

# The annmap Cookbook; Examples and Patterns for annmap

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# 1 Introduction

annmap is a Bioconductor package that provides annotation data and cross-mappings between, amongst other things, genes, transcripts, exons, proteins, domains and Affymetrix probesets. This document provides examples of its usage, and recipes to help you get started.

## 2 Initial Steps

annmap makes use of a MySQL database, annmap, to provide its annotation data (downloadable from the Annmap website<sup>1</sup>). A variety of different species are supported - each with their own separate database.

**This document assumes that you have downloaded and installed one of the available MySQL databases (as described in the INSTALL vignette that came with this package).**

It is also possible to connect to the annmap webservice instead of installing your own database – with some caveats. Please see the section 2.3 for details.

### 2.1 Preamble

This cookbook is designed to walk you through the annmap functions from first principles to you being able to generate plots such as Figure 1 for your Next-Generation Sequencing data.

```
> gene = symbolToGene( 'SHH' )
> strand( gene ) = '*'
> ngsBridgePlot( gene, bamfileData )
```

---

<sup>1</sup><http://annmap.cruk.manchester.ac.uk/download>

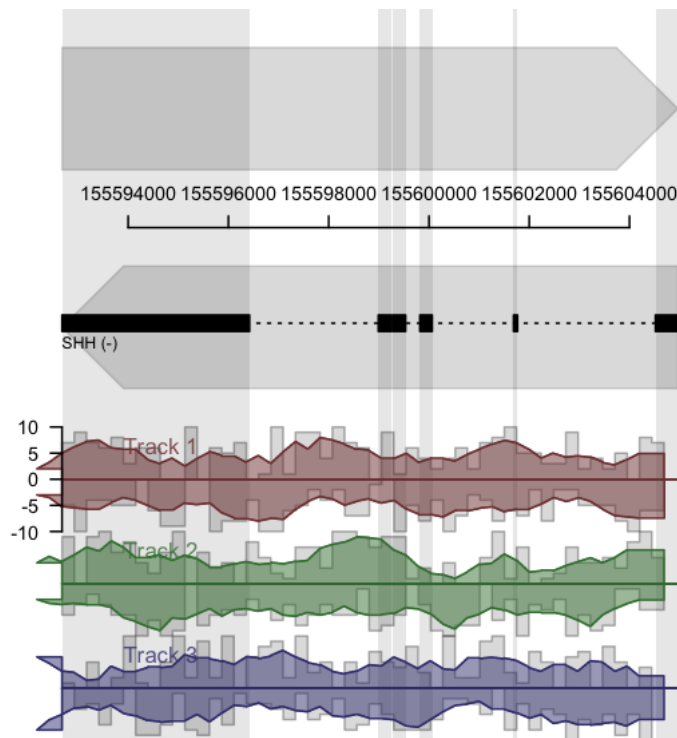


Figure 1: Dummy (in this example) NGS data shown alongside SHH. Grey boxes represent individual values. Coloured regions represent smoothed data.

## 2.2 Loading the `annmap` package and connecting to the database

Assuming everything is installed correctly, you should be able to load the package as normal:

```
> library( annmap )
```

If this is your first time running the package, you might need to create a datasource. These are stored by default in a folder called `.annmap` in your home directory, but this location can be customised by setting an environment variable `ANNMAP_HOME` to point to a folder of your choosing. To add a datasource, we will use the `annmapAddConnection` method.

Assuming you have installed the `annmap_homo_sapiens_66` database into a MySQL instance running on your local machine accessed by the username 'test' and with a blank password, you can run:

```
> annmapAddConnection( 'human66', 'homo_sapiens', '66', username='test' )
```

You then need to tell `annmap` to connect to this annotation database:

```
annmapConnect( 'human66' )
Connected to annmap_homo_sapiens_66 (localhost)
Selected array 'HuEx-1_0' as a default.
```

A typical installation will support a variety of species, each with their own version of the `annmap` database. You can specify which database to use in the function call. Here, for example, `human66` is the name we gave to our new connection. If you enter `annmapConnect()` without any parameters, it will show you a list of possible databases to choose from<sup>2</sup>.

<sup>2</sup>unless you only have one database defined, in which case it will simply connect you to that

## 2.3 Using the annmap webservice

It's also possible to connect to the annmap webservice to get your data.

This is slower than using a locally installed database, less reliable as it relies on the annmap website which is occasionally taken down for updating.

Also, the annmap website operates a 'one in one out' rule, so when a new species version is added, the oldest one is removed to save disk space.

Those caveats aside, it's a great way of testing things out without the need to install MySQL and the database.

To connect to the webservice, simply call:

```
> annmapConnect( use.webservice=TRUE )
```

and you will be presented with a list of available connections to use.

As with the non-webservice connection, you can also do:

```
> annmapConnect( 'homo_sapiens.72', use.webservice=TRUE )
```

To connect to a known named webservice connection (so long as it exists).

## 2.4 Disconnecting from the database

To disconnect from the database, use:

```
annmapDisconnect()
```

```
Disconnecting from annmap_homo_sapiens_66 (localhost)
```

Note that you do not have to disconnect before connecting to another database; annmap will do this automatically for you when `annmapConnect` is called.

## 2.5 Overview

The majority of the calls in annmap fall into one of four categories: 'all' queries that fetch all known instances of an object type, 'details' queries that provide more detailed annotation for a list of IDs, 'to' queries that provide mappings between things (e.g. from genes to exons), and 'range' queries that find the things that lie between a pair of coordinates. These are summarised in table 1.

		TO																
		Gene	Transcript	Exon	EST Gene	EST Transcript	EST Exon	Prediction Transcript	Prediction Exon	Probeset	Probe	Hit	Protein	Domain	Symbol		InRange	Details
From	Gene	N	Y	Y	N	N	N	N	N	Y	N	N	Y	Y	Y		Y	Y
	Transcript	Y	N	Y	N	N	N	N	N	Y	N	N	Y	Y	N		Y	Y
	Exon	Y	Y	N	N	N	N	N	N	Y	N	N	N	N	N		Y	Y
	EST Gene	N	N	N	N	Y	Y	N	N	Y	N	N	N	N	N		Y	Y
	EST Transcript	N	N	N	Y	N	Y	N	N	Y	N	N	N	N	N		Y	Y
	EST Exon	N	N	N	Y	Y	N	N	N	Y	N	N	N	N	N		Y	Y
	Prediction Transcript	N	N	N	N	N	N	N	Y	Y	N	N	N	N	N		Y	Y
	Prediction Exon	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N
	Probeset	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N		Y	Y
	Probe	N	N	N	N	N	N	N	N	Y	N	Y	N	N	N		Y	Y
	Hit	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	N
	Protein	Y	Y	N	N	N	N	N	N	Y	N	N	N	Y	N		Y	Y
	Domain	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N	N		Y	Y
	Symbol	Y	Y	N	Y	Y	N	N	N	N	N	N	N	N	N		N	N

Table 1: The mappings and functions available in annmap database. Mapping queries are of the form XXXToYYY(), searches using genome coordinates, XXXInRange(), and detailed annotation XXXDetails()

### 3 Fetching Data

#### 3.1 Retrieving all instances of a given type.

It is often useful to get a list of, for example, all genes or transcripts in the database. For this we would use functions such as `allGenes` or `allTranscripts`. All the functions of this type are defined in the documentation object `?annmap.all`.

For example, to get a list of all the chromosomes in the human database, we can simply type:

```
annmapConnect( 'human66' )
Connected to annmap_homo_sapiens_66 (localhost)
Selected array 'HuEx-1_0' as a default.

> allChromosomes()
GRanges with 25 ranges and 0 metadata columns:
      seqnames      ranges strand
      <Rle>         <IRanges> <Rle>
 [1]      1 [1, 249250621]      *
 [2]     10 [1, 135534747]      *
 [3]     11 [1, 135006516]      *
 [4]     12 [1, 133851895]      *
 [5]     13 [1, 115169878]      *
 ...     ...
[21]      8 [1, 146364022]      *
[22]      9 [1, 141213431]      *
```

```

[23]      MT [1,      16569]      *
[24]      X [1, 155270560]      *
[25]      Y [1,  59373566]      *
---
seqlengths:
  1 10 11 12 13 14 15 16 17 18 19  2 20 21 22  3  4  5  6  7  8  9 MT  X  Y
  NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA

```

By default these functions all return a `GRanges` object (if the concept of genomic coordinates is appropriate), or a `data.frame` (if a genomic region doesn't exist for the object). It is also possible to just get the names of the chromosomes by passing `as.vector=TRUE` as a parameter:

```

> allChromosomes( as.vector=TRUE )
[1] "11" "21" "7"  "Y"  "2"  "17" "22" "1"  "18" "13" "16" "6"  "X"  "3"  "9"
[16] "12" "14" "15" "20" "8"  "4"  "10" "19" "5"  "MT"

```

### 3.1.1 Possible values for `as.vector` and `as.data.frame`

Almost all of the functions in `annmap` accept an `as.vector` parameter, and will return a vector of identifiers rather than the full table of available information. Setting `as.vector='data.frame'` will return a `data.frame`, rather than a `GRanges` object:

parameter	return type
<code>as.vector=TRUE</code>	=> a character vector of ids
<code>as.vector=FALSE</code>	=> A <code>GRanges</code> object (default)
<code>as.vector='data.frame'</code>	=> A <code>data.frame</code>

All of the `XXXDetails()` functions and functions that map to Hits or coding regions take a parameter `as.data.frame` instead (where a vector would not be possible):

parameter	return type
<code>as.data.frame=FALSE</code>	=> A <code>GRanges</code> object (default)
<code>as.data.frame=TRUE</code>	=> A <code>data.frame</code>

## 3.2 Getting detailed annotation based on database identifiers.

Sometimes, you will have a list of IDs for which you require more detailed annotation. You can use the `?annmapDetails` methods for this.

For example, given a list of chromosome names, we can find their length:

```

> chr.list = c( '1', '2', '3' )
> chromosomeDetails( chr.list )
GRanges with 3 ranges and 0 metadata columns:
      seqnames      ranges strand
      <Rle>        <IRanges> <Rle>
[1]      1 [1, 249250621]      *
[2]      2 [1, 243199373]      *
[3]      3 [1, 198022430]      *
---
seqlengths:
  1  2  3
  NA NA NA

```

Or we can look for the details of a few microarray probes by their sequence:

```
> some.probes = probesetToProbe( '3855295' )
> probeDetails( some.probes )

      sequence probe_hit_count
1 GTCACCCTGCAACCGTGCCACGTGG      1
2 GTCATGTGGCGCCTGTTTCGACGCC      1
3 CAGGCTCTAGGACTGCGCGATGAGC      1
4 ACCAGGTCTGGCTCGCGCGGACGT      1
```

Note that Probe sequences inside the `anmap` database are not the exact physical sequences to be found on the corresponding array. Instead, we always return the sequence of the genomic target.

### 3.2.1 The order of things



When calling the following transformation queries (`XXXToYYY`, and `XXXDetails`), you should not rely on the order of the output being the same as the input order.

If you need to look things up based on the input id you gave them, you should either subset based on the identifier column (ie: `d[ d$stable_id == 'PTEN', ]`), or you can split the results based on a column (see section 3.3.3)

## 3.3 One thing to another

In this section, we look at the available functions for mapping between annotation items. The majority of mappings are available, and all take the form `XXXToYYY`<sup>3</sup> (Table 1)

### 3.3.1 Finding a gene by its symbol

For example, we can fetch a gene by its symbol:

```
> symbolToGene( 'PTEN' )

GRanges with 1 range and 9 metadata columns:
      seqnames      ranges strand |      IN1      stable_id
      <Rle>        <IRanges> <Rle> | <character> <character>
[1]      10 [89622870, 89731687] + |      PTEN ENSG00000171862
      biotype      status
      <character> <character>
[1] protein_coding      KNOWN
      description
      <character>
[1] phosphatase and tensin homolog [Source:HGNC Symbol;Acc:9588]
      db_display_name      symbol      symbol_description
      <character> <character> <character>
[1]      HGNC Symbol      PTEN phosphatase and tensin homolog
      analysis_name
      <character>
[1] ensembl_havana_gene
---
seqlengths:
  10
  NA
```

Or by vector of symbols:

<sup>3</sup>A full list of functions can be found by entering `?anmapTo` in an R console.



```

> symbolToGene( c( 'PTEN', 'SHH' ) )
GRanges with 2 ranges and 9 metadata columns:
      seqnames          ranges strand |          IN1          stable_id
      <Rle>            <IRanges> <Rle> | <character>    <character>
[1]      10 [ 89622870,  89731687]   + |      PTEN ENSG00000171862
[2]       7 [155592680, 155604967]   - |      SHH ENSG00000164690
      biotype          status
      <character> <character>
[1] protein_coding      KNOWN
[2] protein_coding      KNOWN
      description
      <character>
[1] phosphatase and tensin homolog [Source:HGNC Symbol;Acc:9588]
[2]      sonic hedgehog [Source:HGNC Symbol;Acc:10848]
      db_display_name      symbol      symbol_description
      <character> <character>      <character>
[1]      HGNC Symbol      PTEN phosphatase and tensin homolog
[2]      HGNC Symbol      SHH      sonic hedgehog
      analysis_name
      <character>
[1] ensembl_havana_gene
[2] ensembl_havana_gene
---
seqlengths:
  10  7
 NA NA

```

Again, `as.vector` works as before:

```

> symbolToGene( c( 'PTEN', 'SHH' ), as.vector=TRUE )
      PTEN          SHH
"ENSG00000171862" "ENSG00000164690"

```

### 3.3.2 Mapping from a gene to its transcripts

```

> gene = symbolToGene( c( 'PTEN', 'SHH' ) )
> geneToTranscript( gene )
GRanges with 10 ranges and 13 metadata columns:
      seqnames          ranges strand |          IN1          stable_id
      <Rle>            <IRanges> <Rle> |          <character>    <character>
[1]      10 [ 89622870,  89731687]   + | ENSG00000171862 ENST00000371953
[2]      10 [ 89624206,  89626806]   + | ENSG00000171862 ENST00000487939
[3]      10 [ 89624225,  89654083]   + | ENSG00000171862 ENST00000462694
[4]      10 [ 89685280,  89693244]   + | ENSG00000171862 ENST00000498703
[5]      10 [ 89711956,  89721066]   + | ENSG00000171862 ENST00000472832
[6]       7 [155592680, 155604967]   - | ENSG00000164690 ENST00000297261
[7]       7 [155592733, 155601766]   - | ENSG00000164690 ENST00000441114
[8]       7 [155592734, 155601766]   - | ENSG00000164690 ENST00000430104
[9]       7 [155592744, 155601766]   - | ENSG00000164690 ENST00000435425
[10]      7 [155599276, 155600064]   - | ENSG00000164690 ENST00000472308
      biotype          status description      db_display_name
      <character> <character> <character>      <character>

```

```

[1]      protein_coding      KNOWN      <NA> HGNC transcript name
[2] processed_transcript      KNOWN      <NA> HGNC transcript name
[3] processed_transcript      KNOWN      <NA> HGNC transcript name
[4] processed_transcript      KNOWN      <NA> HGNC transcript name
[5] processed_transcript      KNOWN      <NA> HGNC transcript name
[6]      protein_coding      KNOWN      <NA> HGNC transcript name
[7] nonsense_mediated_decay      KNOWN      <NA> HGNC transcript name
[8]      protein_coding      PUTATIVE      <NA> HGNC transcript name
[9] nonsense_mediated_decay      KNOWN      <NA> HGNC transcript name
[10] processed_transcript      KNOWN      <NA> HGNC transcript name

```

```

      symbol      symbol_description      translation_start
<character>      <character>      <integer>
[1] PTEN-001 phosphatase and tensin homolog      1358
[2] PTEN-002 phosphatase and tensin homolog      <NA>
[3] PTEN-003 phosphatase and tensin homolog      <NA>
[4] PTEN-004 phosphatase and tensin homolog      <NA>
[5] PTEN-005 phosphatase and tensin homolog      <NA>
[6] SHH-001      sonic hedgehog      152
[7] SHH-005      sonic hedgehog      91
[8] SHH-003      sonic hedgehog      91
[9] SHH-004      sonic hedgehog      91
[10] SHH-002      sonic hedgehog      <NA>

```

```

      translation_start_exon      translation_end      translation_end_exon
      <integer>      <integer>      <integer>
[1]      8302113      186      8303194
[2]      <NA>      <NA>      <NA>
[3]      <NA>      <NA>      <NA>
[4]      <NA>      <NA>      <NA>
[5]      <NA>      <NA>      <NA>
[6]      8542079      827      8542116
[7]      8542149      80      8542159
[8]      8542149      206      8542173
[9]      8542149      71      8542184
[10]      <NA>      <NA>      <NA>

```

```

      analysis_name
      <character>
[1] ensembl_havana_transcript
[2]      havana
[3]      havana
[4]      havana
[5]      havana
[6] ensembl_havana_transcript
[7]      havana
[8]      havana
[9]      havana
[10]      havana

```

---

```

seqlengths:
10 7
NA NA

```

And with `as.vector`:

```

> transcripts = geneToTranscript( gene, as.vector=TRUE )
> transcripts

```

```

ENSG00000171862   ENSG00000171862   ENSG00000171862   ENSG00000171862
"ENST00000371953" "ENST00000487939" "ENST00000462694" "ENST00000498703"
ENSG00000171862   ENSG00000164690   ENSG00000164690   ENSG00000164690
"ENST00000472832" "ENST00000297261" "ENST00000441114" "ENST00000430104"
ENSG00000164690   ENSG00000164690
"ENST00000435425" "ENST00000472308"

```

We have tried to keep all of the methods in `anmap` "type agnostic" - so this will work passing a `character` vector as the parameter in place of a `GRanges` object:

```

> gene = symbolToGene( 'PTEN', as.vector=TRUE )
> class( gene )
[1] "character"
> geneToTranscript( gene )
GRanges with 5 ranges and 13 metadata columns:
      seqnames          ranges strand |          IN1          stable_id
      <Rle>            <IRanges> <Rle> | <character> <character>
[1]      10 [89622870, 89731687]   + | ENSG00000171862 ENST00000371953
[2]      10 [89624206, 89626806]   + | ENSG00000171862 ENST00000487939
[3]      10 [89624225, 89654083]   + | ENSG00000171862 ENST00000462694
[4]      10 [89685280, 89693244]   + | ENSG00000171862 ENST00000498703
[5]      10 [89711956, 89721066]   + | ENSG00000171862 ENST00000472832
      biotype          status description      db_display_name
      <character> <character> <character> <character>
[1]      protein_coding      KNOWN      <NA>      HGNC transcript name
[2] processed_transcript      KNOWN      <NA>      HGNC transcript name
[3] processed_transcript      KNOWN      <NA>      HGNC transcript name
[4] processed_transcript      KNOWN      <NA>      HGNC transcript name
[5] processed_transcript      KNOWN      <NA>      HGNC transcript name
      symbol          symbol_description translation_start
      <character> <character> <integer>
[1]      PTEN-001 phosphatase and tensin homolog      1358
[2]      PTEN-002 phosphatase and tensin homolog      <NA>
[3]      PTEN-003 phosphatase and tensin homolog      <NA>
[4]      PTEN-004 phosphatase and tensin homolog      <NA>
[5]      PTEN-005 phosphatase and tensin homolog      <NA>
      translation_start_exon translation_end translation_end_exon
      <integer> <integer> <integer>
[1]      8302113      186      8303194
[2]      <NA>      <NA>      <NA>
[3]      <NA>      <NA>      <NA>
[4]      <NA>      <NA>      <NA>
[5]      <NA>      <NA>      <NA>
      analysis_name
      <character>
[1] ensembl_havana_transcript
[2]      havana
[3]      havana
[4]      havana
[5]      havana
---
seqlengths:
  10
 NA

```

### 3.3.3 The IN1 field in 'to' results

The `GRanges` and `data.frame` returned by 'to' queries, contain a column `IN1`. This corresponds to the initial query that led to the row being generated. If we extract the `IN1` column, we get the original 2 genes that we sent to the query:

```
> geneToTranscript( symbolToGene( c( 'PTEN', 'SHH' ) ) )$IN1
[1] "ENSG00000171862" "ENSG00000171862" "ENSG00000171862" "ENSG00000171862"
[5] "ENSG00000171862" "ENSG00000164690" "ENSG00000164690" "ENSG00000164690"
[9] "ENSG00000164690" "ENSG00000164690"
```

This can be very useful when we wish to use, for example, `merge`, to combine a table of expression data with the annotation data supplied by `anmap`.

It can also be used with the `split` method to get the returned data into a `list`, for example, to generate a list, one element per gene, each containing the exons found in that gene:

```
> exons = geneToExon( symbolToGene( c( 'PTEN', 'SHH' ) ) )
> split( exons$stable_id, exons$IN1 )

$ENSG00000164690
[1] "ENSE00001086614" "ENSE00001086617" "ENSE00001149618" "ENSE00001795984"
[5] "ENSE00001760492" "ENSE00001612491" "ENSE00001676036" "ENSE00001758320"
[9] "ENSE00001655961" "ENSE00001800592" "ENSE00001908904" "ENSE00001889423"

$ENSG00000171862
[1] "ENSE00001456562" "ENSE00001156351" "ENSE00001156344" "ENSE00003595610"
[5] "ENSE00001156330" "ENSE00001156327" "ENSE00003601394" "ENSE00001156315"
[9] "ENSE00001456541" "ENSE00001813529" "ENSE00001881230" "ENSE00001926457"
[13] "ENSE00001920439" "ENSE00001837210" "ENSE00003597859" "ENSE00001893700"
[17] "ENSE00001876418" "ENSE00003657126" "ENSE00001939988"
```

### 3.3.4 Microarray probeset mappings

`anmap` also provides probe mappings for Affymetrix microarrays. When you connect to a database, `anmap` will pick an array by default (if a mapping exists for that species).

To select your array of choice, you can use the `arrayType()` method:

```
> arrayType( 'HuEx-1_0' )
Using array 'HuEx-1_0'.
```

As with `anmapConnect()` calling this function with no parameters will give you a menu of available arrays to choose from.

Probesets are treated like any other feature, so:

```
> exonToProbeset( geneToExon( symbolToGene( 'MPI' ) ), as.vector=TRUE)
ENSE00002608534 ENSE00002577963 ENSE00003620329 ENSE00003468114 ENSE00003622704
"3601956" "3601960" "3601963" "3601964" "3601965"
ENSE00001245546 ENSE00002610542 ENSE00001929277 ENSE00003476529 ENSE00003476529
"3601969" "3601971" "3601956" "3601960" "3601959"
ENSE00001245539 ENSE00001816780 ENSE00001816780 ENSE00001816780 ENSE00001816780
"3601971" "3601974" "3601975" "3645143" "3975448"
ENSE00001816780 ENSE00001816780 ENSE00001816780 ENSE00001816780 ENSE00001816780
"2746540" "2880800" "3535951" "2410077" "3655014"
ENSE00001816780 ENSE00001816780 ENSE00001816780 ENSE00001816780 ENSE00001816780
"3462599" "2662284" "3638759" "2766811" "3822407"
```

ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"2830115"	"3585206"	"3825799"	"3961893"	"2735060"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"2646089"	"3471746"	"3720274"	"3017565"	"3328152"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"3248968"	"2713240"	"2685561"	"2328589"	"3415635"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"3869300"	"3067623"	"3259813"	"3707032"	"3731058"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"3190116"	"3384517"	"3235475"	"2676567"	"2796100"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"2923872"	"2591241"	"3056411"	"3474991"	"2756434"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"2813774"	"2861407"	"3601973"	"2544232"	"3336154"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"2393742"	"3318836"	"3470332"	"3841120"	"3553844"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780
"3866317"	"3464331"	"3665054"	"3109991"	"2622458"
ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00001816780	ENSE00002612083
"3970147"	"2396528"	"3454391"	"3472536"	"3601956"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"3601974"	"3601975"	"3645143"	"3975448"	"2746540"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"2880800"	"3535951"	"2410077"	"3655014"	"3462599"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"2662284"	"3638759"	"2766811"	"3822407"	"2830115"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"3585206"	"3825799"	"3961893"	"2735060"	"2646089"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"3471746"	"3720274"	"3017565"	"3328152"	"3248968"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"2713240"	"2685561"	"2328589"	"3415635"	"3869300"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"3067623"	"3259813"	"3707032"	"3731058"	"3190116"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"3384517"	"3235475"	"2676567"	"2796100"	"2923872"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"2591241"	"3056411"	"3474991"	"2756434"	"2813774"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"2861407"	"3601973"	"2544232"	"3336154"	"2393742"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"3318836"	"3470332"	"3841120"	"3553844"	"3866317"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00002594740
"3464331"	"3665054"	"3109991"	"2622458"	"3970147"
ENSE00002594740	ENSE00002594740	ENSE00002594740	ENSE00003507946	ENSE00002619734
"2396528"	"3454391"	"3472536"	"3601956"	"3601964"
ENSE00002609559	ENSE00002591759	ENSE00002585682	ENSE00003519843	ENSE00002602512
"3601963"	"3601964"	"3601965"	"3601956"	"3601960"
ENSE00002577741	ENSE00002586875	ENSE00002588661	ENSE00002582790	ENSE00002582790
"3601965"	"3601963"	"3601965"	"3601956"	"3601957"
ENSE00003499267	ENSE00003499267	ENSE00003469443	ENSE00003511800	ENSE00003522170
"3601960"	"3601959"	"3601963"	"3601964"	"3601965"
ENSE00002576770	ENSE00002576770	ENSE00003475255	ENSE00002588812	ENSE00002588812

```

"3601970"      "3601969"      "3601963"      "3601970"      "3601969"
ENSE00002607384 ENSE00002618236 ENSE00002618236 ENSE00002618236 ENSE00002608338
"3601973"      "3601960"      "3601959"      "3601958"      "3601974"
ENSE00002608338 ENSE00002206295 ENSE00002206295 ENSE00001766858 ENSE00001766858
"3601973"      "3601974"      "3601973"      "3601974"      "3601973"
ENSE00002625217 ENSE00003464705 ENSE00003464705 ENSE00002628390 ENSE00002628048
"3601956"      "3601960"      "3601959"      "3601964"      "3601956"
ENSE00002625319 ENSE00002625319 ENSE00002625319 ENSE00002625319 ENSE00002580706
"3601961"      "3601960"      "3601959"      "3601958"      "3601963"
ENSE00002627095 ENSE00002618421 ENSE00002620219 ENSE00002620219 ENSE00002598250
"3601965"      "3601969"      "3601965"      "3601966"      "3601958"
ENSE00002598250 ENSE00002598250 ENSE00002598250 ENSE00002598739 ENSE00002618779
"3601957"      "3601960"      "3601959"      "3601964"      "3601957"
ENSE00002590001 ENSE00002600867 ENSE00002580471 ENSE00002592096 ENSE00002592096
"3601964"      "3601960"      "3601973"      "3601971"      "3601974"
ENSE00002592096 ENSE00002592096 ENSE00002592096 ENSE00002592096 ENSE00002592096
"3601970"      "3601969"      "3601972"      "3601968"      "3601973"

```

will find all probesets that map to exons in the gene MPI (as a vector).

Omitting the `as.vector=TRUE` parameter in the call will give us the results in a `data.frame`.

Here's the details of the first probeset from our last query:

```

> exonToProbeset(geneToExon(symbolToGene('MPI'))) [1,]
      IN1 stable_id array_name probe_count hit_score gene_score
1 ENSE00002608534 3601956  HuEx-1_0          4          1          1
  transcript_score exon_score est_gene_score est_transcript_score
1                2          2              0                    0
  est_exon_score prediction_transcript_score prediction_exon_score
1                0                    0                    0
  protein_score domain_score
1                0          0

```

As you can see, a probeset's details, comprises a set of scores. These scores are always 0, 1 or 2;

- 0. One or more of the probes miss the item of interest
- 1. All of the probes hit the item of interest once (and only once)
- 2. One or more of the probes hit more than one item of interest

So a `hit_score` of 1 means that each and every probe in the probeset hit the genome once and only once, but a score of 2 for `gene_score` means that one or more of the probes hits more than one gene. Since if it also has a hit score of 1, this means that the match is at a region where 2 genes are overlapping.

### 3.3.5 Some subtleties with probeset mappings

Probeset mappings in `annmap` are done in two ways:

- 1. Probes are mapped to the entire genome, and their match locations recorded, and
- 2. they are also mapped directly to cDNA sequences in order to pick up probes and probesets that fall on exon junctions.

`probesetToCdnatranscript` and `transcriptToCdnaprobeset` provide the mappings needed to retrieve these probesets.

## 4 Searching by genome coordinates

### 4.1 Finding things by their location

It is also possible to provide a set of genomic regions and find the features they contain.

For example:

```
> geneInRange( '7', 1000000, 1060000, 1 )
GRanges with 1 range and 8 metadata columns:
      seqnames      ranges strand |      stable_id      biotype
      <Rle>        <IRanges> <Rle> | <character> <character>
[1]          7 [1022835, 1029276] + | ENSG00000073067 protein_coding
      status
      <character>
[1]      KNOWN
                                     description
                                     <character>
[1] cytochrome P450, family 2, subfamily W, polypeptide 1 [Source:HGNC Symbol;Acc:20243]
      db_display_name      symbol
      <character> <character>
[1]      HGNC Symbol      CYP2W1
                                     symbol_description      analysis_name
                                     <character> <character>
[1] cytochrome P450, family 2, subfamily W, polypeptide 1 ensembl_havana_gene
---
seqlengths:
  7
NA
```

will find all of the genes on the forward strand of chromosome '7' that lie between positions 1000000 and 1060000. XXXInRange queries also accept data.frame, RangedData and GRanges objects (see ?annmapRange):

```
> # Use a data.frame as the initial parameter
> .df = data.frame( chromosome_name='7', start=1000000, end=1060000, strand=1 )
> geneInRange( .df, as.vector=TRUE )
[1] "ENSG00000073067"
> # Use a RangedData object as the initial parameter
> .rd = RangedData( space='7', ranges=IRanges( start=1000000, end=1060000 ), strand=1 )
> geneInRange( .rd, as.vector=TRUE )
[1] "ENSG00000073067"
> # Use a GRanges object as the initial parameter
> .rd = RangedData( space='7', ranges=IRanges( start=1000000, end=1060000 ), strand=1L )
> .rd = as( .rd, 'GRanges' )
> .rd
GRanges with 1 range and 0 metadata columns:
      seqnames      ranges strand
      <Rle>        <IRanges> <Rle>
[1]          7 [1000000, 1060000] +
---
seqlengths:
  7
NA
```

```
> geneInRange( .rd, as.vector=TRUE )
[1] "ENSG00000073067"
```

This means that it is possible to chain InRange queries as you would most of the other queries, ie;

```
> geneToSymbol(           # Return the gene symbols
+   exonToGene(           # get the gene for each exon
+   exonInRange(          # get all exons contained in the GRanges
+   symbolToGene( 'PTEN' ) ) ) ) # get a GRanges object for PTEN
ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000224745
"PTEN" "PTEN" "PTEN" "PTEN" "RP11-380G5.2"
ENSG00000224745 ENSG00000224745 ENSG00000171862 ENSG00000171862 ENSG00000171862
"RP11-380G5.2" "RP11-380G5.2" "PTEN" "PTEN" "PTEN"
ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862
"PTEN" "PTEN" "PTEN" "PTEN" "PTEN"
ENSG00000213613 ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862
"RP11-380G5.3" "PTEN" "PTEN" "PTEN" "PTEN"
ENSG00000171862 ENSG00000171862 ENSG00000171862
"PTEN" "PTEN" "PTEN"
```

For example, to find all probes that target within 1Kb of the 3' end of each gene in genes, we can first alter the range of these objects so they are just covering our region of interest:

```
> genes = geneInRange( '7', 1000000, 1060000, 1 )
> start(genes) = end( genes ) - 1000
```

Before calling probeInRange on the modified GRanges object:

```
> probeInRange( genes )
GRanges with 27 ranges and 2 metadata columns:
      seqnames      ranges strand |          sequence
      <Rle>        <IRanges> <Rle> | <character>
[1]      7 [1028280, 1028304]   + | TCTGTGTTGGGGAGCGCCTGGCCAG
[2]      7 [1028310, 1028334]   + | AGCTCTTCCTGCTGTTTGCCGGCCT
[3]      7 [1028334, 1028358]   + | TCCTGCAGAGGTACCGCCTGCTGCC
[4]      7 [1028398, 1028422]   + | CGCCCGGGCTTTTACCATGAGGCCG
[5]      7 [1028399, 1028423]   + | GCCCGGGCTTTTACCATGAGGCCGA
...
...
[23]      7 [1028980, 1029004]   + | AGCCACTGGGGCCATGCGTATGACT
[24]      7 [1029043, 1029067]   + | AGGCACTGGCGCCAGAGGCTTCCTT
[25]      7 [1029089, 1029113]   + | AGCCCCTGAAGACAAGCAGCACTGC
[26]      7 [1029122, 1029146]   + | AAATGGAACACTGACCCGGTGCGG
[27]      7 [1029125, 1029149]   + | TGGAAACACTGACCCGGTGCGGTGG
      probe_hit_count
      <numeric>
[1]      1
[2]      1
[3]      1
[4]      1
[5]      1
...
[23]      1
[24]      1
[25]      1
```



```

[26]          1
[27]          1
---
seqlengths:
  7
NA

```

As with other queries, the `as.vector` parameter can be used to return more concise results:

```

> probeInRange( genes, as.vector=TRUE )
[1] "TCTGTGTTGGGGAGCGCCTGGCCAG" "AGCTCTTCTGCTGTTTGCCGGCCT"
[3] "TCCTGCAGAGGTACCGCCTGCTGCC" "CGCCCGGGCTTTTACCATGAGGCCG"
[5] "GCCCGGGCTTTTACCATGAGGCCGA" "CCGGGCTTTTACCATGAGGCCGAGG"
[7] "GGGCTTTTACCATGAGGCCGAGGGC" "CCCACAGCTCGGACTGCTCTGGGAG"
[9] "GTCAGCAACTGCTTCCGGTTACACC" "TGCTTCCGGTTACACCCAGGACTAC"
[11] "CTGAAGCTGCACTCCCACCCACCTA" "CTGCAGGGAGACAACGGGTGGCTGC"
[13] "GAGACAACGGGTGGCTGCATCCAGC" "GACAACGGGTGGCTGCATCCAGCCA"
[15] "CTGCATCCAGCCAGAGACAGGCGCA" "TGGGTGTCCTCAGCGTGCGAGCCCT"
[17] "GGTGTCTCAGCGTGCGAGCCCTGC" "TGTCCTCAGCGTGCGAGCCCTGCAC"
[19] "CTCAGCGTGCGAGCCCTGCACCC" "ACTCCATTCCCGCTCCTGGAACACT"
[21] "TCCATTCCCGCTCCTGGAACACTTC" "TGTGCCTGGAGGCAGTCGGCCTGCA"
[23] "AGCCACTGGGGCCATGCGTATGACT" "AGGCACTGGCGCCAGAGGCTTCCTT"
[25] "AGCCCTGAAGACAAGCAGCACTGC" "AAATGGAACACTGACCCGGTGCGG"
[27] "TGGAACACTGACCCGGTGCGGTGG"

```

For example, it is possible to find `estGenes` which overlap known genes as follows:

```

> estGeneInRange( symbolToGene( c( 'lama3', 'tp53', 'shh' ) ), as.vector=TRUE )
[1] "ENSESTG00000004861" "ENSESTG00000004918" "ENSESTG000000032897"
[4] "ENSESTG000000032910" "ENSESTG000000032912" "ENSESTG000000032918"
[7] "ENSESTG000000032924" "ENSESTG000000032930" "ENSESTG000000032952"
[10] "ENSESTG000000033006" "ENSESTG00000010677"

```

## 4.2 Adventures with `annmapRangeApply`

A common task is to iterate down a `GRanges` object and perform a function per row. This functionality is provided by the method `annmapRangeApply()`.

To take a contrived example, lets write a function that takes a `GRanges` object, and returns a character vector of the form `chr:location` (to provide labels for a graph, say):

```

> contrived.function = function( chromosome, location ) {
+   paste( chromosome, ':', location, sep='' )
+ }

```

In a moment, we're going to use `annmapRangeApply` to map this down a set of transcripts, which we'll retrieve now:

```

> transcripts = geneToTranscript( symbolToGene( c( 'shh', 'tp53' ) ) )

```

Before we can do this, though, we need to tell the function which columns in the `GRanges` object to map onto 'chromosome' and 'location', and also to tell it what datatype each of these parameters should be.

This is done using two additional parameters in `annmapRangeApply`: `filter` and `coerce`. The first defines the column names in the `GRanges` object we are interested in, the second, the functions we need to coerce these to the expected types<sup>4</sup>:

```
> contrived.filter = c( chromosome='space', location='start' )
> contrived.coerce = c( as.character, as.numeric )
```

Once these are defined, we can then fire off our apply statement, as follows:

```
> annmapRangeApply( transcripts,
+                   contrived.function,
+                   contrived.filter,
+                   contrived.coerce )
 [1] "17:7565097" "17:7569404" "17:7571720" "17:7571720" "17:7571720"
 [6] "17:7571720" "17:7571722" "17:7571722" "17:7571739" "17:7572887"
[11] "17:7576853" "17:7577535" "17:7577572" "17:7577844" "17:7578434"
[16] "17:7578480" "17:7578547" "7:155592680" "7:155592733" "7:155592734"
[21] "7:155592744" "7:15559276"
```

---

<sup>4</sup>The `coerce` is needed because we can't always rely on R to correctly guess the data type we're expecting ('space' in a `GRanges` object will often be converted to a factor, not a character vector as required by `paste` in our `contrived.function`)

## 5 Affymetrix™ Array Annotation

As well as providing Ensembl annotation, `annmap` also provides mappings for Affymetrix™ arrays, as described in *An annotation infrastructure for the analysis and interpretation of Affymetrix exon array data* Genome Biology 2007, 8:R79, doi:10.1186/gb-2007-8-5-r79.

### 5.1 Exonic, intronic, intergenic and unreliable

Probesets in `annmap` are defined as being either `exonic`, `intronic`, `intergenic` or `unreliable`. These terms are best described by:

- **Exonic:** Probesets are classed as exonic if all of their probes map to the genome only once, and every one of these mappings falls within an exon boundary.
- **Intronic:** Probesets are classed as intronic if all of their probes map to the genome only once, but at least one probe misses an exon region, but still falls within the boundary of a gene.
- **Intergenic:** Probesets are classed as intergenic if all of their probes map to the genome only once, but at least one probe misses all the known genes.
- **Unreliable:** Probesets with one or more multi-targetting probes, or with one or more probes that do not map to the genome, are classed as unreliable.

These categories are a very broad filter. An unreliable probeset may have 3 probes which all hit in a single location and all hit an exon, but the single probe which misses mapping to the genome will result in the entire probeset being classed as to unreliable.

### 5.2 The filtering methods

To filter a collection of probesets by their relevant category, the obvious methods exist. For example, we can look at the probesets that are hitting around the gene for TP53:

```
> g      = symbolToGene('TP53')
> ps     = geneToProbeset( g, as.vector=TRUE )
> length(ps)
[1] 237
```

We can then see numbers of probesets that match each category:

```
> length( exonic( ps ) )
Building probeset specificity cache.....done
[1] 18
> length( intronic( ps ) )
[1] 14
> length( intergenic( ps ) )
[1] 1
> length( unreliable( ps ) )
[1] 204
```

So as you can see, hitting TP53, we have 18 exonic, 14 intronic, 1 intergenic<sup>5</sup> and 204 unreliable probesets.

All of these functions, will take an `exclude` parameter that results in an inverted list. So to get the number of all of the probesets that are not unreliable, we can do:

---

<sup>5</sup>This may seem odd, but note that as only a single probe in a probeset needs to be intergenic for the entire probeset to be categorised as such. So it is possible for a probeset that mostly maps to a gene to be flagged as intergenic.

```
> length( unreliable( ps, exclude=T ) )
[1] 33
```

Since anything that is not unreliable must hit the genome somewhere, the following should evaluate to TRUE:

```
> u.ps = sort( unreliable( ps, exclude=T ) )
> c.ps = sort( c( intronic( ps ), exonic( ps ), intergenic( ps ) ) )
> all( u.ps == c.ps )
[1] TRUE
```

### 5.3 UTR filtering

The function `utrProbesets` can be used to find those probesets that hit UTRs:

```
> gene = symbolToGene( 'TP53' )
> probesets = geneToProbeset( gene )
> utrProbesets( probesets )

[1] "3743926" "3743927" "3743928" "3743929" "3743930" "3743931" "3743932"
[8] "3743933" "3743938" "3743939" "3743940" "3743908" "3743926" "3743927"
[15] "3743928" "3743929" "3743930" "3743931" "3743932" "3743938" "3743939"
[22] "3743940" "3743908" "3743909" "3743910" "3743911" "3743912" "3743935"
[29] "3743926" "3743927" "3743928" "3743929" "3743930" "3743931" "3743932"
[36] "3743938" "3743939" "3743940" "3743908" "3743909" "3743910" "3743911"
[43] "3743912" "3743935" "3743926" "3743927" "3743928" "3743929" "3743930"
[50] "3743931" "3743932" "3743938" "3743939" "3743940" "3743908" "3743921"
[57] "3743922" "3743923" "3743924" "3743925" "3743926" "3743927" "3743928"
[64] "3743929" "3743930" "3743931" "3743932" "3743938" "3743939" "3743940"
[71] "3743926" "3743927" "3743928" "3743929" "3743930" "3743931" "3743932"
[78] "3743938" "3743939" "3743940" "3743926" "3743927" "3743928" "3743929"
[85] "3743930" "3743931" "3743932" "3743938" "3743939" "3743940" "3743926"
[92] "3743927" "3743928" "3743929" "3743930" "3743931" "3743932" "3743938"
[99] "3743939" "3743940" "3743909" "3743926" "3743927" "3743928"
```

In this form, the function will attempt to match probesets to all possible transcripts and then filter by UTR, so any UTR targeting probeset will be found.

You can also pass a vector of transcripts in, in which case the search is limited to these:

```
> transcripts = geneToTranscript( gene, as.vector=TRUE )[1:6]
> transcripts
ENSG00000141510 ENSG00000141510 ENSG00000141510 ENSG00000141510
"ENST00000413465" "ENST00000359597" "ENST00000504290" "ENST00000510385"
ENSG00000141510 ENSG00000141510
"ENST00000504937" "ENST00000269305"
> utrProbesets( probesets, transcripts )
[1] "3743926" "3743927" "3743928" "3743929" "3743930" "3743931" "3743932"
[8] "3743933" "3743938" "3743939" "3743940" "3743908"
```

So these probesets have at least one probe in the UTR regions of the first six transcripts of TP53. It is also possible to limit the search to only the 3' or 5' UTR:

```
> utrProbesets( probesets, transcripts, end='3' )
```

```
[1] "3743908"
> utrProbesets( probesets, transcripts, end='5' )
[1] "3743926" "3743927" "3743928" "3743929" "3743930" "3743931" "3743932"
[8] "3743933" "3743938" "3743939" "3743940"
```

It is also possible to omit the list of probesets and the `utr.probeset` function will generate one for you:

```
> utrProbesets( NULL, transcripts )
[1] "3743908" "3743909" "3743910" "3743911" "3743912" "3743913" "3743914"
[8] "3743915" "3743916" "3743917" "3743918" "3743919" "3743920" "3743935"
[15] "3743936" "3743908" "3743909" "3743910" "3743911" "3743912" "3743913"
[22] "3743914" "3743915" "3743916" "3743917" "3743918" "3743919" "3743920"
[29] "3743935" "3743936" "3743908" "3743909" "3743910" "3743911" "3743912"
[36] "3743913" "3743914" "3743915" "3743916" "3743917" "3743918" "3743919"
[43] "3743920" "3743935" "3743936" "3743908" "3743909" "3743910" "3743911"
[50] "3743912" "3743913" "3743914" "3743915" "3743916" "3743917" "3743918"
[57] "3743919" "3743920" "3743935" "3743936" "3743908" "3743909" "3743910"
[64] "3743911" "3743912" "3743913" "3743914" "3743915" "3743916" "3743917"
[71] "3743918" "3743919" "3743920" "3743935" "3743936" "3743908" "3743909"
[78] "3743910" "3743911" "3743912" "3743913" "3743914" "3743915" "3743916"
[85] "3743917" "3743918" "3743919" "3743920" "3743935" "3743936" "3743926"
[92] "3743927" "3743928" "3743929" "3743930" "3743931" "3743932" "3743933"
[99] "3743938" "3743939" "3743940" "3743908"
```

Or you can get a list of probesets which fall inside the coding region of a transcript by using the complementary `codingProbesets` function:

```
> codingProbesets( NULL, transcripts )
[1] "3743905" "3743907" "3743908" "3743909" "3743910" "3743911" "3743912"
[8] "3743913" "3743914" "3743915" "3743916" "3743917" "3743918" "3743919"
[15] "3743920" "3743921" "3743922" "3743923" "3743924" "3743925" "3743935"
[22] "3743936" "3743907" "3743908" "3743909" "3743910" "3743911" "3743912"
[29] "3743913" "3743914" "3743915" "3743916" "3743917" "3743918" "3743919"
[36] "3743920" "3743921" "3743922" "3743923" "3743924" "3743925" "3743935"
[43] "3743936" "3743909" "3743910" "3743911" "3743912" "3743913" "3743914"
[50] "3743915" "3743916" "3743917" "3743918" "3743919" "3743920" "3743921"
[57] "3743922" "3743923" "3743924" "3743925" "3743935" "3743936"
```

## 5.4 Coding regions and UTRs

It is possible to manipulate a list of transcripts so that you find the genomic location of their coding region or UTR. This is done using the two complementary methods `transcriptToUtrRange` and `transcriptToCodingRange`. Here for example is the normal details for a given transcript ENST00000297261 which falls on the reverse strand of Chromosome 7 in human:

```
> transcriptDetails( 'ENST00000297261' )
GRanges with 1 range and 12 metadata columns:
      seqnames          ranges strand |      stable_id      biotype
      <Rle>            <IRanges> <Rle> | <character> <character>
[1]      7 [155592680, 155604967]   - | ENST00000297261 protein_coding
      status description      db_display_name      symbol
```

```

      <character> <character>          <character> <character>
[1]      KNOWN      <NA> HGNC transcript name      SHH-001
      symbol_description translation_start translation_start_exon
      <character>          <integer>          <integer>
[1]      sonic hedgehog          152          8542079
      translation_end translation_end_exon          analysis_name
      <integer>          <integer>          <character>
[1]          827          8542116 ensembl_havana_transcript
---
seqlengths:
  7
NA

```

We can also get the genomic coordinates of its coding region:

```

> transcriptToCodingRange( 'ENST00000297261' )
GRanges with 1 range and 20 metadata columns:
      seqnames          ranges strand |          IN1 transcript_id
      <Rle>          <IRanges> <Rle> | <character> <numeric>
[1]          7 [155595594, 155604816] - | ENST00000297261      2411254
      stable_id  gene_id chromosome_id      biotype      status
      <character> <numeric>      <numeric> <character> <character>
[1] ENST00000297261      749548          27506 protein_coding      KNOWN
      description synonym_id external_db_id      db_display_name      symbol
      <character> <numeric>      <numeric>      <character> <character>
[1]      <NA>      3788341          50609 HGNC transcript name      SHH-001
      symbol_description translation_start translation_start_exon
      <character>          <integer>          <integer>
[1]      sonic hedgehog          152          8542079
      translation_end translation_end_exon          analysis_name      phase
      <integer>          <integer>          <character> <integer>
[1]          827          8542116 ensembl_havana_transcript      -1
      end.phase
      <integer>
[1]          1
---
seqlengths:
  7
NA

```

Or, we can get the regions covered by the UTR of this same transcript:

```

> transcriptToUtrRange( 'ENST00000297261' )
GRanges with 2 ranges and 4 metadata columns:
      seqnames          ranges strand |          IN1      prime
      <Rle>          <IRanges> <Rle> | <character> <character>
[1]          7 [155604817, 155604967] - | ENST00000297261      5
[2]          7 [155592680, 155595593] - | ENST00000297261      3
      phase translated
      <integer> <logical>
[1]          -1      TRUE
[2]          1      TRUE
---
seqlengths:

```

```
7
NA
```

Again, these two methods take an optional 'end' parameter to specify which end of the transcript you want to work with<sup>6</sup>:

```
> transcriptToCodingRange( 'ENST00000297261', end='3' )
GRanges with 1 range and 20 metadata columns:
      seqnames          ranges strand |          IN1 transcript_id
      <Rle>            <IRanges> <Rle> | <character> <numeric>
[1]      7 [155595594, 155604967]   - | ENST00000297261      2411254
      stable_id  gene_id chromosome_id      biotype      status
      <character> <numeric>      <numeric>      <character> <character>
[1] ENST00000297261      749548          27506 protein_coding      KNOWN
      description synonym_id external_db_id      db_display_name      symbol
      <character> <numeric>      <numeric>      <character> <character>
[1]      <NA>      3788341          50609 HGNC transcript name      SHH-001
      symbol_description translation_start translation_start_exon
      <character>      <integer>      <integer>
[1]      sonic hedgehog          152          8542079
      translation_end translation_end_exon      analysis_name      phase
      <integer>      <integer>      <character> <integer>
[1]          827          8542116 ensembl_havana_transcript      -1
      end.phase
      <integer>
[1]      1
---
seqlengths:
  7
NA
```

```
> transcriptToUtrRange( 'ENST00000297261', end='3' )
GRanges with 1 range and 4 metadata columns:
      seqnames          ranges strand |          IN1      prime
      <Rle>            <IRanges> <Rle> | <character> <character>
[1]      7 [155592680, 155595593]   - | ENST00000297261      3
      phase translated
      <integer> <logical>
[1]      1      TRUE
---
seqlengths:
  7
NA
```

It is also possible to get just those exons (or parts thereof) that are coding or for the UTR:

```
> transcriptToCodingExon( 'ENST00000297261' )
GRanges with 3 ranges and 4 metadata columns:
      seqnames          ranges strand |          IN1      stable_id
      <Rle>            <IRanges> <Rle> | <character> <character>
[1]      7 [155604517, 155604816]   - | ENST00000297261 ENSE00001086614
[2]      7 [155598990, 155599251]   - | ENST00000297261 ENSE00001086617
```

<sup>6</sup>if you pass an 'end' to `transcriptToCodingRange`, you are choosing which UTR to omit from the range, i.e. passing `end='3'` returns you the range that covers both the coding range and the 5' end, but not the 3' end.

```

[3]      7 [155595594, 155596420] - | ENST00000297261 ENSE00001149618
      phase end_phase
      <integer> <integer>
[1]      -1      0
[2]       0      1
[3]       1     -1
---
seqlengths:
  7
NA
> transcriptToUtrExon( 'ENST00000297261' )
GRanges with 2 ranges and 4 metadata columns:
      seqnames      ranges strand |      IN1      stable_id
      <Rle>          <IRanges> <Rle> | <character> <character>
[1]       7 [155604817, 155604967] - | ENST00000297261 ENSE00001086614
[2]       7 [155592680, 155595593] - | ENST00000297261 ENSE00001149618
      phase end_phase
      <integer> <integer>
[1]      -1      0
[2]       1     -1
---
seqlengths:
  7
NA

```

## 5.5 Gene and Transcript coding length

It is possible to get the 'coding length' of a gene by calling:

```

> nonIntronicGeneLength( symbolToGene( c( 'SHH', 'PTEN' ) ) )
ENSG00000164690 ENSG00000171862
      5038      10048

```

This merges all the exons in each gene (overlapping them where applicable) and returns you a sum of all their widths.

For transcripts, you also have the method:

```

> nonIntronicTranscriptLength( geneToTranscript( symbolToGene( 'SHH' ) ) )
ENST00000297261 ENST00000430104 ENST00000435425 ENST00000441114 ENST00000472308
      4454      677      807      888      504

```

In which you may also specify if you wish to only look at coding regions by specifying an 'end':

```

> nonIntronicTranscriptLength( geneToTranscript( symbolToGene( 'SHH' ) ), end='both' )
ENST00000297261 ENST00000430104 ENST00000435425 ENST00000441114
      1389      507      372      381

```



## 6 annmap's local cache

You may have noticed that the first time we called `exonic` on page 19, `annmap` told us that it was 'Building probeset specificity cache.....'. This cache allows us to filter large numbers of probesets much quicker than doing each in turn. However, for smaller queries of less than about 1000 probesets (on our system) it is quicker not to suffer the  $\approx 1s$  load time for the cache to be loaded into memory from disk.

If you are running lots of these filtering queries on small numbers of probesets (in a loop or apply, for example), it might be worth turning the cache off whilst you run them:

```
> annmapToggleCaching()
[1] FALSE
```

Now, the cache (on by default) has been turned off. (This can be seen by the return value of `FALSE`). Calling `toggle` again will turn it back on:

```
> annmapToggleCaching()
[1] TRUE
```

Currently we cache the probeset specificity data used for the filters, described above, and the calls to `allXXX`. The cache is stored in `.annmap/cache`.

## 7 The path to the answer matters

### 7.1 The probeset boundary to commutativity

In general, `annmap` is commutative. For example, if you go from a list of genes to transcripts and back again, you will end up where you started:

```
> genes = symbolToGene( c( 'PTEN', 'SHH' ), as.vector=TRUE )
> genes
           PTEN           SHH
"ENSG00000171862" "ENSG00000164690"
> transcripts = geneToTranscript( genes, as.vector=TRUE )
> transcripts
  ENSG00000171862  ENSG00000171862  ENSG00000171862  ENSG00000171862
"ENST00000371953" "ENST00000487939" "ENST00000462694" "ENST00000498703"
  ENSG00000171862  ENSG00000164690  ENSG00000164690  ENSG00000164690
"ENST00000472832" "ENST00000297261" "ENST00000441114" "ENST00000430104"
  ENSG00000164690  ENSG00000164690
"ENST00000435425" "ENST00000472308"
> genes = transcriptToGene( transcripts, as.vector=TRUE )
> genes
  ENST00000371953  ENST00000487939  ENST00000462694  ENST00000498703
"ENSG00000171862" "ENSG00000171862" "ENSG00000171862" "ENSG00000171862"
  ENST00000472832  ENST00000297261  ENST00000441114  ENST00000430104
"ENSG00000171862" "ENSG00000164690" "ENSG00000164690" "ENSG00000164690"
  ENST00000435425  ENST00000472308
"ENSG00000164690" "ENSG00000164690"
> geneToSymbol( genes )
```

```

ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862
      "PTEN"           "PTEN"           "PTEN"           "PTEN"           "PTEN"
ENSG00000164690 ENSG00000164690 ENSG00000164690 ENSG00000164690 ENSG00000164690
      "SHH"           "SHH"           "SHH"           "SHH"           "SHH"

```

However, with probesets, this commutativity is broken. This is because they (even exonic probesets) can hit genes, transcripts or exons other than those in the list that you passed in – for example:

```

> genes = symbolToGene( c( 'pten', 'shh' ), as.vector=TRUE )
> genes
      PTEN           SHH
"ENSG00000171862" "ENSG00000164690"

> probesets = exonic( geneToProbeset( genes, as.vector=TRUE ) )
> probesets
ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862
      "3256753"      "3256751"      "3256701"      "3256781"      "3256754"
ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862
      "3256783"      "3256703"      "3256706"      "3256726"      "3256702"
ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862
      "3256738"      "3256755"      "3256752"      "3256741"      "3256784"
ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000171862 ENSG00000164690
      "3256740"      "3256716"      "3256782"      "3256756"      "3081218"
ENSG00000164690 ENSG00000164690 ENSG00000164690 ENSG00000164690 ENSG00000164690
      "3081230"      "3081224"      "3081226"      "3081221"      "3081219"
ENSG00000164690 ENSG00000164690 ENSG00000164690 ENSG00000164690 ENSG00000164690
      "3081220"      "3081223"      "3081225"      "3081229"      "3081227"
ENSG00000164690
      "3081222"

> genes = probesetToGene( probesets, as.vector=TRUE )
> genes
      3256753      3256753      3256751      3256751
"ENSG00000171862" "ENSG00000213613" "ENSG00000171862" "ENSG00000213613"
      3256701      3256781      3256754      3256754
"ENSG00000171862" "ENSG00000171862" "ENSG00000171862" "ENSG00000213613"
      3256783      3256703      3256706      3256726
"ENSG00000171862" "ENSG00000171862" "ENSG00000171862" "ENSG00000171862"
      3256702      3256738      3256755      3256755
"ENSG00000171862" "ENSG00000171862" "ENSG00000171862" "ENSG00000213613"
      3256752      3256752      3256741      3256784
"ENSG00000171862" "ENSG00000213613" "ENSG00000171862" "ENSG00000171862"
      3256740      3256716      3256782      3256756
"ENSG00000171862" "ENSG00000171862" "ENSG00000171862" "ENSG00000171862"
      3256756      3081218      3081230      3081224
"ENSG00000213613" "ENSG00000164690" "ENSG00000164690" "ENSG00000164690"
      3081226      3081221      3081219      3081220
"ENSG00000164690" "ENSG00000164690" "ENSG00000164690" "ENSG00000164690"
      3081223      3081225      3081229      3081227
"ENSG00000164690" "ENSG00000164690" "ENSG00000164690" "ENSG00000164690"
      3081222
"ENSG00000164690"

> geneToSymbol( genes )

```

ENSG00000171862	ENSG00000213613	ENSG00000171862	ENSG00000213613	ENSG00000171862
"PTEN"	"RP11-380G5.3"	"PTEN"	"RP11-380G5.3"	"PTEN"
ENSG00000171862	ENSG00000171862	ENSG00000213613	ENSG00000171862	ENSG00000171862
"PTEN"	"PTEN"	"RP11-380G5.3"	"PTEN"	"PTEN"
ENSG00000171862	ENSG00000171862	ENSG00000171862	ENSG00000171862	ENSG00000171862
"PTEN"	"PTEN"	"PTEN"	"PTEN"	"PTEN"
ENSG00000213613	ENSG00000171862	ENSG00000213613	ENSG00000171862	ENSG00000171862
"RP11-380G5.3"	"PTEN"	"RP11-380G5.3"	"PTEN"	"PTEN"
ENSG00000171862	ENSG00000171862	ENSG00000171862	ENSG00000171862	ENSG00000213613
"PTEN"	"PTEN"	"PTEN"	"PTEN"	"RP11-380G5.3"
ENSG00000164690	ENSG00000164690	ENSG00000164690	ENSG00000164690	ENSG00000164690
"SHH"	"SHH"	"SHH"	"SHH"	"SHH"
ENSG00000164690	ENSG00000164690	ENSG00000164690	ENSG00000164690	ENSG00000164690
"SHH"	"SHH"	"SHH"	"SHH"	"SHH"
ENSG00000164690	ENSG00000164690			
"SHH"	"SHH"			

Indeed, any mapping involving hits, probes or probesets, are not commutative.

## 8 Utility methods

### 8.1 Splicing Index

For this test, we have an eSet object stored locally which contains expression data for an MCF7/MCF10A dataset around the gene 'tp53'.

```
> load( file.path( path.package('annmap'), 'rdata', 'HuEx-1_0.tp53.expr.RData' ) )
> x.rma
ExpressionSet (storageMode: lockedEnvironment)
assayData: 240 features, 6 samples
  element names: exprs
protocolData: none
phenoData
  rowNames: ex1MCF7_r1.CEL ex1MCF7_r2.CEL ... ex2MCF10A_r3.CEL (6
  total)
  varLabels: sample group
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: huex10stv1
```

So, we can call our `spliceIndex` method, passing this data, the gene id for 'tp53' and the fact that the first 3 columns are one sample type (mcf7) and the second 3 are the other sample.

```
> spliceIndex( x.rma, symbolToGene( 'TP53' ), gps=list(1:3,4:6) )
$ENSG00000141510
      si      p.score t.statistic  gene.av
3743908 0.112899062 0.22479731  1.43429922 0.02815739
3743909 -0.157154547 0.51591129 -0.71178196 0.02815739
3743912 -0.044769083 0.70715317 -0.40359763 0.02815739
3743913 -0.186660284 0.33324256 -1.09958985 0.02815739
3743915 -0.162931473 0.08576344 -2.26962208 0.02815739
3743917 0.126866306 0.17884429  1.62805226 0.02815739
3743918 0.056591953 0.32816552  1.11278250 0.02815739
3743919 0.062286225 0.46526044  0.80632965 0.02815739
3743920 -0.156910026 0.05743946 -2.64234332 0.02815739
3743922 0.048387256 0.57010143  0.61787261 0.02815739
3743923 -0.102274940 0.36081354 -1.03096096 0.02815739
3743924 0.008979375 0.93770685  0.08317721 0.02815739
3743925 0.006989562 0.95775111  0.05636913 0.02815739
3743928 0.074234272 0.89284542  0.14348489 0.02815739
3743936 -0.865412389 0.11450250 -2.01226730 0.02815739
```

As can be seen, this call returns a list, with one `data.frame` per gene. In this example, we only have passed a single gene for brevity.

### 8.2 Mapping probes into transcript coordinates

To find the location of probes relative to the mature, spliced mRNA sequence (rather than in genome coordinate space), the function `transcriptToTranslatedprobes` joins the exons in a transcript (from the 5' end to the 3' end), and then converts the probe locations so that they are an offset from the 5' end of the transcript.

```
> transcriptToTranslatedprobes( c( 'ENST00000462694', 'ENST00000329958' ) )
```

```
$ENST00000462694
```

```
GRanges with 11 ranges and 3 metadata columns:
```

```
      seqnames      ranges strand |      sequence probe_hit_count
      <Rle> <IRanges> <Rle> | <character> <numeric>
[1]      10 [19, 43]      + | AGAGATCGTTAGCAGAAACAAAAGG      2
[2]      10 [33, 57]      + | GAAACAAAAGGAGATATCAAGAGGA      2
[3]      10 [46, 70]      + | ATATCAAGAGGATGGATTGACTTA      2
[4]      10 [50, 74]      + | CAAGAGGATGGATTGACTTAGACT      2
[5]      10 [56, 80]      + | GATGGATTGACTTAGACTTGACCT      2
...      ...      ...      ...      ...
[7]      10 [137, 161]      + | TACAGGAACAATATTGATGATGTAG      2
[8]      10 [255, 279]      + | AGATACTTTGTGATGTAAACTATTA      1
[9]      10 [290, 314]      + | CTATAATCATTTTTTGGCTTACCGT      1
[10]     10 [305, 329]      + | GGCTTACCGTACCTAATGGACTTCA      1
[11]     10 [331, 355]      + | GGGGATACAGTTCATTTGATAAGAA      1
```

```
IN1
```

```
<character>
```

```
[1] ENSE00001926457
[2] ENSE00001926457
[3] ENSE00001926457
[4] ENSE00001926457
[5] ENSE00001926457
...
[7] ENSE00001920439
[8] ENSE00001920439
[9] ENSE00001920439
[10] ENSE00001920439
[11] ENSE00001920439
```

```
---
```

```
seqlengths:
```

```
10
```

```
NA
```

```
$ENST00000329958
```

```
NULL
```

Please note, that this function does not return probes that span the exon-junctions of the combined transcript sequence.

### 8.3 Converting between Genomic, Transcript and Protein coordinates

In `anmap`, there are two functions that convert between genomic coordinates and the related transcript/protein coordinates.

```
> # Given the tp53 gene
> gene      = symbolToGene( 'tp53' )
> # And the transcripts for this gene
> transcripts = geneToTranscript( gene )
> # And the proteins for this transcript
> proteins   = transcriptToProtein( transcripts )
> # get the transcript coords for the transcripts of this gene, at the start of this gene
> genomeToTranscriptCoords( start( gene ), transcripts )
```

```
GRanges with 1 range and 1 metadata column:
```

```
      seqnames      ranges strand | coord.space
```

```

      <Rle>    <IRanges> <Rle> | <character>
[1] ENST00000413465 [1018, 1018] * | transcript
---
seqlengths:
  ENST00000413465
      NA
> # With as.vector=TRUE
> genomeToTranscriptCoords( start( gene ), transcripts, as.vector=TRUE )
ENST00000413465 ENST00000359597 ENST00000504290 ENST00000510385 ENST00000504937
      1018                NA                NA                NA                NA
ENST00000269305 ENST00000455263 ENST00000420246 ENST00000445888 ENST00000576024
      NA                NA                NA                NA                NA
ENST00000509690 ENST00000514944 ENST00000574684 ENST00000505014 ENST00000508793
      NA                NA                NA                NA                NA
ENST00000604348 ENST00000503591
      NA                NA
> # Get the coding range for the transcripts
> coding = transcriptToCodingRange( transcripts )
> # And then to protein coordinates
> genomeToProteinCoords( start( coding ), proteins )
GRanges with 12 ranges and 2 metadata columns:
      seqnames      ranges strand |      frame coord.space
      <Rle>    <IRanges> <Rle> | <numeric> <character>
[1] ENSP00000269305 [394, 394] * |      2      protein
[2] ENSP00000352610 [344, 344] * |      2      protein
[3] ENSP00000391127 [342, 342] * |      2      protein
[4] ENSP00000391478 [394, 394] * |      2      protein
[5] ENSP00000398846 [347, 347] * |      2      protein
...
...
[8] ENSP00000424104 [166, 166] * |      0      protein
[9] ENSP00000425104 [199, 199] * |      2      protein
[10] ENSP00000426252 [128, 128] * |      1      protein
[11] ENSP00000458393 [ 32,  32] * |      2      protein
[12] ENSP00000473895 [143, 143] * |      2      protein
---
seqlengths:
  ENSP00000269305 ENSP00000352610 ... ENSP00000458393 ENSP00000473895
      NA                NA ...                NA                NA
> # With as.vector=TRUE
> genomeToProteinCoords( start( coding ), proteins, as.vector=TRUE )
ENSP00000410739 ENSP00000352610 ENSP00000269305 ENSP00000398846 ENSP00000391127
      286                344                394                347                342
ENSP00000391478 ENSP00000458393 ENSP00000425104 ENSP00000423862 ENSP00000424104
      394                32                199                156                166
ENSP00000473895 ENSP00000426252
      143                128

```

There are also functions for performing translation in the opposite direction; `proteinCoordsToGenome` and `transcriptCoordsToGenome`.

```

> pos.one = transcriptCoordsToGenome( transcripts, 1, as.vector=TRUE )
> pos.one

```

```

ENST00000413465 ENST00000359597 ENST00000504290 ENST00000510385 ENST00000504937
      7579912          7579912          7578811          7578811          7578811
ENST00000269305 ENST00000455263 ENST00000420246 ENST00000445888 ENST00000576024
      7590856          7590799          7590799          7590805          7576905
ENST00000509690 ENST00000514944 ENST00000574684 ENST00000505014 ENST00000508793
      7590805          7590745          7578437          7590805          7580752
ENST00000604348 ENST00000503591
      7590805          7590745

```

And as we are on the reverse strand, all these locations should match the end of the transcripts:

```

> pos.one == end( transcripts )
ENST00000413465 ENST00000359597 ENST00000504290 ENST00000510385 ENST00000504937
      TRUE          TRUE          TRUE          TRUE          TRUE
ENST00000269305 ENST00000455263 ENST00000420246 ENST00000445888 ENST00000576024
      TRUE          TRUE          TRUE          TRUE          TRUE
ENST00000509690 ENST00000514944 ENST00000574684 ENST00000505014 ENST00000508793
      TRUE          TRUE          TRUE          TRUE          TRUE
ENST00000604348 ENST00000503591
      TRUE          TRUE

```

We can also pass `cds=TRUE` to this method to take the UTRs into account when calculating genomic location:

```

> pos.one = transcriptCoordsToGenome( transcripts, 1, as.vector=TRUE, cds=TRUE )
> pos.one
ENST00000413465 ENST00000359597 ENST00000504290 ENST00000510385 ENST00000504937
      7579912          7579912          NA          NA          NA
ENST00000269305 ENST00000455263 ENST00000420246 ENST00000445888 ENST00000576024
      7579912          7579912          7579912          7579912          7576905
ENST00000509690 ENST00000514944 ENST00000574684 ENST00000505014 ENST00000508793
      7578533          7579912          NA          NA          7579912
ENST00000604348 ENST00000503591
      7579912          7579912

```

## 8.4 Plotting data with `genomicPlot`

`genomicPlot` will plot the genes and exons found at a given locus.

For example, to plot the genomic region around the gene `MPI` (Figure 2):

```

> range = symbolToGene( 'MPI' )
> ranges( range ) = ranges( range ) + 5000
> genomicPlot( range )

```

We can also show the opposite strand, in one of two ways. First, passing `draw.opposite.strand=TRUE`, results in a *'washed out'* view of the opposite strand (Figure 3).

```

> genomicPlot( range, draw.opposite.strand=TRUE )

```

Alternatively setting the `strand` column from the `range` parameter we are passing in (Figure 4) to `'*'` results in both strands being shown.

```

> strand( range ) = '*'
> genomicPlot( range )

```

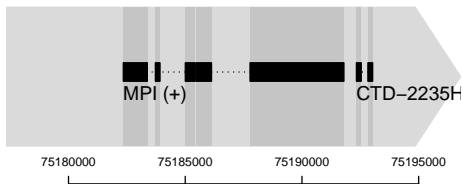


Figure 2: A genomic plot 5000bp either side of MPI.

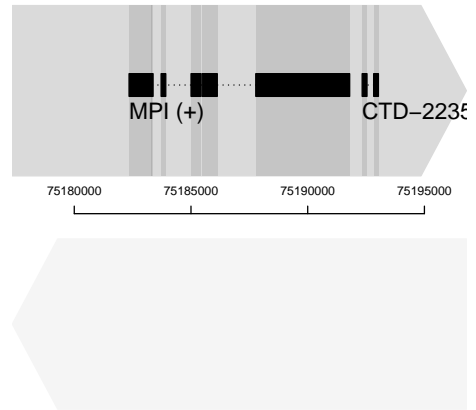


Figure 3: A genomic plot 5000bp either side of MPI with the opposite strand drawn 'washed out'.

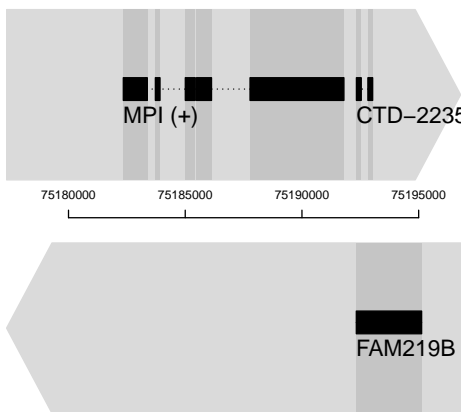


Figure 4: Both strands 5000bp either side of MPI.

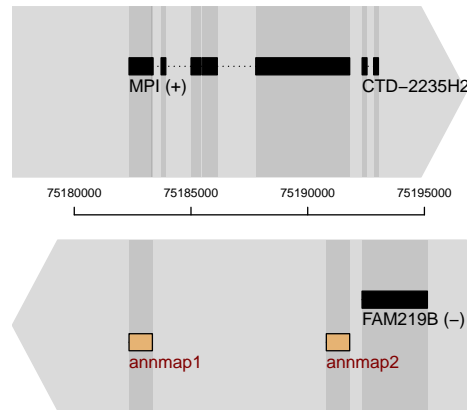


Figure 5: Both strands 5000bp either side of MPI with highlights.

It is also possible to highlight regions of the genome with your own annotation. To do this, you simply need to pass in a `data.frame` containing columns `start`, `end`, `strand` and `name`.

For example, we could add regions to the start and end locations of MPI but on the opposite strand and call them `annmap1` and `annmap2` (Figure 5);

```
> a1          = symbolToGene( 'MPI' )
> end( a1 )   = start( a1 ) + 1000
> a2          = symbolToGene( 'MPI' )
> start( a2 ) = end( a2 ) - 1000
> hig        = data.frame( start =c( as.integer( start( a1 ) ), as.integer( start( a2 ) ) ),
+                             end   =c( as.integer( end( a1 ) ), as.integer( end( a2 ) ) ),
+                             strand=c( strandAsInteger( a1 ), strandAsInteger( a2 ) ) *
+                             name  =c( 'annmap1', 'annmap2' ) )

> genomicPlot( range, highlights=hig )
```



## 8.5 Plotting NGS data with `ngsBridgePlot`

The `ngsBridgePlot` method allows NGS read data to be plotted alongside a `genomicPlot`. This was how Figure 1 was generated. There are many options for this plot function; here we will cover the most important ones.

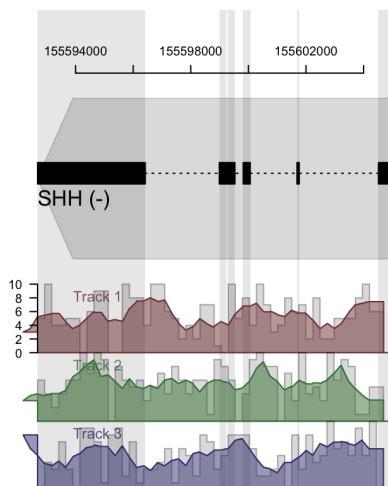


Figure 6: Dummy (in this example) NGS data shown alongside SHH.

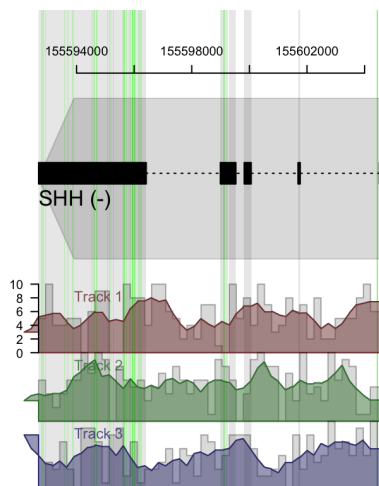


Figure 7: Dummy (in this example) NGS data shown alongside SHH with probes shown.

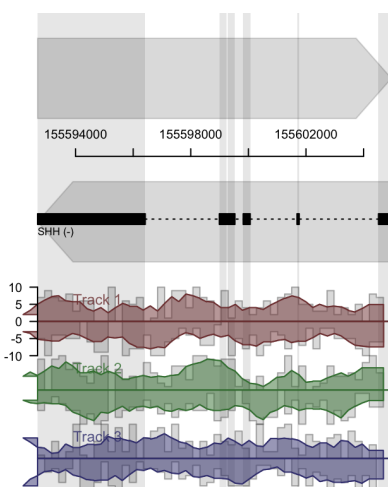


Figure 8: Dummy (in this example) NGS data shown alongside SHH (both strands) with probes shown.

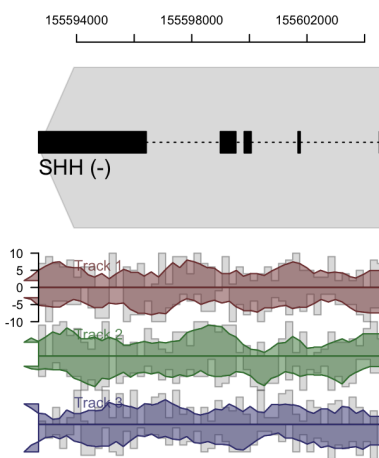


Figure 9: Dummy (in this example) NGS data shown alongside SHH. Both strands of NGS reads are shown. Exon depth plot hidden.

To plot Bamfile data alongside a region (and strand) denoted by a given gene, you can do (Figure 6):

```
> gene = symbolToGene( 'SHH' )  
> ngsBridgePlot( gene, bamfileData )
```

If you want to show where the probes for a given array hit this region, you can set the `probe.plot` parameter to the `genomicProbePlot` function (Figure 7)

```
> gene = symbolToGene( 'SHH' )
> ngsBridgePlot( gene, bamfileData, probe.plot=genomicProbePlot )
```

And as we saw in the preamble to this document, we can set the strand of the given range to '\*' and both strands of the region will be shown (Figure 8).

```
> gene = symbolToGene( 'SHH' )
> strand( gene ) = '*'
> ngsBridgePlot( gene, bamfileData )
```

Or, we can plot the genomicPlot for a single strand, but show the NGS data for both strands side-by-side using the `trace.match.strand` parameter and, for clarity, turn off the `exon.depth.plot` (Figure 9).

```
> gene = symbolToGene( 'SHH' )
> # trace.match.strand=FALSE would have the same effect
> ngsBridgePlot( gene, bamfileData, exon.depth.plot=NULL, trace.match.strand='*' )
```

In all these examples, the `bamfileData` is a synthetic object we have created for the purposes of this vignette. Its structure is:

```
> str( bamfileData )
List of 3
 $ :List of 3
  ..$ name: chr "Track 1"
  ..$ rle :List of 2
  .. ..$ +:Formal class 'Rle' [package "IRanges"] with 4 slots
  .. . . . ..@ values      : int [1:45] 2 7 9 7 6 8 3 6 0 10 ...
  .. . . . ..@ lengths     : int [1:45] 155592680 245 245 245 245 245 245 491 491 245 ...
  .. . . . ..@ elementMetadata: NULL
  .. . . . ..@ metadata    : list()
  .. ..$ -:Formal class 'Rle' [package "IRanges"] with 4 slots
  .. . . . ..@ values      : int [1:39] 3 5 10 4 5 0 6 9 0 10 ...
  .. . . . ..@ lengths     : int [1:39] 155592680 245 245 491 491 245 245 491 491 245 ...
  .. . . . ..@ elementMetadata: NULL
  .. . . . ..@ metadata    : list()
  ..$ col : chr "#804040FF"
 $ :List of 3
  ..$ name: chr "Track 2"
  ..$ rle :List of 2
  .. ..$ +:Formal class 'Rle' [package "IRanges"] with 4 slots
  .. . . . ..@ values      : int [1:45] 4 9 0 9 10 6 8 4 2 6 ...
  .. . . . ..@ lengths     : int [1:45] 155592680 245 245 245 245 245 245 245 245 245 ...
  .. . . . ..@ elementMetadata: NULL
  .. . . . ..@ metadata    : list()
  .. ..$ -:Formal class 'Rle' [package "IRanges"] with 4 slots
  .. . . . ..@ values      : int [1:45] 3 6 4 2 4 6 10 7 10 9 ...
  .. . . . ..@ lengths     : int [1:45] 155592680 245 245 245 245 491 245 245 245 245 ...
  .. . . . ..@ elementMetadata: NULL
  .. . . . ..@ metadata    : list()
  ..$ col : chr "#408040FF"
 $ :List of 3
  ..$ name: chr "Track 3"
  ..$ rle :List of 2
  .. ..$ +:Formal class 'Rle' [package "IRanges"] with 4 slots
```

```

.. .. .@ values      : int [1:49] 6 1 0 5 0 2 10 1 0 5 ...
.. .. .@ lengths     : int [1:49] 155592680 245 245 245 245 245 245 245 245 245 ...
.. .. .@ elementMetadata: NULL
.. .. .@ metadata    : list()
.. .. $ -:Formal class 'Rle' [package "IRanges"] with 4 slots
.. .. .@ values      : int [1:45] 8 0 4 3 7 3 0 9 7 1 ...
.. .. .@ lengths     : int [1:45] 155592680 245 245 245 245 245 245 491 245 245 ...
.. .. .@ elementMetadata: NULL
.. .. .@ metadata    : list()
.. $ col : chr "#404080FF"

```

As you can see, it is a list containing a list for each track. Each of these track lists contain the name of the track, col (its colour), and an rle element which contains a list of Rle elements (one for '+', the forward strand and one for the reverse; '-')

To generate this object from actual BAM file data there is a helper function `generateBridgeData`. Given 3 BAM files you wish to display on the plot – `data1.bam`, `data2.bam` and `data3.bam`:

```

> bamfiles = c( 'data1.bam', 'data2.bam', 'data3.bam' )
> data = generateBridgeData( symbolToGene( 'SHH' ), bamfiles )

```

Will generate the required format with sensible defaults for name and col. These defaults can be overridden by setting the names and colours parameters on the `generateBridgeData` function.

## 8.6 Gviz integration

It is also possible to convert a list of genes to a list of `GeneRegionTrack` for rendering in Gviz (Figure 10).

```

> library( Gviz )
> gene = symbolToGene( 'SHH' )
> genome = 'hg19'
> gtrack = GenomeAxisTrack()
> atrack = geneToGeneRegionTrack( gene, genome, showId=TRUE )
> plotTracks( c( gtrack, atrack ), extend.left=1000, extend.right=1000 )

```

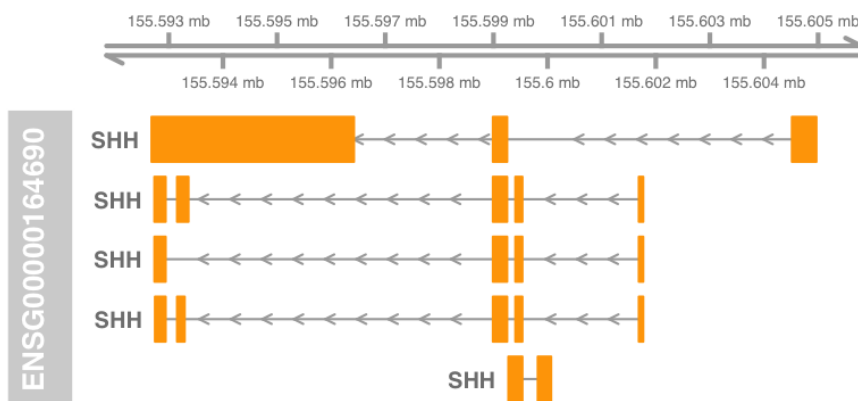


Figure 10: Gviz plot of the gene SHH.