# De novo assembly of bovine genome using PacBio applications

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# *De novo* assembly of bovine genome using PacBio applications

SeqOccIn — Sequencing Occitanie Innovation

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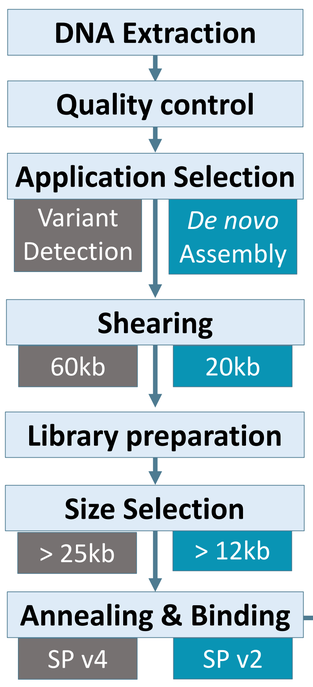
## Background and objectives

GeT-PlaGe is a genomic core facility which provides technologies and expertise in genome sequencing to academic and private research teams. The SeqOccIn (Sequencing Occitanie Innovation) project, supported by Get-PlaGe and Genotoul Bioinfo platforms, was selected by the Occitanie Region as part of the call for projects "Regional Research and Innovation Platforms". The main objective is to acquire expertise on the optimal combination of long fragment sequencing technologies and associated applications to better characterize complex genomes in agronomical field: from SNP and structural variations detection, to the production of a high quality assemblies.
Therefore, to obtain the best genome using PacBio datas, several *de novo* assembly strategies on a Charolais bovine will be presented : CLR datas only, CCS datas only and trio binning approach.
In this poster, we highlight biologicial and bioinformatic aspects taking into account the scientific purpose and different constraints (time, budget, samples qualities).
For this work, we sequenced the same individual (offspring bovine) with different applications: PacBio, ONT and Hi-C, and parents with classical short reads (2x150 pb Illumina).

## Workflow for PacBio library preparation



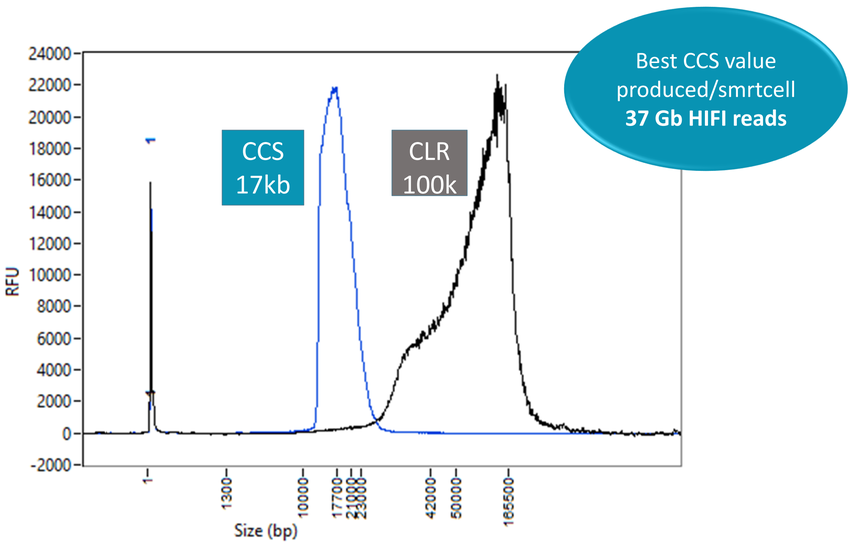




**Figure 1 : Fragment length distribution** of SMRTbell Express libraries of bovine sample (average). (on Agilent Femto Pulse)

| Sequencing | |
|---|---|
| CLR mode 2 runs | CCS mode 6 runs |

| Run QCs | | |
|---|---|---|
| Loading | 50pM | 60-75pM |
| Time | 15h | 2h + 30h |
| Total | 278Gb | 120 Gb Hifi |
| N50 insert | 28Kb | 18kb |

**Complementary informations:** DNA Extraction kit: Promega; Quality control : Femto-Qubit-Nanodrop ; Shearing: Megaruptor 3 ; Library preparation : SMRTbell Express Template Prep Kit 2.0 + Nuclease Treatment of SMRTbell Library, Size Selection: BluePippin

## *De novo* assembly

| | Offspring datas only | | | | | | + parental datas | |
|---|---|---|---|---|---|---|---|---|
| **Data type** | **ONT** | **CLR** | **CLR** | **CCS** | **CCS Hi-C based (Hap1)** | **CCS Hi-C based (Hap2)** | **CCS Trio based (Hap1)** | **CCS Trio based (Hap2)** |
| **Coverage** | 58X | 103X | 47X | 30X | 30X | 31X | 30X | 30X |
| **Assembler** | Wtdbg2 | Wtdbg2 | Wtdbg2 | HifiAsm | HifiAsm | HifiAsm | HifiAsm | HifiAsm |
| **Number of contigs** | 7 226 | 2 937 | 2 857 | 2 175 | 4 418 | 3 553 | 3 943 | 4 056 |
| **Total size (Gb)** | 2.7 | 2.64 | 2.63 | 3.14 | 3.14 | 3.12 | 2.93 | 3.2 |
| **N50 (Mb)** | 23.6 | 22.39 | 16.54 | 82.31 | 30.99 | 38.25 | 49.19 | 66.5 |

**Table 1: Summary of bovine assembly statistics.**
Different primary bovine assemblies (no scaffolding) were produced from Oxford Nanopore Technology (ONT - SQK-LSK109, Guppy version 3.0.6), PacBio CLR and PacBio CCS reads obtained from the same individual. The same assembler (WTDBG2 V2.3) was used for long erroneous reads (ONT and CLR) while CCS reads were assembled using Hifiasm V0.15 in three different ways: based only on the CCS information (CCS column), using the trio binning approach where the two haplotypes are constructed based on short reads sets of the parents (CCS Trio based columns) and a diploid assembly using Hi-C reads (CCS Hi-C based columns). While the assembly size was slightly larger than the expected size (2.8Gb) in all Hifiasm assemblies, the gain in contiguity is significant compared with the long erroneous reads based assemblies. When comparing the two long erroneous sequencing techniques though, the assembler requires a lower coverage with ONT reads than with CLR reads for similar results due to the longer size of ONT reads.

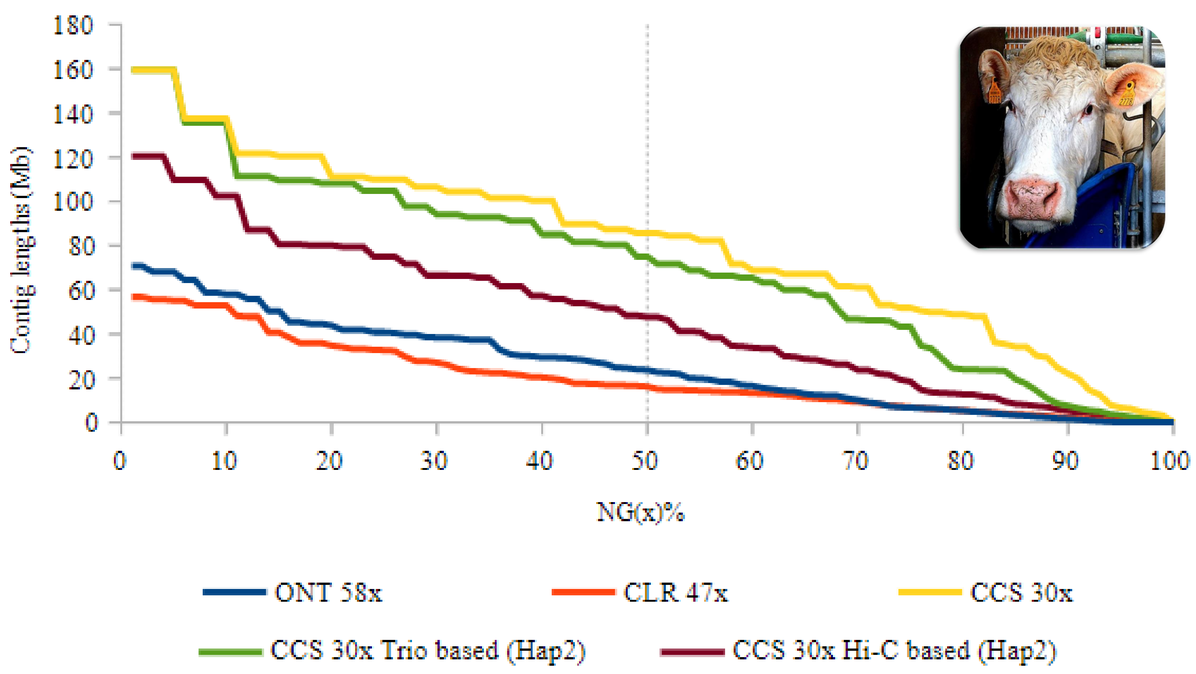



**Figure 2 : NG(X) % values for ONT, CLR and CCS assemblies of the offspring bovine**.
As showed in Table 1, assemblies based on long erroneous reads (PacBio CLR and ONT) produce shorter contigs (NG(50) lower) than CCS assemblies. Between CCS based assemblies, the longest contigs were obtained with CCS stand-alone method and with the trio binning approach. Phasing is more challenging using Hi-C reads than short reads sets with uniform coverage of the individual's parents.

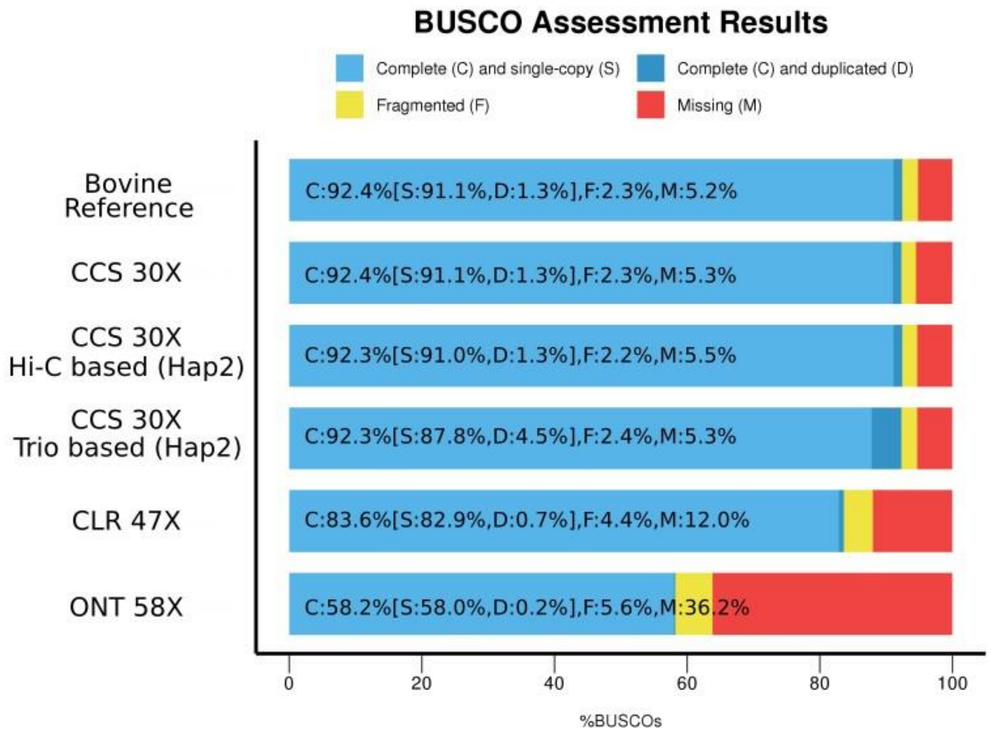



**Figure 3 : Busco statistics of the offspring Bovine assembly versus reference sequence**.
The primary assembly produced with ONT reads contains more errors and therefore fewer genes are found. The CLR approach decreases the number of residual errors and produces a better assembly with higher number of correct genes. Due to the high quality of CCS data, the assembly contains fewer errors and the busco scores are therefore much higher and similar to the refined bovine reference (ARS-UCD1.2), but with significant higher costs.

| | Required Coverage | Extraction type | Min Sample Size | Min Quantity | Lab Time | Required SMRTcells | Cost | Busco Score (C) | N50 | Assembly Total size |
|---|---|---|---|---|---|---|---|---|---|---|
| **CLR** | 50X | HMW | 50kb | 10µg | 3 days +++ | 1 to 2* | + | 83,6% | 16,5 Mb | 2,6 Gb |
| **CCS** | 30X (15x/haplotype) | Simple | 20 kb | 20-30µg | 5 days +++++ | 3 to 6* | +++ | 92,4% | 82 Mb | 3,1 Gb |

**Table 2: Summary of requirements and expected results for CCS and CLR based assemblies.**
* Depending the sample quality

**Thanks to our results we can propose a sequencing strategy to research teams that will fulfill their goal and that will be adapted to their constraints.**

**More about the SeqOccIn program :** https://get.genotoul.fr/seqoccin/
Three majors topics are treated in this project: genome variability analysis, epigenetic mark analysis and metagenome analysis. It's carried out by Get-PlaGe and Genotoul Bioinfo platforms and financed by Feder funds (Programme Opérationnel FEDER-FSE_Midi-Pyrénées et Garonne 2014-2020). The project benefits from the contributions of INRAe research units GenPhySE, MIAT, GABI, GQE. 25 private partners are involved in the project.