	534	100
PH1620	1073	SNV	A	G	No	532	534	99.625468
PH1620	1075	SNV	A	G	No	534	534	100
PH1620	1118	SNV	C	G	No	1402	1407	99.644634
PH1620	1122	Deletion	A	-	No	1395	1407	99.147122
PH1620	1125^1126	Insertion	-	A	No	1398	1408	99.289773
PH1620	1242	SNV	T	G	No	2419	2426	99.711459
PH1620	1318	SNV	A	C	No	1741	1746	99.713631
PH1620	1336	SNV	G	A	No	1497	1498	99.933244
PH1521	270	SNV	A	G	No	7201	7218	99.764478
PH1521	558	SNV	A	G	No	3964	3970	99.848866
PH1521	597	SNV	G	A	No	3291	3293	99.939265
PH1521	600	SNV	C	T	No	3286	3294	99.757134

12. Financial (to be provided to each Funding Agency (at the addresses indicated in 11.2)

a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to the Funders' Funding.
b. Explanation of variances from budget which are greater than 10%.
c. Invoice

13. Final Report Posting Do you consent to a version of this Final Report (with sensitive information removed) to be posted on the funder's website?	☐ Yes - this version can be posted ✓ ☐ Yes - a modified version will be sent ☐ No
14. Research Abstract Posting Do you consent to the 2-3 Research Abstract submitted with this Final Report to be posted on the funders and the Canola Council of Canada's website?	☐ Yes ✓ ☐ No

Please send an electronic copy of this completed document to:

Ellen McNabb
Research Administrator
Canola Council of Canada
400 – 167 Lombard Ave.
Winnipeg, MB R3B 0T6
Phone: (204) 982-2110
Fax: (204) 942-1841
E-Mail: mcnabbe@canolacouncil.org