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# © Evaluating large spontaneous deletions in a bovine cell line selected for bovine viral diarrhea virus resistance

## - MDBK reads mapping to regions in genome not shared with CRIB cell line

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**USMARC Bacterial Systems Biology Working Group** 



Bovine viral diarrhea virus (BVDV) entry into bovine cells involves attachment of virions to cellular receptors, internalization, and pH-dependent fusion with endosomal membranes. The primary host receptor for BVDV is CD46; however, the complete set of host factors required for virus entry is unknown. The Madin-Darby bovine kidney (MDBK) cell line is susceptible to BVDV infection, while a derivative cell line (CRIB) is resistant at the level of virus entry. We performed complete genome sequencing of each to identify genomic variation underlying the resistant phenotype with the aim of identifying host factors essential for BVDV entry. Three large compound deletions in the BVDV-resistant CRIB cell line were identified and predicted to disrupt the function or expression of the genes PTPN12, GRID2, and RABGAP1L. However, CRISPR/Cas9 mediated knockout of these genes, individually or in combination, in the parental MDBK cell line did not impact virus entry or replication. Therefore, resistance to BVDV in the CRIB cell line is not due to the apparent spontaneous loss of PTPN12, GRID2, or RABGAP1L gene function. Identifying the functional cause of BVDV resistance in the CRIB cell line may require more detailed comparisons of the genomes and epigenomes.

DOI

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protocol

<u>Evaluating Large Spontaneous Deletions in a Bovine Cell Line Selected for Bovine Viral Diarrhea Virus Resistance</u>

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#### Inputs

1 Required software

samtools 1.9 👄

source by http://htslib.org/

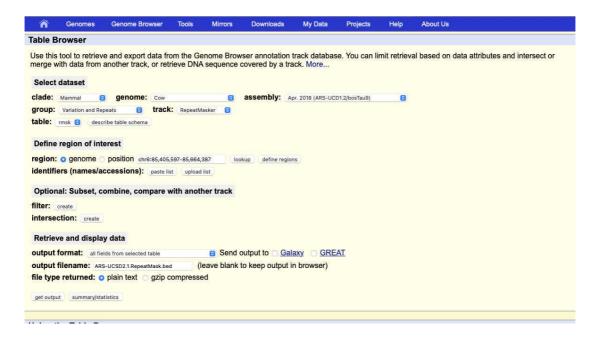
**Bedtools 2.27.1 ☞** 

source by http://quinlanlab.org

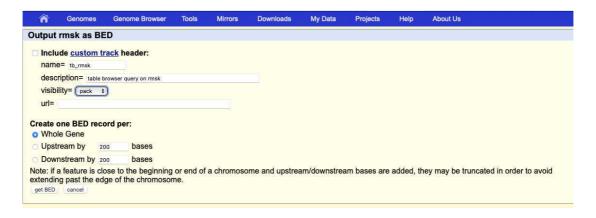
2 Get RepeatMasked ARS-UCSC2.1 RepeatMasked Bovine Genome features

Goto <a href="http://genome.ucsc.edu/cgi-bin/hgTables">http://genome.ucsc.edu/cgi-bin/hgTables</a> to create RepeatMasked ARS-UCSD2.1 bovine genome





Select "Cow" genome and ARS-UCSD 2.1 bovine genome, select "Variation and Repeats" from group and RepeatMasker Track and set other parameters as defined in screenshot above, including outputting to file named ARS-UCD1.2.RepeatMask.bed. Click "get output"



Create BED record "Whole Gene", the click "get BED"

BED file of RepeatMasked Regions of ARS-UCSD bovine genome is

ARS-UCD1.2.RepeatMask.bed

#### **Check BAM Files**

- 3 Cell line library BAM files Illumina libraries of CRIB and MDBK cell lines The library names are
  - LIB14393\_Bovine\_CRIBcells.Bt\_ARS-UCD1.2.realigned.bam
  - LIB14394\_Bovine\_MDBKcells.Bt\_ARS-UCD1.2.realigned.bam



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#### LIB14393\_Bovine\_CRIBcells.Bt\_ARS-UCD1.2.realigned.bam

Check MDBK BAM files

samtools flagstat LIB14394\_Bovine\_MDBKcells.Bt\_ARS-UCD1.2.realigned.bam

OS X



367878117 + 0 in total (QC-passed reads + QC-failed reads)

0 + 0 secondary

1572651 + 0 supplementary

14182468 + 0 duplicates

363791869 + 0 mapped (98.89%: N/A)

366305466 + 0 paired in sequencing

183152733 + 0 read1

183152733 + 0 read2

348244352 + 0 properly paired (95.07%: N/A)

359573946 + 0 with itself and mate mapped

2645272 + 0 singletons (0.72%: N/A)

10766072 + 0 with mate mapped to a different chr

5380851 + 0 with mate mapped to a different chr (mapQ>=5)

BAM file is acceptable

LIB14394\_Bovine\_MDBKcells.Bt\_ARS-UCD1.2.realigned.bam

Check CRIB

samtools flagstat LIB14393\_Bovine\_CRIBcells.Bt\_ARS-UCD1.2.realigned.bam



478473382 + 0 in total (QC-passed reads + QC-failed reads)

0 + 0 secondary

1983826 + 0 supplementary

17840954 + 0 duplicates

469694793 + 0 mapped (98.17%: N/A)

476489556 + 0 paired in sequencing

238244778 + 0 read1

238244778 + 0 read2

451693766 + 0 properly paired (94.80%: N/A)

464266014 + 0 with itself and mate mapped

3444953 + 0 singletons (0.72%: N/A)

11903898 + 0 with mate mapped to a different chr

5382690 + 0 with mate mapped to a different chr (mapQ>=5)

BAM file is acceptable

### Data Conversion

4 Convert BAM to BED

Convert BAM to BED

bamToBed -i LIB14394\_Bovine\_MDBKcells.Bt\_ARS-UCD1.2.realigned.bam > LIB14394\_Bovine\_MDBKcells.Bt\_ARS-UCD1.2.realigned.bed



Convert BAM to BED

bamToBed -i LIB14393\_Bovine\_CRIBcells.Bt\_ARS-UCD1.2.realigned.bam > LIB14393\_Bovine\_CRIBcells.Bt\_ARS-UCD1.2.realigned.bed

#### Groom Data

5 Remove features from the MDBK file that intersect with repeats in ARS-UCD1.2.RepeatMask.bed

bedtools intersect -a LIB14394\_Bovine\_MDBKcells.Bt\_ARS-UCD1.2.realigned.bed -b ARS-UCD1.2.RepeatMask.bed -v > LIB14394\_Bovine\_MDBKcells.Bt\_ARS-UCD1.2.realigned.RepMask.bed

6 Find those features (reads) present in MDBK that do no overlap features (reads) in CRIB

Find reads in MDBK mapping to genome absent in CRIB

nohup bedtools intersect -a LIB14394\_Bovine\_MDBKcells.Bt\_ARS-UCD1.2.realigned.RepMask.bed -b LIB14393\_Bovine\_CRIBcells.Bt\_ARS-UCD1.2.realigned.bed -v > MDBK+\_CRIB-.bed &

Output: MDBK+\_CRIB-.bed

