Sequence Alignment

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1 Python

1.1 Read alignment problem, Exact matching

1.1.1 Naive exact matching

Read genomic data:

```
def readGenome(filename):
    genome = ''
    with open(filename, 'r') as f:
        for line in f:
            if not line[0] == '>':
                genome += line.rstrip()
    return genome

phix_genome = readGenome('Data/phi_x.txt')
print(phix_genome[:20])
```

```
## GAGTTTTATCGCTTCCATGA
```

Naive exact matching:

```
def naive(p, t):
    occurrences = []
    for i in range(len(t) - len(p) + 1):
        match = True
        for j in range(len(p)):
            if not t[i+j] == p[j]:
                match = False
                break
        if match:
            occurrences.append(i)
    return occurrences

occur = naive('AGCG', phix_genome)
print(occur)
```

```
## [334, 723, 917, 1025, 1410, 1640, 2315, 2356, 2361, 3324, 3735, 4032, 4524, 4808]
```

Generate random artificial reads from a genome:

```
import random
def generateReads(genome, numReads, readLn):
    reads = []
    for _ in range(numReads):
        start = random.randint(0, len(genome)-readLn) - 1
        reads.append(genome[start : start+readLn])
    return reads

phix_art_seqs = generateReads(phix_genome, 100, 100)
```

Check how many of reads match the genome:

```
def numMatched(reads, genome):
    numMatched = 0
    for read in reads:
        mtaches = naive(read, genome)
        if len(mtaches) > 0:
            numMatched += 1
    print(f'{numMatched} / {len(reads)} reads matched the genome exactly.')

numMatched(phix_art_seqs, phix_genome)
```

```
## 100 / 100 reads matched the genome exactly.
```

Read real reads from Phi X genome:

```
def readReads(filename):
    sequences = []
    qualities = []
    with open(filename, 'r') as f:
        while True:
            f.readline()
            seq = f.readline().rstrip()
            f.readline()
            qual = f.readline().rstrip()
            if len(seq) == 0:
                break
            sequences.append(seq)
            qualities.append(qual)
    return sequences, qualities

phix_seqs, phix_quals = readReads('Data/ERR266411_1.first1000.txt')
print(phix_seqs[1])
```

```
## AACAAGCAGTAGTAATTCCTGCTTTATCAAGATAATTTTTCGACTCATCAGAAATATACGAAAGTGTTAACTTCTGCGTCATGGACACGAAAAAACTCCC
```

```
print(phix_quals[1])
```

```
## =@@CEBF@BGBG6GF1E<04C3E7E.9G=H<H:HH;HBH;G69F7,7DG((EG8A8,-5-8,?,,,AE>C,B?,,,64$AC'+'='+4'3*4+E322*'(
```

```
numMatched(phix_seqs, phix_genome)
```

```
## 7 / 1000 reads matched the genome exactly.
```

Check how many of the first 30 bases in each read match the genome:

```
def numMatched30(reads, genome):
    numMatched = 0
    for read in reads:
        read = read[:30]
        matches = naive(read, genome)
        if len(matches) > 0:
            numMatched += 1
    print(f'{numMatched} / {len(reads)} reads matched the genome exactly in their initial 30 bases.')

numMatched30(phix_seqs, phix_genome)
```

```
## 459 / 1000 reads matched the genome exactly in their initial 30 bases.
```

Check the exact matches in the antisense strand as well:

```
def readGenome(filename):
    genome = ''
    with open(filename, 'r') as f:
        for line in f:
            if not line[0] == '>':
                genome += line.rstrip()
    return genome

lambda_virus_genome = readGenome('Data/lambda_virus.txt')
print(lambda_virus_genome[:20])
```

```
## GGGCGGCGACCTCGCGGGTT
```

```
def reverseComplement(senseStrand):
    complement = {'A': 'T', 'T': 'A', 'C': 'G', 'G': 'C', 'N': 'N'}
    antisenseStrand = ''
    for base in senseStrand:
        antisenseStrand = complement[base] + antisenseStrand
    return antisenseStrand

lambda_virus_genomeAntisense = reverseComplement(lambda_virus_genome)
print(lambda_virus_genomeAntisense[:20])
```

```
## CGTAACCTGTCGGATCACCG
```

```
def numMatched30BothStrands(reads, genome):
    numMatched = 0
    for read in reads:
        read = read[:30]
        matches = naive(read, genome)
        matches.extend(naive(reverseComplement(read), genome))
        if len(matches) > 0:
            numMatched += 1
    print(f'{numMatched} / {len(reads)} reads matched the genome exactly in their initial 30 bases.')

numMatched30BothStrands(phix_seqs, phix_genome)
```

```
## 932 / 1000 reads matched the genome exactly in their initial 30 bases.
```

1.1.2 Boyer Moore exact matching

```
import string

def z_array(s):
    """ Use Z algorithm (Gusfield theorem 1.4.1) to preprocess s """
    assert len(s) > 1
    z = [len(s)] + [0] * (len(s)-1)
    # Initial comparison of s[1:] with prefix
    for i in range(1, len(s)):
        if s[i] == s[i-1]:
            z[1] += 1
        else:
            break
    r, l = 0, 0
    if z[1] > 0:
        r, l = z[1], 1
    for k in range(2, len(s)):
        assert z[k] == 0
        if k > r:
            # Case 1
            for i in range(k, len(s)):
                if s[i] == s[i-k]:
                    z[k] += 1
                else:
                    break
            r, l = k + z[k] - 1, k
        else:
            # Case 2
            # Calculate length of beta
            nbeta = r - k + 1
            zkp = z[k - l]
            if nbeta > zkp:
                # Case 2a: Zkp wins
                z[k] = zkp
            else:
                # Case 2b: Compare characters just past r
                nmatch = 0
                for i in range(r+1, len(s)):
                    if s[i] == s[i - k]:
                        nmatch += 1
                    else:
                        break
                l, r = k, r + nmatch
                z[k] = r - k + 1
    return z


def n_array(s):
    """ Compile the N array (Gusfield theorem 2.2.2) from the Z array """
    return z_array(s[::-1])[::-1]


def big_l_prime_array(p, n):
    """ Compile L' array (Gusfield theorem 2.2.2) using p and N array.
        L'[i] = largest index j less than n such that N[j] = |P[i:]| """
    lp = [0] * len(p)
    for j in range(len(p)-1):
        i = len(p) - n[j]
        if i < len(p):
            lp[i] = j + 1
    return lp


def big_l_array(p, lp):
    """ Compile L array (Gusfield theorem 2.2.2) using p and L' array.
        L[i] = largest index j less than n such that N[j] >= |P[i:]| """
    l = [0] * len(p)
    l[1] = lp[1]
    for i in range(2, len(p)):
        l[i] = max(l[i-1], lp[i])
    return l
```

```
def small_l_prime_array(n):
    """ Compile lp' array (Gusfield theorem 2.2.4) using N array. """
    small_lp = [0] * len(n)
    for i in range(len(n)):
        if n[i] == i+1:  # prefix matching a suffix
            small_lp[len(n)-i-1] = i+1
    for i in range(len(n)-2, -1, -1):  # "smear" them out to the left
        if small_lp[i] == 0:
            small_lp[i] = small_lp[i+1]
    return small_lp


def good_suffix_table(p):
    """ Return tables needed to apply good suffix rule. """
    n = n_array(p)
    lp = big_l_prime_array(p, n)
    return lp, big_l_array(p, lp), small_l_prime_array(n)


def good_suffix_mismatch(i, big_l_prime, small_l_prime):
    """ Given a mismatch at offset i, and given L/L' and l' arrays,
        return amount to shift as determined by good suffix rule. """
    length = len(big_l_prime)
    assert i < length
    if i == length - 1:
        return 0
    i += 1  # i points to leftmost matching position of P
    if big_l_prime[i] > 0:
        return length - big_l_prime[i]
    return length - small_l_prime[i]


def good_suffix_match(small_l_prime):
    """ Given a full match of P to T, return amount to shift as
        determined by good suffix rule. """
    return len(small_l_prime) - small_l_prime[1]


def dense_bad_char_tab(p, amap):
    """ Given pattern string and list with ordered alphabet characters, create
        and return a dense bad character table.  Table is indexed by offset
        then by character. """
    tab = []
    nxt = [0] * len(amap)
    for i in range(0, len(p)):
        c = p[i]
        assert c in amap
        tab.append(nxt[:])
        nxt[amap[c]] = i+1
    return tab


class BoyerMoore(object):
    """ Encapsulates pattern and associated Boyer-Moore preprocessing. """

    def __init__(self, p, alphabet='ACGT'):
        self.p = p
        self.alphabet = alphabet
        # Create map from alphabet characters to integers
        self.amap = {}
        for i in range(len(self.alphabet)):
            self.amap[self.alphabet[i]] = i
        # Make bad character rule table
        self.bad_char = dense_bad_char_tab(p, self.amap)
        # Create good suffix rule table
        _, self.big_l, self.small_l_prime = good_suffix_table(p)

    def bad_character_rule(self, i, c):
        """ Return # skips given by bad character rule at offset i """
        assert c in self.amap
        ci = self.amap[c]
        assert i > (self.bad_char[i][ci]-1)
        return i - (self.bad_char[i][ci]-1)

    def good_suffix_rule(self, i):
        """ Given a mismatch at offset i, return amount to shift
            as determined by (weak) good suffix rule. """
        length = len(self.big_l)
        assert i < length
        if i == length - 1:
            return 0
        i += 1  # i points to leftmost matching position of P
        if self.big_l[i] > 0:
            return length - self.big_l[i]
        return length - self.small_l_prime[i]

    def match_skip(self):
        """ Return amount to shift in case where P matches T """
        return len(self.small_l_prime) - self.small_l_prime[1]

def boyer_moore(p, p_bm, t):
    """ Do Boyer-Moore matching """
    i = 0
    occurrences = []
    while i < len(t) - len(p) + 1:
        shift = 1
        mismatched = False
        for j in range(len(p)-1, -1, -1):
            if p[j] != t[i+j]:
                skip_bc = p_bm.bad_character_rule(j, t[i+j])
                skip_gs = p_bm.good_suffix_rule(j)
                shift = max(shift, skip_bc, skip_gs)
                mismatched = True
                break
        if not mismatched:
            occurrences.append(i)
            skip_gs = p_bm.match_skip()
            shift = max(shift, skip_gs)
        i += shift
    return occurrences
```

```
t = 'GCTAGCTCTACGAGTCTA'
p = 'TCTA'
p_bm = BoyerMoore(p, alphabet='ACGT') # Preprocessing
boyer_moore(p, p_bm, t) # Boyer Moore function
```

```
## [6, 14]
```

1.1.3 k-mer indexing

```
import bisect
class Index(object):
    def __init__(self, t, k):
        self.k = k
        self.index = []
        for i in range(len(t) - k + 1):
            self.index.append((t[i:i+k], i))
        self.index.sort()

    def query(self, p):
        kmer = p[:self.k]
        i = bisect.bisect_left(self.index, (kmer, -1))
        hits = []
        while i < len(self.index):
            if self.index[i][0] != kmer:
                break
            hits.append(self.index[i][1])
            i += 1
        return hits

def queryIndex(p, t, index):
    k = index.k
    offsets = []
    for i in index.query(p):
        if p[k:] == t[i+k:i+len(p)]:
            offsets.append(i)
    print(offsets)
```

```
# Test
t = 'GCTAGCTCTACGAGTCTA'
p = 'TCTA'

index = Index(t, 2)
queryIndex(p, t, index)
```

```
## [6, 14]
```

1.2 Read alignment problem, Approximate matching

1.2.1 Boyer Moore + Pigenhole principle

Approximate function:

```python
def approximate_match(p, t, n):
    segment_length = round(len(p) / (n+1))
    all_matches = set()

    for i in range(n+1):
        start = i * segment_length
        end = min((i+1) * segment_length, len(p))
        p_bm = BoyerMoore(p[start:end], alphabet='ATCG')
        matches = boyer_moore(p[start:end], p_bm, t)

        for m in matches:
            if m < start or m-start+len(p) > len(t):
                continue

            mismatches = 0
            for j in range(0, start):
                if not p[j] == t[m-start+j]:
                    mismatches += 1
                    if mismatches > n:
                        break

            for j in range(end, len(p)):
                if not p[j] == t[m-start+j]:
                    mismatches += 1
                    if mismatches > n:
                        break

            if mismatches <= n:
                all_matches.add(m - start)

    print(list(all_matches))
```

```python
# Test
p = 'AACTTG'
t = 'CACTTAATTTG'

approximate_match(p, t, 2)
```

```
## [0, 5]
```

1.2.2 Edit distance

Recursive edit distance:

```python
def editDistRecursive(x, y):
    if len(x) == 0:
        return len(y)
    elif len(y) == 0:
        return len(x)
    else:
        distHor = editDistRecursive(x[:-1], y) + 1
        distVer = editDistRecursive(x, y[:-1]) + 1
        if x[-1] == y[-1]:
            distDiag = editDistRecursive(x[:-1], y[:-1])
        else:
            distDiag = editDistRecursive(x[:-1], y[:-1]) + 1
        return min(distHor, distVer, distDiag)
```

Dynamic edit distance:

```
def editDistance(x, y):
    # Create distance matrix
    D = []
    for i in range(len(x)+1):
        D.append([0]*(len(y)+1))
    # Initialize first row and column of matrix
    for i in range(len(x)+1):
        D[i][0] = i
    for i in range(len(y)+1):
        D[0][i] = i
    # Fill in the rest of the matrix
    for i in range(1, len(x)+1):
        for j in range(1, len(y)+1):
            distHor = D[i][j-1] + 1
            distVer = D[i-1][j] + 1
            if x[i-1] == y[j-1]:
                distDiag = D[i-1][j-1]
            else:
                distDiag = D[i-1][j-1] + 1
            D[i][j] = min(distHor, distVer, distDiag)
    # Edit distance is the value in the bottom right corner of the matrix
    return D[-1][-1]
```

```
# Test 1
x = 'ATCGATTGCGTG'
y = 'ATCCTTGC'
editDistRecursive(x, y)
```

```
## 5
```

```
# Test 2
x = 'ATCGATTGCGTG'
y = 'ATCCTTGC'
editDistance(x, y)
```

```
## 5
```

1.2.3 Global alignment

```
def globalAlignment(x, y):
    alphabet = ['A', 'C', 'G', 'T']
    score = [[0, 4, 2, 4, 8],
             [4, 0, 4, 2, 8],
             [2, 4, 0, 4, 8],
             [4, 2, 4, 0, 8],
             [8, 8, 8, 8, 8]]
    # Create distance matrix
    D = []
    for i in range(len(x)+1):
        D.append([0] * (len(y)+1))

    # Initialize first column
    for i in range(1, len(x)+1):
        D[i][0] = D[i-1][0] + score[alphabet.index(x[i-1])][-1]

    # Initialize first row
    for j in range(1,len(y)+1):
        D[0][j] = D[0][j-1] + score[-1][alphabet.index(y[j-1])]

    # Fill rest of the matrix
    for i in range(1, len(x)+1):
        for j in range(1, len(y)+1):
            distHor = D[i][j-1] + score[-1][alphabet.index(y[j-1])]
            distVer = D[i-1][j] + score[alphabet.index(x[i-1])][-1]
            distDiag = D[i-1][j-1] + score[alphabet.index(x[i-1])][alphabet.index(y[j-1])]
            D[i][j] = min(distHor, distVer, distDiag)

    print(D[-1][-1])
```

```
# Test
x = 'TATGTCATGC'
y = 'TATGTCATG'
globalAlignment(x, y)
```

```
## 8
```

1.2.4 Local alignment

```
def localAlignment(x, y):
    alphabet = ['A', 'C', 'G', 'T']
    score = [[+2, -4, -4, -4, -6],
             [-4, +2, -4, -4, -6],
             [-4, -4, +2, -4, -6],
             [-4, -4, -4, +2, -6],
             [-6, -6, -6, -6, -6]]

    # Create distance matrix
    D = []
    for i in range(len(x)+1):
        D.append([0] * (len(y)+1))

    # Initialize first column
    for i in range(1, len(x)+1):
        D[i][0] = D[i-1][0] + score[alphabet.index(x[i-1])][-1]

    # Initialize first row
    for j in range(1,len(y)+1):
        D[0][j] = D[0][j-1] + score[-1][alphabet.index(y[j-1])]
    # Fill rest of the matrix
    for i in range(1, len(x)+1):
        for j in range(1, len(y)+1):
            distHor = D[i][j-1] + score[-1][alphabet.index(y[j-1])]
            distVer = D[i-1][j] + score[alphabet.index(x[i-1])][-1]
            distDiag = D[i-1][j-1] + score[alphabet.index(x[i-1])][alphabet.index(y[j-1])]
            D[i][j] = max(distHor, distVer, distDiag)

    print(D[-1][-1])
```

```
# Test
x = 'TATGTCATGC'
y = 'TATGTCATG'
localAlignment(x, y)
```

```
## 12
```

1.3 Asssembly problem

1.3.1 Overlaps

Find overlaps between reads:

```
def overlap(a, b, min_length=3):
    start = 0
    while True:
        start = a.find(b[:min_length], start)
        if start == -1:
            return 0

        if b.startswith(a[start:]):
            return len(a)-start
        start += 1

# Test
overlap('TTACGT', 'CGTGTGC', min_length=3)
```

```
## 3
```

Naive overlap mapping:

```
from itertools import permutations
def naive_overlap_map(reads, k):
    olaps = {}
    for a, b in permutations(reads, 2):
        olen = overlap(a, b, min_length=k)
        if olen > 0:
            olaps[(a, b)] = olen
    print(olaps)

# Test
reads = ['ACGGATC', 'GATCAAGT', 'TTCACGGA']
naive_overlap_map(reads, 3)
```

```
## {('ACGGATC', 'GATCAAGT'): 4, ('TTCACGGA', 'ACGGATC'): 5}
```

1.3.2 Shortest Common Superstring (SCS)

SCS:

```
import itertools

def scs(ss):
    shortest_sup = None
    for ssperm in itertools.permutations(ss):
        sup = ssperm[0]
        for i in range(len(ss)-1):
            olen = overlap(ssperm[i], ssperm[i+1], min_length=1)
            sup += ssperm[i+1][olen:]
        if shortest_sup is None or len(sup) < len(shortest_sup):
            shortest_sup = sup
    return shortest_sup

# Test
scs(['ACGGATGAGC', 'GAGCGGA', 'GAGCGAG'])
```

```
## 'ACGGATGAGCGAGCGGA'
```

Greedy SCS:

```
def pick_maximal_overlap(reads, k):
    reada, readb = None, None
    best_olen = 0
    for a, b in itertools.permutations(reads, 2):
        olen = overlap(a, b, min_length=k)
        if olen > best_olen:
            reada, readb = a, b
            best_olen = olen
    return reada, readb, best_olen

def greedy_scs(reads, k):
    read_a, read_b, olen = pick_maximal_overlap(reads, k)
    while olen > 0:
        reads.remove(read_a)
        reads.remove(read_b)
        reads.append(read_a + read_b[olen:])
        read_a, read_b, olen = pick_maximal_overlap(reads, k)
    return ''.join(reads)

# Test
greedy_scs(['ABC', 'BCA', 'CAB'], 2)
```

```
## 'CABCA'
```

1.3.3 De Bruijn graphs and Eulerian walks

```
def de_bruijn_ize(st, k):
    """ Return a list holding, for each k-mer, its left
        k-1-mer and its right k-1-mer in a pair """
    edges = []
    nodes = set()
    for i in range(len(st) - k + 1):
        edges.append((st[i:i+k-1], st[i+1:i+k]))
        nodes.add(st[i:i+k-1])
        nodes.add(st[i+1:i+k])
    return nodes, edges

# Test
nodes, edges = de_bruijn_ize("ACGCGTCG", 3)
print(f'Nodes: \n {nodes}')
```

```
## Nodes:
##  {'GT', 'CG', 'TC', 'GC', 'AC'}
```

```
print(f'Edges: \n {edges}')
```

```
## Edges:
##  [('AC', 'CG'), ('CG', 'GC'), ('GC', 'CG'), ('CG', 'GT'), ('GT', 'TC'), ('TC', 'CG')]
```

```
def visualize_de_bruijn(st, k):
    nodes, edges = de_bruijn_ize(st, k)
    dot_str = 'digraph "DeBruijn graph" {\n'
    for node in nodes:
        dot_str += '  %s [label="%s"] ;\n' % (node, node)
    for src, dst in edges:
        dot_str += '  %s -> %s ;\n' % (src, dst)
    return dot_str + '}\n'
```

2 NGS sequencers output

NGS sequencers generate data in FASTQ and CSFASTA (FASTA) formats:

- FASTA files: the most common standard for storing reference or consensus sequence data. FASTA only stores sequences.
- FASTQ files: the most common format for storing raw sequence data. FASTQ stores both sequence and associated sequence quality values (QV).

2.1 FASTQ file

Let's check a FASTQ file:

```
head -n 4 Data/SRR835775_1.first1000.fastq | less -S
```

```
## @SRR835775.1 1/1
## TAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTCACCCTAACCCTAACCCTAACCGTATCCGTCACCCTAACCCTAAC
## +
## ???B1ADDD8??BB+C?B+:AA883CEE8?C3@DDD3)?D2;DC?8?=BAD=@C@(.6.6=A?=?@##################################
```

2.2 FASTA file

Now let's check the FASTA file of human genome chromosome 1:

```
# Assemble parts
gunzip -c Reference/Homo_sapiens.GRCh38.dna.chromosome.1.part1.fa.gz > \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part1.fa
gunzip -c Reference/Homo_sapiens.GRCh38.dna.chromosome.1.part2.fa.gz > \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part2.fa
gunzip -c Reference/Homo_sapiens.GRCh38.dna.chromosome.1.part3.fa.gz > \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part3.fa
gunzip -c Reference/Homo_sapiens.GRCh38.dna.chromosome.1.part4.fa.gz > \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part4.fa
gunzip -c Reference/Homo_sapiens.GRCh38.dna.chromosome.1.part5.fa.gz > \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part5.fa

cat Output/Homo_sapiens.GRCh38.dna.chromosome.1.part1.fa \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part2.fa \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part3.fa \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part4.fa \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part5.fa > \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.fa

rm Output/Homo_sapiens.GRCh38.dna.chromosome.1.part1.fa \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part2.fa \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part3.fa \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part4.fa \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.part5.fa

# Show
head -n 1000 Output/Homo_sapiens.GRCh38.dna.chromosome.1.fa | tail -5 | less -S
```

```
## CCAAATTTCTTTCCAATTCATCTTTGTTCTTCCCTTTCCTTTTTACTCTCTTTAAACATT
## CTATGGACTCTGCCTCCTTCACACTGATATTGAACGCCCATAGTTTCATATTTTGGATTG
## CGATTGTTTTATTTTAAAATGGCAAATGTTCATGTTATAAAGAGAATTTTTCAGTCTTTA
## GACTAATAGGTTCATGTAGTTTGGGATTTTCCTCTTTAAGAAAATTAATTATCACTCACA
## CTCCAAGACAAACACCATTTCAGTAGCAATATGAATTTCAGTAGTAATAGGAATCTCCAA
```

3 Aligned sequences

The SAM file format External link and its compressed version, the BAM format, were developed to store information about the alignment of sequences to a reference genome.

3.1 SAM file

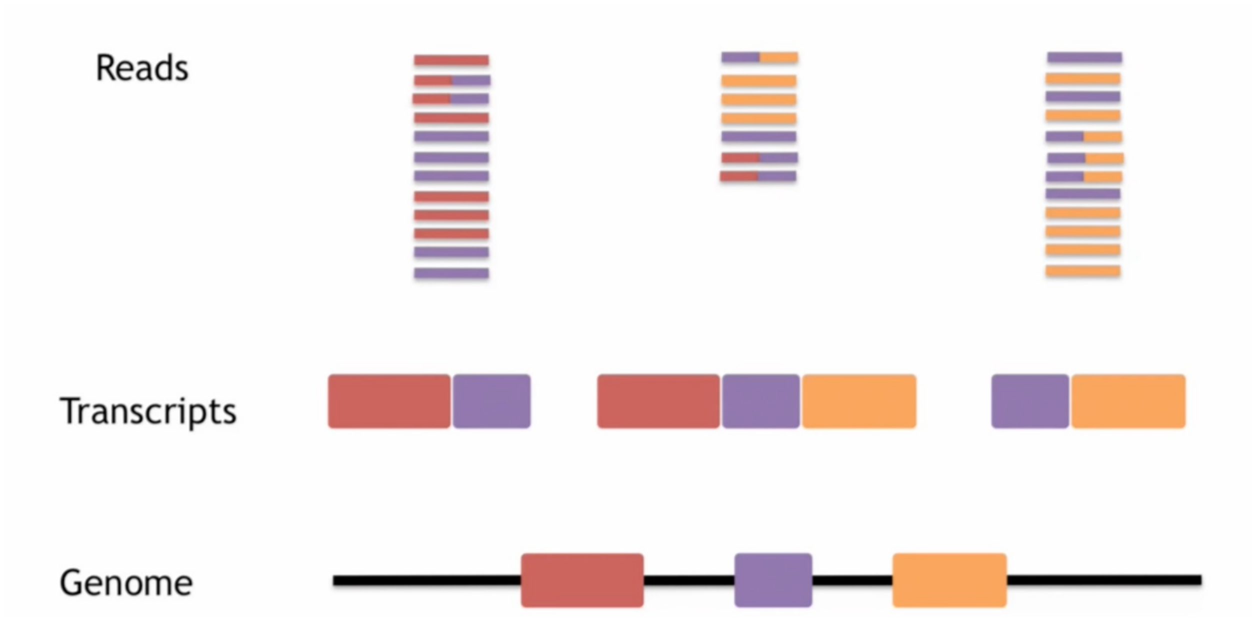
Let's check a SAM file:

```
head -n 10 Data/Sample_sam.sam | tail -1 | less -S
```

```
## SRR1039508.10901400  16  1   32585836    3   43M *   0   0   CTGCTAAAAATACAAAAATCAGCCGGGCCTGGTGGTGTG
```

4 RNA-seq

Initially, we should go from reads to transcripts. Note that a gene might also have different transcripts. Also, it is highly advised to perform some QC and pre-processing before alignment.



Approaches to transcript building:

- Assembly approaches
- Alignment approaches (to a reference genome): BWA, Bowtie2, STAR

Before continuing, let's check the position of a read (which should be on chr16) using a simple BLAST search:

```
library(BSgenome.Hsapiens.UCSC.hg19)
matchPattern(
  "TCGATCCATCGATTGGAAGGCACTGATCTGGACTGTCAGGTTGGTGGTCTTATTTGCAAGTCC",
  Hsapiens$chr16
)
```

```
## Views on a 90354753-letter DNAString subject
## subject: NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
## views:
##          start      end width
##   [1] 72130081 72130143    63 [TCGATCCATCGATTGGAAGGCAC...TGGTGGTCTTATTTGCAAGTCC]
```

Now let's check another read:

```
library(BSgenome.Hsapiens.UCSC.hg19)
matchPattern(
  "TCGCTCTCTCCGTTTCAGGGAAGCCAGCAAGTCCAGTCCGAGTAATGAAGGGCGGGGAGCAGG",
  Hsapiens$chr16
)
```

```
## Views on a 90354753-letter DNAString subject
## subject: NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
## views: NONE
```

Nothing? Let's check if it is from the other strand:

```
library(BSgenome.Hsapiens.UCSC.hg19)
matchPattern(reverseComplement(
  DNAString(
    "TCGCTCTCTCCGTTTCAGGGAAGCCAGCAAGTCCAGTCCGAGTAATGAAGGGCGGGGAGCAGG"
  )
),
Hsapiens$chr16)
```

```
## Views on a 90354753-letter DNAString subject
## subject: NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
## views:
##          start      end width
##   [1] 72130180 72130242    63 [CCTGCTCCCCGCCCTTCATTACT...TTCCCTGAAACGGAGAGAGCGA]
```

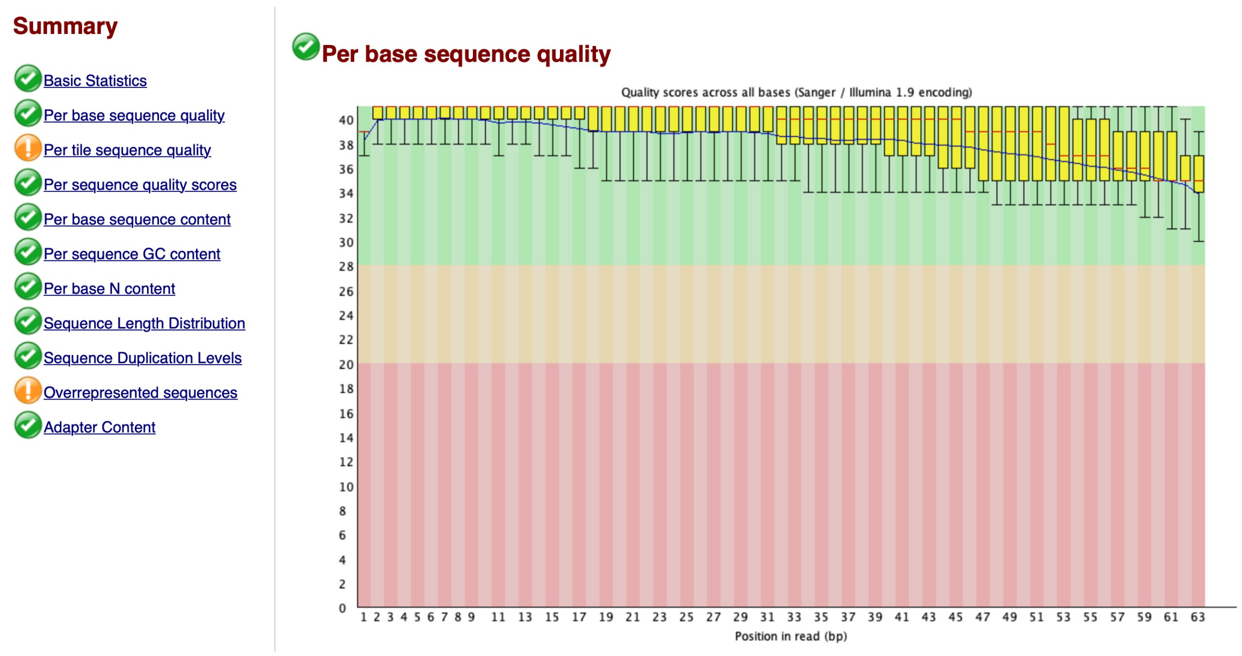
4.1 QC and pre-processing

First, let's run QC using FASTQC algorithm:

```
fastqc --noextract Data/SRR1039508.fastq --outdir Output/
```

```
## null
## Started analysis of SRR1039508.fastq
## Approx 5% complete for SRR1039508.fastq
## Approx 10% complete for SRR1039508.fastq
## Approx 15% complete for SRR1039508.fastq
## Approx 20% complete for SRR1039508.fastq
## Approx 25% complete for SRR1039508.fastq
## Approx 30% complete for SRR1039508.fastq
## Approx 35% complete for SRR1039508.fastq
## Approx 40% complete for SRR1039508.fastq
## Approx 45% complete for SRR1039508.fastq
## Approx 50% complete for SRR1039508.fastq
## Approx 55% complete for SRR1039508.fastq
## Approx 60% complete for SRR1039508.fastq
## Approx 65% complete for SRR1039508.fastq
## Approx 70% complete for SRR1039508.fastq
## Approx 75% complete for SRR1039508.fastq
## Approx 80% complete for SRR1039508.fastq
## Approx 85% complete for SRR1039508.fastq
## Approx 90% complete for SRR1039508.fastq
## Approx 95% complete for SRR1039508.fastq
## Approx 100% complete for SRR1039508.fastq
## Analysis complete for SRR1039508.fastq
```

The output looks like this:


Summary
Basic Statistics
Per base sequence quality
Per tile sequence quality
Per sequence quality scores
Per base sequence content
Per sequence GC content
Per base N content
Sequence Length Distribution
Sequence Duplication Levels
Overrepresented sequences
Adapter Content
Per base sequence quality
Quality scores across all bases (Sanger / Illumina 1.9 encoding)
Position in read (bp)

Now let's do some preprocessing. Steps required include:

- Filter the data by removing adapters.
- Trim low quality cycles.
- Remove low quality reads.

A good program for Illumina data is **trimmomatic**:

```
Java -jar /Users/amirvalizadeh/Trimmomatic/dist/jar/trimmomatic-0.40-rc1.jar \
SE -phred33 Data/SRR1039508.fastq Output/SRR1039508_qc.fastq \
ILLUMINACLIP:/Users/amirvalizadeh/Trimmomatic/adapters/TruSeq3-PE.fa:2:30:10 \
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:50 HEADCROP:10 CROP:80
```

```
## TrimmomaticSE: Started with arguments:
##  -phred33 Data/SRR1039508.fastq Output/SRR1039508_qc.fastq ILLUMINACLIP:/Users/amirvalizadeh/Trimmoma
## Automatically using 4 threads
## Using PrefixPair: 'TACACTCTTTCCCTACACGACGCTCTTCCGATCT' and 'GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT'
## ILLUMINACLIP: Using 1 prefix pairs, 0 forward/reverse sequences, 0 forward only sequences, 0 reverse
## Input Reads: 100000 Surviving: 97309 (97.31%) Dropped: 2691 (2.69%)
## TrimmomaticSE: Completed successfully
```

Notice that:

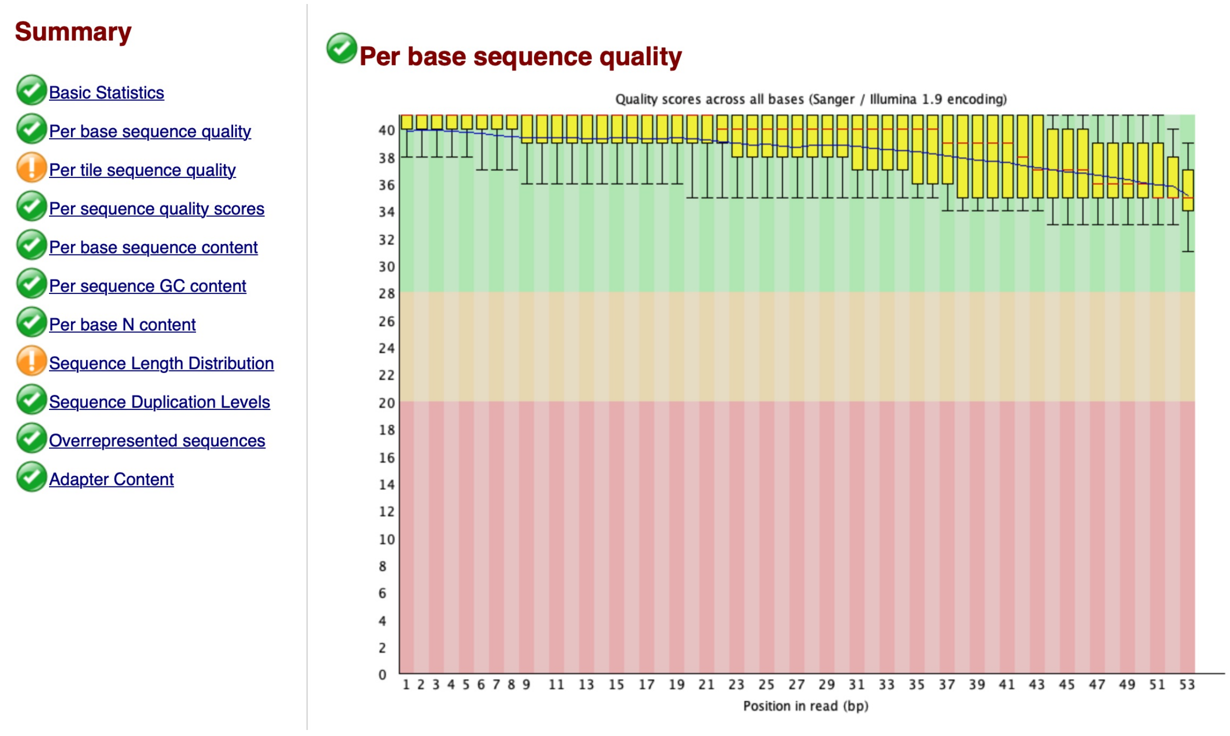
- **SE** means single end reads, for paired-end data use **PE**.
- **phred** is the quality scoring scheme.
- **ILLUMINACLIP** points to the file with the adapters.
- **LEADING** and **TRAILING** remove bases at the beginning and end of the reads below a score of 3.
- **SLIDINGWINDOW** slides across the sequence and removes those inside a window of length 4 that have an average quality below 15.
- **MINLEN** removes sequences that are shorter than 50 after filtering.
- **HEADCROP** removes the first 10 sequences at the start of the run.
- **CROP** deletes everything after the first 80 bases (recall the quality was not so good after 80 cycles).

Now, let's run QC again:

```
fastqc --noextract Output/SRR1039508_qc.fastq --outdir Output/
```

```
## null
## Started analysis of SRR1039508_qc.fastq
## Approx 5% complete for SRR1039508_qc.fastq
## Approx 10% complete for SRR1039508_qc.fastq
## Approx 15% complete for SRR1039508_qc.fastq
## Approx 20% complete for SRR1039508_qc.fastq
## Approx 25% complete for SRR1039508_qc.fastq
## Approx 30% complete for SRR1039508_qc.fastq
## Approx 35% complete for SRR1039508_qc.fastq
## Approx 40% complete for SRR1039508_qc.fastq
## Approx 45% complete for SRR1039508_qc.fastq
## Approx 50% complete for SRR1039508_qc.fastq
## Approx 55% complete for SRR1039508_qc.fastq
## Approx 60% complete for SRR1039508_qc.fastq
## Approx 65% complete for SRR1039508_qc.fastq
## Approx 70% complete for SRR1039508_qc.fastq
## Approx 75% complete for SRR1039508_qc.fastq
## Approx 80% complete for SRR1039508_qc.fastq
## Approx 85% complete for SRR1039508_qc.fastq
## Approx 90% complete for SRR1039508_qc.fastq
## Approx 95% complete for SRR1039508_qc.fastq
## Analysis complete for SRR1039508_qc.fastq
```

The output looks like this:


Summary
✓ Basic Statistics
✓ Per base sequence quality
! Per tile sequence quality
✓ Per sequence quality scores
✓ Per base sequence content
✓ Per sequence GC content
✓ Per base N content
! Sequence Length Distribution
✓ Sequence Duplication Levels
✓ Overrepresented sequences
✓ Adapter Content
Per base sequence quality
Quality scores across all bases (Sanger / Illumina 1.9 encoding)
Position in read (bp)

4.2 Aligning reads to genome with STAR

1- Prepare the reference genome FASTA file (only chromosome 1 for this example). Take a peak:

```
head -n 1000 Output/Homo_sapiens.GRCh38.dna.chromosome.1.fa | tail -10 | less -S
```

```
## CACCTATTCACTCACAAGCTTAAACTCTTAACTTTTCTCCACATATCAGTGACTATTTCC
## TACAGCTTTTCTTTTACTTTCCATGTTTGCAGTGACAATATACATAAACAGTGTATGAAA
## ACTCAAGTAAAATCTACTCTCTCAGGTGTTCATAATGTATCAATGTATATTGCTTTAAGC
## CTGAAGGTAACCTAAGTAAAGATGTACCATGTTCCACCAATGCTTCTTTTGATCATCATT
## TTATCCTGTTTTTTCTTTAGGATTCTTTCTTATTCCTTCCCCTGACCCTTCTTTTATTCT
## CCAAATTTCTTTCCAATTCATCTTTGTTCTTCCCTTTCCTTTTTACTCTCTTTAAACATT
## CTATGGACTCTGCCTCCTTCACACTGATATTGAACGCCCATAGTTTCATATTTTGGATTG
## CGATTGTTTTATTTTAAAATGGCAAATGTTCATGTTATAAAGAGAATTTTTCAGTCTTTA
## GACTAATAGGTTCATGTAGTTTGGGATTTTCCTCTTTAAGAAAATTAATTATCACTCACA
## CTCCAAGACAAACACCATTTCAGTAGCAATATGAATTTCAGTAGTAATAGGAATCTCCAA
```

2- Prepare the reference genome annotation (gtf) file (only chromosome 1 again). Take a peak:

```
# Assemble
gunzip -c Reference/Homo_sapiens.GRCh38.107.chrom1.gtf.gz > \
Output/Homo_sapiens.GRCh38.107.chrom1.gtf

# Show
head -n 1 Output/Homo_sapiens.GRCh38.107.chrom1.gtf | head -c 41 | less -S
```

```
## 1    ensembl_havana  gene    1471765 1497848 .   +
```

3- Run the code below to generate the genome index:

```
STAR --runThreadN 12 \
--runMode genomeGenerate \
--genomeSAindexNbases 12 \
--genomeDir Output \
--genomeFastaFiles Output/Homo_sapiens.GRCh38.dna.chromosome.1.fa \
--sjdbGTFfile Output/Homo_sapiens.GRCh38.107.chrom1.gtf \
--sjdbOverhang 62
```

```
##  STAR --runThreadN 12 --runMode genomeGenerate --genomeSAindexNbases 12 --genomeDir Output --genomeF
##  STAR version: 2.7.10a_alpha_220207   compiled:  :/Users/travis/build/alexdobin/travis-tests/STARcomp
## May 13 20:09:34 ..... started STAR run
## May 13 20:09:34 ... starting to generate Genome files
## May 13 20:09:39 ..... processing annotations GTF
## May 13 20:09:43 ... starting to sort Suffix Array. This may take a long time...
## May 13 20:09:45 ... sorting Suffix Array chunks and saving them to disk...
## May 13 20:13:55 ... loading chunks from disk, packing SA...
## May 13 20:14:02 ... finished generating suffix array
## May 13 20:14:02 ... generating Suffix Array index
## May 13 20:14:23 ... completed Suffix Array index
## May 13 20:14:23 ..... inserting junctions into the genome indices
## May 13 20:14:47 ... writing Genome to disk ...
## May 13 20:14:48 ... writing Suffix Array to disk ...
## May 13 20:14:53 ... writing SAindex to disk
## May 13 20:14:54 ..... finished successfully
```

4- Run the code below to map the reads to the indexed genome:

```
STAR --runThreadN 12 \
--genomeDir Output \
--readFilesIn Output/SRR1039508_qc.fastq

mv Aligned.out.sam Output/Aligned.out.sam
mv Log.final.out Output/Log.final.out
mv Log.progress.out Output/Log.progress.out
mv SJ.out.tab Output/SJ.out.tab
mv Log.out Output/Log.out
```

```
##  STAR --runThreadN 12 --genomeDir Output --readFilesIn Output/SRR1039508_qc.fastq
##  STAR version: 2.7.10a_alpha_220207   compiled:  :/Users/travis/build/alexdobin/travis-tests/STARcom
## May 13 20:14:54 ..... started STAR run
## May 13 20:14:54 ..... loading genome
## May 13 20:14:57 ..... started mapping
## May 13 20:15:24 ..... finished mapping
## May 13 20:15:24 ..... finished successfully
```

Check the log file:

```
cat Output/Log.final.out
```

```
##                                  Started job on |     May 13 20:14:54
##                              Started mapping on |     May 13 20:14:57
##                                     Finished on |     May 13 20:15:24
##        Mapping speed, Million of reads per hour |     12.97
##
##                           Number of input reads |     97309
##                       Average input read length |     52
##                                     UNIQUE READS:
##                    Uniquely mapped reads number |     18097
##                         Uniquely mapped reads % |     18.60%
##                           Average mapped length |     52.39
##                        Number of splices: Total |     1608
##             Number of splices: Annotated (sjdb) |     1521
##                        Number of splices: GT/AG |     1575
##                        Number of splices: GC/AG |     21
##                        Number of splices: AT/AC |     1
##                Number of splices: Non-canonical |     11
##                       Mismatch rate per base, % |     2.47%
##                          Deletion rate per base |     0.02%
##                         Deletion average length |     1.58
##                         Insertion rate per base |     0.02%
##                        Insertion average length |     1.53
##                              MULTI-MAPPING READS:
##         Number of reads mapped to multiple loci |     2255
##              % of reads mapped to multiple loci |     2.32%
##         Number of reads mapped to too many loci |     108
##              % of reads mapped to too many loci |     0.11%
##                                   UNMAPPED READS:
##   Number of reads unmapped: too many mismatches |     0
##        % of reads unmapped: too many mismatches |     0.00%
##            Number of reads unmapped: too short |     76800
##                 % of reads unmapped: too short |     78.92%
##                Number of reads unmapped: other |     49
##                     % of reads unmapped: other |     0.05%
##                                   CHIMERIC READS:
##                        Number of chimeric reads |     0
##                             % of chimeric reads |     0.00%
```

Check some lines of the output:

```
head -n 40 Output/Aligned.out.sam | tail -1 | less -S
```

```
## SRR1039508.8775416   16  1   120152584   255 53M *   0   0   AGGAGCAAATAAGGAAAGAGGTATTACAAACCAAGAGTA
```

Finally, it's better to convert SAM files to BAM:

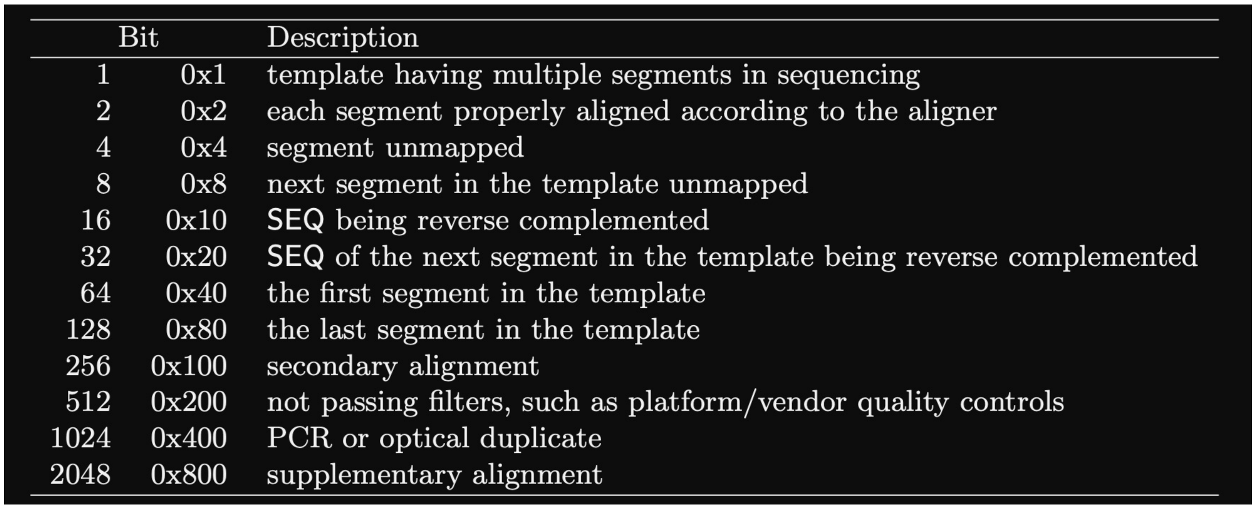
```
samtools view -S -b Output/Aligned.out.sam > Output/Aligned.out.bam
```

Let's import it now and check its data:

```
library(Rsamtools)
bam <- scanBam("Output/Aligned.out.bam")
```

Field	Type	Brief description
QNAME	String	Query template name
FLAG	Int	bitwise flag
RNAME	String	Reference sequence name
POS	Int	1-based leftmost mapping position
MAPQ	Int	Mapping quality
CIGAR	String	CIGAR string
RNEXT	String	Reference name of the mate/next read
PNEXT	Int	Position of the mate/next read
TLEN	Int	Observed template length
SEQ	String	Segment sequence
QUAL	String	ASCII of Phred-scaled base quality

QNAME: Reads/segments having identical QNAME are regarded to come from the same template. A QNAME '' *indicates the information is unavailable.* ***FLAG:*** *This data could be translated using the following website:* http://broadinstitute.github.io/picard/explain-flags.html*



Bit		Description
1	0x1	template having multiple segments in sequencing
2	0x2	each segment properly aligned according to the aligner
4	0x4	segment unmapped
8	0x8	next segment in the template unmapped
16	0x10	SEQ being reverse complemented
32	0x20	SEQ of the next segment in the template being reverse complemented
64	0x40	the first segment in the template
128	0x80	the last segment in the template
256	0x100	secondary alignment
512	0x200	not passing filters, such as platform/vendor quality controls
1024	0x400	PCR or optical duplicate
2048	0x800	supplementary alignment

RNAME: Reference sequence name of the alignment. **POS:** 1-based leftmost mapping POSition of the first CIGAR operation that "consumes" a reference base. The first base in a reference sequence has coordinate 1. POS is set as 0 for an unmapped read without coordinate. **MAPQ:** Mapping quality. A value 255 indicates that the mapping quality is not available. **CIGAR:**

Op	BAM	Description	Consumes query	Consumes reference
M	0	alignment match (can be a sequence match or mismatch)	yes	yes
I	1	insertion to the reference	yes	no
D	2	deletion from the reference	no	yes
N	3	skipped region from the reference	no	yes
S	4	soft clipping (clipped sequences present in SEQ)	yes	no
H	5	hard clipping (clipped sequences NOT present in SEQ)	no	no
P	6	padding (silent deletion from padded reference)	no	no
=	7	sequence match	yes	yes
X	8	sequence mismatch	yes	yes

RNEXT: Reference sequence name of the primary alignment of the next read in the template. For the last read, the next read is the first read in the template. **PNEXT:** 1-based Position of the primary alignment of the next read in the template. Set as 0 when the information is unavailable. This field equals POS at the primary line of the next read. **TLEN:** For primary reads where the primary alignments of all reads in the template are mapped to the same reference sequence, the absolute value of TLEN equals the distance between the mapped end of the template and the mapped start of the template **SEQ:** If not a '*', the length of the sequence must equal the sum of lengths of M/I/S/=/X operations in CIGAR. An '=' denotes the base is identical to the reference base. **QUAL:** Base quality.

Let's check the data for our example:

```
bam[[1]]$qname[1:4]
```

```
## [1] "SRR1039508.12982779" "SRR1039508.15992012" "SRR1039508.18072752"
## [4] "SRR1039508.21513031"
```

```
bam[[1]]$flag[1:4]
```

```
## [1] 16 16  0  0
```

```
bam[[1]]$strand[1:4]
```

```
## [1] - - + +
## Levels: + - *
```

```
bam[[1]]$rname[1:4]
```

```
## [1] 1 1 1 1
## Levels: 1
```

```
bam[[1]]$pos[1:4]
```

```
## [1] 104153271 180189598  55154187  58781345
```

```
bam[[1]]$cigar[1:4]
```

```
## [1] "53M" "53M" "53M" "53M"
```

```
bam[[1]]$seq[1:4]
```

```
## DNAStringSet object of length 4:
##     width seq
## [1]    53 GGGGCCCTTCAGAAGTCGCTGGGCTCAGAAGGCTCTTAGTCGTGCTTGAGAGT
## [2]    53 ATGAATGGCTCAAGAGGCAGAAGAGAAATAAAATTCCCTACAGTTTCTTTAAA
## [3]    53 CACTAAGGATTGTCAGGTGCTCCTCCAAGGCCTGCTGAATAAGGCTACTGGGC
## [4]    53 TCTTAAATATCCTTTCTGGAATTTTCAGAAACAAAACATAAAAAAATTATATA
```

```
bam[[1]]$qual[1:4]
```

```
## PhredQuality object of length 4:
##     width seq
## [1]    53 DDDDDEEEFFFFFFHIIHIHC=JJJJJJJJJJJJJJJIGIJIJJJJJJJJJ
## [2]    53 HHHJJJJJJJJJJJJJJJJJJJJJJJJJIJJJJJJJJGHJJJJJJJJJJJJ
## [3]    53 JJJJJJJJHJIJIJJJJFHIJJJJIJIJJJJJJJJIJJJJJJJJJIIJJJJJI
## [4]    53 JIJJJJJJJJGHIGJJJJHIJJJJJIIIJJJJJJJJJJJIJJJIGHIJJHFE
```

4.3 Aligning reads to transcriptome with RSEM

RSEM expects a GTF file with only exons. So, first let's extract exons from our *gtf* file:

```
awk '$3 == "exon"' Output/Homo_sapiens.GRCh38.107.chrom1.gtf > Output/Homo_sapiens.GRCh38.107.chrom1.ex
```

RSEM will then prepare a reference transcriptome against which to align reads.

```
mkdir Output/rsemGenome
rsem-prepare-reference --gtf Output/Homo_sapiens.GRCh38.107.chrom1.exons.gtf \
Output/Homo_sapiens.GRCh38.dna.chromosome.1.fa \
Output/rsemGenome/GRCh38.107.chrom1
```

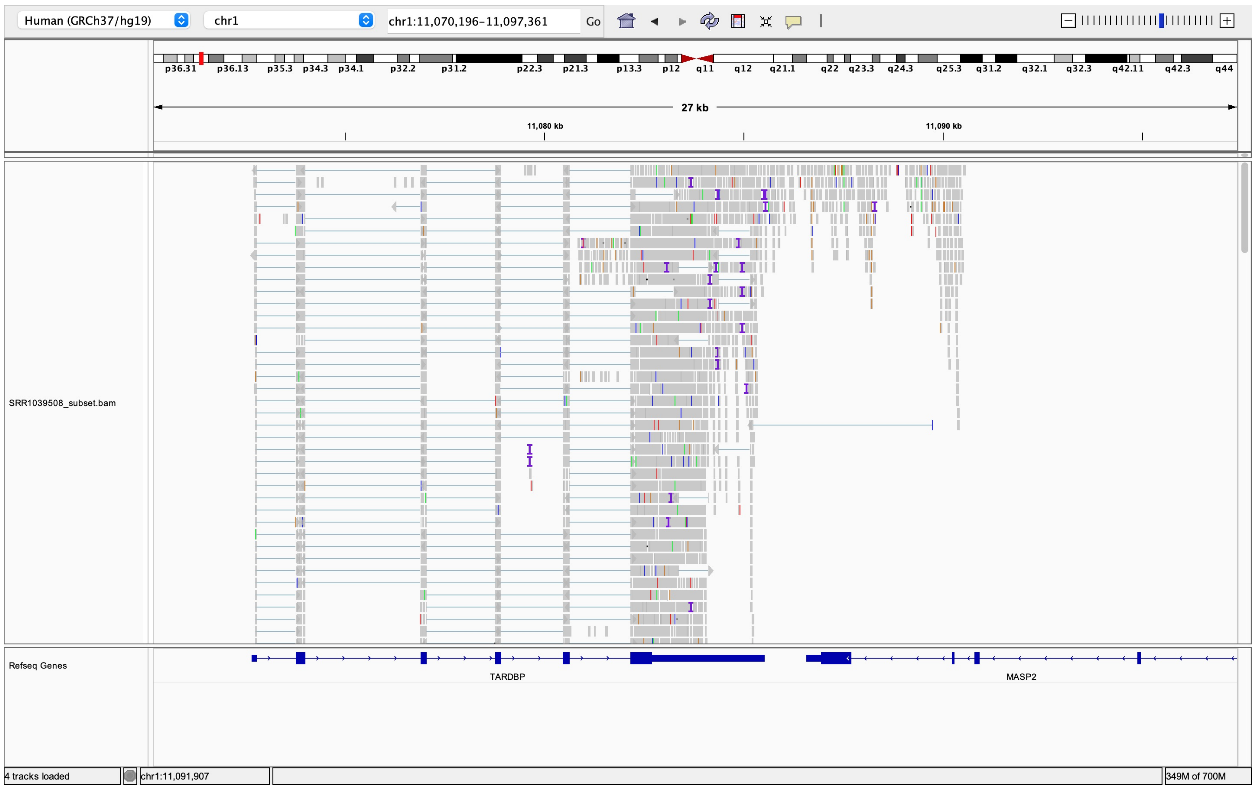
```
## rsem-extract-reference-transcripts Output/rsemGenome/GRCh38.107.chrom1 0 Output/Homo_sapiens.GRCh38.
## Parsing gtf File is done!
## Output/Homo_sapiens.GRCh38.dna.chromosome.1.fa is processed!
## 22393 transcripts are extracted.
## Extracting sequences is done!
## Group File is generated!
## Transcript Information File is generated!
## Chromosome List File is generated!
## Extracted Sequences File is generated!
##
## rsem-preref Output/rsemGenome/GRCh38.107.chrom1.transcripts.fa 1 Output/rsemGenome/GRCh38.107.chrom1
## Refs.makeRefs finished!
## Refs.saveRefs finished!
## Output/rsemGenome/GRCh38.107.chrom1.idx.fa is generated!
## Output/rsemGenome/GRCh38.107.chrom1.n2g.idx.fa is generated!
```

Finally, let's align reads:

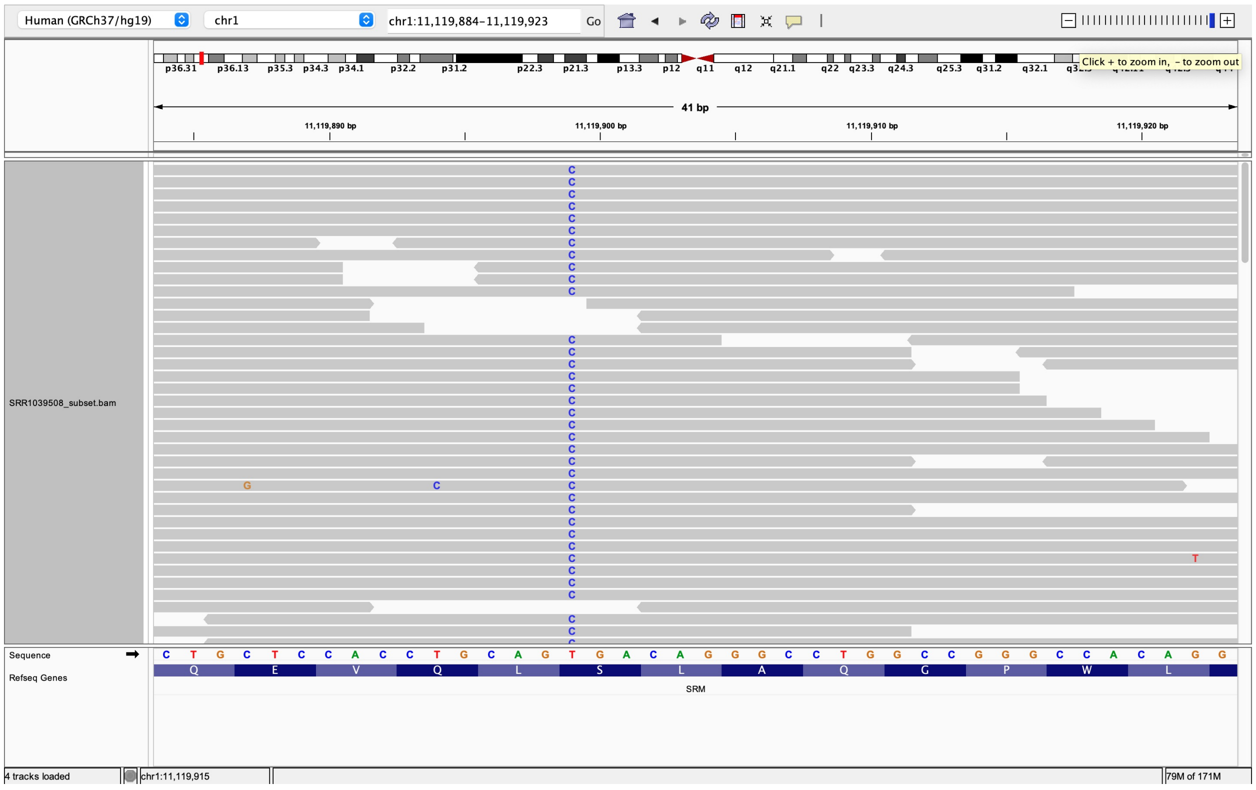
```
rsem-calculate-expression -p 12 Data/SRR1039508.fastq \
Output/rsemGenome/GRCh38.107.chrom1 SRR1039508
```

5 IGV

We can use IGV to visualize sequencing data. In this example, we will take a look at the reads aligned to reference genome in the file *SRR1039508_subset.bam* with its index file named *SRR1039508_subset.bam.bai*.



There is also a SNP at basepair number 11119899:


Human (GRCh37/hg19)
chr1
chr1:11,119,884-11,119,923
Go
Click + to zoom in, – to zoom out
41 bp
11,119,890 bp
11,119,900 bp
11,119,910 bp
11,119,920 bp
SRR1039508_subset.bam
Sequence
Refseq Genes
C T G C T C C A C C T G C A G T G A C A G G G C C T G G C C G G G C C A C A G G
Q E V Q L S L A Q G P W L
SRM
4 tracks loaded
chr1:11,119,915
79M of 171M