Getting Genomics Done with R

Stephen A. Sefick

2017-03-28

Outline

Topic

Motivation

1. How to arrive at analysis ready variants?

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2. GATK HaplotypeCaller is "permissive"

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   - R
   - Bioconductor Project

Bioconductor

1. CRAN

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Topic

GATK Haplotype Caller Annotations

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5. Today concentrate on hard-filtering SNPs

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3. These are recommendations, developed with human data, and might/likely will need to be modified based on the data

Variant	#	Annotation	Remove If
Both	1	DP	min=empirical; max=5 or 6 sigma
	2	GQ (or RGQ)	empirical
SNP	3	QD	< 2.0
	4	MQ	< 40
	5	FS	> 60
	6	SOR	> 3.0
	7	MQRankSum	< -12.5
	8	ReadPosRankSum	< -8.0

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   - QC of the caller will remove reads (MAQ)

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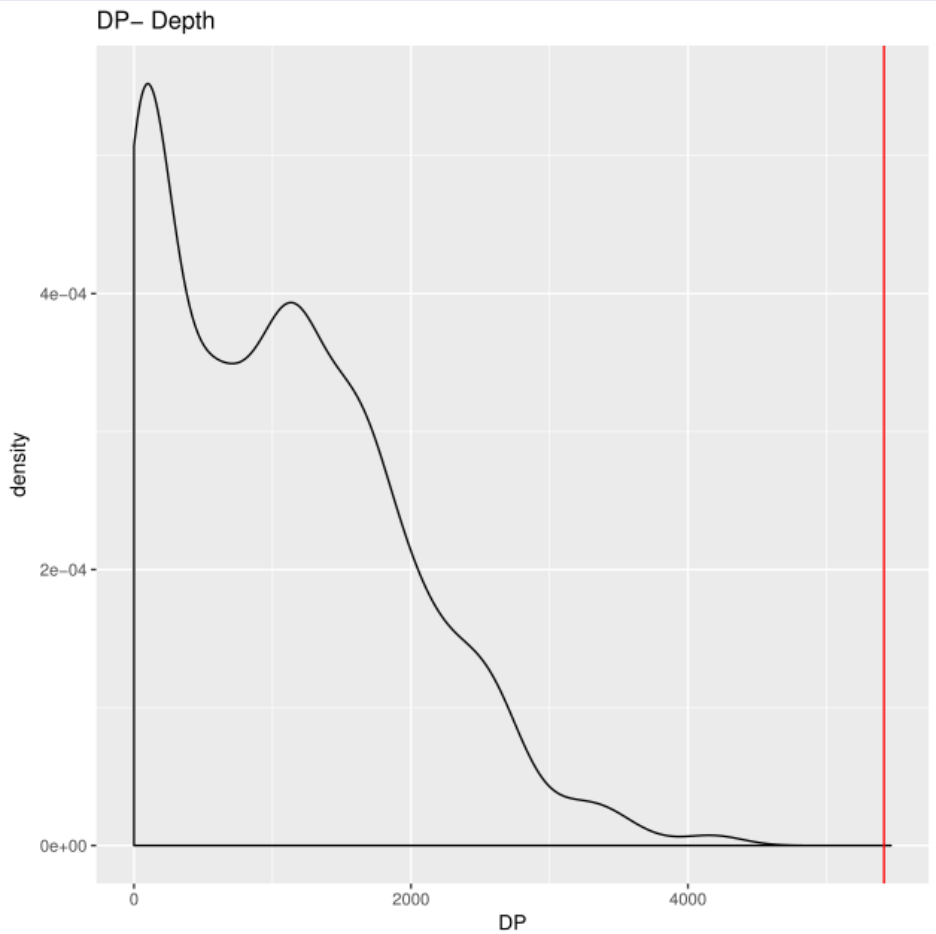
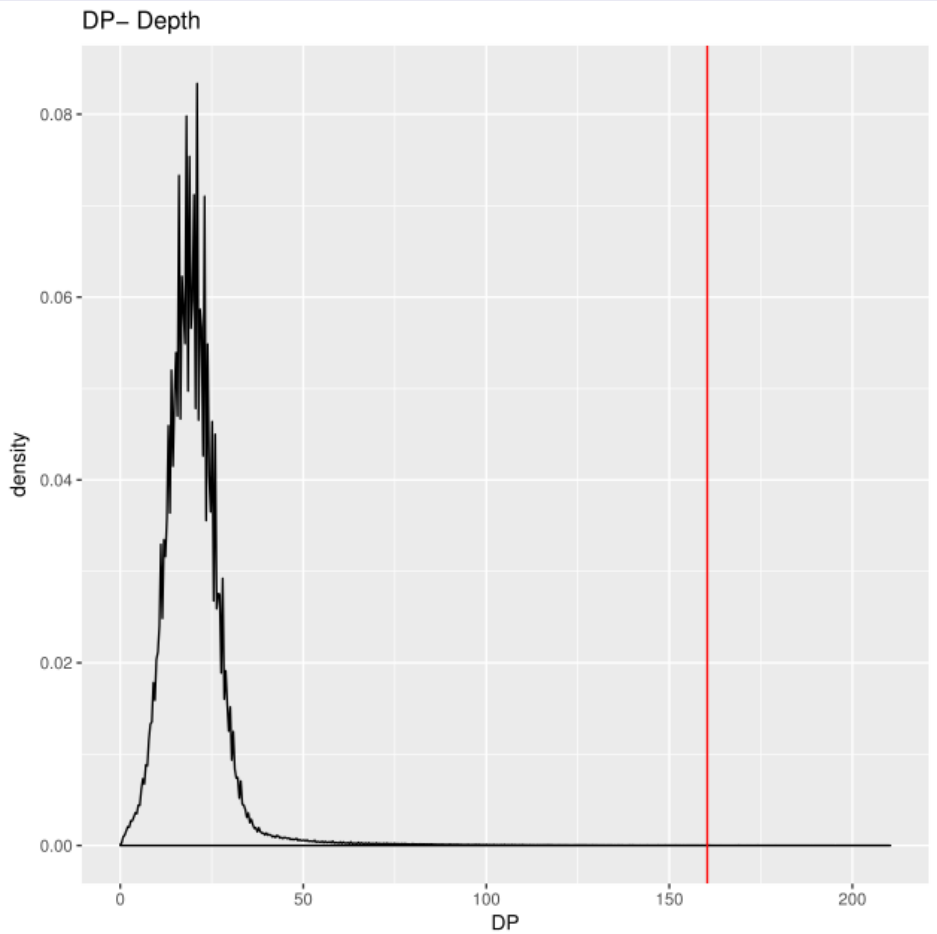
Mitochondria



Chr1-20 and X



2 Genotype quality (GQ); Reference GQ (RGQ)

1. Phred scaled probability of incorrect genotype
   - 20 - 0.01; 30 - 0.001; 40 - 0.0001

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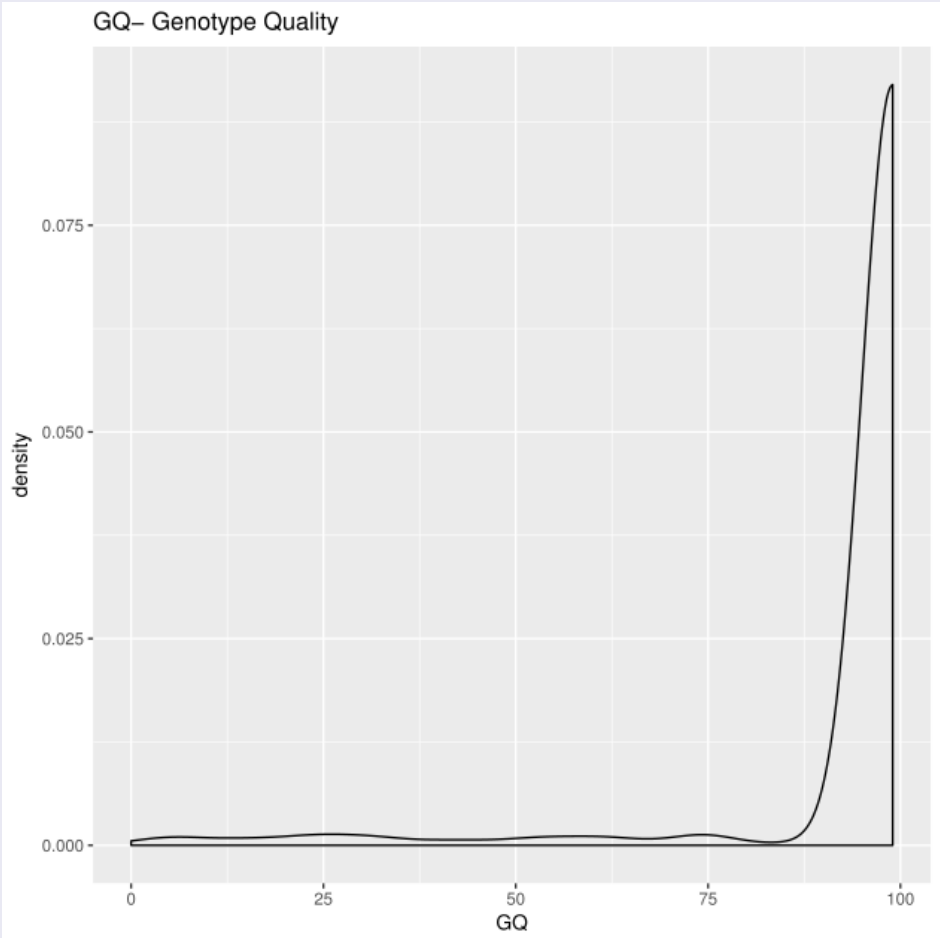

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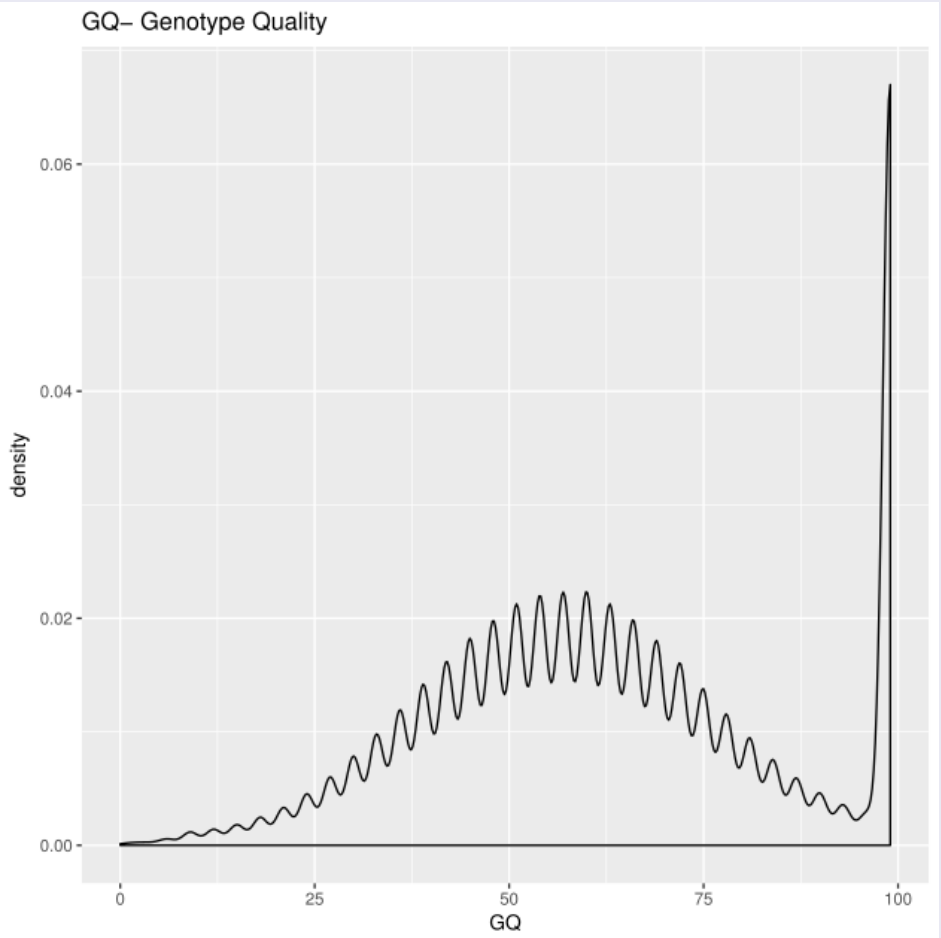
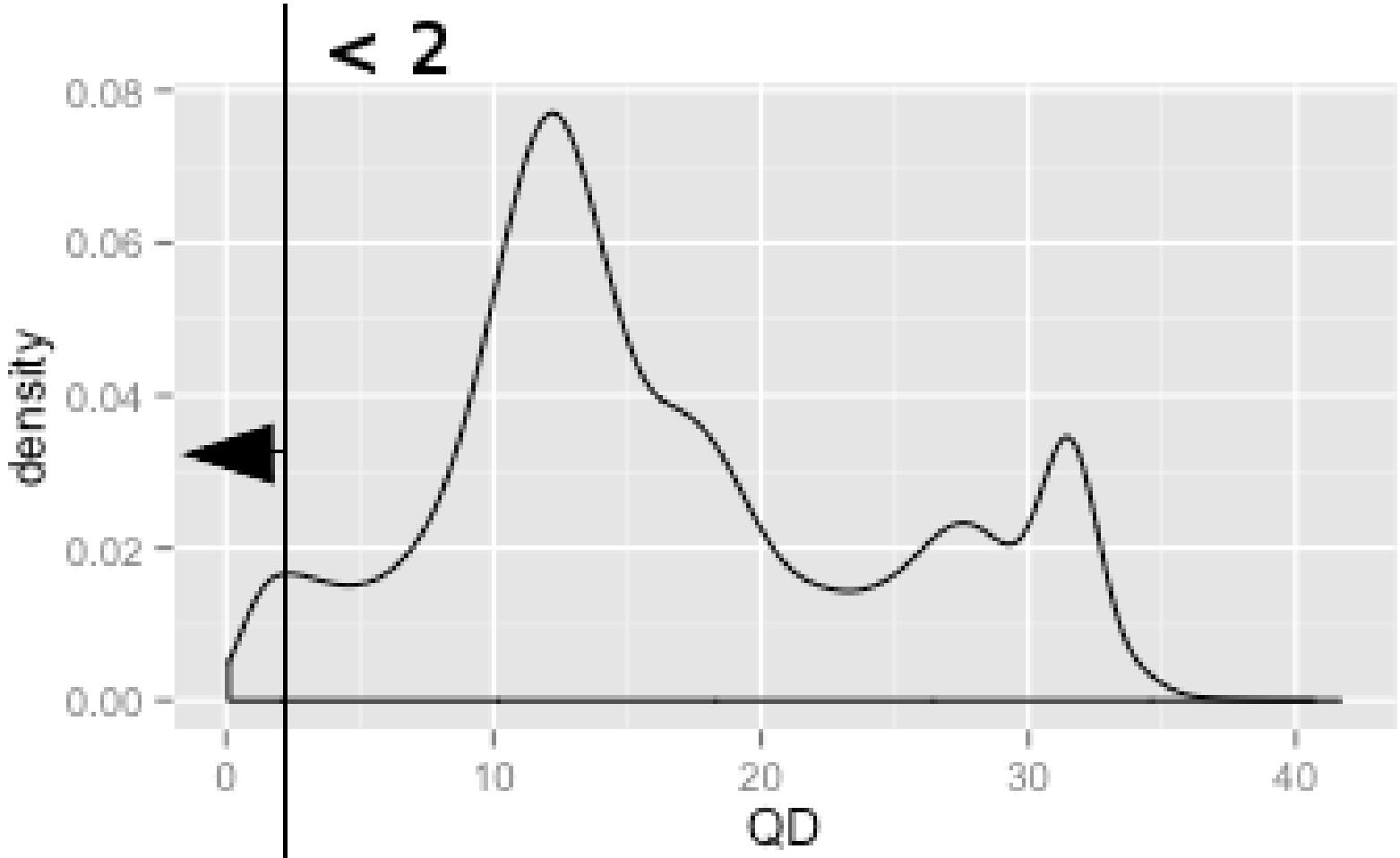


3 Variant quality/allele depth (QD)

1. Variant Quality (QUAL) is the phred scaled probability that the variant is wrong.
2. allele depth is actual depth of each observed allele (How many actual reads; in contrast to DP).



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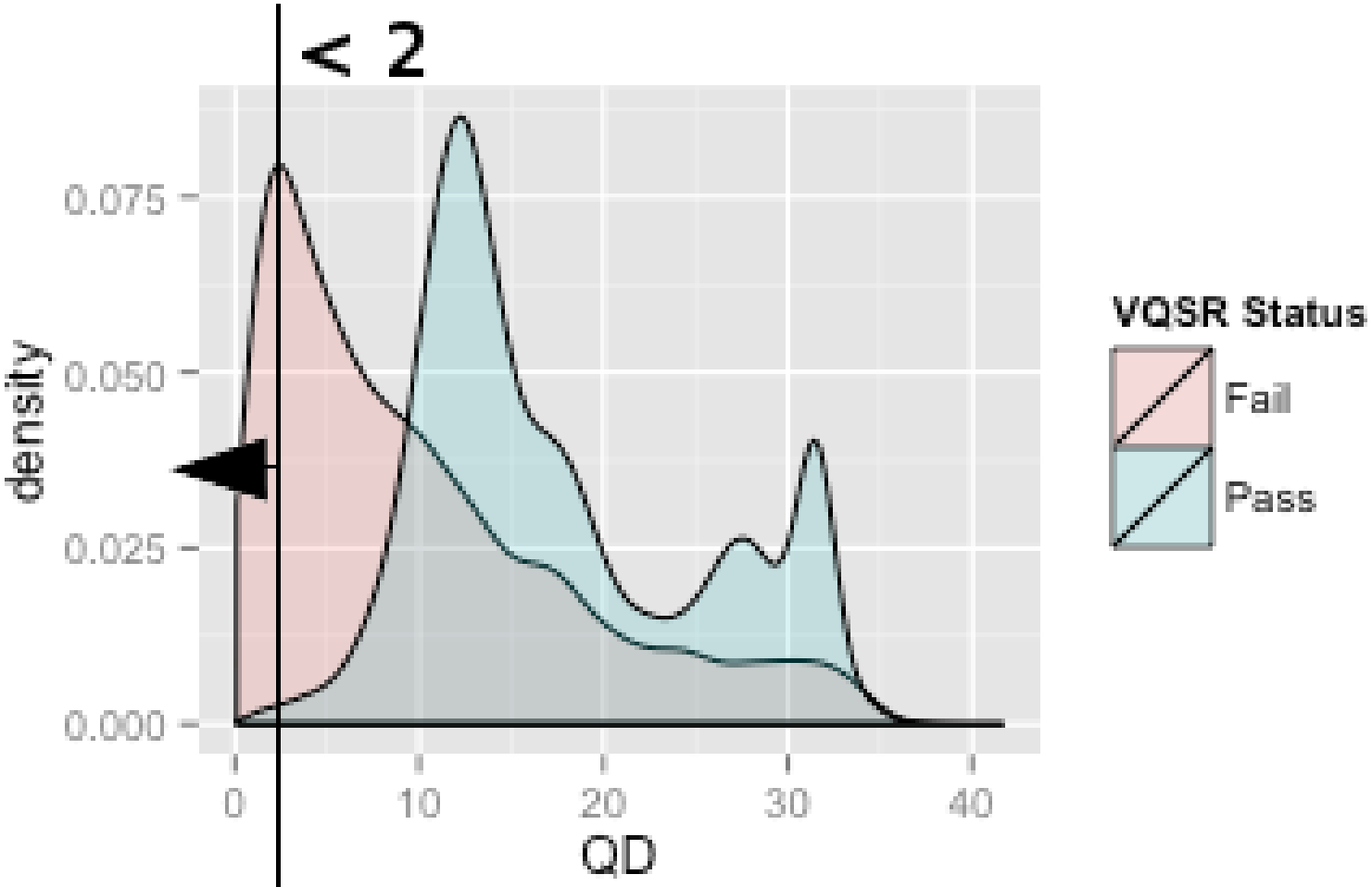

4 Root mean square mapping quality (MQ)

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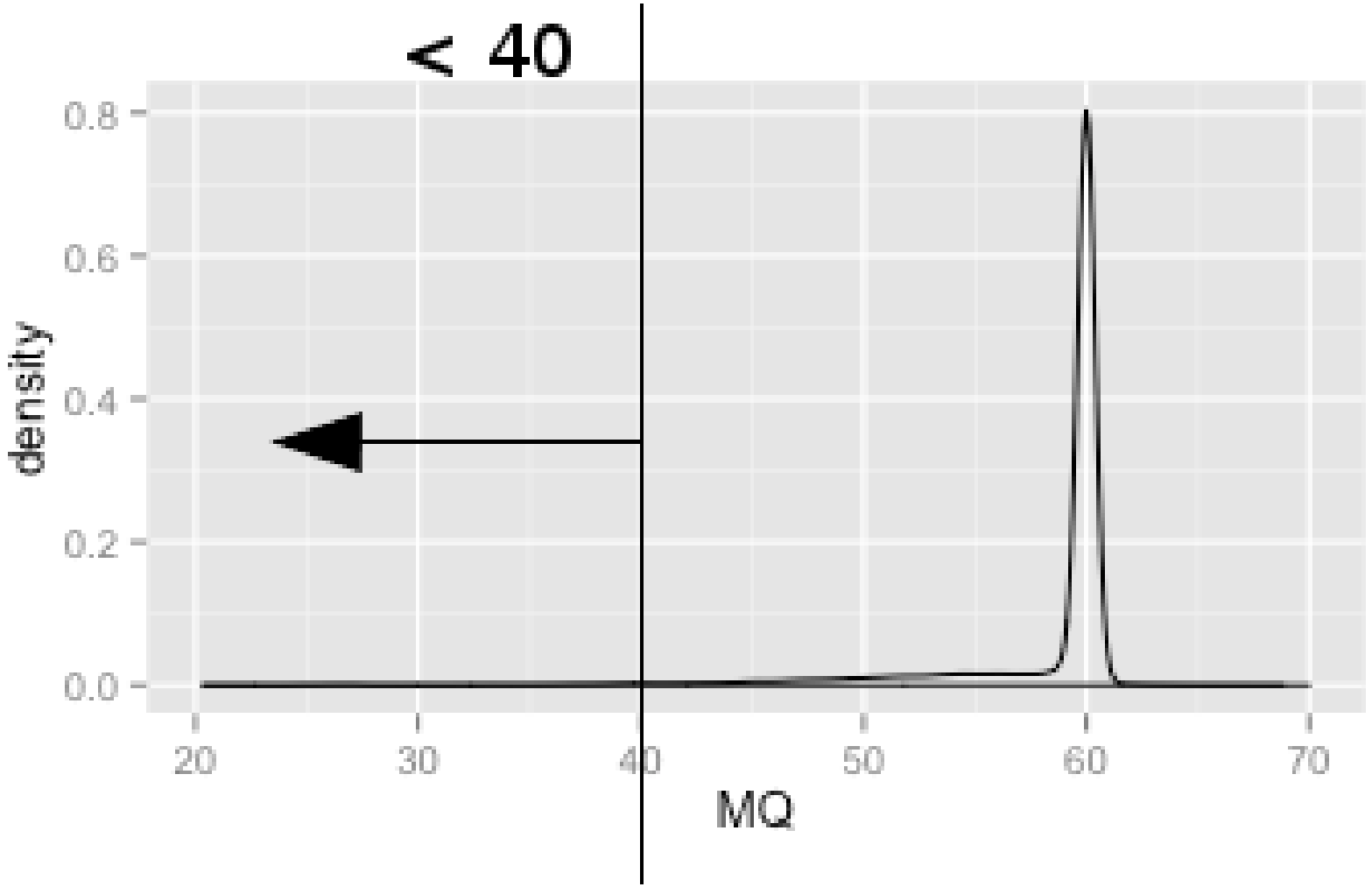

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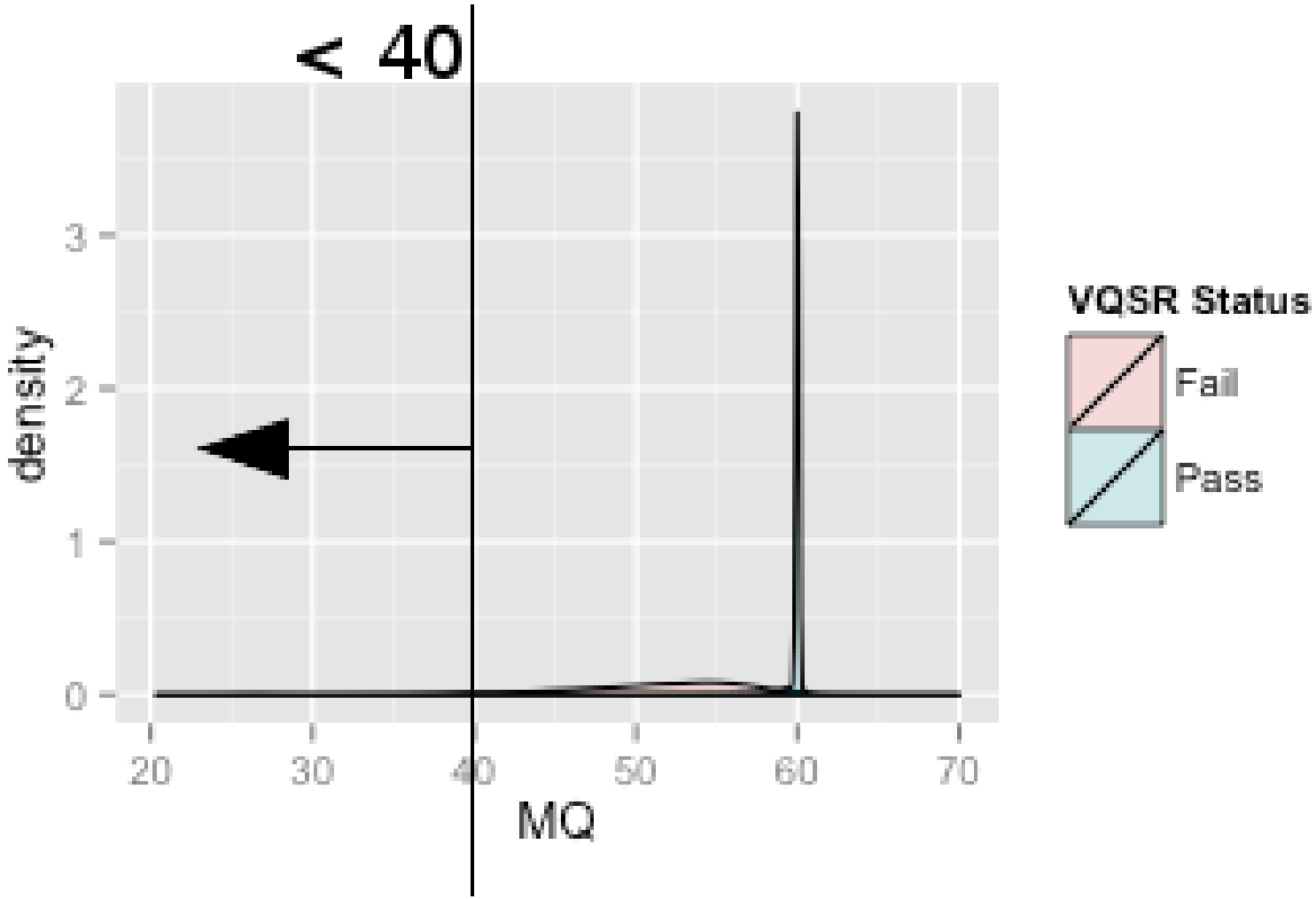

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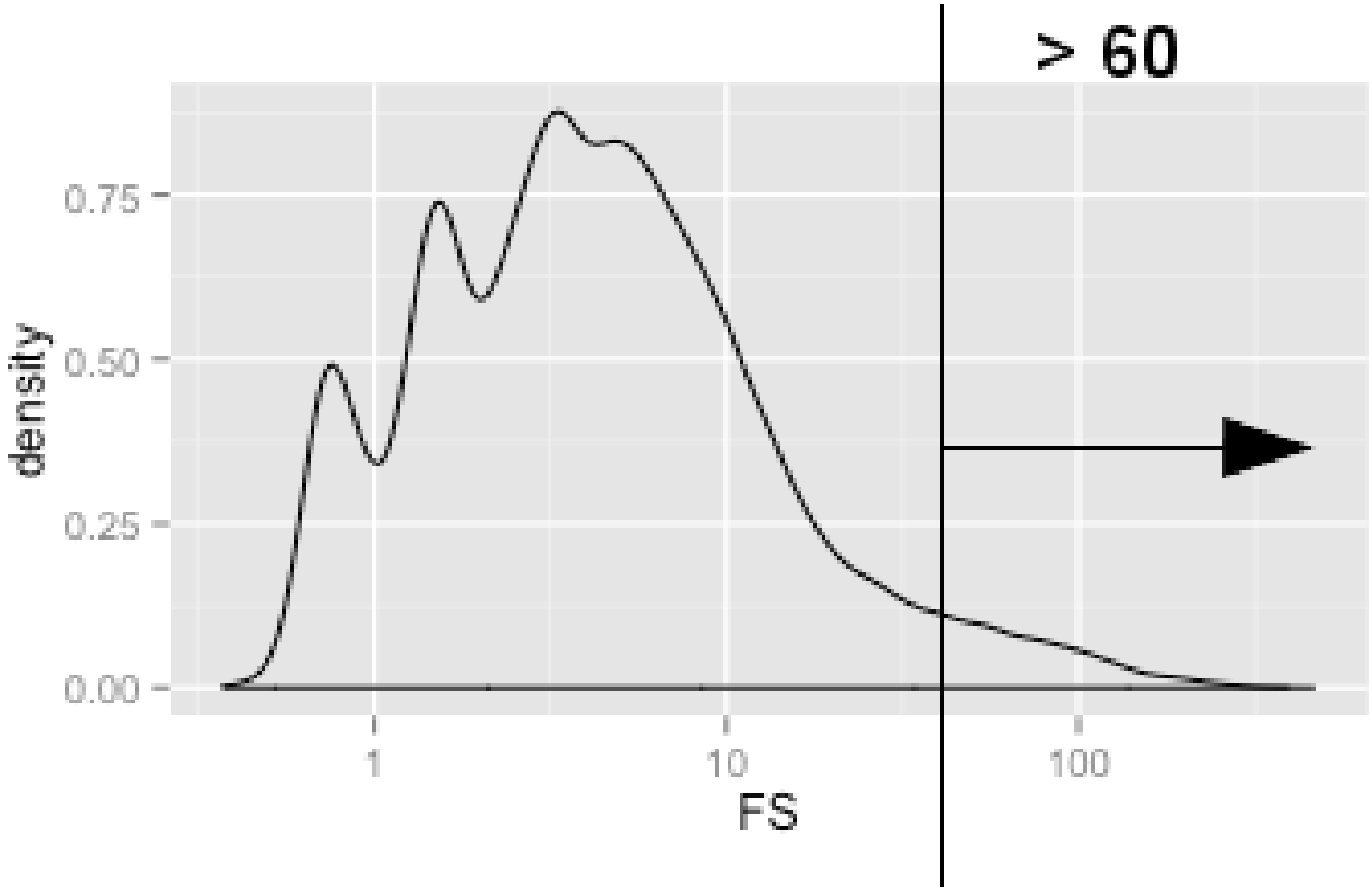

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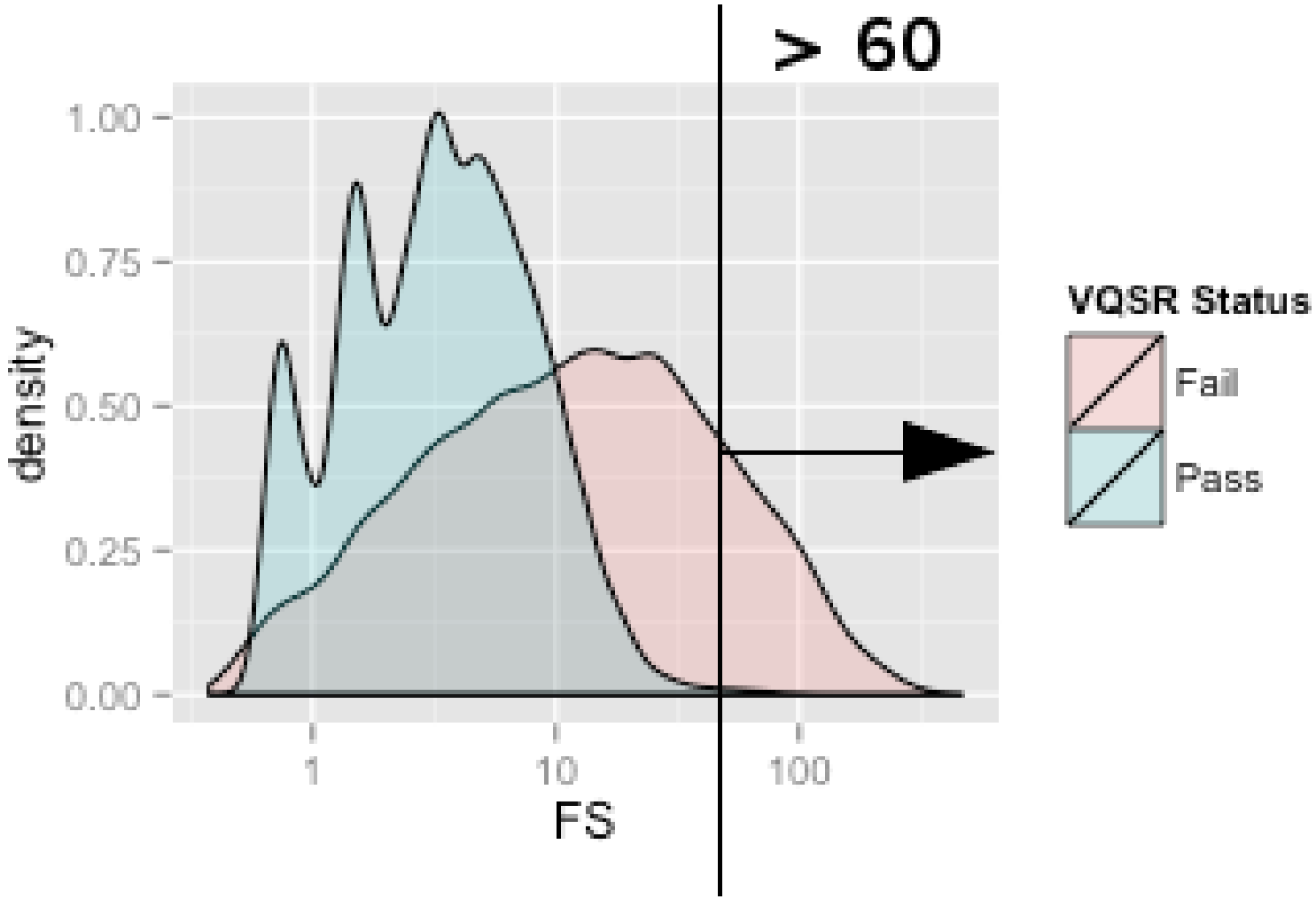

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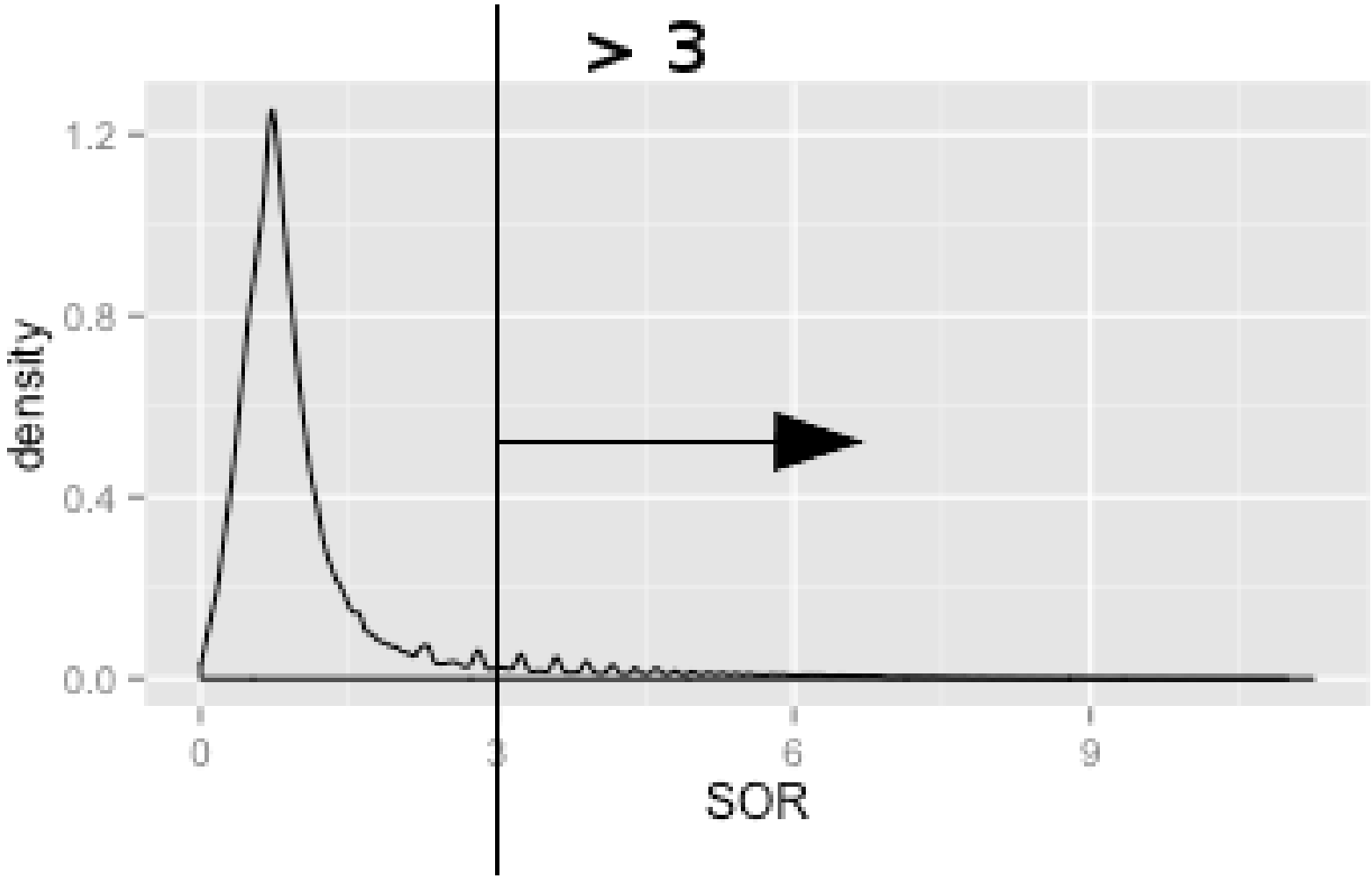

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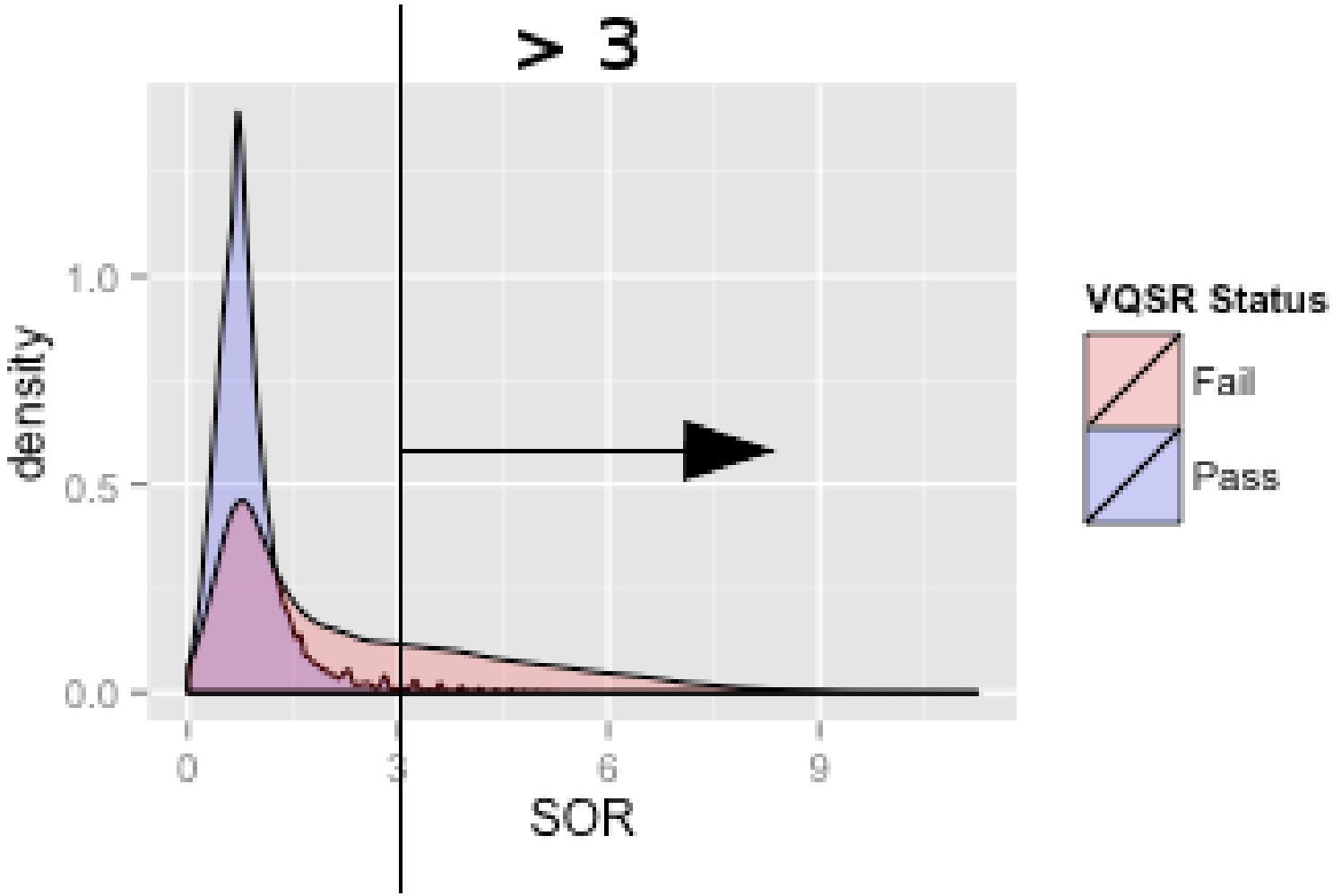

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1. test compares MAQ ALT to REF
   - (-) Alt lower MAQ
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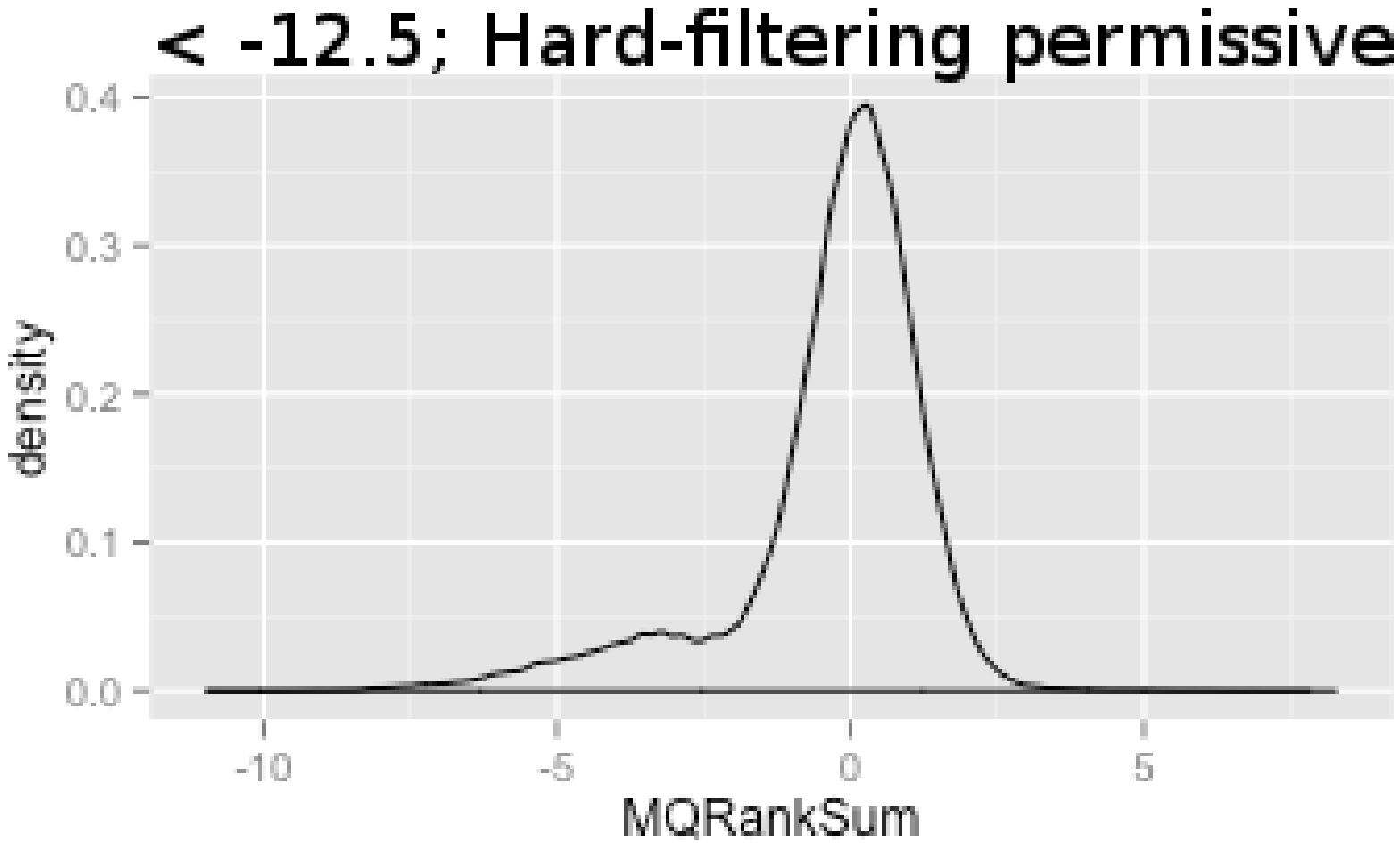

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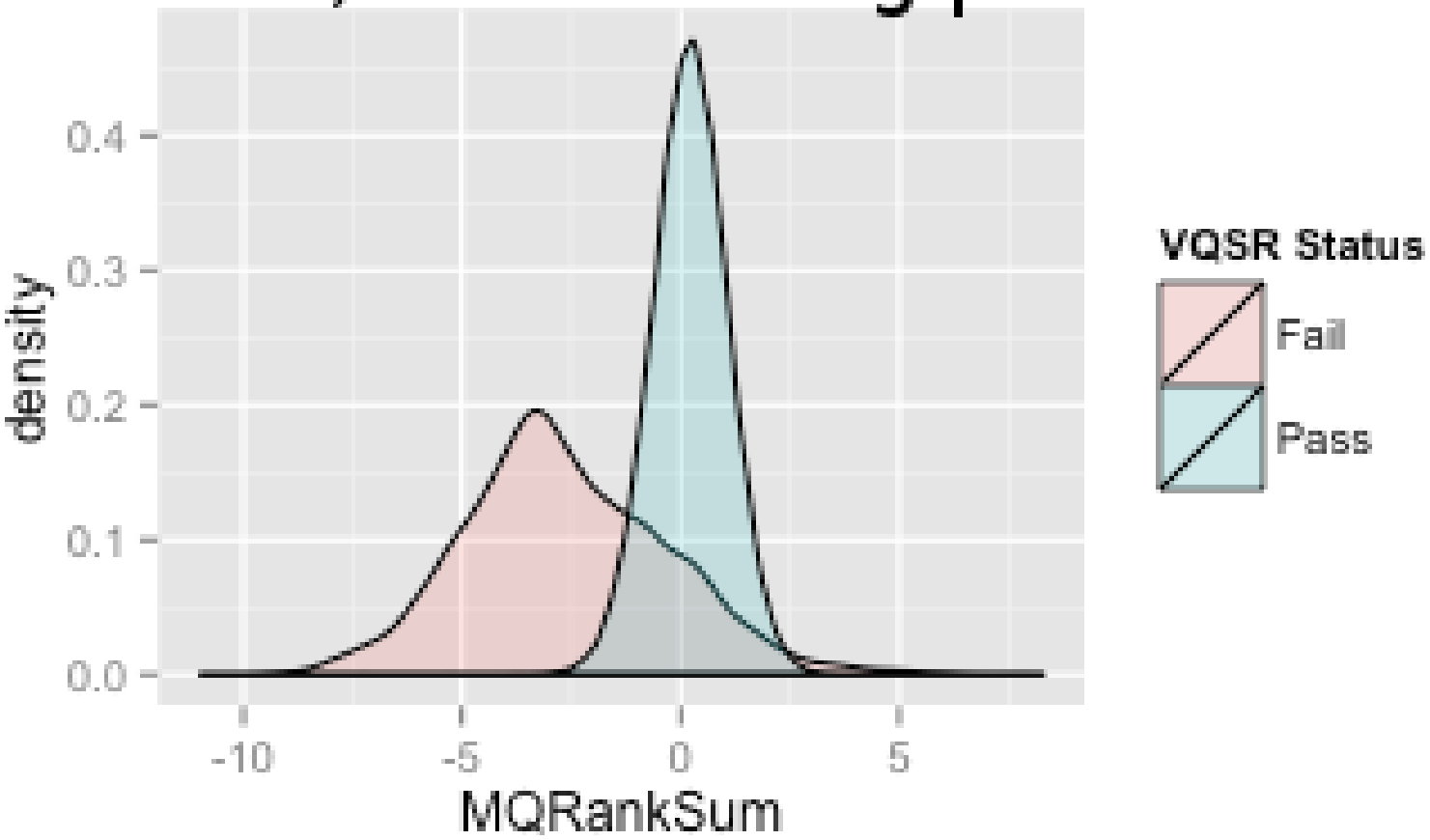
< -12.5; Hard-filtering permissive



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1. test for positional effects
   - (-) Alt close to end of read
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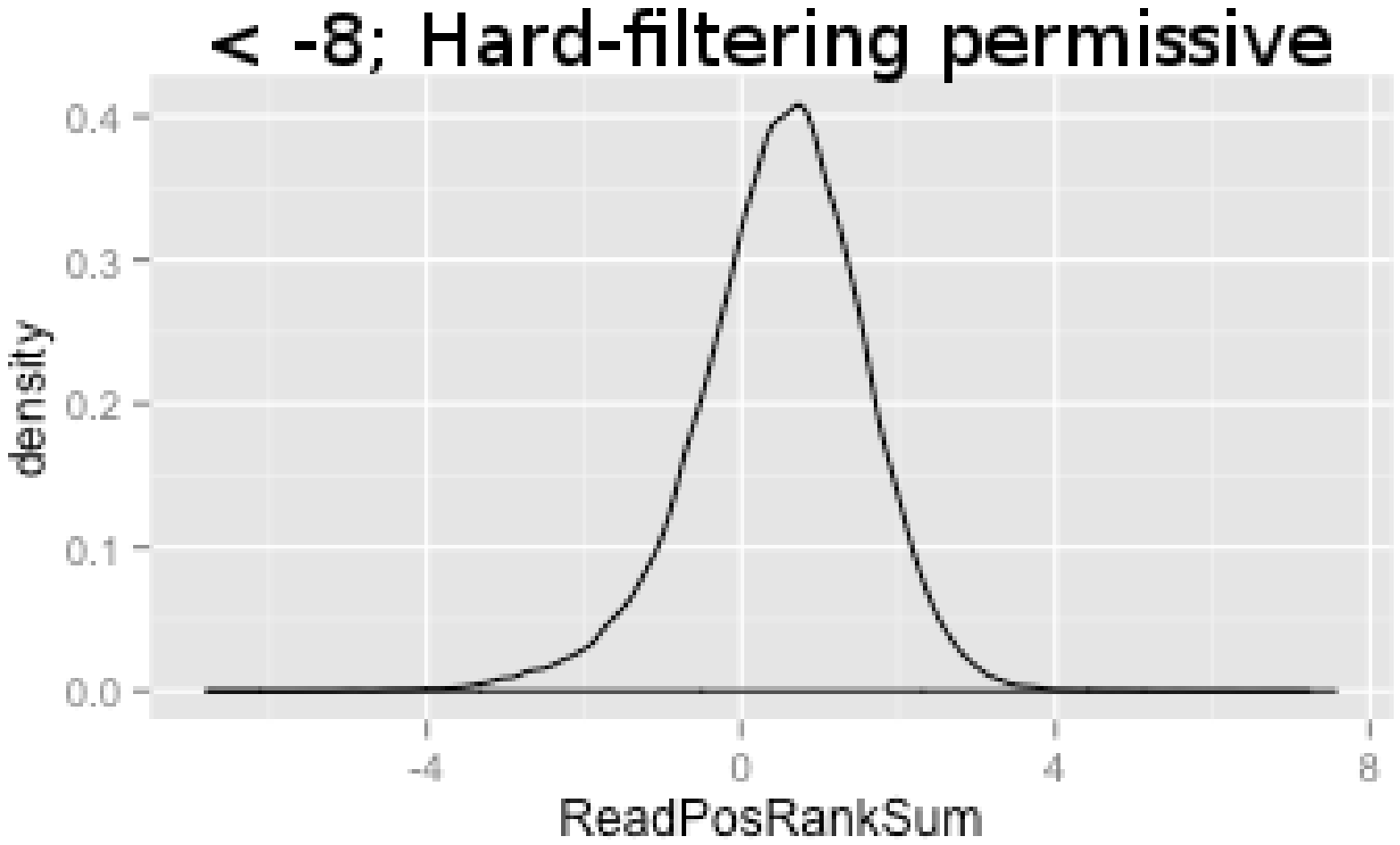

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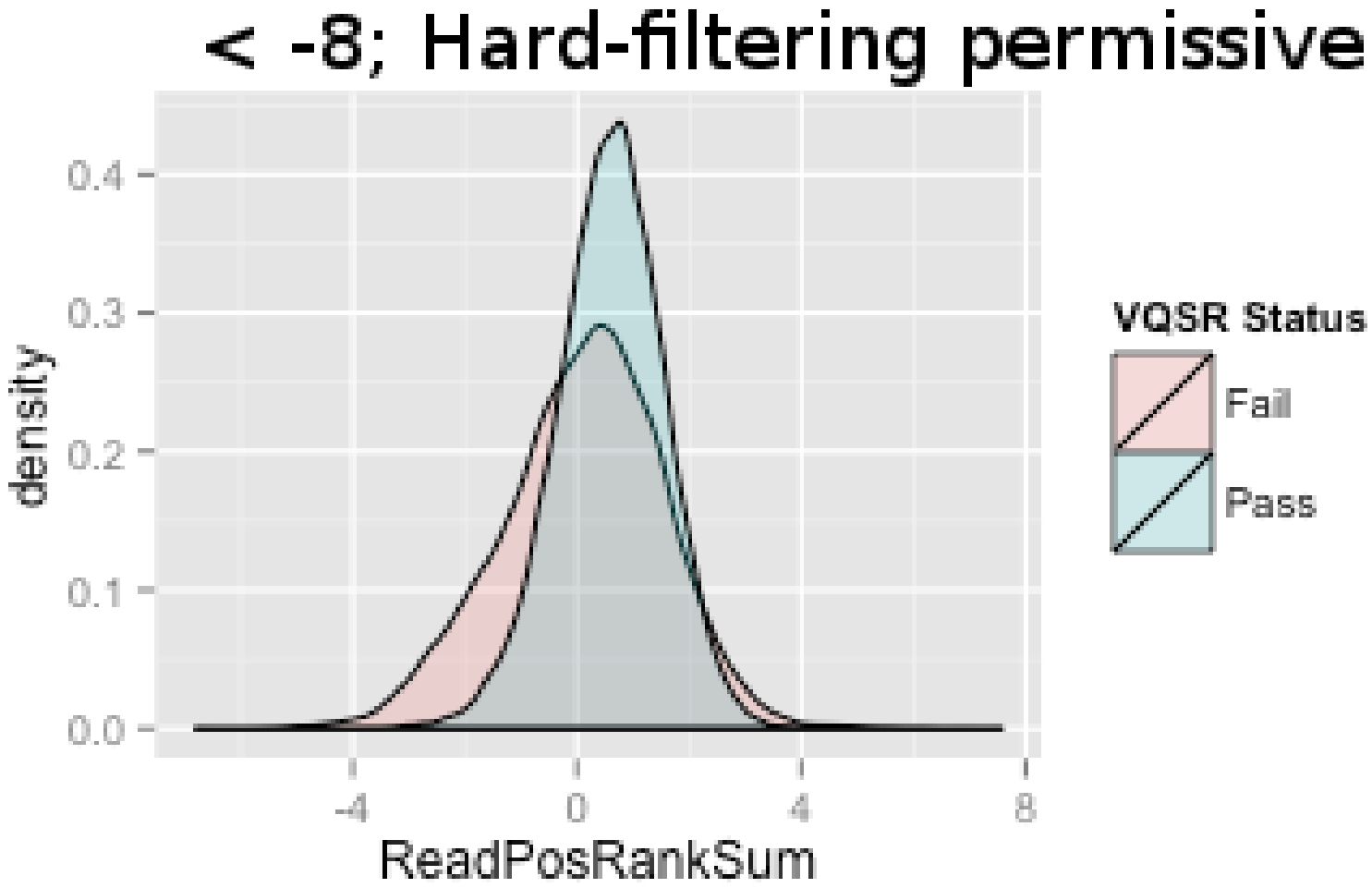

Hard-filtering Summary (SNPs and INDELS)

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	MQ	< 40
	FS	> 60
	SOR	> 3.0
	MQRankSum	< -12.5
	ReadPosRankSum	< -8.0
INDELs	QD	< 2.0
	ReadPosRankSum	< -20.0
	InbreedingCoeff (> 10 samples)	< -8.0
	FS	< 200.0
	SOR	> 10.0

Topic

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4. Custom filters

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   - VariantAnnotation
     - general parsing and filtering
3. Consistent interface
   - Learn 1 piece of software and reuse
4. Custom filters
   - flexible annotations (e.g., RGQ)

Variant Annotation

1. Could write a script in favorite language.
   - Know exactly what you did (+)
   - Time spent engineering software (-)
2. Hard Work already done
   - Bioconductor
   - VariantAnnotation
     - general parsing and filtering
3. Consistent interface
   - Learn 1 piece of software and reuse
4. Custom filters
   - flexible annotations (e.g., RGQ)
   - New annotations just "show up"

Topic

Extract, Filter, and Plot

1. Exercise folder on asc
   - Scripts: 1_initial_annotation_plot.sh; 2_filter_and_plot.sh
   - Data: D_PseudoFS14_16
   - UsefulBioinformaticScripts
2. Edit "Variables" in 1_initial_annotation_plot.sh

```
script_dir=${HOME}/Exercise/UsefulBioinformaticScripts
data_dir=${HOME}/Exercise/D_PseudoFS14_16
out_dir=${HOME}/Exercise
```

3. save script and run
4. Inspect graphs and decide upon filtering thresholds
5. add variable definitions in 2 to 2_filter_and_plot.sh
6. Edit

```
#################################################################################
##Filtering Parameters
##this is
${script_dir}/filter_SNPs_GATK_hard_filter.CHUNKS.R -I ${out_dir}/${vcf1}.gz -T
${out_dir}/${vcf1}.gz.tbi -O ${out_dir}/${vcf1}.filtered.vcf -C 10000 --QD=2
--FS=60 --SOR=3 --MQRankSum=-8 --min_Depth=4 --max_Depth=32 --Genotype_Quality=20

${script_dir}/filter_SNPs_GATK_hard_filter.CHUNKS.R -I ${out_dir}/${vcf2}.gz -T
${out_dir}/${vcf2}.gz.tbi -O ${out_dir}/${vcf2}.filtered.vcf -C 10000 --QD=2
--FS=60 --SOR=3 --MQRankSum=-8 --min_Depth=4 --max_Depth=32 --Genotype_Quality=20
```

7. save script and run
8. inspect graphs and write up.