

```

SetDirectory[NotebookDirectory[]];

Study number of junctions supported by  $\geq K$  samples.

aggregatedJunctionCounts = Drop[Import["hg19.sample.stats.tsv", "TSV"], 1];

totalJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 2}]]];
annotatedJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 3}]]];
exonSkipJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 4}]]];
altStartEndJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 5}]]];
novelJunctions =
  exonSkips = Transpose[Transpose[aggregatedJunctionCounts][[{1, 6}]]];

Proportion of junctions in  $\geq 8k$  samples that are annotated

annotatedJunctions[[16 446 - 7999]]
{8000, 105 624}

annotatedJunctions[[16 446 - 7999]][[2]] / totalJunctions[[16 446 - 7999]][[2]] // N
0.997705

exonSkipAnnotatedJunctions = Transpose[{annotatedJunctions[[All, 1]],
  annotatedJunctions[[All, 2]] + exonSkipJunctions[[All, 2]]}];
someEvidenceJunctions = Transpose[{annotatedJunctions[[All, 1]],
  annotatedJunctions[[All, 2]] + exonSkipJunctions[[All, 2]] +
  altStartEndJunctions[[All, 2]]}];

mathematicaColors = ColorData[97, "ColorList"]
{█, █, █, █, █, █, █, █, █, █, █, █, █, █, █, █}

```

Introduce levels of evidence, and annotated with junction counts for  $\geq 1000$  samples

```

labelForm[x_, y___] := Text[Style[x, FontFamily → "Arial",
  FontSize → Scaled[.033], Bold, TextAlignment → Left], y]

idx = 16 446 - 999
15 447

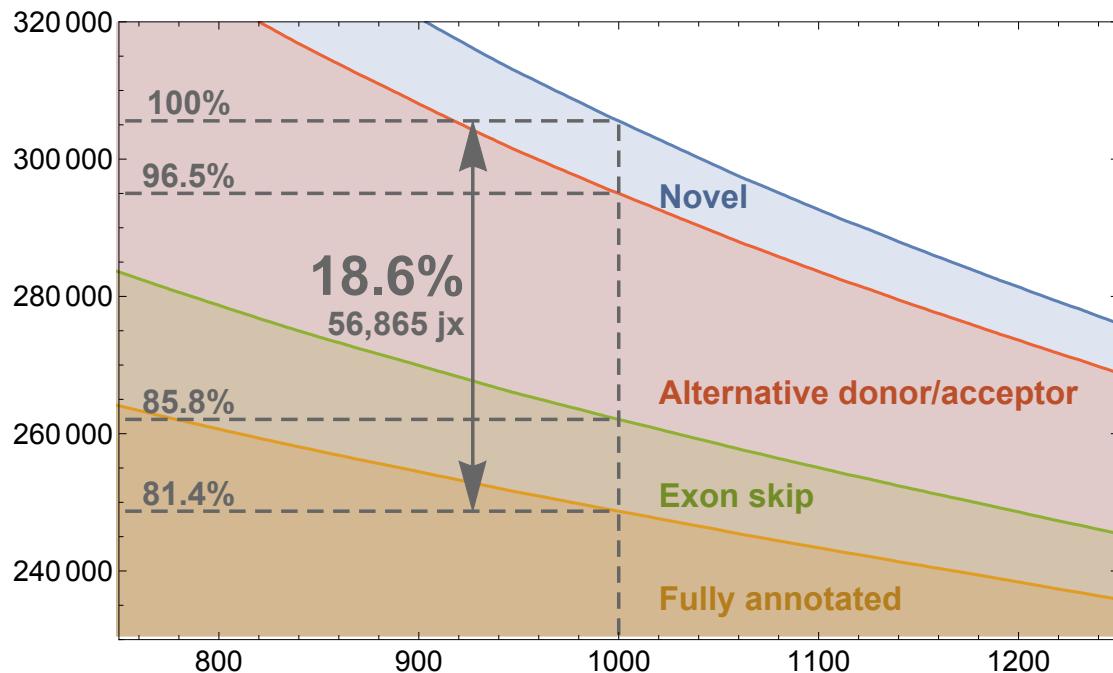
bigLabelForm[x_, y___] := Text[Style[x, FontFamily → "Arial",
  FontSize → Scaled[.055], Bold, TextAlignment → Right], y]

```

```

dashedColor = Darker[Gray, 0.2]; numberStartPos = 785; adjust = 2600;
insetAnnotationPlot = Show[ListPlot[{totalJunctions, annotatedJunctions,
    exonSkipAnnotatedJunctions, someEvidenceJunctions}, Joined -> True,
    PlotRange -> {{750, 1250}, {230 000, 320 000}}, Filling -> Axis, Frame -> True,
    ImageSize -> Large, BaseStyle -> {FontFamily -> "Arial", FontSize -> 15}],
Graphics[{Directive[Thickness[0.0035], Dashing[0.013], dashedColor],
    Line[{{1000, 0}, totalJunctions[[idx]]}],
    Line[{{0, totalJunctions[[idx]][[2]]}, totalJunctions[[idx]]}],
    Line[{{0, annotatedJunctions[[idx]][[2]]}, annotatedJunctions[[idx]]}],
    Line[{{0, exonSkipAnnotatedJunctions[[idx]][[2]]}, exonSkipAnnotatedJunctions[[idx]]}],
    Line[{{0, someEvidenceJunctions[[idx]][[2]]}, someEvidenceJunctions[[idx]]}],
    Directive[{Dashing[None], Arrowheads[{-0.05, 0.05}]}],
    Arrow[{{927, annotatedJunctions[[idx]][[2]]},
        {927, totalJunctions[[idx]][[2]]}}], bigLabelForm[ToString[NumberForm[
            N[100 - annotatedJunctions[[idx, 2]]/totalJunctions[[idx, 2]]*100, 3],
            DigitBlock -> 3]] <> "%", {923, 283 000}, {1, 0}], labelForm[
        ToString[NumberForm[totalJunctions[[idx, 2]] - annotatedJunctions[[idx, 2]],
            DigitBlock -> 3]] <> " jx", {923, 276 000}, {1, 0}],
    labelForm["100%", {numberStartPos, totalJunctions[[idx]][[2]] + adjust}],
    labelForm[ToString[NumberForm[N[someEvidenceJunctions[[idx, 2]]]/
        totalJunctions[[idx, 2]]*100, 3], DigitBlock -> 3]] <> "%",
    {numberStartPos, someEvidenceJunctions[[idx]][[2]] + adjust}],
    labelForm[ToString[NumberForm[N[annotatedJunctions[[idx, 2]]]/
        totalJunctions[[idx, 2]]*100, 3], DigitBlock -> 3]] <> "%",
    {numberStartPos, annotatedJunctions[[idx]][[2]] + adjust}],
    labelForm[ToString[NumberForm[N[exonSkipAnnotatedJunctions[[idx, 2]]]/
        totalJunctions[[idx, 2]]*100, 3], DigitBlock -> 3]] <> "%",
    {numberStartPos, exonSkipAnnotatedJunctions[[idx]][[2]] + adjust}],
    (*Directive[Thickness[0.0035], Arrowheads[.035], Dashing[None], dashedColor],
    Arrow[{{1000, totalJunctions[[idx]][[2]] + 41000},
        {1000, totalJunctions[[idx]][[2]] + 100}}], labelForm[ToString[NumberForm[
            N[100 - annotatedJunctions[[idx, 2]]/totalJunctions[[idx, 2]]*100, 3]] <>
        "% of jx unannotated, but\n" <> ToString[NumberForm[
            someEvidenceJunctions[[idx, 2]]/totalJunctions[[idx, 2]]*100/N, 3]] <>
        "% of jx have donor and/or\nacceptor site in annotation",
            {1005, 330000}, {-1, 0}], *)Darker[mathematicaColors[[1]], 0.2],
    labelForm["Novel", {1020, 294 500}, {-1, 0}],
    Darker[mathematicaColors[[4]], 0.2],
    labelForm["Alternative donor/acceptor", {1020, 266 000}, {-1, 0}],
    Darker[mathematicaColors[[3]], 0.2],
    labelForm["Exon skip", {1020, 251 000}, {-1, 0}],
    Darker[mathematicaColors[[2]], 0.2],
    labelForm["Fully annotated", {1020, 236 000}, {-1, 0}]})]

```

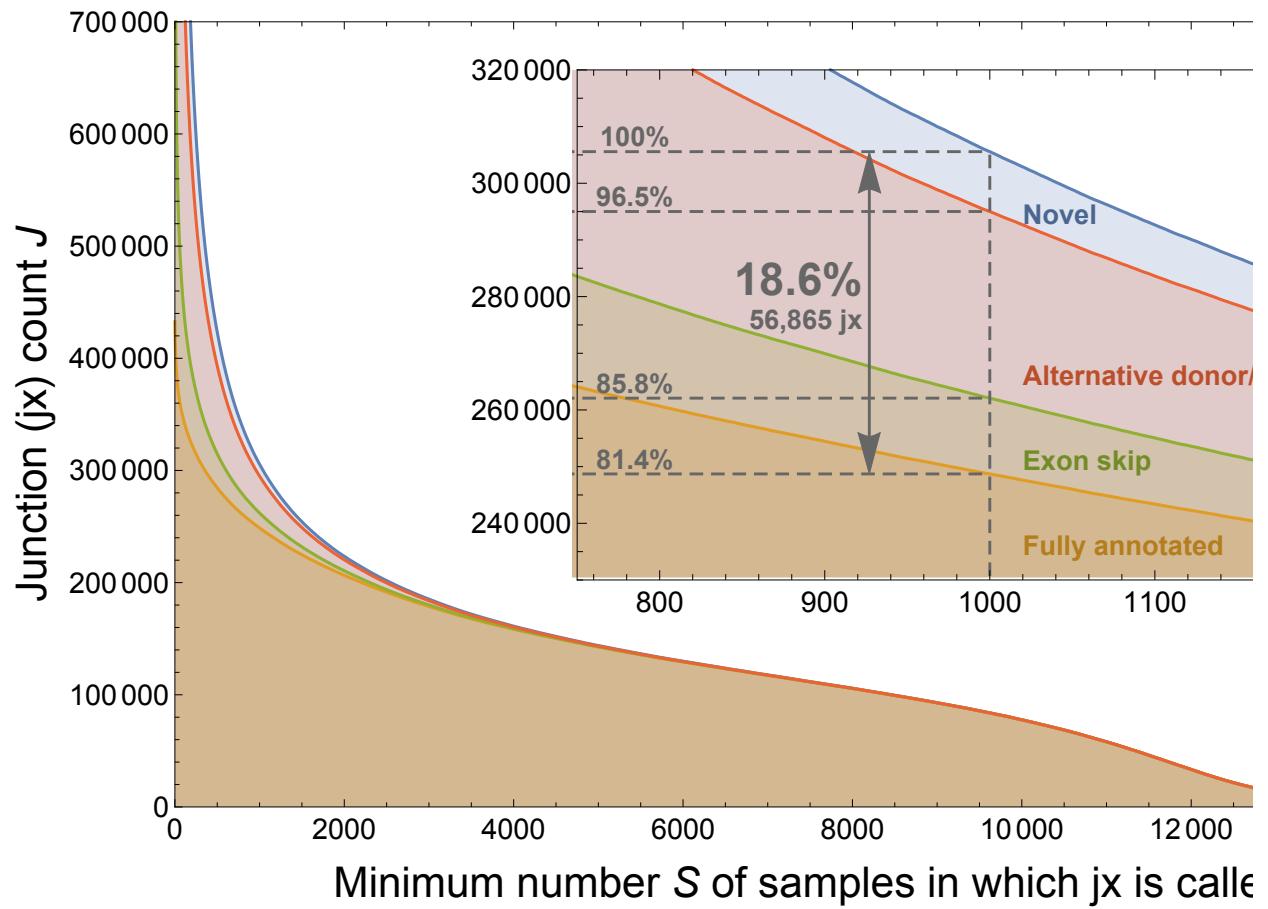


```
baseImageSize = {576, 408} * 1.3  
{748.8, 530.4}
```

```

bigAnnotationPlot = ListPlot[{totalJunctions, annotatedJunctions,
  exonSkipAnnotatedJunctions, someEvidenceJunctions}, Joined → True,
  PlotRange → {{0, 15 000}, {0, 700 000}}, Filling → Axis, Frame → True,
  ImageSize → baseImageSize, BaseStyle → {FontFamily → "Arial", FontSize → 15},
  Epilog → Inset[insetAnnotationPlot, {9000, 420 000}, Automatic, 11000],
  FrameLabel → {Style["Minimum number  $S$  of samples in which jx is called", 22],
  Style["Junction (jx) count  $J$ ", 22]}]

```



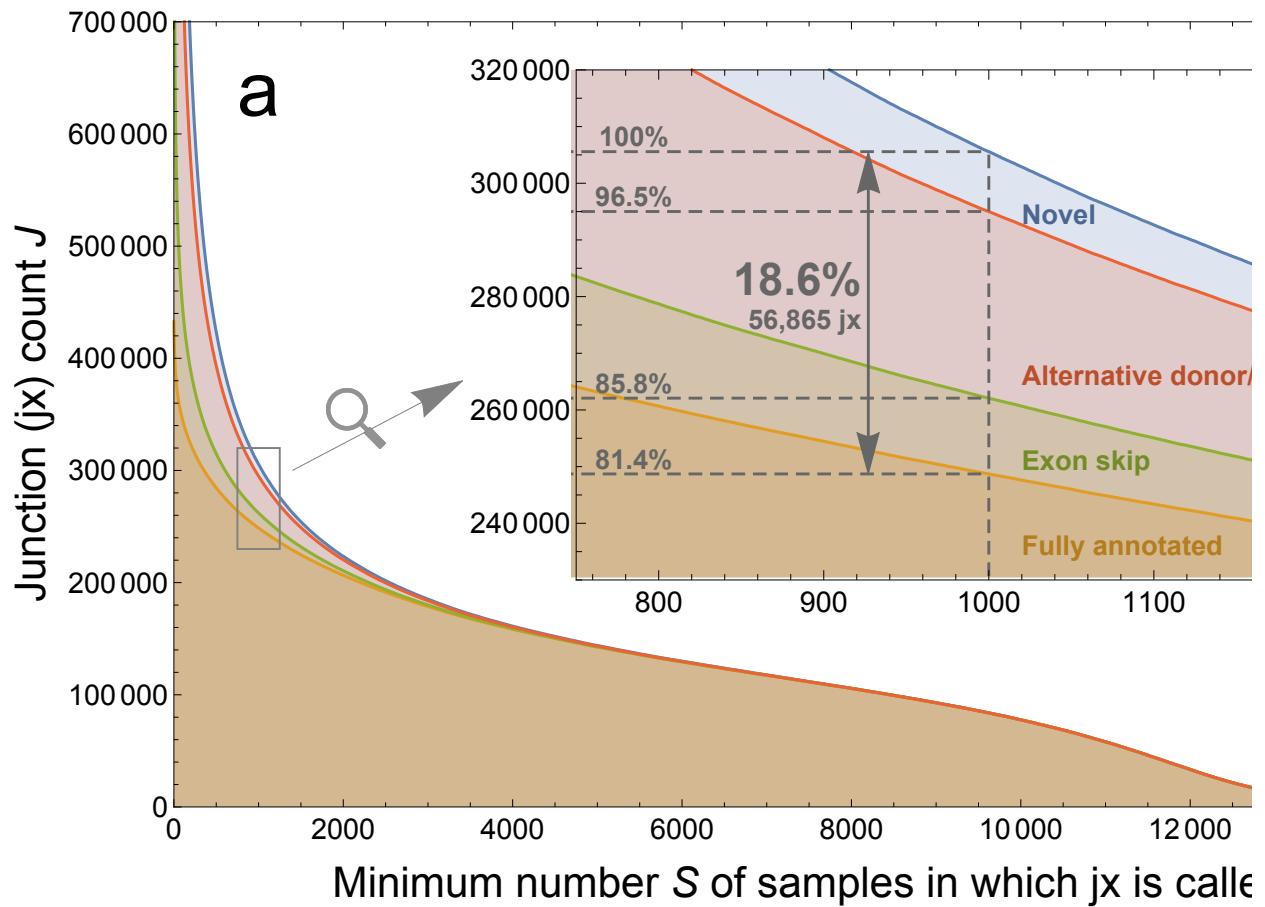
```
magnifyingGlass = Import["mag.png"]
```



```

fig1a = Show[bigAnnotationPlot,
  Graphics[{EdgeForm[Directive[Gray, Thickness[.0015]]],
    Transparent, Rectangle[{750, 230000}, {1250, 320000}]}],
  Graphics[{Gray, Arrow[{{1400, 300000}, {3410, 380000}}]}],
  Graphics[{Opacity[0.5], Inset[magnifyingGlass, {1800, 320000}, {0, 0}, 700]}],
  Graphics[{Black, Text[Style["a", FontFamily -> "Arial", FontSize -> 40],
    {1000, 640000}]}], ImageSize -> baseImageSize]

```



```

statsBySample = Drop[Import["!awk '$6>=100000' " <>
  NotebookDirectory[] <> "hg19.stats_by_sample.tsv", "TSV"], 1];

```

```
jxConsidered = Length[statsBySample]
```

```
10311
```

Overlaps by sample-->

```

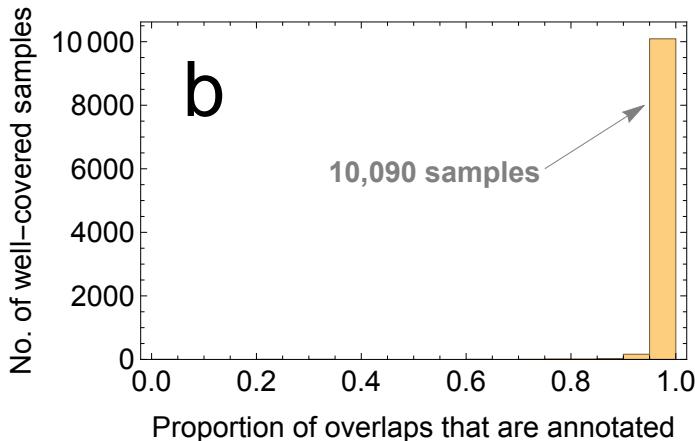
largerLabelForm[x_, y___] := Text[Style[x, FontFamily -> "Arial",
  FontSize -> Scaled[.053], Bold, TextAlignment -> Left], y]

```

```
jxHistList = HistogramList[statsBySample[[All, 11]] / statsBySample[[All, 10]], 15]
{{0, 1/20, 1/10, 3/20, 1/5, 1/4, 3/10, 7/20, 2/5, 9/20, 1/2, 11/20, 3/5, 13/20, 7/10, 3/4, 4/5, 17/20, 9/10, 19/20, 1}, {1, 0, 0, 0, 1, 0, 2, 0, 0, 0, 0, 2, 0, 2, 11, 10, 29, 163, 10090}}

lastBin = jxHistList[[2, 20]]
10090

padding = {{80, 10}, {60, 12}};
fig1b = Show[Histogram[statsBySample[[All, 11]] / statsBySample[[All, 10]] // N,
15, Frame → True, ImageSize → baseImageSize * .5,
BaseStyle → {FontFamily → "Arial", FontSize → 15}, ImagePadding → padding,
FrameLabel → {Style["Proportion of overlaps that are annotated", 15],
Style["No. of well-covered samples", 15]}],
Graphics[{Gray, Arrow[{{0.75, 6000}, {0.94, 8000}}}],
largerLabelForm[ToString[NumberForm[lastBin, DigitBlock -> 3]] <> " samples",
{0.54, 5900}]}, Graphics[
{Black, Text[Style["b", FontFamily → "Arial", FontSize → 40], {0.1, 8500}]}]]]
```

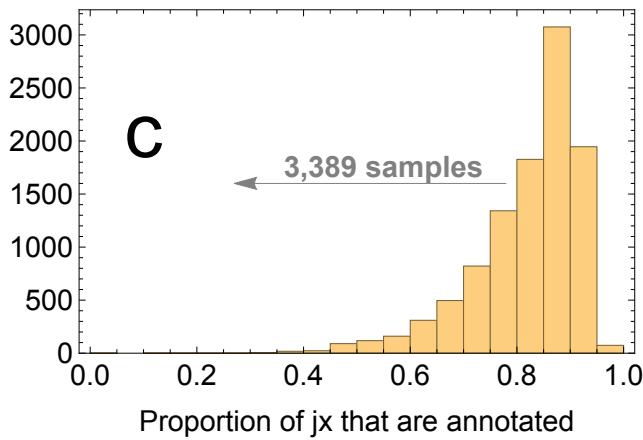


Junctions by sample-->

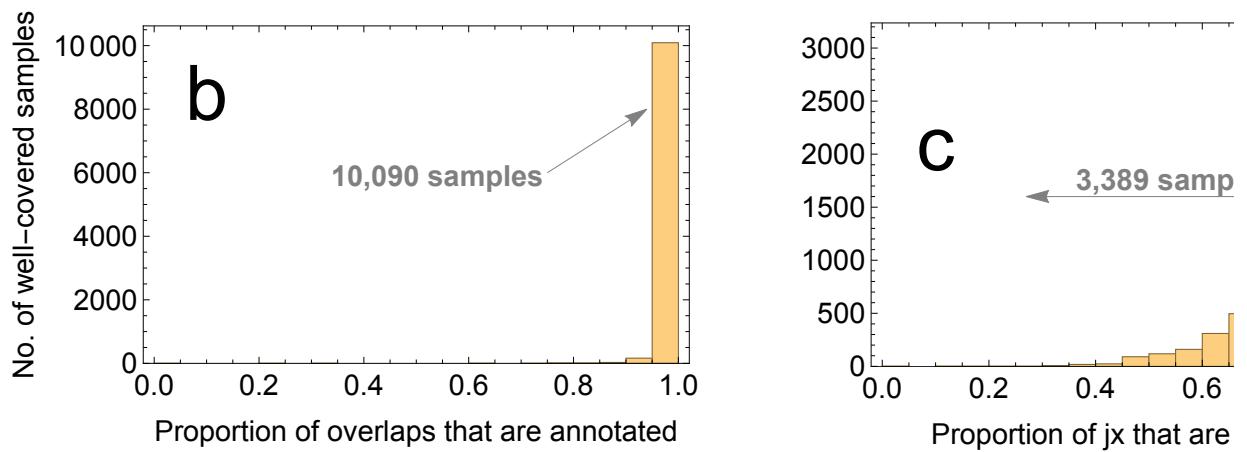
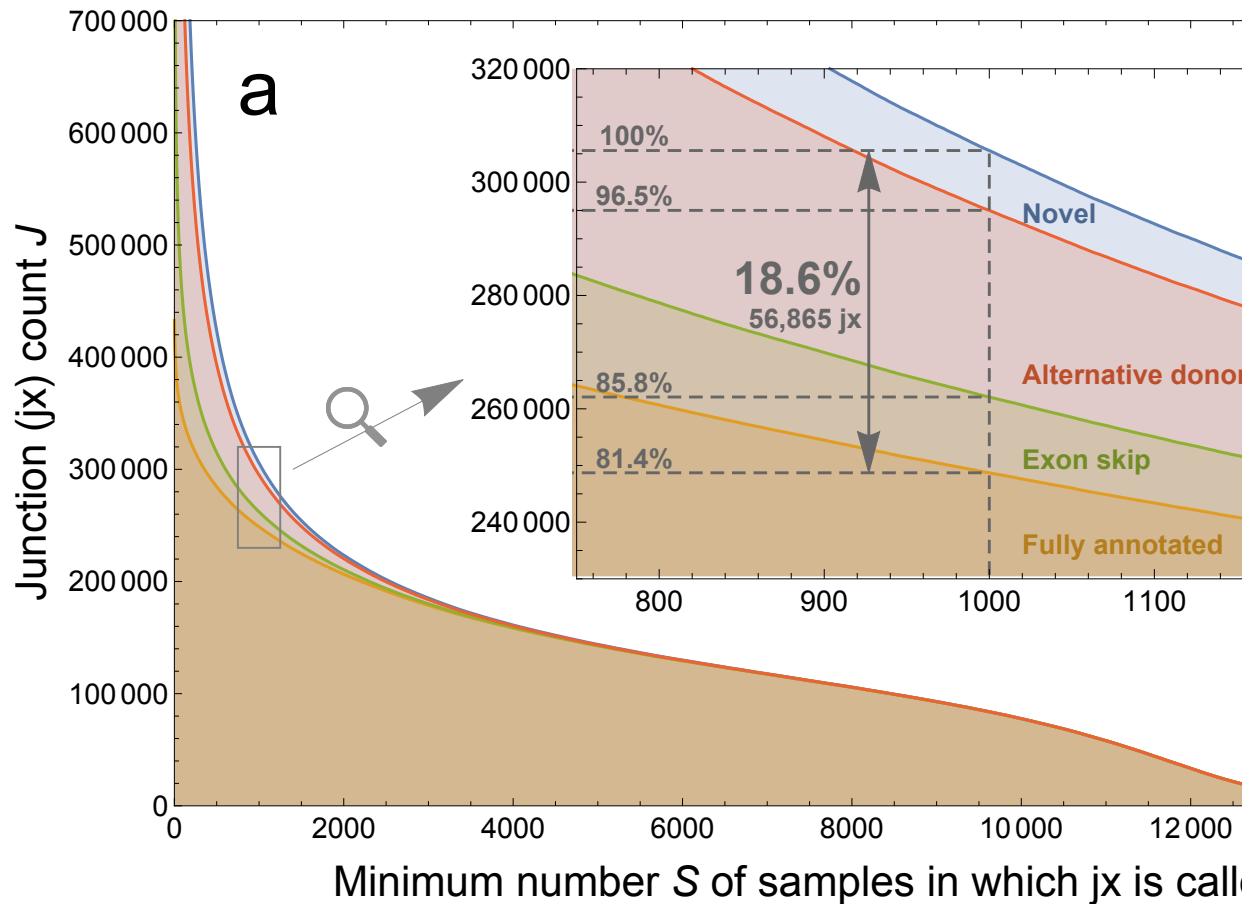
```
jxHistList2 = HistogramList[statsBySample[[All, 7]] / statsBySample[[All, 6]], 15]
{{0, 1/20, 1/10, 3/20, 1/5, 1/4, 3/10, 7/20, 2/5, 9/20, 1/2, 11/20, 3/5, 13/20, 7/10, 3/4, 4/5, 17/20, 9/10, 19/20, 1}, {1, 0, 1, 1, 1, 6, 18, 23, 90, 118, 160, 310, 496, 821, 1342, 1827, 3075, 1946, 74}]

lessThan80 = Total[jxHistList2[[2, Range[1, 16]]]]
3389
```

```
padding2 = {{75, 10}, {60, 12}};  
fig1c = Show[  
  Histogram[statsBySample[[All, 7]] / statsBySample[[All, 6]] // N,  
   15, Frame -> True, ImageSize -> baseImageSize *.5, ImagePadding -> padding2,  
   BaseStyle -> {FontFamily -> "Arial", FontSize -> 15},  
   FrameLabel -> {Style["Proportion of jx that are annotated", 15], None}],  
  Graphics[{Gray, Arrow[{{0.78, 1600}, {0.27, 1600}}]}],  
  largerLabelForm[ToString[NumberForm[lessThan80, DigitBlock -> 3]] <>  
   " samples", {0.55, 1760}]], Graphics[  
  {Black, Text[Style["c", FontFamily -> "Arial", FontSize -> 40], {0.1, 2100}]}]]
```



```
fig1 = Grid[{{fig1a}, {Grid[{{fig1b, fig1c}}]}}]
```



```
Export["jxannotation.pdf", fig1]
jxannotation.pdf

SEQC comparison: subset of 1720 samples out of the 21504 that were aligned by SEQC using magic,
rmake, subread. Shows that when a junction is in a lot of samples, it's found by other aligners.

aggregatedJunctionCounts = Drop[Import["hg19.seqc_sample.stats.tsv", "TSV"], 1];

totalJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 2}]]];
oneAlignerJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 6}]]];
twoAlignerJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 7}]]];
threeAlignerJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 8}]]];

totalJunctions[[1710 - 79]]
{80, 348531}

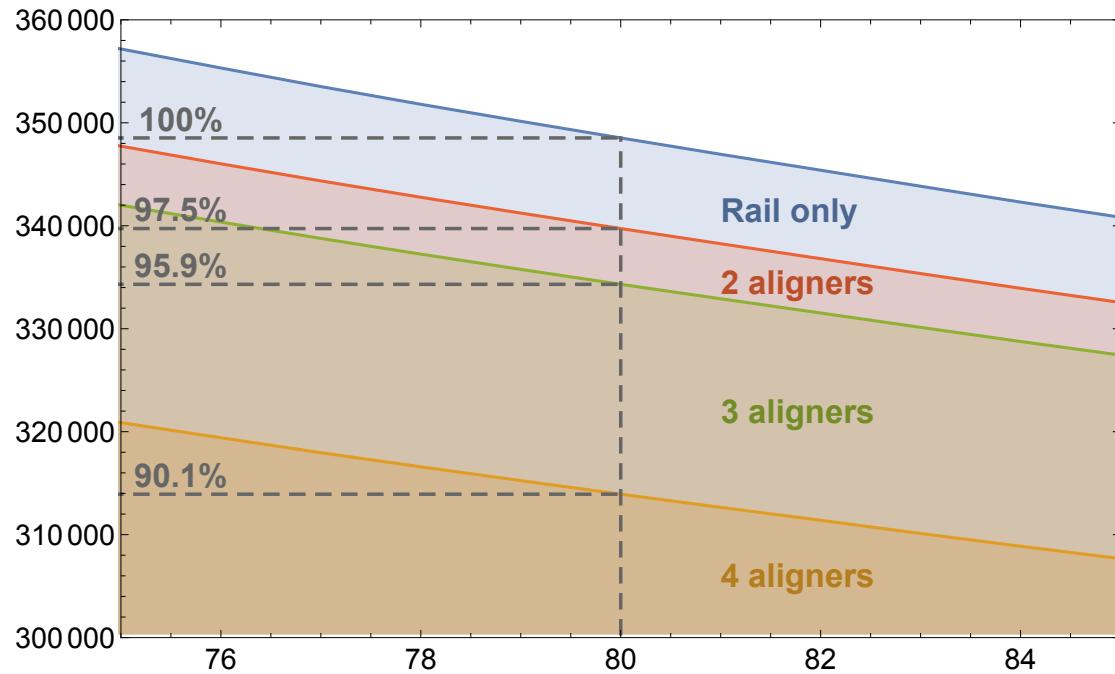
idx = 1710 - 79
1631

atLeastTwoAlignersJunctions = Transpose[{oneAlignerJunctions[[All, 1]],
  threeAlignerJunctions[[All, 2]] + twoAlignerJunctions[[All, 2]]}];
atLeastOneAlignerJunctions = Transpose[
  {oneAlignerJunctions[[All, 1]], oneAlignerJunctions[[All, 2]] +
   twoAlignerJunctions[[All, 2]] + threeAlignerJunctions[[All, 2]]}];
```

```

dashedColor = Darker[Gray, 0.2]; numberStartPos = 75.6;
insetAnnotationPlot = Show[ListPlot[{totalJunctions, threeAlignerJunctions,
    atLeastTwoAlignersJunctions, atLeastOneAlignerJunctions}, Joined -> True,
    PlotRange -> {{75, 85}, {300 000, 360 000}}, Filling -> Axis, Frame -> True,
    ImageSize -> Large, BaseStyle -> {FontFamily -> "Arial", FontSize -> 15}],
Graphics[{Directive[Thickness[0.0035], Dashing[0.013], dashedColor],
    Line[{{80, 0}, totalJunctions[[idx]]}],
    Line[{{0, totalJunctions[[idx]][[2]]}, totalJunctions[[idx]]}],
    Line[{{0, atLeastOneAlignerJunctions[[idx]][[2]]},
        atLeastOneAlignerJunctions[[idx]]}],
    Line[{{0, atLeastTwoAlignersJunctions[[idx]][[2]]},
        atLeastTwoAlignersJunctions[[idx]]}],
    Line[{{0, threeAlignerJunctions[[idx]][[2]]}, threeAlignerJunctions[[idx]]}],
    Directive[{Dashing[None], Arrowheads[{-0.03, 0.03}]}],
    (*Arrow[{{79.63, atLeastOneAlignerJunctions[[idx]][[2]]},
        {79.63, totalJunctions[[idx]][[2]]}}], bigLabelForm[ToString[NumberForm[
        N[100-atLeastOneAlignerJunctions[[idx,2]]/totalJunctions[[idx,2]]*100,3],
        DigitBlock->3]]<>"%",{79.5, 345500}, {1,0}], labelForm[
        ToString[NumberForm[totalJunctions[[idx,2]]-atLeastOneAlignerJunctions[[idx,2]],DigitBlock->3]]<>" jx", {79.5, 341500}, {1,0}],*),
    labelForm["100%", {numberStartPos, totalJunctions[[idx]][[2]] + 1800}],
    labelForm[ToString[NumberForm[N[atLeastOneAlignerJunctions[[idx, 2]]/
        totalJunctions[[idx, 2]] * 100, 3], DigitBlock -> 3]] <> "%",
        {numberStartPos, atLeastOneAlignerJunctions[[idx]][[2]] + 1800}],
    labelForm[ToString[NumberForm[N[threeAlignerJunctions[[idx, 2]]/
        totalJunctions[[idx, 2]] * 100, 3], DigitBlock -> 3]] <> "%",
        {numberStartPos, threeAlignerJunctions[[idx]][[2]] + 1800}],
    labelForm[ToString[NumberForm[N[atLeastTwoAlignersJunctions[[idx, 2]]/
        totalJunctions[[idx, 2]] * 100, 3], DigitBlock -> 3]] <> "%",
        {numberStartPos, atLeastTwoAlignersJunctions[[idx]][[2]] + 1800}],
    (*Directive[Thickness[0.0035], Arrowheads[.035], Dashing[None], dashedColor],
    Arrow[{{1000, totalJunctions[[idx]][[2]]+41000},
        {1000, totalJunctions[[idx]][[2]]+100}}], labelForm[ToString[NumberForm[
        N[100-annotatedJunctions[[idx,2]]/totalJunctions[[idx,2]]*100],3]]<>
        "% of jx unannotated, but\n">>ToString[NumberForm[
        someEvidenceJunctions[[idx,2]]/totalJunctions[[idx,2]]*100//N,3]]]<>
        "% of jx have donor and/or\\nacceptor site in annotation",
        {1005, 330000}, {-1,0}],*)Darker[mathematicaColors[[1]], 0.2],
    labelForm["Rail only", {81, 341500}, {-1, 0}],
    Darker[mathematicaColors[[4]], 0.2],
    labelForm["2 aligners", {81, 334500}, {-1, 0}],
    Darker[mathematicaColors[[3]], 0.2],
    labelForm["3 aligners", {81, 322000}, {-1, 0}],
    Darker[mathematicaColors[[2]], 0.2],
    labelForm["4 aligners", {81, 306000}, {-1, 0}]})]

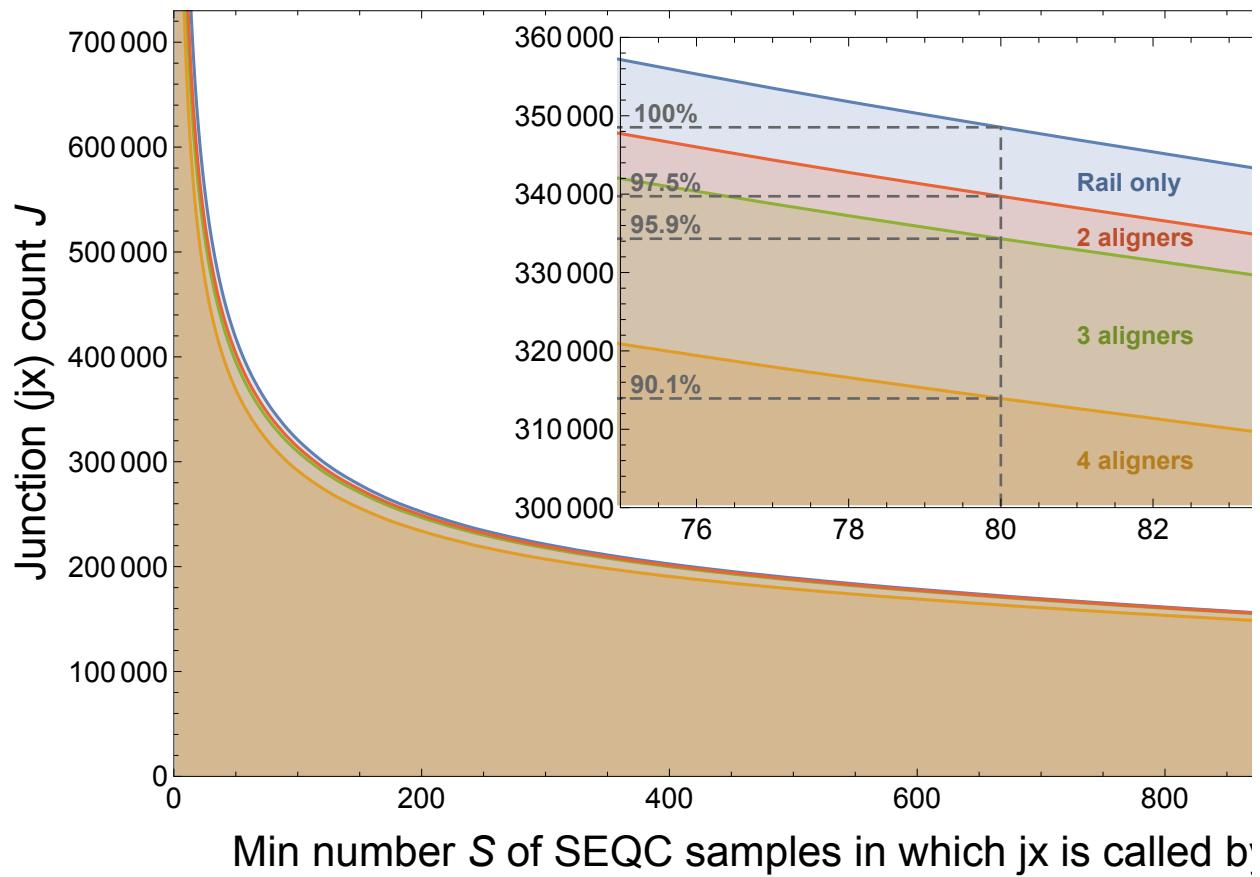
```



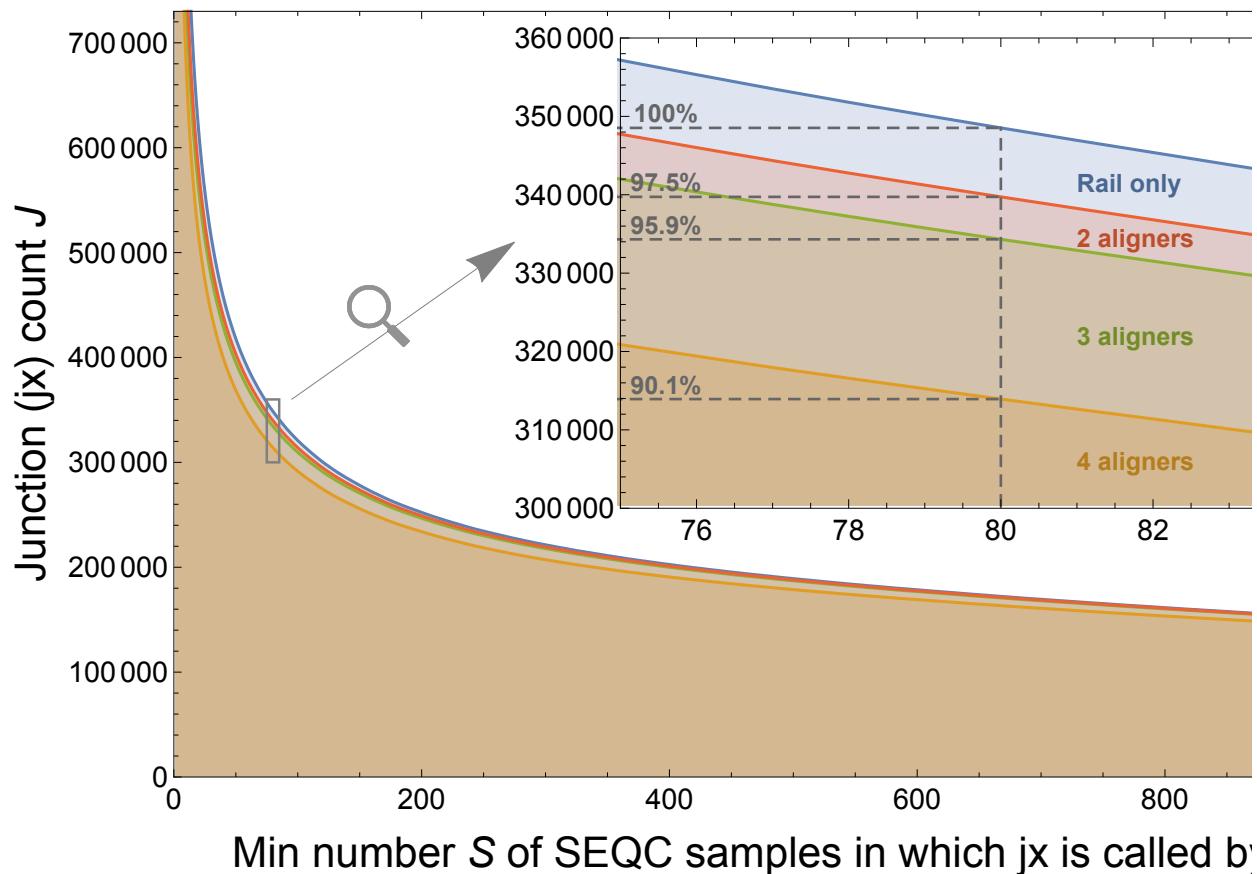
```

seqcPlot = ListPlot[{totalJunctions, threeAlignerJunctions,
  atLeastTwoAlignersJunctions, atLeastOneAlignerJunctions}, Joined -> True,
  PlotRange -> {{0, 1000}, {0, 730 000}}, Filling -> Axis, Frame -> True,
  ImageSize -> baseImageSize, BaseStyle -> {FontFamily -> "Arial", FontSize -> 15},
  Epilog -> Inset[insetAnnotationPlot, {625, 470 000}, Automatic, 700], FrameLabel ->
  {Style["Min number  $S$  of SEQC samples in which  $j_x$  is called by Rail", 22],
  Style["Junction ( $j_x$ ) count  $J$ ", 22}]}

```



```
suppfigseqc = Show[seqcPlot, Graphics[{EdgeForm[Directive[Gray, Thickness[.002]]], Transparent, Rectangle[{75, 300000}, {85, 360000}]}], Graphics[{Gray, Arrow[{{95, 360000}, {275, 510000}}]}], Graphics[{Opacity[0.5], Inset[magnifyingGlass, {140, 410000}, {0, 0}, 50]}], ImageSize → baseImageSize]
```



```
Export["seqc.pdf", suppfigseqc]
```

```
seqc.pdf
```

Repeat Figure 1a, except at project level for supplement.

```
aggregatedJunctionCounts = Drop[Import["hg19.project.stats.tsv", "TSV"], 1];

totalJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 2}]]];
annotatedJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 3}]]];
exonSkipJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 4}]]];
altStartEndJunctions = Transpose[Transpose[aggregatedJunctionCounts][[{1, 5}]]];
novelJunctions =
exonSkips = Transpose[Transpose[aggregatedJunctionCounts][[{1, 6}]]];
```

```

exonSkipAnnotatedJunctions = Transpose[{annotatedJunctions[[All, 1]],
    annotatedJunctions[[All, 2]] + exonSkipJunctions[[All, 2]]}];
someEvidenceJunctions = Transpose[{annotatedJunctions[[All, 1]],
    annotatedJunctions[[All, 2]] + exonSkipJunctions[[All, 2]] +
    altStartEndJunctions[[All, 2]]}];

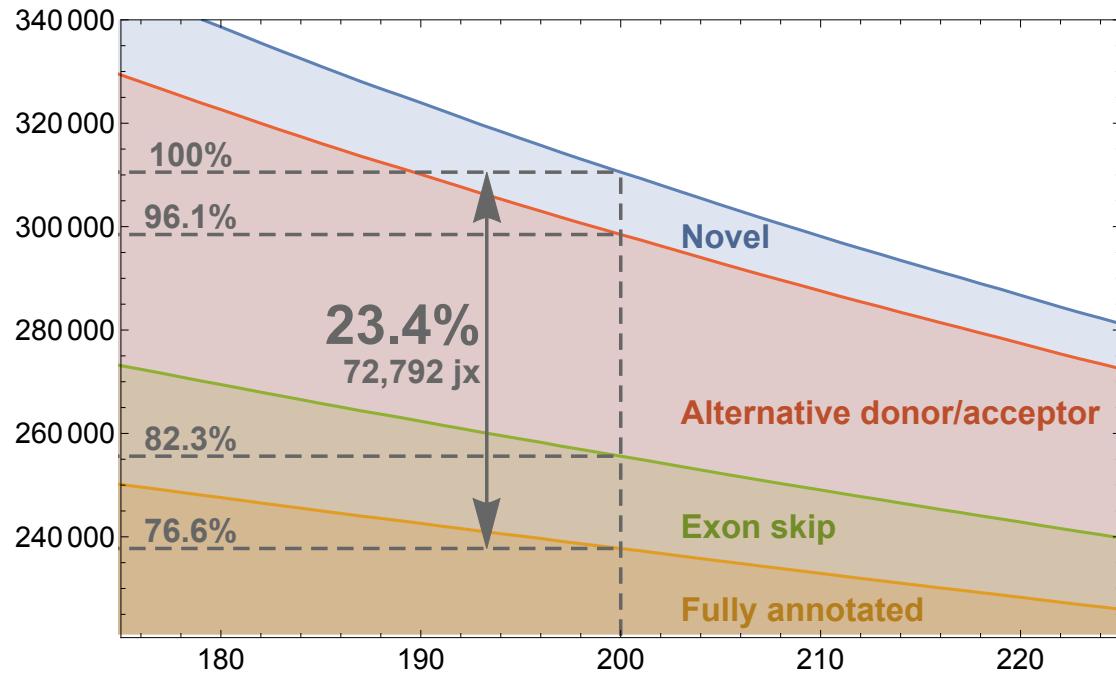
Introduce levels of evidence, and annotated with junction counts for ≥ 30 samples.

totalJunctions[[784 - 199]]
{200, 310 536}

idx = 784 - 199
585

dashedColor = Darker[Gray, 0.2]; numberStartPos = 178.5; adjust = 3400;
insetAnnotationPlot = Show[ListPlot[{totalJunctions, annotatedJunctions,
    exonSkipAnnotatedJunctions, someEvidenceJunctions}, Joined → True,
    PlotRange → {{175, 225}, {220 500, 340 000}}, Filling → Axis, Frame → True,
    ImageSize → Large, BaseStyle → {FontFamily → "Arial", FontSize → 15}],
    Graphics[{Directive[Thickness[0.0035], Dashing[0.013], dashedColor],
        Line[{{200, 0}, totalJunctions[[idx]]}],
        Line[{{0, totalJunctions[[idx]][[2]]}, totalJunctions[[idx]]}],
        Line[{{0, annotatedJunctions[[idx]][[2]]}, annotatedJunctions[[idx]]}],
        Line[{{0, exonSkipAnnotatedJunctions[[idx]][[2]]},
            exonSkipAnnotatedJunctions[[idx]]}],
        Line[{{0, someEvidenceJunctions[[idx]][[2]]}, someEvidenceJunctions[[idx]]}],
        Directive[{Dashing[None], Arrowheads[{-0.05, .05}]}],
        Arrow[{{193.3, annotatedJunctions[[idx]][[2]]},
            {193.3, totalJunctions[[idx]][[2]]}}], bigLabelForm[ToString[NumberForm[
                N[100 - annotatedJunctions[[idx, 2]] / totalJunctions[[idx, 2]] * 100, 3],
                DigitBlock → 3]] <> "%", {193, 281 000}, {1, 0}], labelForm[
                ToString[NumberForm[totalJunctions[[idx, 2]] - annotatedJunctions[[idx, 2]],
                    DigitBlock → 3]] <> " jx", {193, 272 000}, {1, 0}],
        labelForm["100%", {numberStartPos, totalJunctions[[idx]][[2]] + adjust}],
        labelForm[ToString[NumberForm[N[someEvidenceJunctions[[idx, 2]]] /
            totalJunctions[[idx, 2]] * 100, 3], DigitBlock → 3]] <> "%",
        {numberStartPos, someEvidenceJunctions[[idx]][[2]] + adjust}],
        labelForm[ToString[NumberForm[N[annotatedJunctions[[idx, 2]]] /
            totalJunctions[[idx, 2]] * 100, 3], DigitBlock → 3]] <> "%",
        {numberStartPos, annotatedJunctions[[idx]][[2]] + adjust}],
        labelForm[ToString[NumberForm[N[exonSkipAnnotatedJunctions[[idx, 2]]] /
            totalJunctions[[idx, 2]] * 100, 3], DigitBlock → 3]] <> "%",
        {numberStartPos, exonSkipAnnotatedJunctions[[idx]][[2]] + adjust}],
        Darker[mathematicaColors[[1]], 0.2],
        labelForm["Novel", {203, 298 000}, {-1, 0}],
        Darker[mathematicaColors[[4]], 0.2],
        labelForm["Alternative donor/acceptor", {203, 264 000}, {-1, 0}],
        Darker[mathematicaColors[[3]], 0.2],
        labelForm["Exon skip", {203, 242 000}, {-1, 0}],
        Darker[mathematicaColors[[2]], 0.2],
        labelForm["Fully annotated", {203, 226 000}, {-1, 0}]}]
    }]
]

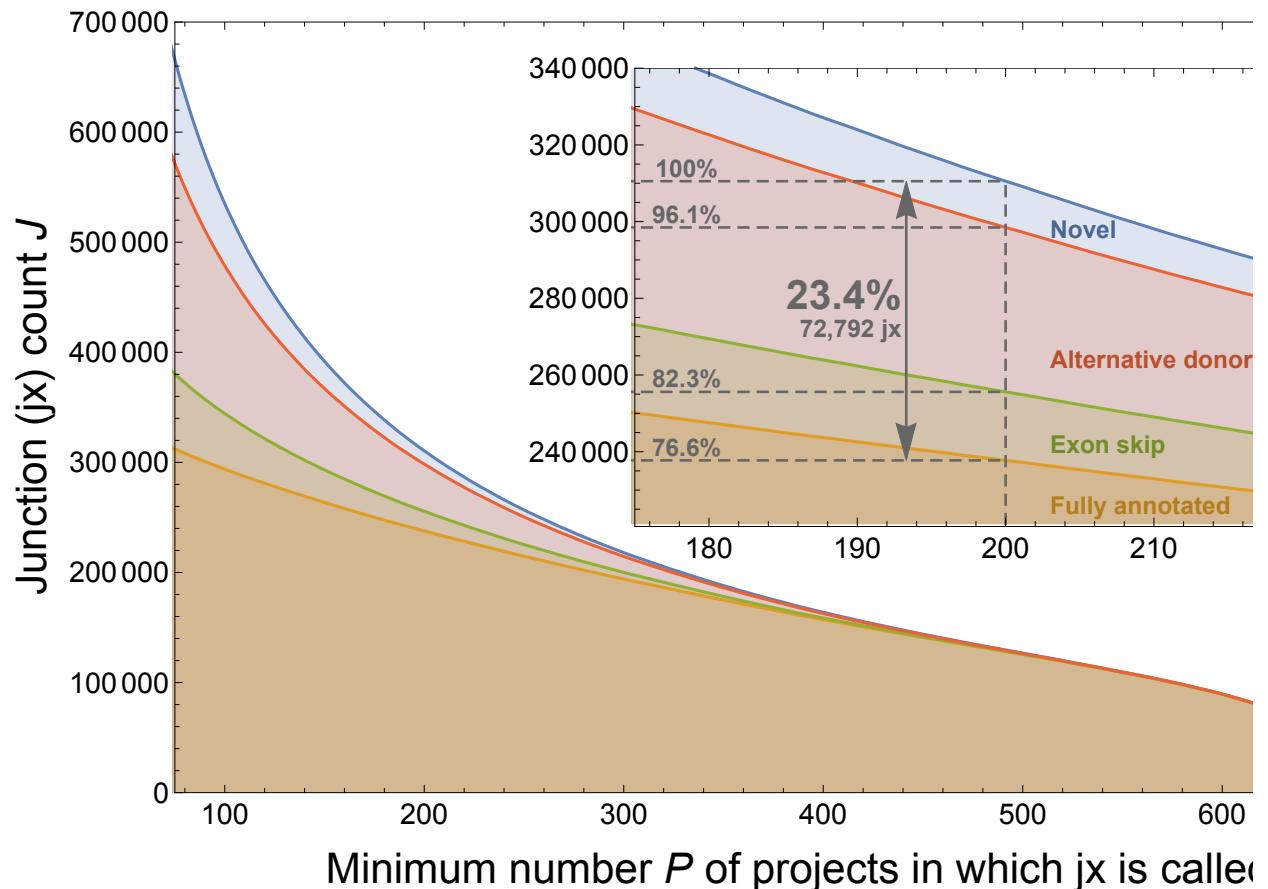
```



```

bigAnnotationPlot = ListPlot[{totalJunctions,
  annotatedJunctions, Transpose[{Transpose[annotatedJunctions][[1]],
    Transpose[annotatedJunctions][[2]] + Transpose[exonSkipJunctions][[2]]}],
  Transpose[{Transpose[annotatedJunctions][[1]],
    Transpose[annotatedJunctions][[2]] + Transpose[exonSkipJunctions][[2]] +
    Transpose[altStartEndJunctions][[2]]}],
  Joined -> True, PlotRange -> {{75, 700}, {0, 700 000}}, Filling -> Axis,
  Frame -> True, ImageSize -> baseImageSize,
  BaseStyle -> {FontFamily -> "Arial", FontSize -> 15},
  Epilog -> Inset[insetAnnotationPlot, {465, 440 000}, Automatic, 425],
  FrameLabel -> {Style["Minimum number  $P$  of projects in which jx is called", 22],
  Style["Junction (jx) count  $J$ ", 22]}]

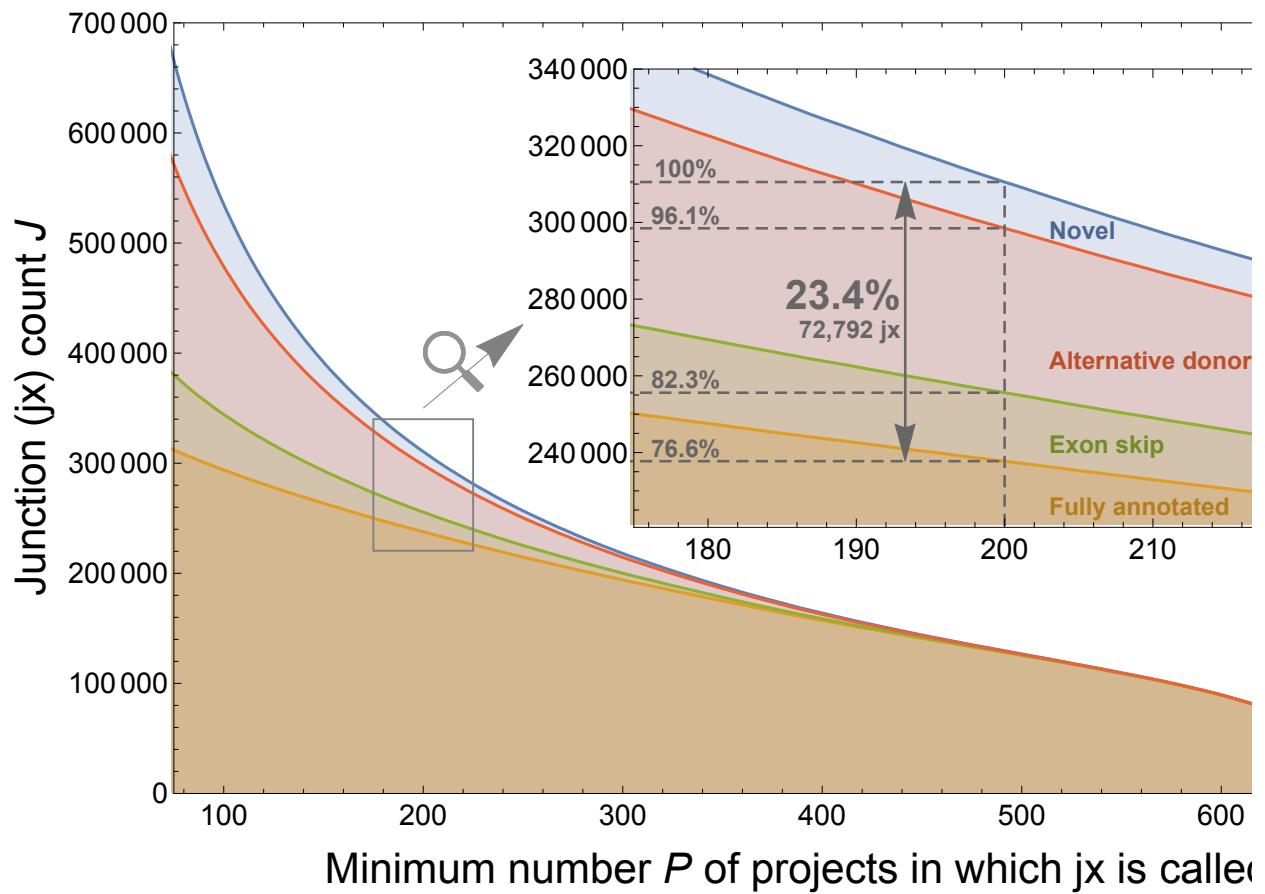
```



```

suppfigproj =
  Show[bigAnnotationPlot, Graphics[{EdgeForm[Directive[Gray, Thickness[.0015]]],
  Transparent, Rectangle[{175, 220500}, {225, 340000}]}], 
  Graphics[{Gray, Arrow[{{200, 350000}, {250, 426000}}]}], 
  Graphics[{Opacity[0.5], Inset[magnifyingGlass, {200, 365000}, {0, 0}, 30]}], 
  ImageSize → baseImageSize]

```



```
Export["projlevel.pdf", suppfigproj]
```

```
projlevel.pdf
```

For how many runs are we missing Biosample submission dates? We ran the command  
`cat index_to_SRA_accession.tsv | grep -vwFf <(cat biosample_tags.tsv | cut-f10 | tail-n+2)`  
`>missing_biosample_dates.tsv`

in the sra/hg19 directory of the repo nellore/runs to obtain that dates were missing for only  
 $77/21504=0.3\%$  of runs. Our analysis is reasonably complete if we ignore them.

```

junctionsEvidenceVsDatesGeq20 = Drop[
  Import["!gzip -cd hg19.sample_count_submission_date_overlap_geq_20.tsv.gz",
  "TSV"], 1];

```

```

junctionsEvidenceVsDatesGeq[x_, y_: junctionsEvidenceVsDatesGeq20] :=
  Select[y, #[[1]] ≥ x &];
talliedJunctionsGeq[x_, y_: junctionsEvidenceVsDatesGeq20] :=
  SortBy[Tally[junctionsEvidenceVsDatesGeq[x, y][[All, 4]]], First];
accumulatedJunctionsGeq[x_, y_: junctionsEvidenceVsDatesGeq20] :=
  (talliedJunctions = talliedJunctionsGeq[x, y];
   Transpose[{talliedJunctions[[All, 1]], Accumulate[talliedJunctions[[All, 2]]]}])

```

Convert from days after 2/27/2009 to dates.

```
Clear[daysToDate]
```

```
daysToDate[x_] := DatePlus[DateObject[{2009, 02, 27}], x]
```

When were junctions supported by reads in  $\geq 20, 40, 80, 160$  reads across samples found?

```
twentyThirteen = 1404; daysToDate[twentyThirteen]
```

 Tue 1 Jan 2013

```
dateFormat = {"Month", "/", "Day", "/", "YearShort"};
```

Design ticks to intersect 1/1/2013.

```
dateTicks = ({#,DateString[daysToDate[#], dateFormat]} & /@ Range[0, 2070, 234])
{{0, 02/27/09}, {234, 10/19/09}, {468, 06/10/10}, {702, 01/30/11}, {936, 09/21/11},
 {1170, 05/12/12}, {1404, 01/01/13}, {1638, 08/23/13}, {1872, 04/14/14}}
```

```
lastDay = Max[junctionsEvidenceVsDatesGeq20[[All, 4]]]
```

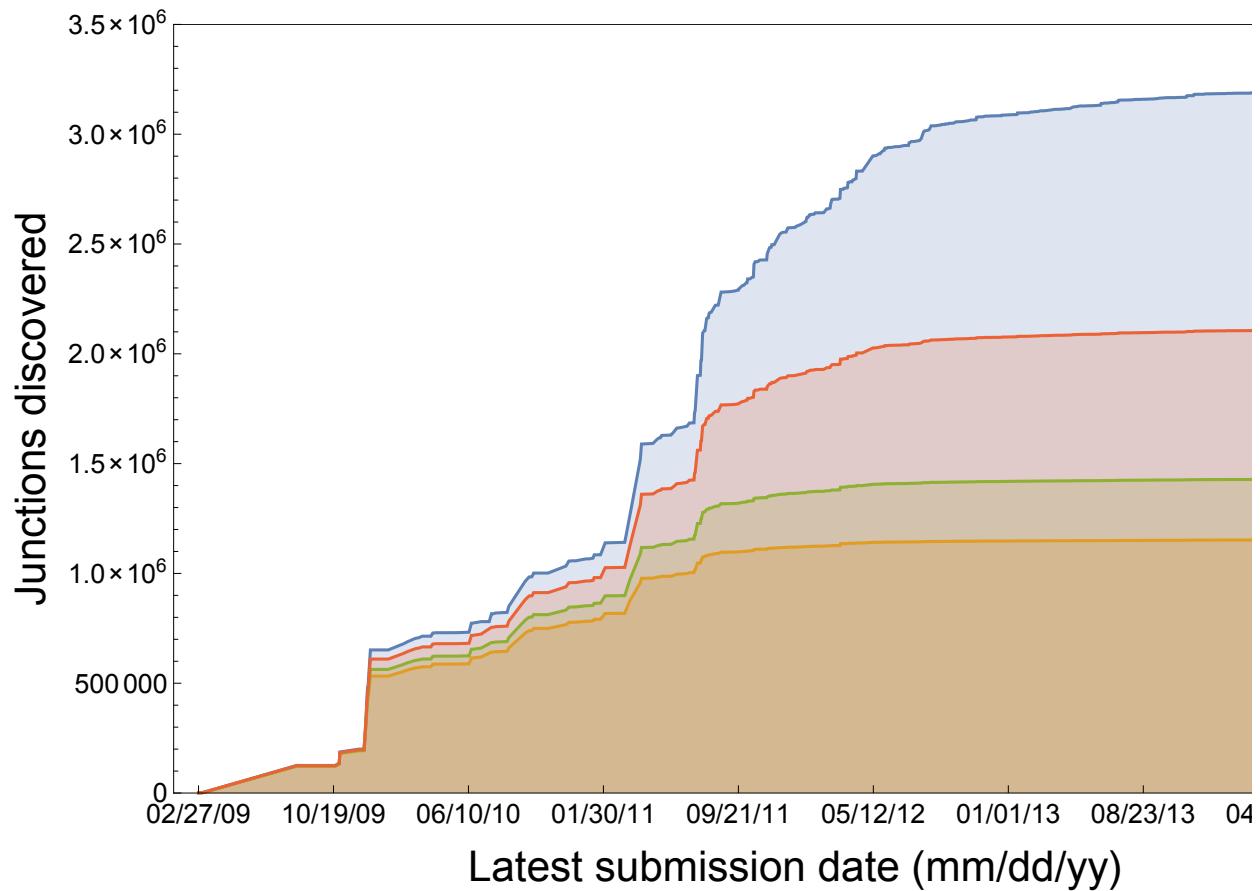
2070

```
dateTicks =
```

```
Append[dateTicks, {lastDay, DateString[daysToDate[lastDay], dateFormat]}]
{{0, 02/27/09}, {234, 10/19/09}, {468, 06/10/10},
 {702, 01/30/11}, {936, 09/21/11}, {1170, 05/12/12}, {1404, 01/01/13},
 {1638, 08/23/13}, {1872, 04/14/14}, {2070, 10/29/14}}
```

```
baseJunctionsPlotData = accumulatedJunctionsGeq /@ {20, 120, 80, 40};
```

```
baseJunctionsPlot = ListPlot[baseJunctionsPlotData, Joined → True, Filling → Axis,
  Frame → True, FrameTicks → {{Automatic, None}, {dateTicks, None}},
  BaseStyle → {FontFamily → "Arial", FontSize → 14},
  FrameLabel → {Style["Latest submission date (mm/dd/yy)", 22],
  Style["Junctions discovered", 22]},
  ImageSize → baseImageSize, PlotRange → {All, {0, 3.5 * 10^6}}]
```



```
sortedDays = Sort[junctionsEvidenceVsDatesGeg20[[All, 4]]];
junctionsInCommons = Count[sortedDays, #] & /@ Commonest[sortedDays, 7]
{123 759, 124 121, 155 069, 163 007, 124 664, 252 628, 162 196}
```

These correspond to, respectively....

```
daysToDate /@ Commonest[sortedDays, 7]
```

```
{Sun 16 Aug 2009, Mon 14 Dec 2009, Thu 17 Dec 2009,
Tue 22 Dec 2009, Thu 17 Mar 2011, Mon 4 Apr 2011, Tue 12 Jul 2011}
```

Some of these dates correspond to jumps in the plot above. Grepping for the submission dates in biosample\_tags.tsv in sra/hg19 gives samples in the following projects:

1. who cares
2. who cares
3. Study of 69 LCLs (2)(Understanding mechanisms underlying human gene expression variation with RNA sequencing, by Pickrell et al.) (SRP001540) 17 Dec 2009
4. Study of 41 Coriell cell lines (SRP001563)(Polymorphic cis-and trans-regulation of human gene expression, by Cheung et al.) 22 Dec 2009
5. Illumina bodyMap2 (ERP000546) 17-Mar-2011
6. University of Washington Human Reference Epigenome Mapping Project (SRP001371)(total RNA, fetal tissues, contributed most junctions on a single day (4 April 2011)); note also that on this day, there are two more projects: SRP005309, a microRNA study with negligible # junctions, and SRP005846, for which grepping hg19.stats\_by\_sample.tsv gives ~95 annotated jx, < 50k each of 4 samples. So overwhelmingly dominant contribution on 4 April 2011 is UW.
7. ENCODE long RNA-seq from CSHL (SRP007461) 12-Jul-2011

Annotate plot with the top 5 projects (3,4,5,6, and 7 above); the 1st, 2nd, and 5th are about the same size but have many fewer junctions than the top 4 contributors. However, bodyMap 2 is interesting in that GENCODE incorporated it in its annotation.

Find GEUVADIS. Grepping biosample\_tags.tsv gives that the GEUVADIS submission date was 2012-11-07. This was

```
daysToDate[1349]
```

 Wed 7 Nov 2012

A glance at the tallies below shows that just 11294 novel junctions were contributed on the day GEUVADIS samples were submitted to Biosample!

```
tallied = Tally[sortedDays]
```

```
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Find GEUV day's rank:

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{1950, 3}, {1901, 3}, {1900, 3}, {1790, 3}, {1740, 3}, {1056, 3}, {1928, 2},
{1918, 2}, {1842, 2}, {1693, 2}, {1633, 2}, {1492, 2}, {1421, 2}, {1399, 2},
{2014, 1}, {2010, 1}, {1977, 1}, {1967, 1}, {1958, 1}, {1890, 1}, {1886, 1},
{1869, 1}, {1817, 1}, {1777, 1}, {1719, 1}, {1711, 1}, {1644, 1}, {1628, 1},
{1589, 1}, {1537, 1}, {1369, 1}, {1228, 1}, {1092, 1}, {1083, 1}, {936, 1}, {504, 1}}

```

**Position[Reverse[SortBy[tallied, Last]], {1349, 11294}]**

{55}

GEUV is at 55!

```

geuvDate = 1349
1349

arrowLabelForm [x_, y___] := Text[Style[x, FontFamily -> "Arial",
  FontSize -> Scaled[.03], Bold, TextAlignment -> Left], y]

smallerLabelForm [x_, y___] := Text[Style[x, FontFamily -> "Arial",
  FontSize -> Scaled[.02], Bold, TextAlignment -> Left], y]

biggerLabelForm [x_, y___] := Text[Style[x, FontFamily -> "Arial",
  FontSize -> Scaled[.04], Bold, TextAlignment -> Left], y]

altLabelForm [x_, y___] := Text[Style[x, FontFamily -> "Arial",
  FontSize -> Scaled[.045], Bold, TextAlignment -> Left], y]

accJunc = accumulatedJunctionsGeq[20];

maxAtTwentyThirteen = Select[accJunc, #[[1]] <= twentyThirteen &][[-1]][[2]]
3 087 471

maxAtEnd = accJunc[[-1]][[2]]
3 211 228

```

How many junctions covered by  $\geq 20$  reads are there? Should agree with maxAtEnd.

```

Length[junctionsEvidenceVsDatesGeq20]
3 211 228

```

From the command line and in the runs/sra directory, run

```

join -2 3 <(cut -f10,11 hg19/biosample_tags.tsv | tail -n +2 | cut -dT' -f1 | sort-k1,1) <(sort -k3,3 intropo-
lis.idmap.v1.hg19.tsv) | awk '$2 < "2013-01-01"' | wc -l

```

to get that 7426 samples are before 2013, and change the  $<$  to a  $\geq$  in the awk command to get that 14801 samples are  $\geq 2013$ . The 77 missing samples don't have Biosample submission dates, and they're ignored.

```

before2013 = 7426; after2013 = 14 001;

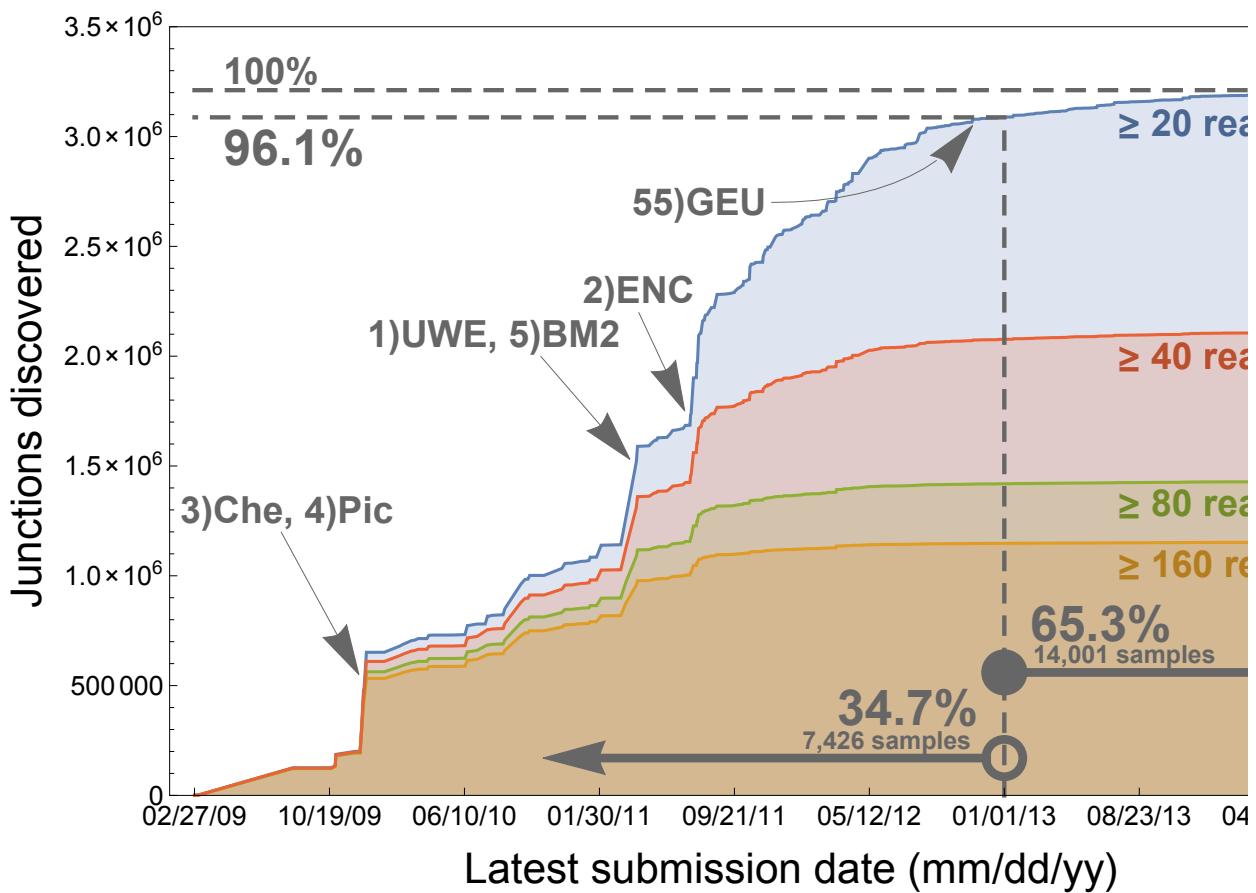
before2013 / (before2013 + after2013) // N
0.346572

```

```

labelColor = Darker[Gray, 0.2]; leftPos = 1600;
botPos = 110000; fig2 = Show[baseJunctionsPlot,
  Graphics[{labelColor, Arrow[{{150, 1.2 * 10^6}, {285, 550000}}], arrowLabelForm[
    "3)Che, 4)Pic", {160, 1.3 * 10^6}], Arrow[{{600, 2 * 10^6}, {755, 1.53 * 10^6}}],
  arrowLabelForm["1)UWE, 5)BM2", {600, 2.1 * 10^6}],
  Arrow[{{770, 2.2 * 10^6}, {850, 1.75 * 10^6}}],
  Arrow[BezierCurve[{{1000, 2.7 * 10^6}, {1249, 2.7 * 10^6}, {1349, 3.05 * 10^6}}]],
  arrowLabelForm["2)ENC", {770, 2.3 * 10^6}], Directive[Thickness[0.0035],
  Dashing[0.013], labelColor], arrowLabelForm["55)GEU", {875, 2.7 * 10^6}],
  Directive[Thickness[0.0035], Dashing[0.013], labelColor],
  Line[{{twentyThirteen, 0}, {twentyThirteen, maxAtTwentyThirteen}}],
  Line[{{twentyThirteen, maxAtTwentyThirteen}, {0, maxAtTwentyThirteen}}],
  Line[{{lastDay, maxAtEnd}, {lastDay, 0}}],
  Line[{{0, maxAtEnd}, {lastDay, maxAtEnd}}],
  arrowLabelForm["100%", {50, 3.3 * 10^6}, {-1, 0}], biggerLabelForm[ToString[
    NumberForm[N[maxAtTwentyThirteen / maxAtEnd * 100, 3], DigitBlock -> 3]] <> "%",
  {50, 2.95 * 10^6}, {-1, 0}], Darker[mathematicaColors[[2]], 0.2],
  arrowLabelForm["≥ 160 reads", {leftPos, 1.055 * 10^6}, {-1, 0}],
  Darker[mathematicaColors[[3]], 0.2],
  arrowLabelForm["≥ 80 reads", {leftPos, 1.33 * 10^6}, {-1, 0}],
  Darker[mathematicaColors[[4]], 0.2], arrowLabelForm["≥ 40 reads",
  {leftPos, 2 * 10^6}, {-1, 0}], Darker[mathematicaColors[[1]], 0.2],
  arrowLabelForm["≥ 20 reads", {leftPos, 3.06 * 10^6}, {-1, 0}],
  Dashing[None], Thickness[.007], Darker[Gray, .2], Arrowheads[{0, .05}],
  smallerLabelForm[ToString[NumberForm[after2013, DigitBlock -> 3]] <> " samples",
  {twentyThirteen + 50, botPos + 530000}, {-1, 0}],
  biggerLabelForm[ToString[NumberForm[
    N[after2013 / (after2013 + before2013) * 100, 3], DigitBlock -> 3]] <> "%",
  {twentyThirteen + 45, botPos + 670000}, {-1, 0}],
  smallerLabelForm[ToString[NumberForm[before2013, DigitBlock -> 3]] <>
  " samples", {twentyThirteen - 350, 249000}, {-1, 0}],
  biggerLabelForm[ToString[NumberForm[N[before2013 / (after2013 + before2013) *
  100, 3], DigitBlock -> 3]] <> "%", {twentyThirteen - 290, 400000},
  {-1, 0}], Arrow[{{twentyThirteen + 18, botPos + 450000},
  {twentyThirteen + 610, botPos + 450000}}],
  Disk[{twentyThirteen, botPos + 450000}, {40, 105000}],
  Arrow[{{twentyThirteen - 23, 170000}, {twentyThirteen - 800, 170000}}],
  Circle[{twentyThirteen, 170000}, {32, 85000}]]]

```



```
Export["dateplot.pdf", fig2]
```

```
dateplot.pdf
```

Format of next list is {GENCODE index, date}.

```
earliestGencodes =
  {#[[2]], #[[1, 1, 1]]} & /@ Select[{Position[#[[Range[5, 22]]], 1], #[[4]]} & @
    junctionsEvidenceVsDatesGeq20, Length[#[[1]]] > 0 &];
```

Freeze dates taken from <http://www.gencodegenes.org/releases/>.

```
daysAfterDate [y_] := DateDifference[DateObject[{2009, 2, 27}], y]
```

```
gencodeFreezeDates =
  {DateObject[{2009, 7}], DateObject[{2009, 7}], DateObject[{2010, 1}],
   DateObject[{2010, 4}], DateObject[{2010, 11}], DateObject[{2010, 12}],
   DateObject[{2011, 3}], DateObject[{2011, 5}], DateObject[{2011, 7}],
   DateObject[{2011, 10}], DateObject[{2011, 12}], DateObject[{2012, 3}],
   DateObject[{2012, 6}], DateObject[{2012, 8}], DateObject[{2012, 11}],
   DateObject[{2013, 2}], DateObject[{2013, 4}], DateObject[{2013, 7}]};
```

```

gencodeAppearDates =
  {DateObject[{2009, 9}], DateObject[{2010, 3}], DateObject[{2010, 5}],
   DateObject[{2010, 11}], DateObject[{2011, 2}], DateObject[{2011, 4}],
   DateObject[{2011, 6}], DateObject[{2011, 9}], DateObject[{2011, 12}],
   DateObject[{2012, 2}], DateObject[{2012, 5}], DateObject[{2012, 7}],
   DateObject[{2012, 10}], DateObject[{2013, 1}], DateObject[{2013, 4}],
   DateObject[{2013, 6}], DateObject[{2013, 9}], DateObject[{2013, 12}]};

gencodeFreezeDays = QuantityMagnitude /@ daysAfterDate /@ gencodeFreezeDates
{124, 124, 308, 398, 612, 642, 732, 793, 854,
 946, 1007, 1098, 1190, 1251, 1343, 1435, 1494, 1585}

gencodeAppearDays = QuantityMagnitude /@ daysAfterDate /@ gencodeAppearDates
{186, 367, 428, 612, 704, 763, 824, 916, 1007,
 1069, 1159, 1220, 1312, 1404, 1494, 1555, 1647, 1738}

appearDateFormat = {"Month", "/", "YearShort"};

gencodeAppearDateTicks =
  {#,DateString[daysToDate[#], appearDateFormat]} & /@ gencodeAppearDays
{{186, 09/09}, {367, 03/10}, {428, 05/10}, {612, 11/10},
 {704, 02/11}, {763, 04/11}, {824, 06/11}, {916, 09/11}, {1007, 12/11},
 {1069, 02/12}, {1159, 05/12}, {1220, 07/12}, {1312, 10/12},
 {1404, 01/13}, {1494, 04/13}, {1555, 06/13}, {1647, 09/13}, {1738, 12/13}};

discoveryDaysToGencodeDays =
  {#[[1]], gencodeAppearDays[[#[[2]]]]} & /@ earliestGencodes;

toAcc = SortBy[Tally[#[[1]] & /@ earliestGencodes], First];
accumulatedAnnotated = Transpose[{toAcc[[All, 1]], Accumulate[toAcc[[All, 2]]]}];

baseImageSize
{748.8, 530.4}

toBoxWhisker = Table[#[[1]] & /@ Select[discoveryDaysToGencodeDays, #[[2]] == i &],
  {i, gencodeAppearDays}];

toBar = Length /@ toBoxWhisker
{251810, 1145, 10593, 5478, 7188, 9319, 3455,
 3119, 2579, 4879, 2088, 3196, 4599, 3265, 553, 515, 542, 667}

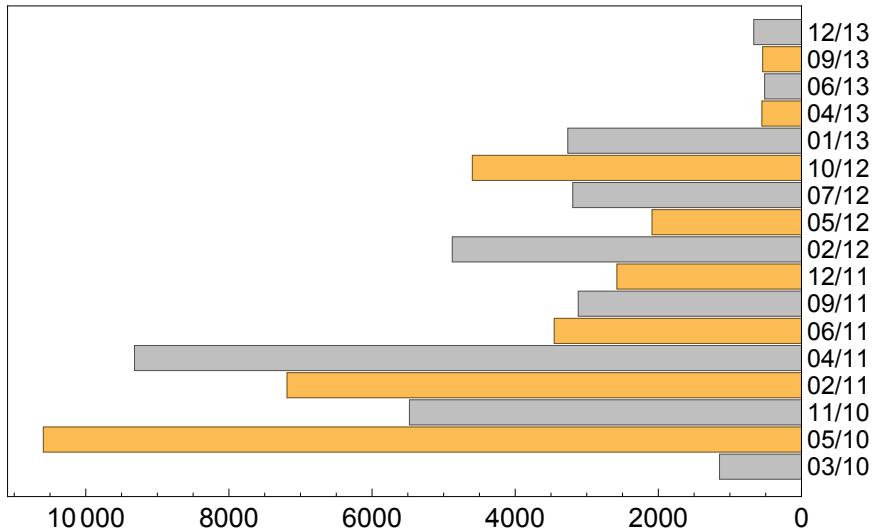
chartColors = {█, Lighter[Gray, .5]}
{█, █}

```

```

insetBars = BarChart[toBar[[Range[2, 18]]], ImageSize -> baseImageSize * 0.6,
Frame -> True, FrameTicks -> {{None, Automatic}, {Automatic, None}},
ChartLabels -> {Style[#, 13] & /@ gencodeAppearDateTicks[[Range[2, 18], 2]]},
BaseStyle -> {FontFamily -> "Arial", FontSize -> 14},
ChartStyle -> {chartColors[[2]], chartColors[[1]]},
PlotRangePadding -> {{500, 0}, {0.3, 0.5}}, BarOrigin -> Right]

```



```

toBar = Length /@ toBoxWhisker
{251810, 1145, 10593, 5478, 7188, 9319, 3455,
3119, 2579, 4879, 2088, 3196, 4599, 3265, 553, 515, 542, 667}

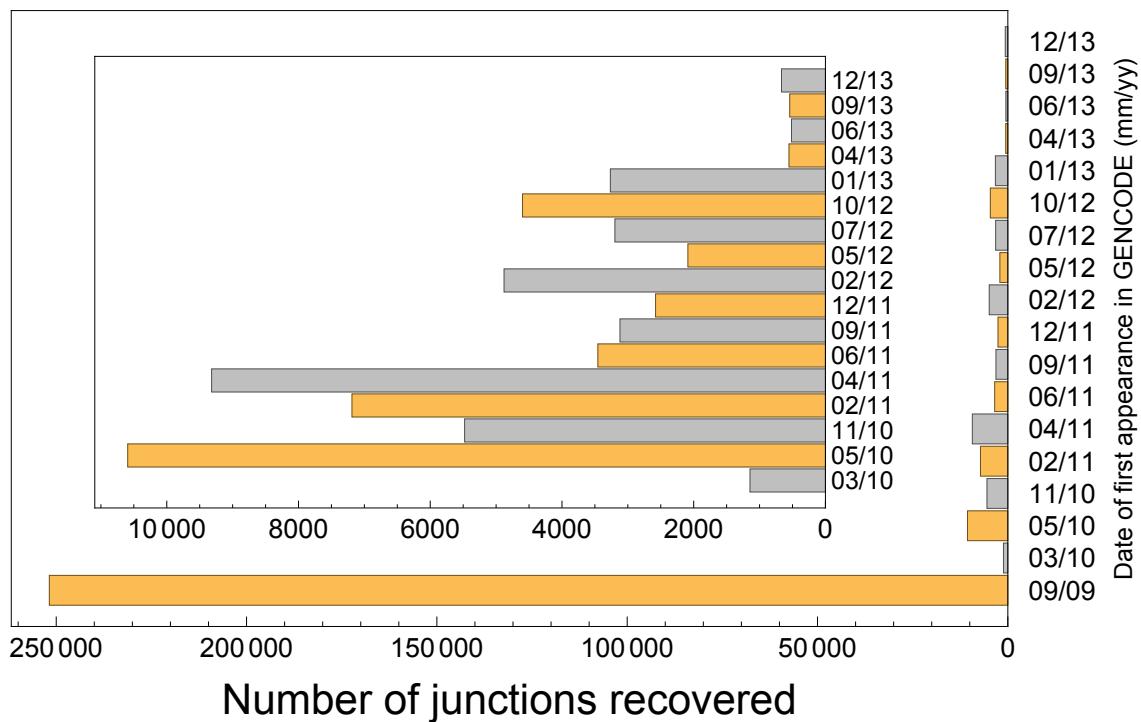
toBar[[1]] / Total[toBar] // N
0.799422

```

```

padding = {{10, 70}, {50, 0}};
barsWithInset = BarChart[toBar, ImageSize -> baseImageSize *.8,
  Frame -> True, FrameTicks -> {{None, Automatic}, {Automatic, None}},
  PlotRangePadding -> {{10000, 0}, {.3, .5}},
  ChartLabels -> {StringJoin[" ", #] & /@ gencodeAppearDateTicks[[All, 2]]},
  BaseStyle -> {FontFamily -> "Arial", FontSize -> 14},
  FrameLabel -> {{None, Style["Date of first appearance in GENCODE (mm/yy)", 13]}, {Style["Number of junctions recovered", 22], None}},
  ChartStyle -> chartColors, ImagePadding -> padding, BarOrigin -> Right,
  Epilog -> Inset[insetBars, {-135000, 10}, Automatic, 210000]]

```



```
magFlipped = Import["magflipped.png"]
```



```
origDateTicks =
  ({#,DateString[daysToDate[#], dateFormat]} & /@ Range[0, 2070, 329.3])
{{0., 02/27/09}, {329.3, 01/22/10}, {658.6, 12/17/10}, {987.9, 11/11/11},
 {1317.2, 10/06/12}, {1646.5, 08/31/13}, {1975.8, 07/26/14}}


newDateTicks = {{0.^, "02/27/09"}, {329.3^, "01/22/10"},
  {658.6^, "12/17/10"}, {987.900000000001^, "11/11/11"}, {1317.2^, "10/06/12"},
  {1646.5^, "08/31/13"}, {1975.800000000002^, "07/26/14"}}
{{0., 02/27/09}, {329.3, 01/22/10}, {658.6, 12/17/10},
 {987.9, 11/11/11}, {1317.2, 10/06/12}, {1646.5, 08/31/13}, {1975.8, 07/26/14}}


sampleCountsInFirstGencode =
  #[[2]] & /@ Select[junctionsEvidenceVsDatesGeq20, #[[5]] == 1 &];
sampleCountsInOtherGencodes = #[[2]] & /@ Select[junctionsEvidenceVsDatesGeq20,
  #[[5]] == 0 && Length[Position[#[[Range[5, 22]]], 1]] != 0 &];

anotherLabelForm [x_, y___] :=
  Text[Style[x, FontFamily -> "Arial", FontSize -> 14, Bold, TextAlignment -> Left], y]
```

```
daysToDate[329]
Fri 22 Jan 2010

proportionOfTotalAt329 = ToString[
  NumberForm[N[Select[accumulatedJunctionsGeq[20], #[[1]] <= 329 &][[-1]][[2]] / 
    accumulatedJunctionsGeq[20][[-1]][[2]] * 100, 3], DigitBlock -> 3]]
20.3

totBound = Select[accumulatedJunctionsGeq[20], #[[1]] <= 329 &][[-1]][[2]]
651 644

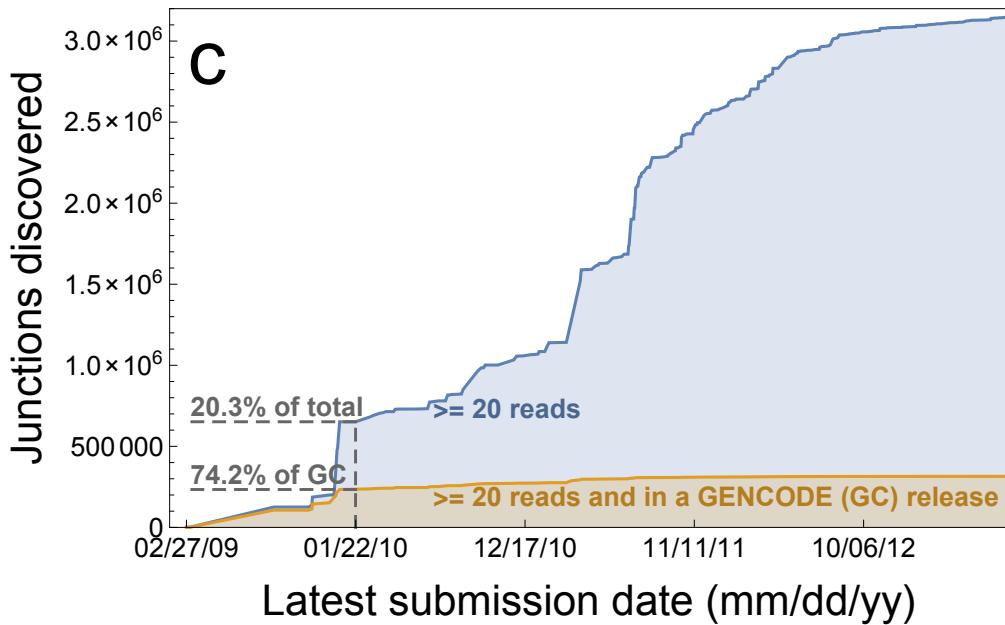
proportionOfAnnotatedAt329 =
  ToString[NumberForm[N[Select[accumulatedAnnotated, #[[1]] <= 329 &][[-1]][[2]] / 
    accumulatedAnnotated[[-1]][[2]] * 100, 3], DigitBlock -> 3]]
74.2

annBound = Select[accumulatedAnnotated, #[[1]] <= 329 &][[-1]][[2]]
233 834
```

```

annJunctionsPlot = ListPlot[{accumulatedJunctionsGeg[20], accumulatedAnnotated},
  Joined → True, Filling → Axis, Frame → True,
  FrameTicks → {{Automatic, None}, {origDateTicks, None}},
  BaseStyle → {FontFamily → "Arial", FontSize → 14}, FrameLabel →
  {Style["Latest submission date (mm/dd/yy)", 22, TextAlignment → Left],
   Style["Junctions discovered", 22]}, ImageSize → baseImageSize * 0.7,
  PlotRange → {{Automatic, 1600}, {0, 3.2 * 10^6}}];
annJunctionsComplete = Show[annJunctionsPlot,
  Graphics[{Darker[mathematicaColors[[1]]], 0.2}, anotherLabelForm[">= 20 reads",
    {480, 735000}, {-1, 0}], Darker[mathematicaColors[[2]]], 0.2},
  anotherLabelForm[">= 20 reads and in a GENCODE (GC) release", {480, 187000},
    {-1, 0}], Directive[Thickness[0.0035], Dashing[0.013], labelColor],
  Line[{{329, 0}, {329, totBound}}], Line[{{329, annBound}, {0, annBound}}],
  anotherLabelForm[ToString[proportionOfAnnotatedAt329] <> "% of GC",
    {8, annBound + 90000}, {-1, 0}], Line[{{329, totBound}, {0, totBound}}],
  anotherLabelForm[ToString[proportionOfTotalAt329] <> "% of total",
    {8, totBound + 90000}, {-1, 0}]], Graphics[
  {Black, Text[Style["c", FontFamily → "Arial", FontSize → 40], {40, 2.9 * 10^6}]}]]

```



```

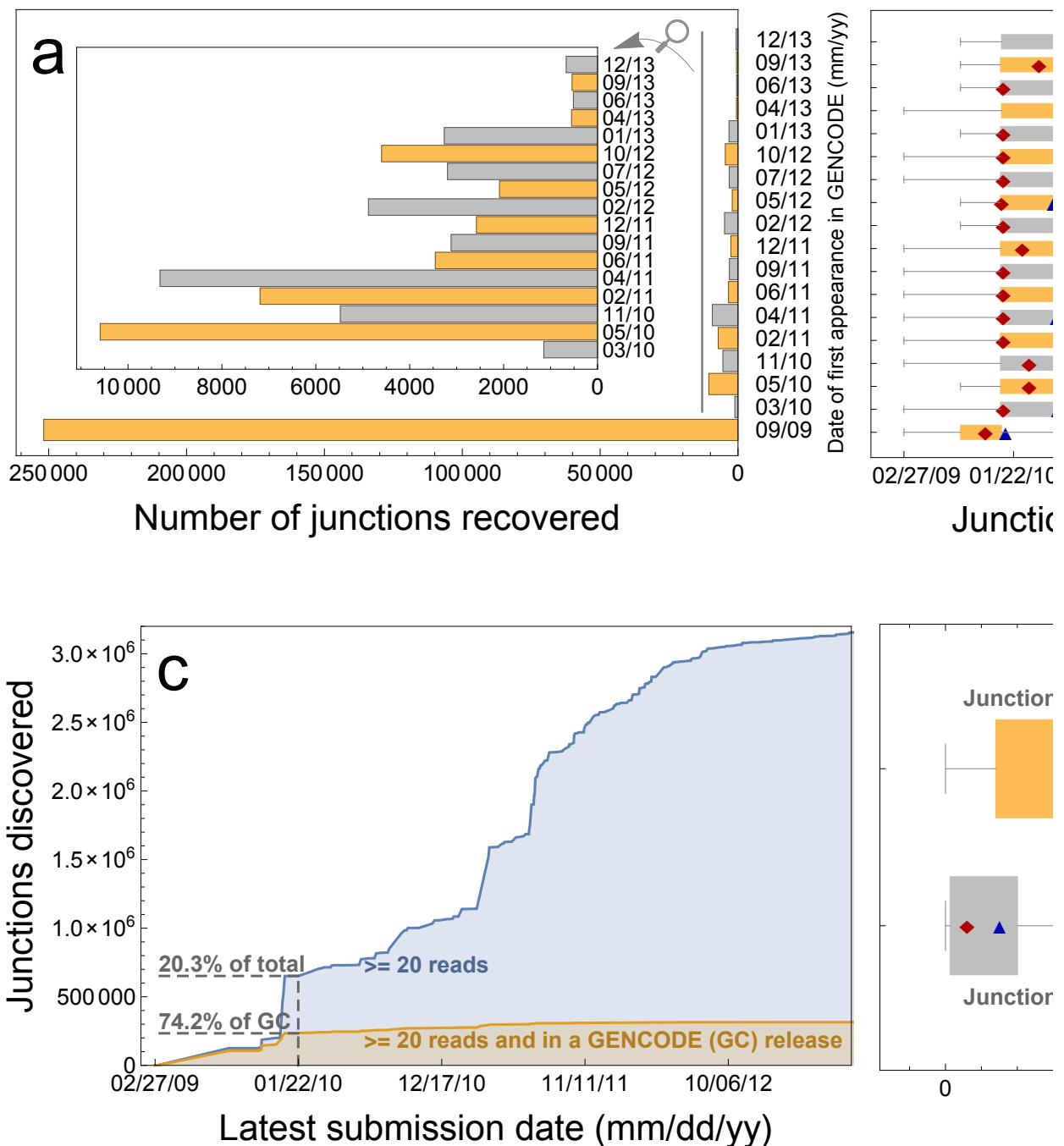
altLabelForm [x_, y___] := Text[Style[x, FontFamily → "Arial",
  FontSize → Scaled[.02], Bold, TextAlignment → Left], y]

```

```

sampleBoxPlot =
Show[BoxWhiskerChart[{sampleCountsInOtherGencodes, sampleCountsInFirstGencode},
  {"MeanMarker", "▲", Darker[Blue, 0.3]},
  {"MedianMarker", "◆", Darker[Red, 0.3]}}, ImageSize → baseImageSize * .613,
ChartStyle → {chartColors[[2]], chartColors[[1]]},
BarOrigin → Left, BarSpacing → Medium, Frame → True,
FrameLabel → {Style["Sample count", 22], None},
BaseStyle → {FontFamily → "Arial", FontSize → 14}],
Graphics[{Darker[Gray, 0.2], anotherLabelForm[
  "Junctions first appearing in first GENCODE release", {500, 2.45}, {-1, 0}],
anotherLabelForm["Junctions first appearing in other GENCODE releases",
{500, .55}, {-1, 0}]}], Graphics[
{Black, Text[Style["d", FontFamily → "Arial", FontSize → 40], {17 000, 2.65}]}]];
leftOff = -145 000; upOff = 90 000; suppevleft =
Show[barsWithInset, Graphics[
{Black, Text[Style["a", FontFamily → "Arial", FontSize → 40], {-250 000, 17.5}],
Gray, Arrow[BezierCurve[{{-16 000, 16.6}, {-30 000, 18.8}, {-45 000, 17.6}}]],
Thickness[.003], Line[{{-13 000, 18.3}, {-13 000, 1.8}}]}],
Graphics[{Opacity[0.5], Inset[magFlipped, {-30 000, 17.4}, {0, 0}, 12 000]}],
ImageSize → baseImageSize * 0.7];
otherpadding = {{0, 10}, {50, 0}};
suppevright =
Show[BoxWhiskerChart[tоБoxWhisker, {"MeanMarker", "▲", Darker[Blue, 0.3]},
 {"MedianMarker", "◆", Darker[Red, 0.3]}}, ImageSize → baseImageSize * .613,
ChartStyle → chartColors, BarOrigin → Left, BarSpacing → Medium, Frame → True,
PlotRange → {Automatic, Automatic}, PlotRangePadding → {{0.1, 0.9}, {0.3, 0.5}},
ImagePadding → otherpadding, FrameTicks → {{None, None}, {newDateTicks, None}},
BaseStyle → {FontFamily → "Arial", FontSize → 14},
FrameLabel → {Style["Junction discovery date (mm/dd/yy)", 22], None}], Graphics[
{Black, Text[Style["b", FontFamily → "Arial", FontSize → 40], {1985, 17.8}]}]];
suppeval = Grid[{{Grid[{{suppevleft, suppevright}}]}},
{Grid[{{annJunctionsComplete, sampleBoxPlot}}]}]

```



```
Export["ev.pdf", suppeval1]
```

```
ev.pdf
```

Assess strength of correlation between discovery date and Gencode date. Even rank correlation is small.

```
SpearmanRankTest[discoveryDaysToGencodeDays[[All, 1]],
discoveryDaysToGencodeDays[[All, 2]], "TestDataTable"] // N
```

Statistic	P-Value
Spearman Rank	$0.356502 \times 10^{-9300}$

Exclude 2/28/09; relationship between it and the rest may be the dominant effect.

```
daysToDate[186]
```

 Tue 1 Sep 2009

```
discoveryDaysToGencodeDaysNoFirst =
Select[discoveryDaysToGencodeDays, #[[2]] != 186 &];

SpearmanRankTest[discoveryDaysToGencodeDaysNoFirst[[All, 1]],
discoveryDaysToGencodeDaysNoFirst[[All, 2]], "TestDataTable"] // N
```

Statistic	P-Value
Spearman Rank	-0.0148139
	0.00019633

... and this is true. Now let's load and plot PCs.

```
pcData =
Import["!cat pcs_unannotated_with_pd.tsv | tr -s '[:blank:]' '\t'", "TSV"];

pcHeader = pcData[[1]]
{PC1, PC2, PC3, PC4, PC5, brain, lcl, blood, seqc_abrf, geuvadis, mixture}

pcData = pcData[[Range[2, Length[pcData]]]];

In[380]:= brain = Select[pcData, #[[7]] == 1 &]; lcl = Select[pcData, #[[8]] == 1 &];
blood = Select[pcData, #[[9]] == 1 &]; oneToZero = Select[pcData, #[[-1]] == "1:0" &];
threeToOne = Select[pcData, #[[-1]] == "3:1" &];
oneToThree = Select[pcData, #[[-1]] == "1:3" &];
zeroToOne = Select[pcData, #[[-1]] == "0:1" &];
others = Select[pcData, #[[7]] != 1 && #[[8]] != 1 && #[[9]] != 1 && #[[-1]] == "NA" &];
geuvadis = Select[pcData, #[[-2]] == 1 &];
abrf = Select[pcData, #[[-3]] == 2 &]; seqc = Select[pcData, #[[-3]] == 1 &];

mathematicaColors
{
```

```
In[432]:= arrowLabelForm[x_, y___] :=
  Text[Style[x, FontFamily -> "Arial", FontSize -> Scaled[.03],
    Bold, TextAlignment -> Left], y]; labelColor = Darker[Gray, 0.2];
pcaPlot = Show[ListPlot[Transpose[{#[[All, 2]], #[[All, 3]]}]] & /@ {others, blood,
  lcl, brain, oneToZero, oneToThree, threeToOne, zeroToOne}, Frame -> True,
  ImageSize -> baseImageSize, PlotRange -> {{-.008, .015}, {-0.02, .023}}, Axes -> None,
  FrameLabel -> {Style["PC1", 22, TextAlignment -> Left], Style["PC2", 22]},
  BaseStyle -> {FontFamily -> "Arial", FontSize -> 14},
  PlotStyle -> {{PointSize[.002], Gray}, {PointSize[.006], mathematicaColors[[4]]},
    {PointSize[.006], Magenta}, {PointSize[.006], Cyan}, {PointSize[.01],
      mathematicaColors[[15]]}, {PointSize[.01], PointSize[.01], PointSize[.01]}},
Graphics[{Opacity[0.25], Polygon[{{-.005, -.002}, {-0.005, .003},
  {-0.001, .0073}, {0.0005, -0.0015}}], Polygon[
  {{.0075, .004}, {.0103, .012}, {.0048, .016}, {.01, .026}, {.0147, .016}, {0.015
  , 0.005}}], Polygon[{{.0054, -.015}, {.005, -.008},
  {.0095, -.013}, {.01, -.019}}], Opacity[1],
  labelColor, Arrow[{{-.006, .008}, {-0.0035, .005}}],
  arrowLabelForm["ABRF", {-0.0069, .0092}, {-1, 0}],
  Arrow[BezierCurve[{{.002, .016}, {.004, .01}, {.007, .0142}}]],
  arrowLabelForm["SEQC", {.001, .017}, {-1, 0}],
  Arrow[BezierCurve[{{0, -.016}, {.002, -.013}, {.004, -.024}, {.0063, -.016}}]],
  arrowLabelForm["GEU", {-0.001, -.0172}, {-1, 0}]}
```

