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# Looking for mutations in PacBio cancer data: an alignment-free method

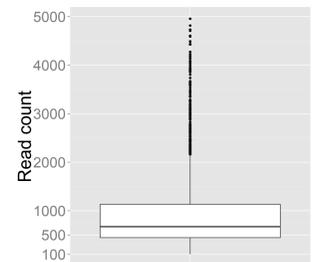
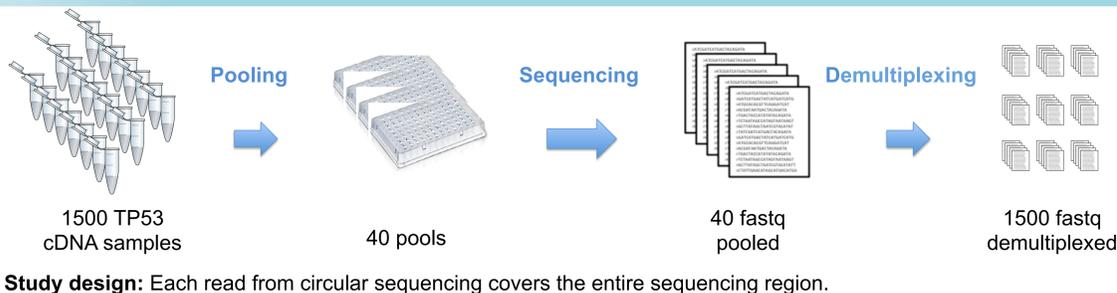
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## Abstract

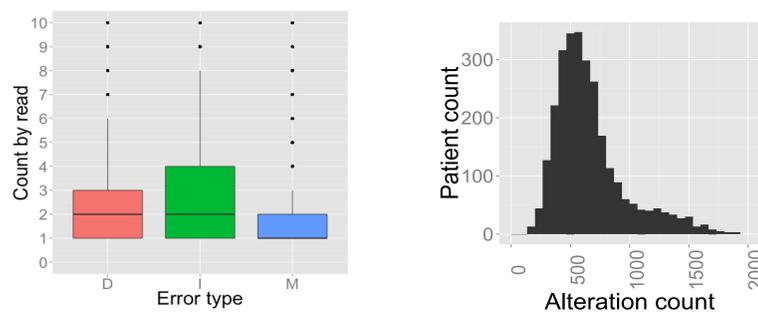
To determine the **TP53 mutations** present in a patient cohort (~1500 patients), breast tumor TP53 mRNA was sequenced with **PacBio** technology. However, none of the existing tools, e.g. VarScan, GATK, has proven to be suitable for this type of data. Indeed, in addition to the **high sequencing error rate** generated by PacBio (~15%), the tumor biopsies are **contaminated** by healthy tissue. This makes it difficult to differentiate real mutations as they are lost within the high background noise. To circumvent this problem, we have developed a method for detecting mutations using **De Bruijn graphs: MICADo** for Mutations In Cancer Data.

## Data



**Read number by patient:** varies between 100 and 5000

## Extremely noisy



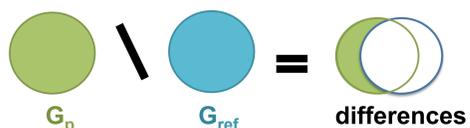
We expect **1-2 mutations by patient** but the number of different alterations per patient is very high.

## MICADo

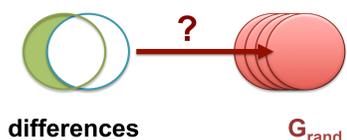
De Bruijn Graph based method that identifies paths coding for genomic alterations and distinguishes those that are specific to a patient from those common to the whole cohort.

- G<sub>reference</sub>** 3 splicing variants and 4 known SNPs
- G<sub>patient</sub>** read set of one patient
- G<sub>random</sub>** sample of reads from the whole cohort

**Step 1:** compute differences between **G<sub>p</sub>** and **G<sub>ref</sub>**



**Step 2:** check if differences are frequent in the cohort

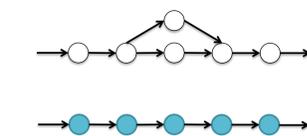


### Step 1

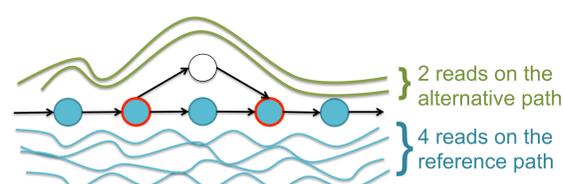
$$G_p = \langle V_p, E_p \rangle$$

$$G_{ref} = \langle V_{ref}, E_{ref} \rangle$$

$$G^* = \langle V_p \cup V_{ref}, E_p \setminus E_{ref} \rangle = \langle V^*, E^* \rangle$$



$\forall v_1, v_2 \in V_{ref} \cap V_p$ , compute paths between  $v_1$  &  $v_2$  in  $G^*$

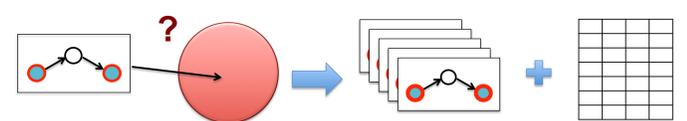


Read counts for paths

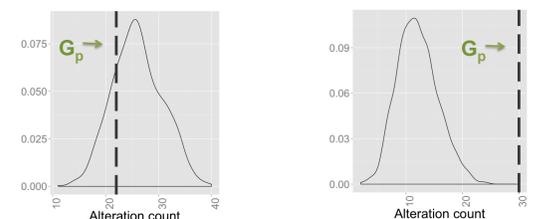
### Step 2



Construct 1000 random graphs from 1000 samples of **random reads**



Check the **presence of the paths** in the 1000 random graphs and compute associated read counts.



Compare read counts between **G<sub>p</sub>** and **G<sub>rand</sub>** and deduce a **p.value**

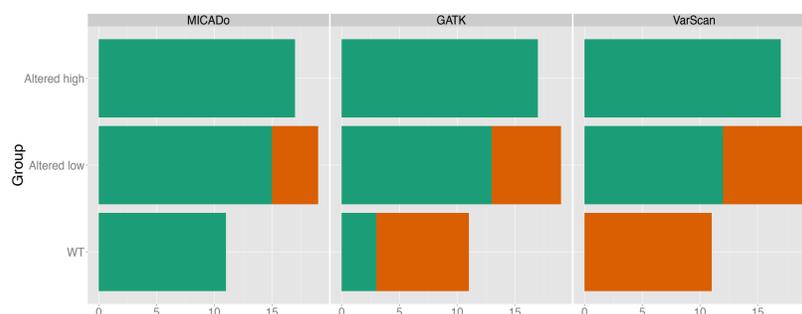
## Results

**Comparison** between VarScan, GATK and MICADo on one pool (48 PacBio samples) previously sequenced by 454 technology.

**Three types of samples:**

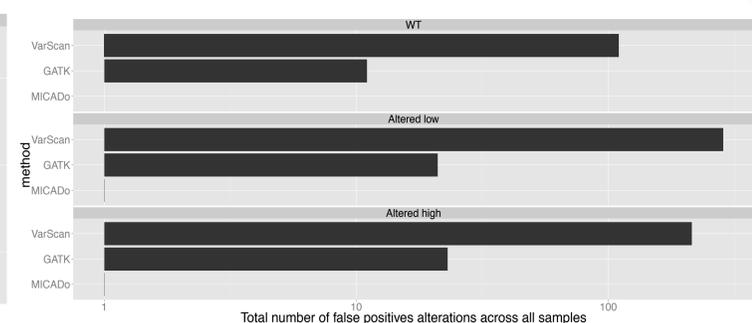
- Wild type: no mutation
- Altered low: few reads carry the true mutation
- Altered high: many reads carry the true mutation

→ **Better results with MICADo**



**Well classified:** no mutation found in wild type; true mutation found in mutated samples

**Badly classified:** mutation found in wild type; no mutation or not the right one in mutated samples



## Conclusion

**MICADo:** Mutations In Cancer Data  
 Suited to PacBio data for **gene-level** analyses

**Overcomes bias** linked to PacBio error rate for the **detection of mutations**

## Perspectives

Test **MICADo** on **other data**

Consider **paths** in which starting node or end node is not present in the reference graph  
 Define a **biological quality score** of samples

