Introduction to bioinformatics (NGS data analysis)

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Got your sequencing data - now, what to do with it?

- File size: several Gb
- Number of lines: >1,000,000

```
@M02443:17:000000000-ABPBW:1:1101:12675:1533 1:N:0:1
TCGATAATTCTTACTTTCTCTCTGGTCTGAGCGTTTCACATCAACGACAAGCTCGA
TTCTTCCTTTTCTCTTTTTTTCTTCTCTTCCTCTTTTTTCCTTTTCTCCCTCTTCT
TTTTTTTTTCTTCTT
+
8B6-@-,CFFED9CFAE@@C6;@,CFEEF9<@6FGGF9F<CC,,CB,@::8CF,6+
,,3733>>@@,,,388@,,8*,773333,3,333738,*,,,,,,76,,2,,2,,2
0*).1.))(0*)***
@M02443:17:000000000-ABPBW:1:1101:18658:1535 1:N:0:1
TCCCTAATTCTCTGTCTTCAAATTTTCCTTCTCTAAATCGTCCCTCGTTTCTACCT
TTTCTTGTTTTTTTATTTCCTCCTCTTCCTTTTTTACTTCCACCTTCTTTTCTGCC
TTTTCTTCTTTTTCT
+
-<<9-@CCEF9CE-<,,,,,;,,<C,=,6,C9,C<=C,,,;,86C,6:C,,,;<;,,
,,,,5,5:,,9++4,,,:,,,,,,,,,,,38,853,5,,3,,7,,,6,,,,,7,,,,
+0,()+++)11.*)*
```

Before library preparation

What you need to know to steer your way through the analysis

- Research question
  - Identify adaptive genes
  - *De novo* genome assembly
  - Population genetic structure
  - Phylogenetic relation
- Experimental design
  - Number of individuals
  - Treatment of samples (e.g. heat stress)
- Sample collection
  - Samples degraded (e.g. stored in Formalin)
  - Tissue (reproductive, vegetative)

Library preparation

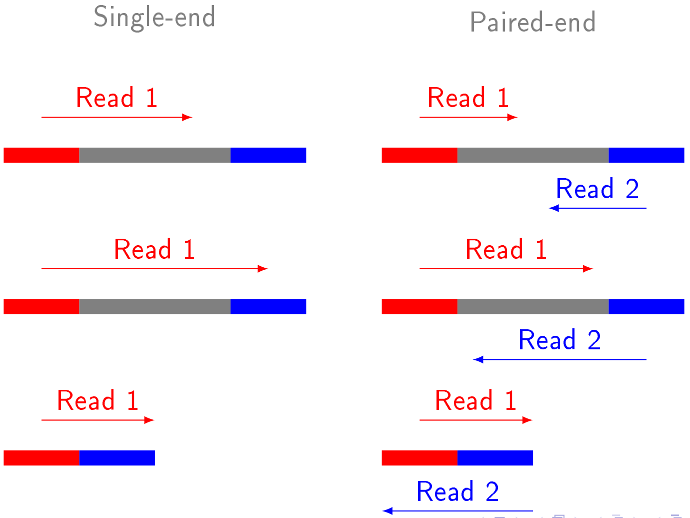
- DNA-seq, RNA-seq, Bis-Seq, Chip-Seq. . .
  - RNA reads (which lack introns) require splice-aware mappers.
  - Bis-seq changes GC ratio (bisulphite converts cytosine to uracil, but leaves 5-methylcytosine unaffected)
  - Chip-Seq enriches binding-sites of DNA-associated proteins
- Pooled samples?
  - Demultiplexing
  - Remove barcodes
- Adapter sequences that have to be trimmed off?
- Targeted coverage

Single- or Paired end sequencing, read length

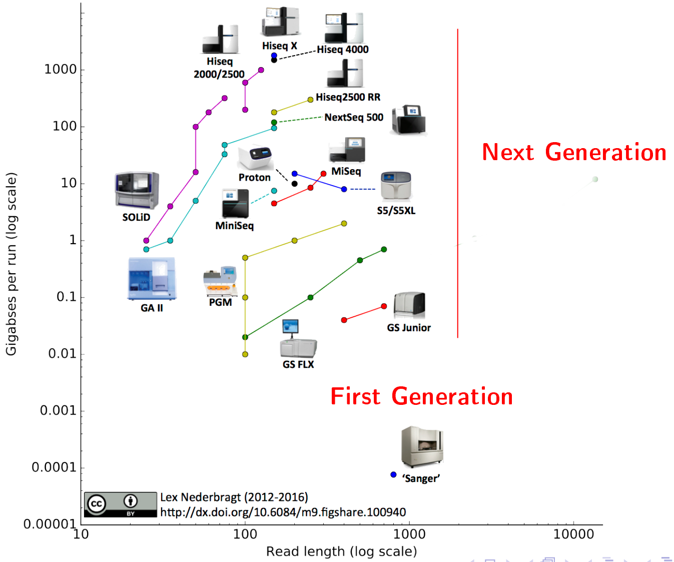
Library fragment

Adapter	Adapter
Flowcell/bead binding sequences	Flowcell/bead binding sequences
Amplification primers	Amplification primers
Sequencing primers	Sequencing primers
Barcodes	Barcodes

Single- or paired-end sequencing, read length - why does it matter

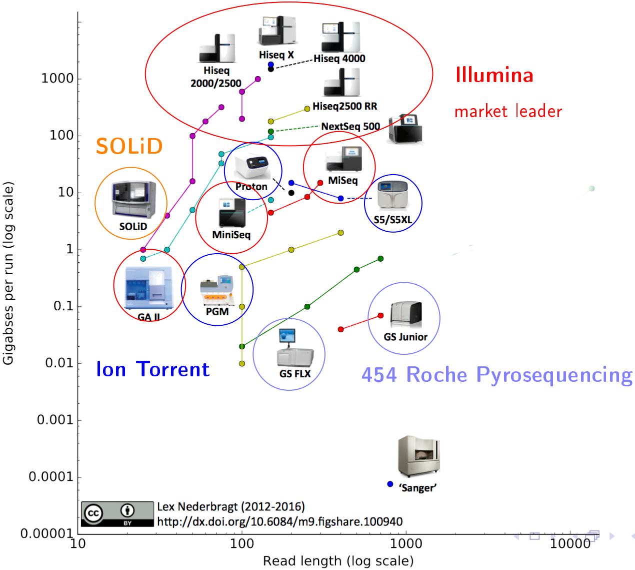


NGS platforms differ in throughput and read length

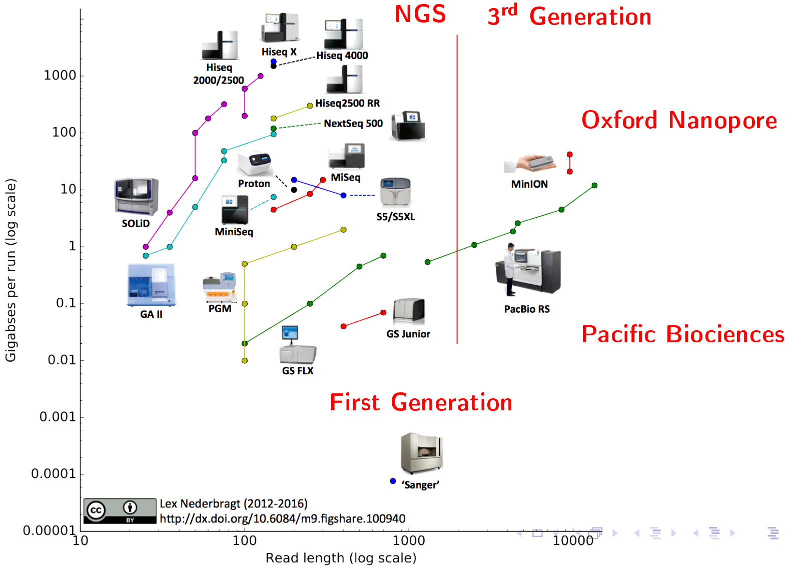


Lex Nederbragt (2012-2016)
http://dx.doi.org/10.6084/m9.figshare.100940

NGS platforms differ in throughput and read length



Third Generation Sequencing



Lex Nederbragt (2012-2016)
http://dx.doi.org/10.6084/m9.figshare.100940

Primary analysis

- Demultiplexing
- Adapter trimming
- Quality control

Demultiplexing of pooled samples (if barcoded inline)

AATTANNNNNNNNNNNNNNN	File 1
AGTCGNNNNNNNNNNNNNNN	File 2
AGTCGNNNNNNNNNNNNNNN	File 2
GCCATNNNNNNNNNNNNNNN	File 3
AATTANNNNNNNNNNNNNNN	File 1
GCCATNNNNNNNNNNNNNNN	File 3
AGTCGNNNNNNNNNNNNNNN	File 2

Trimmig: Adapter removal

Mostly 3'adapters disturb assembly and alignment

GATTTGGGGTTCAANNNNNNNATTAGTATCGAT

GATTTGGGGTTCAANNNNNNNATTAGTATCGAT

TTGGGGTTCAANNNNNNNATTAGTATCGAT

GATTTGGGGTTCAANNNNNNNATTAGTATCGAT

ATTTGGGGTTCAANNNNNNNATTAGTATCGAT

GATTTGGGGTTCAANNNNNNNATTAGTATCGAT

Fastq file - 4 lines for each read

```
@HWI-ST141_0365:2:1101:2983:2114#TTAGGC/1
GATTTGGGGTTCAAATTAGTATCGATCAAATAGTAAATCCATTTGTTCAACTC
+
!’’*((((***+))%%%++)(%%%%).1***-+*’’))**55CCF>>>>>>CC
```

1. sequence id (specifications can differ slightly between sequencing platforms)
   - =@=instrument name : flowcell lane : tile number: flowcell x coordinate : flowcell y coordinates : #barcode sequence: pair number for paired-end sequencing
2. sequence
3. + optionally followed by sequence identifier again
4. quality scores

Trimmig of low-quality bases

- Trim bases with a Phred quality score <20
- $Quality = -10 * log_{10} P$

Phred Score	Probability of incorrect base	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%

Fastq file contains both sequence reads and base quality scores

Fastq file

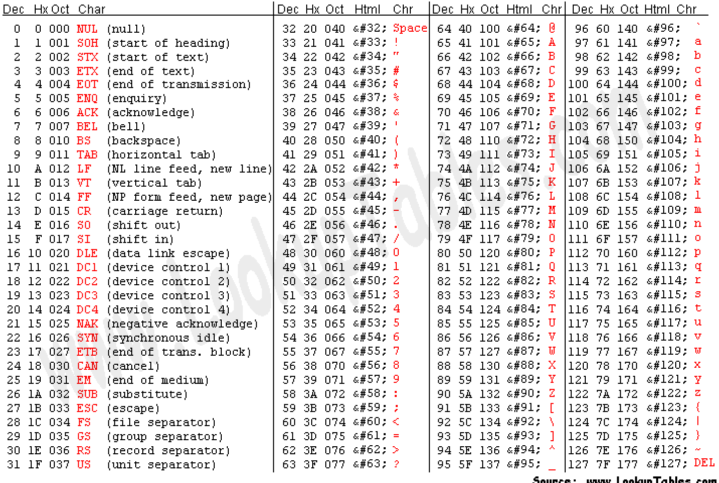
```
@SEQ_ID
GATTTGGGGTTCAAATTAGTATCGATCAAATAGTAAATCCATTTGTTCAACTC
+
!’’*((((***+))%%%++)(%%%%).1***-+*’’))**55CCF>>>>>>CC
```

Fasta file

```
>SEQ_ID
GATTTGGGGTTCAAATTAGTATCGATCAAATAGTAAATCCATTTGTTCAACTC
```

Base qualities are encoded in ascii format

ASCII stands for American Standard Code for Information Interchange. An ASCII code is the numerical representation for a character.

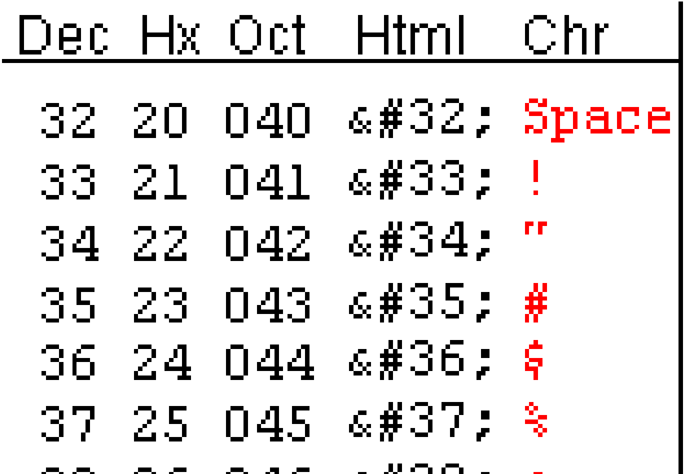


Dec	Hx	Oct	Char		Dec	Hx	Oct	Html	Chr	Dec	Hx	Oct	Html	Chr	Dec	Hx	Oct	Html	Chr
0	0	000	NUL	(null)	32	20	040	&#32;	Space	64	40	100	&#64;	@	96	60	140	&#96;	`
1	1	001	SOH	(start of heading)	33	21	041	&#33;	!	65	41	101	&#65;	A	97	61	141	&#97;	a
2	2	002	STX	(start of text)	34	22	042	&#34;	"	66	42	102	&#66;	B	98	62	142	&#98;	b
3	3	003	ETX	(end of text)	35	23	043	&#35;	#	67	43	103	&#67;	C	99	63	143	&#99;	c
4	4	004	EOT	(end of transmission)	36	24	044	&#36;	$	68	44	104	&#68;	D	100	64	144	&#100;	d
5	5	005	ENQ	(enquiry)	37	25	045	&#37;	%	69	45	105	&#69;	E	101	65	145	&#101;	e
6	6	006	ACK	(acknowledge)	38	26	046	&#38;	&	70	46	106	&#70;	F	102	66	146	&#102;	f
7	7	007	BEL	(bell)	39	27	047	&#39;	'	71	47	107	&#71;	G	103	67	147	&#103;	g
8	8	010	BS	(backspace)	40	28	050	&#40;	(	72	48	110	&#72;	H	104	68	150	&#104;	h
9	9	011	TAB	(horizontal tab)	41	29	051	&#41;	)	73	49	111	&#73;	I	105	69	151	&#105;	i
10	A	012	LF	(NL line feed, new line)	42	2A	052	&#42;	*	74	4A	112	&#74;	J	106	6A	152	&#106;	j
11	B	013	VT	(vertical tab)	43	2B	053	&#43;	+	75	4B	113	&#75;	K	107	6B	153	&#107;	k
12	C	014	FF	(NP form feed, new page)	44	2C	054	&#44;	,	76	4C	114	&#76;	L	108	6C	154	&#108;	l
13	D	015	CR	(carriage return)	45	2D	055	&#45;	-	77	4D	115	&#77;	M	109	6D	155	&#109;	m
14	E	016	SO	(shift out)	46	2E	056	&#46;	.	78	4E	116	&#78;	N	110	6E	156	&#110;	n
15	F	017	SI	(shift in)	47	2F	057	&#47;	/	79	4F	117	&#79;	O	111	6F	157	&#111;	o
16	10	020	DLE	(data link escape)	48	30	060	&#48;	0	80	50	120	&#80;	P	112	70	160	&#112;	p
17	11	021	DC1	(device control 1)	49	31	061	&#49;	1	81	51	121	&#81;	Q	113	71	161	&#113;	q
18	12	022	DC2	(device control 2)	50	32	062	&#50;	2	82	52	122	&#82;	R	114	72	162	&#114;	r
19	13	023	DC3	(device control 3)	51	33	063	&#51;	3	83	53	123	&#83;	S	115	73	163	&#115;	s
20	14	024	DC4	(device control 4)	52	34	064	&#52;	4	84	54	124	&#84;	T	116	74	164	&#116;	t
21	15	025	NAK	(negative acknowledge)	53	35	065	&#53;	5	85	55	125	&#85;	U	117	75	165	&#117;	u
22	16	026	SYN	(synchronous idle)	54	36	066	&#54;	6	86	56	126	&#86;	V	118	76	166	&#118;	v
23	17	027	ETB	(end of trans. block)	55	37	067	&#55;	7	87	57	127	&#87;	W	119	77	167	&#119;	w
24	18	030	CAN	(cancel)	56	38	070	&#56;	8	88	58	130	&#88;	X	120	78	170	&#120;	x
25	19	031	EM	(end of medium)	57	39	071	&#57;	9	89	59	131	&#89;	Y	121	79	171	&#121;	y
26	1A	032	SUB	(substitute)	58	3A	072	&#58;	:	90	5A	132	&#90;	Z	122	7A	172	&#122;	z
27	1B	033	ESC	(escape)	59	3B	073	&#59;	;	91	5B	133	&#91;	[	123	7B	173	&#123;	{
28	1C	034	FS	(file separator)	60	3C	074	&#60;	<	92	5C	134	&#92;	\	124	7C	174	&#124;	|
29	1D	035	GS	(group separator)	61	3D	075	&#61;	=	93	5D	135	&#93;	]	125	7D	175	&#125;	}
30	1E	036	RS	(record separator)	62	3E	076	&#62;	>	94	5E	136	&#94;	^	126	7E	176	&#126;	~
31	1F	037	US	(unit separator)	63	3F	077	&#63;	?	95	5F	137	&#95;	_	127	7F	177	&#127;	DEL

Source: www.LookupTables.com

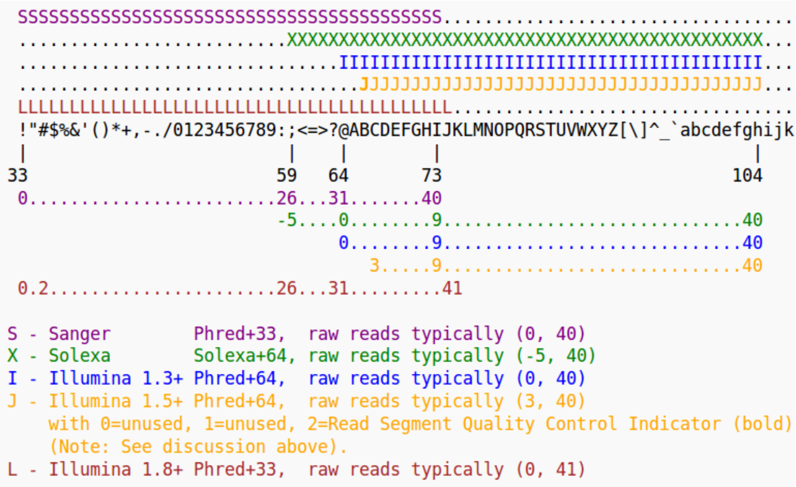
Base qualities are encoded in ascii format

ASCII stands for American Standard Code for Information Interchange. An ASCII code is the numerical representation for a character.



Dec	Hx	Oct	Html	Chr
32	20	040	&#32;	Space
33	21	041	&#33;	!
34	22	042	&#34;	"
35	23	043	&#35;	#
36	24	044	&#36;	$
37	25	045	&#37;	%

ASCII encodings of sequencing platforms



```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS..................................
 ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX...
 ...............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII...
 .................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ...
 LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.................................
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijk
 |                         |    |        |                              |
33                        59   64       73                            104
 0........................26...31.......40
                          -5....0........9.............................40
                                0........9.............................40
                                   3.....9.............................40
 0.2......................26...31.........41

S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

Figure: Quality score encodings

Quality control tool: FastQC

Informs on:

- Base quality
- Duplication
- Overrepresentation of sequences
    - contamination?
    - adapters?
- GC content (should be around 50%, in Bis-Seq lower)

Quality before trimming

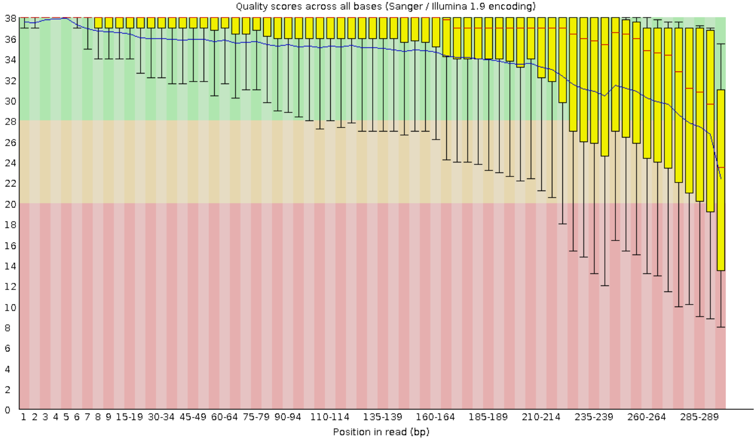


Figure: Base-quality generally decreases with increasing sequencing length

Quality after trimming

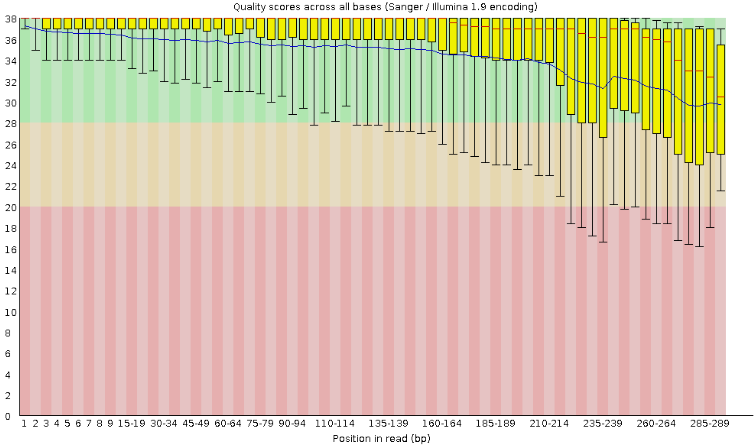
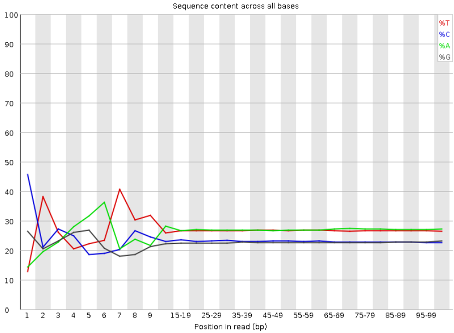


Figure: Quality after trimming

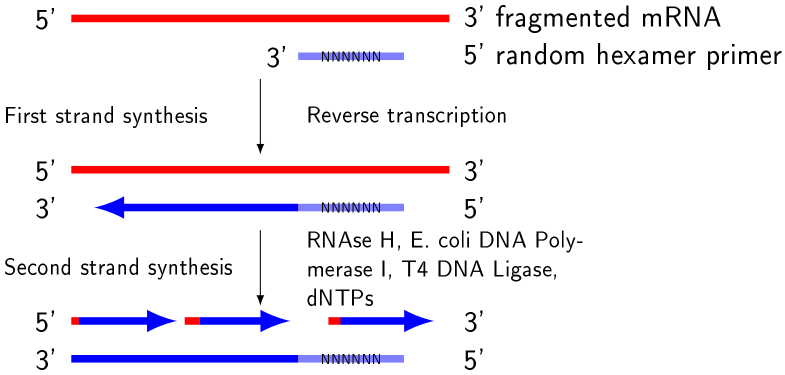
Sequence bias

For example in:

- First bases of Illumina RNAseq due to 'random' hexamer primers for reverse transcription
- RADseq fragments (cutting sites)



Hexamer primers for cDNA synthesis cause sequence bias



PCR Duplicates

Duplicates are generally removed in quantitative analyses (e.g. RNA-seq)

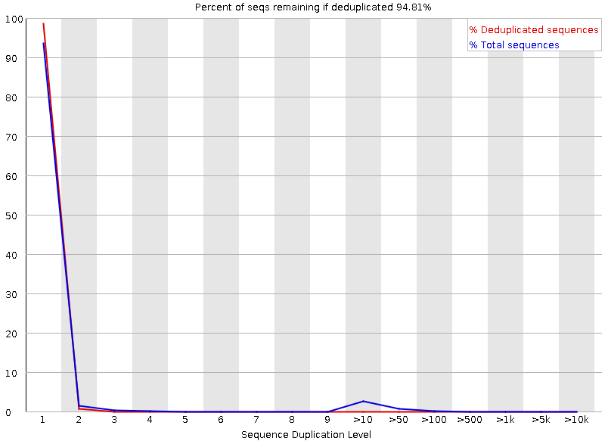


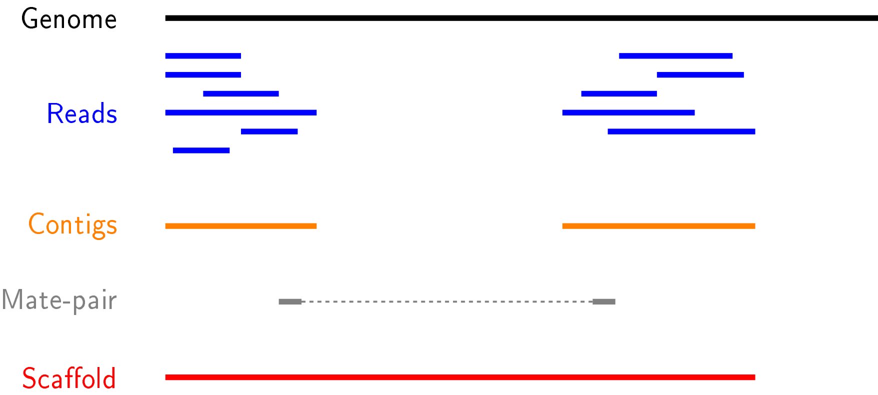
Figure: Duplication levels (FastQC output)

De novo assembly

Task: Look for overlapping regions and create contigs (contiguous sequences)

- Genome assembly software
  - SOAP de NOVO
  - Velvet
  - MIRA (we use this one in the course)
- Transcriptome assembly software
  - Review: Martin and Wang, (2011)
  - Trinity
  - MIRA

De novo assembly: Step by step



De novo assembly: The N50 metric

N50 is a single measure of the contig length size distribution in an assembly

- Sort contigs in descending length order
- Size of contig above which the assembly contains at least 50% of the total length of all contigs

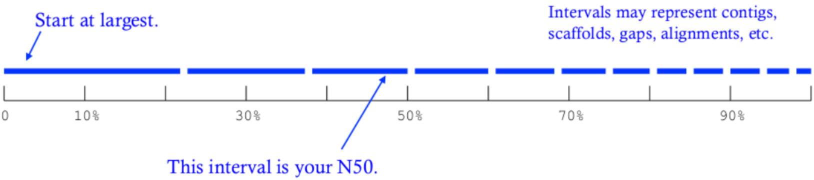
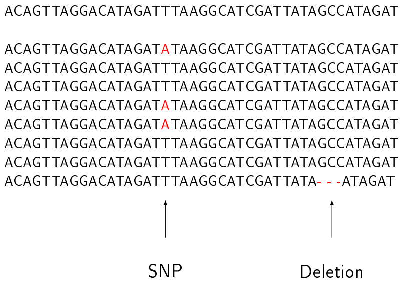

Start at largest.
Intervals may represent contigs, scaffolds, gaps, alignments, etc.
0
10%
30%
50%
70%
90%
This interval is your N50.

Figure: From Kane, N.C.

Mapping against reference genome/transcriptome

- Main purposes:
  - Identify variants (SNPs, InDels)

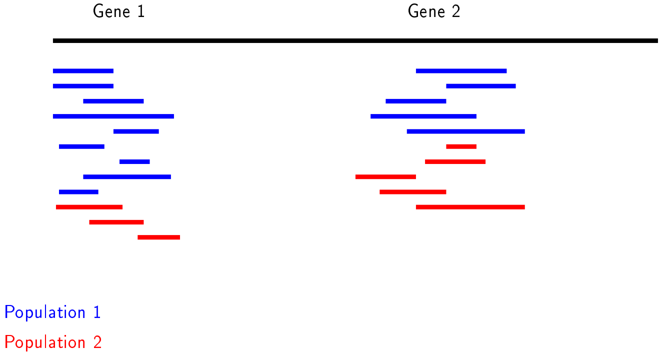


```
ACAGTTAGGACATAGATTTAAGGCATCGATTATAGCCATAGAT

ACAGTTAGGACATAGATATAAGGCATCGATTATAGCCATAGAT
ACAGTTAGGACATAGATTTAAGGCATCGATTATAGCCATAGAT
ACAGTTAGGACATAGATTTAAGGCATCGATTATAGCCATAGAT
ACAGTTAGGACATAGATATAAGGCATCGATTATAGCCATAGAT
ACAGTTAGGACATAGATATAAGGCATCGATTATAGCCATAGAT
ACAGTTAGGACATAGATTTAAGGCATCGATTATAGCCATAGAT
ACAGTTAGGACATAGATTTAAGGCATCGATTATAGCCATAGAT
ACAGTTAGGACATAGATTTAAGGCATCGATTATA- - -ATAGAT
```

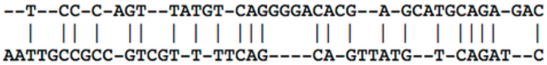
Mapping against reference genome/transcriptome

- Main purposes:
    - Quantify gene expression



Mapping: global alignment

- Implemented in e.g. BWA, Bowtie2
- Needleman-Wunsch algorithm
- Aligns sequences in their full length
- Used for multiple sequence alignment when sequences are similar

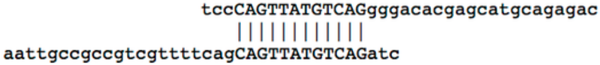


```
--T--CC-C-AGT--TATGT-CAGGGGACACG--A-GCATGCAGA-GAC
  |  || |  ||  | | | |||    || |  | |  | ||||   |
AATTGCCGCC-GTCGT-T-TTCAG----CA-GTTATG--T-CAGAT--C
```

Figure: Global alignment from rosalind.info

Mapping: local alignment

- Smith-Waterman algorithm
- Clipping of terminal unmatched bases
- Only aligned bases contribute to the alignment's score
- Used to target smaller portions of genes with high similarity



```
                  tccCAGTTATGTCAGggacacgagcatgcagagac
                     ||||||||||||
aattgccgccgtcgttttcagCAGTTATGTCAGatc
```

Figure: Local alignment from rosalind.info

Splice-aware alignment of RNAseq reads to the genome

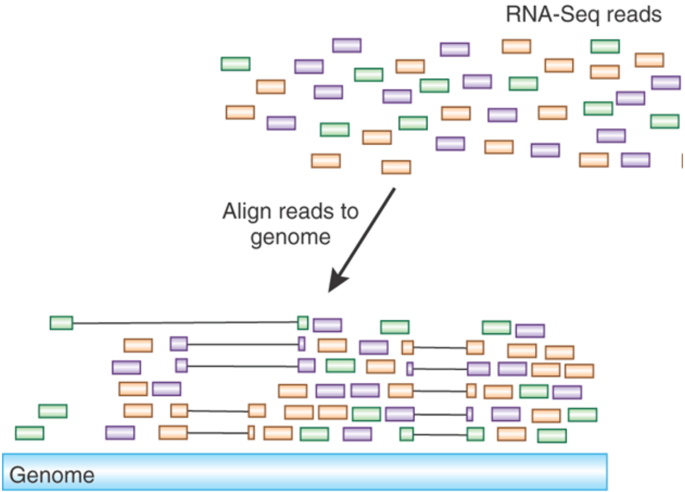
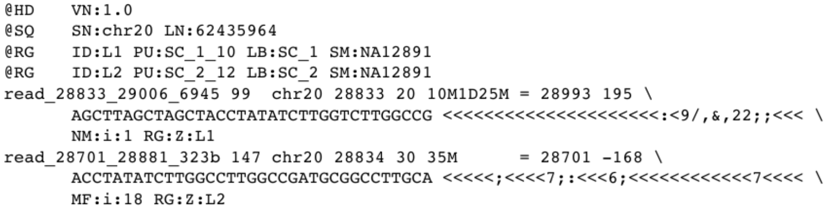


Figure: Adapted from Haas and Zody, (2010)

Mapping: SAM/BAM files example

Output format of most alignment programs

- Header lines preceded by @
- One tab-delimited line per read

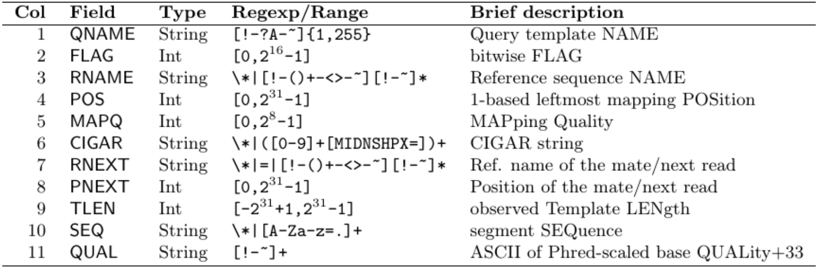


```
@HD    VN:1.0
@SQ    SN:chr20 LN:62435964
@RG    ID:L1 PU:SC_1_10 LB:SC_1 SM:NA12891
@RG    ID:L2 PU:SC_2_12 LB:SC_2 SM:NA12891
read_28833_29006_6945 99  chr20 28833 20 10M1D25M = 28993 195 \
       AGCTTAGCTAGCTACCTATATCTTGGTCTTGGCCG <<<<<<<<<<<<<<<<<<<<<:<9/,&,22;;<<< \
       NM:i:1 RG:Z:L1
read_28701_28881_323b 147 chr20 28834 30 35M      = 28701 -168 \
       ACCTATATCTTGGCCTTGGCCGATGCGGCCTTGCA <<<<<;<<<<7;:<<<6;<<<<<<<<<<<<7<<<< \
       MF:i:18 RG:Z:L2
```

Figure: Example from `http://samtools.sourceforge.net/SAM1.pdf`

- SAM files are large
- BAM: Compressed binary versions, not human-readable

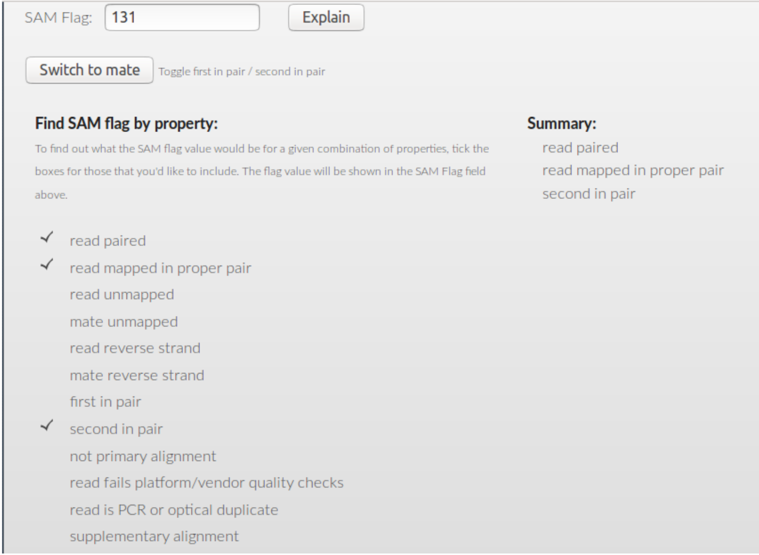
Mapping: Mandatory fields in SAM files



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^{31}-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 read
8	PNEXT	Int	[0,2^{31}-1]	Position of the mate/next read
9	TLEN	Int	[-2^{31}+1,2^{31}-1]	observed Template LENgth
10	SEQ	String	*|[A-Za-z=.]+	segment SEQuence
11	QUAL	String	[!-~]+	ASCII of Phred-scaled base QUALity+33

Explanation of the flag field (click here: Link1, Link2)

Mapping: Easy decoding of SAM flags



SAM Flag: 131 Explain

Switch to mate Toggle first in pair / second in pair

Find SAM flag by property:

To find out what the SAM flag value would be for a given combination of properties, tick the boxes for those that you'd like to include. The flag value will be shown in the SAM Flag field above.

- ✓ read paired
- ✓ read mapped in proper pair
- read unmapped
- mate unmapped
- read reverse strand
- mate reverse strand
- first in pair
- ✓ second in pair
- not primary alignment
- read fails platform/vendor quality checks
- read is PCR or optical duplicate
- supplementary alignment

Summary:

read paired
read mapped in proper pair
second in pair

Mapping: CIGAR string in SAM files

Op	BAM	Description
M	0	alignment match (can be a sequence match or mismatch)
I	1	insertion to the reference
D	2	deletion from the reference
N	3	skipped region from the reference
S	4	soft clipping (clipped sequences present in SEQ)
H	5	hard clipping (clipped sequences NOT present in SEQ)
P	6	padding (silent deletion from padded reference)
=	7	sequence match
X	8	sequence mismatch

Mapping: CIGAR string example

RefPos:	1	2	3	4	5	6	7		8	9	10	11	12	13	14	15	16
Ref:	C	C	A	T	A	C	T		G	A	A	C	T	G	A	C	T
Read:					A	C	T	A	G	A	A		T	G	G	C	T

CIGAR: 3M1I3M1D5M

Variant calling

Consistent mismatches in the alignment indicate:

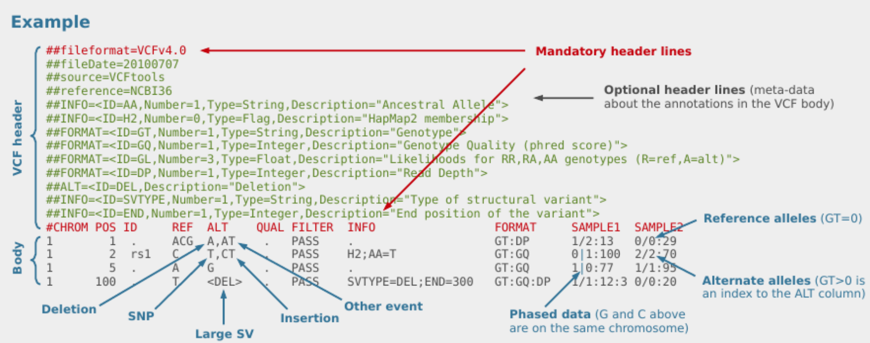
- Single Nucleotide Polymorphisms (SNPs)
- Insertions/Deletions (InDels)

VCF file format

Variant call format

- described in `http://www.1000genomes.org/node/101`
- informs on location and quality of each SNP

VCF file information



Example

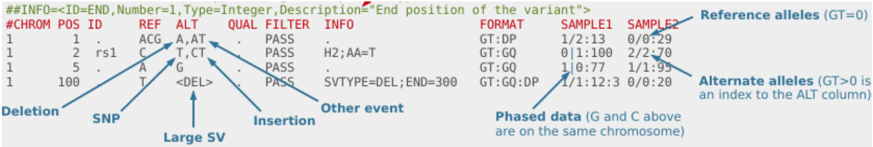
```
##fileformat=VCFv4.0
##fileDate=20100707
##source=VCFtools
##reference=NCBI36
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality (phred score)">
##FORMAT=<ID=GL,Number=3,Type=Float,Description="Likelihoods for RR,RA,AA genotypes (R=ref,A=alt)">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
#CHROM POS ID     REF ALT    QUAL FILTER  INFO                 FORMAT     SAMPLE1   SAMPLE2
1        1 .      ACG A,AT   .    PASS    .                    GT:DP      1/2:13    0/0:29
1        2 rs1    C   T,CT   .    PASS    H2;AA=T              GT:GQ      0|1:100   2/2:70
1        5 .      A   G      .    PASS    .                    GT:GQ      1|0:77    1/1:95
1      100 .      T   <DEL>  .    PASS    SVTYPE=DEL;END=300   GT:GQ:DP   1/1:12:3  0/0:20
```

VCF header
Body
Mandatory header lines
Optional header lines (meta-data about the annotations in the VCF body)
Reference alleles (GT=0)
Alternate alleles (GT>0 is an index to the ALT column)
Deletion
SNP
Large SV
Insertion
Other event
Phased data (G and C above are on the same chromosome)

Figure: VCF file info from `http://vcftools.sourceforge.net/VCF-poster.pdf`

Phased alleles are on the same chromosome strand

VCF file information



```
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
#CHROM POS ID     REF  ALT    QUAL FILTER  INFO                 FORMAT      SAMPLE1   SAMPLE2
1        1  .     ACG  A,AT    .   PASS    .                    GT:DP       1/2:13    0/0:29
1        2  rs1   C    T,CT    .   PASS    H2;AA=T              GT:GQ       0|1:100   2/2:70
1        5  .     A    G       .   PASS    .                    GT:GQ       1|0:77    1/1:95
1      100  .     T    <DEL>   .   PASS    SVTYPE=DEL;END=300   GT:GQ:DP    1/1:12:3  0/0:20
```

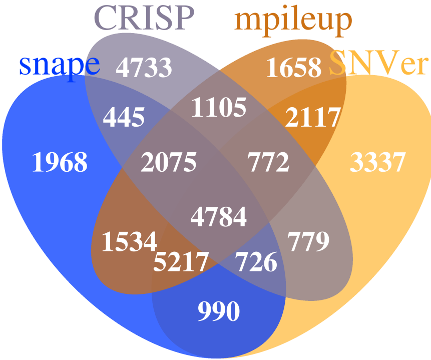
Reference alleles (GT=0)
Alternate alleles (GT>0 is an index to the ALT column)
Deletion
SNP
Large SV
Insertion
Other event
Phased data (G and C above are on the same chromosome)

Figure: VCF file info from `http://vcftools.sourceforge.net/VCF-poster.pdf`

Phased alleles are on the same chromosome strand

Identified SNPs vary between programs/algorithms

Venn diagram of the number of SNPs (coverage >400) called with four programs from the same alignment file (ddRAD tags mapped against the genome of Guppy).



Differential gene expression analysis

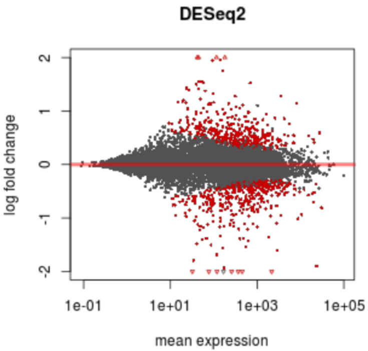


Figure: Log2 fold-change of expression over the mean of counts normalized by size factors. Differentially expressed genes (p<0.1) are red.

From the DESeq2 R package documentation

Clustering

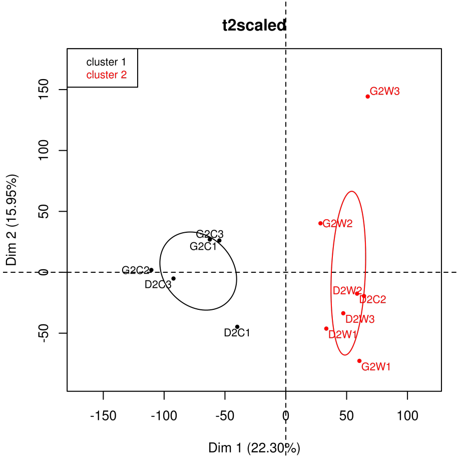


Figure: Multivariate grouping of stressed (W) and control (C) seagrass samples. Most variation is explained by the first principle component

Visualizing differential expression

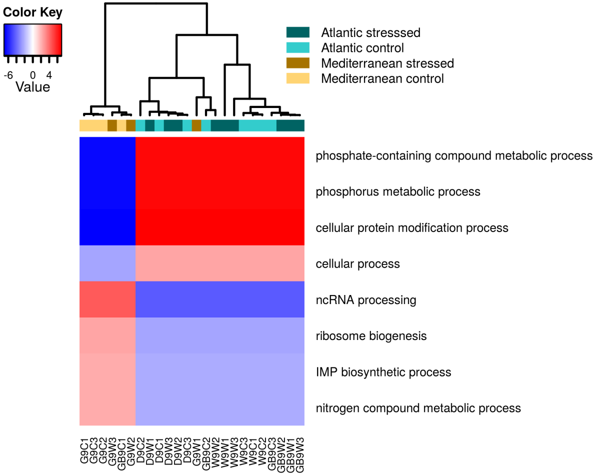
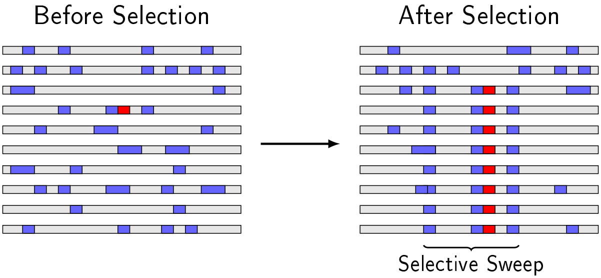


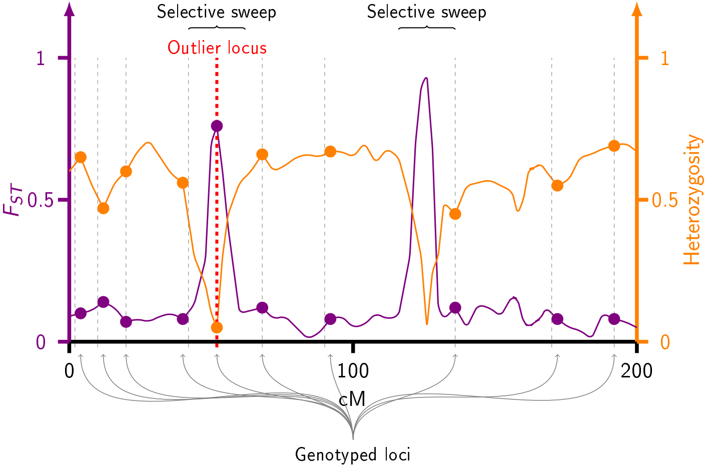
Figure: Heatmap of functions that were differentially expressed between Atlantic and Mediterranean seagrass samples.

Outlier analysis



Based on Vitti2012

Outlier detection



Gene ontologies

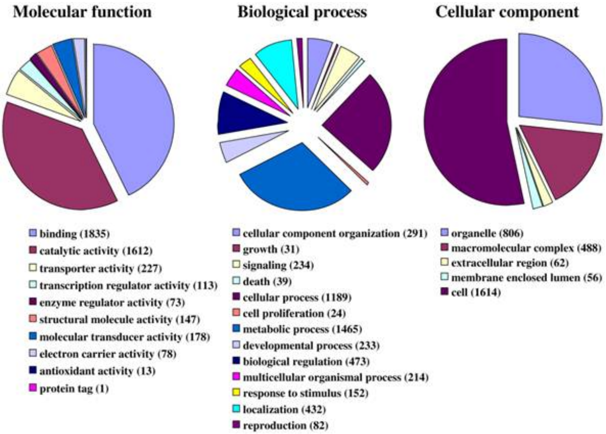


Figure: GO terms of unigenes in a moth genome

(Jacquin-Joly et al., 2012)

Cloud of GO term enrichments

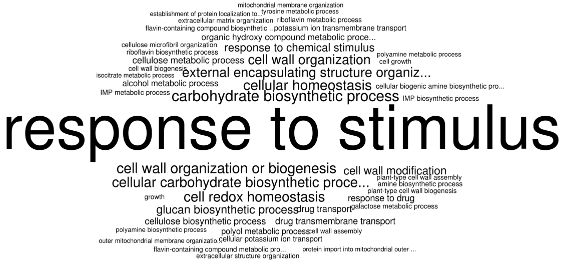


Figure: Term cloud of heat-responsive functions in seagrass

Bioinformatics-Practical

- Unix Tools
- Trimming and Quality Control
- Genome Assembly
- Mapping and Variant Calling

References I

Haas, BJ and MC Zody (2010). “Advancing RNA-Seq analysis”. In: *Nat. Biotechnol.* 28.5, pp. 421–423.

Jacquin-Joly, E, F Legeai, N Montagné, C Monsempes, MC François, J Poulain, et al. (2012). “Candidate chemosensory genes in female antennae of the noctuid moth Spodoptera littoralis.” In: *Int. J. Biol. Sci.* 8.7, pp. 1036–1050.

Martin, JA and Z Wang (2011). “Next-generation transcriptome assembly”. In: *Nat. Rev. Genet.* 12.10, pp. 671–682.