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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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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.

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<https://dx.doi.org/10.17504/protocols.io.bvv3n68n>



protocol

[Evaluating Large Spontaneous Deletions in a Bovine Cell Line Selected for Bovine Viral Diarrhea Virus Resistance](#)

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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 <http://genome.ucsc.edu/cgi-bin/hgTables> 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

- 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

check files

[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: