De novo identification of SNPs from RNA-seq data in non-model species

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N. Kremer, F. Vavre, M. Sagot, V. Lacroix

Lyon 1

Colib'read

informatics mathematics

LBBE
BIOMETRIE ET BIOLOGIE EVOLUTIVE

Single Nucleotide Polymorphism

…ATTGTGCATGAATTCTGAATACGGCTACGCCAT…

…ATTGTGCATGAATTCTAAATACGGCTACGCCAT…

genetic marker for association studies, population structure etc.

Single Nucleotide Polymorphism and NGS

…ATTGTGCATGAATTCTGAATACGGCTACGCCAT…

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genetic marker for association studies, population structure etc.

With NGS : massive access to sequencing, cheaper and easier

Single Nucleotide Polymorphism and NGS

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genetic marker for association studies, population structure etc.

With NGS : massive access to sequencing, cheaper and easier

Methods well developped for model species :

- rely on a (good) reference genome
- mostly for genome (DNAseq) re-sequencing

Why working with RNAseq ?

- Lower cost
- SNPs from **expressed regions**
- SNPs with a more direct **functional impact**

How to detect SNPs in RNAseq data ?

How to detect SNPs in RNAseq data ? for non model species ?

SNP identification from RNAseq

reads from RNAseq

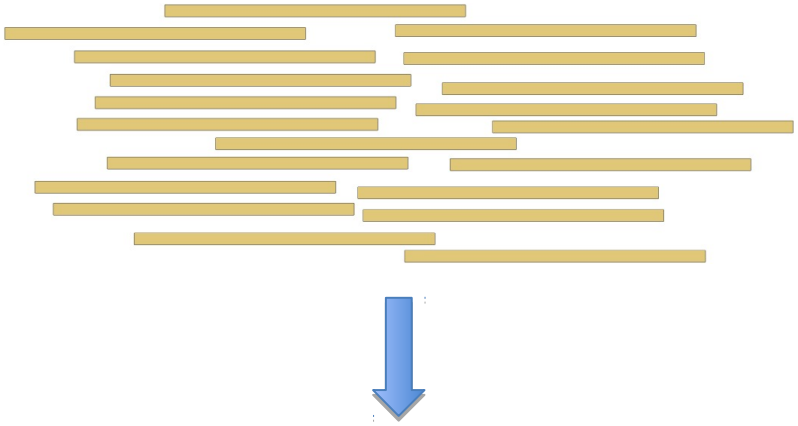
SNP identification from RNAseq
1) with a reference genome

reads from RNAseq

reference genome

SNP identification from RNAseq
1) with a reference genome

reads from RNAseq



reference genome

Several softwares :

- SNPiR
- GATK
- SAMtools mpileup

etc.

SNP identification from RNAseq
2) with an assembled transcriptome

reads from RNAseq

transcriptome assembly :

Trinity

Oases

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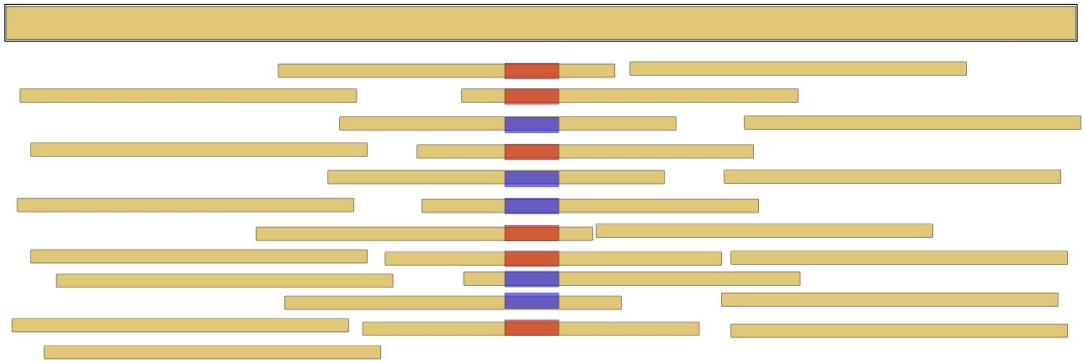

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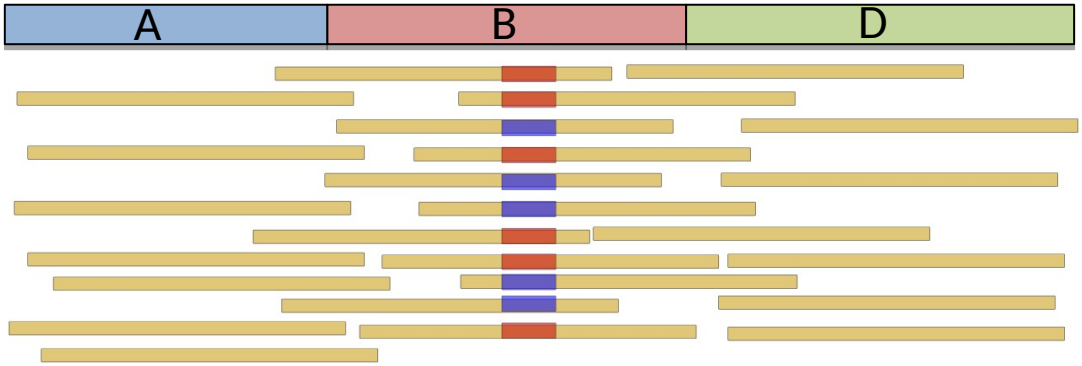

SNP identification from RNAseq
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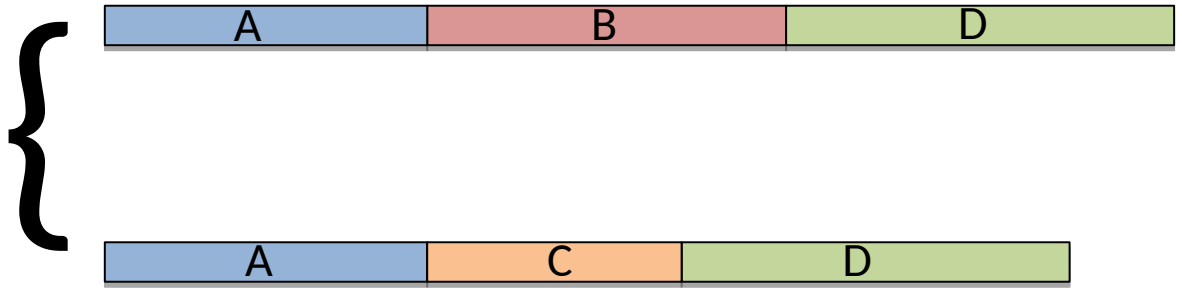
transcriptome assembly :

two assembled transcripts
from one gene
due to alternative splicing



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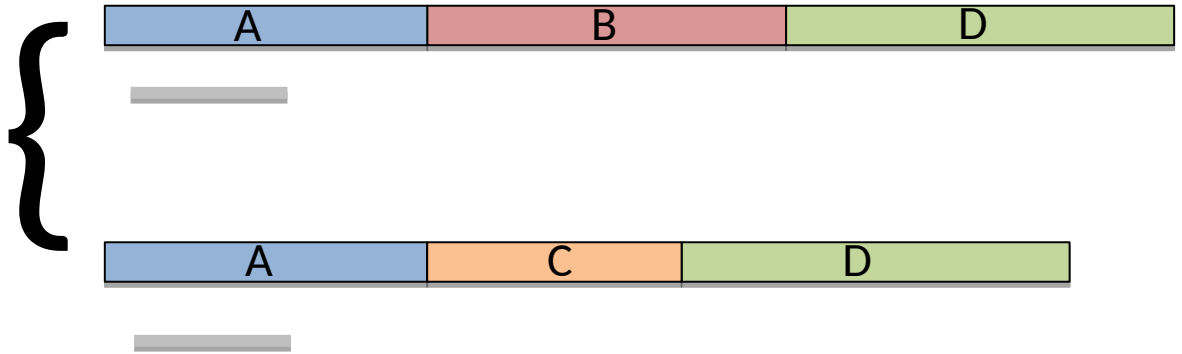

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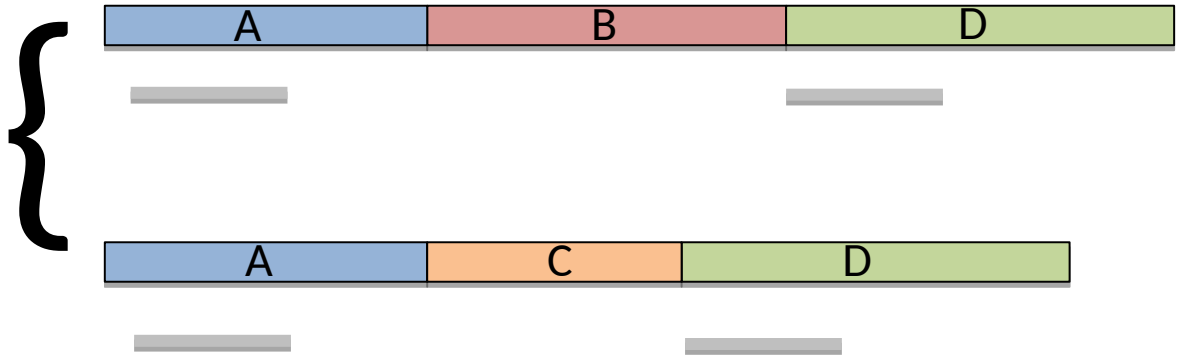

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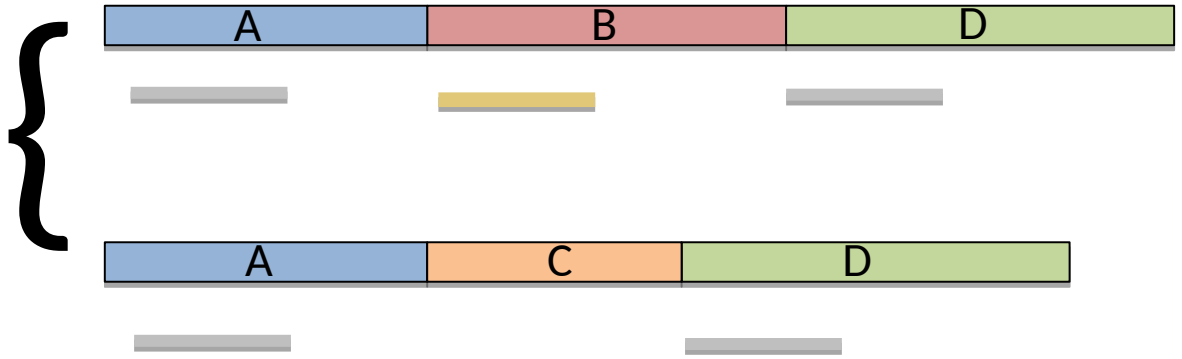

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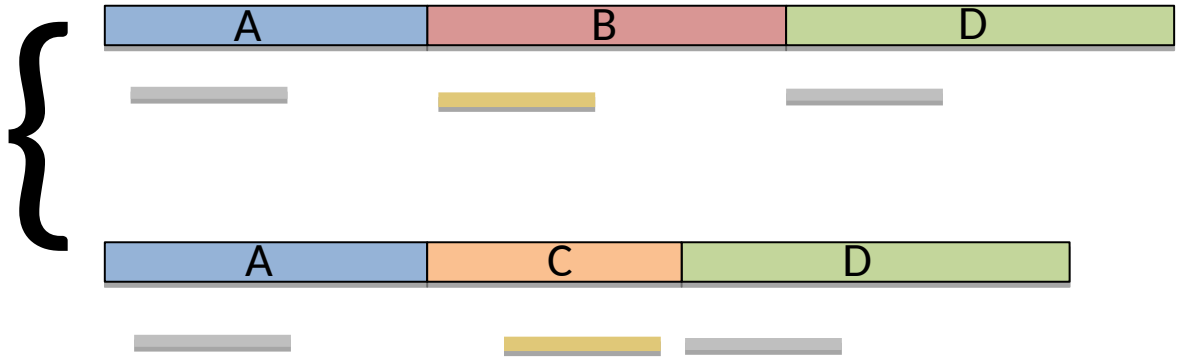

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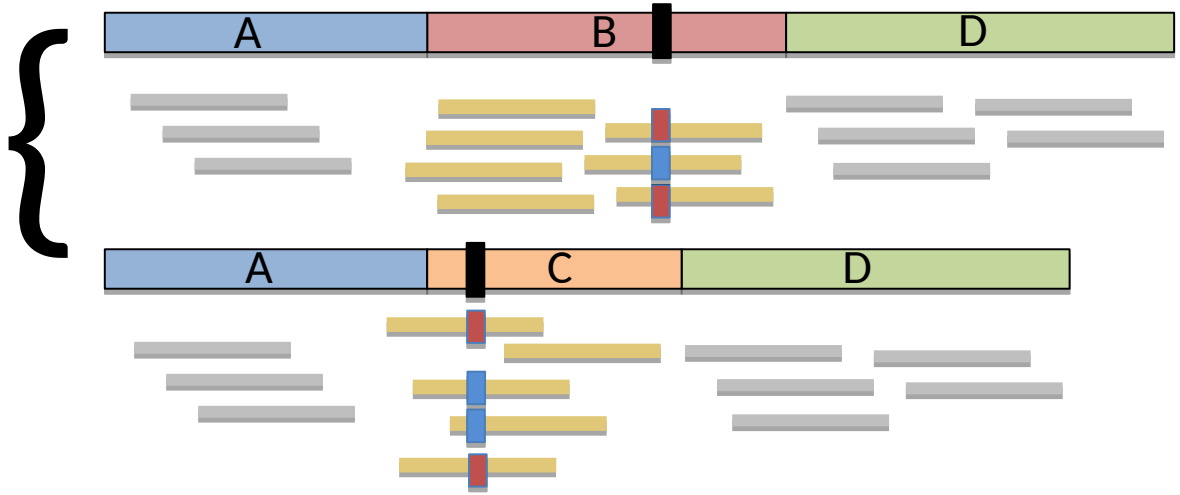

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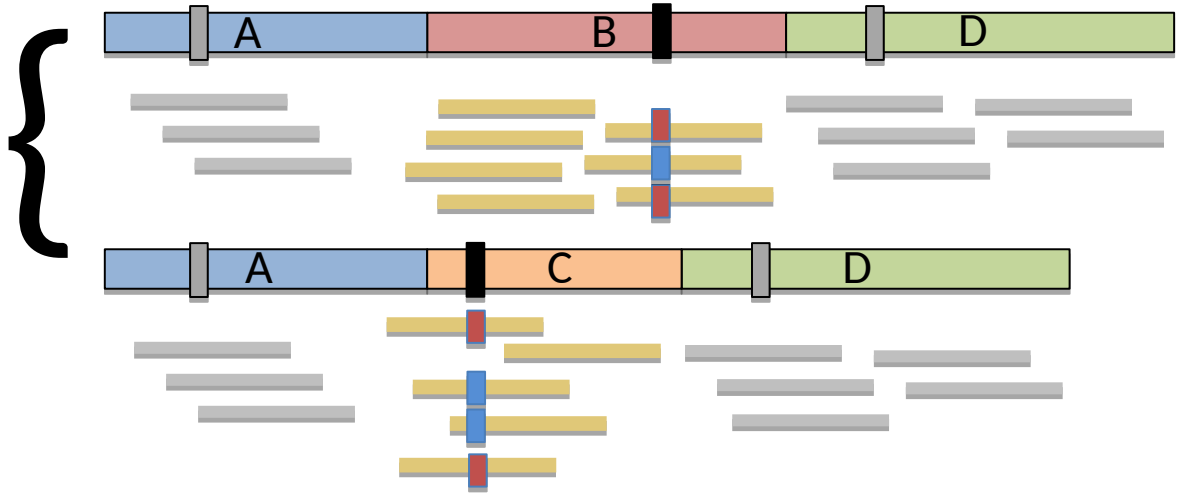

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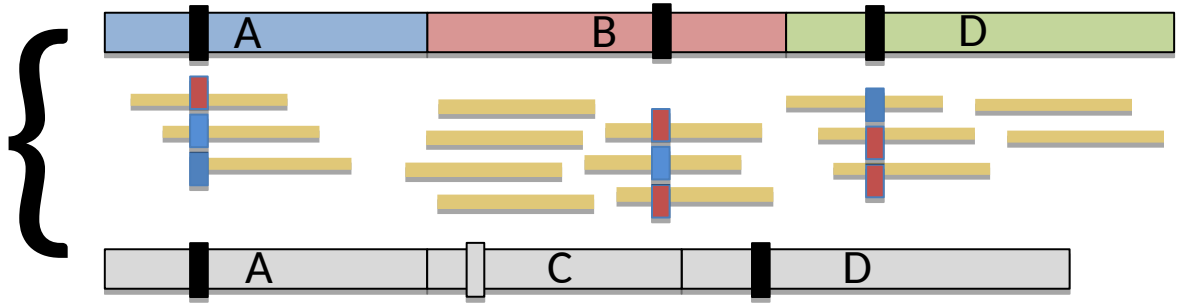
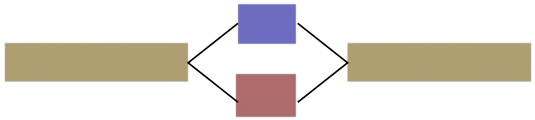


SNP identification from RNAseq
3) from a De Bruijn graph

reads from RNAseq

local assembly



SNP identification from RNAseq
3) from a De Bruijn graph

ATTCCTGCAATAC

GGTAGGTACATCGTGT

GTTGTGCATGAATTCTG

TGCATGAATTCTACAATA

ATGAGGTACATGCAT

GCATGAATTCTGCAATAC

GTTGTGCATGAATTCTA

ATTCCTACAATAC

KisSplice

SNP identification from RNAseq
3) from a De Bruijn graph

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GGTAGGTACATCGTGT

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ATGAGGTACATGCAT

GCATGAATTCTGCAATAC

GTTGTGCATGAATTCTA

ATTCCTACAATAC

ATT
TTC
TCC
CCT
...

KisSplice

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GTTGTGCATGAATTCTG

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KisSplice

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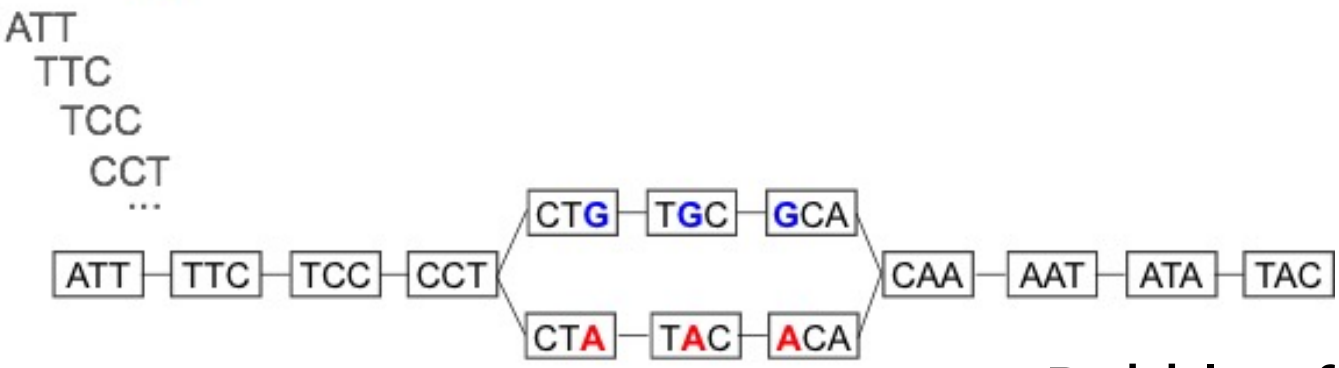

Bubble of size = 2k+1

SNP identification from RNAseq
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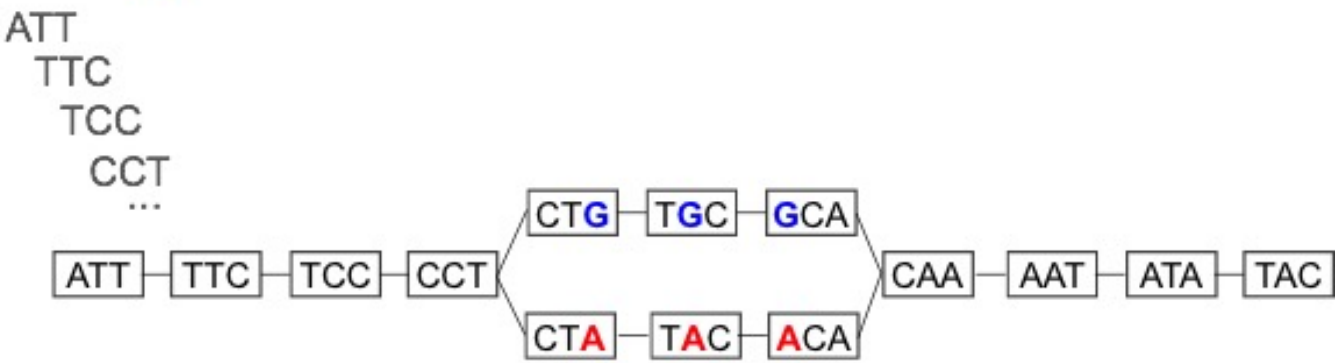

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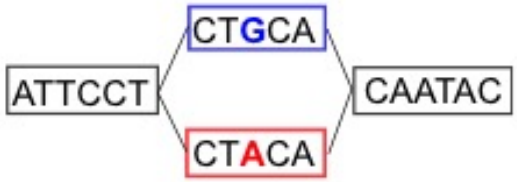

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GCATGAATTCTGCAATAC

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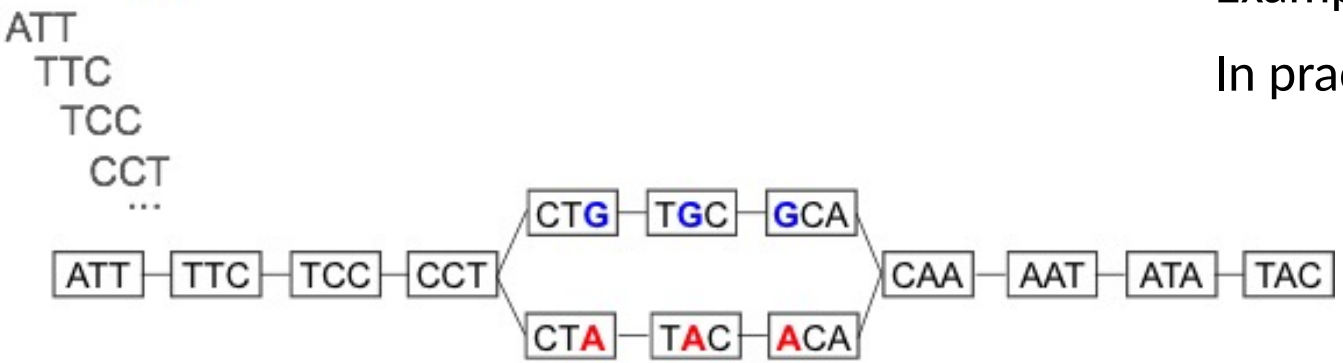
KisSplice

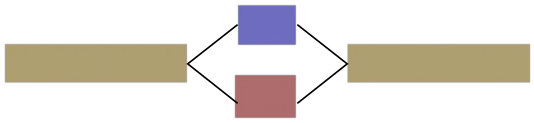
ATTCCTACAATAC

Example with k=3

In practice k=41 (default)



Bubble of size = 2k+1



SNP identification from RNAseq

1) with a reference genome

2) with an assembled transcriptome

3) directly from the De Bruijn graph

Validation of the method

Recall : Out of all the « True » SNPs, the proportion predicted by the method

Precision : Out of all the predicted SNPs, the proportion that is correctly predicted

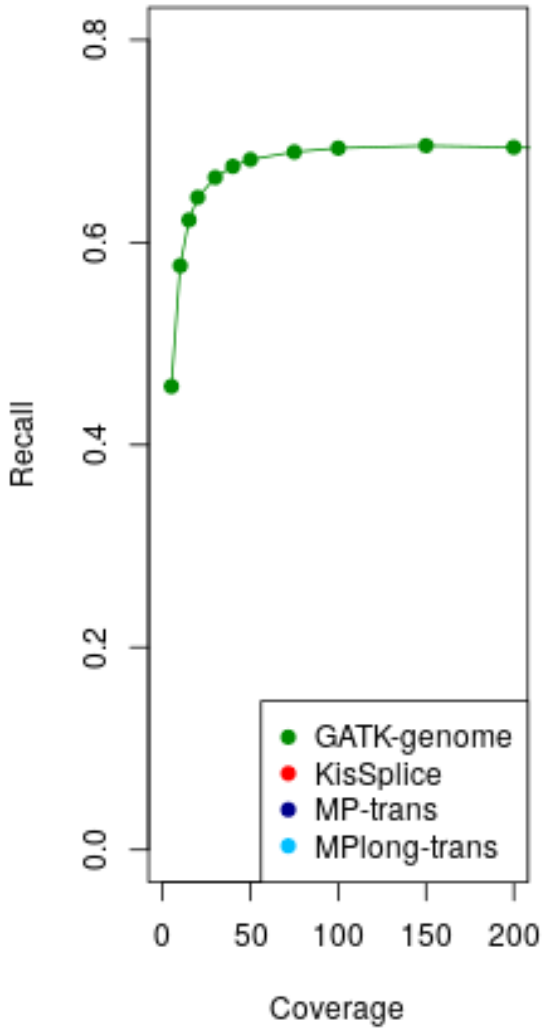
Validation of the method

Human dataset

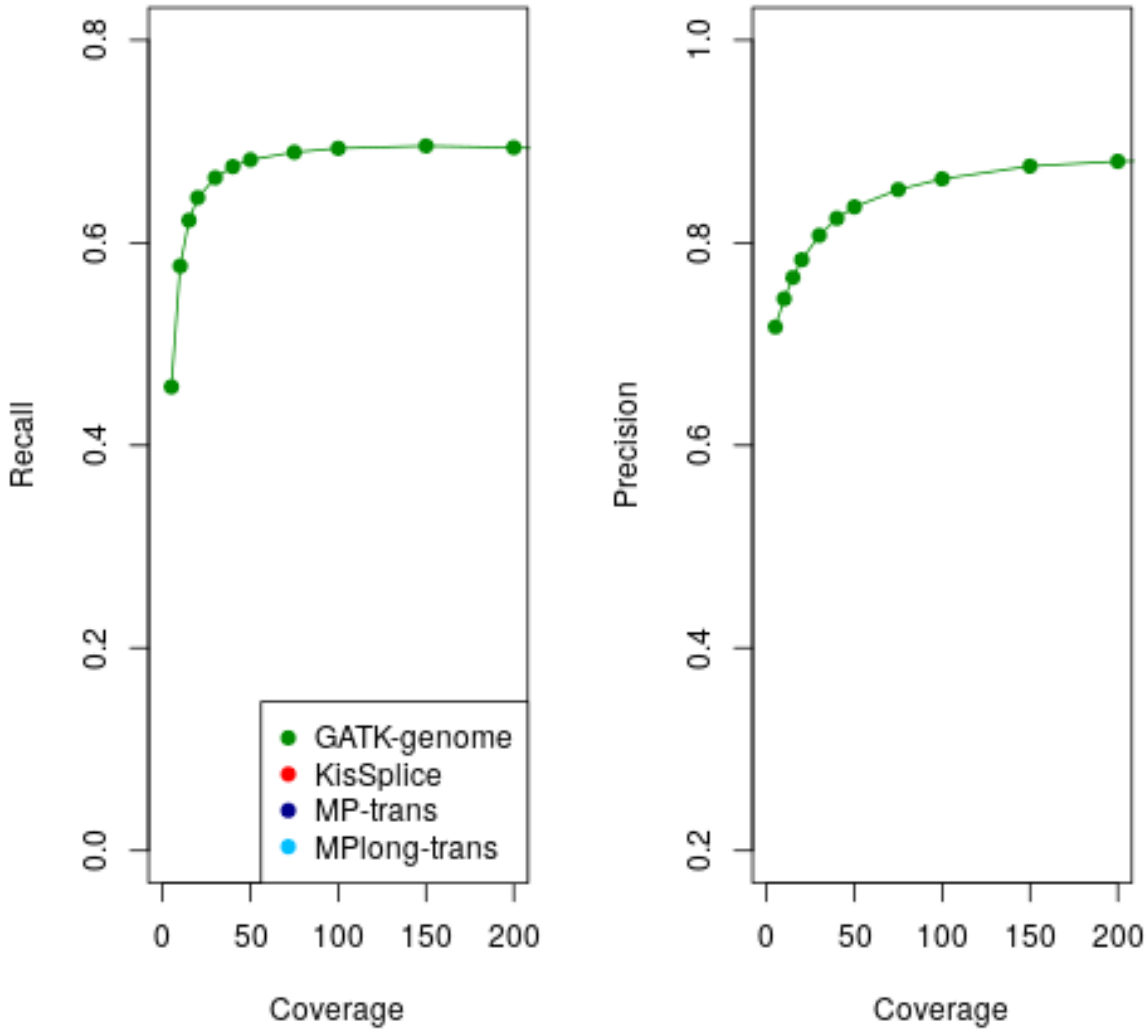
- genome and annotation available
- **1000G** project : genotype of 1000 individuals
- **Geuvadis** : RNAseq of these individuals

RNAseq from 2 x 5 central europeans and 2 x 5 toscans

Recall


Recall
0.0
0.2
0.4
0.6
0.8
0
50
100
150
200
Coverage
GATK-genome
KisSplice
MP-trans
MPlong-trans

Recall and precision



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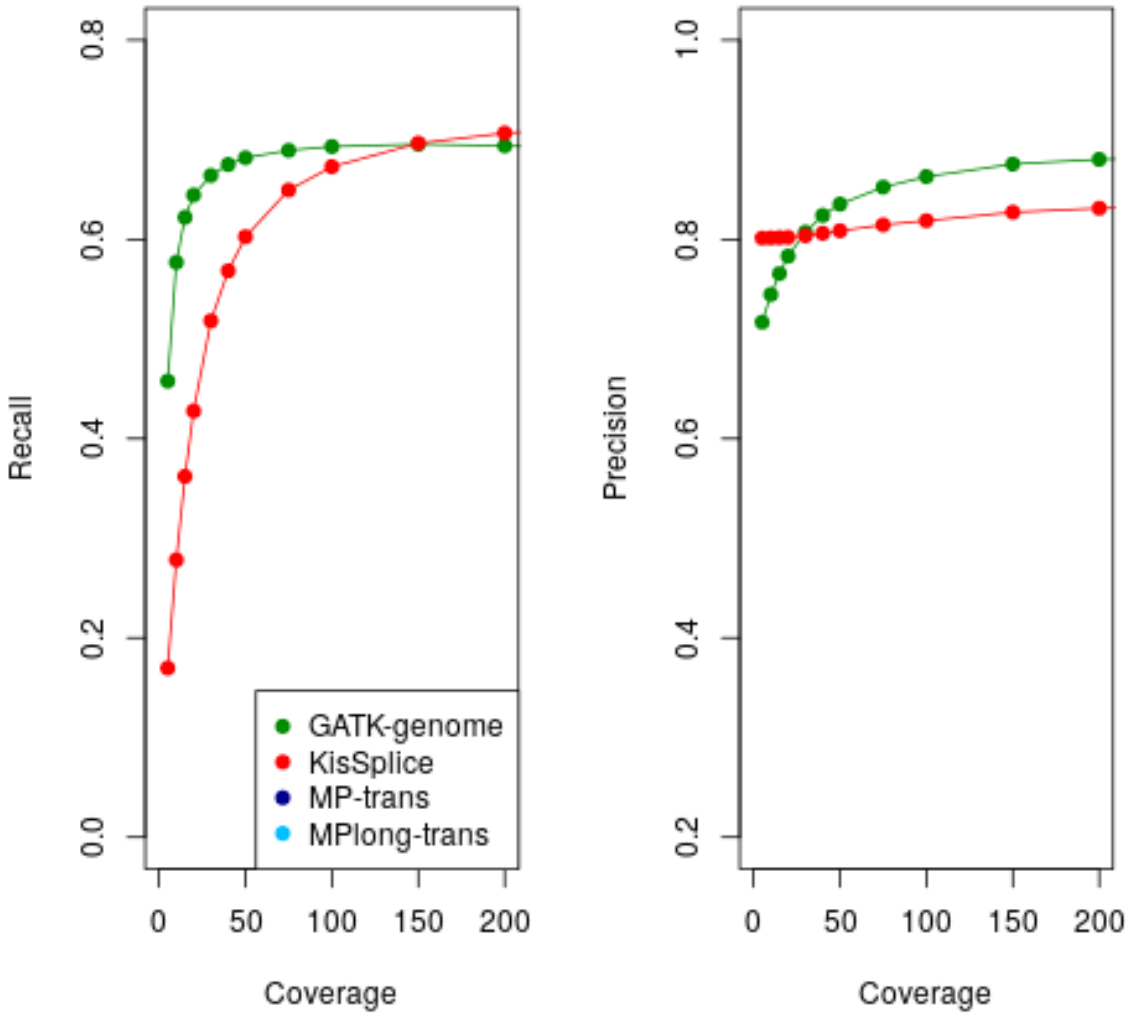

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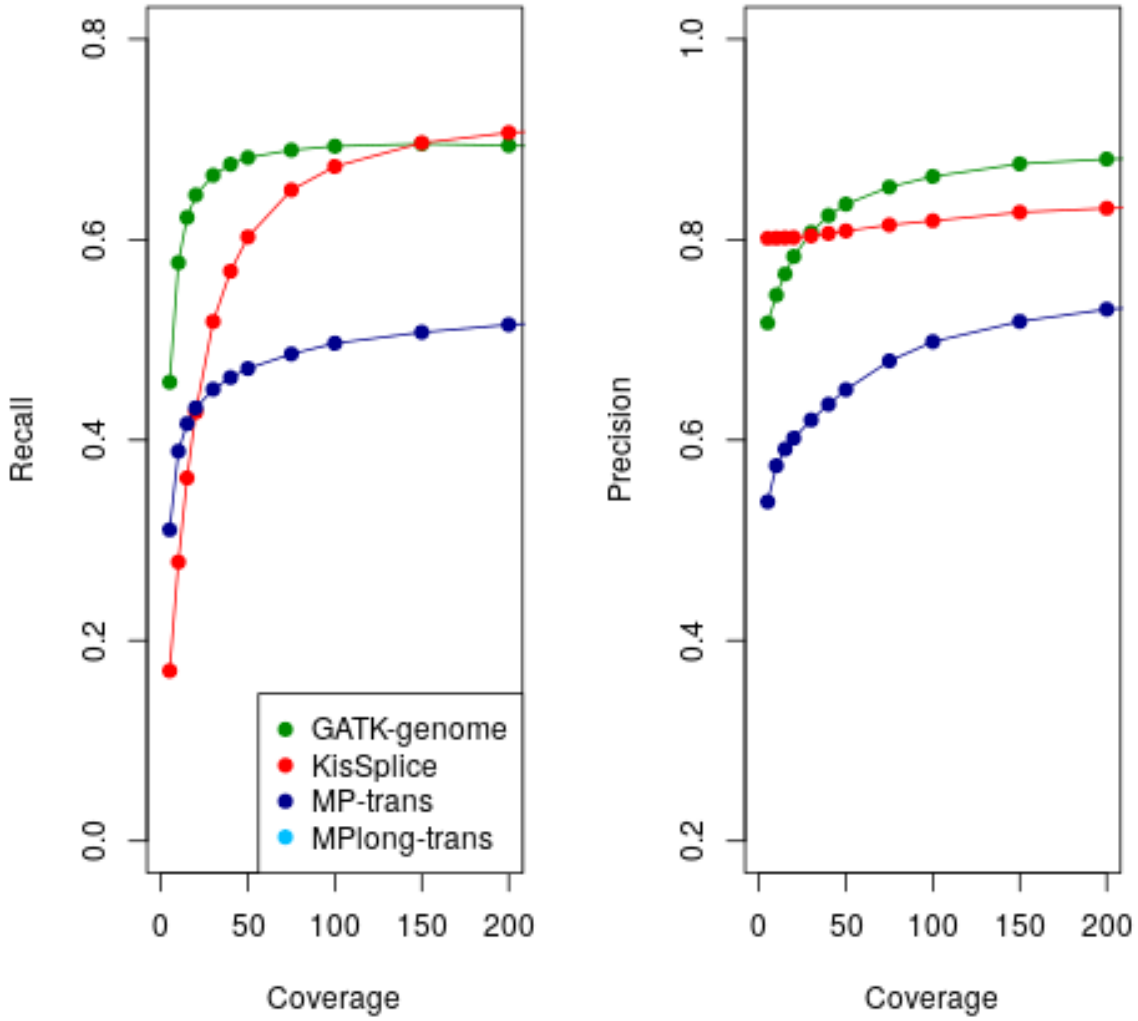

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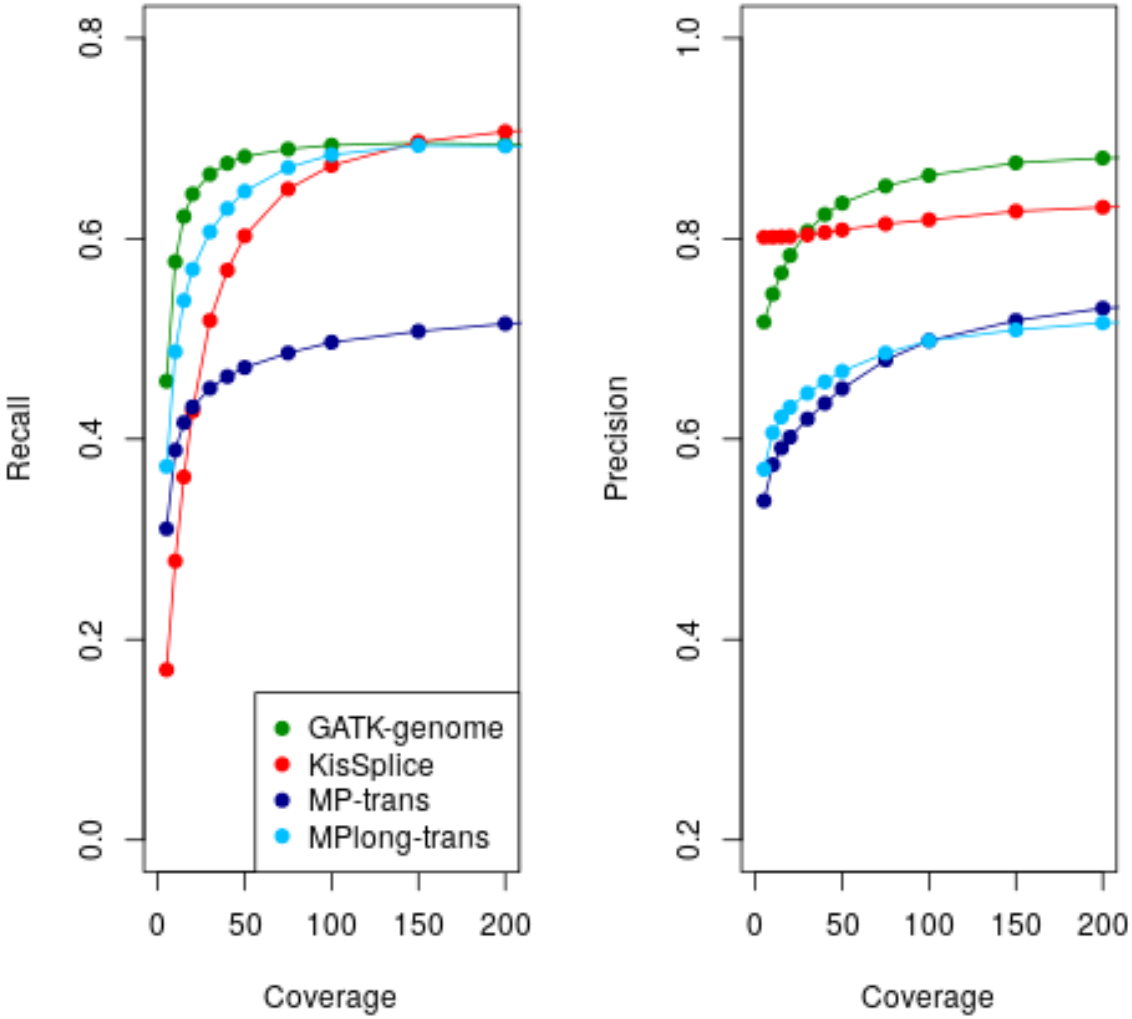


Recall and precision

- GATK > KisSplice > Mplong > MP
- False negatives include rare variants, and SNP clusters
- False positives include sequencing errors and inexact repeats

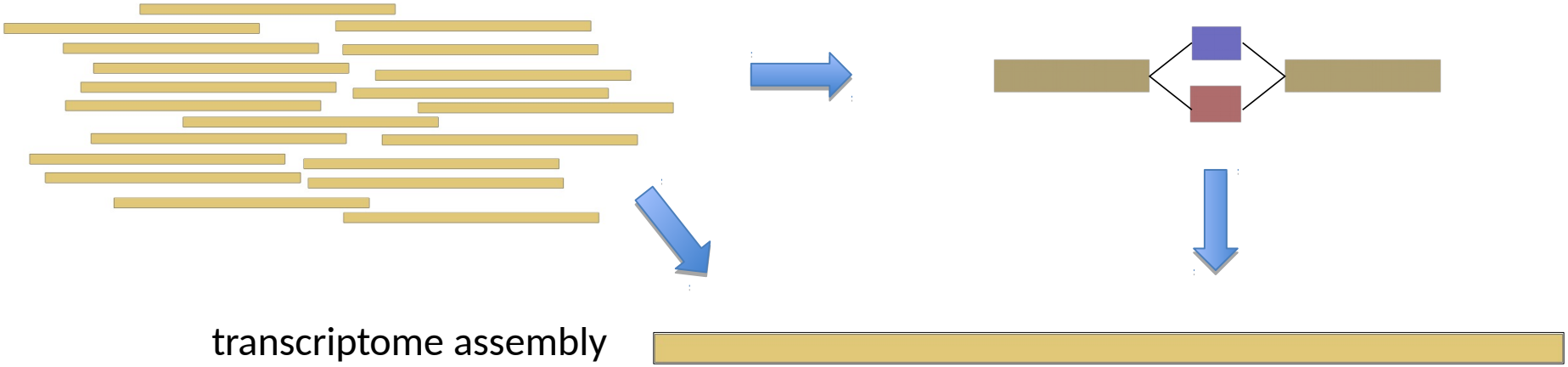
Downstream analyses

i) What is the impact on the protein ?

ii) Is my variant associated to a given biological condition ?

Prediction of the amino acid changes

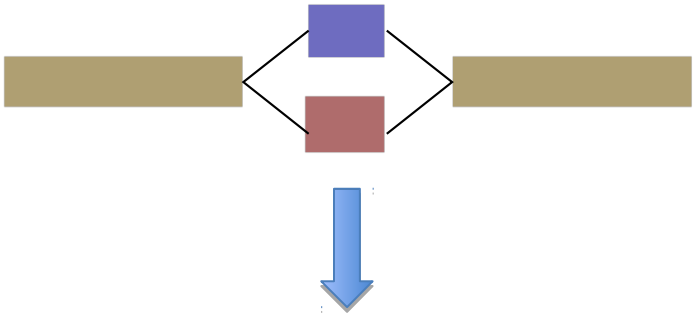
reads from RNAseq



transcriptome assembly

Prediction of the amino acid changes

KisSplice2refTranscriptome



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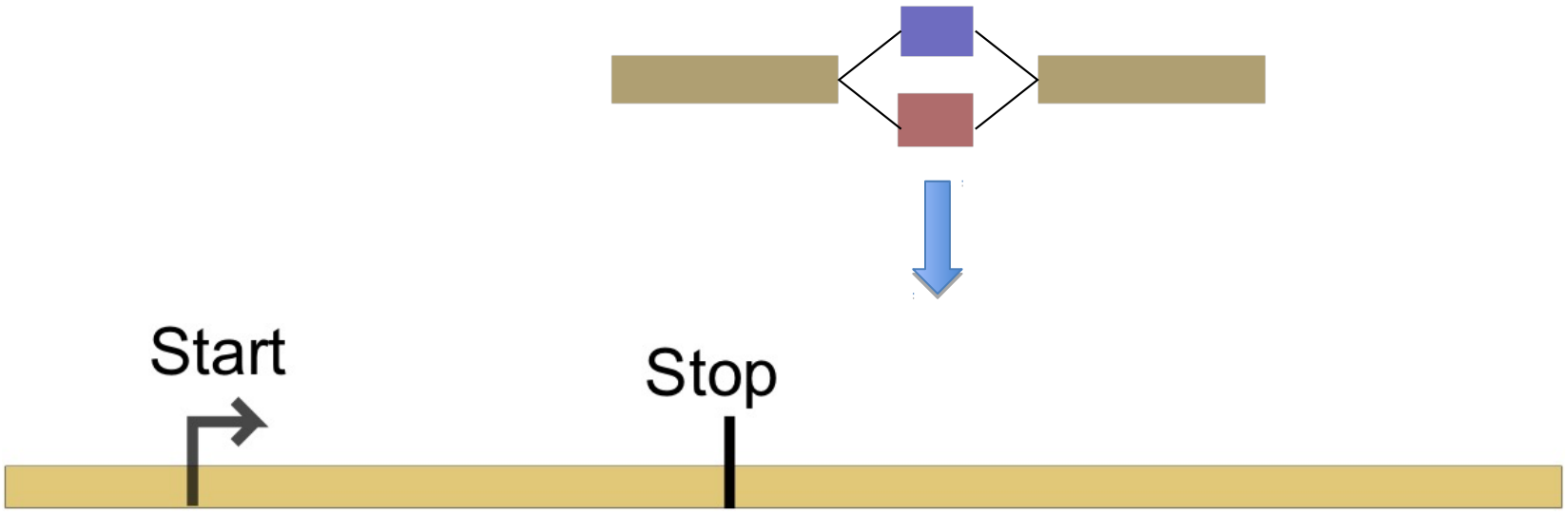

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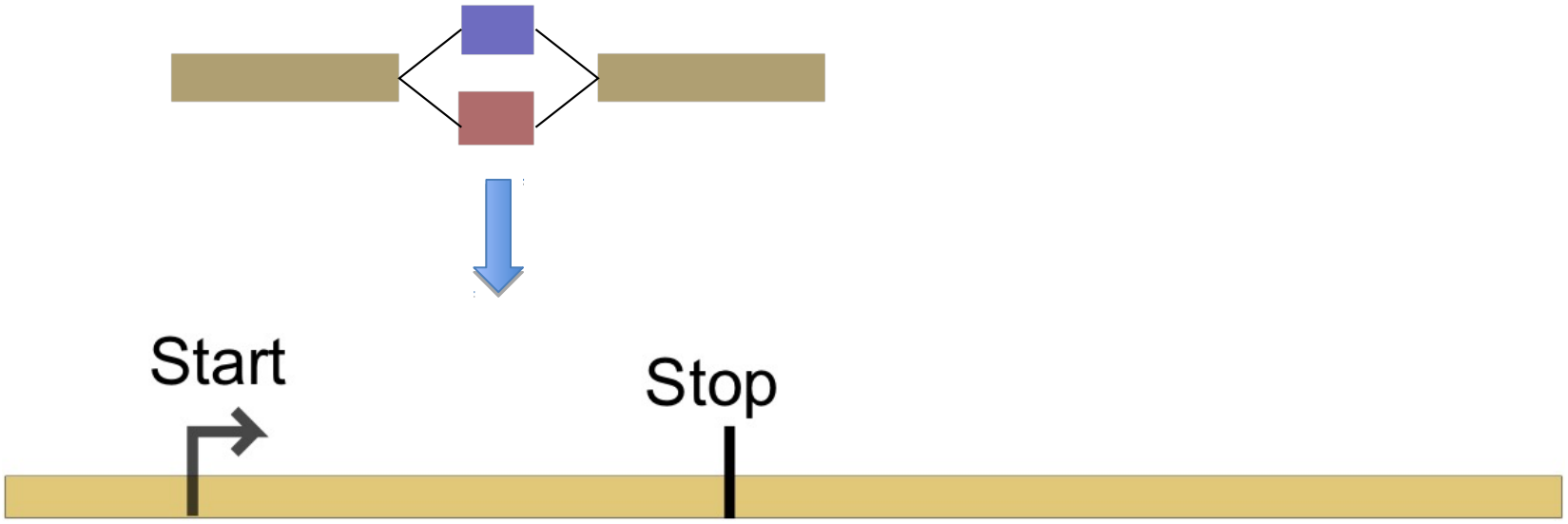

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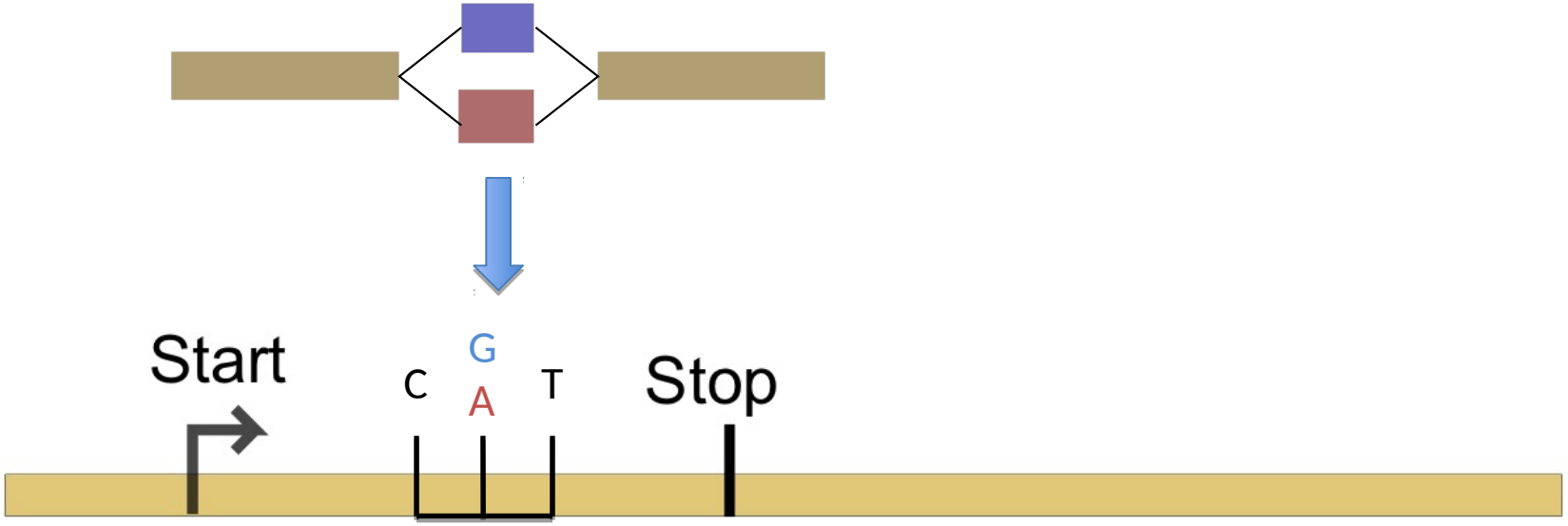

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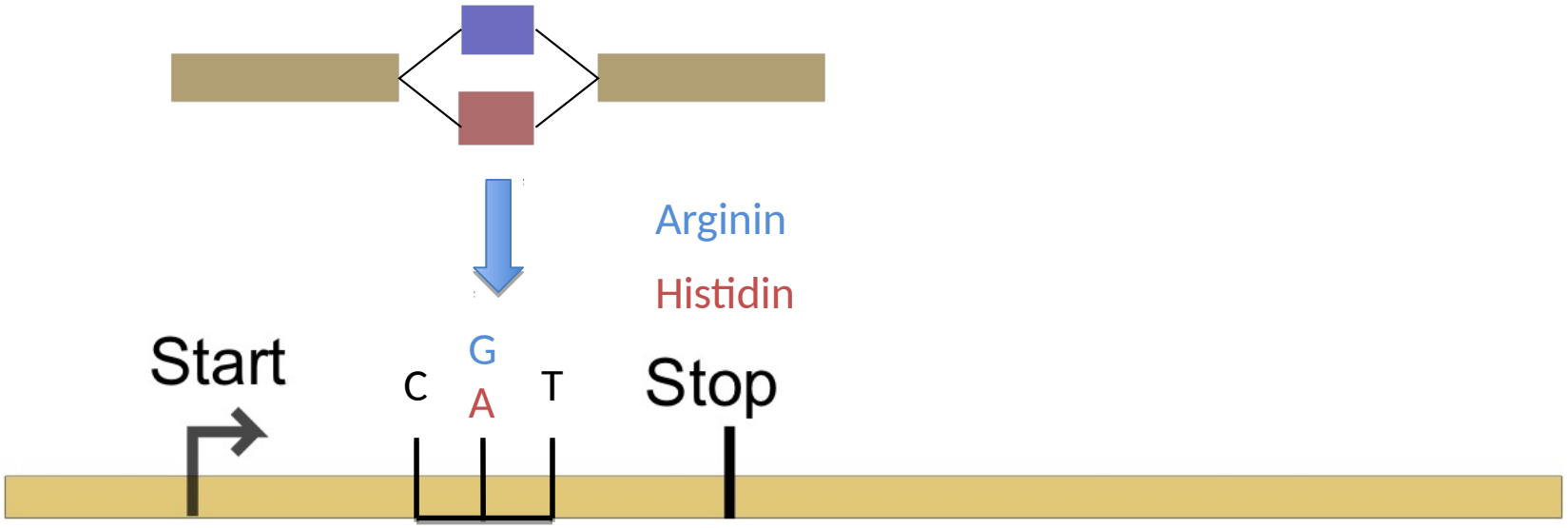

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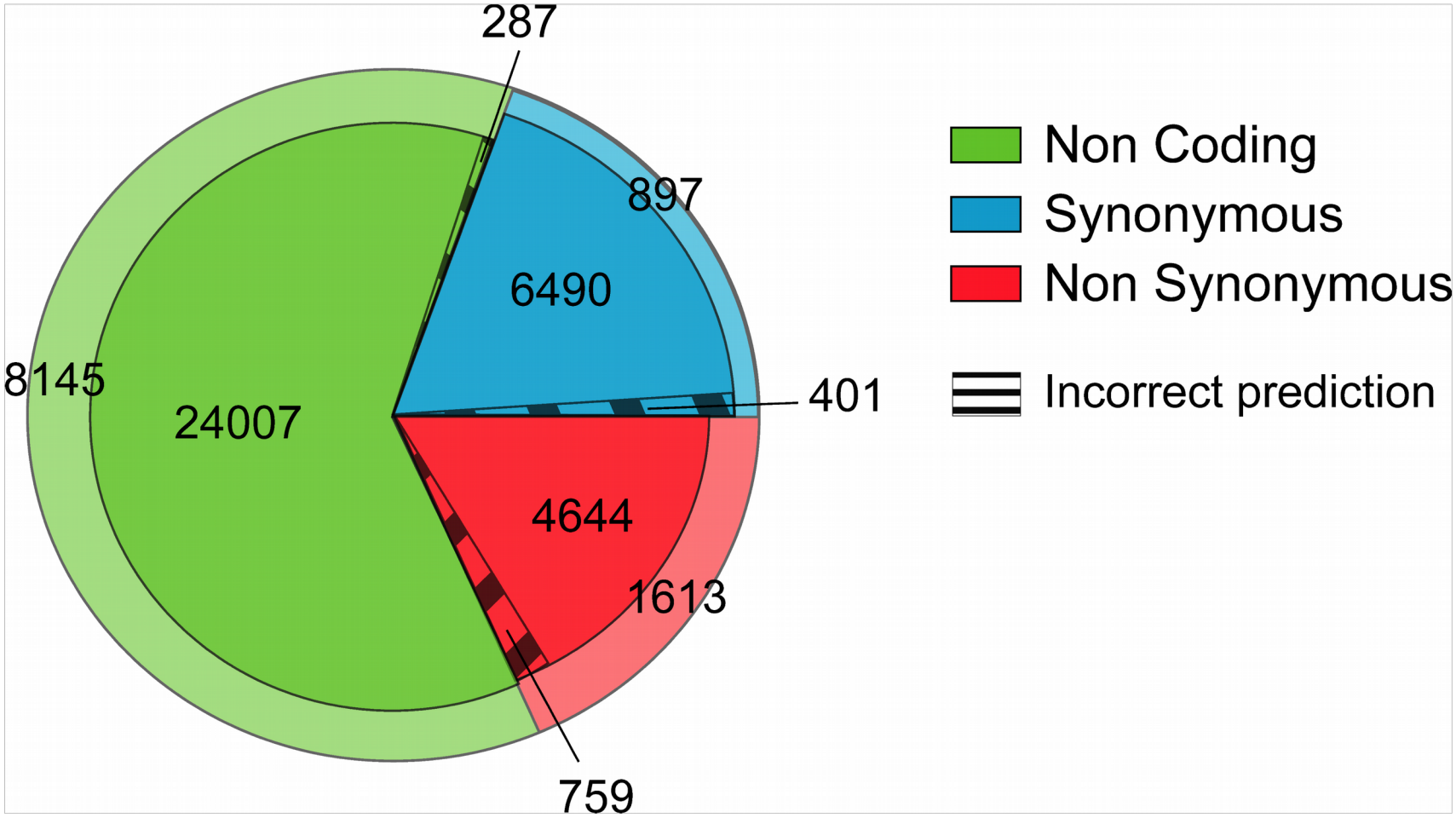
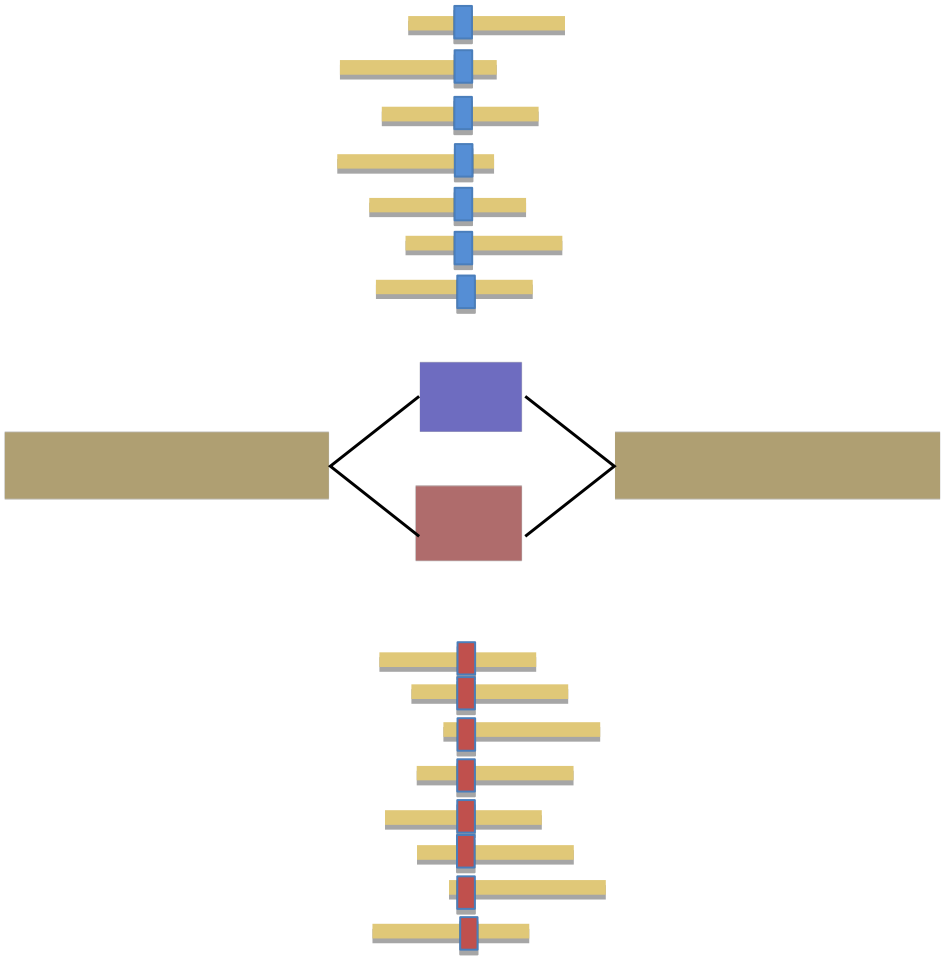


Quantification of variants



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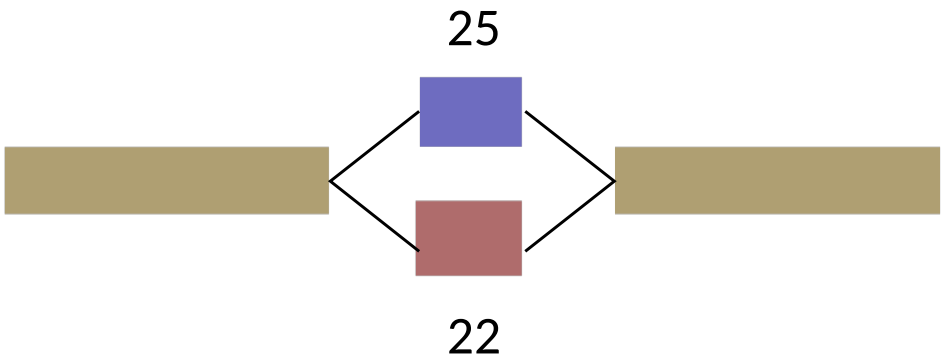
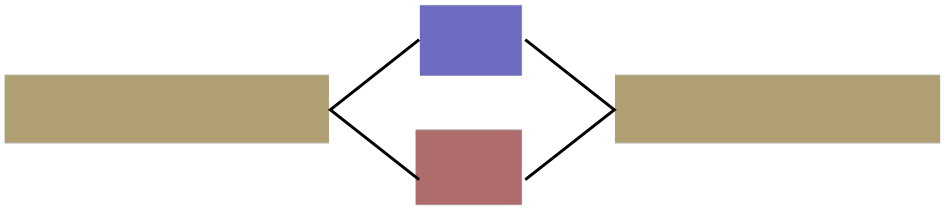


Is my variant specific to a condition ?

Condition 1

Condition 2



KissDE

Statistical Analysis

- Count regression with negative binomial distribution
- Generalised linear model, 2 way design, with interaction

$$\log \lambda_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}$$

Mean gene expression

Contribution of variant i

Contribution of condition j

Interaction term

$$H_0 : \{(\alpha\beta)_{ij} = 0\}$$

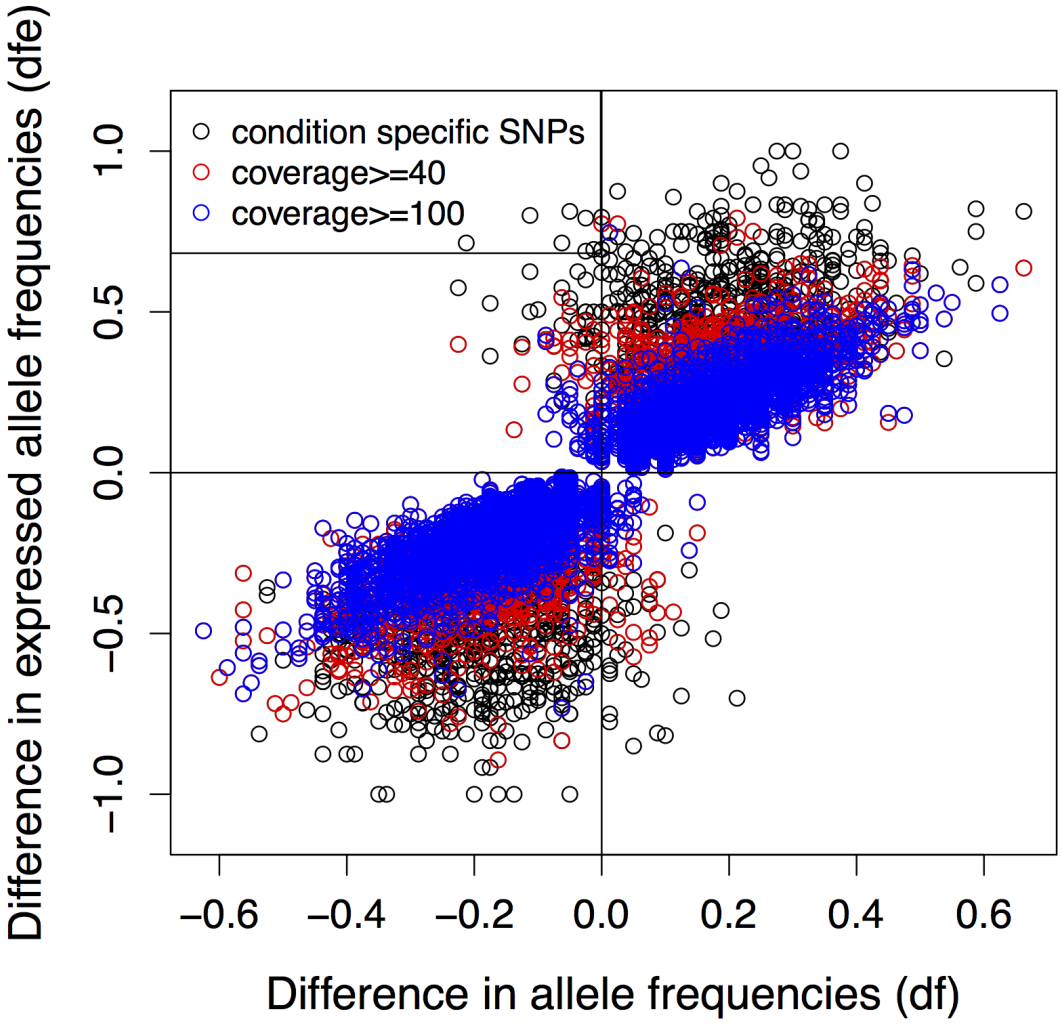
- Target hypothesis:
- Likelihood ratio test
- P-values adjusted with benjamini-hochberg procedure

Magnitude of the effect : Dfe

	C1	C2
Var. 1	23 20	2 1
Var. 2	4 2	18 16

Frequency of variant 1 in C1 : 90%
Frequency of variant 1 in C2 : 10%
Difference of allele frequency: 90 – 10 = 80 %

Dfe is a good proxy for Df when genes are highly expressed



Pipeline : Input

fastq files :

@HWI-D00393:39:C3PHDACXX:2:1101:8716:2263 1:N:0:CTATAC
TCAGTCTTTTTCTCTCTCCTAGTCCCATAATGCCTTGACGAACATCAGTCTGTCCCATAATGCTCTCCTATTA
+
CCCCCCCCCCCCCCCCCCCBBBBBBBBBBBBBBFFFFFFFFFFFFFFFFFJJJJJJJJJJJJJJJJJJJJJJJJJJJHHI92:?EGIJ
@HWI-D00393:39:C3PHDACXX:2:1101:11867:2372 1:N:0:CTATAC
TACGTCGGAAATATATAAAAAAATGGCACTGGAAGAATTCACTGGCACTGGAAGAATTCATGGCACTGGA
+
CCCCCCCCCCCCCCCCCCCCCB?BCBBBBBBBBCBBCBBFFFFFHHHHHJJJJJJIJJJHFHGIJJIIIHBGHIHDFH
@HWI-D00393:39:C3PHDACXX:2:1101:3047:2629 1:N:0:CTATAA
CCGTGGTCCCATAATGCCTTGACACGGTCTTGGCACTGGAAGAATTCAAGAATTCATGGCACTGGAAGAA
+
CCCCCCCCCCCCCCCBBBBBBBB?BBBBBBBBBBBBBBBBBBBCBBDDDDHHHHHJJJJJJJJJJJJIJFHIJJIJDH;
@HWI-D00393:39:C3PHDACXX:2:1101:8009:2719 1:N:0:CTATAC
CCCGACTGAAGGTGTACTCATGGCCAGGTCCCATAATGCCTTGACCTCTCCTATTACTGAAGGGGTGTACT
+
CCCCCCCCCCCCCCCCCCBCCCCCCCCCCBC?CBBBBBBBBBBBBBCFFFFFGGHHFHHHHHHHHJJIIJIIJJJI

Pipeline : Output

KisSplice + KissDE + Kissplice2refTranscriptome output :

c54770_g1_i3	1128	bcc_487|Cycle_259CAA	CCA	Q	P	NonSyn.	No

Pipeline : Output

KisSplice + KissDE + Kissplice2refTranscriptome output :

c54770_g1_i3	1128	bcc_487|Cycle_259	CAA	CCA	Q	P	NonSyn.	No	
c54495_g1_i1	1245	bcc_6275|Cycle_1	GCA	GCC		A	A	Syn.	No

Pipeline : Output

KisSplice + KissDE + Kissplice2refTranscriptome output :

c54770_g1_i3	1128	bcc_487|Cycle_259	CAA	CCA	Q	P	NonSyn.	No	
c54495_g1_i1	1245	bcc_6275|Cycle_1	GCA	GCC	A	A	Syn.	No	
c59969_g1_i1	1073	bcc_9888|Cycle_0	AGC	TGC	S	C	NonSyn.	Yes	

Pipeline : Output

KisSplice + KissDE + Kissplice2refTranscriptome output :

c54770_g1_i3	1128	bcc_487|Cycle_259	CAA	CCA	Q	P	NonSyn.	No	
c54495_g1_i1	1245	bcc_6275|Cycle_1	GCA	GCC		A	A	Syn.	No
c59969_g1_i1	1073	bcc_9888|Cycle_0	AGC	TGC		S	C	NonSyn.	Yes
c65293_g5_i2	847	bcc_6446|Cycle_0	N/A	N/A		N/A	N/A	NonCod.	Yes

Experimental Validations

Asobara tabida

ovaries infected with Wolbachia

wildtype

Asobara tabida

ovaries infected with Wolbachia

2 lines with variable dependency

no eggs

some sterile eggs

Asobara tabida

ovaries infected with Wolbachia
2 lines with variable dependency

RNAseq (ovaries) pool of 30 individuals

no eggs

some sterile eggs

Asobara tabida

ovaries infected with Wolbachia
2 lines with variable dependency

RNAseq (ovaries) pool of 30 individuals

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some sterile eggs

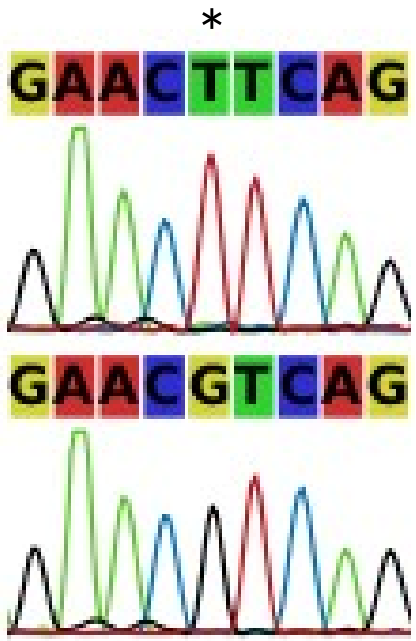
54532 SNPs of which 28392 condition specific

36/36 SNPs validated (rt-PCR + sequencing)
of which 7 predicted by Kissplice only

Experimental validation

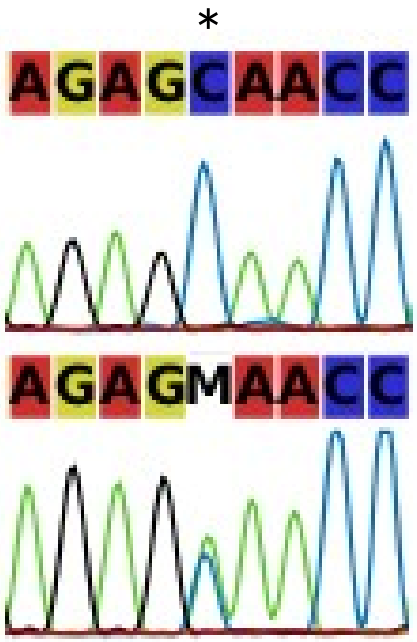
SFR2

SFR3



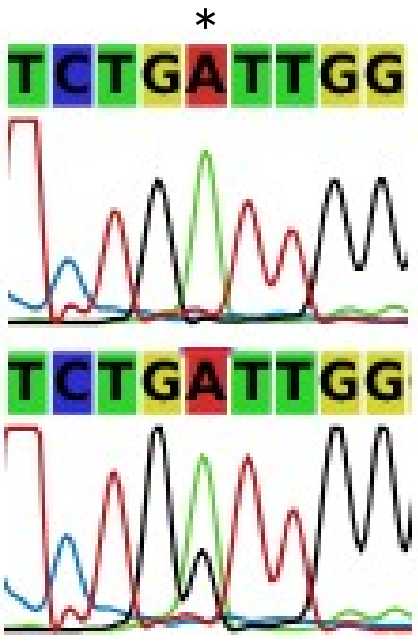
Nitric oxide synthase

SFR2: 0% / SFR3: 100%



Protein ovarian tumor

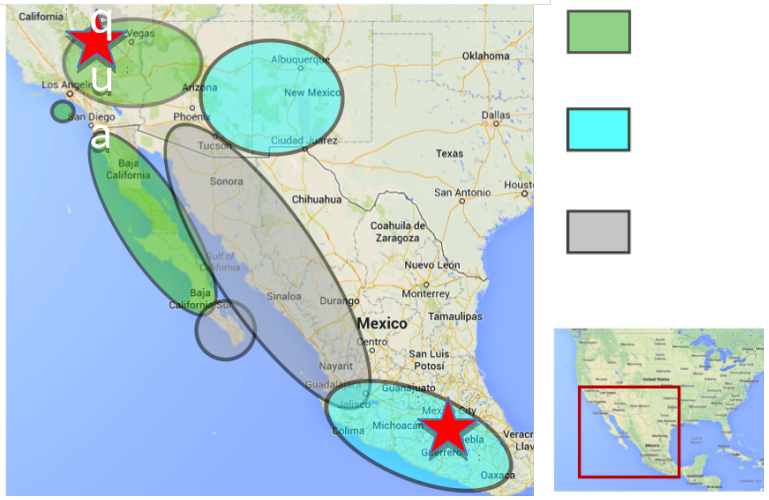
SFR2: 0% / SFR3: 33%



Pyrimidodiazepine synthase

SFR2: 100% / SFR3: 67%

Drosophila mojavensis & *D. arizonae*



D. mojavensis

D. arizonae

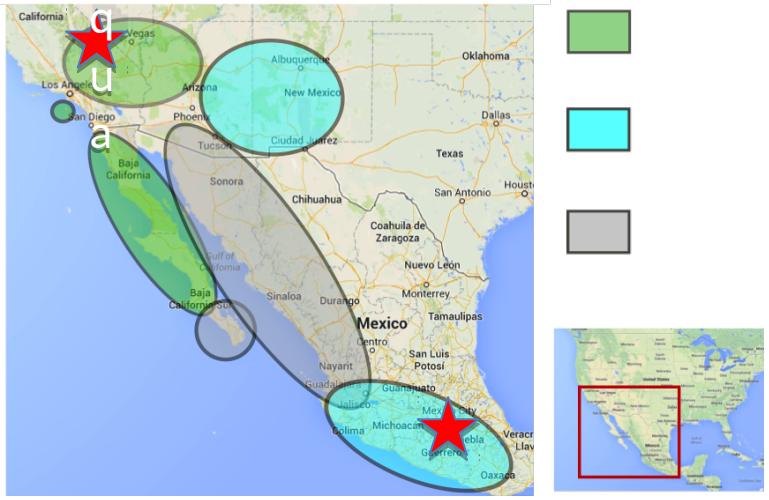
Sympatry

Divergence < 0.5 MYA

D. mojavensis

D. arizonae

Drosophila mojavensis & *D. arizonae*



D. mojavensis

D. arizonae

Sympatry

Divergence < 0.5 MYA

D. mojavensis *D. arizonae*

25 SNPs choosen for validation :

- 3/4 inexact repeats validated
- 19/21 SNPs and divergent sites validated
- 3 non validated cases

Conclusion

Detecting SNPs in non model species from (pooled) RNAseq data is possible

- Similar performances (precision and recall) compared to methods using a ref. genome

- Average precision is 77 %
- Filtering out SNPs which are not condition specific increases the precision to 96 %

Lopez-Maestre et al. NAR 2016 - http://kissplice.prabi.fr/TWAS/

Open questions

Two main challenges for SNP detection

1- Rare variants Vs sequencing errors

2- Inexact repeats Vs Close SNPs

What is the recall and precision of our method in species with large effective population size ?

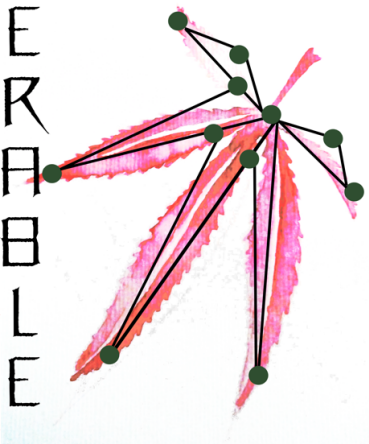
People

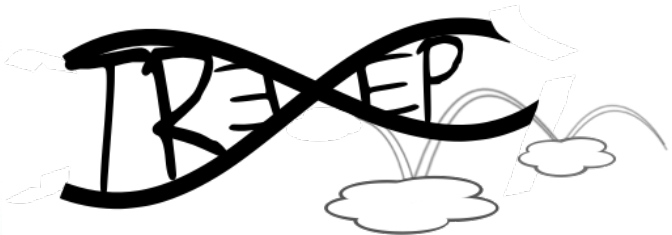
- KisSplice models and algorithms
  - Leandro Ishi, Gustavo Sacomoto, Rayan Chikhi, Pierre Peterlongo, Marie-France Sagot
- KisSplice2RefTranscriptome
  - Hélène Lopez-Maestre, Mathilde Boutigny
- KissDE
  - Camille Marchet, Clara Benoit, Audric Cologne, Janice Kielbassa, Lilia Brinza, Franck Picard
- Data analysis & Experimental Validation
  - Cristina Vieira, Hélène Lopez-Maestre (Drosophila)
  - Fabrice Vavre, David Monin, Natacha Kremer (Asobara)

Thanks to

H. Lopez-Maestre, L. Brinza,
C. Marchet, J. Kielbassa,
S. Bastien, M. Boutigny,
D. Monin, A. El Filali,
C. Carareto, C. Vieira, F. Picard,
N. Kremer, F. Vavre, M. Sagot,
V. Lacroix

ANR Colib'read

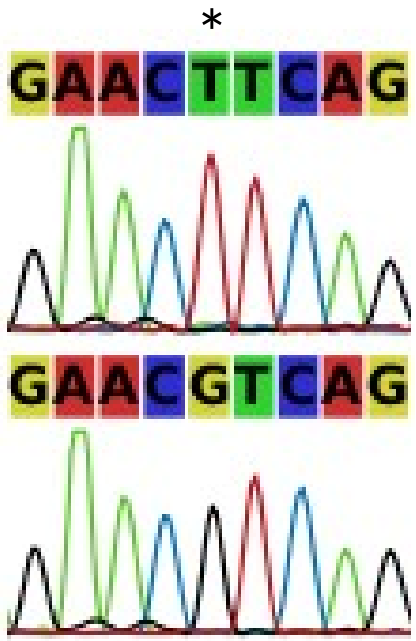




Experimental validation

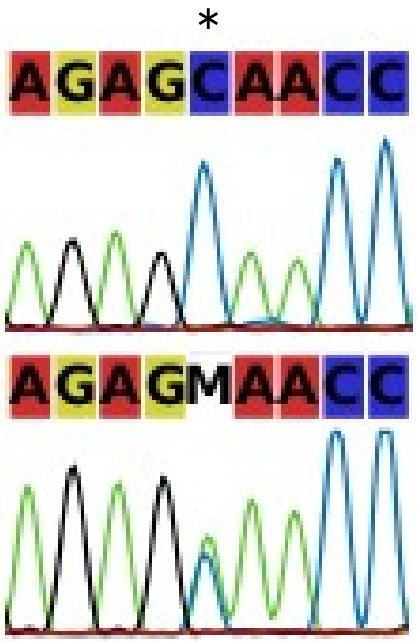
SFR2

SFR3



Nitric oxide synthase

SFR2: 0% / SFR3: 100%



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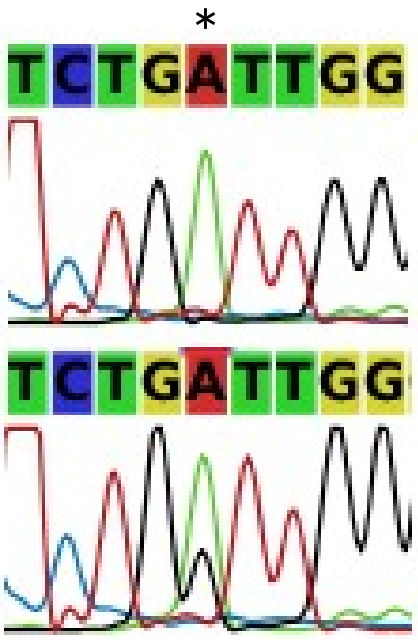


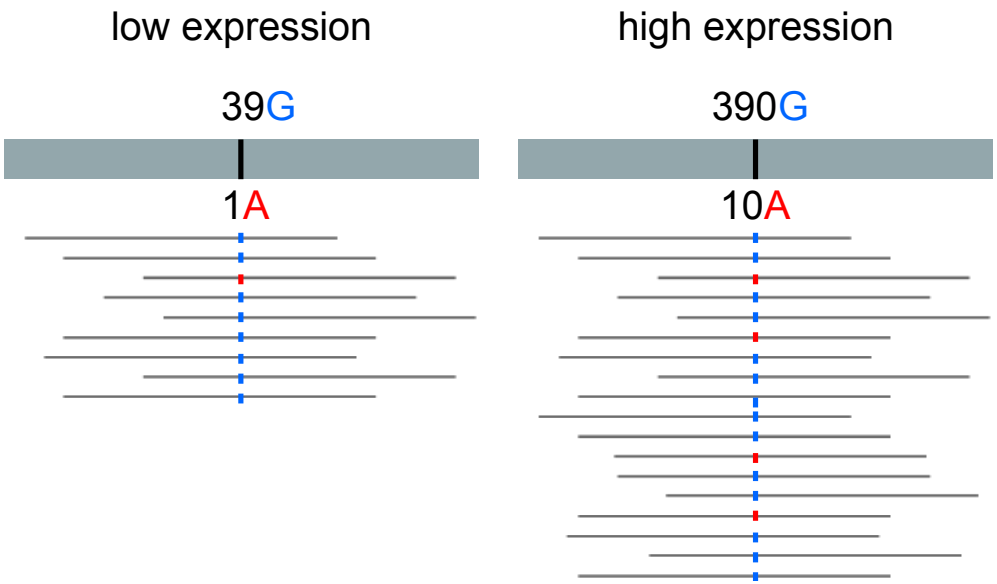
Pyrimidodiazepine synthase

SFR2: 100% / SFR3: 67%

Kissplice Filtres

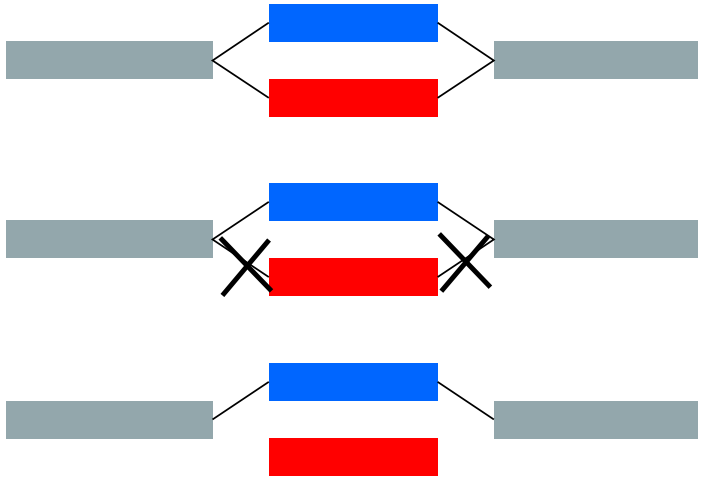
- Sequencing errors
- Inexacts repeats

Sequencing errors



absolute cutoff

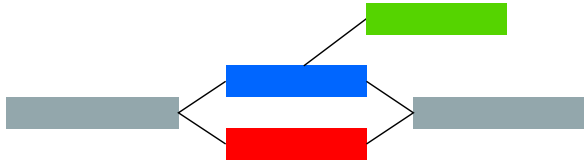
relative cutoff



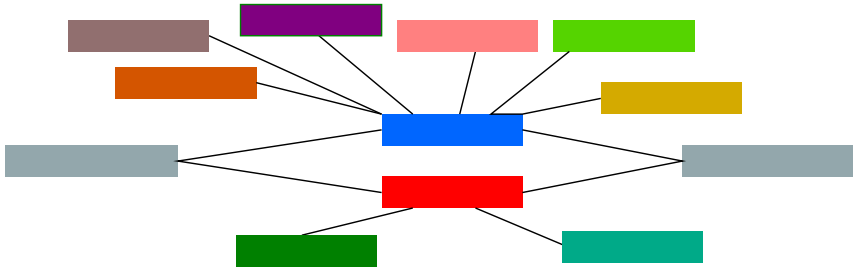
Inexacts repeats

...ATTGCTGCAATTC.....ATTGCTACAATTC...
...ATTGCTGCAATTC.....ATTGCTACAATTC...

...ATTGCTGCACTTC.....ATTGCTGCAATTC.....ATTGCTACAATTC...
...ATTGCTGCACTTC.....ATTGCTGCAATTC.....ATTGCTACAATTC...



X10 copies



Multiple SNPs

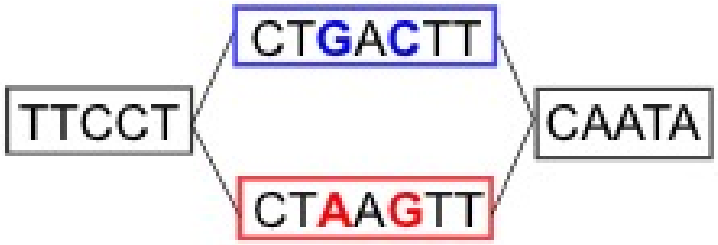
Exclus de l‘étude :

- Fort taux de faux positifs
- Probablement des répétitions inexactes

Close SNPs : phased

...TTCCTGACTTTGA...
...TTCCTAAGTTTGA...

distance <k



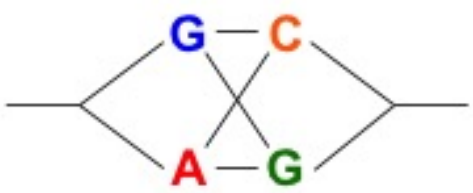
Bubble of size >2k+1

Close SNPs : non-phased

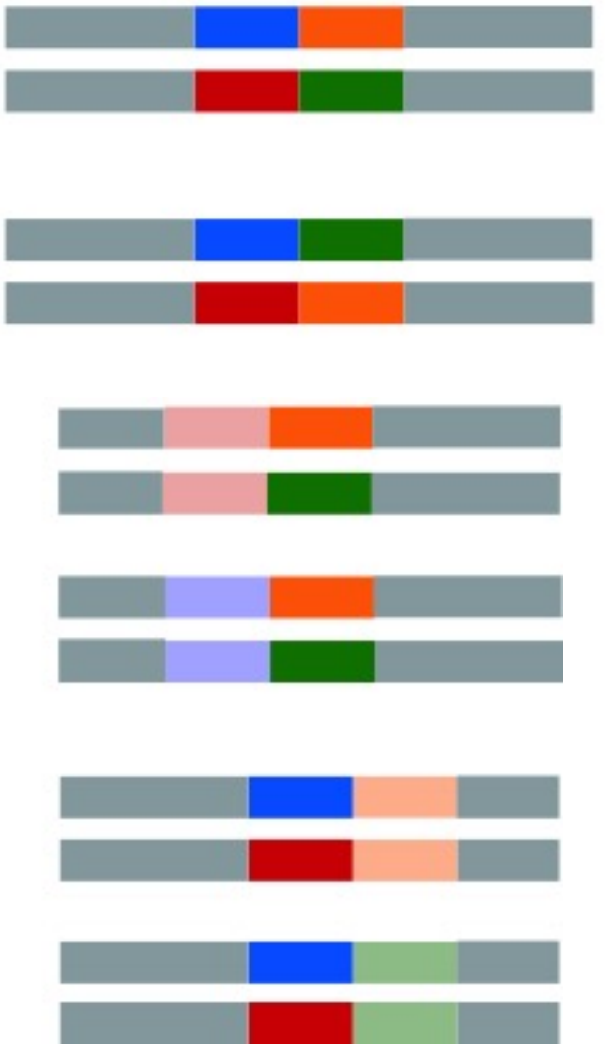
A)

...TCCTGACTTTG...
...TCCTAAGTTTG...
...TCCTGAGTTTG...
...TCCTAACTTTG...

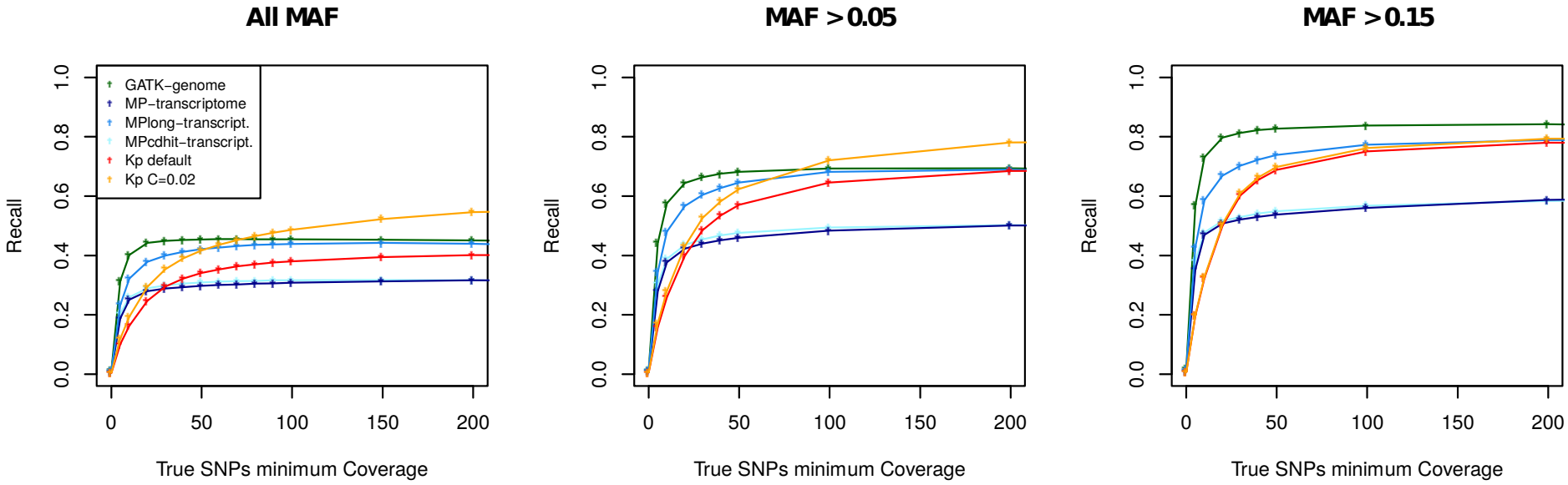
B)



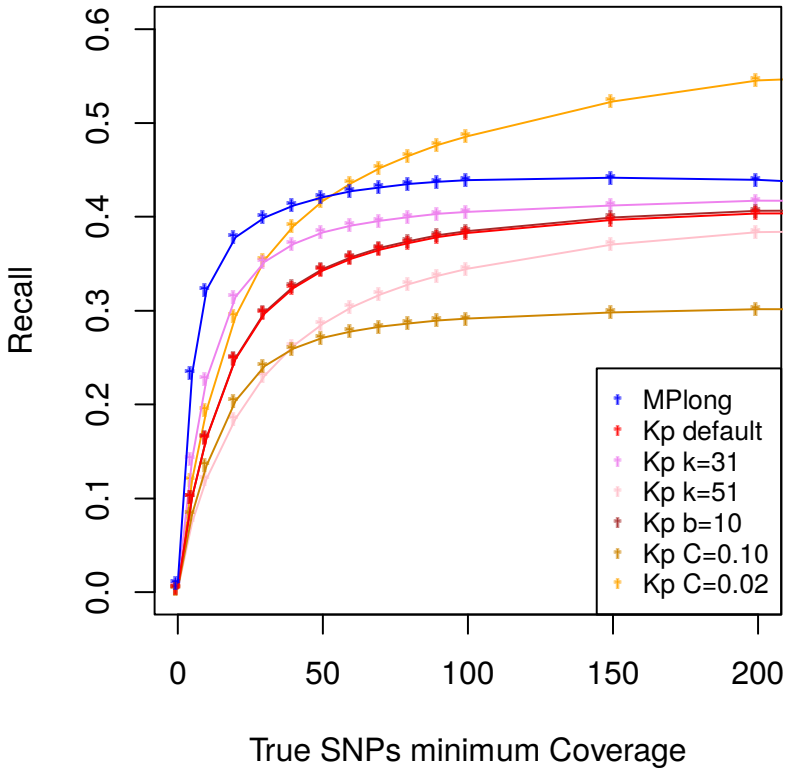
C)

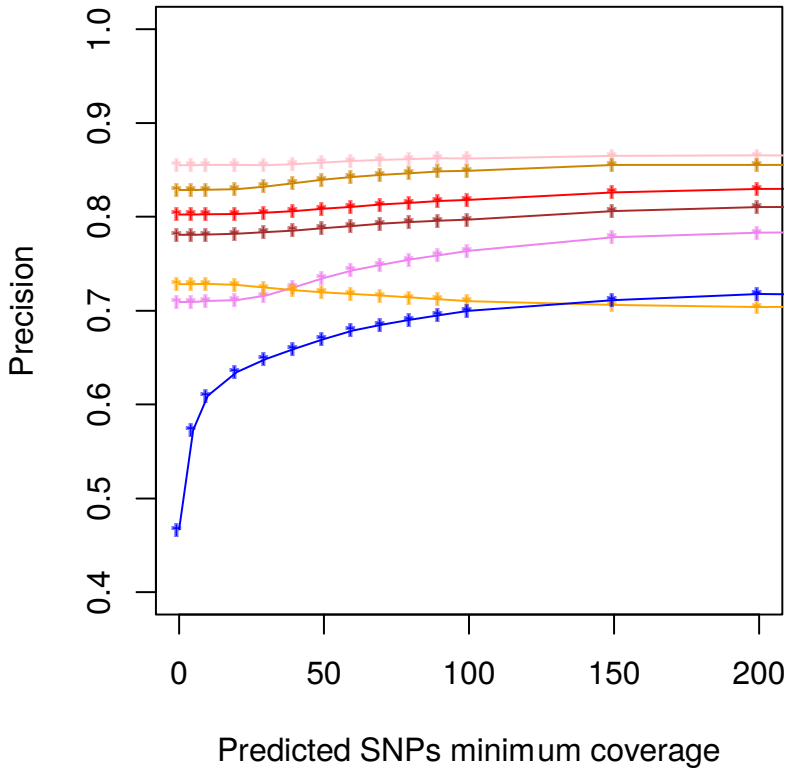


Recall vs MAF



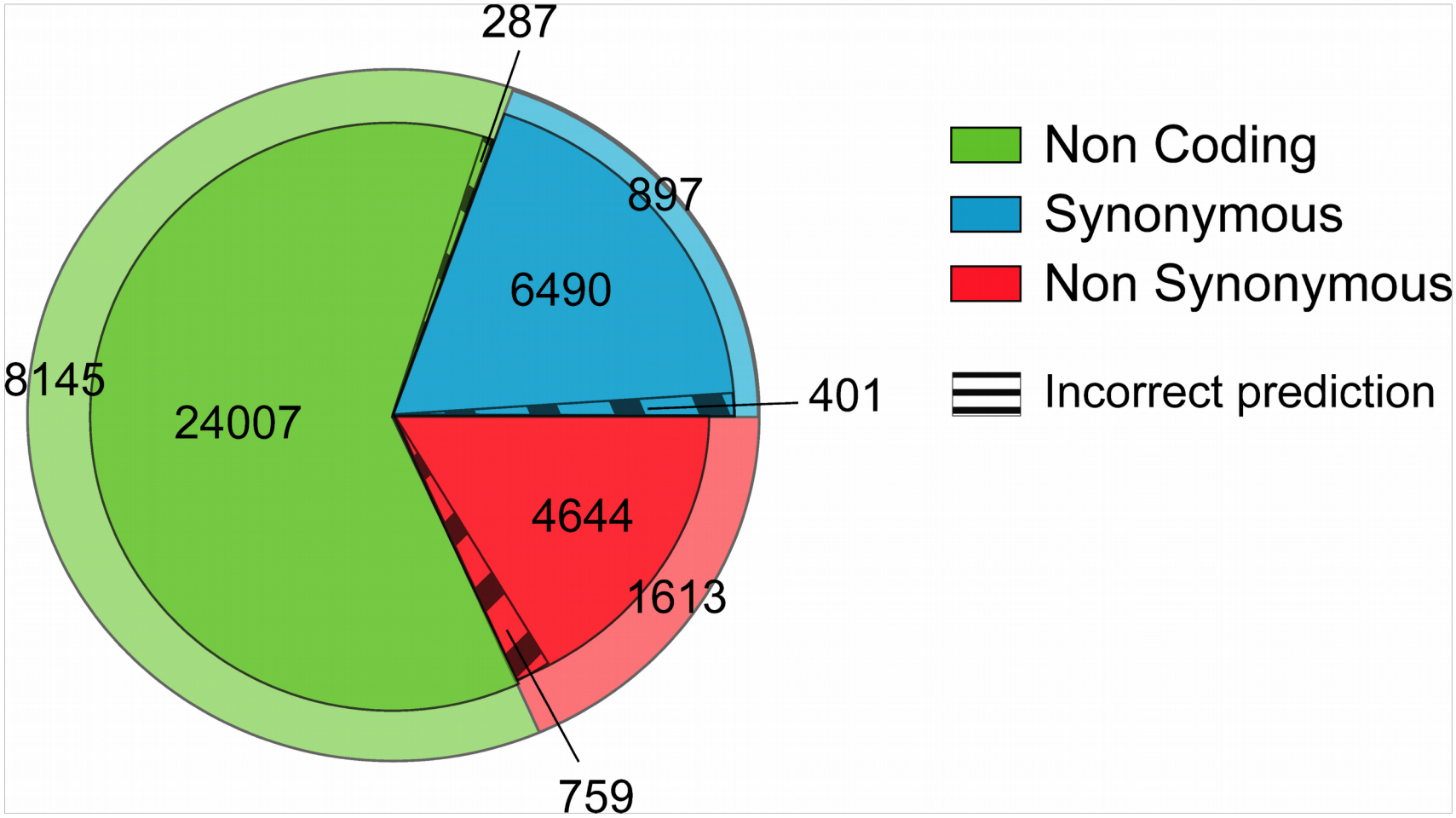
Kissplice parameters



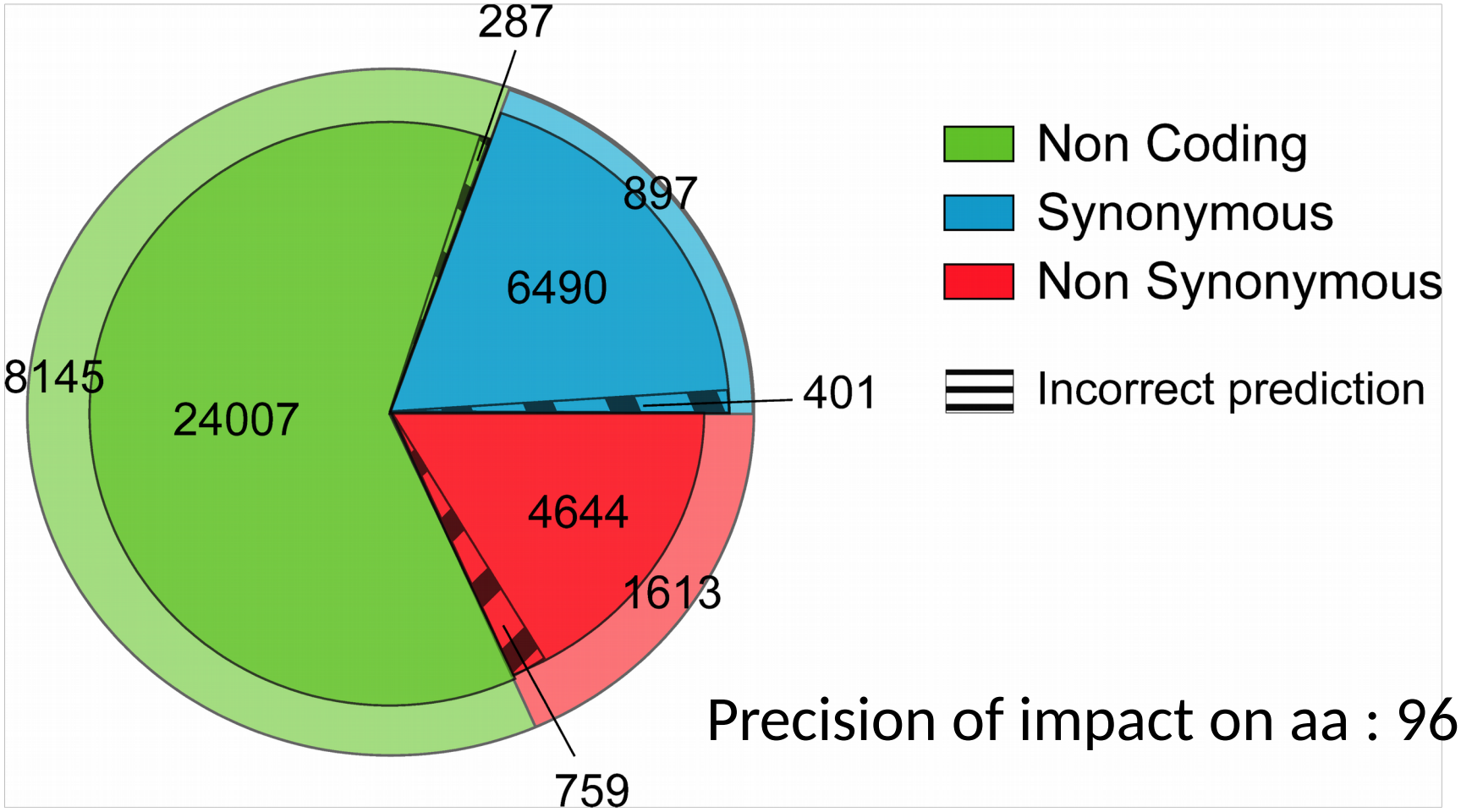


Impact on amino acids

Impact on amino acids

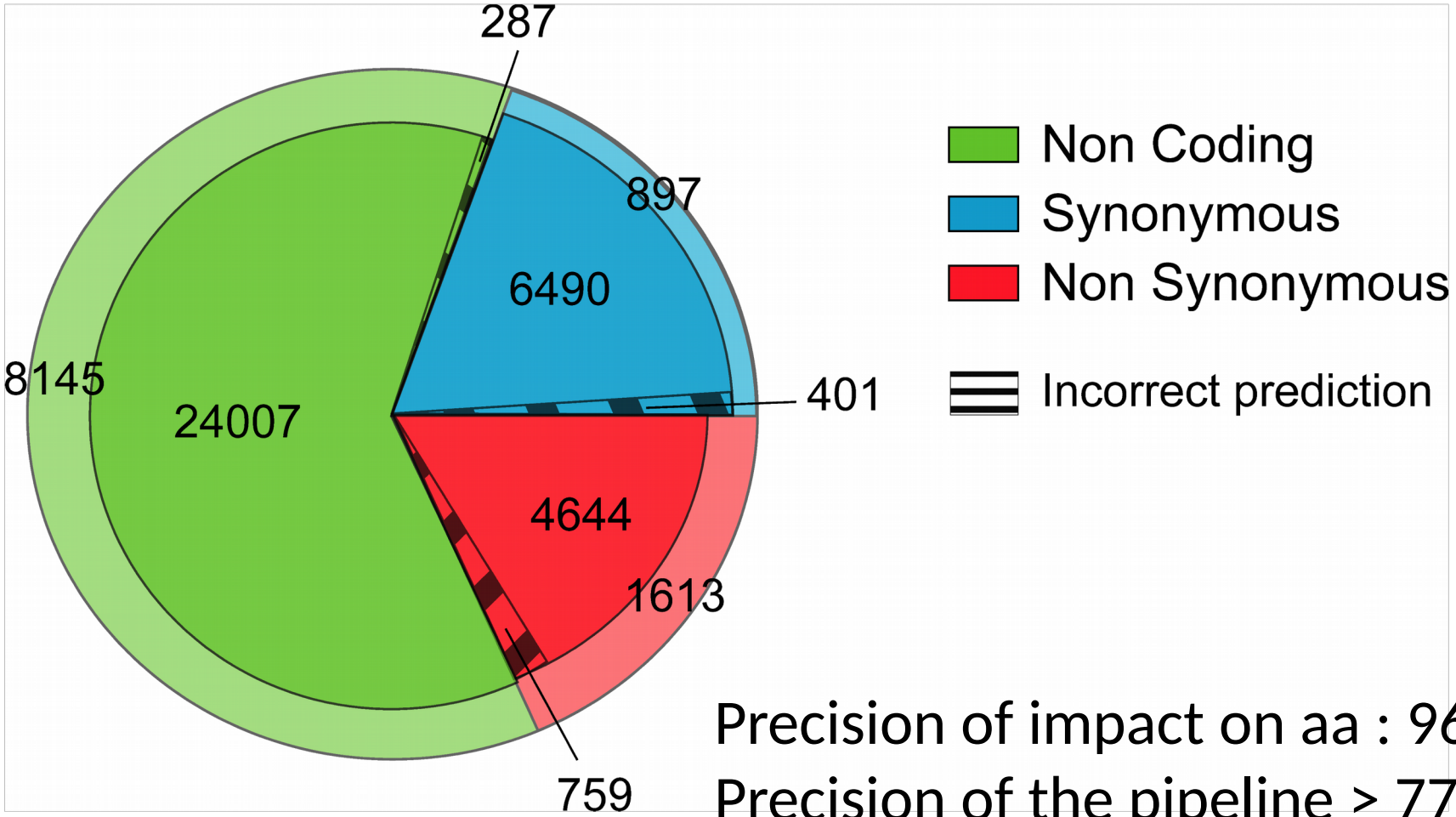


Impact on amino acids



Precision of impact on aa : 96%

Impact on amino acids



Precision of impact on aa : 96%

Precision of the pipeline > 77%

KissDE

	C1	C2
Var. 1	21	0
Var. 2	0	23

100% – 0%

KissDE

	C1	C2
Var. 1	21	0
Var. 2	0	23

100% – 0%

	C1	C2
Var. 1	21	7
Var. 2	0	16

100% – 30%

KissDE

	C1	C2
Var. 1	21	0
Var. 2	0	23

100% – 0%

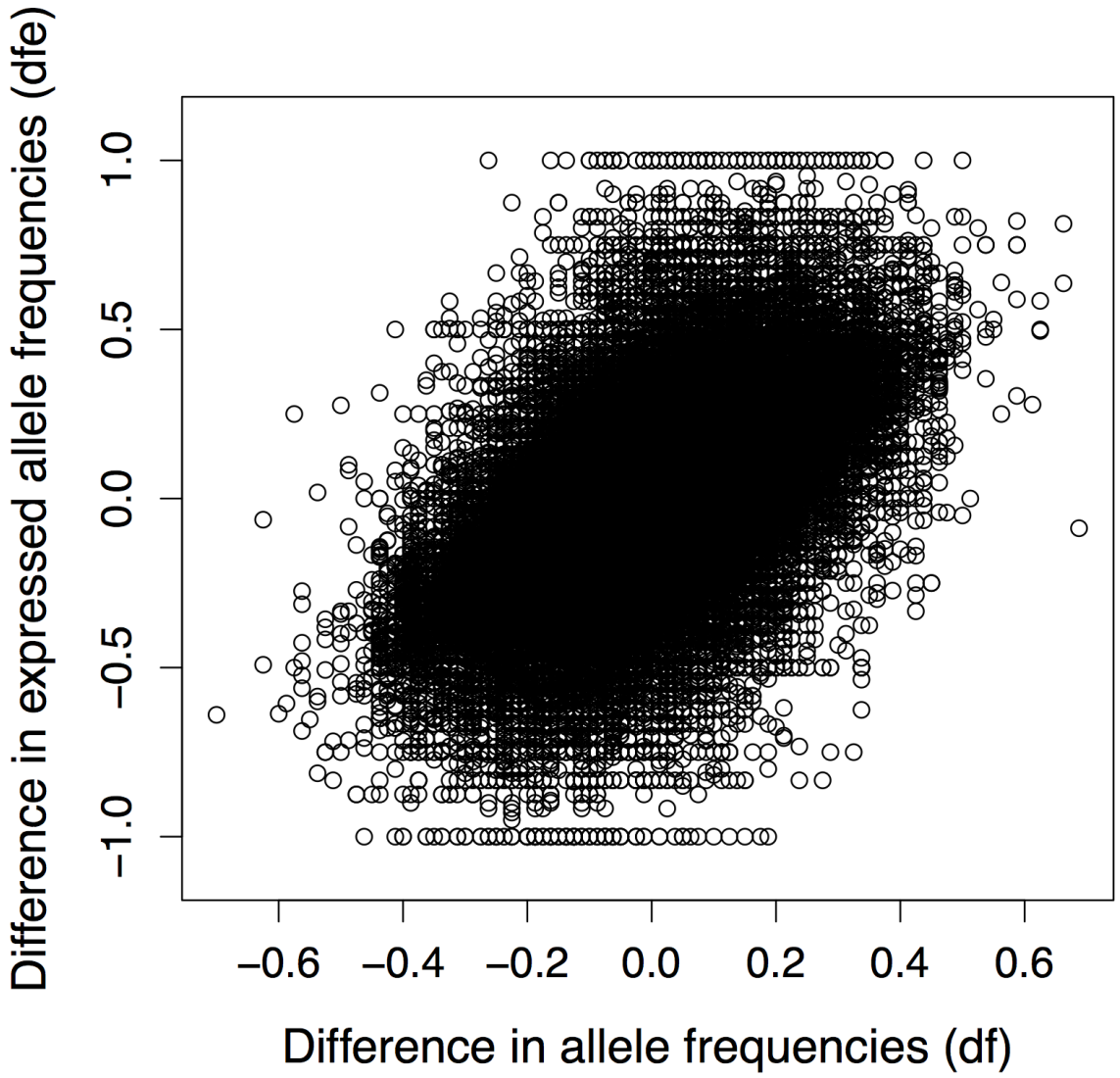
	C1	C2
Var. 1	21	7
Var. 2	0	16

100% – 30%

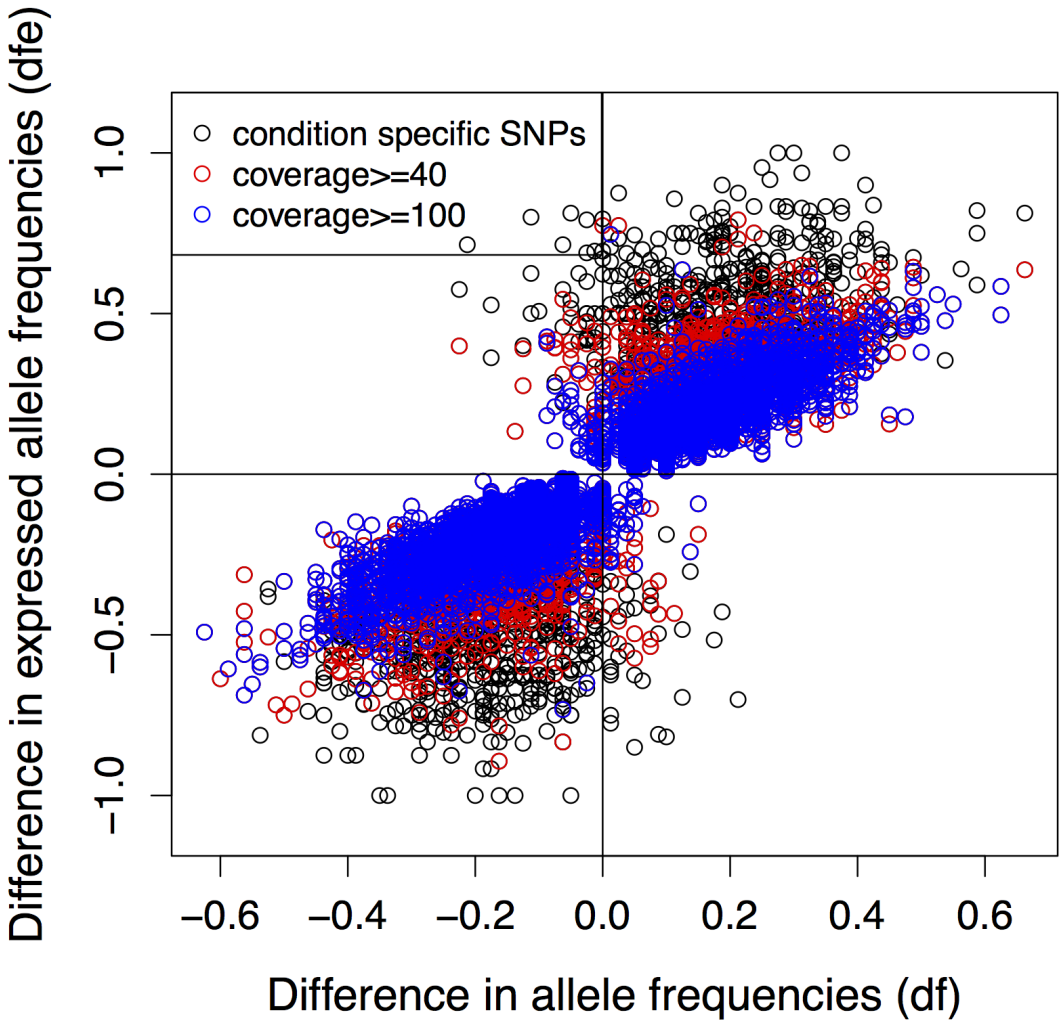
	C1	C2
Var. 1	15	7
Var. 2	6	16

71% – 30%

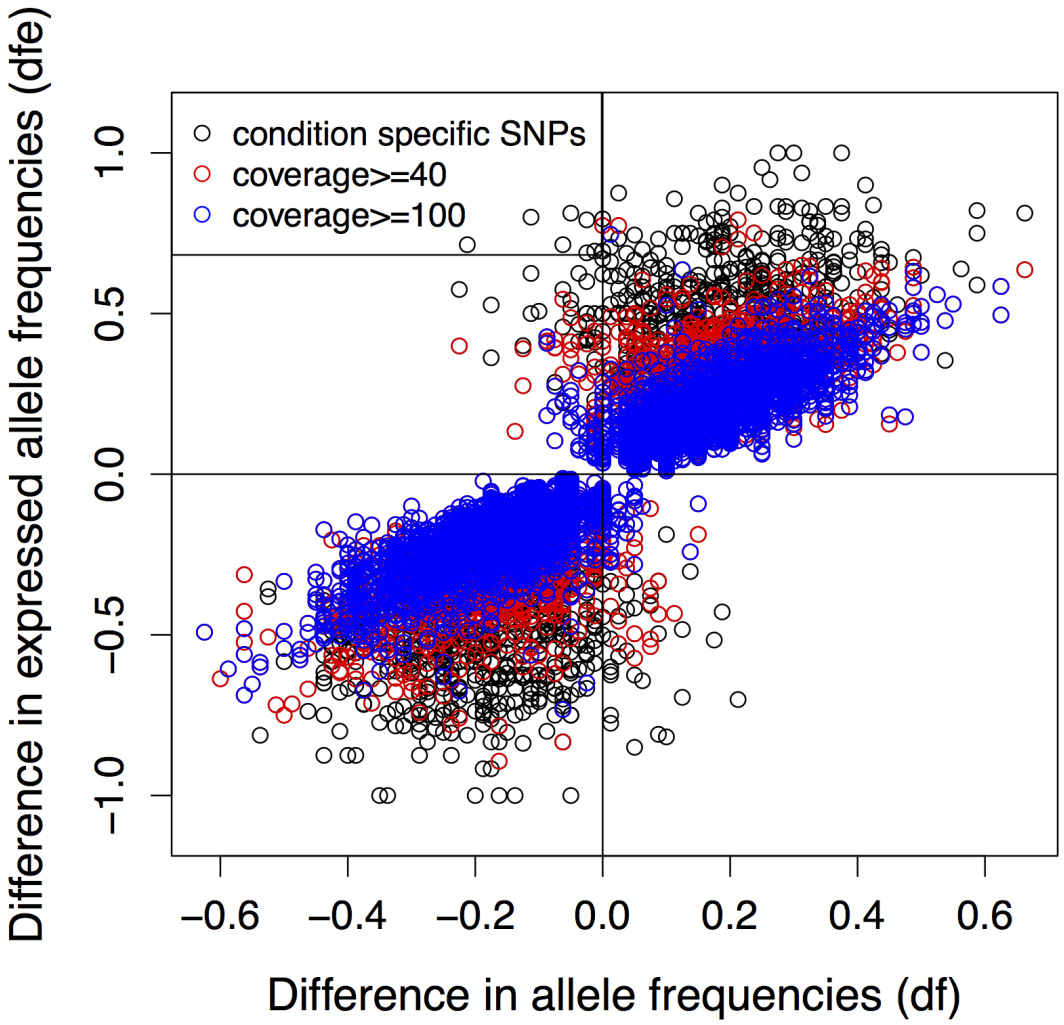
Difference in allele frequency



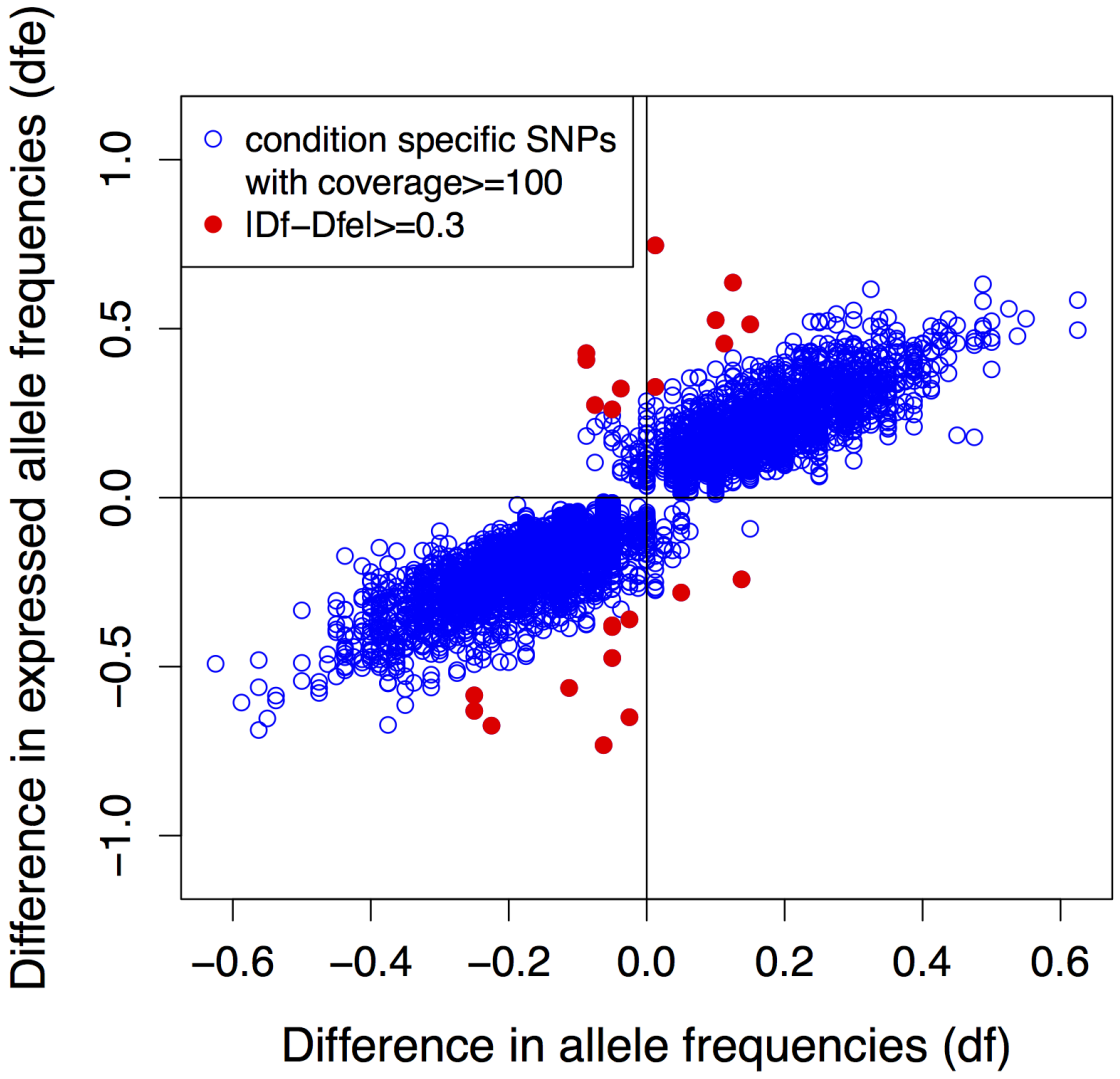
Difference in allele frequency



Difference in allele frequency



Difference in allele frequency



Pvalue ≤ 0.05

#reads ≥ 100