File formats

Wrapping your data in the right package

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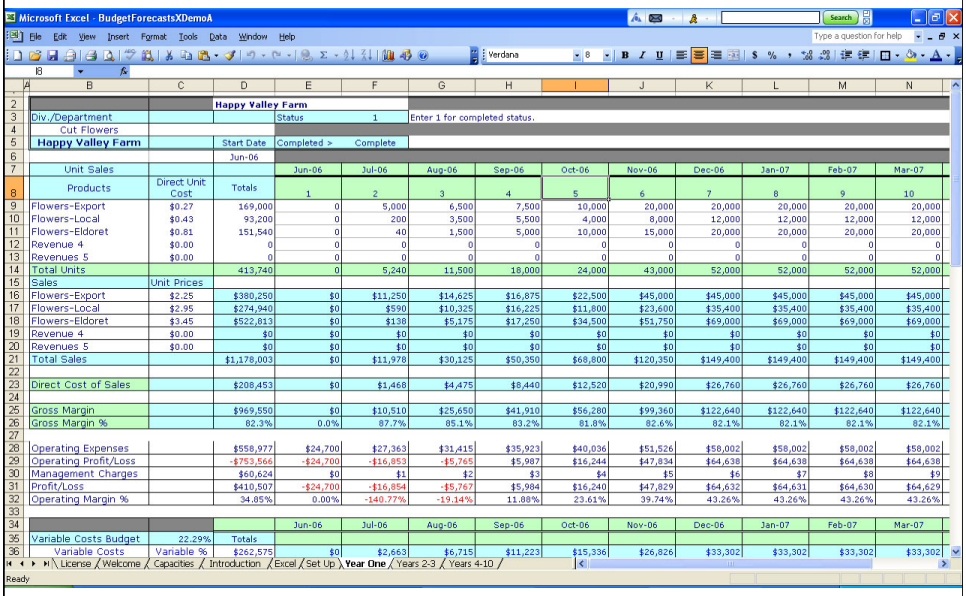
Leading the search for tomorrow's cures

Short Course in Medical Genetics 2014



http://www.downloadsoftfree.com/windows/Business/Business-Finance/Budgeting-Spreadsheets-for-Excel-1-2-13-4345-1-0-0.html

```
<Styles>
 <Style ss:ID="Default" ss:Name="Normal">
  <Alignment ss:Vertical="Bottom"/>
  <Borders/>
  <Font ss:FontName="Arial"/>
  <Interior/>
  <NumberFormat/>
  <Protection/>
 </Style>
 <Style ss:ID="s16">
  <Alignment ss:Vertical="Bottom" ss:WrapText="1"/>
  <Font ss:FontName="Arial" ss:Bold="1"/>
 </Style>
 <Style ss:ID="s17">
  <Alignment ss:Horizontal="Right" ss:Vertical="Bottom" ss:WrapText="1"/>
  <Font ss:FontName="Arial" ss:Bold="1"/>
 </Style>
 <Style ss:ID="s18">
  <NumberFormat ss:Format="#,##0"/>
 </Style>
 <Style ss:ID="s19">
  <Alignment ss:Horizontal="Left" ss:Vertical="Bottom"/>
  <NumberFormat ss:Format="#,##0"/>
 </Style>
 <Style ss:ID="s20">
  <Alignment ss:Horizontal="Right" ss:Vertical="Bottom"/>
 </Style>
 <Style ss:ID="s21">
  <Alignment ss:Horizontal="Left" ss:Vertical="Bottom"/>
 </Style>
</Styles>
```

```
track name="tb_gap" description="table browser query
chr1 167280   217280
chr1 257582   307582
chr1 461231   511231
chr1 2624080  2674080
chr1 3835128  3895128
```


```
track name="tb_gap" descript
chr1^I167280^I217280$
chr1^I257582^I307582$
chr1^I461231^I511231$
chr1^I2624080^I2674080$
chr1^I3835128^I3895128$
```

```
track name="tb_gap" description="table browser query
chr1    167280  217280
chr1    257582  307582
chr1    461231  511231
chr1    2624080     2674080
```


```
track name="tb_gap" description
chr1  167280  217280$
chr1  257582  307582$
chr1  461231  511231$
chr1  2624080     2674080$
chr1  3835128     3895128$
```

Control Characters: invisible to you but not to software

Carriage return (CR): \r or ^M

Line feed (LF): \n or ^J

Unix/Linux: uses LF character

Macs: uses CR character

Windows: uses CR followed by LF

http://danielmiessler.com/study/crlf/

Most bioinformatics packages expect:

- A plain text file
  - Not a word or excel document
- A particular field delimiter
  - often tab or comma, sometimes pipe
  - Unix style line terminators

Read file specifications!*

* Even though they may not be complete

Vince Buffalo
@vsbuffalo

Following

If I had one thing to tell biologists learning bioinformatics, it would be "write code for humans, write data for computers".

NCBI data representation:

- Uses ASN.1
- Not easily human readable
- Limited flexibility
- Robust validation tools
- Not easily parsed by Perl/Python

```
Seq-entry ::= seq {
  id {
    general {
      db "WGS:AMYH01" ,
      tag
        str "chr1_315417" } ,
    genbank {
      accession "JH976292" ,
      version 1 } ,
    gi 409188728 } ,
  descr {
    title "Homo sapiens chromosome 1 genomic scaffold" ,
    source {
      genome genomic ,
      org {
        taxname "Homo sapiens" ,
        common "human" ,
        db {
          {
            db "taxon" ,
            tag
              id 9606 } } ,
        orgname {
          name
            binomial {
              genus "Homo" ,
              species "sapiens" } ,
            lineage "Eukaryota; Metazoa; Chordata; Craniat
 Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Pri
 Catarrhini; Hominidae; Homo" ,
            gcode 1 ,
            mgcode 2 ,
            div "PRI" } } ,
      subtype {
        {
          subtype chromosome ,
          name "1" } ,
        {
          subtype cell-line ,
          name "CHM1htert" } ,
        {
          subtype tissue-type ,
          name "hydatidiform mole" } ,
```

Typical bioinformatics data representation:

Tab delimited file

```
track name="tb_snp135Common" description="table browser query on snp135Common" visibility=3 url=
chr1  10491 10492 rs55998931 0    +
chr1  10582 10583 rs58108140 0    +
chr1  14929 14930 rs75454623 0    +
chr1  20244 20245 rs71262674 0    -
chr1  20303 20304 rs71262673 0    -
chr1  33494 33495 rs75468675 0    +
```

- Flexible
  - Good: with rapidly changing data/tech (but don't change/add columns!)
  - Poor: validation
- Human Readable
  - Convenient for de-bugging
  - Computer doesn't care!

Putting the data in the right package

- Sequences
  - FASTA
  - FASTQ
  - SAM/BAM
- Alignments
  - SAM/BAM
  - MAF
- Annotations
  - Genes
    - GFF3
    - GTF
  - Variation
    - VCF
    - GVF
    - HGVS
  - General
    - GFF3
    - BED

http://deannachurch.github.io/BHSC_Bioinformatics/formats.html

FASTA

```
>OTTMUST00000058732 cdna:all chromosome:VEGA44:6:17256335:17291324:1 Gene:OTTMUSG00000024023
GCCCCCCGCCAGCGCCTTTCCCCCCTCTATACAATACAAGATCTTCCTTCCTCAGTTCTC
TTAAATCACAGCCCAGGGAAACCTCCTCAGAGCCTGCAGCCAGCCACGCGCCAGCATGTC
TGGGGGCAAATACGTAGACTCCGAGGGACATCTCTACACTGTTCCCATCCGGGAACAGGG
CAACATCTACAAGCCCAACAACAAGGCCATGGCAGACGAGGTGACTGAGAAGCAAGTGTA
TGACGCGCACACCAAGGAGATTGACCTGGTCAACCGCGACCCCAAGCATCTCAACGACGA
CGTGGTCAAGATTGACTTTGAAGATGTGATTGCAGAACCAGAAGGGACACACAGTTTCGA
CGGCATCTGGAAGGCCAGCTTCACCACCTTCACTGTGACAAAATATTGGTTTTACCGCTT
```

FASTQ

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

FASTQ Details

Sequence data format

- Text based
  - Encodes sequence calls and quality scores with ASCII characters
  - Stores minimal information about the sequence read
  - 4 lines per sequence
    - Line 1: begins with @; followed by sequence identifier and optional description
    - Line 2: the sequence
    - Line 3: begins with the "+" and is followed by sequence identifiers and description (both are optional)
    - Line 4: encoding of quality scores for the sequence in line 2

References
http://maq.sourceforge.net/fastq.shtml
Cock et al. (2009) Nuc Acids Res 38: 1767-1771

FASTQ Example

For analysis, it may be necessary to convert to the Sanger form of FASTQ.

```
@SRR014849.1 EIXKN4201CFU84 length=93
GGGGGGGGGGGGGGGCTTTTTTTGTTTGGAACCGAAAGG
GTTTTGAATTTCAAACCCTTTTCGGTTTCCAACCTTCCAA
AGCAATGCCAATA
+SRR014849.1 EIXKN4201CFU84 length=93
3+&$#""""""""""""7F071,'";C?,B;?6B;:EA1EA
1EA5'9B:?:#9EA0D02EA5':>5?:%A;A8A;?9B;D0
/=<?7=9<2A8==
```

@title and optional description
sequence line(s)
+optional repeat of title line
quality line(s)

FASTQ example from Cock et al., 2009

Quality Scores

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90 %
20	1 in 100	99 %
30	1 in 1000	99.9 %
40	1 in 10000	99.99 %
50	1 in 100000	99.999 %

Q = Phred Quality Scores
P = Base-calling error probabilities

Quality Scores

Not always directly comparable between to programs/pipelines

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

```
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
 |                         |    |        |                              |                     |
33                        59   64       73                            104                   126
```

Format/Platform	QualityScoreType	ASCII encoding	
Sanger	Phred: 0-93	33-126	
Solexa	Solexa:-5-62	64-126	
Illumina 1.3	Phred: 0-62	64-126	
Illumina 1.5	Phred: 0-62	64-126	
Illumina 1.8	Phred: 0-62	33-126	*** Sanger format!

Need to know what your program is expecting
Likely to change again (to improve compressing data)

SAM (Sequence Alignment/Map)

Alignment data format

- Standard output of aligners that map reads to a reference genome
  - Tab delimited w/ header section and alignment section
    - Header sections begin with @ (are optional)
    - Alignment section has 11 mandatory fields
  - BAM is the binary format of SAM

http://samtools.sourceforge.net/

Mandatory Alignment Fields

Col	Field	Type	Regexp/Range	Brief description
1	QNAME	String	`[!-?A-~]{1,255}`	Query template NAME
2	FLAG	Int	$[0,2^{16}-1]$	bitwise FLAG
3	RNAME	String	`\*|[!-()+-<>-~][!-~]*`	Reference sequence NAME
4	POS	Int	$[0,2^{29}-1]$	1-based leftmost mapping POSition
5	MAPQ	Int	$[0,2^{8}-1]$	MAPping Quality
6	CIGAR	String	`\*|([0-9]+[MIDNSHPX=])+`	CIGAR string
7	RNEXT	String	`\*|=|[!-()+-<>-~][!-~]*`	Ref. name of the mate/next fragment
8	PNEXT	Int	$[0,2^{29}-1]$	Position of the mate/next fragment
9	TLEN	Int	$[-2^{29}+1,2^{29}-1]$	observed Template LENgth
10	SEQ	String	`\*|[A-Za-z=.]+`	fragment SEQuence
11	QUAL	String	`[!-~]+`	ASCII of Phred-scaled base QUALity+33

http://samtools.sourceforge.net/SAM1.pdf

Alignments example

```
Coor     12345678901234  5678901234567890123456789012345
ref      AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT

+r001/1        TTAGATAAAGGATA*CTG
+r002         aaaAGATAA*GGATA
+r003       gcctaAGCTAA
+r004                     ATAGCT..............TCAGC
-r003                            ttagctTAGGC
-r001/2                                       CAGCGCCAT
```

CIGAR string -> 8M2I4M1D3M

Alignments in SAM format

```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002   0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003   0 ref  9 30 5H6M       *  0   0 AGCTAA   *   NM:i:1
r004   0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003  16 ref 29 30 6H5M       *  0   0 TAGGC    *   NM:i:0
r001  83 ref 37 30 9M         =  7 -39 CAGCGCCAT         *
```

http://samtools.sourceforge.net/SAM1.pdf

Annotation Formats

- Mostly tab delimited files that describe the location of genome features (i.e., genes, etc.)
- Also used for displaying annotations on standard genome browsers
- Important for associating alignments with specific genome features
- Descriptions
- Knowing format details can be important to translating results!
  - BED is zero based/exclusive
  - GTF/GFF are one based/inclusive

BED: zero based, start inclusive, stop exclusive

```
chr1 10491     10492     rs55998931     0     +
chr1 10582     10583     rs58108140     0     +
```

- First base on the chromosome is 0
- Length = stop - start

GTF/GFF: one based, inclusive

```
chr1 snp135Com exon 10492     10492     0.000
chr1 snp135Com exon 10583     10583     0.000
```

- First base on the chromosome is 1
- Length = stop – start+1

BED format

Annotation data format

Required (1-3) Optional (4-12) →

```
chr1   86114265    86116346    nsv433165
chr2   1841774     1846089     nsv433166
chr16  2950446     2955264     nsv433167
chr17  14350387    14351933    nsv433168
chr17  32831694    32832761    nsv433169
chr17  32831694    32832761    nsv433170
chr18  61880550    61881930    nsv433171
```

```
chr1   16759829    16778548    chr1:21667704 270866 -
chr1   16763194    16784844    chr1:146691804      407277 +
chr1   16763194    16784844    chr1:144004664      408925 -
chr1   16763194    16779513    chr1:142857141      291416 -
chr1   16763194    16779513    chr1:143522082      293473 -
chr1   16763194    16778548    chr1:146844175      284555 -
chr1   16763194    16778548    chr1:147006260      284948 -
chr1   16763411    16784844    chr1:144747517      405362 +
```

GFF3

Annotation data format

```
 0  ##gff-version 3
 1  ##sequence-region   ctg123 1 1497228
 2  ctg123 . gene            1000  9000  .  +  .  ID=gene00001;Name=EDEN

 3  ctg123 . TF_binding_site 1000  1012  .  +  .  ID=tfbs00001;Parent=gene00001

 4  ctg123 . mRNA            1050  9000  .  +  .  ID=mRNA00001;Parent=gene00001;Name=EDE
 5  ctg123 . mRNA            1050  9000  .  +  .  ID=mRNA00002;Parent=gene00001;Name=EDE
 6  ctg123 . mRNA            1300  9000  .  +  .  ID=mRNA00003;Parent=gene00001;Name=EDE

 7  ctg123 . exon            1300  1500  .  +  .  ID=exon00001;Parent=mRNA00003
 8  ctg123 . exon            1050  1500  .  +  .  ID=exon00002;Parent=mRNA00001,mRNA0000
 9  ctg123 . exon            3000  3902  .  +  .  ID=exon00003;Parent=mRNA00001,mRNA0000
10  ctg123 . exon            5000  5500  .  +  .  ID=exon00004;Parent=mRNA00001,mRNA0000
    ctg123 . exon            7000  9000  .  +  .  ID=exon00005;Parent=mRNA00001,mRNA0000

    ctg123 . CDS             1201  1500  .  +  0  ID=cds00001;Parent=mRNA00001;Name=eden
```

Fixed columns:
Column 1: Sequence Id
Column 2: Source
Column 3: Feature type
Column 4: Start (1-based)
Column 5: End
Column 6: Score
Column 7: Strand
Column 8: Phase (0,1,2)

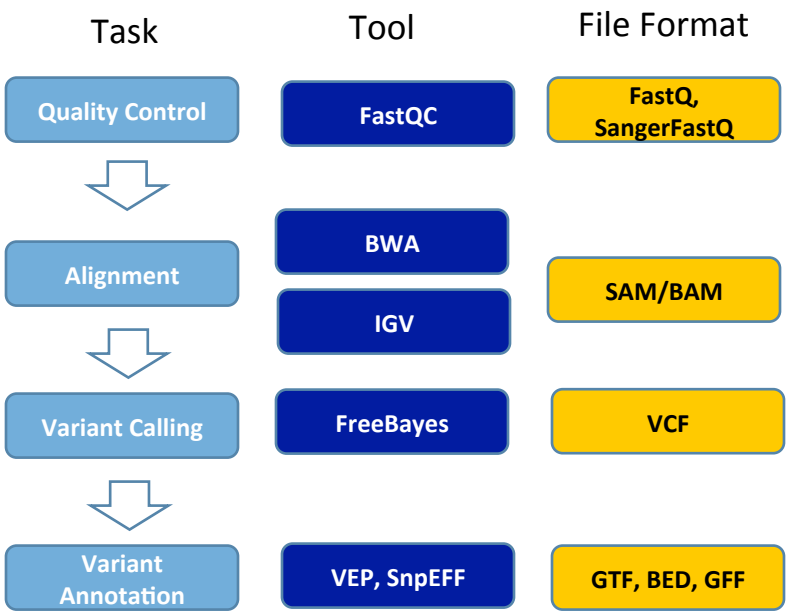
Flexible column:
Column 9: attributes

Semi-colon delimited tag=value pairs. Some tags are reserved (ID, Name, etc).

http://www.sequenceontology.org/resources/gff3.html

DNASeq Tasks, Tools and File Formats

We'll re-visit this on Friday during the Galaxy tutorial.



Take home messages

- Understand how your tools work
  - What is the tool expecting?
  - What type of data am I representing?
  - What type of data will it produce
- Output of programs/pipelines are not always comparable
  - Score values
- Know how to count (starting at 0 or 1)
- Just because 2 files are of the same type (BED, GFF3) it does not mean they are identical or 'standard'.

What to do next

- Work on the file format exercises on the workshop web site
- Explore the links on the File Formats section of the course web site
- The file formats that will be most relevant to you this week:
  - Fastq
  - SAM/BAM
  - BED
  - VCF