De novo Deletion Detection

In Case-Parent Targeted Sequencing Trios

What have we learned from sequencing data?

- Lots of different types of variation
  - Substitutions, deletions, insertions, translocations, inversions...

- Much variation between people
  - 1000 Genomes project [2015]
  - 4-5 million locations affected
  - 2100-2500 structural variants (covering 20Mb)

- What are genetic differences that cause/contribute to disease?

The data at hand

- Oral cleft is a birth defect affecting about 1 in 700 births (WHO)

- Decades of genetic studies have pointed to the same regions
    - Targeted sequencing of these 13 regions, 6.3Mb*
    - 1,018 case-parent trios (3,054 individuals)
    - Goal: look for *de novo* copy-number deletions that could be causal

- Why look for *de novo* deletions in case-parent trios?
    - Parents are phenotypically normal, while the child is not
    - Deletions can readily cause loss-of-function
    - Evidence of *de novo* CNV burden in ASD
    - The trio data structure is perfectly suited for finding *de novo* variants

* https://www.ncbi.nlm.nih.gov/pubmed/25704602 [Leslie et al 2015]

The challenge and our approach

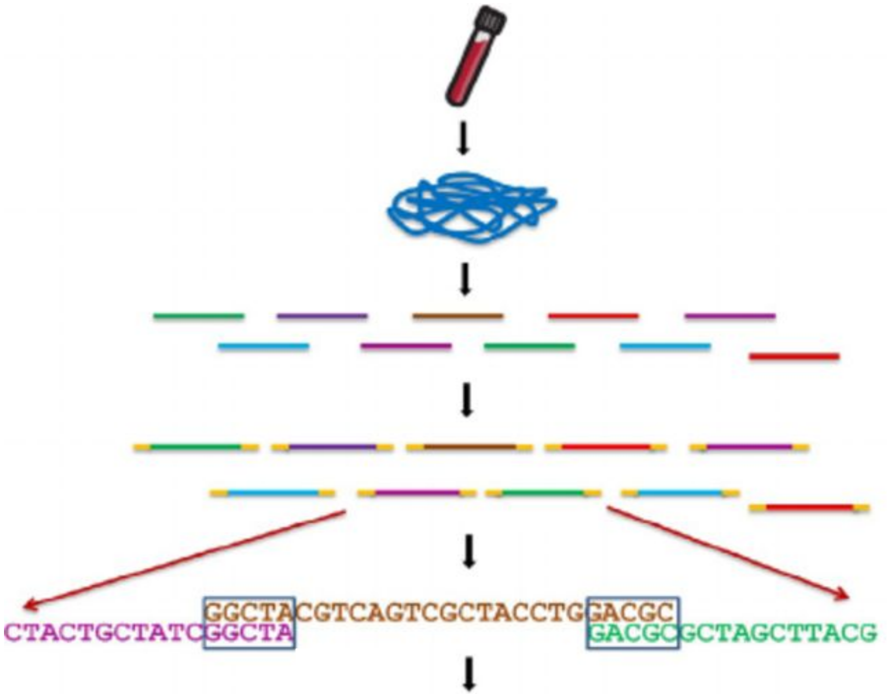
- **High** false-positive rate of CNV/deletion calling methods

- No existing method takes account of trio structure AND characteristics of targeted sequencing
    - De novo deletion calling using trio structure
        - TrioCNV
    - Deletion calling for targeted sequencing
        - CANOES

- Minimum Distance for Targeted Sequencing (MDTS)
    - 2 innovations
        - Explicitly account for trio structure of data
        - Flexibly model the unique challenges of TS

    - Resulting in high positive predictive value (PPV) while maintaining sensitivity

Targeted Sequencing



Sample

Genetic material

Fragmentation

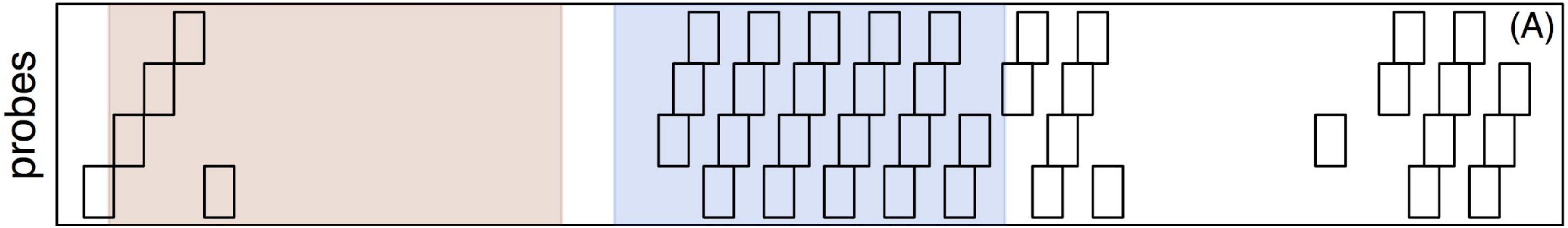
Target capture

Sequencing

Alignment

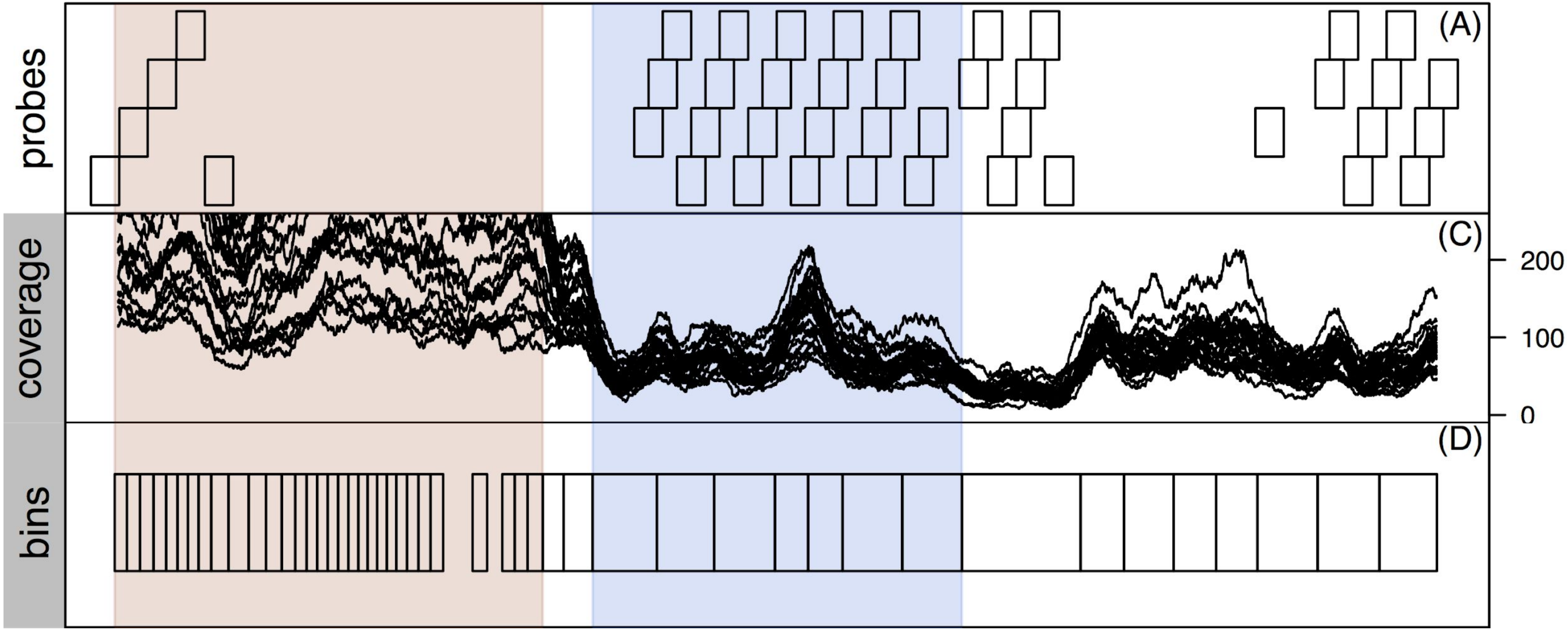
CTACTGCTATCGGCTACGTCAGTCGCTACCTGGACGCGCTAGCTTACG Reference

Target capture in theory

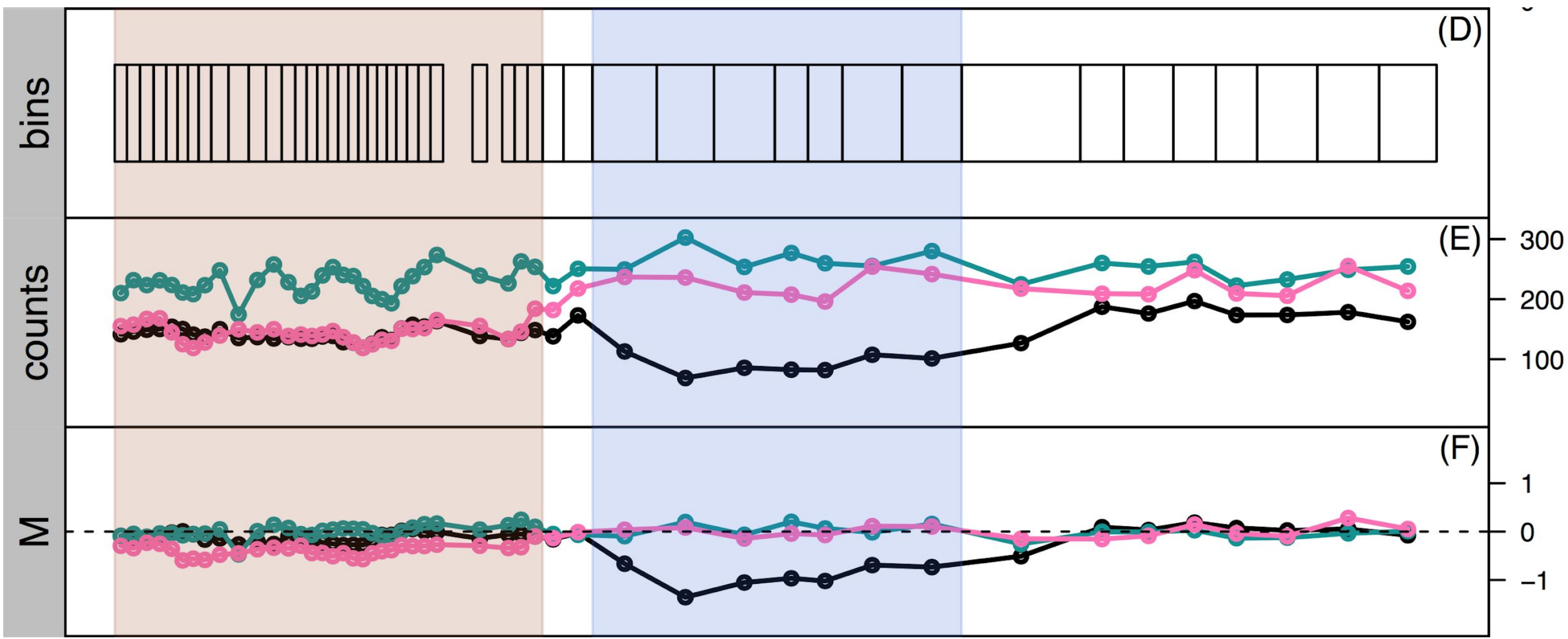


- 209.944 Mb - 209.948 Mb region of chromosome 1 (4kb)
- Each rectangle is a probe (~120bp)
- Expectation that observed coverage is perfectly dictated by probe locations

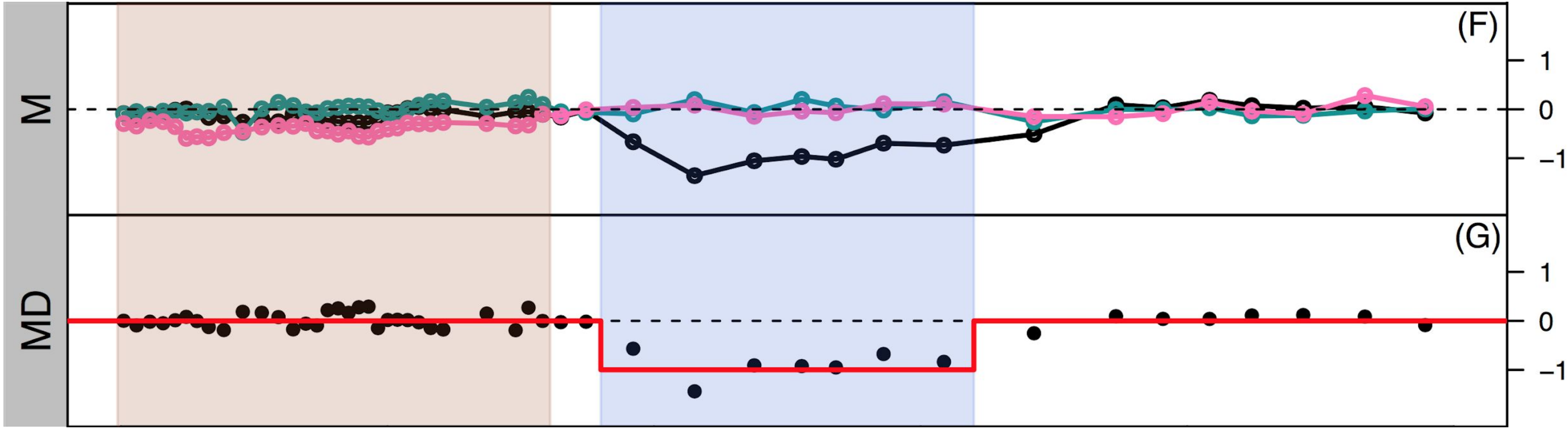
Target capture in practice



Counting and normalization



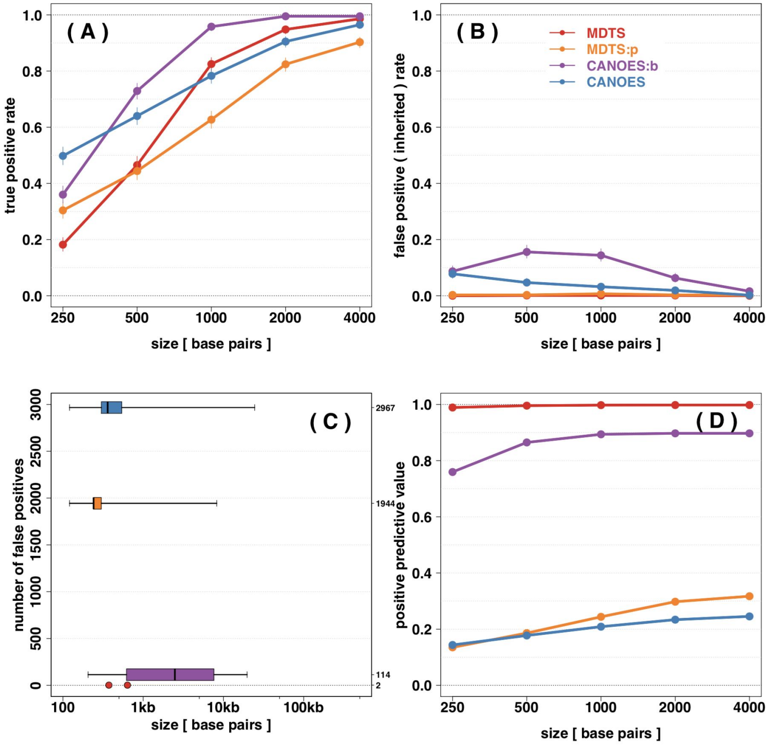
The minimum distance statistic



Performance on simulated data

- Try to create simulation data that is as realistic as possible
- Simulated 1000 repetitions
- For each repetition, sample a trio (with replacement from 1,018 trios)
    - Spike in 5 *de novo* deletions
        - 250, 500, 1000, 2000, 4000 bp
        - Remove reads from real sequencing data in a binomial process with p=0.5 in child ONLY
    - Spike in 5 inherited deletion
        - 250, 500, 1000, 2000, 4000 bp
        - Remove reads from real sequencing data in a binomial process with p=0.5 in child AND one parent

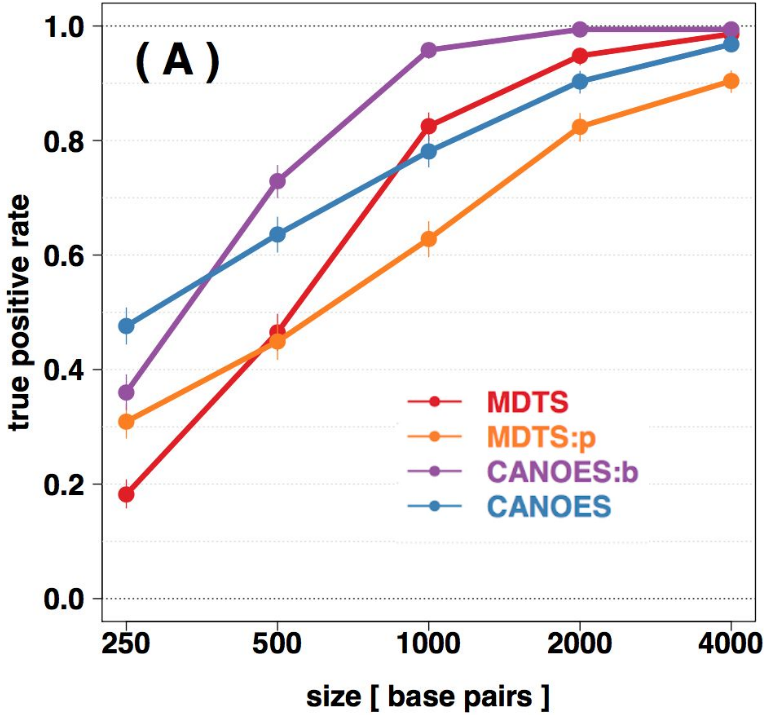
Performance on simulated data



- Methods should have high sensitivity and low false positives
- TrioCNV produced 0 calls (not graphed)
- To isolate bin-effect vs MD-effect:
  - MDTS
  - MDTS with probe-based bins (MDTS:p)
  - CANOES with MDTS bins (CANOES:b)
  - CANOES (as published)

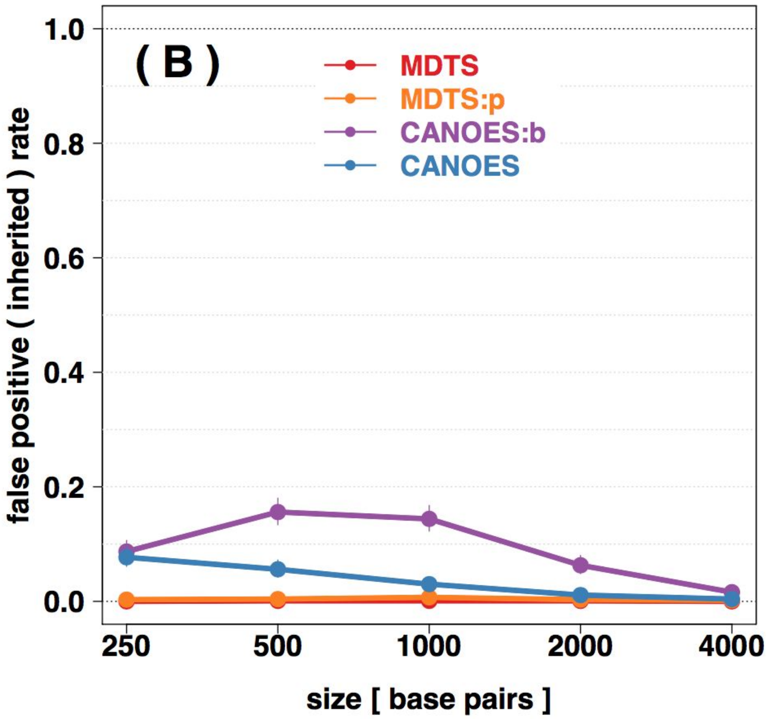
- (A) sensitivity of methods
- (B) false positive inherited deletions
- (C) other false positive deletions
- (D) positive predictive value

Sensitivity



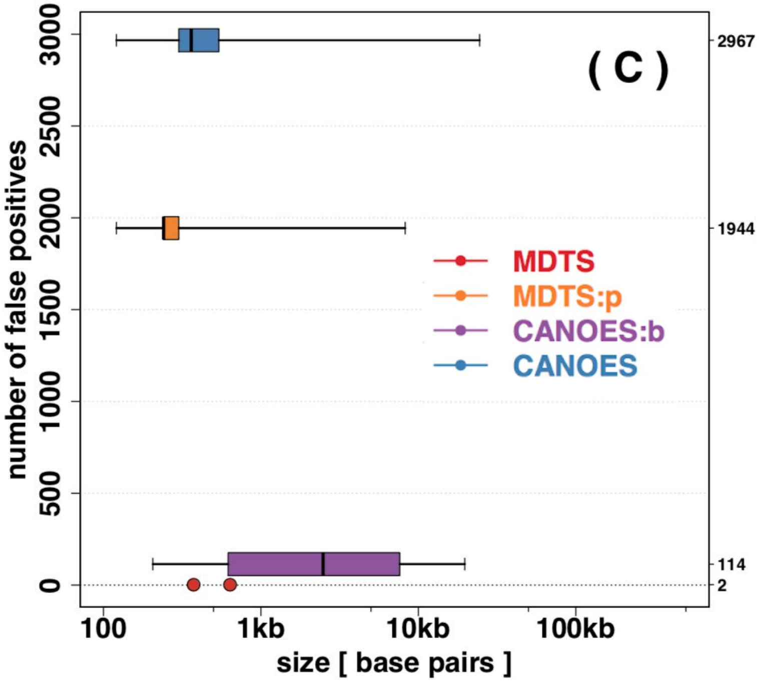
- Bin-effect
    - MDTS vs MDTS:p
    - CANOES vs CANOES:b
    - Significant bumps to sensitivity (deletions >250bp)

False positive inherited deletions



- Minimum Distance-effect
  - Regardless of binning scheme, our method is able to have negligible false positive identification of inherited deletions
  - Direct result of the use of the Minimum Distance statistic
  - CANOES exhibits false positives

Other false positives



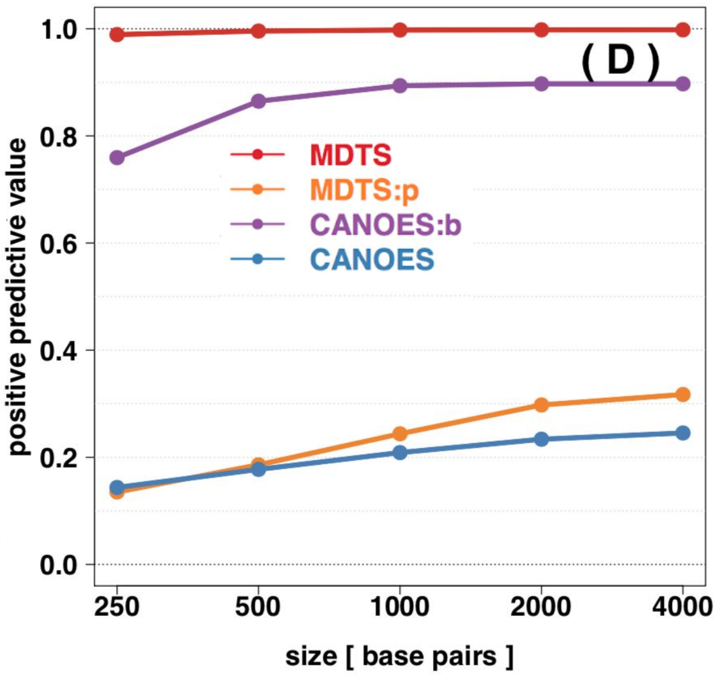
- No deletions were spiked-in for these identified regions

- Expected ~0.16 *de novo* structural variant per generation across ENTIRE GENOME*

- Finding >100 *de novo* deletions in 1/500 of the genome in 1000 repetitions/generations seems unreasonable

* https://www.ncbi.nlm.nih.gov/pubmed/25883321

Positive predictive value



- Positive predictive value (PPV)
  - A/(A+C)

- MDTS
  - ~100% PPV

- CANOES
  - High number of false positive calls

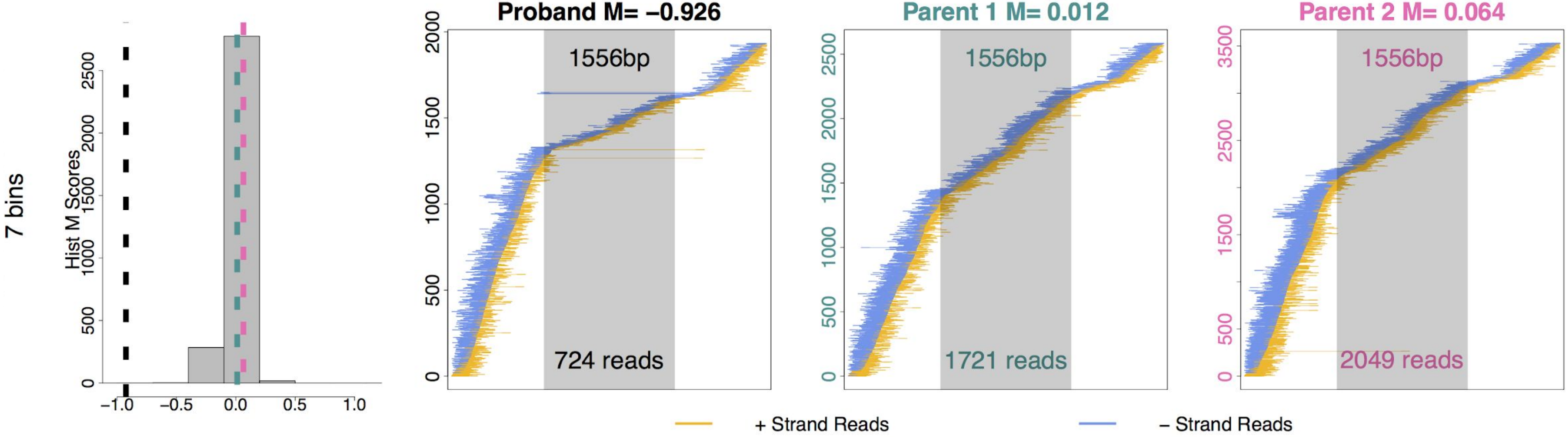
- CANOES:b
  - Significant boost to CANOES by using our dynamic bins

Performance in oral cleft data

- Only 3 signals
  - 1,018 trios
  - 6.3Mb targeted sequencing

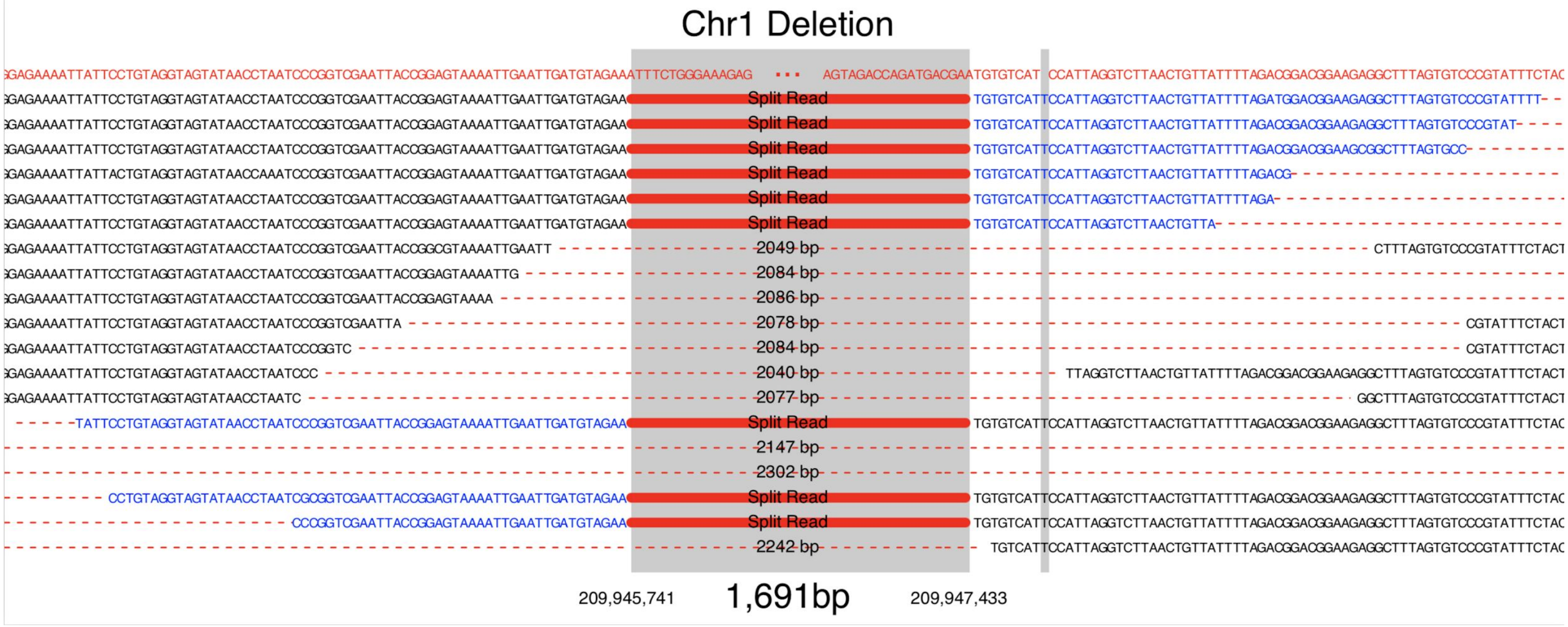
1) Definitive
2) Possible
3) Inherited deletion

1) Definitive

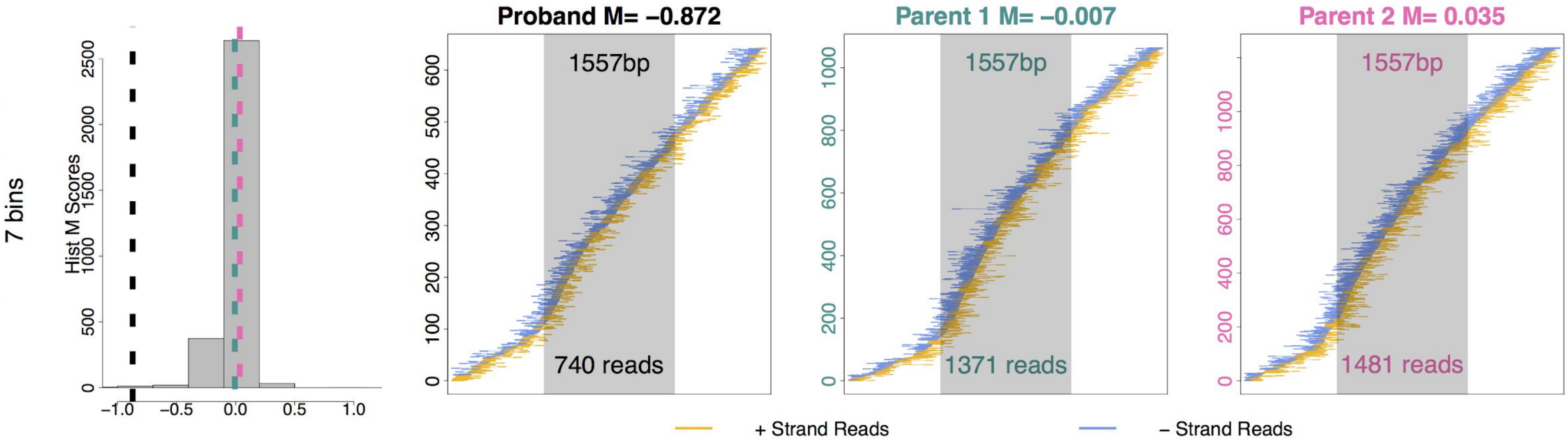


- Family DS10826
- MD = -0.9
- [Chr1: 209,945,655-209,947,210]

Supporting WGS data

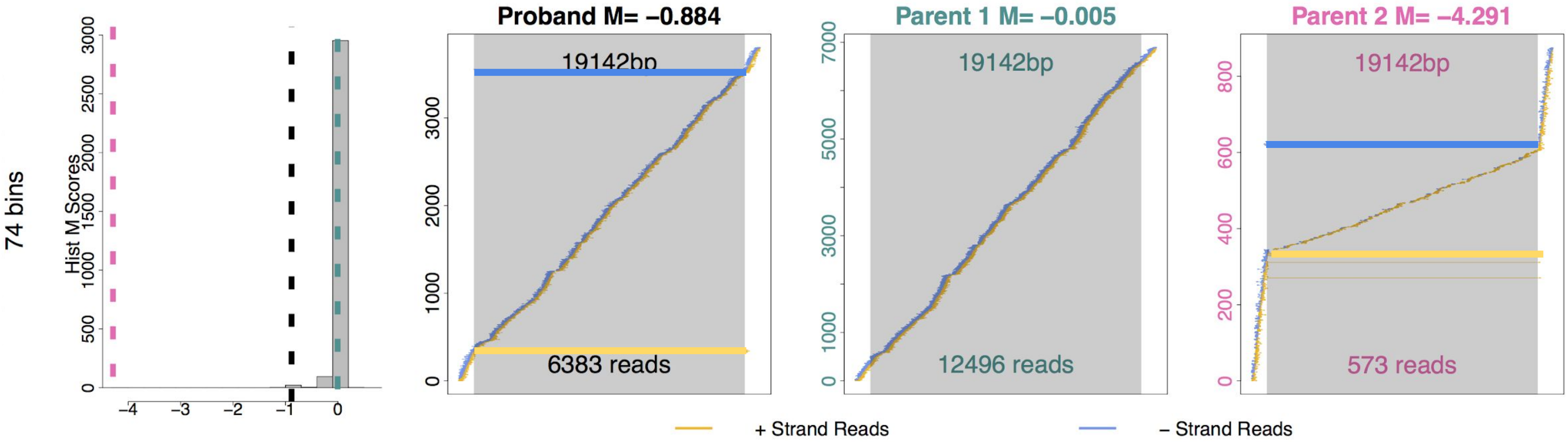

Chr1 Deletion
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAAATTTCTGGGAAAGAG ... AGTAGACCAGATGACGAATGTGTCAT CCATTAGGTCTTAACTGTTATTTTAGACGGACGGAAGAGGCTTTAGTGTCCCGTATTTCTAC
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTATTTTAGATGGACGGAAGAGGCTTTAGTGTCCCGTATTTT
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTATTTTAGACGGACGGAAGAGGCTTTAGTGTCCCGTAT
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTATTTTAGACGGACGGAAGCGGCTTTAGTGCC
GGAGAAAATTATTACTGTAGGTAGTATAACCAAATCCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTATTTTAGACG
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTATTTTAGA
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTA
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGCGTAAAATTGAATT 2049 bp CTTTAGTGTCCCGTATTTCTACT
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAATTG 2084 bp
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAA 2086 bp
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTA 2078 bp CGTATTTCTACT
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCCGGTC 2084 bp CGTATTTCTACT
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATCCC 2040 bp TTAGGTCTTAACTGTTATTTTAGACGGACGGAAGAGGCTTTAGTGTCCCGTATTTCTACT
GGAGAAAATTATTCCTGTAGGTAGTATAACCTAATC 2077 bp GGCTTTAGTGTCCCGTATTTCTACT
TATTCCTGTAGGTAGTATAACCTAATCCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTATTTTAGACGGACGGAAGAGGCTTTAGTGTCCCGTATTTCTAC
2147 bp
2302 bp
CCTGTAGGTAGTATAACCTAATCGCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTATTTTAGACGGACGGAAGAGGCTTTAGTGTCCCGTATTTCTAC
CCCGGTCGAATTACCGGAGTAAAATTGAATTGATGTAGAA Split Read TGTGTCATTCCATTAGGTCTTAACTGTTATTTTAGACGGACGGAAGAGGCTTTAGTGTCCCGTATTTCTAC
2242 bp TGTCATTCCATTAGGTCTTAACTGTTATTTTAGACGGACGGAAGAGGCTTTAGTGTCCCGTATTTCTAC
209,945,741
1,691bp
209,947,433

2) Possible



- Family DS12329
- MD = -0.82
- [Chr8: 129,614,522-129,616,078]

3) Unusual inherited hemizygous deletion



- Family DS11025
- MD = -0.88
- Chr8: 130,113,612-130,132,753

Performance in oral cleft data

	True *De Novo*	False Positives
MDTS	1	0
CANOES	1	2969
CANOES:b	1	89
TrioCNV	0	0
TrioCNV:b	0	24

Future directions

- A framework to rank identified candidates
- Extension to WGS and/or WES
- Statistical evaluation of bin depth/size tuning
    - Formal recommendations on how to choose the median number of reads falling into each bin

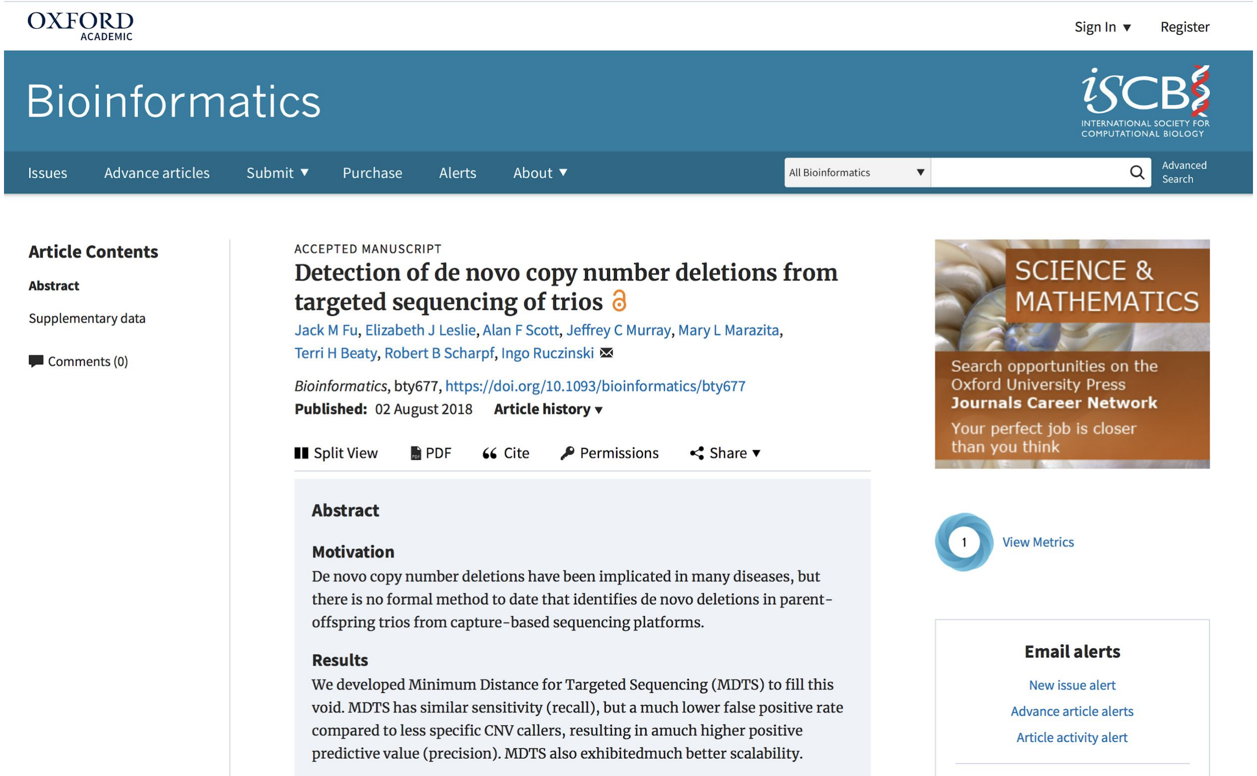
Summary

- *De Novo* copy number changes/deletions can have disease implications

- Understanding and accommodating the characteristics of sequencing is vital for downstream analysis

- Joint analysis of family data preferable to post-hoc comparisons

For the details…



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ACCEPTED MANUSCRIPT

Detection of de novo copy number deletions from targeted sequencing of trios

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Abstract

Motivation
De novo copy number deletions have been implicated in many diseases, but there is no formal method to date that identifies de novo deletions in parent-offspring trios from capture-based sequencing platforms.

Results
We developed Minimum Distance for Targeted Sequencing (MDTS) to fill this void. MDTS has similar sensitivity (recall), but a much lower false positive rate compared to less specific CNV callers, resulting in amuch higher positive predictive value (precision). MDTS also exhibitedmuch better scalability.

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