Parallelization of whole genome analysis on a Cray XE6
Next Generation sequencing is Massively parallel: fast

Red = A  Blue = G  Yellow = C  Green = T
The cost of sequencing a genome is dropping quickly

Cost per Genome

Moore's Law

NIH National Human Genome Research Institute

genome.gov/sequencingcosts
Targeted gene sequencing

- Clinical and research applications
- Analyzes 1-100’s of genes
- Identifies variation in genes previously established as disease causing
- Analyzes coding region of genes
- Costly (several $1000s)
Whole exome sequencing (WES)

- Relies on predetermined exon identification.
- High coverage 50-100X
- Only includes ~1-2% of genome
- Does not include regulatory regions
- Approximately $1000 (research setting)
Whole Genome Sequencing

150 base pair sequences $\times$ 3 billion base pairs $\times$ 40 fold coverage

$= 800,000,000$ sequences to align per genome
Whole genome sequencing

- Comprehensive
  - Single nucleotide polymorphisms (SNPs), insertion/deletion (indels) polymorphisms, splice site variants, structural variation
- Potential to identify new genes
- Potential to identify multiple pathologic variants as modifiers
- Cost ~ $3,000
Gene Panel
50X coverage
1,581,742 bp
0.16Gb

Exome
50X coverage
62Mbase genome
6.2Gb

Genome
35X coverage
2.8 Gbase genome
125Gb

1 Gigabyte = 10 yards of books
Whole genome (research) costs for Whole Genome Sequencing and Whole Exome Sequencing:

- Whole genome (research) costs have decreased significantly from $5B in 2000 to around $1K in 2013.
- Clinical Genome and Clinical Exome costs have also decreased but at a slower rate compared to whole genome sequencing.

The graph illustrates the dramatic decrease in costs over time, highlighting the rapid advancement in genomic sequencing technology and its associated financial accessibility.
A

Data (Gigabytes)

100
50
0

B

Variants x 10^6

4
3
2
1
0

C

Clinical Cost ($)

9000
6000
3000
0

D

Cost/Variant ($)

1.60
1.20
0.80
0.40
0

Gene Panel
50X coverage
1,581,742 bp
0.16Gb output

Exome
50X coverage
62Mbase genome
6.2Gb output

Genome
35X coverage
2.8 Gbase genome
125Gb output

Puckelwartz and McNally Genes 2014
Generate Reads

Align to Reference

ATCGACCCTAGCGCGCTAACGTAATTGCTAGCTAAGCTAAGCTACTGATGCGGTT

TAGCTGGCATCGCGCATTGCAATTACGATCGATTGATTCTCGATGACTACGCGCAA
Generate Reads

Align to Reference

......ATCGACCCTAGCGCGCTAACGTAATTGCTAGCTAAGCTAATAGCTGGCATCGCGCCTGCATCTAAGCTACTGATGCGCGTT......

......TAGCTGGCATACTGCGCGATTGCAATTACGATCGATTGCATTTGCTGACTACCGCGCTAACGAA......
Why use a supercomputer?

• Whole genome analysis is severely limited by time constraints (many steps, many of which are computationally expensive)
• A parallel machine’s size and speed allows for efficient analysis of multiple genomes
• A parallel machine allows for testing of new methods, algorithms, parameters and for comparing with old ones
A Parallel workflow: MegaSeq

• Uses a Cray XE6 to achieve the parallelization required for multiple genome analysis

• Relies on open source software (free, at least to academia), BWA, SAMTOOLS, BAMTOOLS, PICARD, GATK

• Employs a MapReduce approach and multithreading to take advantage of the distributed nodes.
Aligning and calling variants with Megaseq

BAM file

ReadGroup1 ReadGroup2 ReadGroup n

Step

Sam2Fastq by Readgroup
Align
Compress
Sort
(BWA mem)
Merge ReadGroups
Split by Chromosome

Mark Duplicates (Picard)
IndelRealigner (GATK)
Recalibrate Scores (GATK)

Call Variants (Haplotype Caller)
Generate VCF files
Merge VCF files

Puckelwartz et al. Bioinformatics 2014;30:1508-1513
Raw data extraction phase

- Extract raw sequence from bam files, only necessary when fastq not provided
  - Picard Suite SamtoFastq
- Split patient sequences by readgroup
  - each ~150bp sequence has a unique identifier based on machine, sample, library, lane and flow cell location
  - provides easy “data packet” for downstream analysis
- ~12 hours
- Other approaches are possible (bamutil, biobambambam)
Alignment-BWA

- Burrows-Wheeler Aligner (BWA) uses gapped alignment
- One node per read group
- Alignment scales perfectly (linear speedup with the number of cores used)
- Aln/sampe:
  - Trimmed all short sequence reads to a quality of 30
  - Convert alignment files to readable format using BWA-tpx. Conversion does not scale perfectly
  - ~10 hours
- mem:
  - no need for trimming
  - No need for conversion
  - no scaling issues
  - <<~ 3 hours, depending on number of readgroups
Cleaning Computation Requirements

- After alignment, readgroups per genome are merged, then each genome is split by chromosome
- For cleaning, each step was performed on 25 cores concurrently
  - (3 nodes, plus one core – 24 chromosomes + mitochondria)
- Threading was used, where available
- 58GB memory/number of jobs per node for Java programs
- Java programs were also given 2 threads for GC which better managed memory issues allowing us to pack more jobs per node
Cleaning alignments

• Picard & Samtools process the aligned reads to prepare for variant calling
  – Mark Duplicates (Picard) identifies and flags duplicates that can be produced during library preparation
  – Megaseq1a: Samtools does all the splitting & sorting business – very fast, neat
  – Megaseq1b: splitting is done directly after bwa mem, without any step to disk; bamtools

• Megaseq1a: ~9 hours; Megaseq1b: ~1-3 hours?
Genome Analysis ToolKit (GATK)

• Broad Institute

• Local realignment around indels:
  – alignment is performed using each sequence read individually
  – uses multiple alignments at the suspected indel to identify mismatches

• Base quality score recalibration:
  – more closely matches the actual probability of mismatching the referent genome
  – corrects for any variation in quality between machine cycle and sequence context

• ~7-12 hours
Variant Calling

- Haplotype Caller (GATK) calls SNVs (single variants) and insertion/deletion variants simultaneously
- ~3 nodes with 25 X concurrency per genome
- Variants were filtered based on quality metrics including quality score, depth and others
- ~1-4 hours
CPU and real time constraints of Whole Genome Analysis (WGA)

Puckelwartz et al. Bioinformatics 2014;30:1508-1513

![Graph showing CPU and real time constraints for whole genome analysis. The graph includes data points for 3-node cluster real time and Cray real time. The x-axis represents the number of genomes, while the y-axis represents CPU time in hours. The data points are marked with labels indicating time periods such as 47.2 years, 11.8 years, 7.1 months, 17.9 days, 2.4 years, and 2 days.](image)
Disk space constraints of Genome Analysis (1TB/genome)

- VCF (1GB)
- Logs (78GB)
- fastq (250GB)
- Processed bams (365GB)
- Readgroup & Chromosome bams (274GB)
- SAI (20GB)
Megaseq covers more of the genome with fewer variants

Megaseq

ELAND/CASAVA

Deeper coverage

Covers more genome

Fewer SNVs

Fewer Indels
Individual Genomes

- ~3-4 million SNPs differ from the reference per genome
- 130-400 rare non-synonymous variants per genome
- 10-20 Loss of function
- 0-8 variants per genome are predicted “highly damaging”
Acknowledgements

Elizabeth McNally, PI
Megan Roy-Puckelwartz
Alexis Demonbreun
Dave Barefield
Eugene Wyatt
Ellis Kim
Joshua DeJong
Maddie Allen
Brandon Gardner
Quan Gao
Bridget Biersmith
Andy Vo
Kay Marie Lamar
Michele Hadhazy
Judy Earley
Will Montag
Jessie Golbus

Argonne Nat’l Labs/CI
Ian Foster

Gerald Dorn - Wash U
Euan Ashley - Stanford
Rick Dewey - Stanford
Sharlene Day - Michigan
Tom Cappola - Penn