Institut Pasteur

# Characterization of Genome Coverage and Identification of Genomic Regions of Interest (ROIs)

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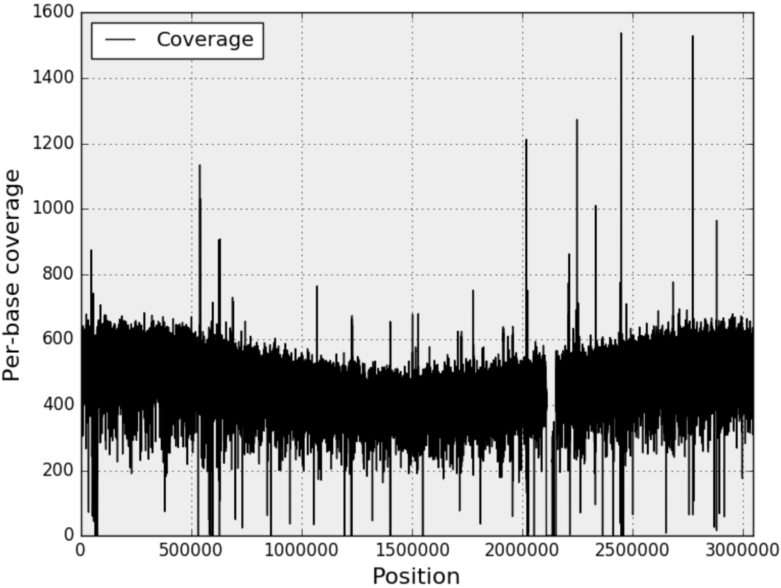
# Genome coverage

**Definition:** The number of reads mapped to a specific position, $b$, within the reference genome.

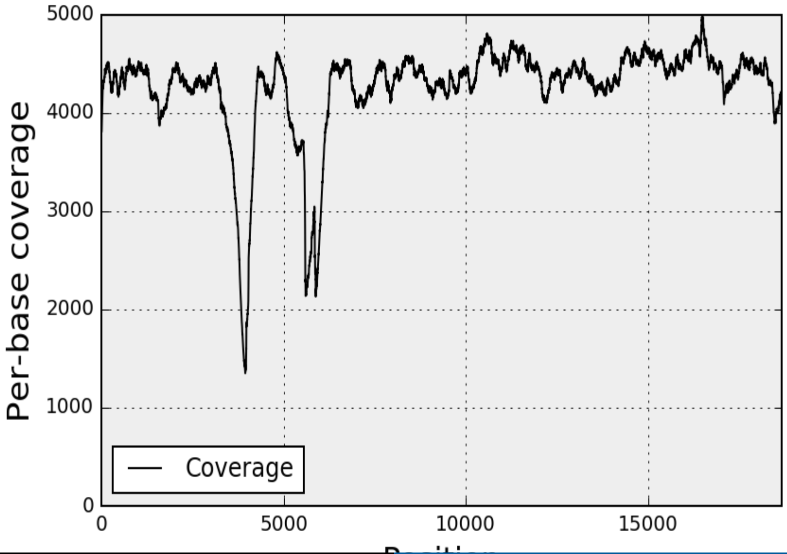
**Notation:** $C(b)$ also denoted $C_b$

**Theoretical distribution:** Poisson distribution but in practice over dispersed. The poisson parameter is distributed according to a Gamma hence leading to a negative binomial (See e.g., Linder et al 2013).

# Bacteria case (low/high $\mu$ components and del. region)



# Virus case


Per-base coverage
5000
4000
3000
2000
1000
0
0
5000
10000
15000
Coverage
Position

Question: how to automatically detect and characterise under and over covered genomic regions

# The algorithm

1. Detrending (running median)
2. Mixture model estimation (Gaussian approximation)
3. Set a statistics (z-score)
4. Clustering (double threshold)

# 1. Detrending

We divide $C_b$ by its moving average (MA), or even better its running median (RM) defined as

$$RM_W(b) = \mathrm{median}\left(C(b-V), \ldots, C(b+V)\right)$$

$W$ is the running window and $V = (W-1)/2$.

### The normalised genome coverage

$$\widetilde{C}_b = \frac{C_b}{RM_W(b)}$$

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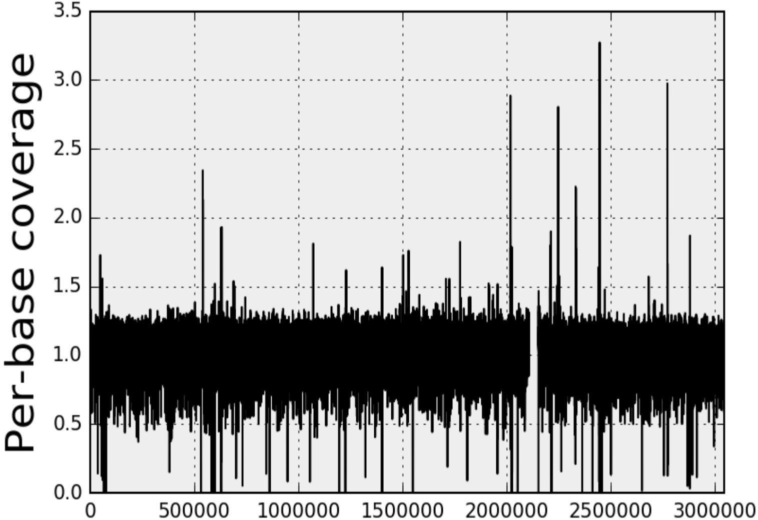
## Computational note

Running median is slow due to the sorting task.
**Solution:** a rolling window + a bisection method to insert new element in a sorted list + efficient insert/deletion in a list (B-tree) helps: **Only a few seconds to scan a 3Mb-length genome.**

# Normalised coverage



# 2. Building a statistics

**Definition:** the normalised data can be decomposed into a **central** distribution $\widetilde{C}^0$ and a set of **outliers** $\widetilde{C}^1$ (above and below the central distribution)

$$\widetilde{C}_b = \left\{ \widetilde{C}_b^0, \{ \widetilde{C}_b^+, \widetilde{C}_b^- \} \right\}$$

**Hypothesis 1:** Central distribution is predominant.

$$\left| \widetilde{C}_b^0 \right| > \left| \widetilde{C}_b^1 \right|$$

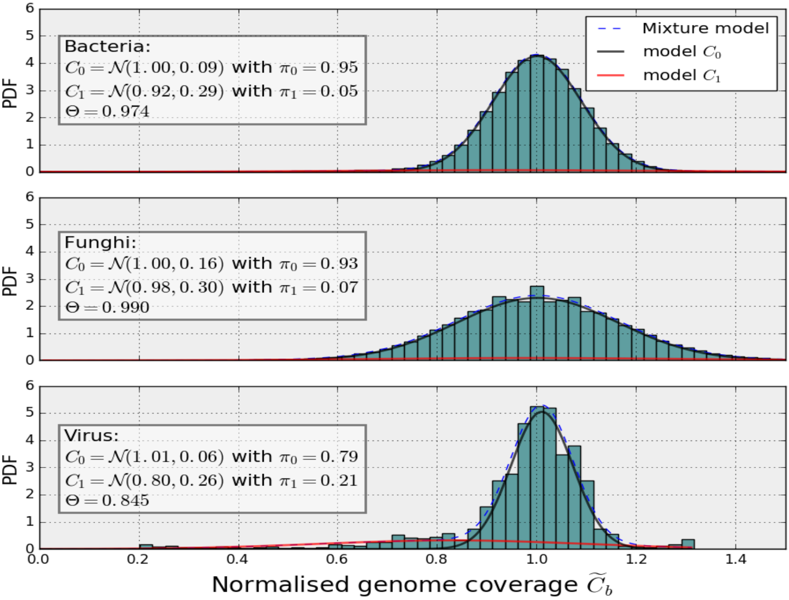
**Hypothesis 2:** The normalised genome coverage follows a Gaussian distribution in particular the central distribution

$$PDF(\widetilde{C}_b^0) \sim \mathcal{N}(\mu_0, \sigma_0)$$

We consider regime with $\delta \gg 1$. However, the algorithm works for low values: $\delta \sim 5 - 10$.

The central distribution $C_0$ will be fitted to a Gaussian distribution while the outliers will be fitted to another distribution.

- With an EM algorithm using $k = 2$ distributions we can estimate the parameters.
- The parameters of the outliers components are not used.
- The average of the central distribution is 1 (by definition)
- Note that the average of the outliers can be around 1 as well if the weights of C+ and C- are equivalent


Mixture model
model $C_0$
model $C_1$
Bacteria:
$C_0 = \mathcal{N}(1.00, 0.09)$ with $\pi_0 = 0.95$
$C_1 = \mathcal{N}(0.92, 0.29)$ with $\pi_1 = 0.05$
$\Theta = 0.974$
Funghi:
$C_0 = \mathcal{N}(1.00, 0.16)$ with $\pi_0 = 0.93$
$C_1 = \mathcal{N}(0.98, 0.30)$ with $\pi_1 = 0.07$
$\Theta = 0.990$
Virus:
$C_0 = \mathcal{N}(1.01, 0.06)$ with $\pi_0 = 0.79$
$C_1 = \mathcal{N}(0.80, 0.26)$ with $\pi_1 = 0.21$
$\Theta = 0.845$
PDF
PDF
PDF
0
1
2
3
4
5
6
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Normalised genome coverage $\widetilde{C}_b$

# C. From a constant to adaptative $z$-score

Assuming that the central distribution is the null hypothesis, we can now assign a $z$-score in the normalised space:

$$z(b) = \frac{\widetilde{C}(b) - \widetilde{\mu}_0}{\widetilde{\sigma}_0}$$

We can replace $\widetilde{C}_b$ by its expression $(C_b/RM_W(b))$ and express $C_b$ as a function of the running median, the $z(b)$ and the parameters of the central distribution:

$$C(b) = (\widetilde{\mu}_0 + z(b)\widetilde{\sigma}_0)\, RM_W(b).$$

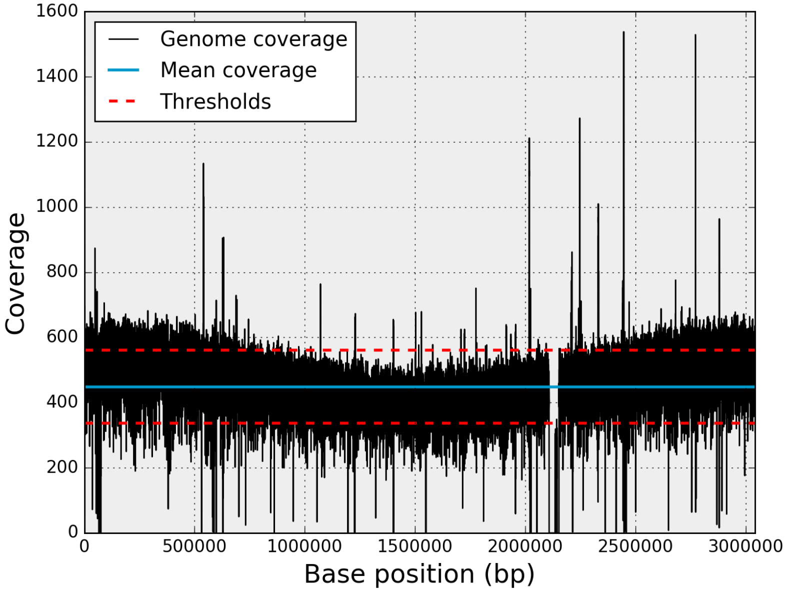
# C. From a constant to adaptative *z*-score

We can now set a constant threshold in the normalised space (e.g. $\lambda \pm 3$) and get an adaptative threshold in the original space.

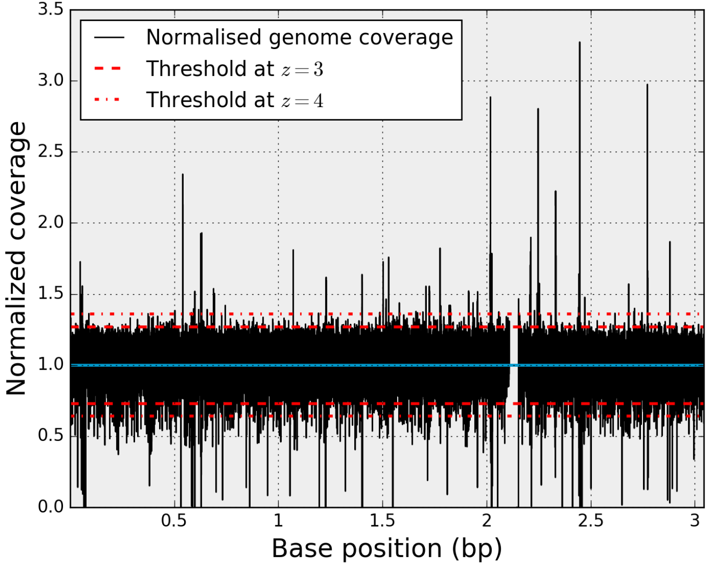
We can derive an adaptative threshold in the original space that is function of the genome position:

$$\tilde{\delta}^{\pm}(b) = \left(\widetilde{\mu}_0 \pm \lambda^{\pm} \times \widetilde{\sigma}_0\right) RM_W(b). \tag{1}$$

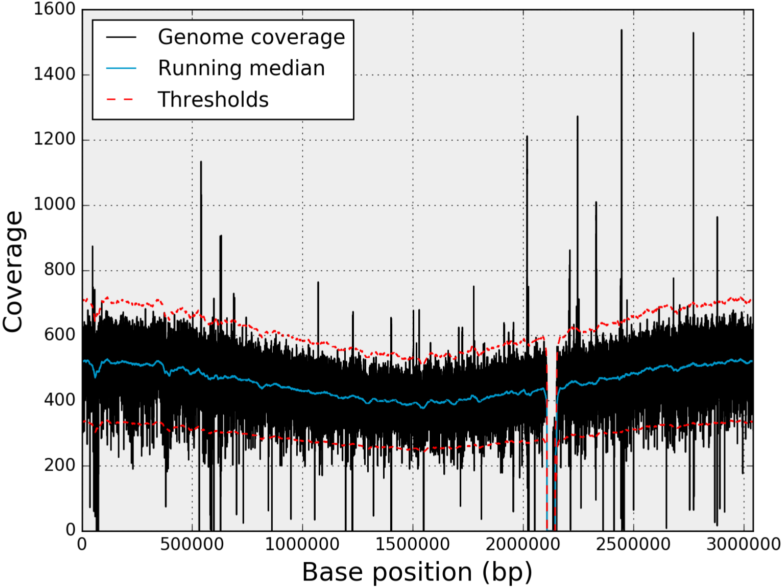
# constant thresholds in the original space



# constant thresholds in the normalised space



# adaptative thresholds in the original space



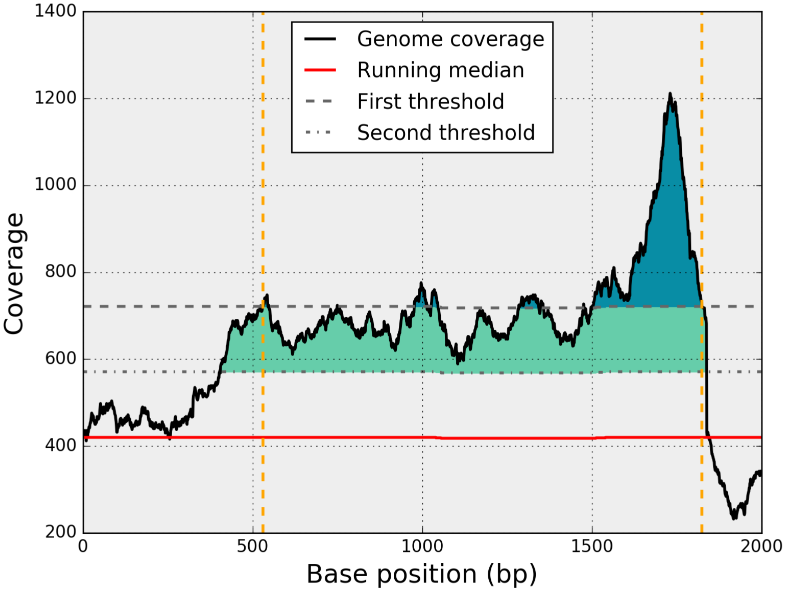
# D. Regions of interest (clustering)

On a 3Mb genome with low thresholds the number of outliers are high. $\lambda = 3$ means about 4000 events by pure chance.

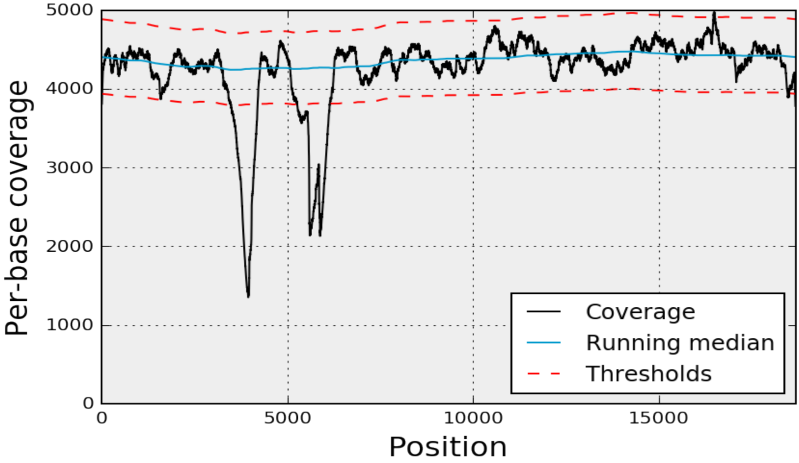
We need a strategy to reduce the number of interesting events. This is achieved by clustering the data.

Besides, to cluster close-by events further, we proceed with a double-thresholds approach.

# Double thresholds



```
sequana_coverage --download-genbank JB409847
sequana_coverage --input JB409847.bed -w 3001 --circular
                 --genbank JB409847.gbk --show-html
```



# Summary

- A robust and fast algo. to detect under/over covered regions.
- The algorithm is made of 3 steps:
  1. Normalisation (running median)
  2. Set a statistics using EM to estimate mixture model
  3. Clustering of events in original space
- Implementation in Sequana as a standalone
  - HTML reports with ROIs provided as sortable tables
  - Identify repeated regions
  - A genbank can be provided to annotate the report

Dimitri Desvillechabrol, Christiane Bouchier, Sean Kennedy, Thomas Cokelaer *Detection and characterization of low and high genome coverage regions using an efficient running median and a double threshold approach.* bioRxiv 092478; doi: http://dx.doi.org/10.1101/092478