Genotype Refinement Workflow

Using additional data to improve genotype calls and likelihoods

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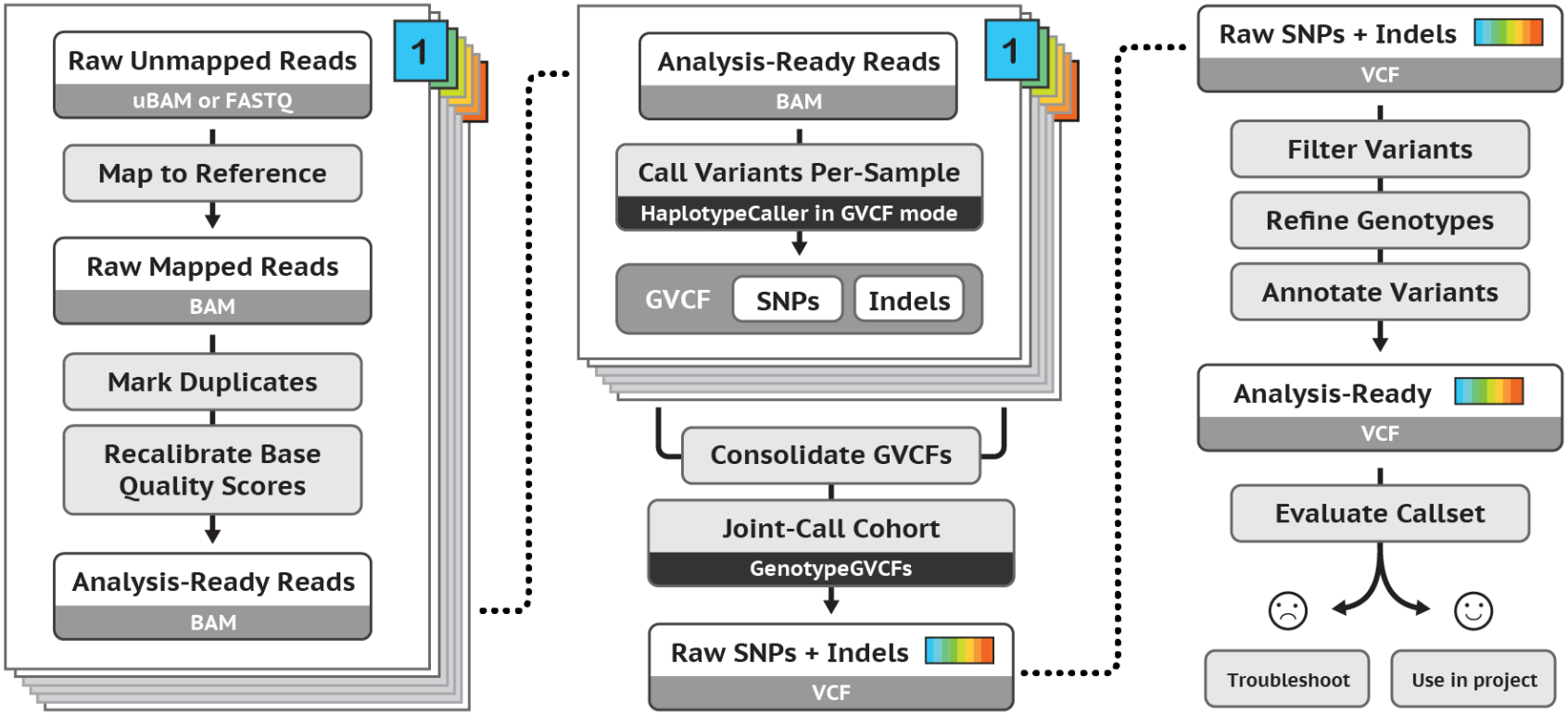
gatk

Best Practices for Germline SNP & INDEL Discovery



Why care about genotypes?

- Medical geneticists need genotypes for patients
  - Do any patients have two copies of a LOF mutation?
  - Are the parents of a diseased child likely to have more afflicted children?

- Population geneticists need genotypes for association studies
  - How does the number of copies of an allele affect the phenotype?

Variant call vs. Genotype call

Genotype call quality is important!

- Some sites/samples have poor genotype calls
  - Can be ambiguous due to low confidence
  - Might be entirely wrong!

- Can additional (independent) data improve genotype calls?
  - Use high quality data (like 1000G) as priors
  - Use pedigree (if available)
  - Calculate posterior genotype probabilities

Review of Bayes's Rule

Given that your coworker just walked in with an umbrella, what is the probability that it is raining?

- Observation = umbrella
- Θ = probability of rain

posterior probability

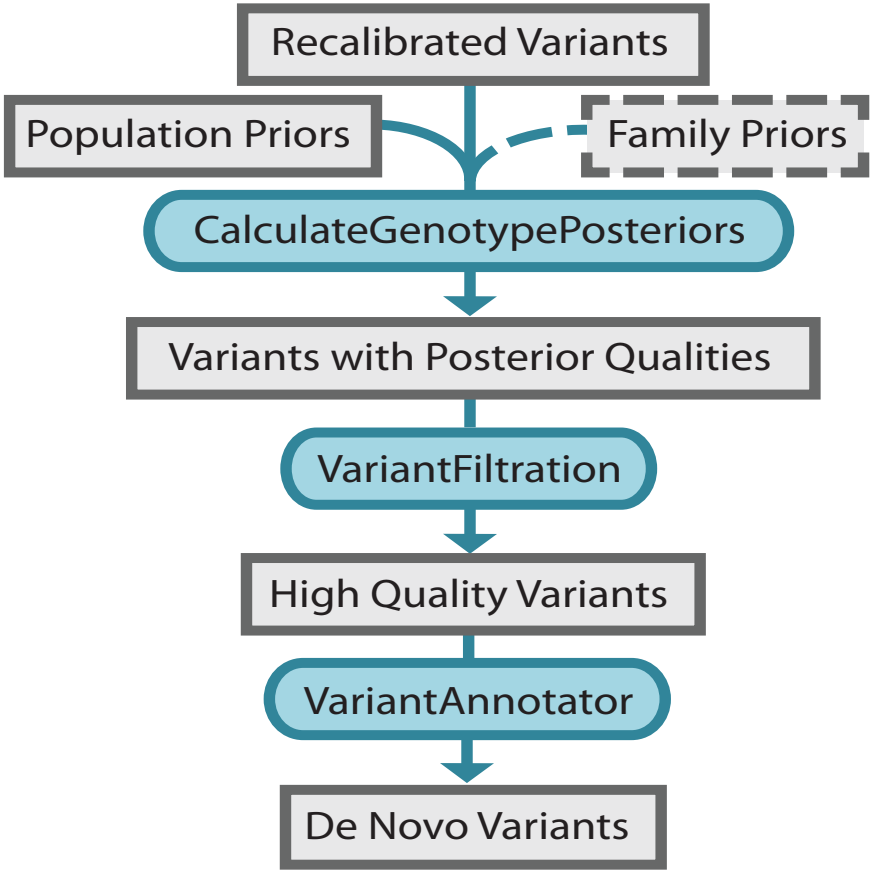
likelihood

prior

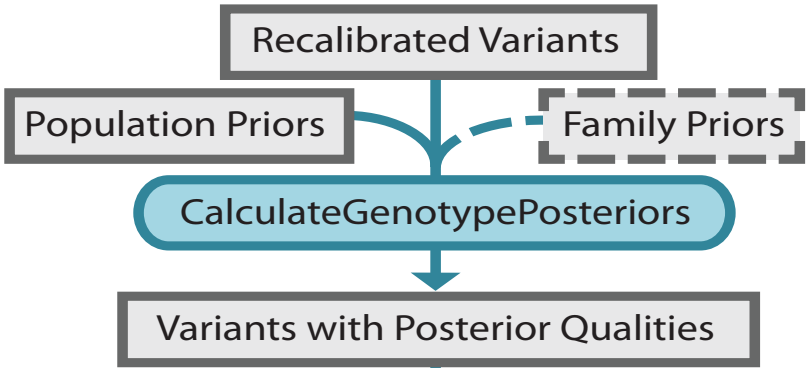
$$P(\theta|Obs) = \frac{P(Obs|\theta)P(\theta)}{\sum_{\theta} P(Obs|\theta)P(\theta)}$$

(normalize)

Genotype Refinement Workflow

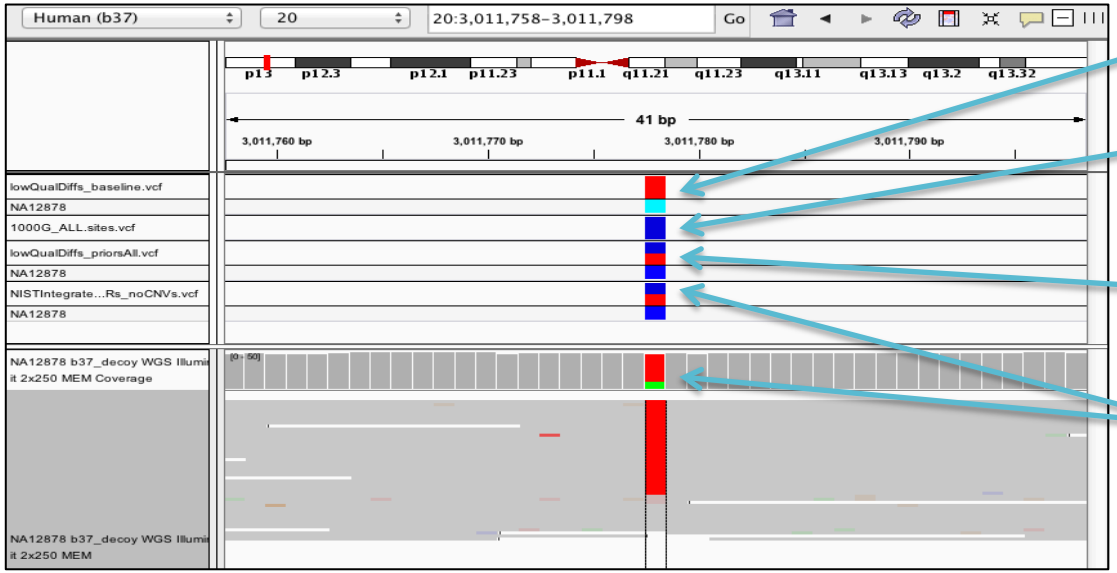


CalculateGenotypePosteriors




```
gatk CalculateGenotypePosteriors \
    -R reference.fasta \
    -V input.vcf \
    -ped family.ped \
    -supporting population.vcf \
    -O output.vcf
```

Case 1: HOM_VAR Call w/ Low Frequency Priors



1) Baseline HOM_VAR call

2) Priors w/low allele frequency applied

3) Posterior genotype called HET

4) In agreement w/NIST and BAMs

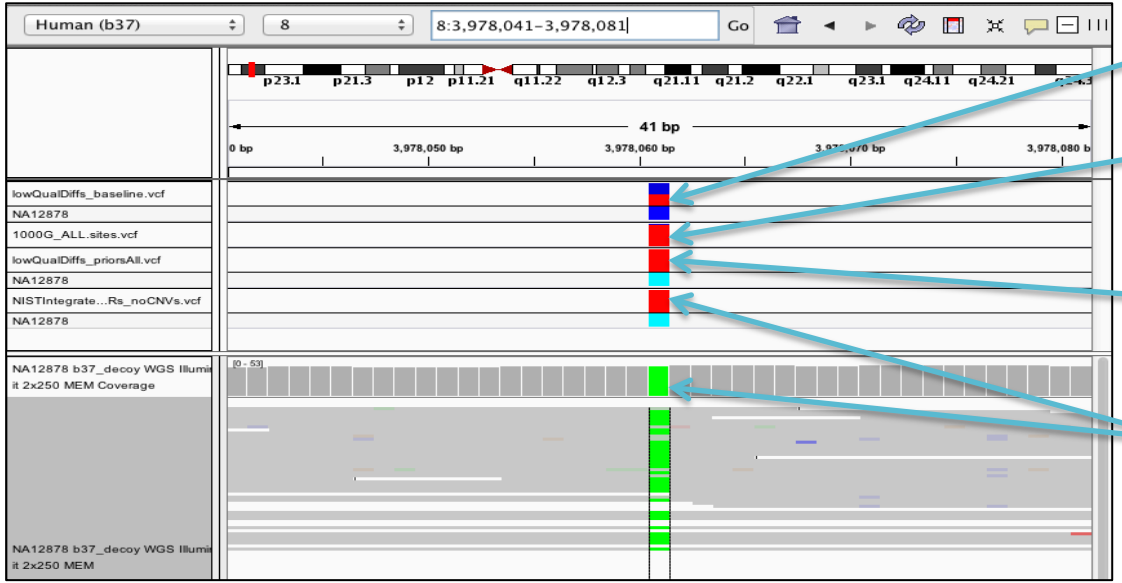
Likelihoods x Priors = Posterior Probabilities

[895,3,0] AF=0.002 [868,0,27]

[HOM_REF, HET, HOM_VAR] [HOM_REF, HET, HOM_VAR]

Genotype corrected
Confidence improved
from Q3 to Q27

Case 2: HET Call with High Frequency Priors



1) Baseline HET call

2) Priors w/high allele frequency applied

3) Posterior genotype called HOM_VAR

4) In agreement w/NIST and BAMs

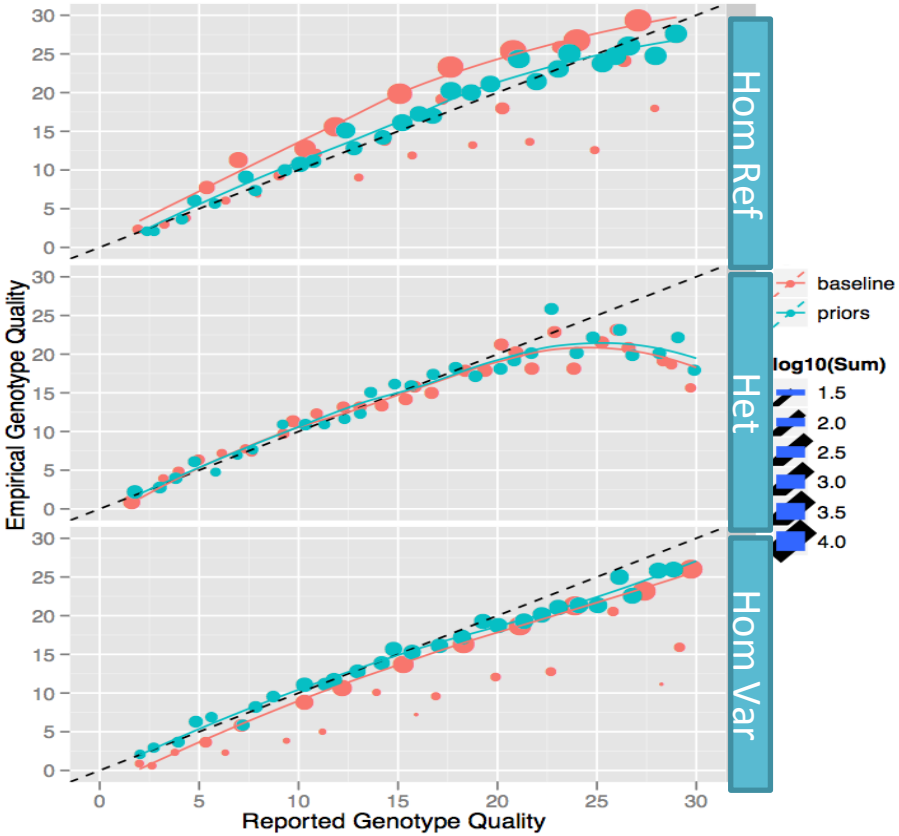
Likelihoods x Priors = Posterior Probabilities

[894,0,0] AF=0.987 [932,16,0]

[HOM_REF, HET, HOM_VAR] [HOM_REF, HET, HOM_VAR]

Genotype corrected
Confidence improved
from Q0 to Q16

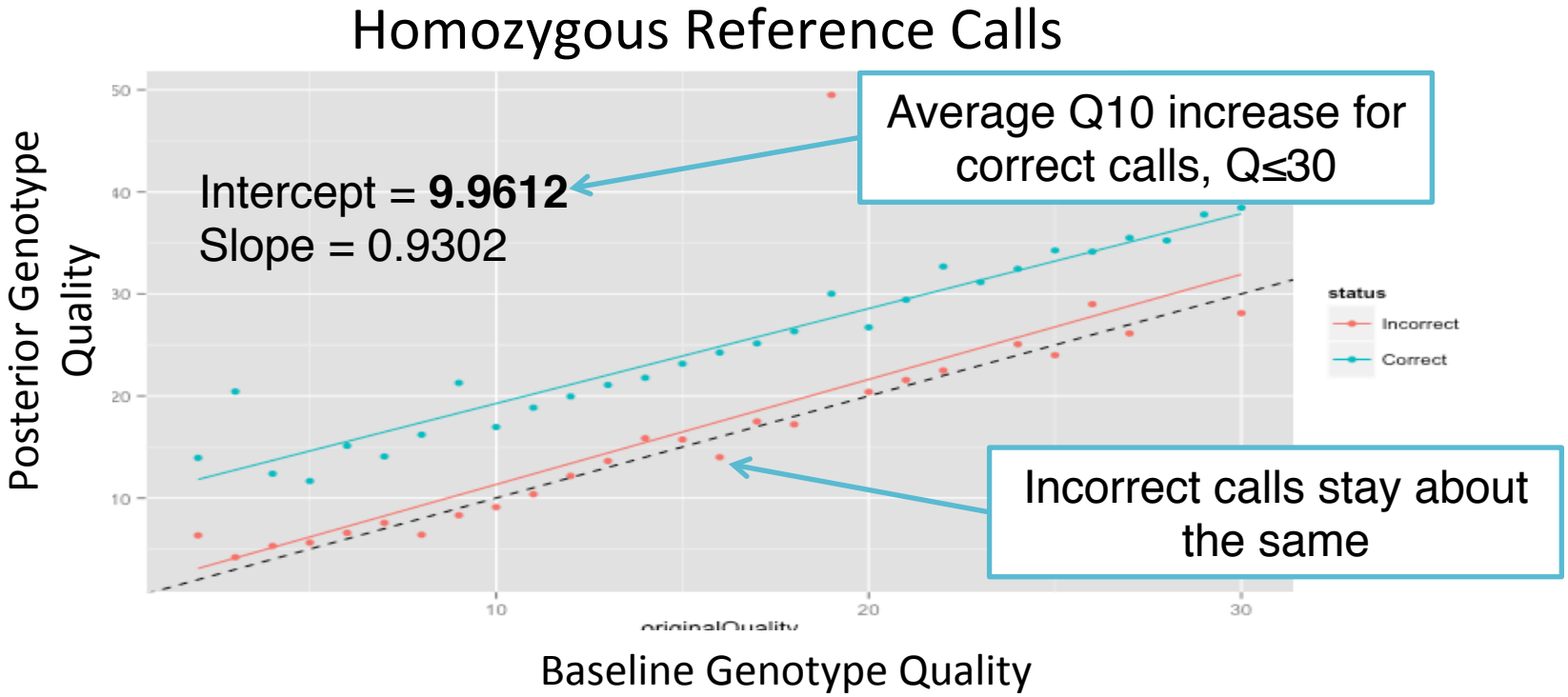
Population priors improve genotype confidence



Baseline HomRef calls are under confident, but posterior calls are more accurate

Baseline HomVar calls are over confident, but posterior calls are improved

Assessing confidence and correctness



Parental genotypes inform child genotypes

- Child can only inherit alleles present in parents
- Parent genotypes determine possible child genotypes (assuming no mutations)

Child	Mother	Father
HR	HR	HR
HR	HR	HET
HR	HET	HR
HR	HET	HET

Child	Mother	Father
HET	HET	HET
HET	HR	HET
HET	HET	HR
HET	HV	HET
HET	HET	HV
HET	HR	HV
HET	HV	HR

Child	Mother	Father
HV	HV	HV
HV	HV	HET
HV	HET	HV
HV	HET	HET

- HaplotypeCaller gives $P(G_C|D_C)$
- Given trio data we can derive $P(G_C|D_C, D_M, D_F)$

Bayesian priors applied to trios

- Recall Bayes's Rule: $$P(\theta|Obs) = \frac{P(Obs|\theta)P(\theta)}{\sum_\theta P(Obs|\theta)P(\theta)}$$

- Establish genotype configuration probabilities

$$P(G_M, G_F, G_C) = P(\vec{G}) \begin{cases} \mu, 1MV \\ \mu^2, 2MVs \\ 1 - 10\mu - 2\mu^2, non - MV \end{cases}$$

- Apply family priors

likelihood

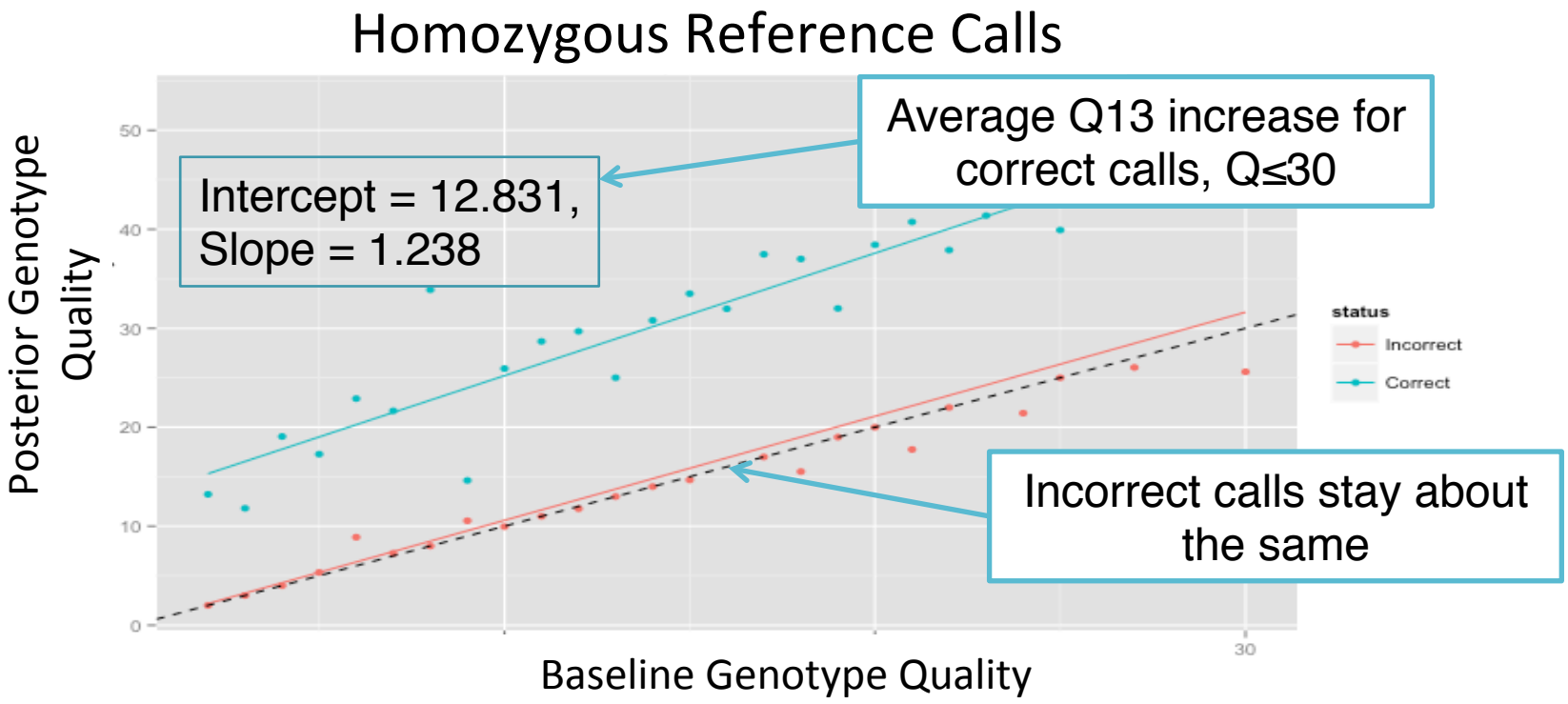
posterior

$$P(G_C = HR|\vec{D}) = \frac{L_C(G_C = HR)\sum_{G_F,G_M} L_F(G_F)L_M(G_M)P(\vec{G})}{\sum_{\vec{H}} P(\vec{D}|\vec{H})P(\vec{H})}$$

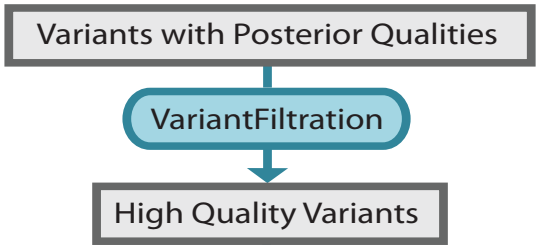
apply prior

normalize

Assessing confidence and correctness



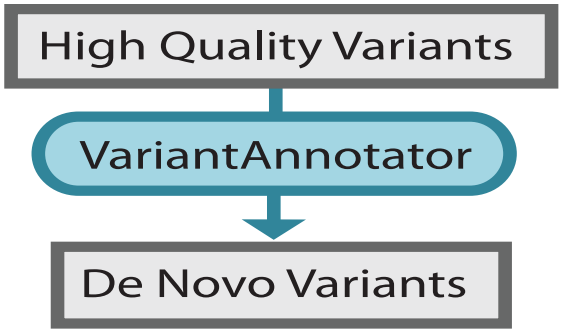
Filter low confidence GQs




```
gatk VariantFiltration \
    -R reference.fasta \
    -V input.vcf \
    --filter-expression "GQ<20" \
    --filter-name "lowGQ" \
    -O output.vcf
```

- Use VariantFiltration to filter ambiguous, low-confidence calls
- Recommended threshold is GQ = 20
  - GQ 20 is Phred-scaled 99% confidence
- Restrict further analysis to high-quality data

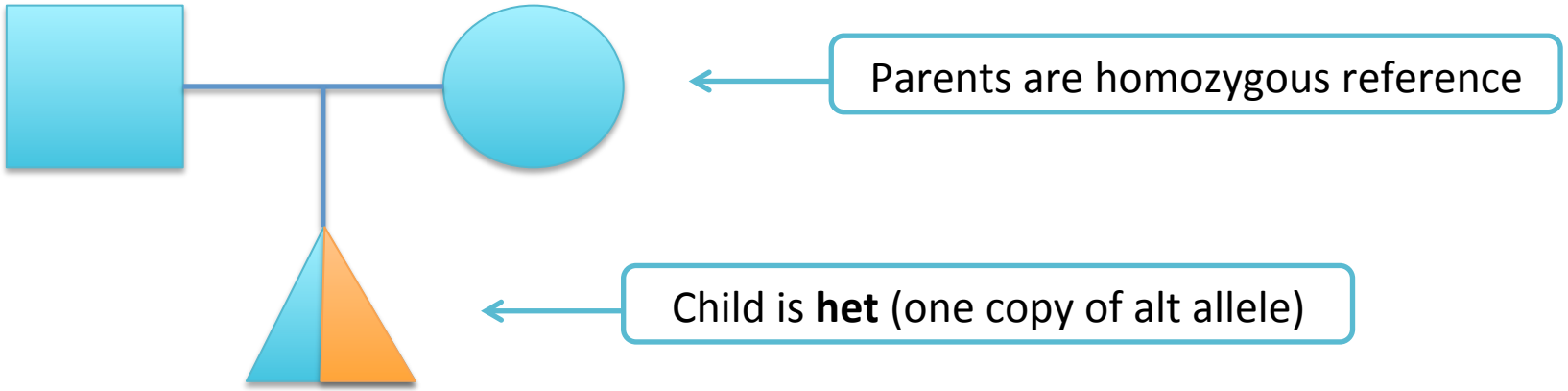
VariantAnnotator




```
gatk VariantAnnotator \
    -R reference.fasta \
    -V input.vcf \
    -A PossibleDeNovo \
    -O output.vcf
```

What are *De Novo* mutations?

- Culprits in many rare Mendelian disorders
- ~30 *de novo* mutations occur per human genome



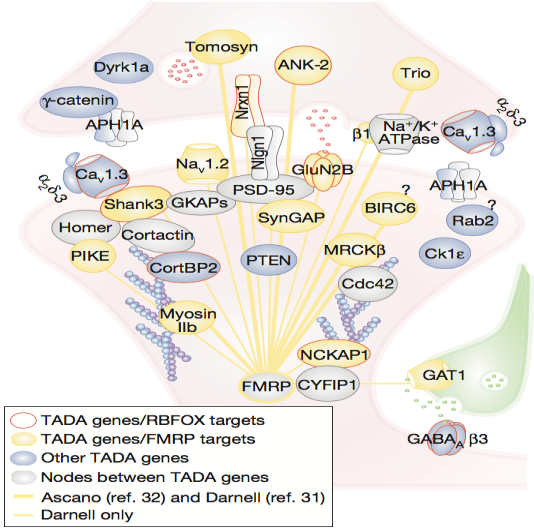
Properties of sequenced De Novos

- **Novelty**
  - Child has only alt allele in trio, not inherited
- **Rarity**
  - Allele frequency across all samples sequenced is low
- **Confidence**
  - Set GQ threshold for parents and child
  - (GQ improvement tools help A LOT here!)

ARTICLE

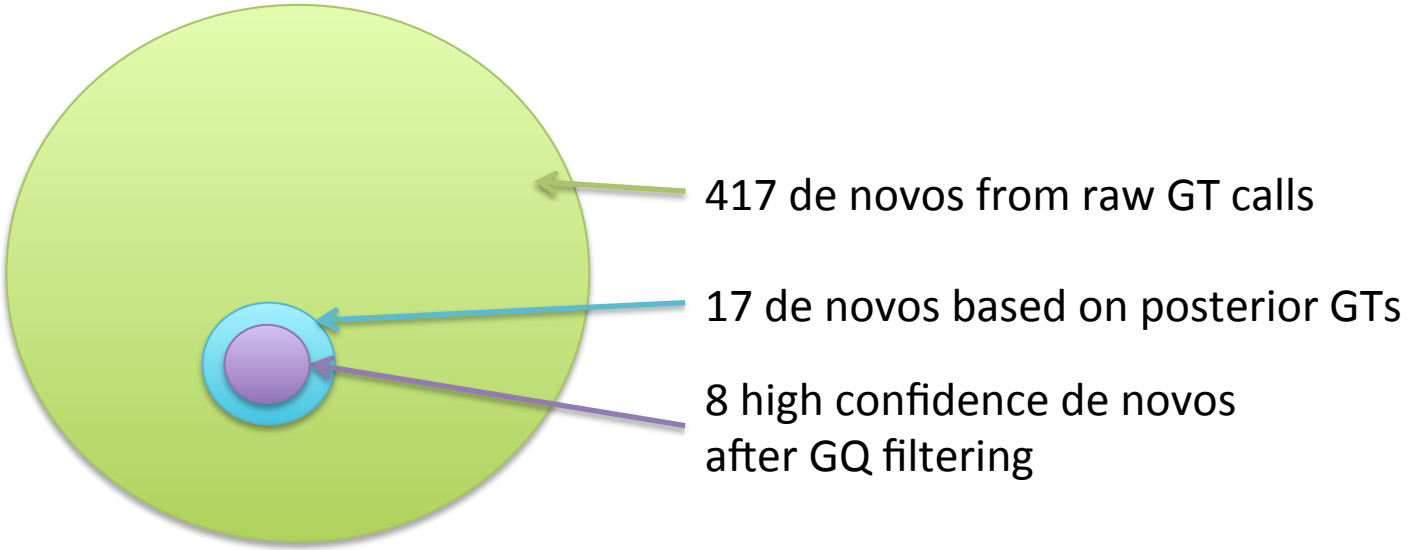
doi:10.1038/nature13772

Synaptic, transcriptional and chromatin genes disrupted in autism



Example of a clinical case

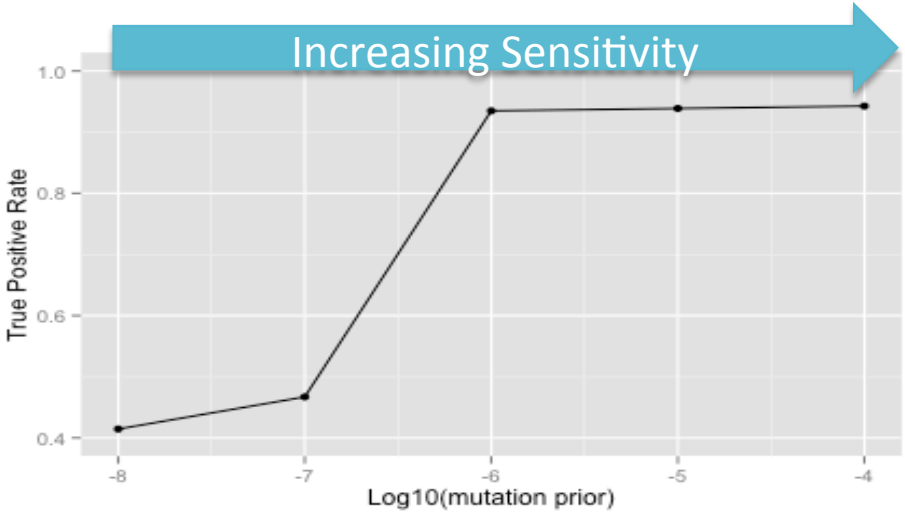
- Real clinical data
- Suspected de novo mutation in offspring



Priors can be tuned for sensitivity

Mutation prior is a parameter in genotype configuration probability:

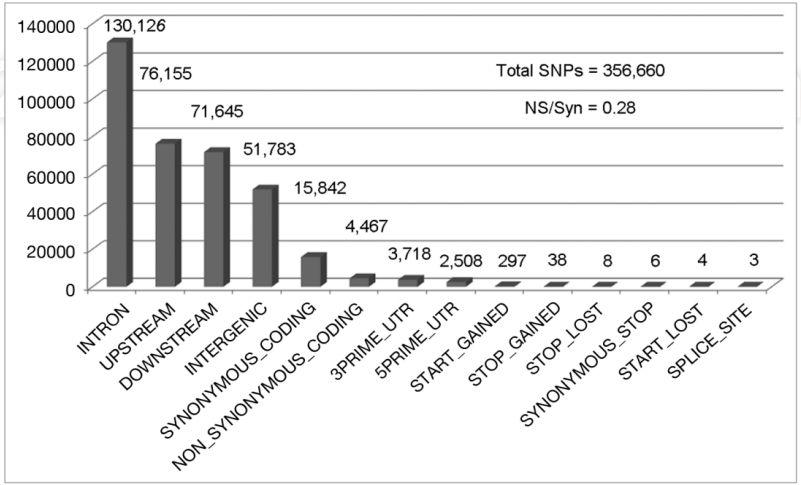
$$P(G_M, G_F, G_C) = P(\vec{G}) \begin{cases} \mu, 1MV \\ \mu^2, 2MVs \\ 1 - 10\mu - 2\mu^2, non - MV \end{cases}$$



Sensitivity and specificity can be tuned as in VQSR

Genotype refinement yields more high-quality genotypes

- Initial genotype calls may be ambiguous or wrong
- Applying population + family priors improves confidence
- More high confidence genotypes -> more data for downstream analysis!
- External tools can be used for further variant annotation (e.g. SnpEff)



*SnpEff is not supported by GATK

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