Hybrid and non hybrid error correction for long reads: LoRDEC and LoRMA

Eric Rivals

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Eric Rivals

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7th Nov. 2016

Outline

1. Introduction
2. LoRDEC algorithm
3. LoRDEC experimental results
    - Impact of parameters
    - Scalability
    - Correction of transcriptomic reads (RNA-seq)
    - Correction of Oxford Nanopore MINIon reads
4. LoRDEC*+LoRMA
5. LoRMA experimental results
6. Conclusion and future works

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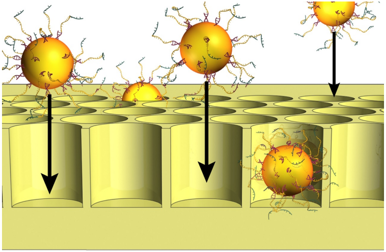
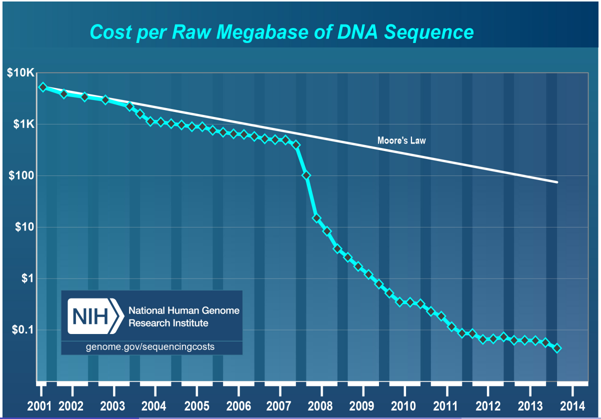
Revolution in DNA sequencing




Cost per Raw Megabase of DNA Sequence
$10K
$1K
$100
$10
$1
$0.1
Moore's Law
NIH National Human Genome Research Institute
genome.gov/sequencingcosts
2001
2002
2003
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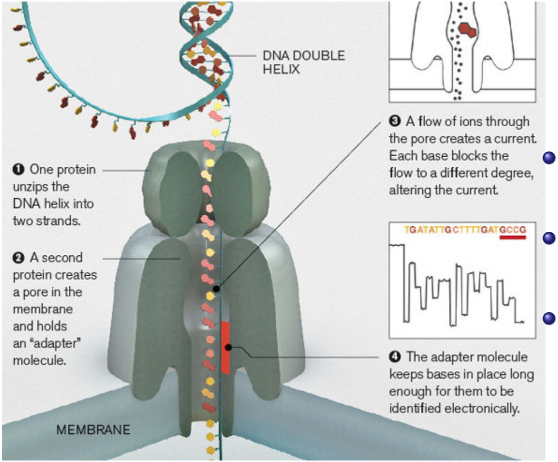
2014

Third generation technologies



❶ One protein unzips the DNA helix into two strands.

❷ A second protein creates a pore in the membrane and holds an "adapter" molecule.

❸ A flow of ions through the pore creates a current. Each base blocks the flow to a different degree, altering the current.

❹ The adapter molecule keeps bases in place long enough for them to be identified electronically.

©Oxford Nanopore

- PacBio: Pacific Biosciences up to 25 Kbp
- Oxford Nanopore MINion up to 50 Kbp
- Moleculo synthetic reads up to 10 Kbp

Overview of sequencing techniques

Name	Read Lg	Time	Gb/run	pros / cons
454 GS Flex	700	1 d	0.7	long / indels
Illumina HiSeq X	2*300	3 d	200	short/cost
Illumina NextSeq 500	2*300	3 d	150	PE, single/idem
SOLID (LifeSc)	85	8 d	150	long time
Ion Proton	200	2 h	100	new
Illumina TrueSeq	10-8500	—	4	synthetic reads
PacBio Sciences	10-40000	0.3 d	3	high error rate
Oxford MINion	10-50000	1 d	0.8	high error rate

The vast majority of errors for PacBio and Oxford are insertions & deletions.

Context

- 3rd generation sequencing technologies yield longer reads
- PacBio Single Molecule Real Time sequencing:
  much longer reads (up to 25 Kb) but much higher error rates
- Error correction is required
  1. self correction: using long reads only
  2. hybrid correction: using short reads to correct long reads

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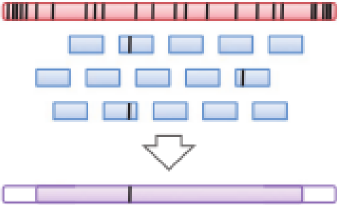
LoRDEC

Hybrid Fast Furious

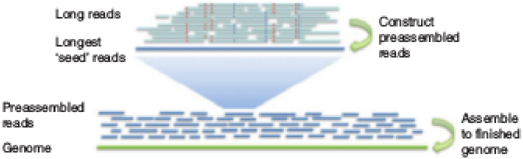
Hybrid correction methods



[Koren et al, Nat. Bio. 2012]

- Short reads are aligned to long reads
- a consensus is applied to correct part of the long read

Self correction methods



[Chin et al, Nat. Met. 2013]

Long reads are corrected with shorter reads from same technology

Other hybrid PacBio error correction programs

- PacBioToCA [Koren et al. 2012]
- AHA [Bashir et al. 2012]
  inside the assembler
- LSC [Au et al. 2012]
  compress homopolymers before alignment

All follow an alignment based strategy (e.g. BLAST like)

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All follow an alignment based strategy (e.g. BLAST like)

- proovread [Hackl et al. 2014]: alignment & chimera detection
- Jabba [Miclotte et al. 2015]: LoRDEC's approach + MEM based alignment
  variable length seeds for anchoring the LR on graph
- CoLoRMap [Haghshenas et al. 2016]: alignment & local assembly

Hybrid correction and assembly

- ECtools [Lee et al. bioRxiv 2014]
  assemble SR into unitigs, assemble unitigs and LR with Celera
- Nanocorr [Goodwin et al. bioRxiv 2014]
  recruit SR for a LR using BLAST,
  select SR with Longest Increasing Subsequence (LIS)
  compute consensus
  assembly with Celera
- NaS (Nanopore) [Madoui et al BMC Genomics 2015]
  recruit SR for each LR and reassemble the LR sequence
  complex pipeline

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All need to assemble SR

Motivation

LR correction programs "require high computational resources and long running times on a supercomputer even for bacterial genome datasets".

[Deshpande et al. 2013]

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LR correction programs "require high computational resources and long running times on a supercomputer even for bacterial genome datasets".

[Deshpande et al. 2013]

For a 1 Gb plant genome, correction of 18x PacBio with 160x Illumina required 600000 *CPU hours with EC-tools !*

Contributions

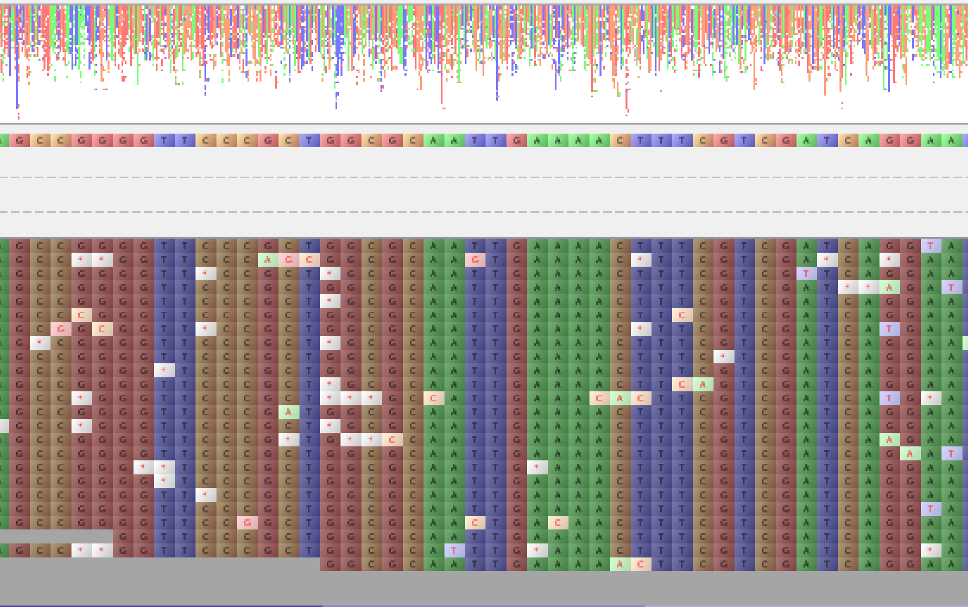
LoRDEC

- a new and efficient hybrid correction algorithm
- based on De Bruijn Graphs (DBG) of short reads
- avoids the time consuming alignments (of SR on LR)

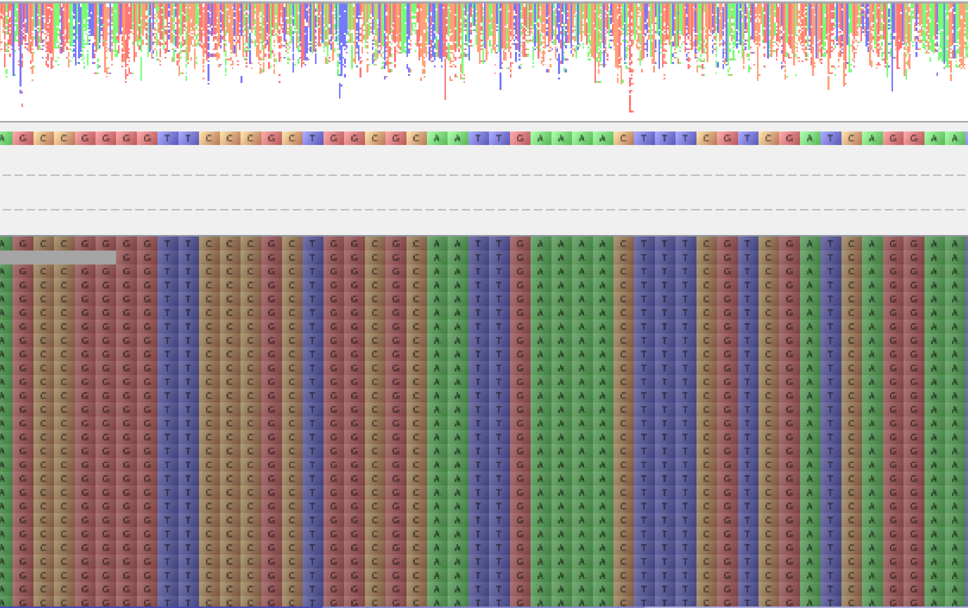
LoRMA

- a complementary tool to LoRDEC for self correction of long reads
- a pipeline that iterates LoRDEC and apply LoRMA

Aperçu of raw and corrected PacBio reads


G C C G G G G T T C C C G C T G G C G C A A T T G A A A A C T T T C G T C G A T C A G G A A

Aperçu of raw and corrected PacBio reads


A G C C G G G G T T C C C G C T G G C G C A A T T G A A A A C T T T C G T C G A T C A G G A A

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Algorithm overview

1. build a de Bruijn graph of the short reads

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   the graph represents the short reads in compact form

   GATB

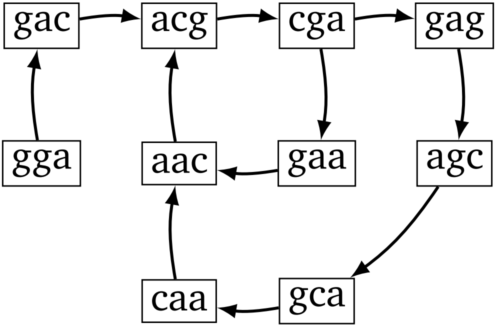
   The Genome Assembly & Analysis Tool Box

2. take each long read in turn and attempt to correct it

   1. correct internal regions,

   2. correct end regions of the long read

Example of short read DBG of order 3



$S = \{ggacgaa, cgaac, gacgag, cgagcaa, gcaacg\}$

The DBG is built from the set of short reads (Illumina)

using the GATB library.

Filtering *k*-mers of short reads

Filtering *k*-mer rationale

Because errors are randomly positioned

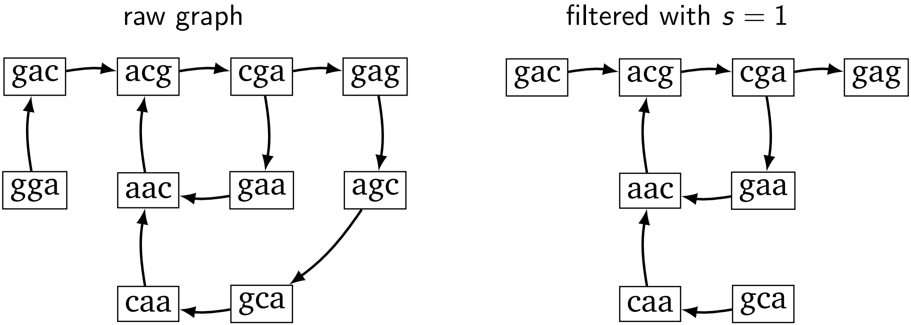
Erroneous *k*-mers have low expected occurrence numbers

Threshold based filter *s*: minimum number of occurrences in short reads

All *k*-mers present more than *s* times are called solid *k*-mers

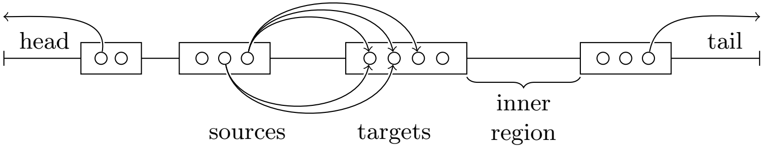
and kept in the de Bruijn Graph

Example of filtered short read DBG of order 3



$S = \{ggacgaa, cgaac, gacgag, cgagcaa, gcaacg\}$

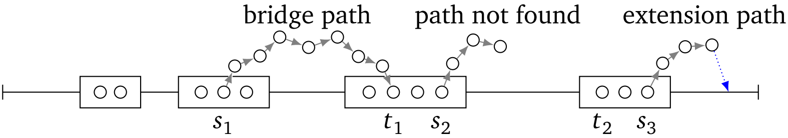
Long read sequence is partitioned



○: solid *k*-mers of the long read

- Solid *k*-mers are a priori correct piece of the sequences
- we correct the region between two solid *k*-mers

Long read is corrected with DBG

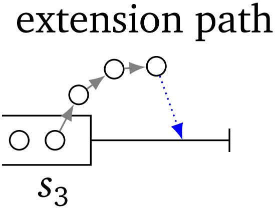


For each putative region of a long read:

- align the region to paths of the de Bruijn graph
- find best path according to edit distance
- limited path search

LoRDEC: Correcting read ends

- Find a path in DBG starting from the extreme solid *k*-mer
- Maximize length of the prefix of the end to correct
- Minimize edit distance between the path and the prefix of the end
- Find best extension maximizing an alignment score



Correction algorithm

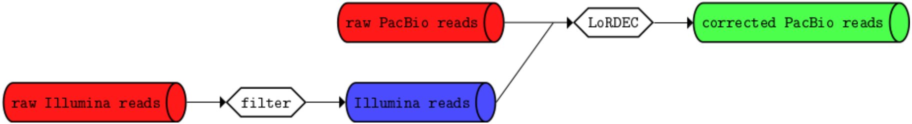
1. Correct inner region:
    1. depth first search traversal of paths between source and target *k*-mers
    2. node wise: minimal edit distance computation with seq region
2. Correct end region:
3. Paths optimisation:
    1. build a graph of all correction paths for current read
    2. finding a shortest path between the first and last solid k-mers
       Dijkstra algorithm

Trimming and splitting (optional)

- Classify each base as solid if it belongs to at least one solid *k*-mer and weak otherwise
- LoRDEC outputs solid bases in upper case characters and weak ones in lower case characters
- Corrected reads can be trimmed and/or split:
  1. Trim weak bases from both ends of the read
  2. Extract all runs of solid bases from the corrected reads

- Output of LoRDEC:
  >read1
  acgtgaGTAGTCGAGTagcgtagG
  TGGATCGAGCTAGggggt
- Trimmed read:
  >read1
  GTAGTCGAGTagcgtagGTGGATCG
  AGCTAG
- Trimmed and split reads:
  >read1_1
  GTAGTCGAGT
  >read1_2
  GTGGATCGAGCTAG

LoRDEC correction pipline



- Filtering short-reads data for quality value and adapter presence `cutadapt` [Martin, 2012]

- Long reads correction with LoRDEC.
  Two parameters must be set :
  - k-mer length – default $k = 19$
  - threshold : minimum abundance for a k-mer to be solid
    that is, to be included in the de Bruijn graph

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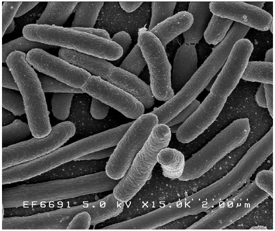
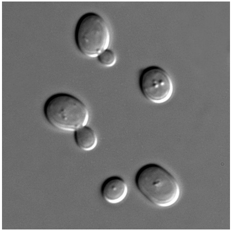
Data sets

	E. coli	Yeast	Parrot
Genome size	4.6 Mbp	12 Mbp	1.23 Gbp
PacBio coverage	21x	129x	5.5x
Illumina coverage	50x	38x	28x

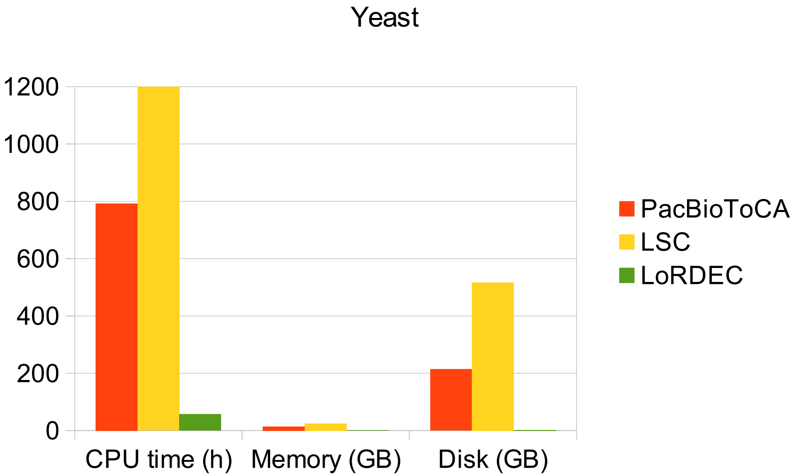




Results: time and memory

Data	Method	CPU time	Elapsed time	Memory	Disk
E. coli	PacBioToCA	45 h 18 min	3 h 12 min	9.91	13.59
	LSC	39 h 48 min	2h 56 min	8.21	8.51
	LoRDEC	2 h 16 min	10 min	0.96	0.41
Yeast	PacBioToCA	792 h 41 min	21 h 57 min	13.88	214
	LSC	1200 h 46 min	130 h 16 min	24.04	517
	LoRDEC	56 h 08 min	3 h 37 min	0.97	1.63
Parrot	LoRDEC	568 h 48 min	29 h 7 min	4.61	74.85

Runtime, memory and disk usage



Evaluation methods

Two ways:

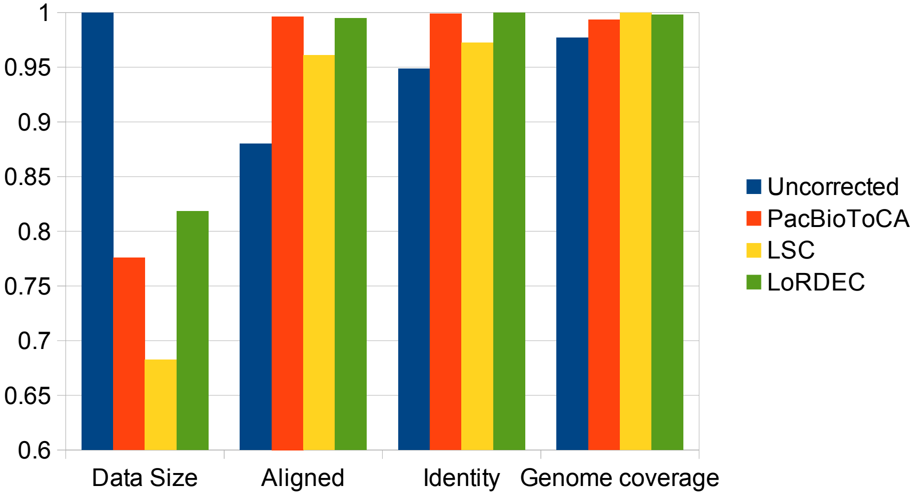
1. how do the reads align to the genome?
2. how do raw and corrected reads differ in their alignments?

Using the Error Correction Toolkit [Yang et al. 2013] we compute

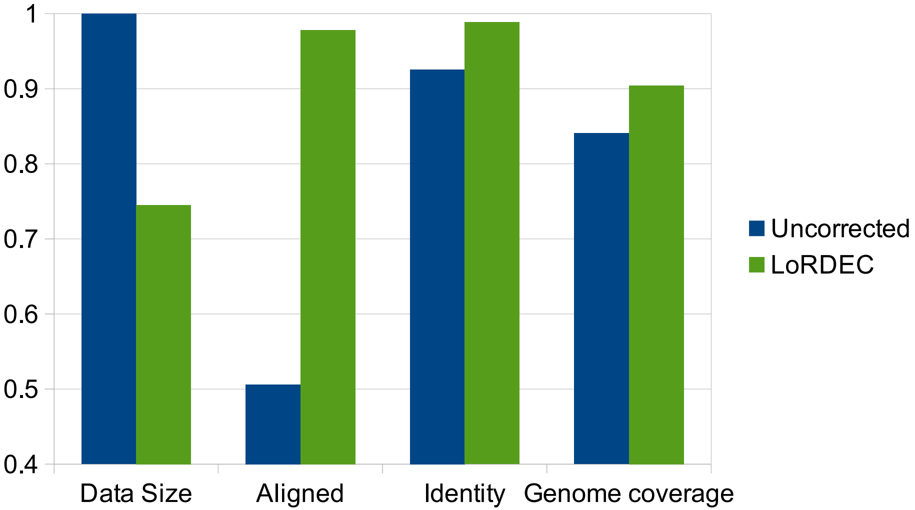
- Sensitivity = TP/(TP+FN)
  how well does the tool recognise erroneous positions?

- Gain = (TP-FP)/(TP+FN)
  how well does the tool remove errors without introducing new ones?

Error correction performance: *E. coli*



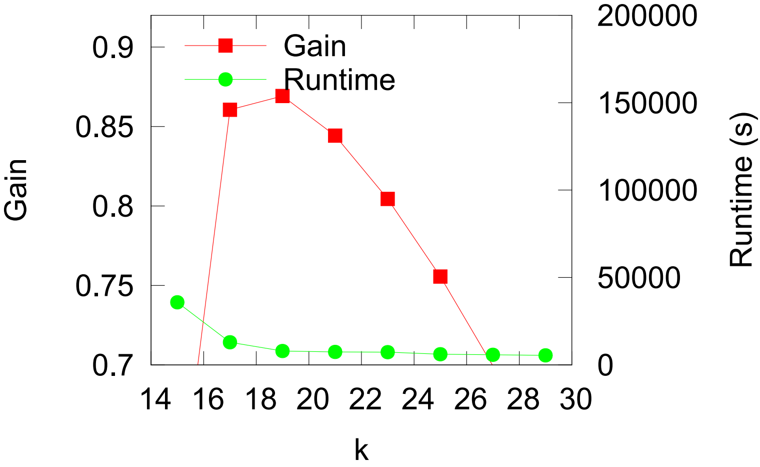
Error correction performance: Parrot



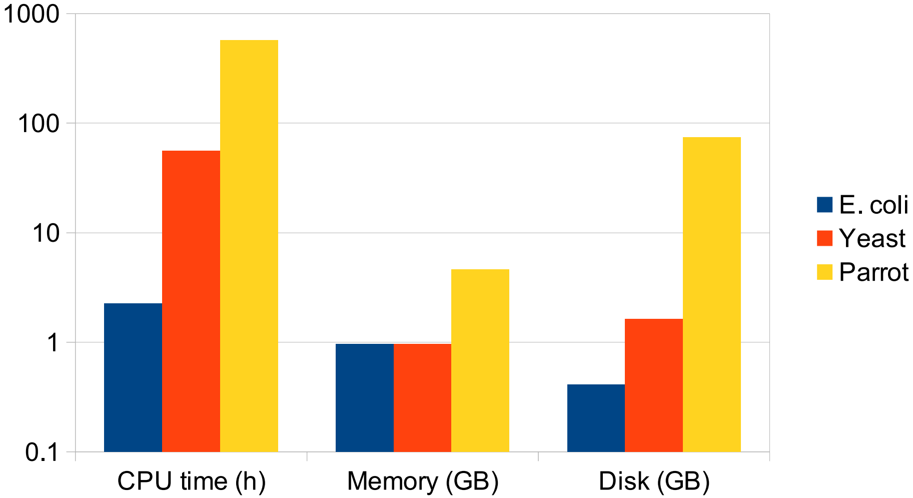
Sensitivity and gain results

Data	Method	Sensitivity	Gain
E. coli	PacBioToCA	NA	NA
	LSC	0.2865	0.2232
	LoRDEC	0.9090	0.8997
Yeast	PacBioToCA[1]	NA	NA
	LSC	0.3246	0.2596
	LoRDEC	0.8427	0.8194
Parrot	LoRDEC	0.8962	0.8544

Parameters: E. coli



Scalability of LoRDEC



Scalability of LoRDEC

Mais transcriptome data

- Illumina HiSeq : 194 million of reads, 29 Tbp
- PacBio : 276000 reads, 168 Gbp
- LoRDEC time: 12 hours
- LoRDEC memory: 5 Gbytes

Chicken transcriptome with PacBio

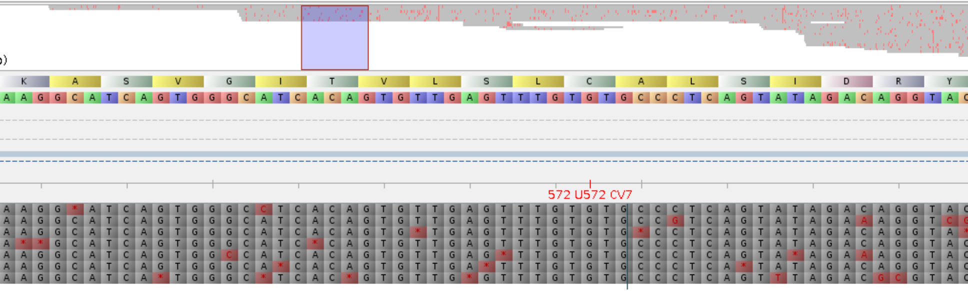
PacBio data	Raws	Corrected and trimmed
# reads (x1000)	1 849	1 848
# reads > 1Kbp (x1000)	687	569
Max length of reads (kbp)	12.2	11.9
Total length (Gbp)	1.98	1.77
%GC	48.08	47.28
Avg length (bp)	1 075	960

Chicken transcriptome with PacBio

After correction and mapping with BWA-MEM [Li H., 2013]
on ref. transcriptome (1 RNA per gene)

- 5% more transcripts covered with uniquely mapping reads
- 80% id in alignments vs 66% before correction

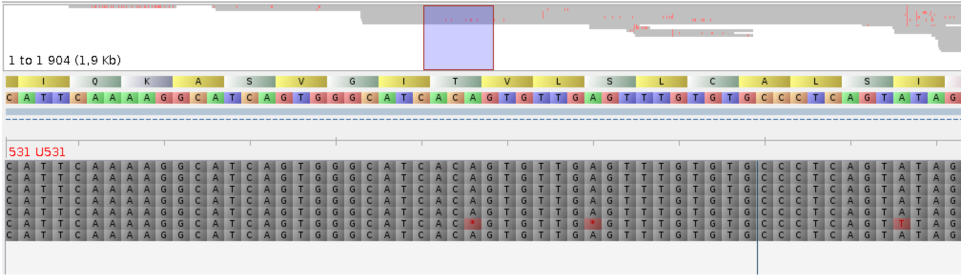
Aperçu of raw and corrected PacBio RNA reads



```
K A S V G I T V L S L C A L S I D R Y
A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G A C A G G T A C

A A G G * A T C A G T G G G C C T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G A C A G G T A C
A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C G T C A G T A T A G A A A G G T C G
A A G G C A T C A G T G G G C A T C A C A G T G * T G A G T T T G T G T G * C C T C A G T A T A G A C A G G T A *
A * * G C A T C A G T G G G C A T C * C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G A C A G G T A C
A A G G C A T C A G T G G C C A T C A C A G T G T T G A G * T T G T G T G C C C T C A G T A * A G A A A G G T A C
A A G G C A T C A G T G G G C A * C A C A G T G T T G A * T T T G T G T G C C C T C A * T A T A G A C A G G T A C
A A G G C A T C A * T G G G C * T C A C * G T G T T G * G T T T G T G T G C C C T C A G T T T A G A C G C G T A C
```

Aperçu of raw and corrected PacBio RNA reads



1 to 1 904 (1,9 Kb)

```
I  Q  K  A  S  V  G  I  T  V  L  S  L  C  A  L  S  I
C A T T C A A A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G

531 U531
C A T T C A A A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G
C A T T C A A A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G
C A T T C A A A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G
C A T T C A A A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G
C A T T C A A A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G
C A T T C A A A A G G C A T C A G T G G G C A T C A C * G T G T T G * G T T T G T G T G C C C T C A G T T T A G
C A T T C A A A A G G C A T C A G T G G G C A T C A C A G T G T T G A G T T T G T G T G C C C T C A G T A T A G
```

Correcting E. coli Nanopore MINIon data

- Raw reads + quast
- Corrected reads + quast

Nanopore data	Raw	Corrected
Nb reads	3463	2749
Nb reads $\geq$ 1kbp	3420	2685
Total length (Mbp)	22	17
Unaligned bases (%)	99.99	7.60
Genome fraction (%)	0.02	96.59

Quast [Gurevich et al. 2013]

MINion S. aureus data

Mapping of reads with BWA-MEM onto the reference genome with appropriate options

- ref génome: 2.8 Mbp
- MINIon sequencing coverage $14x$
- gain for $k = 17$ and $s = 2$ reaches 69%
- $99,9$ % genome covered by corrected reads
- 65 % genome at median coverage 8x
- 79% identity instead of 66 % without correction

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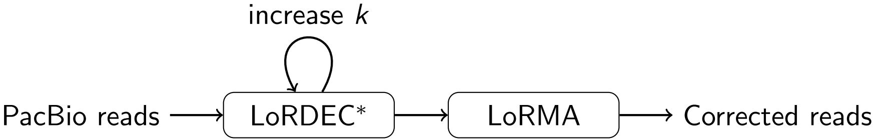
Overview of LoRDEC*+LoRMA

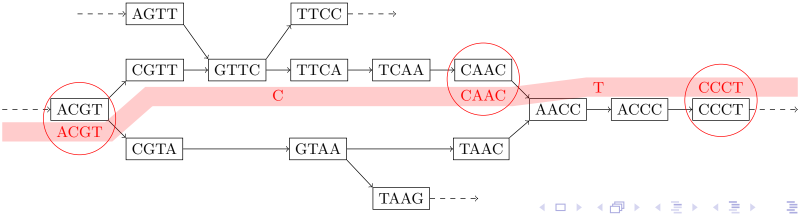
- Modify LoRDEC to run on long reads only $\implies$ LoRDEC*
- Run LoRDEC* iteratively with increasing k
- Polish the result with multiple alignments $\implies$ LoRMA



LoRDEC

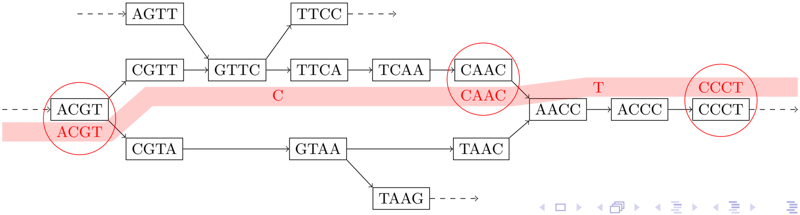
- Build a de Bruijn graph of the short reads

- For each long read:
    - Classify *k*-mers: solid (= in the DBG) and weak
    - Find paths in the DBG between the solid *k*-mers
    - Minimize edit distance between the long read and the path's string



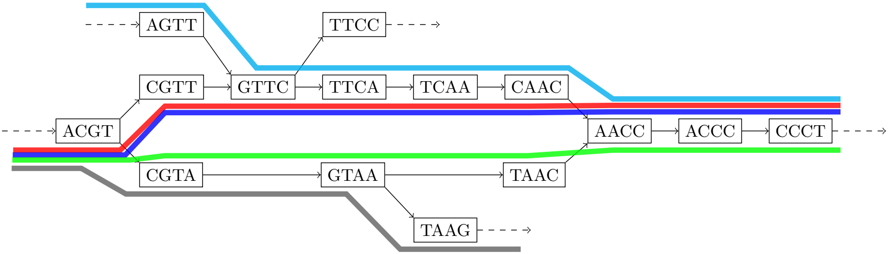
LoRDEC*

- Build a de Bruijn graph of the LONG reads
  - Use a small k such that the genomic k-mers are expected to be found in the reads
  - Use an abundancy threshold to differentiate between correct and erroneous k-mers
- For each long read:
  - Classify k-mers: solid (= in the DBG) and weak
  - Find paths in the DBG between the solid k-mers
  - Minimize edit distance between the long read and the path's string
  - Select a correcting path only if all possibilities have been explored.



LoRMA

- Build a de Bruijn graph of the reads
- Annotate the graph by threading each read through the graph
- For each read find its friends, i.e. the most similar reads
- Use a multiple alignment of a read and its friends to correct the read



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3. LoRDEC experimental results
   - Impact of parameters
   - Scalability
   - Correction of transcriptomic reads (RNA-seq)
   - Correction of Oxford Nanopore MINIon reads
4. LoRDEC*+LoRMA
5. LoRMA experimental results
6. Conclusion and future works

Evaluation method

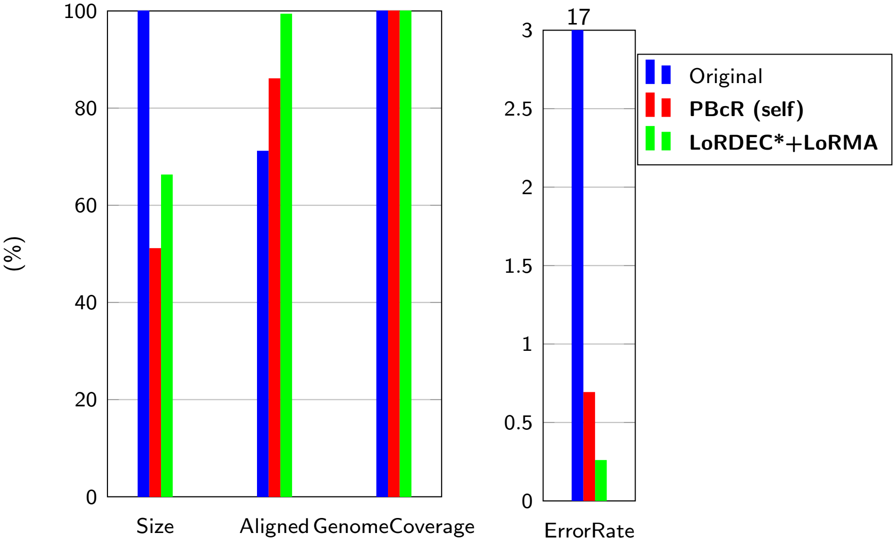
Process

1. Align the raw and corrected reads to the genome with `BLASR` [Chaisson et Tesler, 2012]
2. Consider a single best alignment.

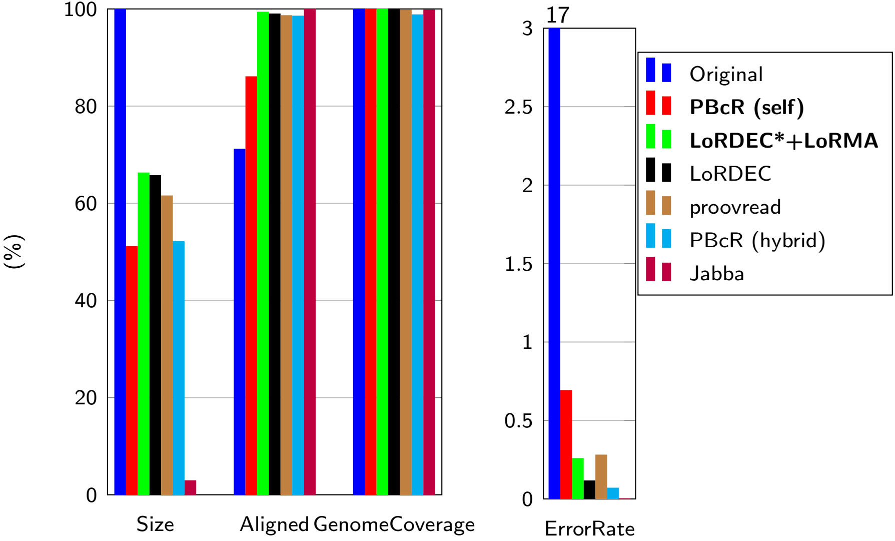
Compute following metrics

- total size of corrected reads
- total aligned size of corrected
- error rate of aligned regions (nb erroneous positions / aligned length)
- genome coverage

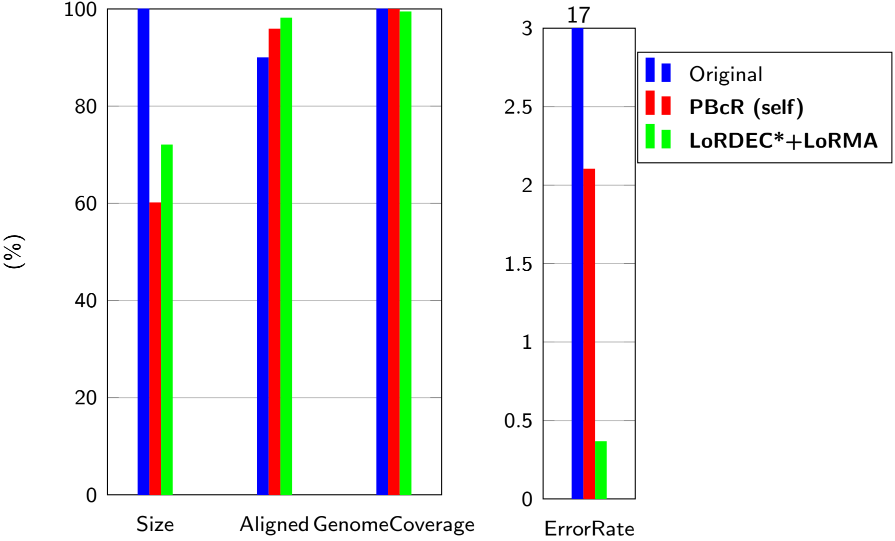
Selfcorrection: *E. coli* with $k = 19, 40, 61$



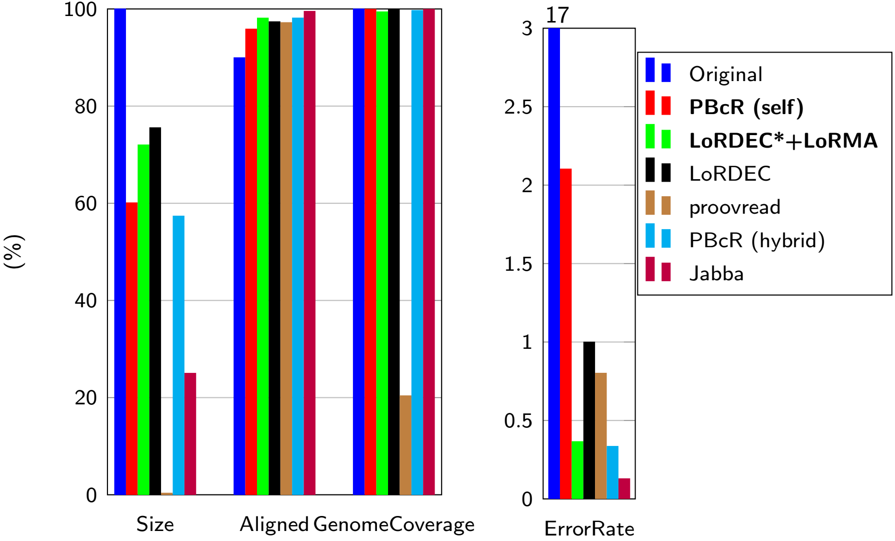
Selfcorrection and hybrid correction: *E. coli*



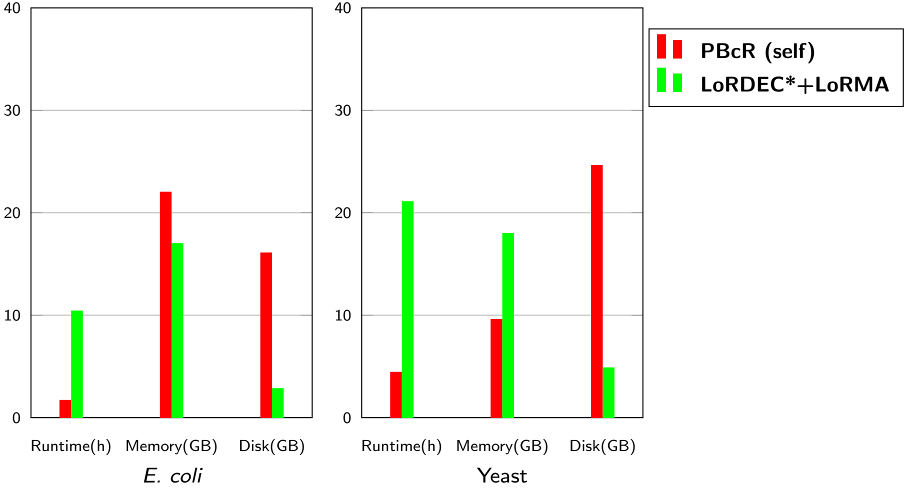
Selfcorrection: Yeast



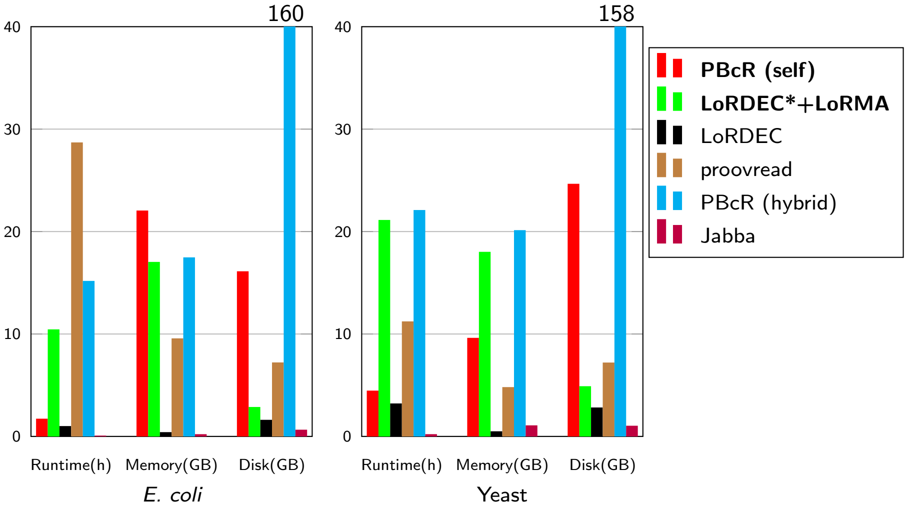
Selfcorrection and hybrid correction: Yeast



Selfcorrection: Resources



Selfcorrection and hybrid correction: Resources



Outline

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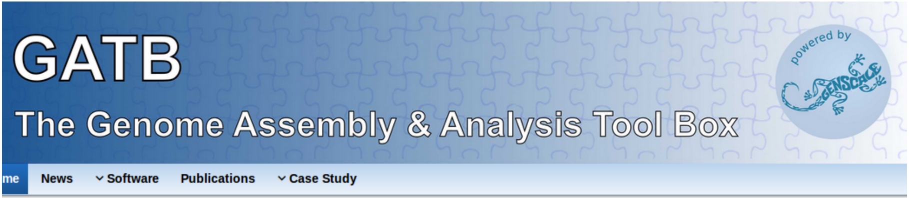
Take home message

LoRDEC is

- at least 6 times faster than previous methods
- uses at least 93% less memory than previous methods
- corrects both PacBio & Nanopore reads
- scales up to vertebrate cases
- achieves similar accuracy as state-of-the-art methods.

LoRDEC is freely available at http://atgc.lirmm.fr/lordec/

LoRDEC and LoRMA use GATB



http://gatb.inria.fr

Conclusions

LoRDEC*+LoRMA [Bioinformatics 2016]:

- DBG based initial correction of sequencing errors in long read data
- Further polishing with multiple alignments
- Accurate selfcorrection method, needs high coverage (75×)
- Future: improve memory footprint and running time
- Freely available at http://www.cs.helsinki.fi/u/lmsalmel/LoRMA/

LoRDEC and LoRMA publications

LoRDEC: accurate and efficient long read error correction

L. Salmela, E. Rivals

Bioinformatics, doi:10.1093/bioinformatics/btu538, 30 (24): 3506-3514, 2014.

Accurate selfcorrection of errors in long reads using de Bruijn graphs

L. Salmela, R. Walve, E. Rivals, E. Ukkonen

Bioinformatics, doi: 10.1093/bioinformatics/btw321, 2016.

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Thank you for your attention!

Questions?

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