Restriction site-associated DNA sequencing

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What is Restriction site-associated DNA sequencing?

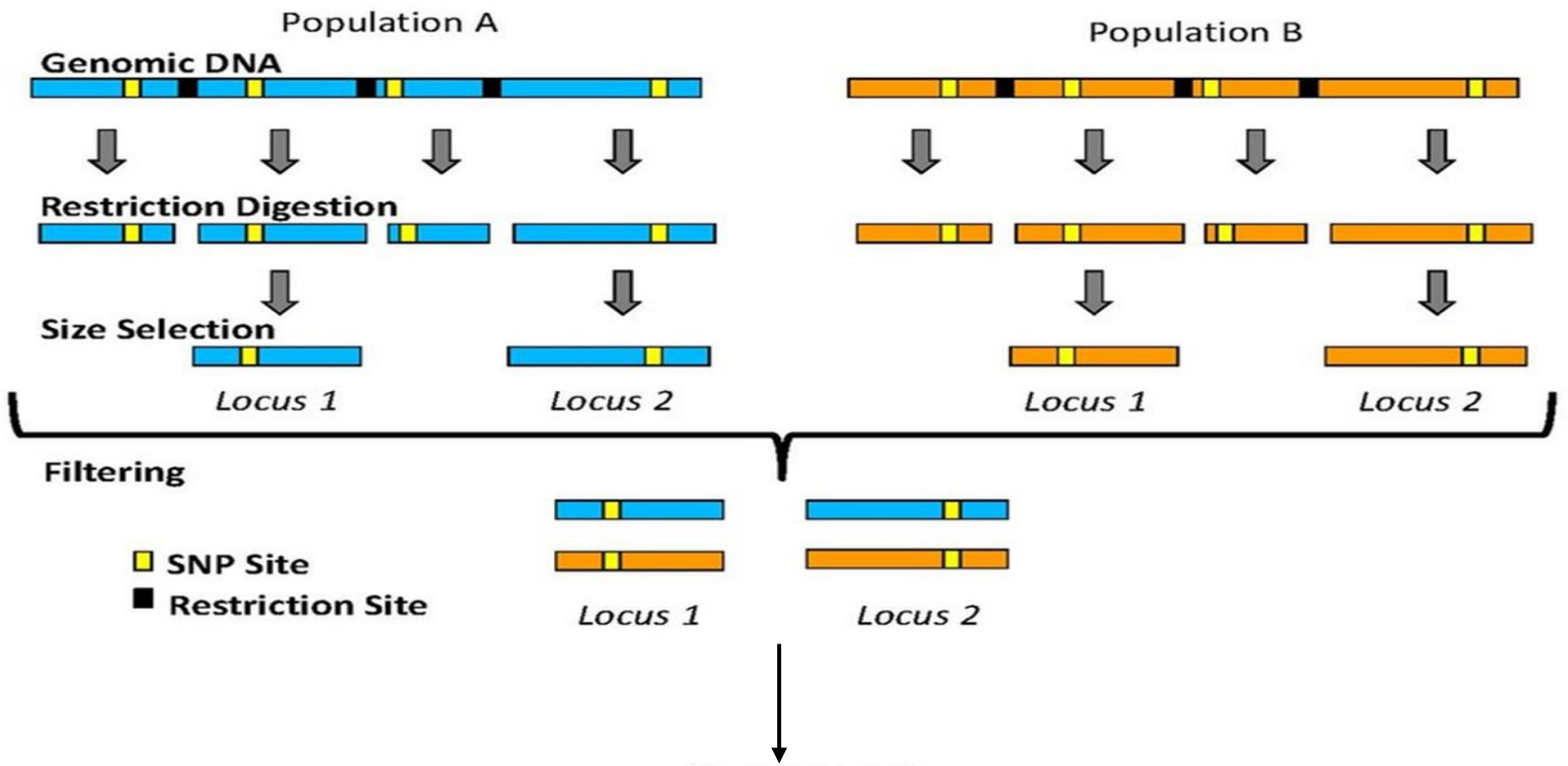
- Restriction site-associated DNA sequencing (RAD-seq or similar genotyping-by-sequencing approaches) is a method to sequence DNA next to restriction sites.
- Normally it is useful for association mapping, QTL-mapping, population genetics, ecological genetics and evolution.
- The rad sequencing essentially targets a subsets of genome for greater depth of coverage per locus yet have the ability to sequence more samples at once.

- The process is divided into different parts: -
    a. Digestion of DNA
    b. Ligation of adapters
    c. Size selection
    d. Amplification
    e. Sequencing

The process of RAD- sequencing

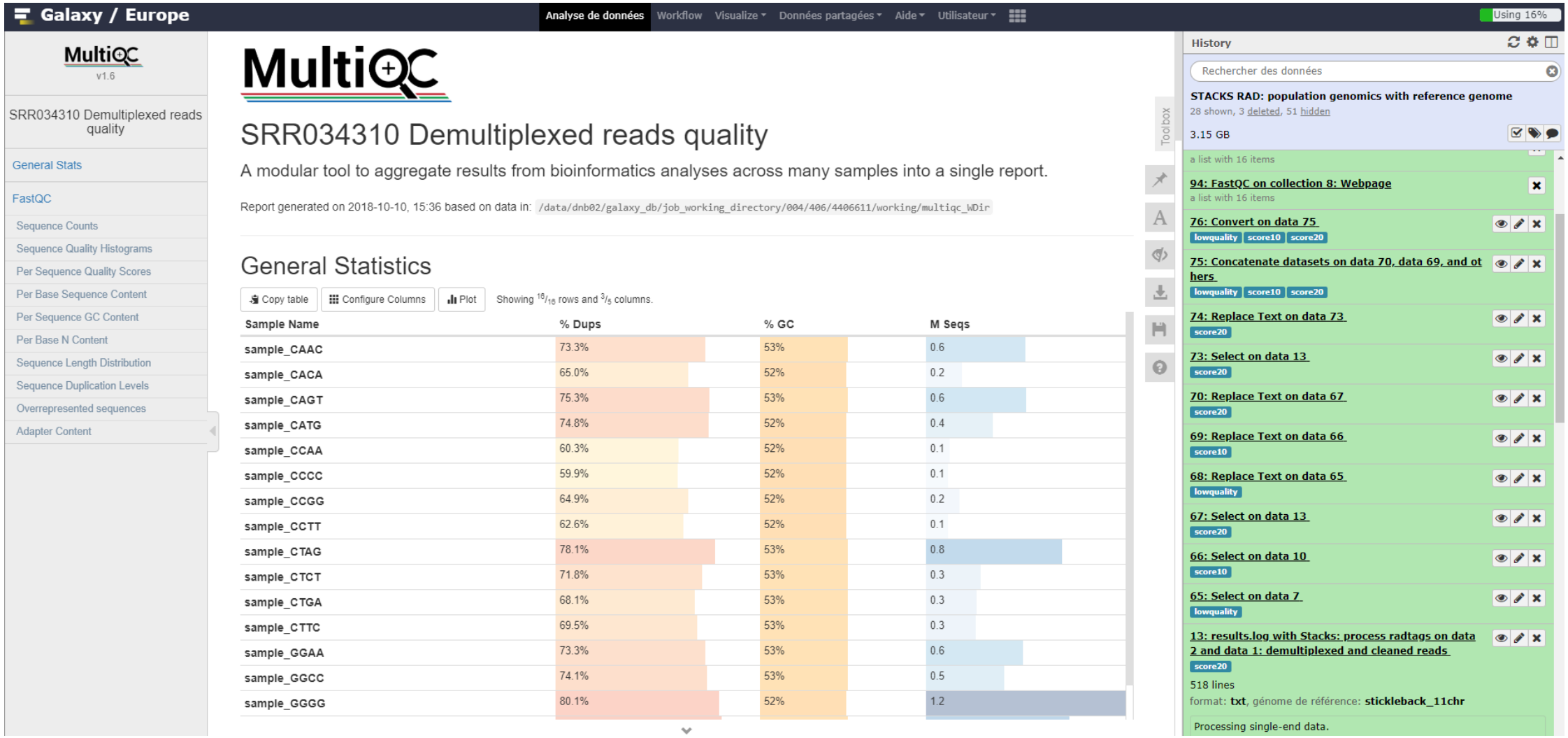
- DNA from an individual is cut with the chosen restriction enzyme, producing a set of sticky-ended fragments.
- To be sequenced on an Illumina machine, these fragments are ligated to adapters that will bind to an Illumina flow cell.
- The tagged restriction fragments from a number of individuals are pooled, and then sheared randomly to generate fragments with a mean length of a few hundred base pairs.
- These sheared, sequencer-ready fragments are then size selected (approximately 200–500 base fragments are isolated) and this RADSeq library is then sequenced on the Illumina platform.

Restriction-site Associate DNA Sequencing (RADseq)



Illumina sequencing

Data analysis



Analysis of Sequenced data

Conclusion

- The Illumina platform currently permits sequencing out to 150 bases, and thus approximately 300 bases on both sides of each restriction site can be screened for polymorphisms.
- Unlike other arrays the knowledge of sequences priorly and number of SNPs markers in the given populations are not needed.
- Deep sequencing allows accurate SNPs discovery and genotyping.