# Interactive software for the integrated analysis and identification of rare and undiagnosed diseases using NGS data

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# NGS for rare / undiagnosed diseases



HiSeq 3000/HiSeq 4000 Systems

NextSeq

MiSeq
PGM
Proton

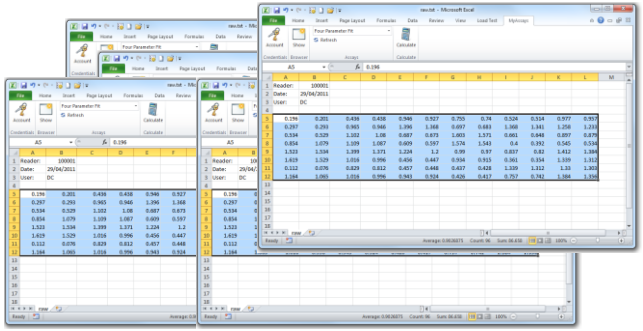
# Output

| VarChr | VarStart | VarEnd | DNAChange | VarType | VarClass | VarPercenta | RefCov | VarCov | dbSnpId | Transcript | VarId | TotalCov |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| chr13 | 32890572 | 32890572 | G>A | SNP | R | 100 | 0 | 184 | rs1799943 | NM_000059 | 16 | 184 |
| chr13 | 32890572 | 32890572 | G>A | SNP | R | 100 | 0 | 249 | rs1799943 | NM_000059 | 37 | 249 |
| chr13 | 32890572 | 32890572 | G>A | SNP | R | 99,62 | 0 | 530 | rs1799943 | NM_000059 | 37 | 532 |
| chr13 | 32890572 | 32890572 | G>A | SNP | R | 100 | 0 | 98 | rs1799943 | NM_000059 | 13 | 98 |
| chr13 | 32890572 | 32890572 | G>A | SNP | R | 99,62 | 0 | 1294 | rs1799943 | NM_000059 | 40 | 1299 |
| chr13 | 32899388 | 32899388 | A>C | SNP | R | 99,37 | 0 | 631 | rs11571610 | NM_000059 | 23 | 635 |
| chr13 | 32900933 | 32900933 | T>A | SNP | R | 99,76 | 0 | 1681 | rs3752451 | NM_000059 | 14 | 1685 |
| chr13 | 32900933 | 32900933 | T>A | SNP | R | 99,13 | 0 | 227 | rs3752451 | NM_000059 | 13 | 229 |
| chr13 | 32900933 | 32900933 | T>A | SNP | R | 99,81 | 0 | 1584 | rs3752451 | NM_000059 | 15 | 1587 |
| chr13 | 32900933 | 32900933 | T>A | SNP | R | 99,44 | 0 | 536 | rs3752451 | NM_000059 | 13 | 539 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,7 | 0 | 673 | rs206073 | NM_000059 | 7 | 675 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,89 | 0 | 936 | rs206073 | NM_000059 | 10 | 937 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,44 | 0 | 530 | rs206073 | NM_000059 | 18 | 533 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 650 | rs206073 | NM_000059 | 18 | 650 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 543 | rs206073 | NM_000059 | 16 | 543 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,74 | 0 | 780 | rs206073 | NM_000059 | 10 | 782 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 463 | rs206073 | NM_000059 | 16 | 463 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,81 | 0 | 530 | rs206073 | NM_000059 | 9 | 531 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 636 | rs206073 | NM_000059 | 14 | 636 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,85 | 0 | 645 | rs206073 | NM_000059 | 19 | 646 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 109 | rs206073 | NM_000059 | 9 | 109 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 72 | rs206073 | NM_000059 | 14 | 72 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 107 | rs206073 | NM_000059 | 7 | 107 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,89 | 0 | 920 | rs206073 | NM_000059 | 24 | 921 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 783 | rs206073 | NM_000059 | 16 | 783 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,88 | 0 | 868 | rs206073 | NM_000059 | 9 | 869 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,76 | 0 | 842 | rs206073 | NM_000059 | 16 | 844 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,87 | 0 | 777 | rs206073 | NM_000059 | 17 | 778 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 671 | rs206073 | NM_000059 | 16 | 671 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 1272 | rs206073 | NM_000059 | 8 | 1272 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 1341 | rs206073 | NM_000059 | 15 | 1341 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 791 | rs206073 | NM_000059 | 8 | 791 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,84 | 0 | 613 | rs206073 | NM_000059 | 20 | 614 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,72 | 0 | 351 | rs206073 | NM_000059 | 16 | 352 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 99,72 | 0 | 702 | rs206073 | NM_000059 | 9 | 704 |
| chr13 | 32905265 | 32905265 | G>A | SNP | R | 100 | 0 | 828 | rs206073 | NM_000059 | 8 | 828 |

# Finding the “needle in the haystack“

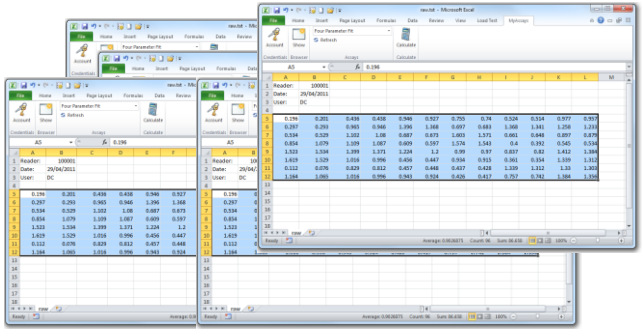
# Software design

Instead of lots of Excel files



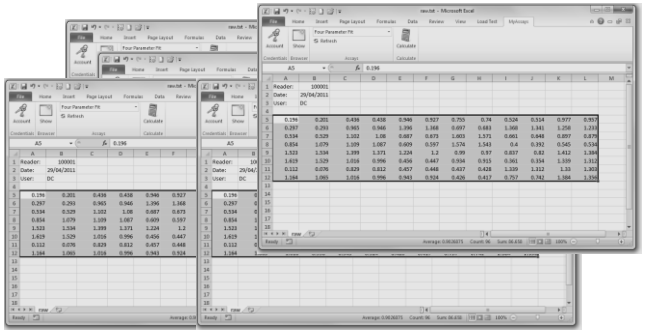
# Software design

Instead of lots of Excel files → Results stored in one place

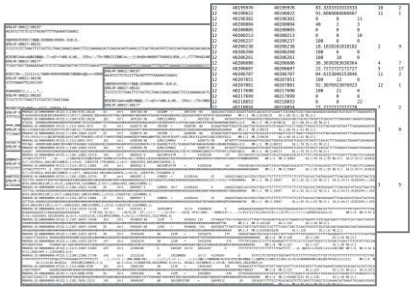


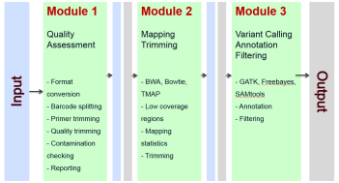
# Software design

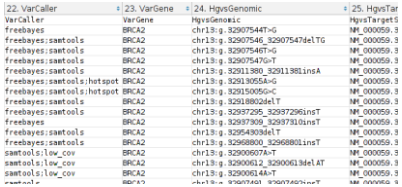
Instead of lots of Excel files → Results stored in one place



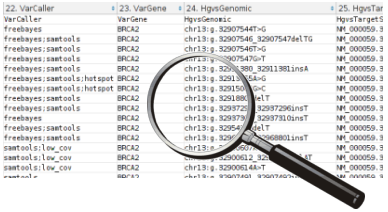
Raw data → Analysis → List of variants → Identification





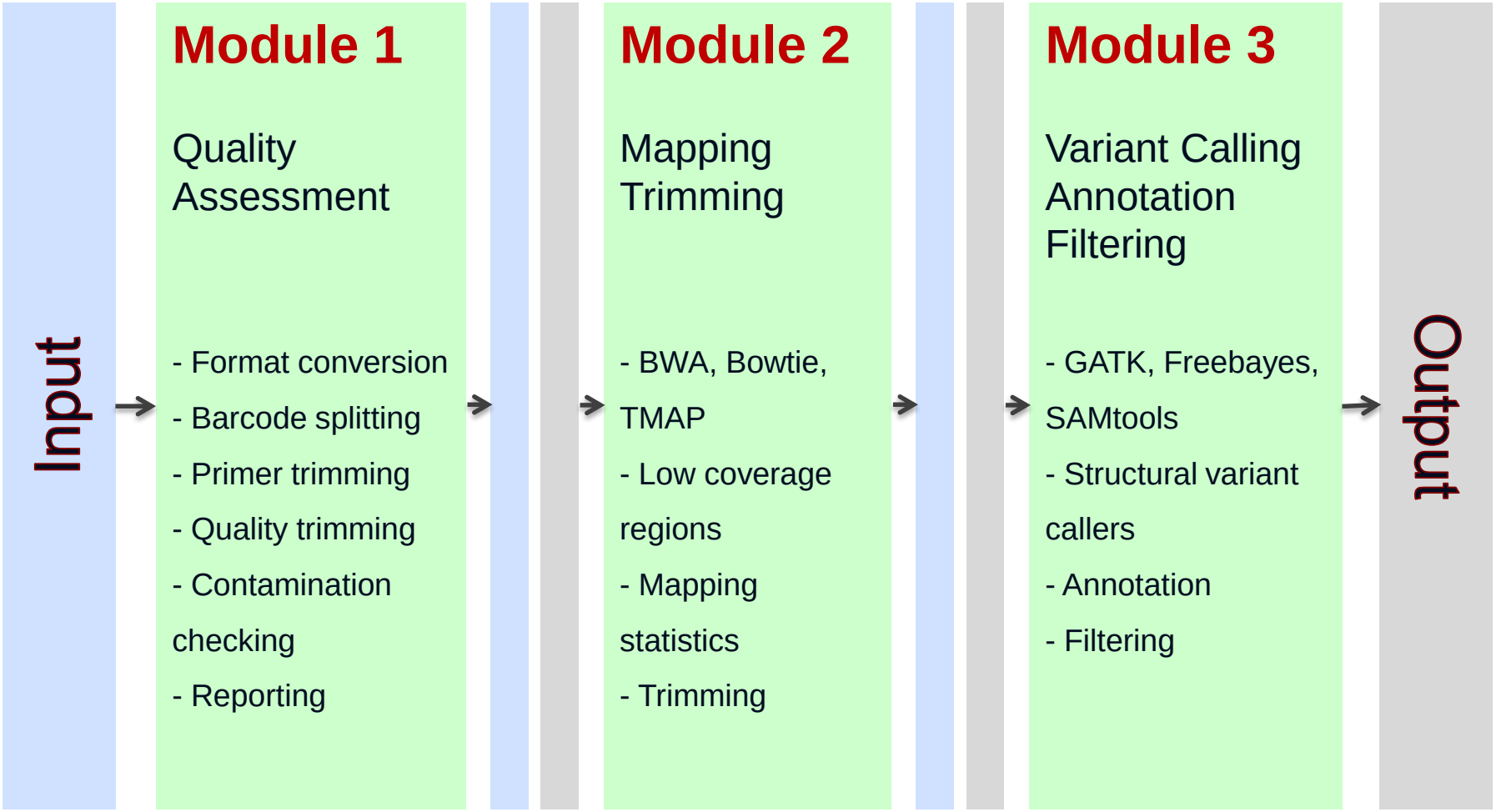



| 22. VarCaller | 23. VarGene | 24. HgvsGenomic | 25. HgvsTar |
|---|---|---|---|
| VarCaller | VarGene | HgvsGenomic | HgvsTargetS |
| freebayes | BRCA2 | chr13:g.32907544T>G | NM_000059.3 |
| freebayes;samtools | BRCA2 | chr13:g.32907546_32907547delTG | NM_000059.3 |
| freebayes;samtools | BRCA2 | chr13:g.32907546T>G | NM_000059.3 |
| freebayes;samtools | BRCA2 | chr13:g.32907547G>T | NM_000059.3 |
| freebayes;samtools | BRCA2 | chr13:g.32911380_32911381insA | NM_000059.3 |
| freebayes;samtools;hotspot | BRCA2 | chr13:g.32913055A>G | NM_000059.3 |
| freebayes;samtools;hotspot | BRCA2 | chr13:g.32915005G>C | NM_000059.3 |
| freebayes;samtools | BRCA2 | chr13:g.32918802delT | NM_000059.3 |
| freebayes;samtools | BRCA2 | chr13:g.32937295_32937296insT | NM_000059.3 |
| freebayes | BRCA2 | chr13:g.32937309_32937310insT | NM_000059.3 |
| freebayes;samtools | BRCA2 | chr13:g.32954303delT | NM_000059.3 |
| freebayes;samtools | BRCA2 | chr13:g.32968800_32968801insT | NM_000059.3 |
| samtools;low_cov | BRCA2 | chr13:g.32900607A>T | NM_000059.3 |
| samtools;low_cov | BRCA2 | chr13:g.32900612_32900613delAT | NM_000059.3 |
| samtools;low_cov | BRCA2 | chr13:g.32900614A>T | NM_000059.3 |



# Multistep Application

Accepts input from all big NGS technologies (Illumina, Ion Torrent, 454 ...)



# Reproducibility & Configuration

## Logging

- Complete log of all used tools, references, annotation databases, and versions

## Storing

- Storage of output and input data → Run and re-run analyses

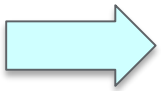
## Accessing

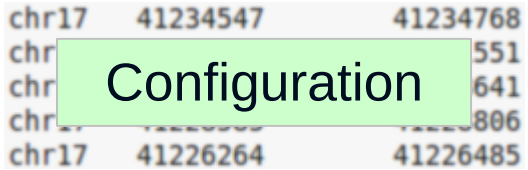
- Get all data from all samples at any time

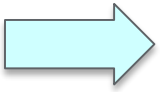
## Configuring

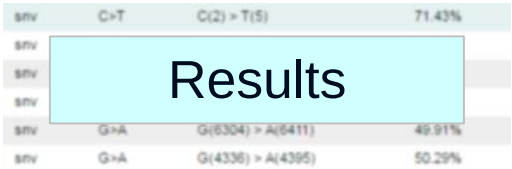
- Specify exactly which genes/regions should be analyzed

BMPR2
ELN
SCNN1A







# Features

Supports AmpliconSeq, WES, WGS

Uses proven open-source packages and frameworks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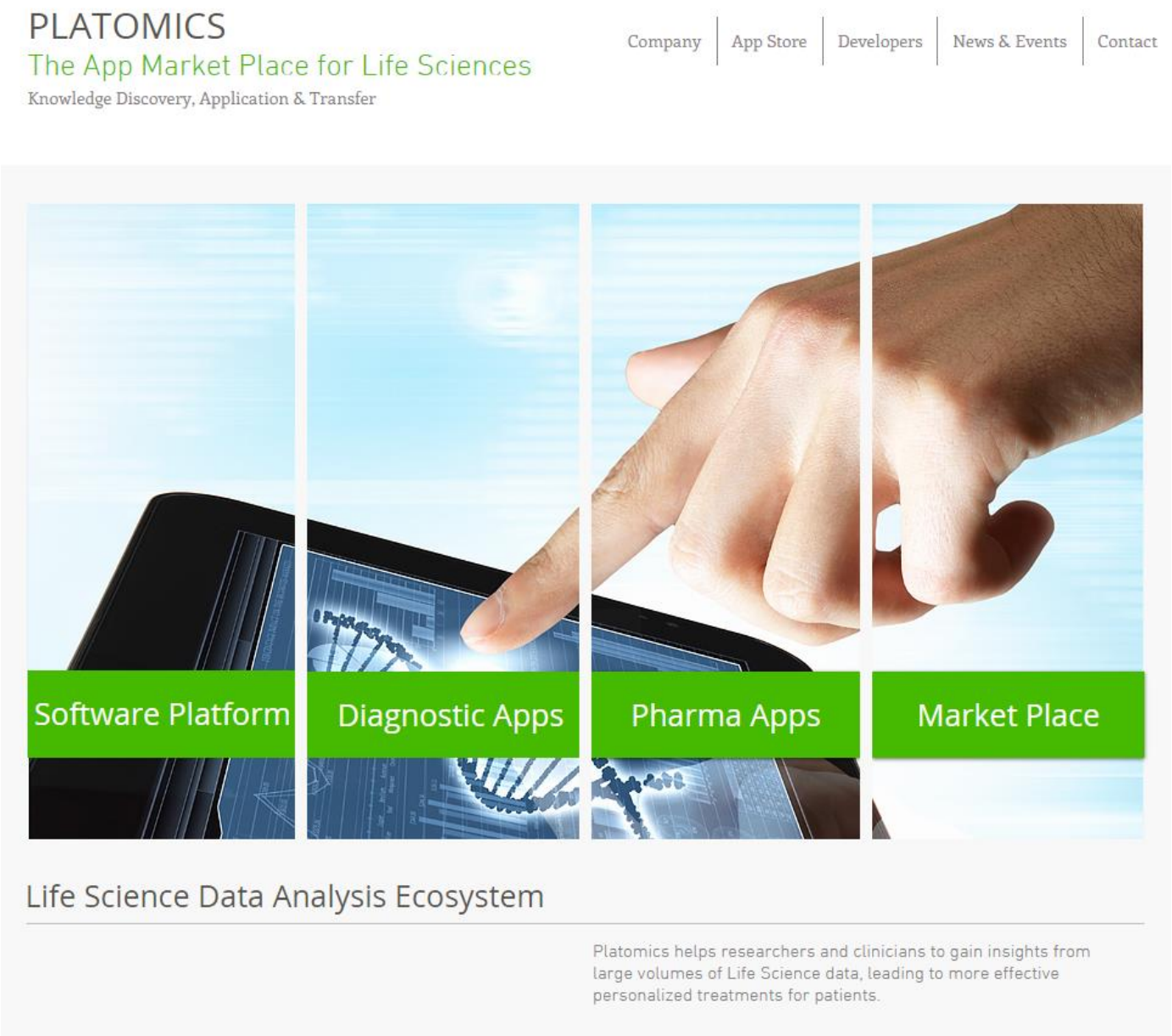
Transformation of variant coordinates into Transcript HGVS

Variant identification with multiple tools

→ Merging of variants from different callers

| | | | | | | |
|---|---|---|---|---|---|---|
| C(330) > T(29 | 47.45% | het | NM_000059.3:c.9038C>T | chr13:g.32953971C>T | non_syn_coding | F;S |
| A(952) > C(8 | 47.15% | het | NM_000059.3:c.*105A>C | chr13:g.32973012A>C | UTR_3_prime | F;S |
| C(1391) > T(9 | 40.57% | het | NM_007294.3:c.4956G>A | chr17:g.41222975C>T | non_syn_coding | F;S |

# Integration into Platomics Platform



# Integration into Platomics Platform

Web-based

Easy installation of server part

App-store concept

Customization

NGS Rare diseases Software

# Platomics

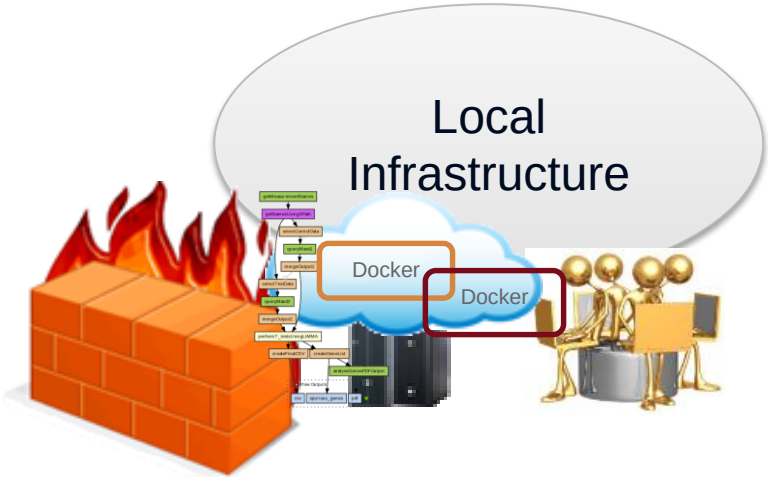
## Remote deployment

- Data access secured through user management
- Sharing of data

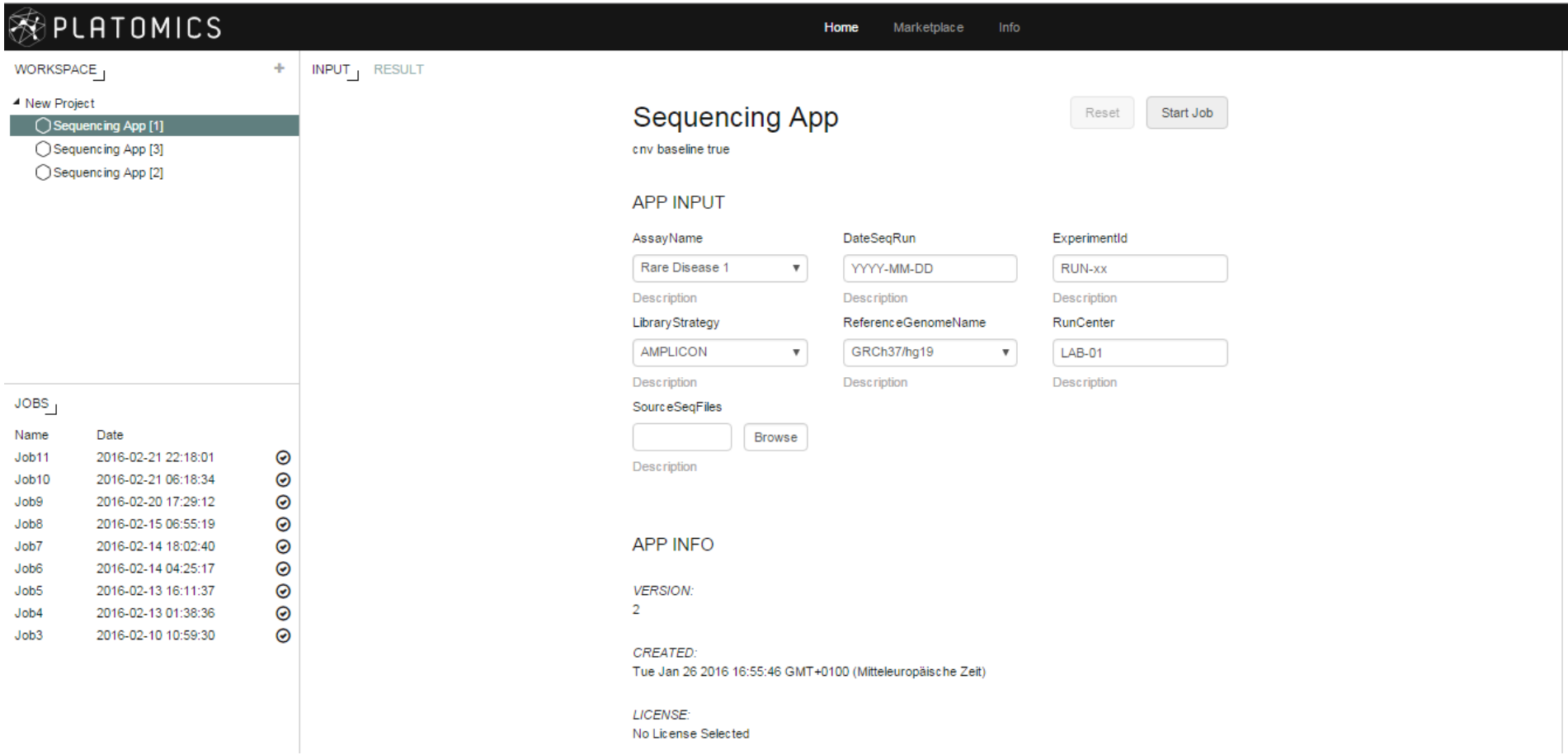

## Local deployment

- Only accessible through local network
- Data stored on local infrastructure

→ data security



# Integration into Platomics platform



# Results

INPUT RESULT

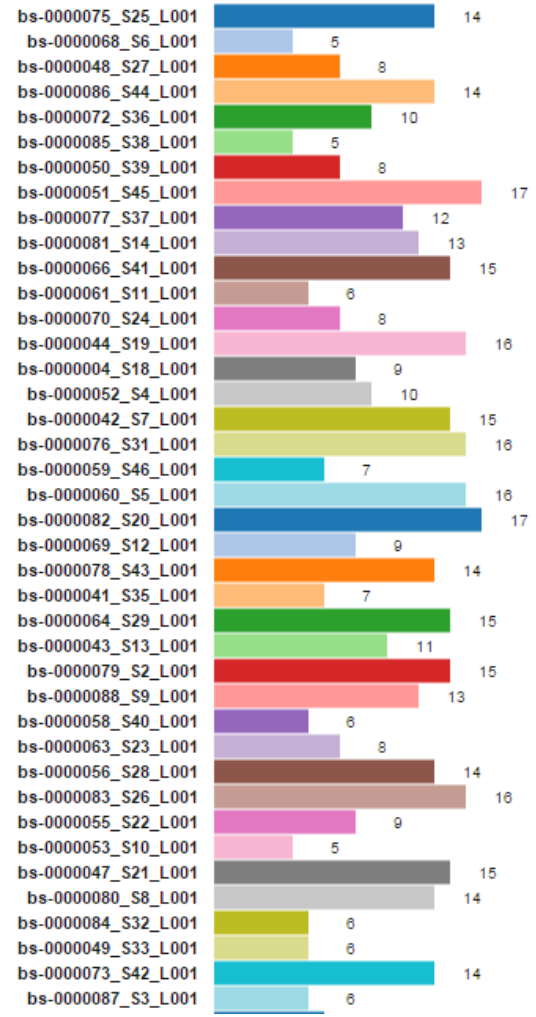
EXPERIMENT PATIENTS VARIANTS CNV GENES VALIDATION

Info

| | |
|---|---|
| **Experiment Id:** | RUN-12 |
| **Assay Name:** | BRCA1&2 CNV [as-3] |
| **Run Center:** | LAB-01 |
| **Date Seq Run:** | YYYY-MM-DD |
| **SeqPlatform:** | Illumina_MiSeq |
| **DateSeqAnalysis:** | 2016-02-14 |

Pathogenic serverity variants

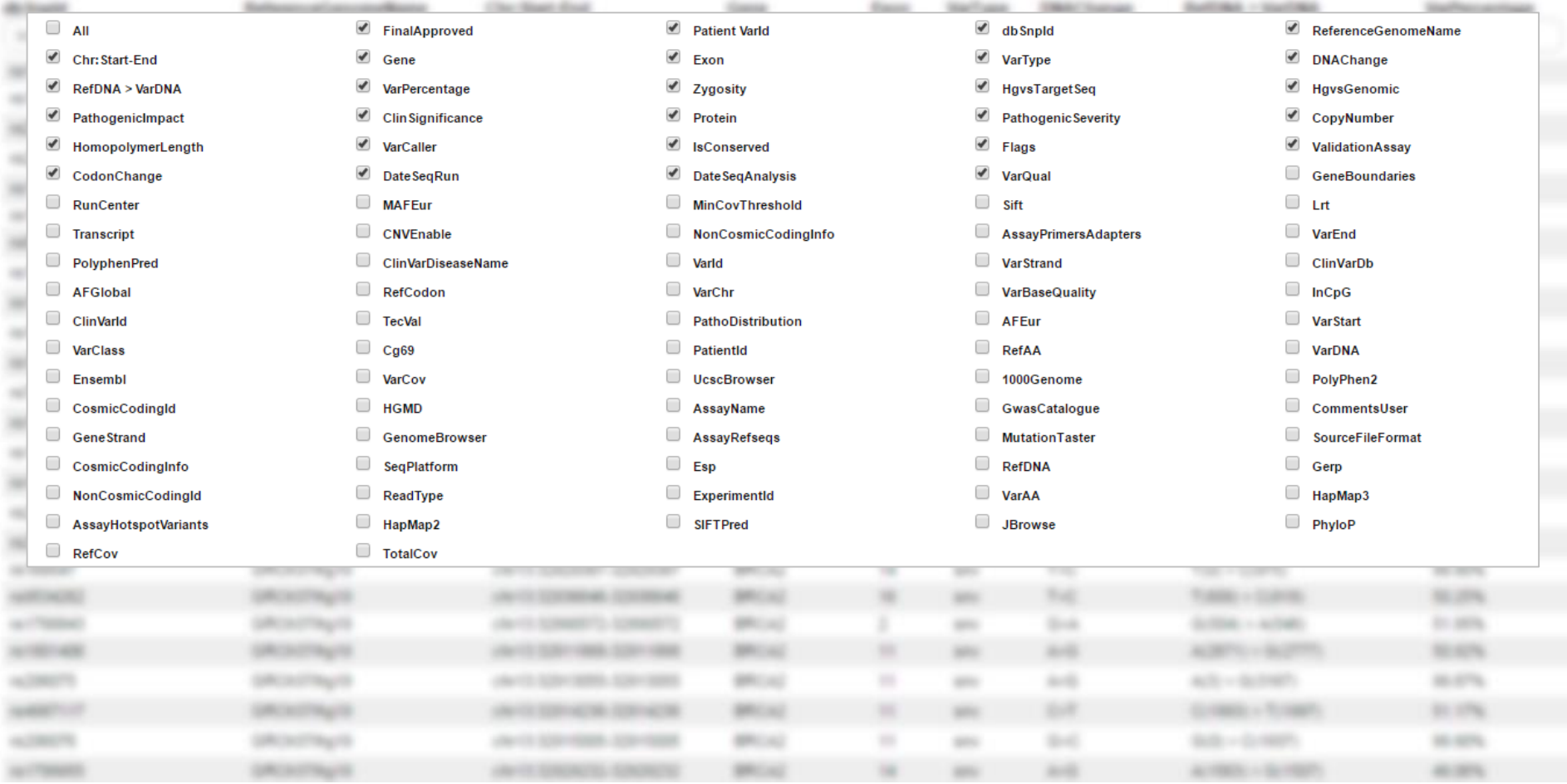

Variants per Patient



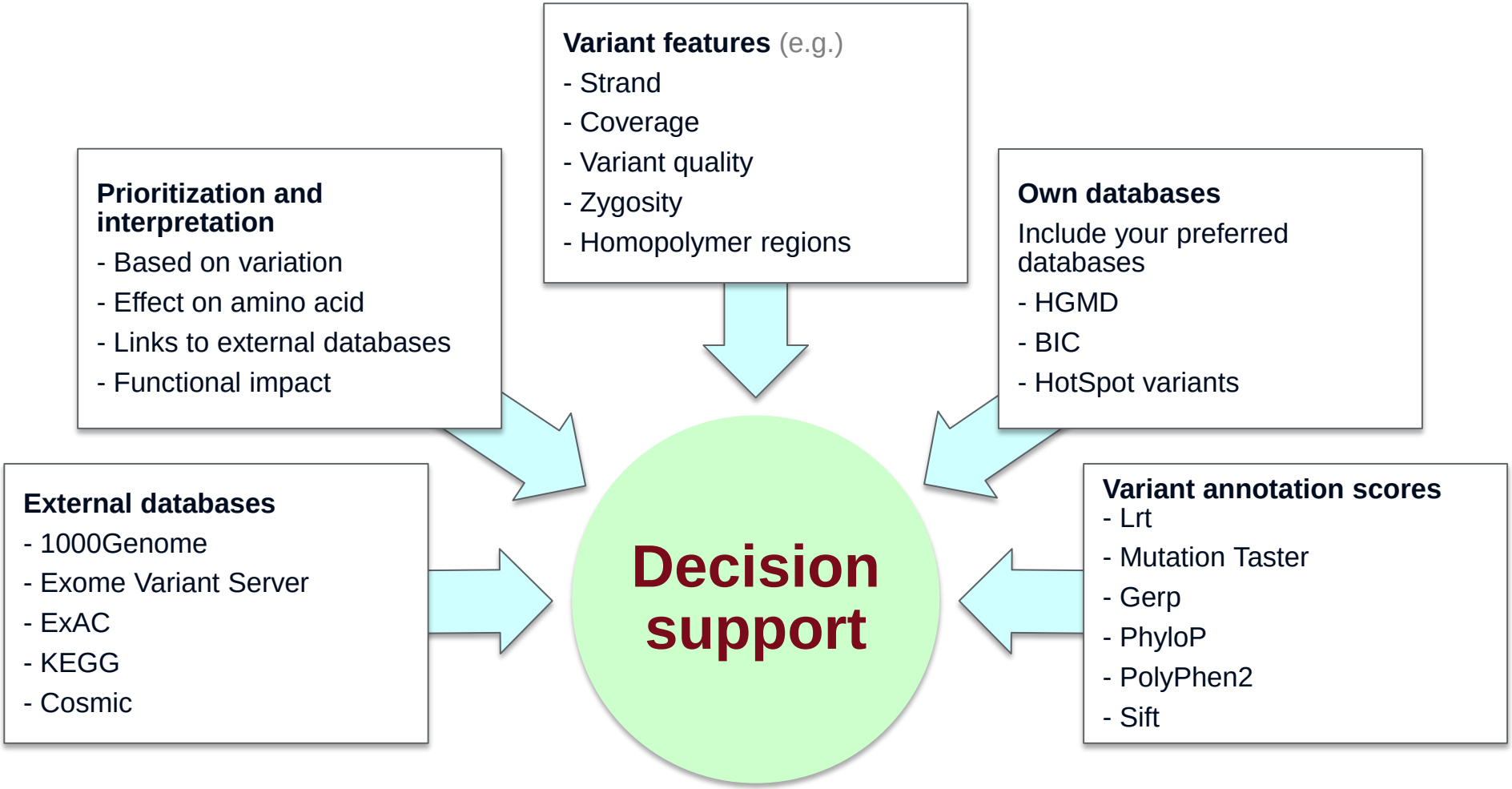
# Results

| dbSnpId | ReferenceGenomeName | Chr:Start-End | Gene | Exon | VarType | DNAChange | RefDNA > VarDNA | VarPercentage |
|---|---|---|---|---|---|---|---|---|
| search... | search... | search... | search... | searc | search.. | search... | search... | search... |
| rs1799943 | GRCh37/hg19 | chr13:32890572-32890572 | BRCA2 | 2 | snv | G>A | G(0) > A(1304) | 99.54% |
| rs1801406 | GRCh37/hg19 | chr13:32911888-32911888 | BRCA2 | 11 | snv | A>G | A(20) > G(7821) | 99.67% |
| rs206075 | GRCh37/hg19 | chr13:32913055-32913055 | BRCA2 | 11 | snv | A>G | A(2) > G(3868) | 99.92% |
| rs206076 | GRCh37/hg19 | chr13:32915005-32915005 | BRCA2 | 11 | snv | G>C | G(1) > C(2728) | 99.85% |
| rs1799955 | GRCh37/hg19 | chr13:32929232-32929232 | BRCA2 | 14 | snv | A>G | A(2066) > G(1974) | 48.79% |
| rs169547 | GRCh37/hg19 | chr13:32929387-32929387 | BRCA2 | 14 | snv | T>C | T(4) > C(1094) | 99.64% |
| rs9534262 | GRCh37/hg19 | chr13:32936646-32936646 | BRCA2 | 16 | snv | T>C | T(986) > C(966) | 49.46% |
| rs1799966 | GRCh37/hg19 | chr17:41223094-41223094 | BRCA1 | 16 | snv | T>C | T(0) > C(909) | 100.00% |
| rs1060915 | GRCh37/hg19 | chr17:41234470-41234470 | BRCA1 | 12 | snv | A>G | A(0) > G(1283) | 99.92% |
| rs16942 | GRCh37/hg19 | chr17:41244000-41244000 | BRCA1 | 10 | snv | T>C | T(15) > C(7544) | 99.67% |
| rs16941 | GRCh37/hg19 | chr17:41244435-41244435 | BRCA1 | 10 | snv | T>C | T(15) > C(6083) | 99.75% |
| rs799917 | GRCh37/hg19 | chr17:41244936-41244936 | BRCA1 | 10 | snv | G>A | G(14) > A(3740) | 99.44% |
| rs16940 | GRCh37/hg19 | chr17:41245237-41245237 | BRCA1 | 10 | snv | A>G | A(17) > G(9433) | 99.76% |
| rs1799949 | GRCh37/hg19 | chr17:41245466-41245466 | BRCA1 | 10 | snv | G>A | G(26) > A(4910) | 98.38% |
| rs1801406 | GRCh37/hg19 | chr13:32911888-32911888 | BRCA2 | 11 | snv | A>G | A(2945) > G(3037) | 50.67% |
| rs206075 | GRCh37/hg19 | chr13:32913055-32913055 | BRCA2 | 11 | snv | A>G | A(8) > G(3069) | 99.71% |
| rs206076 | GRCh37/hg19 | chr13:32915005-32915005 | BRCA2 | 11 | snv | G>C | G(0) > C(1963) | 99.95% |
| rs169547 | GRCh37/hg19 | chr13:32929387-32929387 | BRCA2 | 14 | snv | T>C | T(0) > C(975) | 99.90% |
| rs9534262 | GRCh37/hg19 | chr13:32936646-32936646 | BRCA2 | 16 | snv | T>C | T(809) > C(818) | 50.25% |
| rs1799943 | GRCh37/hg19 | chr13:32890572-32890572 | BRCA2 | 2 | snv | G>A | G(504) > A(546) | 51.95% |
| rs1801406 | GRCh37/hg19 | chr13:32911888-32911888 | BRCA2 | 11 | snv | A>G | A(2671) > G(2777) | 50.92% |
| rs206075 | GRCh37/hg19 | chr13:32913055-32913055 | BRCA2 | 11 | snv | A>G | A(3) > G(3167) | 99.87% |
| rs4987117 | GRCh37/hg19 | chr13:32914236-32914236 | BRCA2 | 11 | snv | C>T | C(1893) > T(1997) | 51.17% |
| rs206076 | GRCh37/hg19 | chr13:32915005-32915005 | BRCA2 | 11 | snv | G>C | G(0) > C(1937) | 99.90% |
| rs1799955 | GRCh37/hg19 | chr13:32929232-32929232 | BRCA2 | 14 | snv | A>G | A(1563) > G(1507) | 49.06% |

# Results – fully customizable



# Module 3 – Annotation & Prioritization

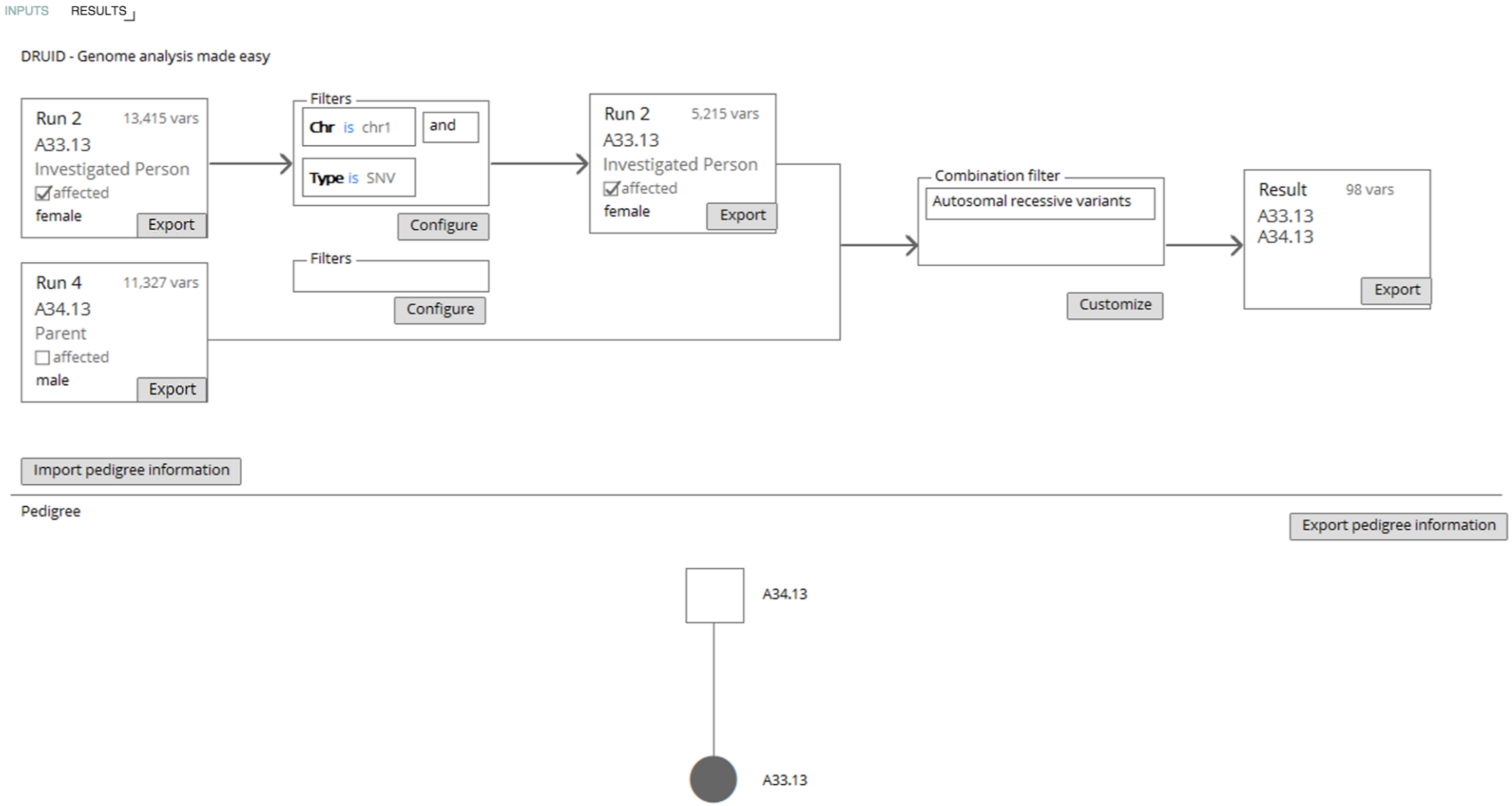


# Results

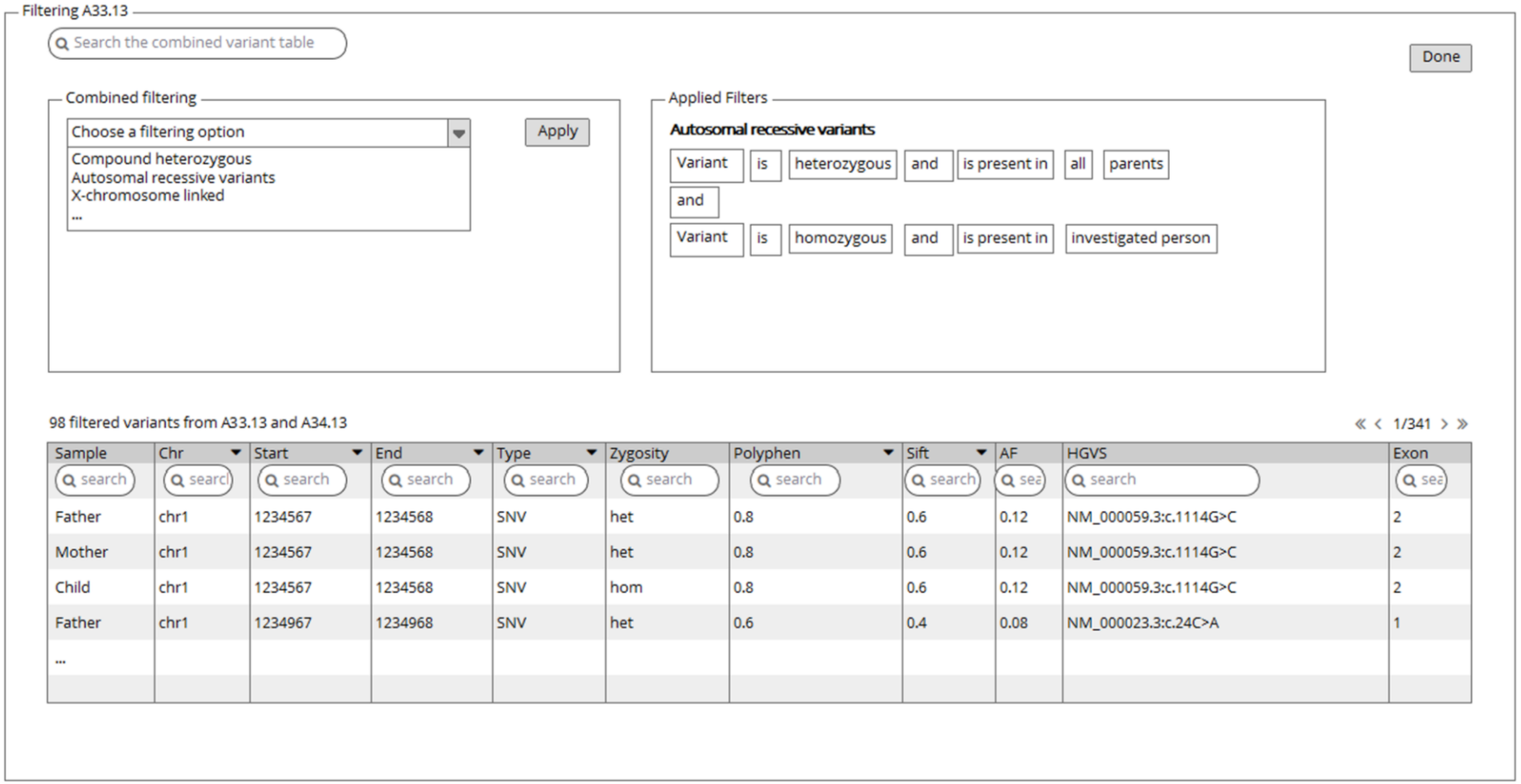
| dbSnpId | ReferenceGenomeName | Chr:Start-End | Gene | Exon | VarType | DNAChange | RefDNA > VarDNA | VarPercentage |
|---|---|---|---|---|---|---|---|---|
| search... | search... | search... | search... | searc | search... | search... | search... | search... |
| rs1799943 | GRCh37/hg19 | chr13:32890572-32890572 | BRCA2 | 2 | snv | G>A | G(0) > A(1304) | 99.54% |
| rs1801406 | GRCh37/hg19 | chr13:32911888-32911888 | BRCA2 | 11 | snv | A>G | A(20) > G(7821) | 99.67% |
| rs206075 | GRCh37/hg19 | chr13:32913055-32913055 | BRCA2 | 11 | snv | A>G | A(2) > G(3868) | 99.92% |
| rs206076 | GRCh37/hg19 | | | | | | G(1) > C(2728) | 99.85% |
| rs1799955 | GRCh37/hg19 | | | | | | A(2066) > G(1974) | 48.79% |
| rs169547 | GRCh37/hg19 | | | | | | T(4) > C(1094) | 99.64% |
| rs9534262 | GRCh37/hg19 | | | | | | T(966) > C(966) | 49.46% |
| rs1799966 | GRCh37/hg19 | chr17:41223094-41223094 | BRCA1 | 16 | snv | T>C | T(0) > C(909) | 100.00% |
| rs1060915 | GRCh37/hg19 | chr17:41234470-41234470 | BRCA1 | 12 | snv | A>G | A(0) > G(1283) | 99.92% |
| rs16942 | GRCh37/hg19 | chr17:41244000-41244000 | BRCA1 | 10 | snv | T>C | T(15) > C(7544) | 99.67% |
| rs16941 | GRCh37/hg19 | chr17:41244435-41244435 | BRCA1 | 10 | snv | T>C | T(15) > C(6083) | 99.75% |
| rs799917 | GRCh37/hg19 | chr17:41244936-41244936 | BRCA1 | 10 | snv | G>A | G(14) > A(3740) | 99.44% |
| rs16940 | GRCh37/hg19 | chr17:41245237-41245237 | BRCA1 | 10 | snv | A>G | A(17) > G(9433) | 99.76% |
| rs1799949 | GRCh37/hg19 | chr17:41245466-41245466 | BRCA1 | 10 | snv | G>A | G(26) > A(4910) | 98.38% |
| rs1801406 | GRCh37/hg19 | chr13:32911888-32911888 | BRCA2 | 11 | snv | A>G | A(2945) > G(3037) | 50.67% |
| rs206075 | GRCh37/hg19 | chr13:32913055-32913055 | BRCA2 | 11 | snv | A>G | A(8) > G(3069) | 99.71% |
| rs206076 | GRCh37/hg19 | chr13:32915005-32915005 | BRCA2 | 11 | snv | G>C | G(0) > C(1963) | 99.95% |
| rs169547 | GRCh37/hg19 | chr13:32929387-32929387 | BRCA2 | 14 | snv | T>C | T(0) > C(975) | 99.90% |
| rs9534262 | GRCh37/hg19 | chr13:32936646-32936646 | BRCA2 | 16 | snv | T>C | T(809) > C(818) | 50.25% |
| rs1799943 | GRCh37/hg19 | chr13:32890572-32890572 | BRCA2 | 2 | snv | G>A | G(504) > A(546) | 51.95% |
| rs1801406 | GRCh37/hg19 | chr13:32911888-32911888 | BRCA2 | 11 | snv | A>G | A(2871) > G(2777) | 50.92% |
| rs206075 | GRCh37/hg19 | chr13:32913055-32913055 | BRCA2 | 11 | snv | A>G | A(3) > G(3167) | 99.87% |
| rs4987117 | GRCh37/hg19 | chr13:32914236-32914236 | BRCA2 | 11 | snv | C>T | C(1893) > T(1997) | 51.17% |
| rs206076 | GRCh37/hg19 | chr13:32915005-32915005 | BRCA2 | 11 | snv | G>C | G(0) > C(1937) | 99.90% |
| rs1799955 | GRCh37/hg19 | chr13:32929232-32929232 | BRCA2 | 14 | snv | A>G | A(1563) > G(1507) | 49.06% |

… still, a long list of variants

# Filtering


INPUTS
RESULTS
DRUID - Genome analysis made easy
Run 2
13,415 vars
A33.13
Investigated Person
affected
female
Export
Run 4
11,327 vars
A34.13
Parent
affected
male
Export
Filters
Chr is chr1
and
Type is SNV
Configure
Filters
Configure
Run 2
5,215 vars
A33.13
Investigated Person
affected
female
Export
Combination filter
Autosomal recessive variants
Customize
Result
98 vars
A33.13
A34.13
Export
Import pedigree information
Pedigree
Export pedigree information
A34.13
A33.13

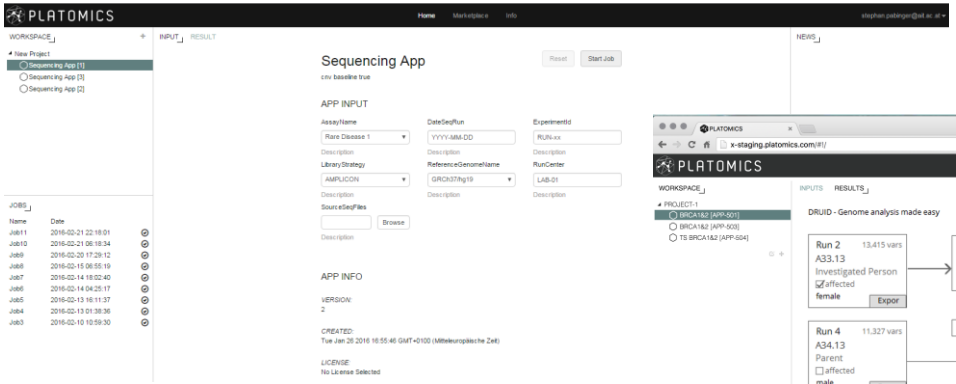
# Interactive filtering and prioritization of variants

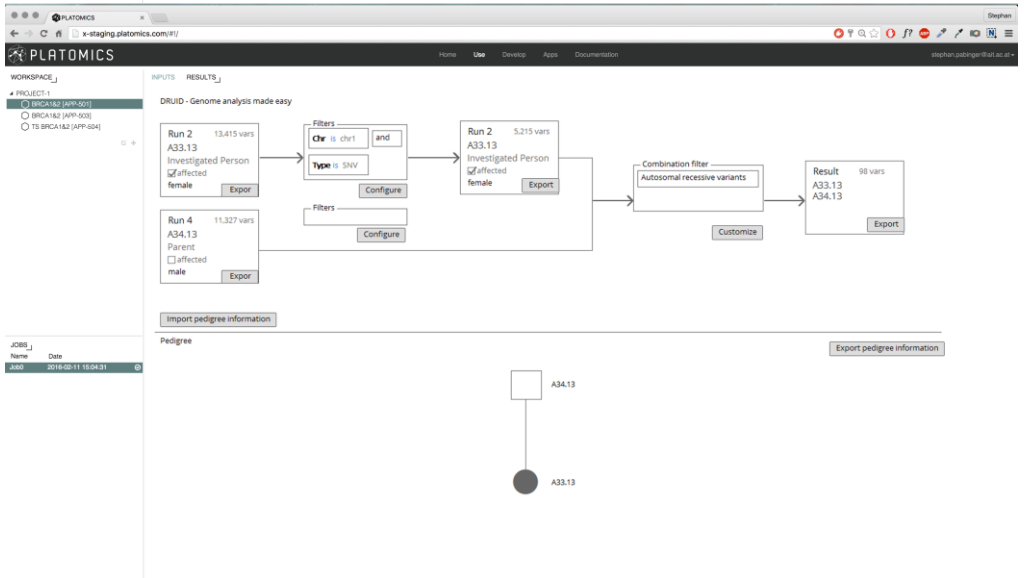



| Sample | Chr | Start | End | Type | Zygosity | Polyphen | Sift | AF | HGVS | Exon |
|---|---|---|---|---|---|---|---|---|---|---|
| Father | chr1 | 1234567 | 1234568 | SNV | het | 0.8 | 0.6 | 0.12 | NM_000059.3:c.1114G>C | 2 |
| Mother | chr1 | 1234567 | 1234568 | SNV | het | 0.8 | 0.6 | 0.12 | NM_000059.3:c.1114G>C | 2 |
| Child | chr1 | 1234567 | 1234568 | SNV | hom | 0.8 | 0.6 | 0.12 | NM_000059.3:c.1114G>C | 2 |
| Father | chr1 | 1234967 | 1234968 | SNV | het | 0.6 | 0.4 | 0.08 | NM_000023.3:c.24C>A | 1 |
| ... | | | | | | | | | | |

# Summary

- Software for variant identification and annotation
- Integration into a web-based system (Platomics)
- Intuitive filtering mechanism



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www.ait.ac.at

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www.cemm.at

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www.platomics.com

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- Gregor Rosenauer
- Albert Kriegner

# Collaboration oportunities

**Horizon 2020**

PM03 - Diagnostic characterisation of rare diseases

PM08 - New therapies for rare diseases


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