Final Project Demonstration

Application of Machine Learning Techniques to Next Generation Sequencing Quality Control

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Aims

Report on state of the current quality control system at Sanger

- Working with the Wellcome Trust Sanger Institute's Human Genetics Informatics Team
- `auto_qc` classifies samples as pass, fail or warn
- Current classifier consists of hard-coded simple thresholds
- `auto_qc` also requires timely human intervention

Goals

- Apply learning techniques to replicate current human rules
- Attempt to improve efficiency of current "warning" handling
- Identify new or unused parameters that improve classification

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Input Data and Format

Input: **Lanelet** QC Data

- Access to two of the largest studies at the institute
- 13,455 "lanelets"; aggregated clusters of a sample in one lane
- `auto_qc` **pass** 9,154 (68%), **fail** 1,542 (11%) **warn** 2,759 (21%)

Input Format: **"BAMcheckR'd"** Text Files

- Key-value statistical summary numbers from `samtools stats`
- `samtools stats` also generates tab-delimited dataframes measuring some metrics over cycle time
- Additional summary numbers gained by passing output of `samtools stats` through internal Sanger tool, `bamcheckr`

Handling Data

Introducing **Frontier**

- A Python package providing interfaces for reading, storage and retrieval of machine learning data sets
- Users write their own reader classes but need only provide implementations of two functions so any file can be used as input
- Presents an API for manipulation and extraction of stored data-target pairs, allowing filter by parameters or classes
- Supports 'any' machine learning problem – user merely provides simple definitions of the labels
- Returns data via the API in efficient **NumPy** containers for direct use with the **scikit-learn** framework
- Quick and easy logging of machine learning experiments

Frontier Example Usage

```
from Frontier import frontier
from Frontier.IO import DataReader, TargetReader

data_dir = "/home/sam/Projects/owl_classifier/data/"
target_path = "/home/sam/Projects/owl_classifier/targets.txt"

CLASSES = {
        "hoot": {
            "names": ["owl", "owls"],
            "code": 1,
        },
        "unhoot": {
            "names": ["cat", "dog", "pancake"],
            "code": 0,
        },
}

statplexer = frontier.Statplexer(data_dir,
                                 target_path,
                                 CLASSES,
                                 DataReader,
                                 TargetReader)
```

Introduction to the Frontier API

Feature Inspection

- **list_parameters**
  Return a sorted list of all parameters
- **find_parameters**
  Return parameters which contain any of the input strings as a substring
- **exclude_parameters**
  Return parameters which do not contain any of the input strings as a substring

Data-Target Extraction

- **get_data_by_parameters**
  Return data for all observations, but only include columns for each parameter in a given list
- **get_data_by_target**
  Return data for observations that have been classified as one of the targets specified and only return columns for the parameters in the given list

Contributions to Current QC System

- `bamcheckr`; an in-house tool written in R
- Supplements `samtools stats` key-value summary numbers which are then used by the current `auto_qc` system

What did I do?

- Patched a bug that prevented plotting of diagnostic graphs
- Authored additional routines to recover "missing" percentage and ratio based quality parameters that were typically calculated outside of bamcheckr...
- ...although **Frontier** turned out to be more efficient for this task

Testing Parameter Sets

Set	#	CV ± SD	SCV ± SD	Depth	Most Important Feature
ALL	86	90 ± 4	97 ± 1	38	T-percent-max-baseline-deviation (27%)
AQC	27	87 ± 4	95 ± 1	36	T-percent-max-baseline-deviation (31%)
AQCN	21	86 ± 4	95 ± 1	39	max-max-baseline-deviation (31%)
ERROR	1	60 ± 6	61 ± 2	53	error-rate (100%)
NO_ERROR	85	90 ± 4	97 ± 1	38	T-percent-max-above-baseline(27%)
BASELINE	34	82 ± 5	89 ± 1	46	T-percent-max-above-baseline(28%)
NOBASELINE	52	72 ± 10	91 ± 1	31	error-rate (24%)
MARP	47	87 ± 4	95 ± 1	39	T-percent-max-above-baseline (27%)
NO_MARP	39	75 ± 7	87 ± 1	38	max-max-baseline-deviation (34%)

Table : **Parameter Set Cross Validation Scores**: Results of classifying testing data into one of three classes; pass, fail or warn. Columns left to right; parameter set name, number of parameters included, average cross-validation score (max 100) ± std. deviation, average stratified cross-validation score (max 100) ± std. deviation, average depth of the generated tree and the most important parameter by Gini importance (max 100). Tree depth and parameter importance was estimated on experiments using the stratified data.

- No surprise that parameter superset **ALL** validates well
- **AQC** and **AQCN** score highly despite far smaller models
- Generally good performance, possibly reflecting simple linear nature of underlying rules or possible bias (lots of passes)

ALL vs. AQC

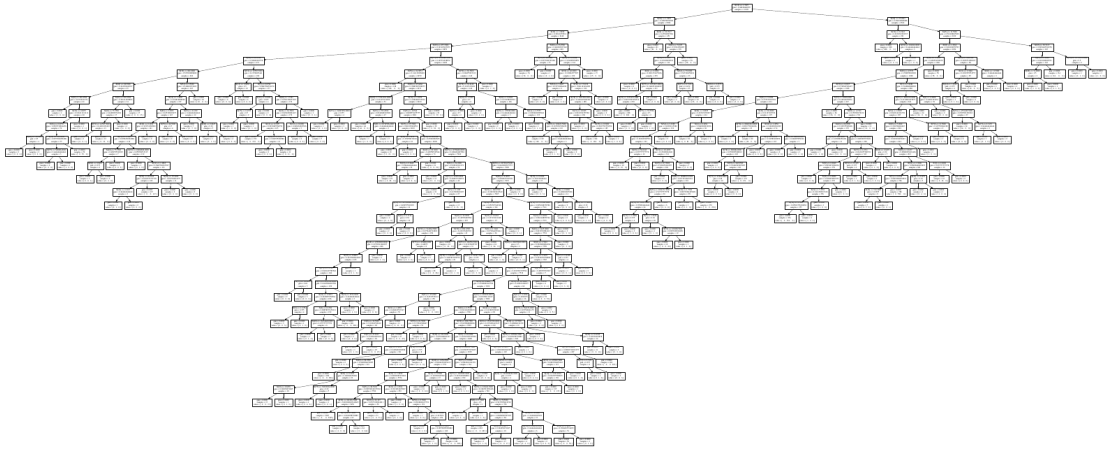

Figure : **ALL Set Decision Tree**

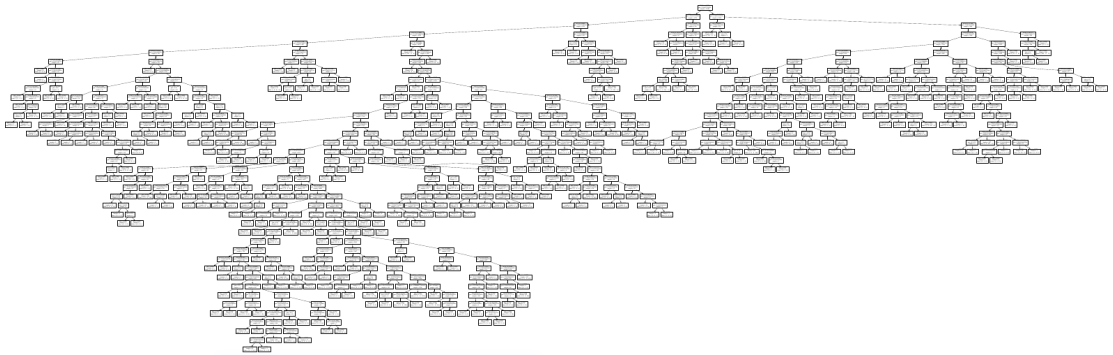

Figure : **AQC Set Decision Tree**

https://github.com/SamStudio8/frontier/tree/master/results/front/

Overfitting

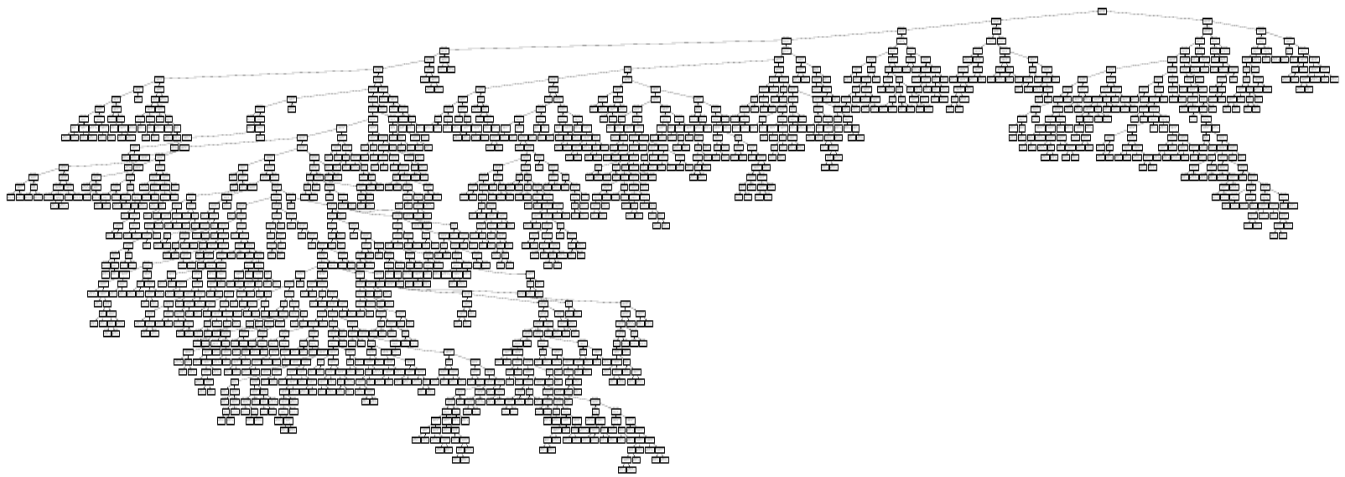

Figure : **BASELINE Set Decision Tree**

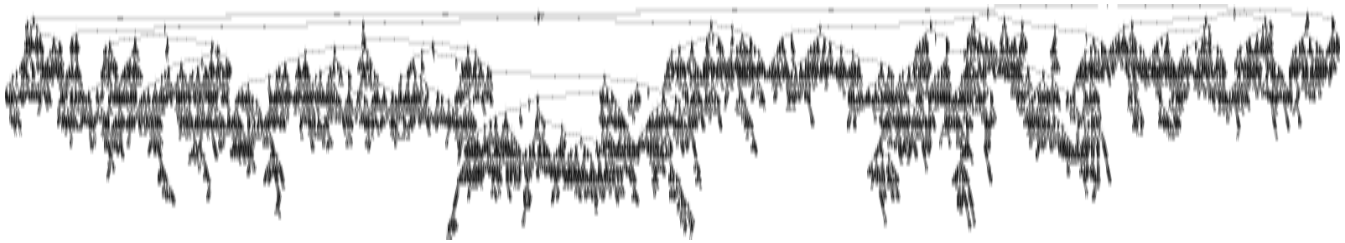

Figure : **ERROR Set Decision Tree**

https://github.com/SamStudio8/frontier/tree/master/results/front/

Augmenting Warning Handling

PSet	DSet	#	CV $\pm$ SD	SCV $\pm$ SD	Depth	Most Important Feature
ALL	IGNWARN	86	96 $\pm$ 3	99 $\pm$ 0	22	error-rate (43%)
ALL	WARNPASS	86	95 $\pm$ 3	99 $\pm$ 0	33	quality-dropoff-rev-mean-runmed -decline-low-value (32%)
AQC	IGNWARN	27	94 $\pm$ 4	98 $\pm$ 1	26	error-rate (44%)
AQC	WARNPASS	27	92 $\pm$ 4	98 $\pm$ 1	33	quality-dropoff-rev-mean-runmed -decline-low-value (33%)

Table : **Parameter Set Cross Validation Scores using Alternative Warning Handling**: Results of classifying testing data. Columns left to right; parameter set name, data set name, number of parameters included, average cross-validation score (max 100) $\pm$ std. deviation, average stratified cross-validation score (max 100) $\pm$ std. deviation, average depth of the generated tree and the most important parameter by Gini importance (max 100). Tree depth and parameter importance was estimated on experiments using the stratified data. *N.B.* **IGNWARN** and **WARNPASS** data sets perform classifications on two classes (pass and fail) rather than three.

- **IGNWARN** discards, **WARNPASS** recodes as pass
- Very high validation, appears noise has been significant reduced when compared to the previously tabulated results
- Average maximum depth reduced

Backward Elimination

A-percent-mean-below-baseline
duplicate-mapped-ratio
fwd-percent-insertions-above-baseline
insert-size-average
max-max-baseline-deviation
quality-dropoff-fwd-mean-runmed-decline-low-value
quality-dropoff-rev-mean-runmed-decline-low-value
rev-percent-insertions-above-baseline
rev-percent-insertions-below-baseline

Table : **TOP9 Parameter Set**: Features selected by a backward elimination experiment providing all observations to an iterative decision tree classifier and removing the least important feature until cross-validation fell below a threshold.

- Borrowed a method from statistical model design
- Repeatedly refitted trees after removing the least important feature until cross-validation fell below some percentage of the running average
- Would be very interesting to repeat this process for various augmentations of the warnings class handling

TOP9 Experiment Results

PSet	DSet	#	CV $\pm$ SD	SCV $\pm$ SD	Depth	Most Important Feature
RTOP9	ALL	9	87 $\pm$ 6	95 $\pm$ 1	32	max-max-baseline-deviation (32%)
RTOP9	IGNWARN	9	98 $\pm$ 1	99 $\pm$ 0	20	rev-pct-insertions-above-baseline (38%)
RTOP9	WARNPASS	9	95 $\pm$ 3	99 $\pm$ 1	24	quality-dropoff-rev-mean-runmed -decline-low-value (34%)

Table : **Backward Elimination Parameter Set Cross Validation Scores**: Results of classifying testing data. Columns left to right; parameter set name, data set name, number of parameters included, average cross-validation score (max 100) $\pm$ std. deviation, average stratified cross-validation score (max 100) $\pm$ std. deviation, average depth of the generated tree and the most important parameter by Gini importance (max 100). Tree depth and parameter importance was estimated on experiments using the stratified data. *N.B.* **IGNWARN** and **WARNPASS** data sets perform classifications on two classes (pass and fail) rather than three.

- Impressive validation results considering only 9 parameters
- Average maximum tree depth upper bound briefly overlaps the lower bound of the results found from the naive parameter sets
- Clearly we can gain accuracy on class prediction without the need for including every parameter in the model, **TOP9** is even smaller than the **AQC** set!

TOP9 Decision Tree with All Data

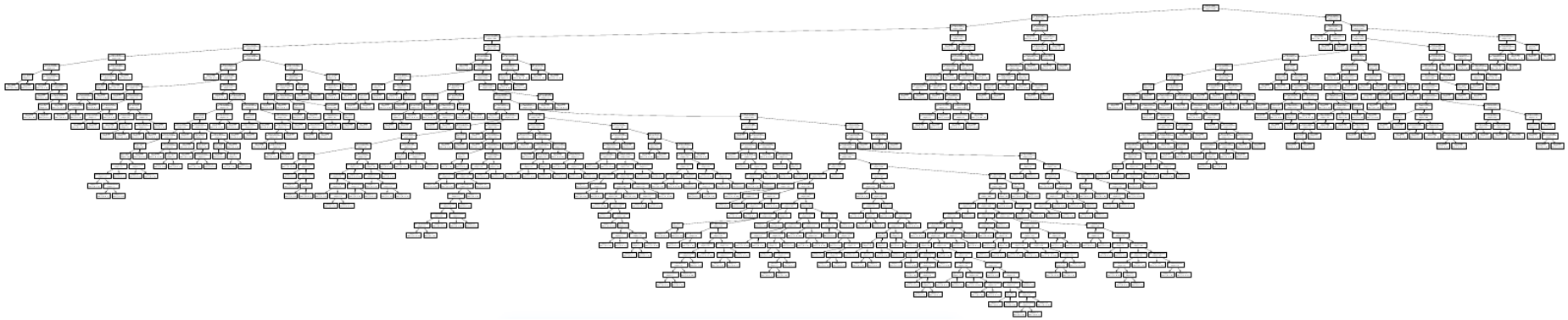

Figure : **TOP9 Set Decision Tree with ALL Data**

https://github.com/SamStudio8/frontier/tree/master/results/front/

- More complex structure and larger size than previous figures that include all parameters
- Further experimentation with the stopping criteria of the backward elimination process should be considered

TOP9 Decision Trees with Warning Augmentations

Figure : **TOP9 Set Decision Tree with IGNWARN Data**

Figure : **TOP9 Set Decision Tree with WARNPASS Data**

https://github.com/SamStudio8/frontier/tree/master/results/front/

TOP9 Example Decision Path

Figure : **TOP9 Set Decision Tree with IGNWARN Data**

- Paths still exhibit some element of arbitrariness
- Need to further investigate criteria for backward elimination
- Surprised that the set is much smaller than **AQC**
- Sensible to investigate some form of pruning to further remove smaller leaves

https://github.com/SamStudio8/frontier/tree/master/results/front/

Aims

Identify lanelet properties that affect downstream variant calling

- For better QC we need an idea of "good" and "bad"
- How does quality affect analyses performed after sequencing?

Goals

- Will leaving out a sample during variant calling affect the result?
- Select a "representative" region of the human genome for analysis
- Compare calls on whole genome samples to "SNP chips"
- Determine what is actually "good" and "bad" for QC

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Searching for Goldilocks

Introducing **Goldilocks**

- Need to locate an appropriate region for the pipeline to minimise computational time and resources
- Representative region – not too many or too few variants
- Need to handle variant data from two different types of study
- A Python module capable of parsing files containing chromosome-position pairs and conducting a variant census
- Filter and ranks censused regions of a genome based on the number of variants contained
- Presents an API to allow users to call any desired part of the module from other programs and scripts

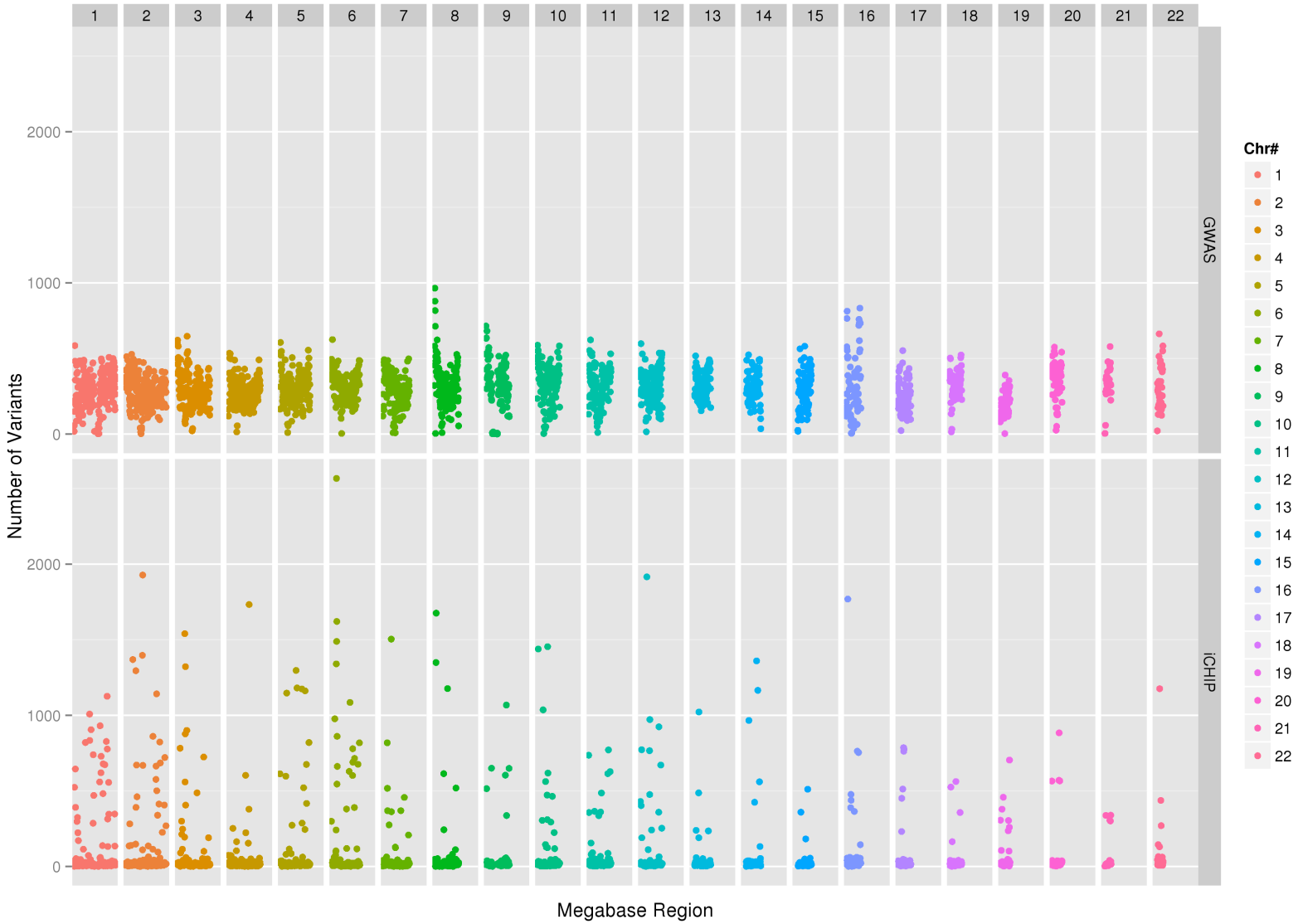
Top 25 1Mnt Goldilocks Regions

i	GWAS	iCHIP	Chr	Start	End
0234	297	470	1	117,000,001	118,000,000
1074*	294	1540	3	46,000,001	47,000,000
5222	294	336	21	16,500,001	17,500,000
3125	298	310	10	60,000,001	61,000,000
0880	293	344	2	191,500,001	192,500,000
3560	299	772	12	9,000,001	10,000,000
4407	299	512	15	78,500,001	79,500,000
1036	292	300	3	27,000,001	28,000,000
2734	300	515	9	5,000,001	6,00,0000
3426	300	486	11	76,000,001	77,000,000
0015	291	1029	1	7,500,001	8,500,000
0365	301	487	1	182,500,001	183,500,000
3415	301	419	11	70,500,001	71,500,000
1581	290	802	4	102,500,001	103,500,000
3554	290	403	12	6,000,001	7,000,000
3184	302	449	10	89,500,001	90,500,000
1580	289	603	4	102,000,001	103,000,000
1948	288	1297	5	96,000,001	97,000,000
2215	288	622	7	49,500,001	50,500,000
0414	288	346	1	207,000,001	208,000,000
2055	304	1377	5	149,500,001	150,500,000
0384	287	827	1	192,000,001	193,000,000
0959	306	406	2	231,000,001	232,000,000
4214	286	393	14	88,500,001	89,500,000
0320	307	620	1	160,000,001	161,000,000

- Filtered by median GWAS (297), ranked by maximum iCHIP
- Initially required over an hour to process the human genome, now completes the task in less than 20 seconds
- Avoided chromosome 6 due to presence of HLA system
- Suitable candidate 1074(*) located on chromosome 3

GWAS vs. iCHIP Variant Densities

GWAS vs. iCHIP Variant Densities



Pipeline Components

- **Extraction**
  Extract the Goldilocks region for all **GWAS** study lanelets
- **Indexing**
  Create indexes for the extracted Goldilocks regions
- **Merge**
  Merge the data from each extracted region into one file
- **Pileup**
  Calculate genotype likelihoods based on the reads seen across all the extracted regions
- **Call**
  Use the genotype likelihood scores to call the variants for each position of interest in each of the Goldilocks regions
- **Compare**
  For each pair of **GWAS** and **iCHIP** samples, measure the concordance of called variants

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Pipeline Difficulties: Pileup and Calling

Scaling **samtools mpileup**

- Executing a pileup is a performance intensive task
- Initial test runs required 6.5 hours of CPU time with 1GB RAM
- Significant overhead with thousands of sample files, couldn't have performed this step multiple times without merging

Compatibility with **bcftools call**

- Produced only standard header information and no data as the pileup task had not included an appropriate reference sequence
- Using `-M` flag for *masked reference* caused software to segmentation fault instead
- Needed to rebuild all libraries due to compatibility trouble

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Documentation for **samtools merge**

- Filed pull request to document feature allowing a file of filenames to be provided as an input instead of listing on command line

Memory Leaks in **samtools merge**

- Merge jobs repeatedly killed for excessive memory use by LSF
- Discovered several memory leaks whose severity increased proportionally to the number of input files – fixed by author
- During testing I tracked down and patched several memory leaks in both the **merge** and **split** testing harnesses using **valgrind**
- Merge jobs then repeatedly killed for exceeding time limits

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Pipeline Difficulties: Merging

Time Sinks in **samtools merge**

- Experimented with **callgrind** to search for heavily used functions or particularly costly calls
- Found many expensive calls to **zlib** – an open source compression library – when compressing the output
- Turned out to be proportionally insignificant, using uncompressed output still led to long execution times
- Tried **gprof** to look at actual execution time rather than CPU instruction count, found 50% of execution time was spent searching for tags in data structures
- Number of files causes non-linear increase in time, now currently believe implementation of header parsing is very inefficient and just cannot scale in current form

Conclusion

Critical Evaluation

- **Frontier** greatly assisted the analysis conducted in Part I
- Happy with choice of Python and **scikit-learn**, project benefits from mutual use of **NumPy** containers and functions
- Many interesting lines of questioning introduced in Part I results
- Demonstrated in brief that decision trees generated exhibit similar behaviour to the currently existing **auto_qc** system
- Encouraging progress in both Part I and II but ultimately cut short due to unexpected difficulties with the pipeline components

Conclusion

In summary this project...

- Introduced **Frontier**, a Python package providing users with interfaces for reading, storage and retrieval of large machine learning data sets
- Used **Frontier** and **scikit-learn** to conduct preliminary analysis as to whether behaviour of the current QC system could be recovered via machine learning
- Identified parameters (**TOP9**) that contribute to accurate classification and showed ignoring warnings reduces noise
- Created **Goldilocks** for locating an appropriate genomic region for use in Part II analysis
- Outlined a pipeline for processing thousands of samples
- Produced contributions to widely used bioinformatics tools

Conclusion

Future Plans

- Complete the assembly of the analysis pipeline (most likely requiring substantial additions to **samtools merge**)
- Use concordance results from Part II to inform new lines of inquiry where Part I left off
- Continue development of **Frontier** and submit to **PyPi**
- Explore use of other machine learning algorithms and frameworks and their application to the task of quality control classification
- Investigate post-pruning algorithms and ensemble methods as decision trees cannot promise optimality

Conclusion

Questions?