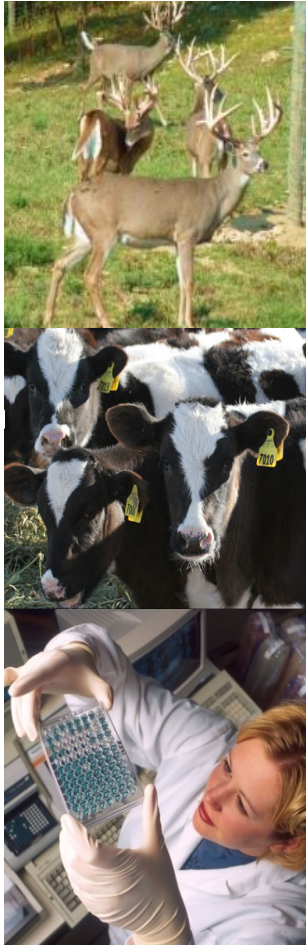




United States Department of Agriculture

Veterinary Services



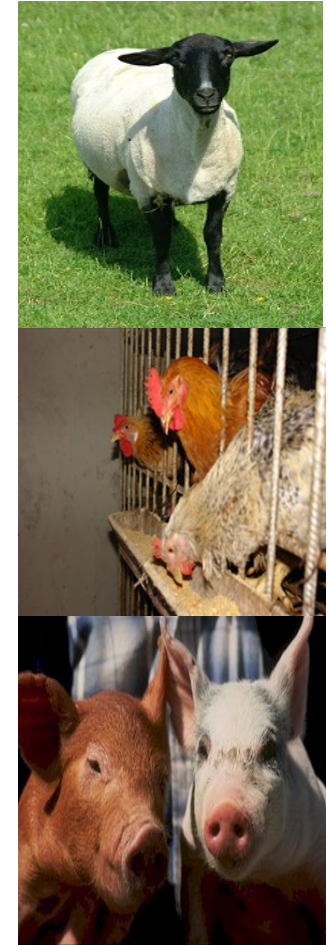
Mycobacterial Diseases of Livestock

Current techniques and advances in diagnostics for mycobacterial diseases (organism detection)

Suelee Robbe-Austerman, DVM, PhD
Mycobacteria Brucella Section Head
National Veterinary Services Laboratories

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services

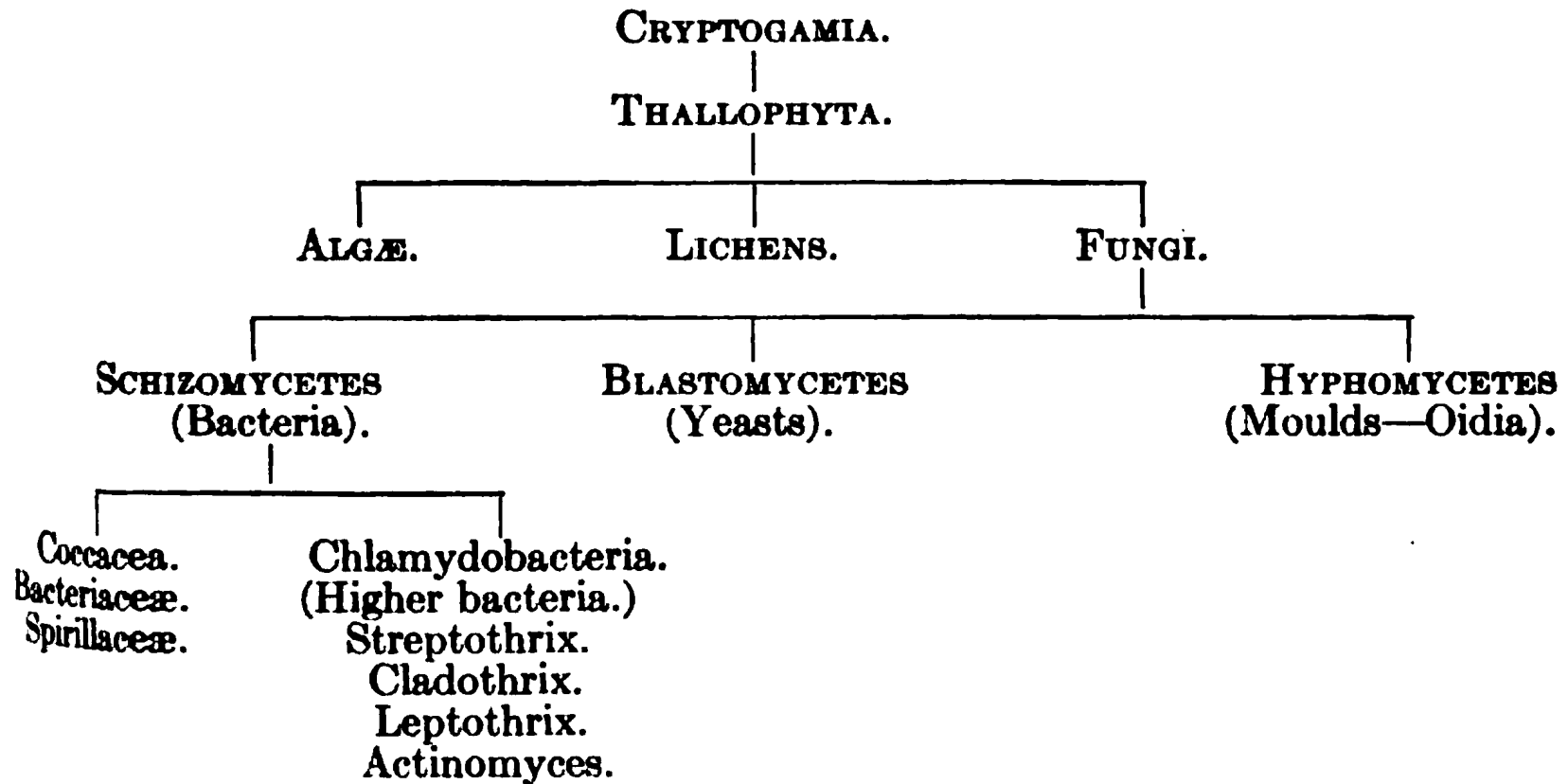
STAS
10/18/2014



Objectives- Mycobacterial organism detection assays

- Brief history
- Advances in mycobacterial culture techniques
- Advances in culture independent techniques for detection/identification
- Advances in genotyping – WGS
- Future directions

The turn of the century-1900





The impact of cracking the DNA code

“Today clinical laboratories have to deal with more than 25 other mycobacterial species.”

LB Heifets, 1989

Current list (10/06/2014) standing nomenclature

– 169 species, 13 subspecies...

Approx. 20 - 30% of mycobacteria isolated at NVSL still appear to be unnamed.

Bacterial culture of Mycobacteria

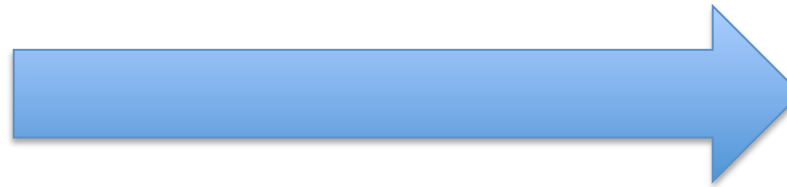
- Challenges
 - Slow growing
 - Fastidious, some require specialized media, temperatures or ingredients- pyruvate, mycobactin j, heme, etc...
 - Many are non-culturable: *M. leprae*, *mungi*, cutaneous lesions.
- Advances: Liquid culture systems

Culture independent – Direct detection

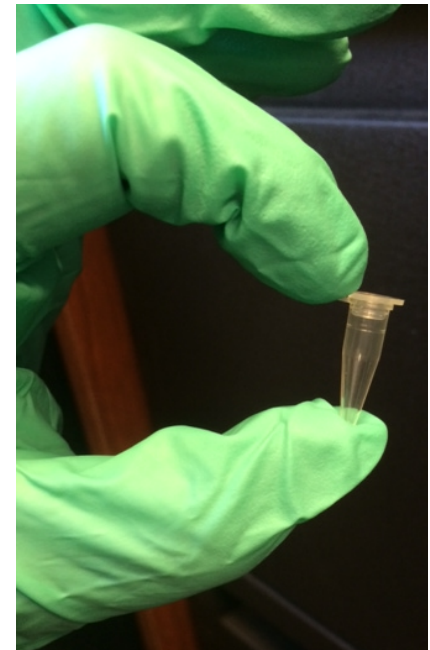
- Direct PCR



How to go from this



To this!





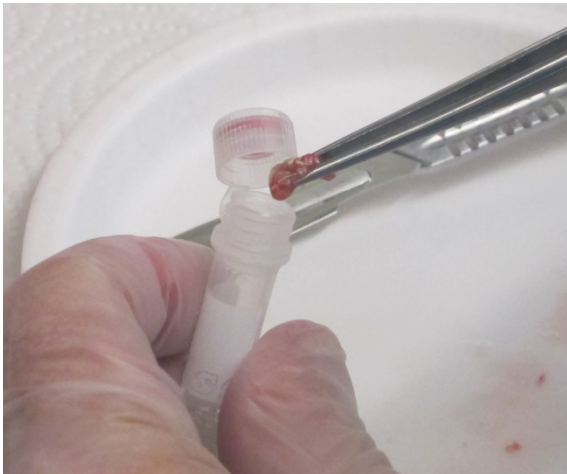
A new chapter in diagnostics

- PCR was developed ~1983, real time PCR in the 1990's.. Why is this a “new thing”?
 - Has been an adjunct test in the laboratory for a long time.
- Significant decreases in costs.
- Significant improvements in extraction protocols, especially difficult sample types.
- Significant improvements in chemistry.
- AAVLD currently developing guidelines

Single PCR workflow?

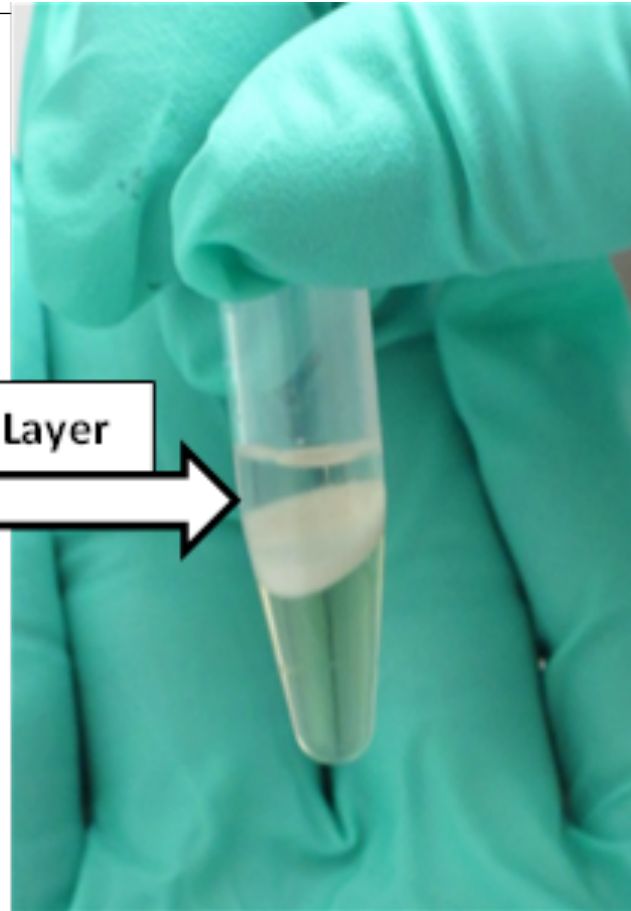

- Tissue extraction
- Fecal/environmental extraction
- Culture extraction

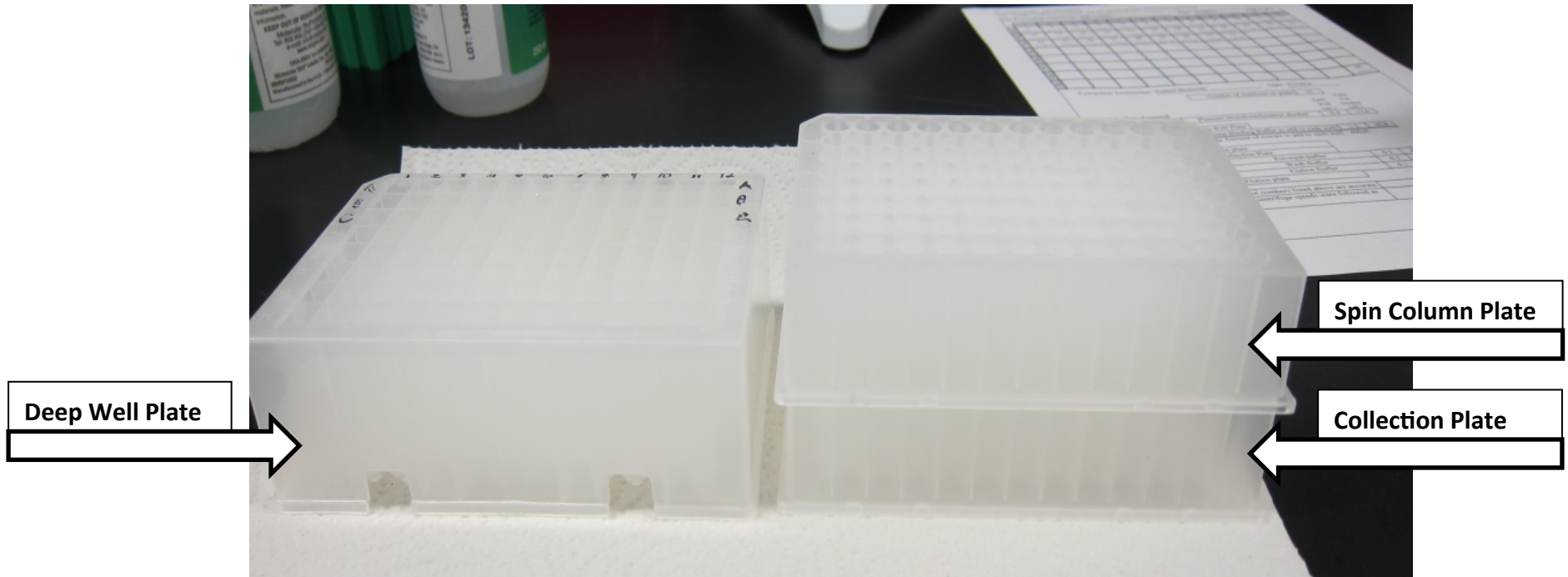
- Different primer/probe combinations that are optimized for the same Thermocycler settings

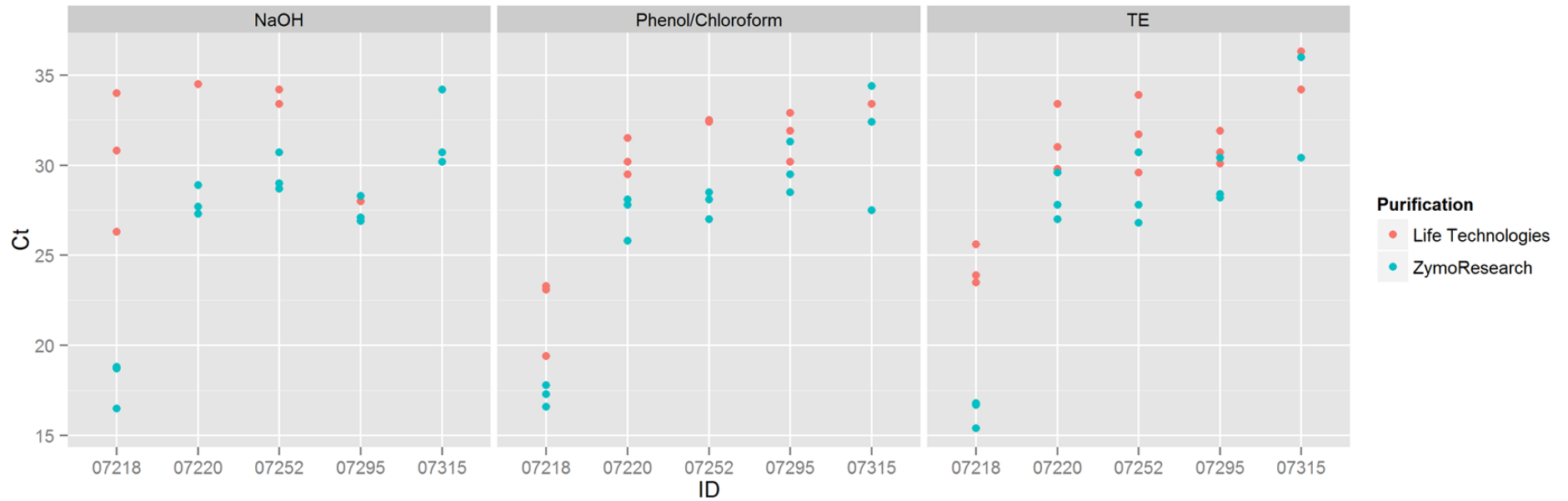




Aqueous Layer







a: LifeTechnologies, MagMAX™ Express-96 Deep Well Magnetic Particle Processor
 b: Zymo Research, customized format of ZR-96 Fecal DNA Kit

Building a PCR to manage error

- Tuberculosis – False positives and false negatives both result in major consequences
 - Confirm a positive with a different target
 - Use Mtb-avirulent strain as positive control every 5-10 wells
 - Confirm with an *M. bovis* specific primer/probe
 - Culture all positive (non-negative samples)



Analysis by: Suelee Robbe-Austerman

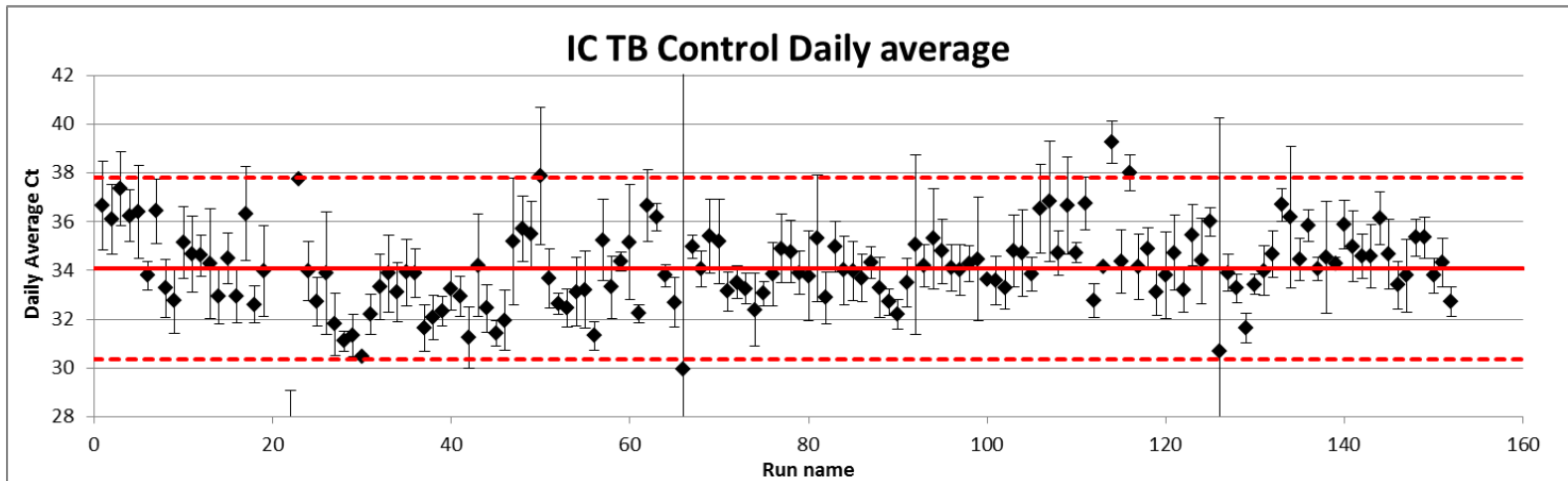
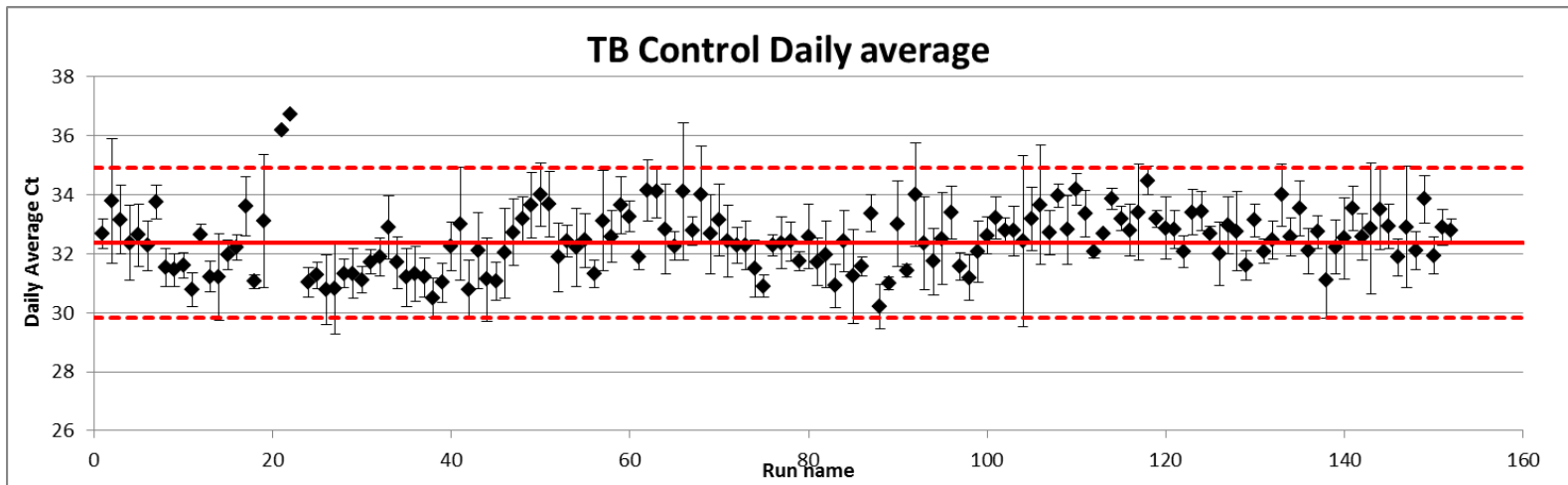
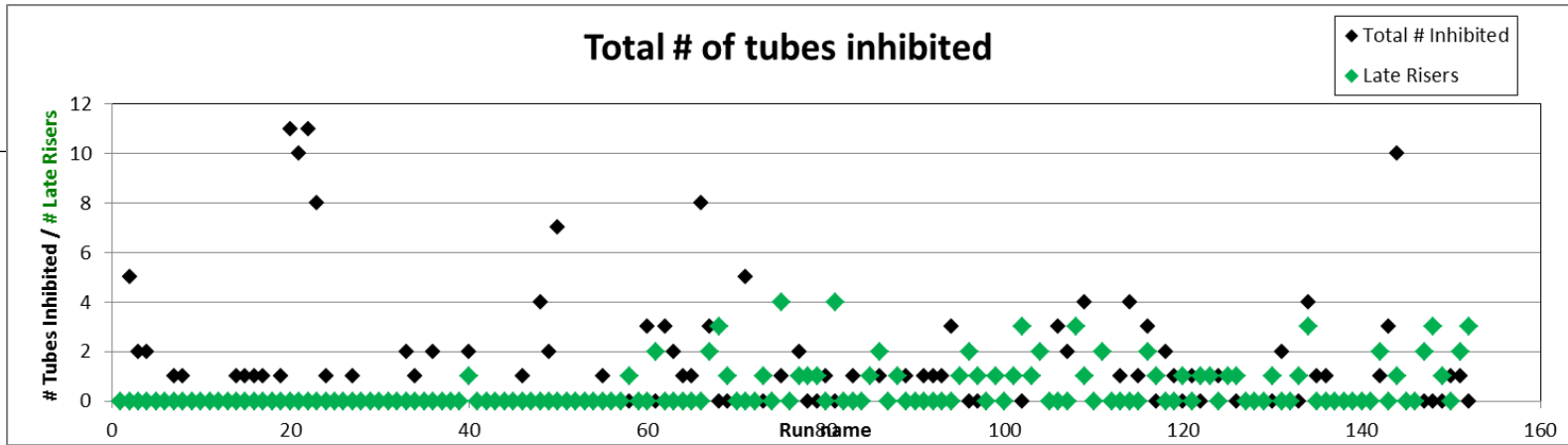
Date: 10/1/2014

File name: 141001_1_MTBC

Is Run Valid? **Yes**

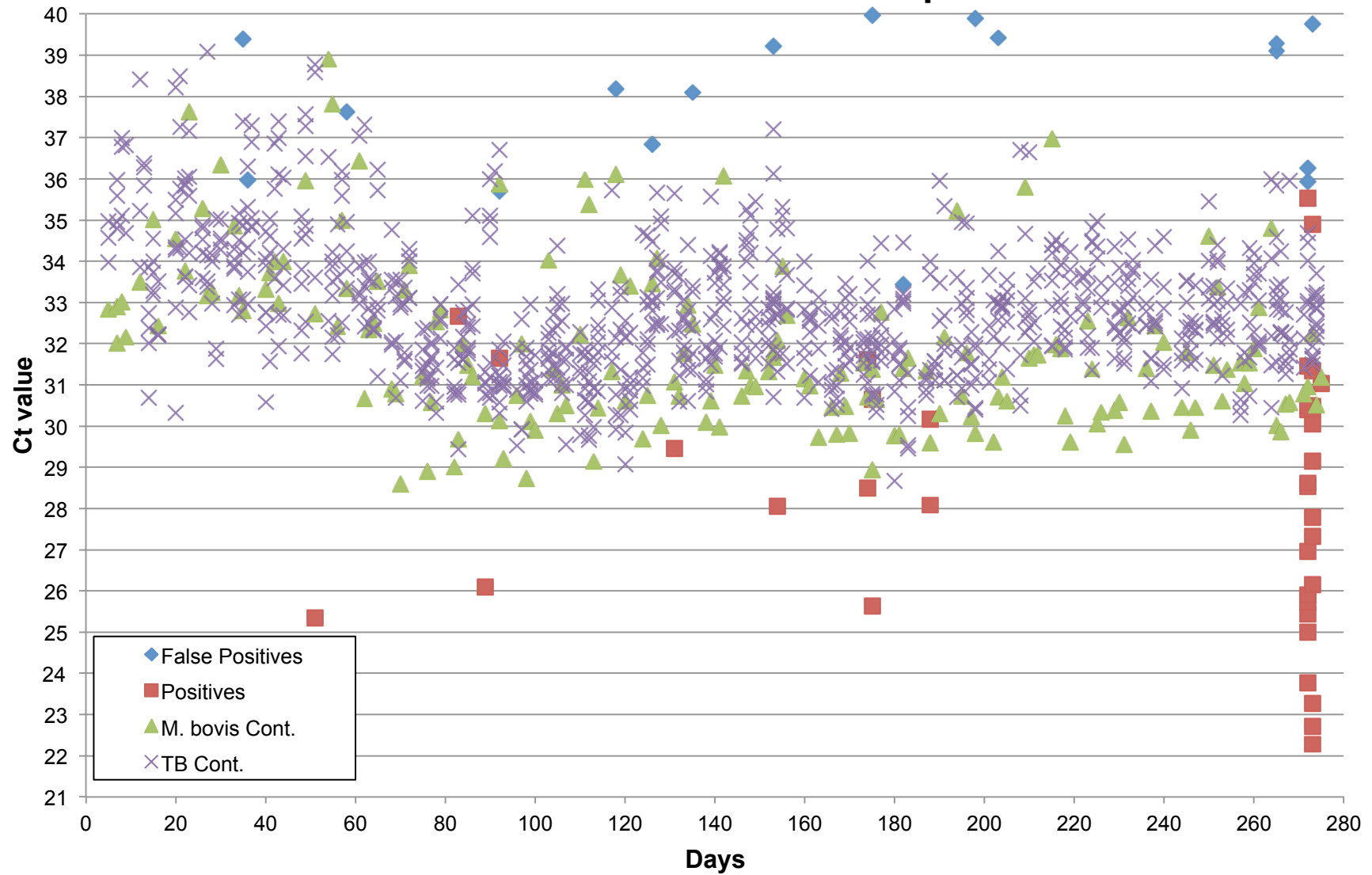
Page 6 of 7

Well	Accession #	Re-Test	Notes	Ct		Result	Redo (X)	L3 (X)
				IC	Bovis			
A1	Neg.Cont.			35.9	U	Negative		X
A2	Pos.Cont.			33.5	31.9	Non-Neg		X
A3	14-031386			35.4	39.8	Non-Neg	X	X
A4	14-031593		Two huge tissues with lesions	34.1	U	Negative		X
A5	14-031594		Tissues with many gritty lesions	34.3	U	Negative		X
A6	14-031678		Tissue with only one tiny lesion noted	35.8	U	Negative		X
A7	14-031757			34.7	U	Negative		X
A8	Neg.Cont.			39.0	U	Inhibited		X
A9	Pos.Cont.			34.4	31.6	Non-Neg		X
A10	14-031758		Two tissues	36.2	34.9	Non-Neg		X
A11	14-031759		Huge tissue	35.5	30.5	Non-Neg		X
A12	14-031761		Huge tissue	35.5	U	Negative		X
B1	14-031762		Huge tissue	33.6	U	Negative		X
B2	14-031763			35.0	U	Negative		X
B3	Neg.Cont.			32.7	U	Negative		X
B4	Pos.Cont.			33.9	31.5	Non-Neg		X
B5	14-031764			36.2	U	Negative		X
B6	14-031765			35.1	U	Negative		X
B7	14-031766			35.0	U	Negative		X
B8	14-031769		Tissue with suspicious lesions	37.5	27.8	Non-Neg		X
B9	14-031771			35.3	U	Negative		X
B10	Neg.Cont.			35.8	U	Negative		X
B11	Pos.Cont.			34.7	31.6	Non-Neg		X
B12	14-031772			34.3	U	Negative		X
C1	14-031773			34.5	31.3	Non-Neg		X
C2	14-031775			35.1	U	Negative		X
C3	14-031784		Five tiny tissues, NGL	34.0	U	Negative		X
C4	14-031785		lesion	33.1	U	Negative		X
C5	Neg.Cont.			33.4	U	Negative		X
C6	Pos.Cont.			33.6	33.2	Non-Neg		X
C7	14-031786		protuding lesions LN color	33.1	U	Negative		X
C8	14-031790		enlarged LN small pinpoint lesions	35.4	26.2	Non-Neg		X
C9	14-031791			34.8	U	Negative		X
C10	14-031792		pinpoint lesions	33.3	30.0	Non-Neg		X
C11	14-031794		small lesions all over LN	8.8	22.7	Non-Neg		X
C12	14-031795			36.5	29.2	Non-Neg		X
D1	14-031796		one lesion	33.6	U	Negative		X
D2	14-031797		lesion with exudate	35.0	U	Negative		X
D3	14-031798		large lesion (looks like corn beard)	32.5	U	Negative		X
D4	14-031800		small tissue with lesion	34.3	U	Negative		X





Scatter Plot of Positive Samples





NVSL MTBC Performance

Validation

	Histology/ Culture +	Histology/ Culture -	Total
Direct PCR +	57	6	63
Direct PCR -	2	2907	2909
Total	59	2913	2972

Sensitivity = **96.61 %**

Specificity = **99.79 %**

6 mo. in practice

	Histology/ Culture +	Histology/ Culture -	Total
Direct PCR +	35	17	52
Direct PCR -	0	4673	4673
Total	35	4690	4725

Sensitivity = **100.00 %**

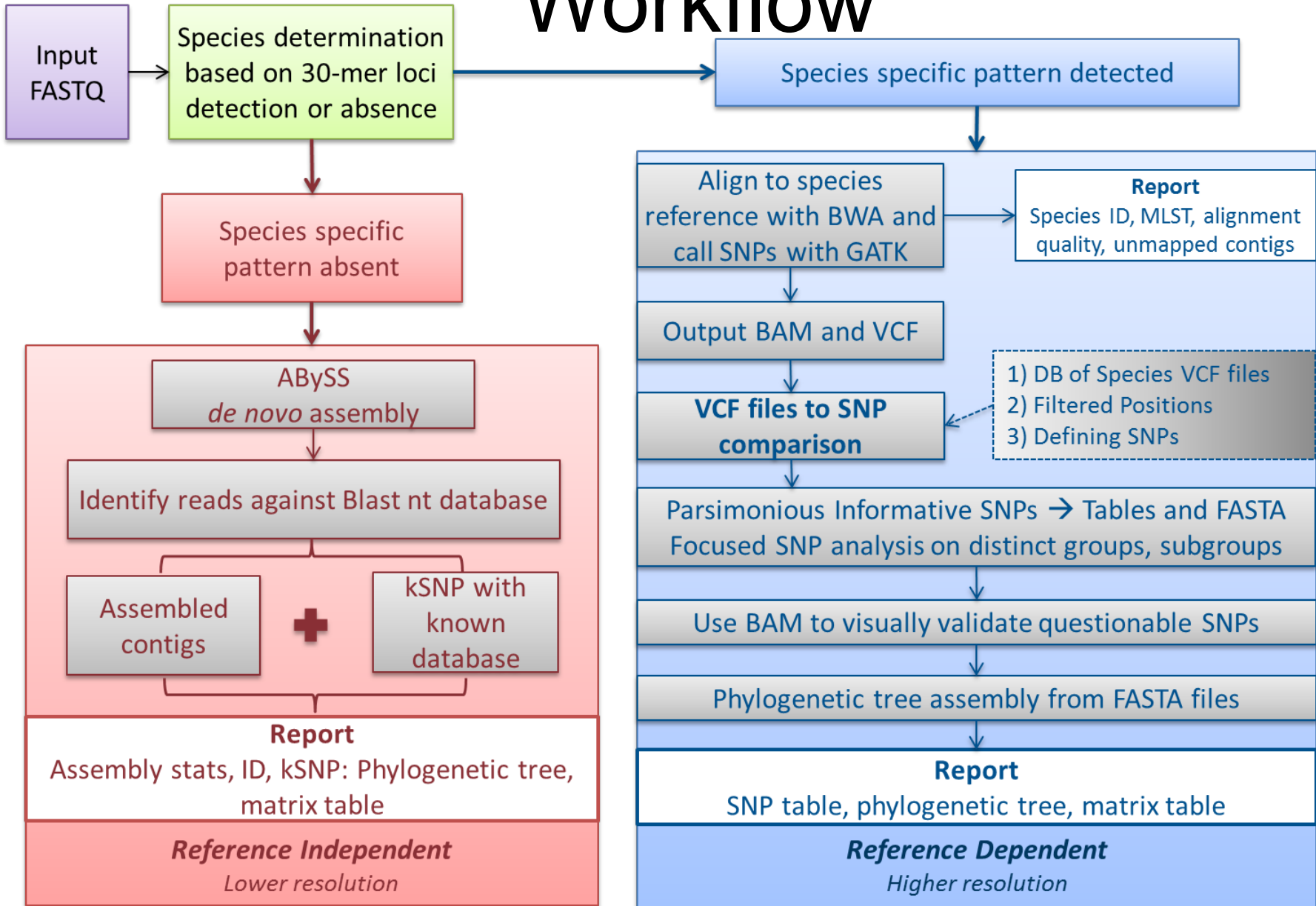
Specificity = **98.89 %**

Advances in genotyping

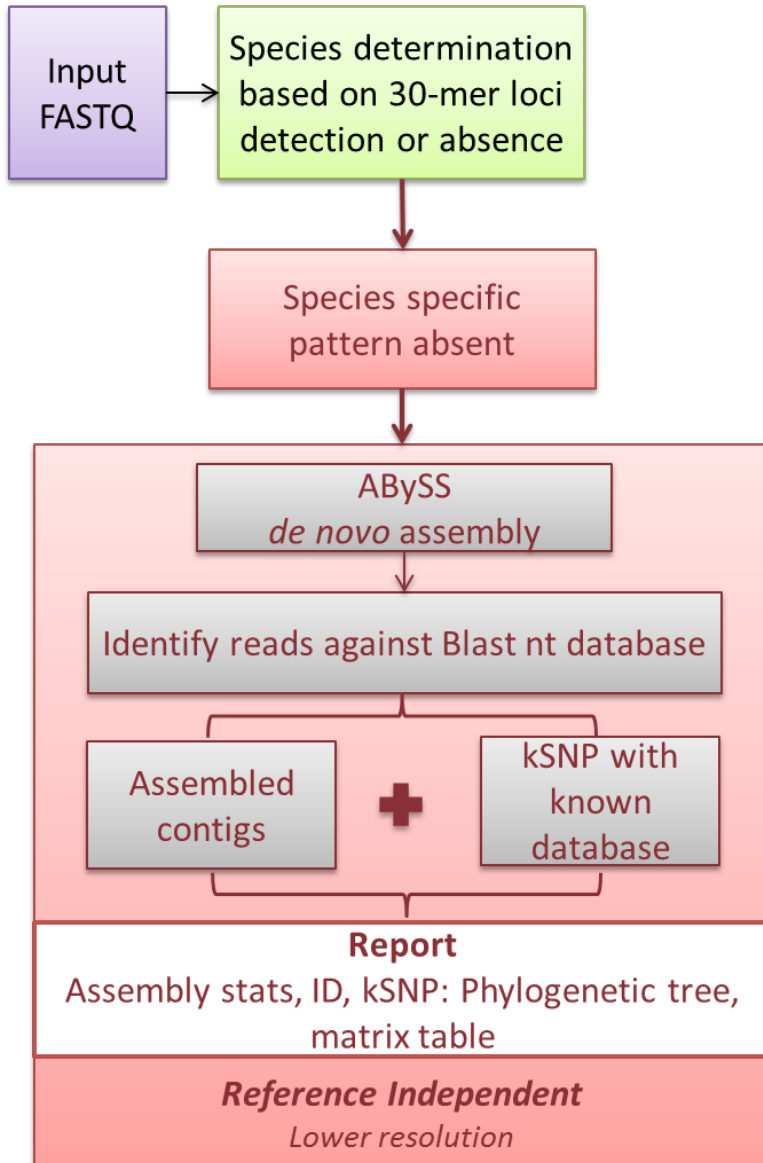
- Whole genome sequencing



Workflow



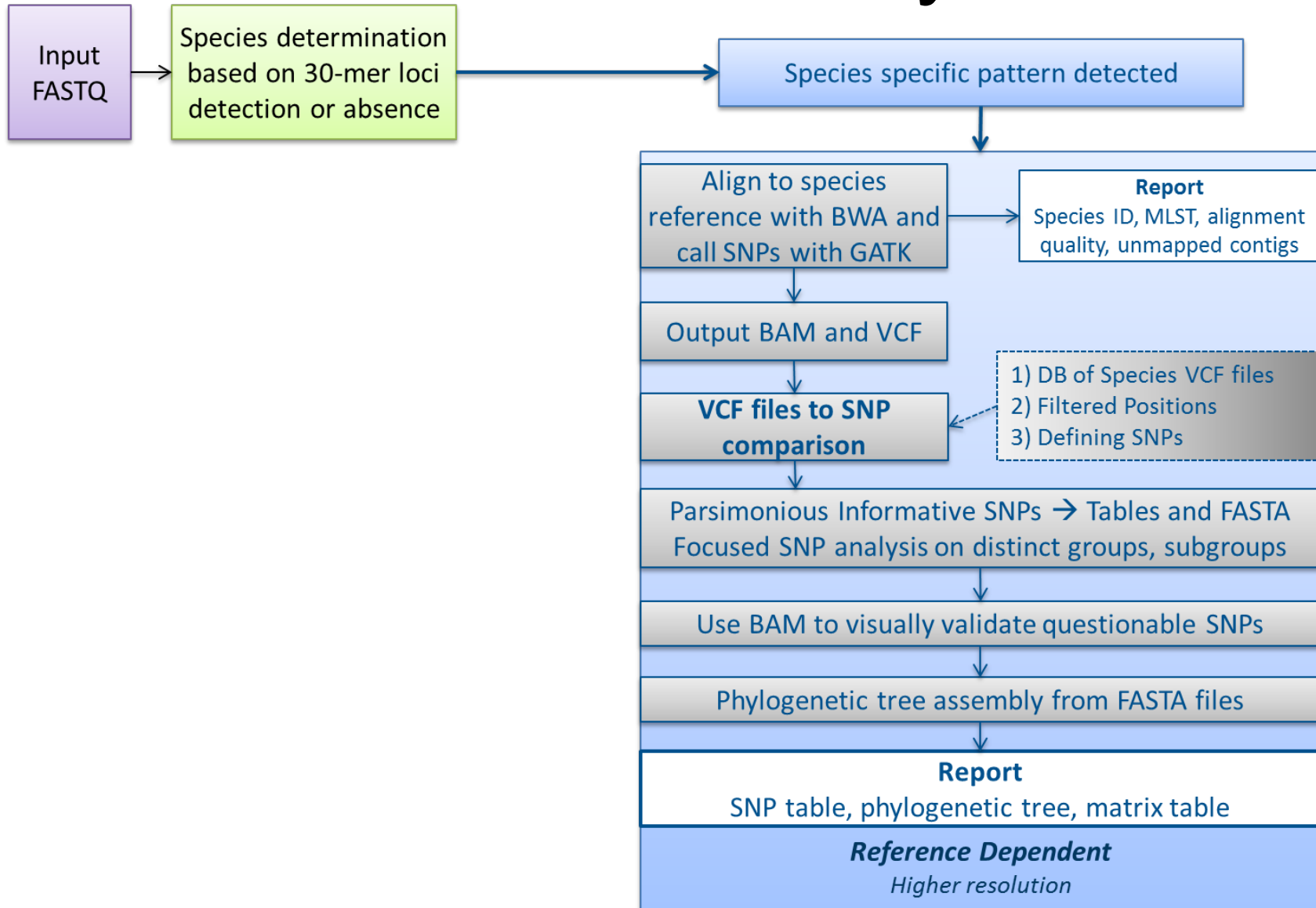
Reference Independent



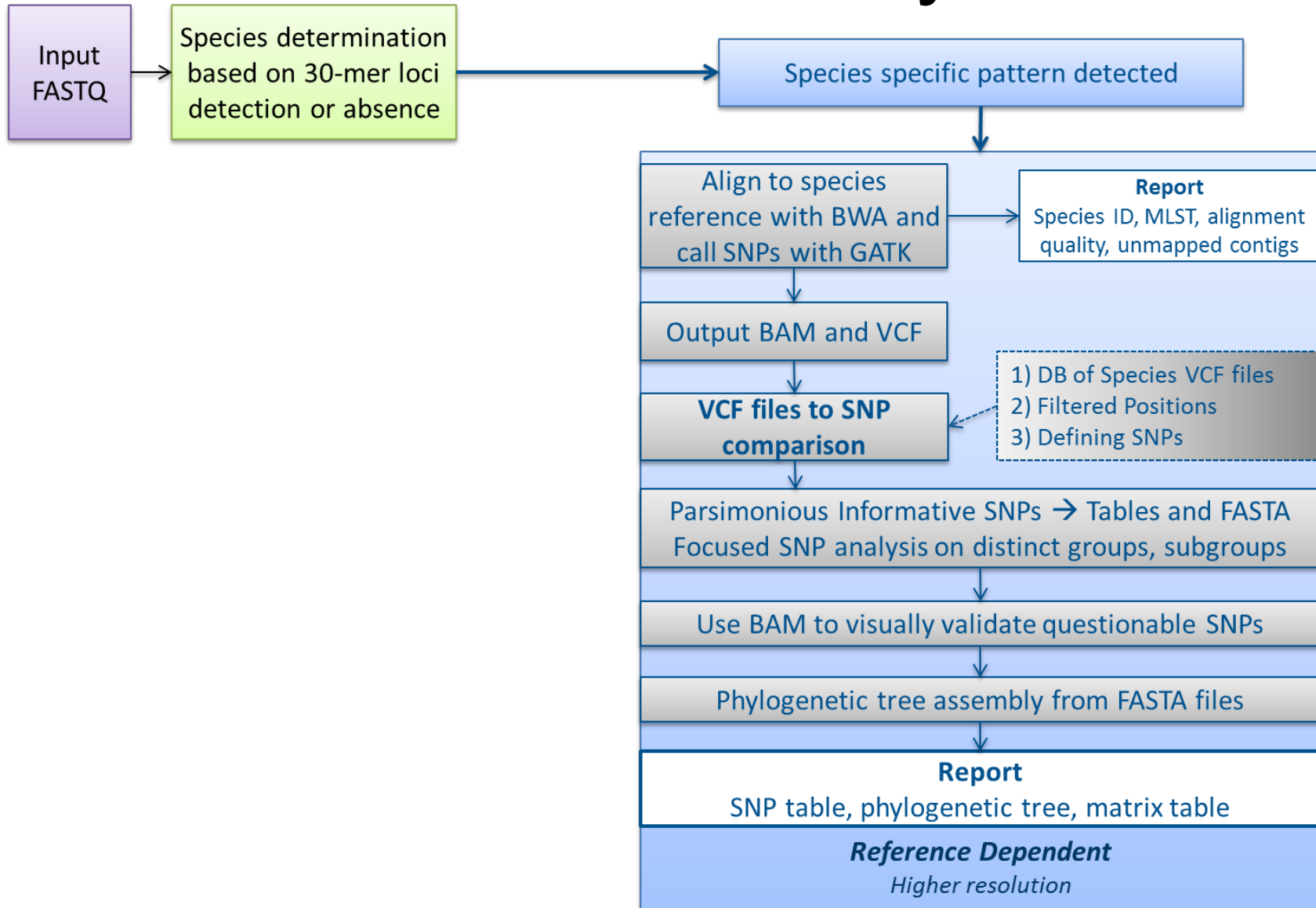
Outputs from kSNP

- Annotation_summary
- ClusterInfo.core
- ClusterInfo.majority0.5
- ClusterInfo.ML
- ClusterInfo.parsimony
- core_SNPs
- core_SNPs_matrix
- core_SNPs_matrix.fasta
- COUNT_coreSNPs
- COUNT_Homoplastic_SNPs.core
- COUNT_Homoplastic_SNPs.majority0.5
- COUNT_Homoplastic_SNPs.ML
- COUNT_Homoplastic_SNPs.parsimony
- COUNT_SNPs
- fastainput
- finished_genomes
- genbank_from_NCBI.gbk
- Homoplasy_groups.core
- Homoplasy_groups.majority0.5
- Homoplasy_groups.ML
- Homoplasy_groups.parsimony
- Node_SNP_counts.core
- Node_SNP_counts.majority0.5
- Node_SNP_counts.ML
- Node_SNP_counts.parsimony
- nonCore_SNPs
- Protein_Annotation_counts
- SNP_annotations
- SNPs_all
- SNPs_all_annotated
- SNPs_all_matrix
- SNPs_all_matrix.fasta
- SNPs_in_majority0.5
- SNPs_in_majority0.5_matrix
- SNPs_in_majority0.5_matrix.fasta
- tip_SNP_counts.core
- tip_SNP_counts.majority0.5
- tip_SNP_counts.ML
- tip_SNP_counts.parsimony
- tree.core.tre
- tree.majority0.5.tre
- tree.ML.tre
- tree.parsimony.tre
- tree_AlleleCounts.core.NodeLabel.tre
- tree_AlleleCounts.core.tre
- tree_AlleleCounts.majority0.5.NodeLabel.tre
- tree_AlleleCounts.majority0.5.tre
- tree_AlleleCounts.ML.NodeLabel.tre
- tree_AlleleCounts.ML.tre
- tree_AlleleCounts.parsimony.NodeLabel.tre
- tree_AlleleCounts.parsimony.tre
- tree_nodeLabel.core.tre
- tree_nodeLabel.majority0.5.tre
- tree_nodeLabel.ML.tre
- tree_nodeLabel.parsimony.tre
- tree_tipAlleleCounts.core.tre
- tree_tipAlleleCounts.majority0.5.tre
- tree_tipAlleleCounts.ML.tre
- tree_tipAlleleCounts.parsimony.tre
- unassembled_genomes

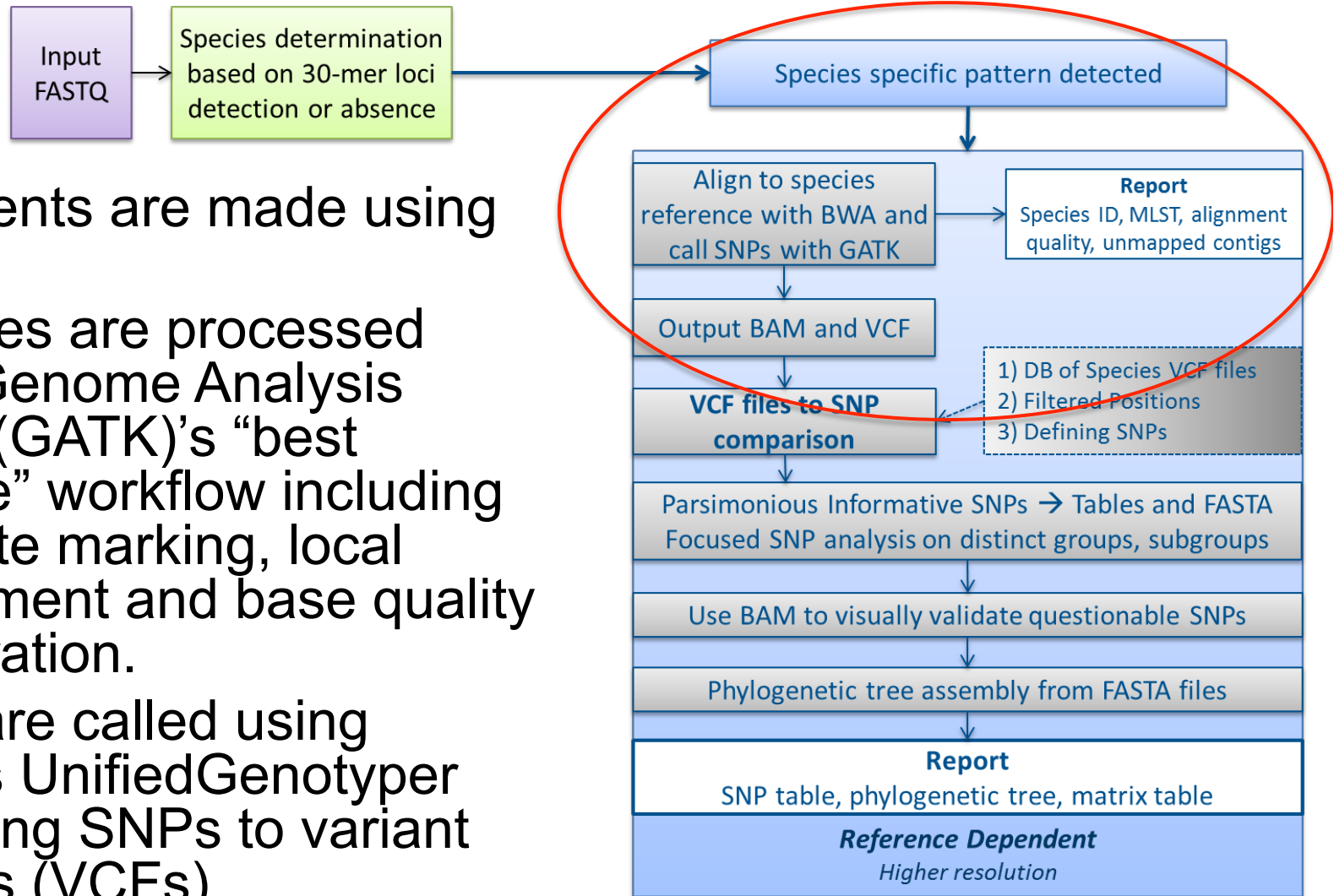
Reference Analysis



Reference Analysis



Alignment and VCF



- Alignments are made using BWA
- BAM files are processed using Genome Analysis Toolkit (GATK)'s "best practice" workflow including duplicate marking, local realignment and base quality recalibration.
- SNPs are called using GATK's UnifiedGenotyper outputting SNPs to variant call files (VCFs).



Script 1 output contains 3 sections

From: Tod Stuber [mailto:tod.p.stuber@usda.gov]
 Sent: Saturday, October 04, 2014 12:08 PM
 To: Robbe Austerman, Suelee - APHIS
 Subject: WGS results

Start Time: Sat Oct 4 10:43:04 CDT 2014 End Time: Sat Oct 4 12:08:08 CDT 2014 Run time: 5104 seconds

(85 min for 24 isolates)

Isolate	Total_Bases	AveDep	%>Q15
Sample identified and ran as: suis1			
B14-0518	293296306	88.47	99.9
Sample identified and ran as: bovis			
05-6593	323525919	74.45	98.0
Sample identified and ran as: suis1			
B14-0539	396478324	119.60	100.0
Sample identified and ran as: bovis			
11-2687	375415537	86.39	97.6
Sample identified and ran as: bovis			
03-2617	392443746	90.31	98.2
Sample identified and ran as: ab1			
B14-0124	477776473	145.38	100.0
Sample identified and ran as: bovis			
07-7140	477702076	109.93	98.4
Sample identified and ran as: bovis			
03-3568	522640682	120.27	98.5
Sample identified and ran as: ab1			
B14-0121	553461068	168.41	100.0



WG Spoligo Check

<---- 05-6593 ---->
 WGSpoligo: 676773677777600
 <---- Ravenal ---->
 WGSpoligo: 656573777777600
 <---- 11-2687 ---->
 WGSpoligo: 776377777760771
 <---- 03-2617 ---->
 WGSpoligo: 676713677777600
 <---- 97-25MIDNRdeerAlc ---->
 WGSpoligo: 640013777777600
 <---- 03-2994 ---->
 WGSpoligo: 676713677777600
 <---- 03-2995 ---->
 WGSpoligo: 664073777777600
 <---- 07-7140 ---->
 WGSpoligo: 640013777777600
 <---- 03-3568 ---->
 WGSpoligo: 676573777077600

Brucella MLST Check

B14-0518 --> MLST type 14
 B14-0539 --> MLST type 14
 B14-0517 --> MLST type 14
 B14-0124 --> MLST type 01
 B14-0121 --> MLST type 01



<----- 03-3568 bovis ----->

Total reads: 2412163
Mapped reads: 2412163 (100%)
Forward strand: 1206338 (50.0106%)
Reverse strand: 1205825 (49.9894%)
Paired-end reads: 2412163 (100%)
Both pairs mapped: 2407810 (99.8195%)
Read 1: 1208294
Read 2: 1203869
Singletons: 4353 (0.18046%)

fastq.gz file sizes:

229M

249M

Unmapped fastq file sizes:

455K

457K

Unmapped contig count:

1

Average coverage: 120.594X

Reference with coverage: 99.5163%

Mean_Insert_Size Standard_Deviation:

319.011586 157.696348

Mean_Read_Length:

227.078289

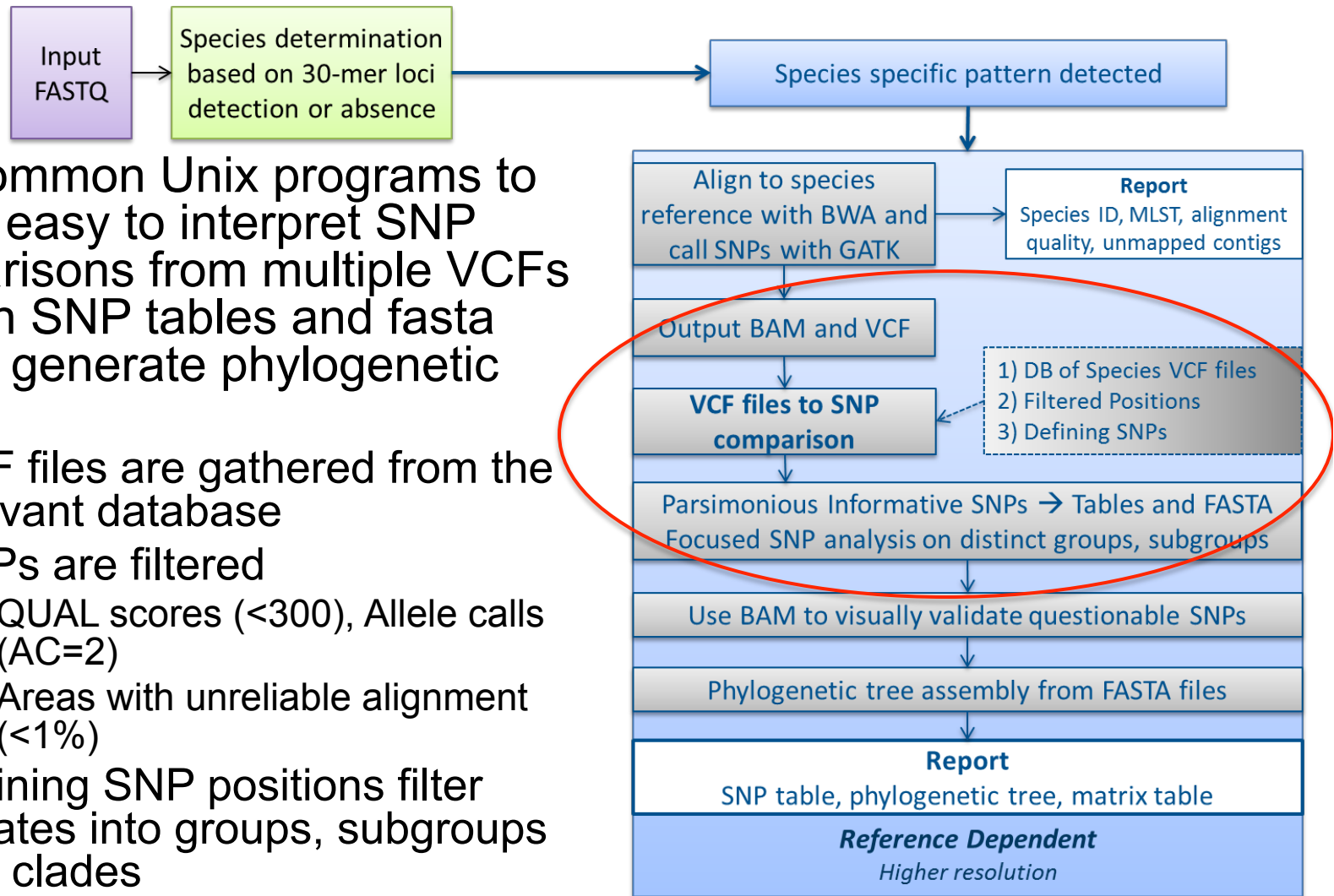
Number of SNPs and Map-zero in ready-mem.vcf:

28225

SNPs of AC2 and QUAL > 150:

547

VCF to FASTA



- Use common Unix programs to output easy to interpret SNP comparisons from multiple VCFs as both SNP tables and fasta files to generate phylogenetic trees.
 - VCF files are gathered from the relevant database
 - SNPs are filtered
 - QUAL scores (<300), Allele calls (AC=2)
 - Areas with unreliable alignment (<1%)
 - Defining SNP positions filter isolates into groups, subgroups and clades



Start Time: Sat Oct 4 21:39:09 CDT 2014

End Time: Sat Oct 4 23:14:38 CDT 2014

Run time: 5729 seconds

Script 2 output contains 3 sections

Script vcftofasta.sh ran using M. bovis variables

(95 min for 1200 VFC files)

These files did not get renamed:

03-2614	97-27MIDNRdeerAlp	99-35MIDNRdeerMontm
03-2617	97-29MIDNRdeerAlc	99-56MIDNRdeerAlc
03-2994	97-30MIDNRdeerPI	AF2122-1
03-2995	97-39MIDNRdeerAlc	AF2122-2
07-01820	97-40MIDNRdeerAlc	AF-61-03335-12
09-4486-2	97-41MIDNRdeerMontm	AFH-16-01309-13
10-05288	98-14MIDNRdeerAlc	Human-Bovis-07
14-2506	98-16MIDNRdeerAlc	ourBCG
16-2185-11	98-18MIDNRdeerAlp-1	Ravenal
61-1967-01	98-45MIDNRdeerOsco	TB1089
94-04770	98-53MIDNRdeerAlc	TB1099
97-23MIDNRdeerAlc	98-71MIDNRdeerAlc	TB1159
97-25MIDNRdeerAlc	99-20MIDNRdeerAlp	TB1185
97-26MIDNRdeerMontm	99-22MIDNRdeerAlc-2	

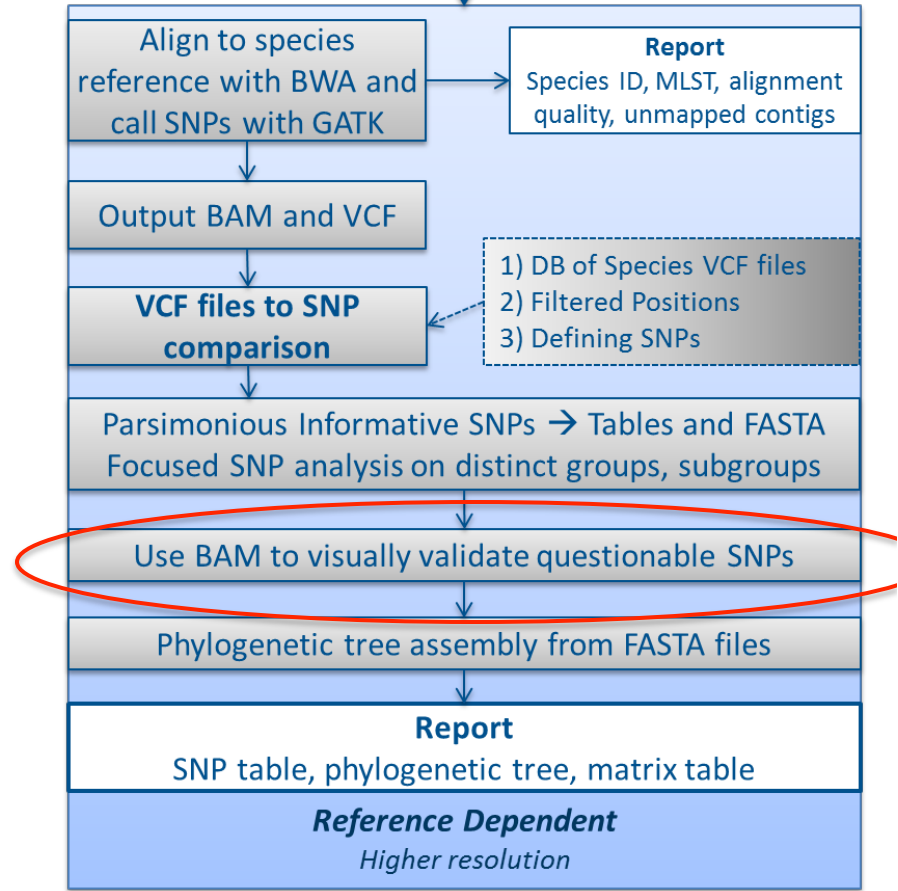
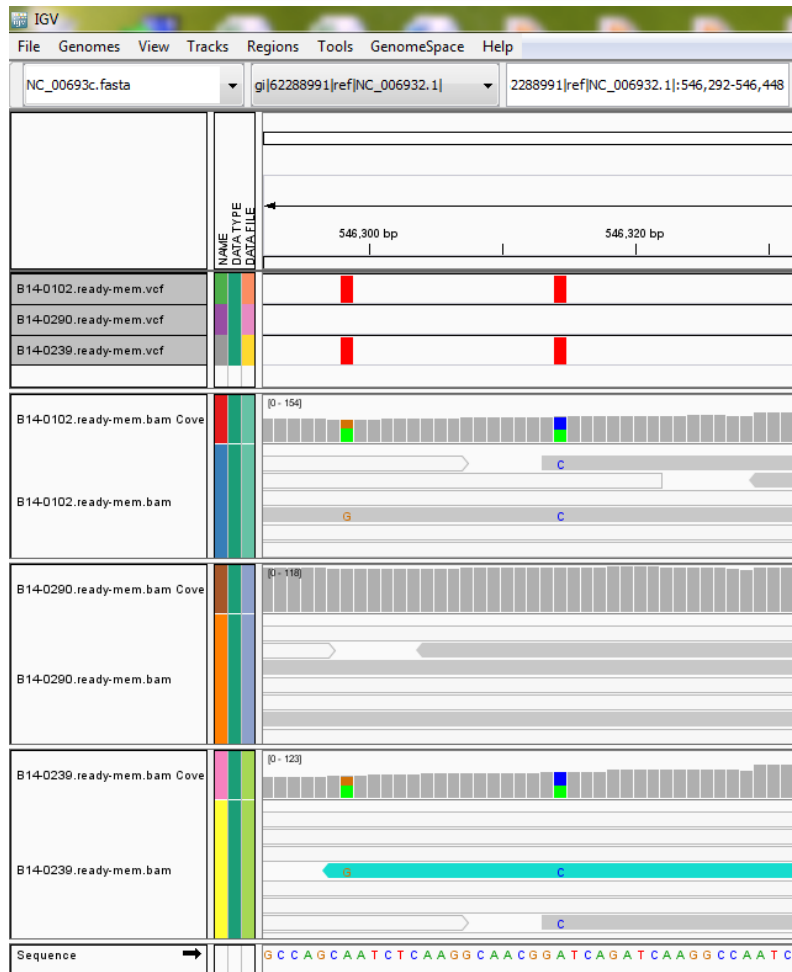
Possible Mixed Isolates, Defining SNPs called AC=1

01-5639_MEX_TX_Fed.vcf	Pos: 4246065	QUAL: 710.79	AC=1	AF=0.500
07-5172_MI_Wildlife_Opossum_37.vcf	Pos: 1458346	QUAL: 2227.77	AC=1	AF=0.500
07-5172_MI_Wildlife_Opossum_37.vcf	Pos: 2406621	QUAL: 2688.77	AC=1	AF=0.500
07-5173_MI_Wildlife_Opossum_37.vcf	Pos: 1458346	QUAL: 2038.77	AC=1	AF=0.500



NAME	GROUP	SUBGROUP	CLADE
00-0121_WI_Cervid_99-A-Val	14	14B	
00-2684_MI_Pres_Dairy_5-Val	1	1A	1A-4
00-3221_MI_Wildlife_Bobcat-Val	1	1C	
00-3692_DUN_KS_Fed	24	16A	
00-3942_MI_Alco_Beef_7-Val	1	1D	
00-4177_DUS_TX_Fed	6	6A	
00-5559_MI_Alco_Beef_9-Val	1	1D	
00-6095_MI_Alco_Beef_11-Val	1	1D	
01-0050_MEX_TX_Fed	15		
01-0423_UNK_NE_Event_Roping	16	16B	16B-2
01-0467_CHI_TX_Fed	16	16B	
01-0843_MEX_TX_Fed	24	16A	
01-0865_MEX_KS_Fed	23	16C	16C-4
01-1360_NL_TX_Fed	2		
01-1473_TAM_TX_Fed-Val	23	16C	16C-1
01-1787_FL_Zoo_Jaguar	17		
01-1890_SD_Untraceable_Beef-Val	14		
01-1920_NL_TX_Fed-Val	24	16A	
01-1951_PA_Lab_01-A	21		
01-1952_PA_Lab_01-A	21		
01-1954_PA_Lab_01-A	21		
01-2011.vcf	Grp	not	found

SNP Verification



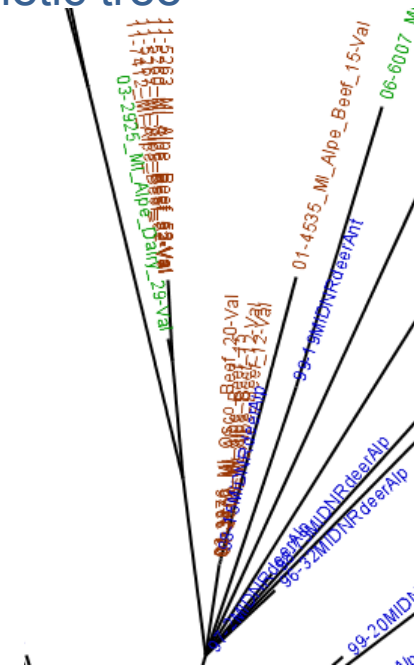


Name	Date modified	Type	Size
fasta	10/4/2014 11:17 PM	File folder	
marked_files	10/4/2014 11:17 PM	File folder	
starting_files	10/4/2014 11:17 PM	File folder	
clean_total_pos	10/4/2014 11:17 PM	File	10 KB
Group-1.organizedTable	10/4/2014 11:17 PM	TXT File	346 KB
Group-1.sortedTable	10/4/2014 11:17 PM	TXT File	346 KB
Group-1.table	10/4/2014 11:17 PM	TXT File	346 KB
Group-1_alignment	10/4/2014 11:17 PM	FASTA File	173 KB

Into Excel template for SNP table

	A	CN	CO	CP	CQ	CR	CS	CT
1	reference_pos	chrom1-2824728	chrom1-1126954	chrom1-3005112	chrom1-3816582	chrom1-1258903	chrom1-2913998	chrom1-862227
2	reference_call	C	C	G	A	G	C	A
85	97-2MIDNRdeerAlp	T	T	G	A	G	C	A
86	96-32MIDNRdeerAlp	T	T	T	A	G	C	A
87	98-15MIDNRdeerAlp	T	T	G	C	G	C	A
88	01-2508_MI_Alpe_Beef_12-Val	T	T	G	C	G	C	A
89	01-2509_MI_Alpe_Beef_12-Val	T	T	G	C	G	C	A
90	02-3376_MI_Osco_Beef_20-Val	T	T	G	C	G	C	A
91	01-3872_MI_Alpe_Beef_15-Val	T	T	G	C	G	C	A
92	01-4535_MI_Alpe_Beef_15-Val	T	T	G	C	A	A	G

Into Alignment program for Phylogenetic tree



Future direction

- Sequence the Mycobacteria directly from sample.
 - Host removal techniques need improvement
- Taxonomy naming conventions for strains
- Database curation and validation of isolates and sequences



Total reads: 11712
Mapped reads: 11712 (100%)
Forward strand: 5851 (49.9573%)
Reverse strand: 5861 (50.0427%)
Paired-end reads: 11712 (100%)
Both pairs mapped: 11631 (99.3084%)
Read 1: 5903
Read 2: 5809
Singletons: 81
(0.691598%)

fastq file sizes:

3.1M

3.1M

Unmapped fastq file sizes:

4.8K

4.9K

Unmapped contig count: 8

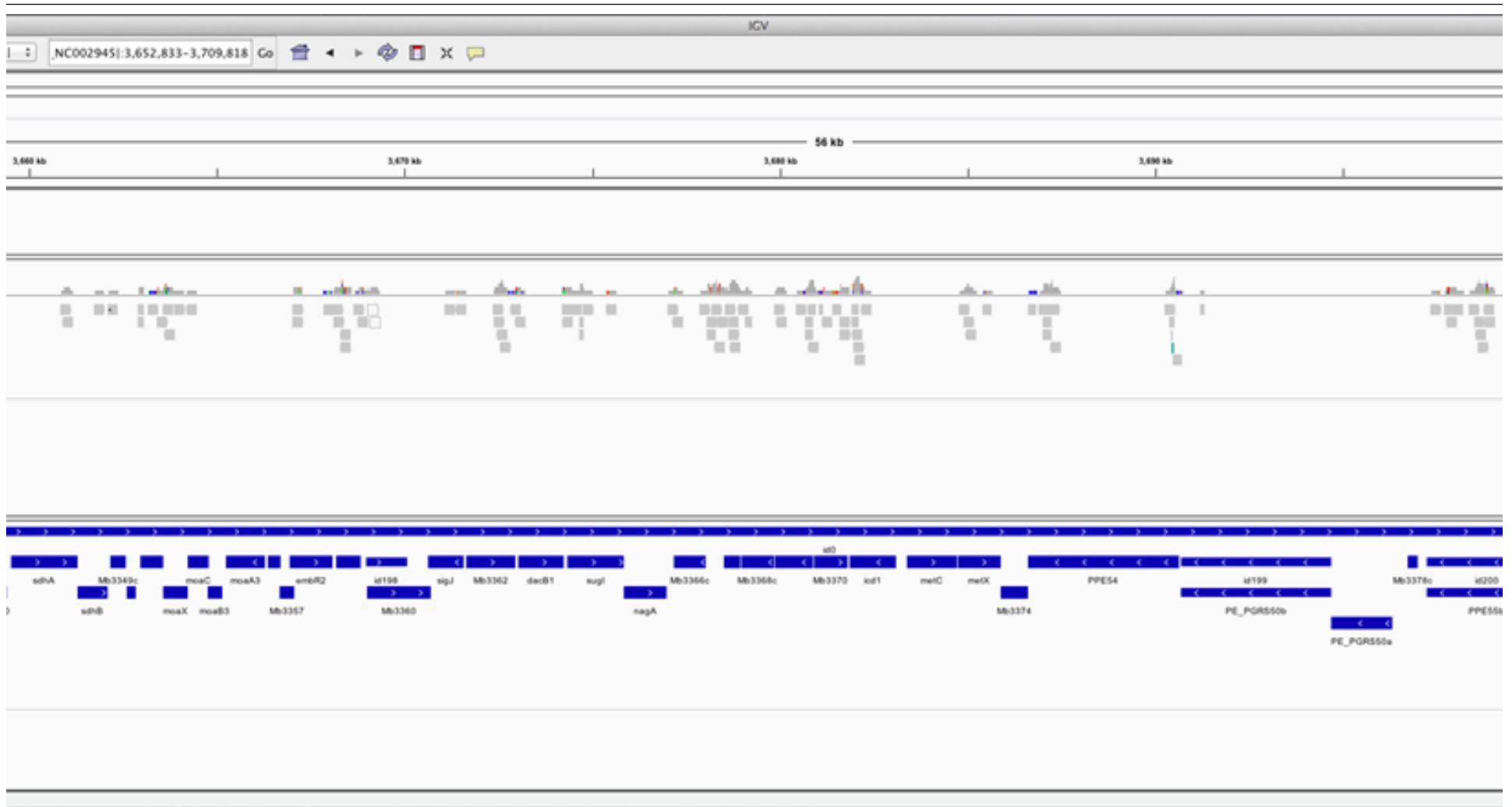
Average coverage: 0.62975X

Reference with coverage: 42.4536%

Quality metrics
Sequencing directly
from a granuloma with
no host removal
1 MiSeq 500 cycle chip

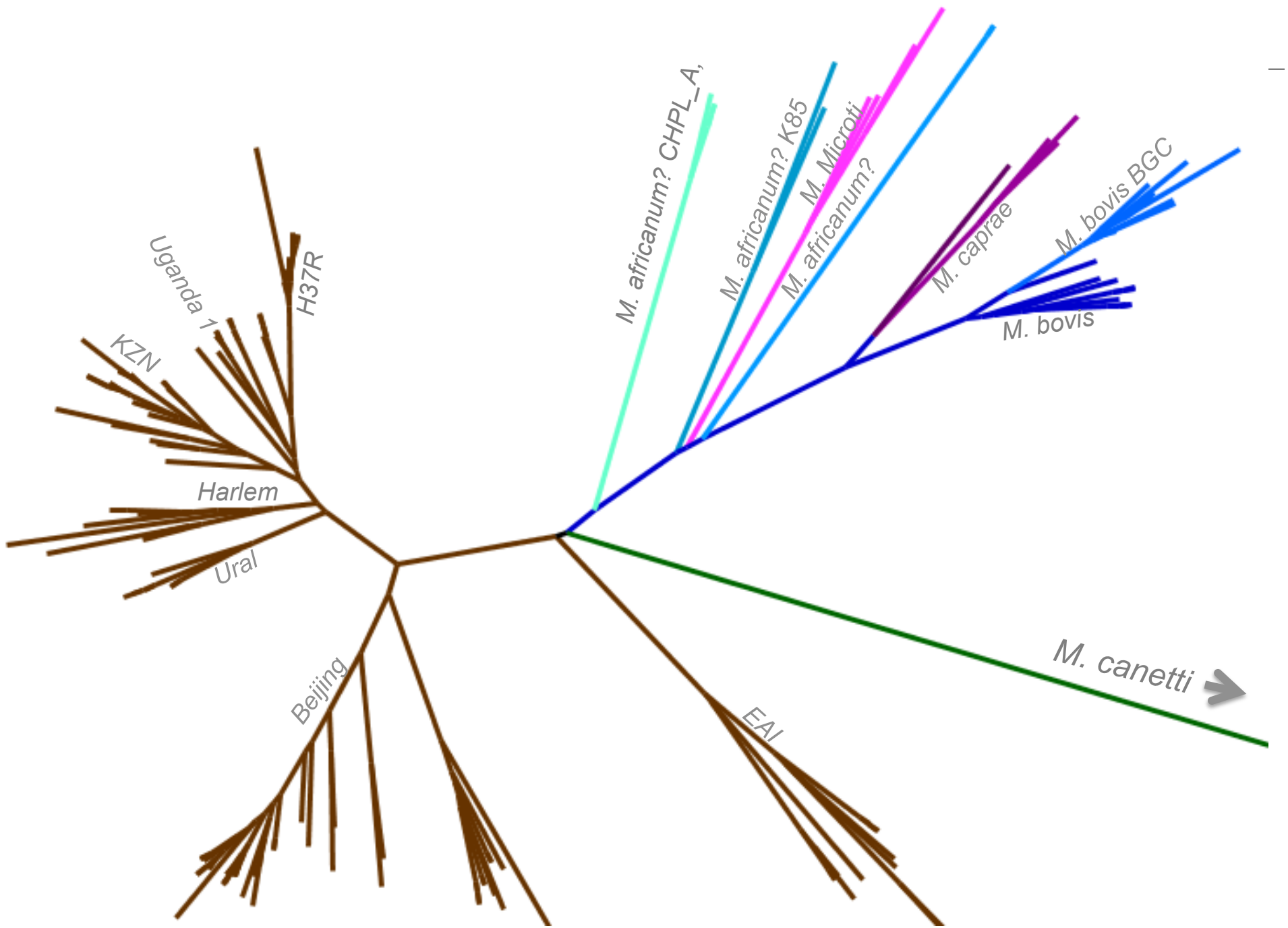
**MBTC direct PCR of
granuloma
Ct= 22.3**

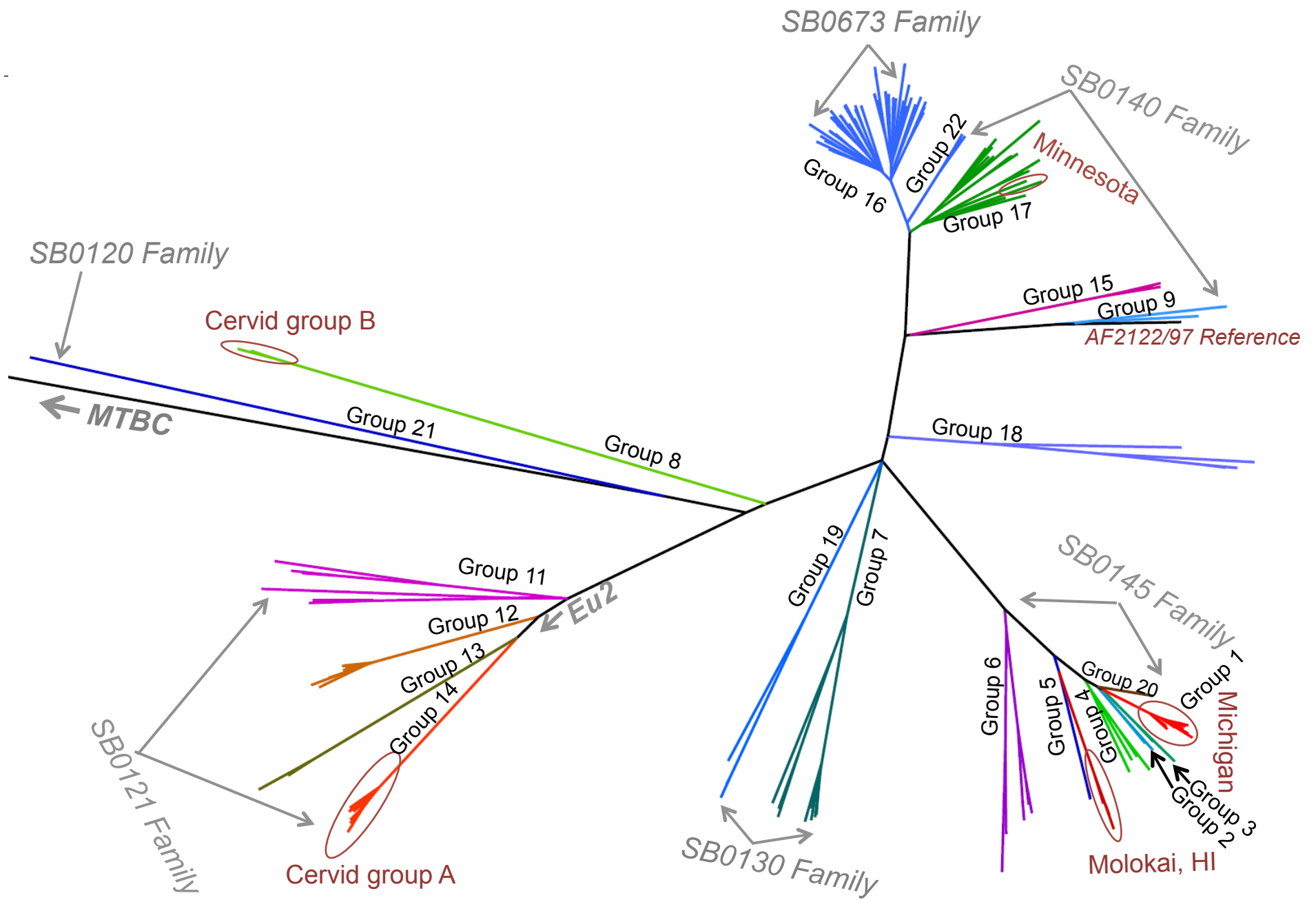
reference_pos	chrom 1-2486675	chrom 1-3681326	chrom 1-249090	chrom 1-3039283	chrom 1-3484416	chrom 1-3668428	chrom 1-642172	chrom 1-1005800	chrom 1-1240726	chrom 1-2424289	chrom 1-2735780	chrom 1-3098045	chrom 1-1962932	chrom 1-2174565	chrom 1-2518995	chrom 1-691874	chrom 1-935221	chrom 1-2067418	chrom 1-2327649	chrom 1-3958374	chrom 1-2681279	chrom 1-4105788	chrom 1-1389360	chrom 1-2709705	chrom 1-972557	chrom 1-2520914	chrom 1-2139205	chrom 1-4275119
reference_call	T	G	C	G	C	C	G	G	C	T	G	G	A	T	G	C	T	A	G	G	G	G	C	G	C	T	A	G
14-031794 from tissue	n	G	n	A	n	T	A	n	n	n	n	G	n	n	n	C	n	n	n	n	G	G	n	n	n	n	n	n
03-1057_MI_Alco_Beef_27-Val	C	T	C	G	C	C	G	G	C	T	G	G	A	T	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-3970_MI_Wildlife_Opossum_47-Val	T	G	T	A	T	T	A	A	T	C	A	A	A	T	G	C	T	A	G	G	G	G	C	G	C	T	A	G
14-2481_MI_Huro_HO_Steer_F3	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
14-2239_MI_Huro_HO_Steer_F3-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
14-2092_MI_Saga_Cat_56-A-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
14-2091_MI_Saga_Cat-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
14-2088_MI_Saga_Cat_56-A-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
14-1593_Huro_MI_HO_Steer_F3	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-6216_MI_Aren_Fed_Heifer-58A	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-5489_MI_Saga_Dairy_56-J_45-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2894_MI_Saga_Dairy_56-J_39-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2691_MI_Saga_Dairy_56-A_38-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2673_MI_Saga_Dairy_56-A_21THX-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2656_MI_Saga_Dairy_56-A_42-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2543_MI_Saga_Dairy_56-J_15AB-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2541_MI_Saga_Dairy_56-J_15HD-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2534_MI_Saga_Dairy_56-J_13AB-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2531_MI_Saga_Dairy_56-J_12AB-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-1941_MI_Saga_Dairy_56-A_6-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	A	G	G	G	G	C	G	C	T	A	G
13-3082_MI_Midl_Beef_59-J_1-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	A	C	T	A	G	G	G	G	C	G	C	T	A	G
13-2540_MI_Saga_Dairy_56-J_32THX-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	T	A	G	G	G	G	G	C	G	C	T	A	G
13-2522_MI_Saga_Dairy_56-J_43-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	C	A	G	G	G	G	C	G	C	T	A	G
13-2533_MI_Saga_Dairy_56-J_13THX	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	G	G	G	G	G	C	G	C	T	A	G
13-1416_MI_Saga_Dairy_56-A_1-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	G	T	G	G	G	C	G	C	T	A	G
14-2111_MI_HO_Steer-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	G	T	A	G	G	C	G	C	T	A	G
13-2745_MI_Saga_Dairy_56-A_40-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	G	T	G	A	G	C	G	C	T	A	G
14-2738_MI_NE_Fed	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	G	T	G	G	A	T	G	C	T	A	G
13-1932_MI_Saga_Dairy_56-A_3-Val	T	G	T	A	T	T	A	G	C	T	G	G	G	A	G	C	T	G	T	G	G	G	C	G	C	T	A	G



Future direction

- Sequence the Mycobacteria directly from sample.
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Pipeline Available

- https://github.com/stuber/SNP_analysis





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Questions?

