

# Exploring the Complete Genomics Diversity panel

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# 1 Introduction

Complete Genomics Inc. distributes a collection of data on deeply sequenced genomes (from Coriell cell lines) from 11 different human populations.

```
> library(cgdv17)
> data(popvec)
> popvec[1:5]

NA19700 NA19020 NA19701 NA19025 NA19703
  "ASW"   "LWK"   "ASW"   "LWK"   "ASW"

> table(popvec)
```

```
popvec
ASW CEU CHB GIH JPT LWK MKK MXL TSI YRI
  5   5   4   4   4   4   4   5   4   7
```

The data are distributed with many details; VJC obtained the masterVar TSV files from the Complete Genomics ftp2 site, converted these to VCF 4.0 in Oct. 2011, using a tool noted at

<http://community.completegenomics.com/tools/m/cgtools/219.aspx>

The conversion tool used was released with various caveats. Perhaps the whole conversion should be redone with official tools.

The purpose of this note is to explore some basic structural features of the data, so that relevant genetic structures can be identified for analytic programming and interpretation. We focus on variant calls on chromosome 17.

Formal restrictions on publications related to these data are as follows.

1. The Coriell and ATCC Repository number(s) of the cell line(s) or the DNA sample(s) must be cited in publication or presentations that are based on the use of these materials.
2. You must reference our Science paper (R. Drmanac, et. al. Science 327(5961), 78. [DOI: 10.1126/science.1181498])
3. You must provide the version number of the Complete Genomics assembly software with which the data was generated. This can be found in the header of the summary.tsv file (\# Software\_Version).

## 2 Contents of a VCF header

There is a lot of redundancy among the headers for the 46 files, so one was isolated for distribution.

```
> data(h1)
```

```
> h1
```

```
$NA21767_17.vcf.gz
```

```
SimpleList of length 3
```

```
names(3): Reference Sample Header
```

```
> h1[[1]]$Sample
```

```
[1] "GS21767-1100-37-ASM"
```

```
> h1[[1]]$Header$META
```

```
DataFrame with 5 rows and 1 column
```

	Value
	<character>
fileformat	VCFv4.1
fileDate	20111102
source	masterVar2VCFv40
reference	build37.fa.bz2
phasing	partial

```
> h1[[1]]$Header$INFO
```

```
DataFrame with 3 rows and 3 columns
```

	Number	Type	Description
	<character>	<character>	<character>
NS	1	Integer	Number of Samples With Data
DP	1	Integer	Total Depth
DB	0	Flag	dbSNP membership, build 131

```
> h1[[1]]$Header$FORMAT
```

```
DataFrame with 12 rows and 3 columns
```

	Number	Type	Description
	<character>	<character>	<character>
GT	1	String	Genotype
GQ	1	Integer	Genotype Quality
DP	1	Integer	Read Depth

HDP	2	Integer	Haplotype Read Depth
HQ	2	Integer	Haplotype Quality
PS	2	Integer	Phase Set
GENE	.	String	Overlapping Genes
mRNA	.	String	Overlapping mRNA
rmsk	.	String	Overlapping Repeats
segDup	.	String	Overlapping segmentation duplication
rCov	1	Float	relative Coverage
cPd	1	String	called Ploidy(level)

## 3 Variant calls for chromosome 17

### 3.1 Recording structural variation for an individual

We created a provisional container for the call data on chromosome 17. Tabix facilities were used to filter and index the data from the full VCF to all of chromosome 17.

At present it is not clear how to model a collection of deeply sequenced chromosomes. I have used `VariantAnnotation::readVcf`, which must be applied separately for each individual, given the Complete Genomics distribution. The focus is on structural information in the `rowData` component of the VCF object returned by `readVcf`, which is a `GRanges` instance. From the `elementMetadata` I removed `FILTER` and added `geno()``$GT` information. This gives us information to specific variants and phase for some variants, depending on the string content of the GT information.

The `getRVS` function will collect file references for the serialized `GRanges`.

```
> rv = getRVS("cgdv17")
> rv
```

```
raggedVariantSet instance with 46 elements.
some sampleNames: NA06985 NA06994 ... NA21737 NA21767
```

Data on one individual can be extracted using `getrd()`. We will confine attention to variants with quality score in the top quartile of its distribution for this individual.

```
> R85 = getrd(rv, "NA06985")
> length(R85)
```

```
[1] 174744
```

```
> summary(elementMetadata(R85)$QUAL)
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
0	0	98	117	166	1714

```
> kp = which(elementMetadata(R85)$QUAL >= 166)
> R85hiq = R85[kp]
```

A small excerpt gives us a sense of the sorts of variation to be encountered:

```
> elementMetadata(R85hiq)[11:20,]
```

DataFrame with 10 rows and 5 columns

	REF	ALT	QUAL
	<DNAStringSet>	<CompressedCharacterList>	<numeric>
rs7209783	T	C	309

rs7209943	T	C	417
rs7220384	T	C	187
rs7210283	A	G	187
rs7220537,rs35411518	CTG	CCA,CCG	203
rs8075072	A	G	380
chr17:20080	C	T	272
rs2294074	G	A	324
chr17:23182	TAGT	TGGG	237
chr17:23722	T	G	396

	geno	depth
	<character>	<integer>
rs7209783	1 0	66
rs7209943	1/0	63
rs7220384	1/1	61
rs7210283	1/0	48
rs7220537,rs35411518	1/2	46
rs8075072	1/1	26
chr17:20080	1/0	45
rs2294074	1/0	45
chr17:23182	1/0	62
chr17:23722	1 0	50

```
> refs = elementMetadata(R85hiq)$REF
> alts = elementMetadata(R85hiq)$ALT
> genos = elementMetadata(R85hiq)$geno
> table(nchar(refs))
```

1	2	3	4	5	6	7	8	9	10	11	12	13
41848	646	645	347	193	63	33	21	25	17	16	18	15
14	15	16	17	18	19	20	21	22	23	24	25	26
9	10	7	5	3	1	2	2	1	2	2	2	1
27	29	30	31	32	34	135						
2	1	2	1	2	1	1						

```
> alts[grep(",",unlist(alts))]
```

CompressedCharacterList of length 120

```
[[1]] CCA,CCG
[[2]] CCC,CCG
[[3]] A,C
[[4]] ACA,ATG
[[5]] CTCG,CNCN
[[6]] GCA,GTG
```

```
[[7]] CGCA,CGCG
[[8]] C,G
[[9]] TGG,TCA
[[10]] CAAG,CGAG
...
<110 more elements>
```

Summary: references are recorded as DNAStrings, alternatives are compressed character strings with commas, and the phasing of the individual-level calls can be derived by parsing the `geno` component.

### 3.2 Isolating variants in the vicinity of a gene, for an individual

We are interested in gene `ORMDL3`. We will use the `hg19` transcriptDb to obtain the locations and tabulate higher quality variants observed 100kb up and downstream of the transcript.

```
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)
> tx19 = TxDb.Hsapiens.UCSC.hg19.knownGene
> library(org.Hs.eg.db)
> get("ORMDL3", revmap(org.Hs.egSYMBOL))
```

```
[1] "94103"
```

```
> ortx = transcriptsBy(tx19, "gene")$"94103"
> seqlevels(R85hiq) = "chr17"
> aro = subsetByOverlaps(R85hiq, ortx+100000)
> table(elementMetadata(aro)$geno)
```

```
0/. 0|. 1/0 1/1 1|0 1|2
  2   1 121  10  65   1
```

```
> alts = unlist(elementMetadata(aro)$ALT)
> alts[nchar(alts)>1]
```

```
[1] "AGTG"      "CC"        "GG"        "CTGC"      "AT"        "AGG"       "AA"
[8] "ACAC"      "GG"        "ACA,ACG"   "GAA"
```

There are deletion and insertion events, but I don't see any simple way of isolating and counting them at the moment. Some code will be added to address this.

We can use `VariantAnnotation` to obtain structural contexts. It takes over a minute to use `locateVariants`, so I just show the code and results here for now.

```
mycache = new.env(hash=TRUE)
lvaro = locateVariants(aero, tx19, cache=mycache)
lvaro[1:4,]
table(lvaro$loc, sapply((lvaro$geneID), function(x) strsplit(x, ",")[[1]][1])))
DataFrame with 4 rows and 5 columns
```

	queryID	location	txID	geneID	cdsID
	<integer>	<factor>	<integer>	<CompressedCharacterList>	<integer>
1	1	intron	60959	22806	189661
2	1	intron	60960	22806	189661
3	1	intron	60961	22806	189661
4	1	intron	60962	22806	189661

	124626	1440	22806	284110	2886	55876	5709	94103	9862
3'UTR	0	0	0	0	0	0	0	0	20
5'UTR	0	0	0	1	0	8	0	0	10
coding	0	0	0	6	0	0	0	0	10
intergenic	34	1	10	4	1	2	25	10	0
intron	28	0	105	42	0	138	99	6	0

We see that this search for variants near ORMDL3 identifies variants affecting other nearby genes.

## 4 Filtering and analyzing variants on multiple individuals

The analysis of a ragged variant set requires infrastructure. We will illustrate with a focused analysis of variants in the vicinity of ORMDL3. We have used the GGdata and hmyriB36 packages to collect expression data on 12 individuals in the diversity cohort, in the CY17 smlSet instance. This includes expression on all genes on chr17, and the HapMap phase 2 genotypes as well.

```
> suppressPackageStartupMessages(library(GGtools))
> data(CY17)
> CY17
```

```
SnpMatrix-based genotype set:
number of samples: 12
number of chromosomes present: 1
annotation: illuminaHumanv1.db
Expression data dims: 1291 x 12
Total number of SNP: 83889
Phenodata: An object of class 'AnnotatedDataFrame'
```



```

rowNames: NA06985 NA06994 ... NA19129 (12 total)
varLabels: mothid fathid isFounder male
varMetadata: labelDescription

> sn = sampleNames(CY17)

```

## 4.1 Sample filtering

The ragged variant set can be filtered to these individuals.

```

> rv17 = rv[, sn]
> rv17

```

```

raggedVariantSet instance with 12 elements.
some sampleNames: NA06985 NA06994 ... NA18517 NA19129

```

## 4.2 Counting variants in a specified region, with quality filtering

The variant counting function takes two key parameters in addition to the variant set: a region within which to count, and a lower bound on call quality for retained variants. A third additional parameter tells how to iterate over samples with an lapply-like function.

Since ORMDL3 is on the minus strand, the upstream region is to the right. We will create a region from start site to 50k upstream.

```

> if (length(ortx)>1) ortx = ortx[2]
> ortss = end(ortx)
> ortup50 = GRanges("chr17", IRanges(ortss, width=50000))
> cv50k = countVariants(rv17, ortup50, 160, lapply )

> cv50k

```

NA06985	NA06994	NA07357	NA10851	NA12004	NA18501	NA18502	NA18504	NA18505	NA18508
65	0	51	10	9	26	72	68	56	56
NA18517	NA19129								
81	58								

We see that the second sample seems to have a quality problem. We will now drop it from both the expression and variant structures.

```

> if (length(sampleNames(rv17))==12) rv17 = rv17[,-2]
> if (length(sampleNames(CY17))==12) CY17 = CY17[,-2]
> #redo

> cv50k = countVariants(rv17, ortup50, 160, lapply )

```

We can acquire the full data on variants in the region under the quality constraint using variantGRanges.

```
> vv50k = variantGRanges( rv17, ortup50, 160, lapply )
```

```
> vv50k[[1]][1:5]
```

GRanges with 5 ranges and 5 metadata columns:

	seqnames	ranges	strand	REF
	<Rle>	<IRanges>	<Rle>	<DNAStringSet>
rs12946393	17	[38087429, 38087429]	*	T
rs35557848	17	[38087439, 38087439]	*	C
rs56199421	17	[38090808, 38090808]	*	C
rs7207600	17	[38091660, 38091660]	*	G
rs6503525	17	[38095174, 38095174]	*	G

	ALT	QUAL	geno	depth
	<CompressedCharacterList>	<numeric>	<character>	<integer>
rs12946393	G	339	1/1	70
rs35557848	T	339	1/0	56
rs56199421	T	270	1/0	48
rs7207600	A	470	1/0	66
rs6503525	C	440	1/0	69

---

seqlengths:

17

NA

```
> sapply(vv50k,length)
```

```
NA06985 NA07357 NA10851 NA12004 NA18501 NA18502 NA18504 NA18505 NA18508 NA18517
      65      51       10        9       26       72       68       56       56      81
NA19129
      58
```

As a naive hint of a connection of “variant burden” with ORMDL3 expression, consider the following display.

```
> ORMDL3ex = as.numeric(exprs(CY17[genesym("ORMDL3"),]))
> ygr = ifelse((1:11)<=4, "red", "green")
> plot(ORMDL3ex~cv50k, col=ygr, pch=19,
+       ylab="variant count from 50kb upstream to TSS")
> legend(10, 8.5, pch=19, col=c("red", "green"), legend=c("CEU", "YRI"))
> summary(lm(ORMDL3ex~cv50k*factor(ygr)))
```

Call:

```
lm(formula = ORMDL3ex ~ cv50k * factor(ygr))
```

Residuals:

Min	1Q	Median	3Q	Max
-0.29969	-0.13281	-0.02538	0.12717	0.32077

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	8.912418	0.355770	25.051	4.12e-08 ***
cv50k	-0.010792	0.005763	-1.873	0.103
factor(ygr)red	-0.520704	0.412601	-1.262	0.247
cv50k:factor(ygr)red	0.008317	0.007625	1.091	0.311

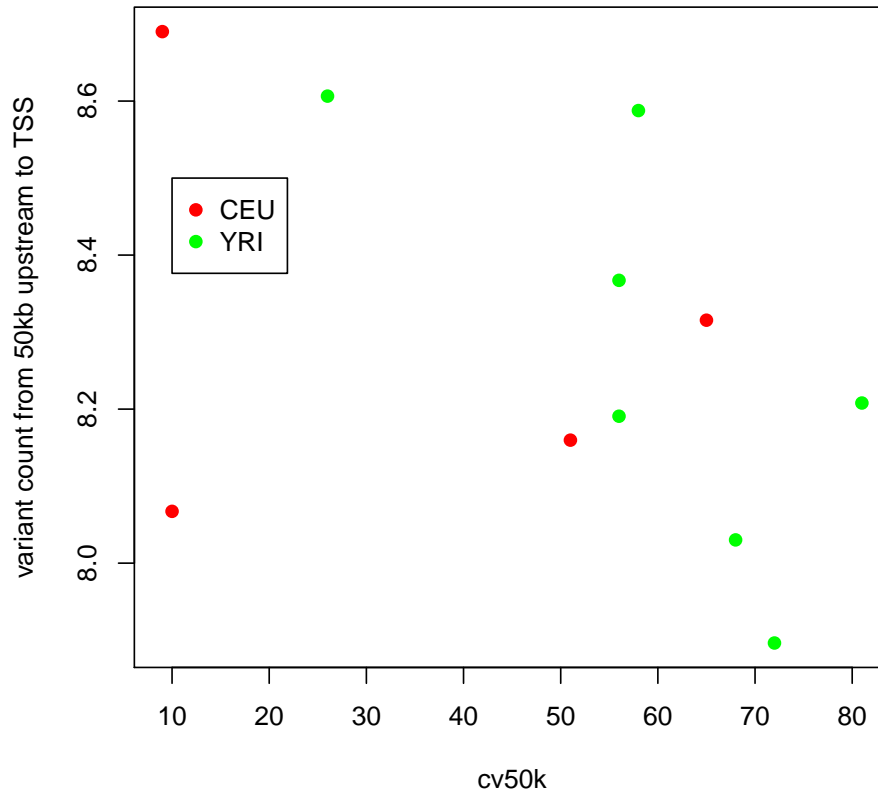
---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.2472 on 7 degrees of freedom

Multiple R-squared: 0.3528, Adjusted R-squared: 0.07538

F-statistic: 1.272 on 3 and 7 DF, p-value: 0.3557



### 4.3 Enumerating variants by structural context

Now we focus on variants in the ORMDL3 coding region.

```
> library(parallel)
> options(mc.cores=max(c(2, parallel::detectCores()-2)))
> vv = variantGRanges( rv17, ortx, 160, mclapply )
> vvv = lapply(vv, function(x) renameSeqlevels(x, c("17"="chr17")))
> mycache = new.env(hash=TRUE)
> locs = lapply(vvv, function(x) {
+   locateVariants(x, tx19, CodingVariants(), cache=mycache)
+ })
```

Further work: illustrate the predictCoding behavior, streamline the catalog of variants relevant to a given gene over the 46 individuals. Relate to population membership, and, where available, to expression variation.