Tiling Path File (TPF) Specification v1.9 Date: Feb. 25, 2019

What it is: A TPF specifies the order of a set of sequences along a larger molecule(s). Gap types and sizes can be specified, along with the desired orientation of the component sequences. The molecule can be as large as a chromosome and as small as a sequence contig made up of one smaller sequence. A TPF can have one or more sequences/clones.

What it is not: A TPF does not specify the particular version of a sequence, nor does it specify the switch points between adjacent clones in a given scaffold/contig.

Header Information: Header information is provided in a key-value type of structure. Each header field will be prefixed with ##. These fields are available:

- ##Organism (Required)
- ##Assembly Name (Required)
- ##Chromosome (Required)
- ##Strain/Haplotype/Cultivar (Optional)
- ##Type (controlled vocabulary) (Required)
- ##Version (Calculated by Database, Required)
- ##Comment (Optional)

Additional header information will be calculated upon submission.

- ##Submitter
- ##Create date
- ##Update date

```
Here is an example header:
##ORGANISM: Mus musculus
##ASSEMBLY NAME: Reference
##CHROMOSOME: 1
##STRAIN/HAPLOTYPE/CULTIVAR: C57BL/6J
##TYPE: Complete Chromosome
##Version: 1
##Comment: some useful information here.
##SUBMITTER: Tina Graves, WUGSC
##CREATE DATE: Nov 3 2006 12:16PM
##UPDATE DATE: Nov 5 2006 12:12PM
```

The data defining the actual tiling path should be flanked by header and footer lines to minimize the chance of file truncation. The header and footer lines shall have the following format:

```
All header "## TAG: value" pairs must occur before this line:
##=== Beginning of TPF Data ===
Required after the TPF data rows:
##=== End of TPF Data ===
```

Column definitions for sequence lines:

Column 1: accession number. This must be a valid accession assigned by the International DNA Sequence Database (GenBank/EMBL/DDBJ). No version number should be used. It is assumed that the current version of the accession is what is intended. **OPTIONAL**, use "?" if unknown.

Column 2: clone name. If the sequence is defined by a clone, then the clone name should be specified here. Ideally, standard Clone DB nomenclature will be used. **OPTIONAL**.

- If a sequence is derived from more than one clone, the clone name can be given as "MULTIPLE".
- Use "?" if clone name unknown or component is not a clone.

Column 3: local contig identifier. The name given to the scaffold/contig generated by assembling the listed sequences. **REQUIRED.**

NOTE: A local contig identifier may only be used once per assembly.

Column 4: contained status or component orientation. This column is only used to specify clones that are known to be contained within another clone on the TPF, or to indicate the desired orientation of a component within the contig.

- For contained components, the only values allowed are "CONTAINED", "CONTAINED_TURNOUT" and "CONTAINED_SUBSEQ". Use: REQUIRED for contained components, otherwise OPTIONAL.
- When indicating orientation, the only allowed values are "PLUS" and "MINUS". **Use: OPTIONAL.**
 - Note: If no orientation is specified for a singleton component, a default orientation of "PLUS" is assumed
 - Note: Orientation cannot be specified for components marked as contained

Column 5: accession number. This must be a valid accession assigned by the INSDC. No version number should be used. It is assumed that the current version of the accession is what is intended. The accession provided in this column must be in column 1 on another line of the same TPF and must also

belong to the same local contig (column 3) as the contained clone. See below for requirements. REQUIRED if COLUMN 4 is CONTAINED or CONTAINED TURNOUT or CONTAINED SUBSEQ, otherwise not valid.

Column 6: clone name. The clone name used in this column should correspond to the accession listed in column 5, if the sequence is derived from a clone. Ideally, standard Clone DB nomenclature will be used. See below for requirements. REQUIRED IF COLUMN 4 is CONTAINED or CONTAINED_TURNOUT or CONTAINED_SUBSEQ, otherwise not valid.

- NOTE1: Columns 5 and 6- these are similar to columns 1 and 2 in that at least one, but not both, requires a value. However, if both are known, please supply both values.
- NOTE2: If column 4 is not populated with CONTAINED or CONTAINED TURNOUT or CONTAINED SUBSEQ in a sequence line, do not populate columns 5 or 6. If column 4 is populated with CONTAINED or CONTAINED TURNOUT or CONTAINED SUBSEQ, then provide values or "?" for columns 5 and 6.

Column 7: Sequence start. This column must contain a positive numerical value. This column is only used if column 4 is populated with CONTAINED SUBSEQ. This column is only used to provide the START coordinate of accession in column 1 at which switch occurs from CONTAINER to CONTAINED_SUBSEQ (in 1-base coordinates).

Column 8: Sequence stop. This column must contain a positive numerical value. This column is only used if column 4 is populated with CONTAINED SUBSEQ. This column is only used to provide the STOP coordinate of accession in column 1 at which switch occurs from CONTAINED SUBSEQ to CONTAINER (in 1-base coordinates).

Column definitions for non-sequence lines:

Column 1: GAP. This is the term used and it should always be capitalized. Used to note the gap lines. **REQUIRED**

Column 2: gap type. Specifies the type of gap. **REQUIRED** Values are:

- TYPE-1: [Deprecated]- was a place-holder for a picked clone.
- TYPE-2: clone gap
- TYPE-3: contig gap- unable to close using available technology
- Biological Gap: If there is a biological gap such as a centromere, etc. then use the name rather than type-4. This is a controlled vocabulary:
 - → CENTROMERE
 - → TELOMERE

- **↔** HETEROCHROMATIN
- → SHORT-ARM
- PAR: (Y-chromosome only) The pseudoautosomal regions of the Y chromosome are represented by PAR gaps. Accessions for chr. Xderived PAR sequences are omitted from the chr. Y TPF, but are present in the chr. Y AGP. Switch points for PAR boundary clones will be curated manually.
 - o Note: The comments section of the header should be used to provide the bp positions at which the PAR region begins and ends in the relevant accessions. Header comments are entered via the web-based form at the time of submission. This information will be inserted into the ##COMMENT line of the header. It will also be stored in the database and can be retrieved for future use.
- Biological Feature: If there are TPF components providing representation of a biological feature (e.g. centromere, heterochromatin), use the following terms to flank the first and last such components on the TPF. NOTE: No gap will be inserted at these lines; they are used for TPF mark-up only.
 - CENTROMERE DATA START
 - CENTROMERE DATA END
 - o HETEROCHROMATIN DATA START
 - HETEROCHROMATIN DATA END

Column 3:

- For TYPE-2, TYPE-3 or Biological gap: Gap size. Estimated size of gap. In the absence of submitted gap sizes, a pre-defined species-specific gap size will be used for clone and contig gaps. **OPTIONAL**, unless the gap type is 'Biological' then required.
- For PAR gap: accession number of the first sequence to contain the PAR region, as defined on the X chromosome (this accession may contain both non-PAR and PAR sequence)

Column 4:

- For TYPE-2, TYPE-3 or Biological gap: Method used to determine gap size. If a gap size has been estimated experimentally, the method should be noted here. Currently acceptable values:
 - FISH
 - OPTICAL MAP
 - RADIATION HYBRID
 - PCR
 - FINGERPRINT
 - PAIRED ENDS
 - ALIGN GENUS
 - ALIGN XGENUS

ALIGN TRNSCPT

- Multiple methods may be entered for a single gap. Methods should be separated by a semi-colon (Example: FISH; OPTICAL MAP; ALIGN GENUS).
- REQUIRED if column 3 is populated and gap type is not biological, otherwise not valid.
- For PAR gap: accession number of the last sequence to contain the PAR region, as defined on the X chromosome (this accession may contain both non-PAR and PAR sequence)

All columns are tab delimited and lines are terminated by a newline. Lines beginning with a single "#" can occur anywhere within the file, are comments and can be ignored by parsers. Lines beginning with "##" can be ignored by simple parsers but contain structured information about the TPF. Each file should have only one header, so all of the objects described in a file

Validation:

For sequence based lines:

should have common header information.

- If column 1 is populated with a valid accession, column 2 may be unpopulated (using "?" as a placeholder). Ideally, this will only occur in the case where a sequence is not based on a defined clone and has no other easily identified name.
- If an accession is supplied, it must be valid according to the IDNSC.
- A given accession cannot be used more than once per assembly, but may be used in >1 assembly. (The assembly name is listed in row 3 of the TPF header).
- If column 1 is not populated (using "?" as a placeholder), then column 2 should be populated.
- If there is not enough information to populate column 1 or 2, then a gap line should be used.
- If column 4 = CONTAINED or CONTAINED_TURNOUT or CONTAINED_SUBSEQ, then columns 5 and 6 must be populated.
- If column 4 = CONTAINED_SUBSEQ, then columns 7 and 8 must be populated.
- If column 4 = CONTAINED_SUBSEQ, then columns 7 and 8 must be numerical values.
- If column 4 = CONTAINED_SUBSEQ, then columns 7 and 8 cannot be zero or negative values.
- If column 4 = CONTAINED_SUBSEQ, then numerical value in column 7 must be smaller than numerical value in column 8.
- If column 4 = CONTAINED_SUBSEQ, then numerical values in columns 7 and 8 must be within range of sequence specified in column 1.

- If column 4 = PLUS or MINUS, then columns 5, 6, 7 and 8 must not be populated.
- If column 4 is not populated, then columns 5, 6, 7 and 8 must not be populated.
- If column 4 = CONTAINED or CONTAINED TURNOUT or CONTAINED SUBSEQ, the accession provided in column 5 must be present in the same local contig (column 3) as the accession in column 1.

For gap lines:

- Gap type-1 lines are no longer allowed.
- Gap type-2 lines will default to a species-specific preset size if no sizing data is provided.
- Gap type-2 lines may not be adjacent to one another.
- Gap type-3 lines will default to a species-specific preset size if no sizing is provided.
- Gap type-3 lines may not be adjacent to one another.
- Gap type-2 and type-3 lines may not be adjacent to one another.
- TYPE-3 gaps may not be contained within a contig
- Biological gaps must provide a size estimate in column 3; they do not need to have a method in column 4.
- Biological gaps may not be contained within a contig
- There can be consecutive biological gap lines of different types.
- If a gap size is provided in column 3, the method used to determine this size must be provided in column 4, unless column 2 is a biological gap.
- PAR gaps are only permitted on the Y chromosome TPF.
- If the gap type is PAR, the beginning and ending accessions for this region must be listed in columns 3 and 4, respectively.
- If the gap type is PAR, columns 3 and 4 must both contain valid accessions that are found in column 1 on the chromosome X TPF.

CONTAINED COMPONENTS

Types of Contained Components

A component is considered "contained" when its entire sequence can be aligned to another component on the TPF (excluding any internal alignment gaps). A contained component MUST be marked on a TPF if it is to be included in the corresponding AGP. There are three designators that can be used to mark contained components on the TPF: CONTAINED, CONTAINED_TURNOUT, and CONTAINED_SUBSEQ. The designators differ in the switch point selection rules applied to the contained component during AGP production. The rule of full alignment of contained to container does not apply to CONTAINED_SUBSEQ.

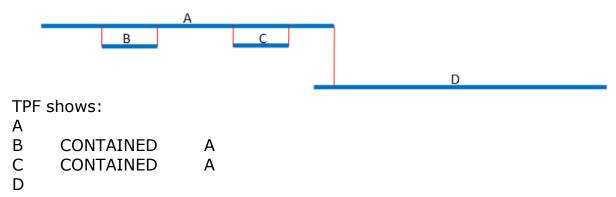
A. CONTAINED

TPF components marked with the "CONTAINED" designator will follow the default switching rules during AGP production. Examples for usage are shown.

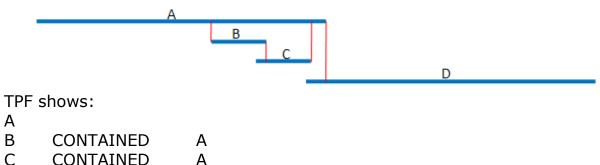
Example A1: Single contained clone



Example A2: Multiple non-overlapping contained clones



Example A3: Multiple overlapping contained clones



C CONTAINED D

Note that the TPF mark-ups for examples A2 and A3 are identical. The software that produces the AGPs will determine the relationship between clones B and C and produce the appropriate switch points.

IMPORTANT: If the last clone in a chain of contained clones will switch back to the container, all clones in the chain should be marked CONTAINED.

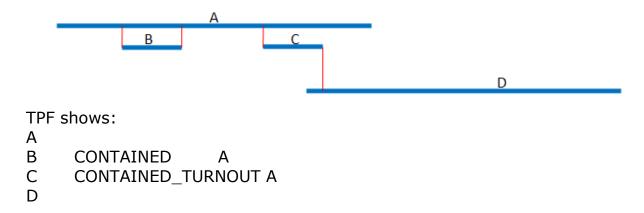
B. CONTAINED_TURNOUT

TPF components marked with the "CONTAINED_TURNOUT" designator will follow alternate switching rules during AGP production. Examples for usage are shown.

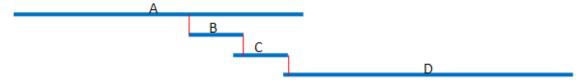
Example B1: Single contained clone



Example B2: Multiple non-overlapping contained clones



Example B3: Multiple overlapping contained clones



TPF shows:

Α В CONTAINED TURNOUT A C CONTAINED_TURNOUT A D

IMPORTANT: In Example B3, note the use of CONTAINED TURNOUT for both components, and contrast to Example A3. If the last clone in a chain of contained clones will not switch back to the container, the entire chain should be marked as CONTAINED_TURNOUT.

C. CONTAINED_SUBSEQ

TPF components marked with the "CONTAINED SUBSEQ" designator will follow using the switch points provided in columns 7 and 8 during AGP production. Example for usage is shown.

Example C1: Small sequence of a long-read inserted into a clone



The software will use the switch points provided in columns 7 and 8 to produce the AGP.

<u>Additional Info for TPF Representation of Contained Components</u>

- A component can only be contained by another component present in the same TPF contig
 - If column 4 = CONTAINED or CONTAINED TURNOUT or CONTAINED SUBSEQ, the accession provided in column 5 must be present in the same local contig (column 3) as the accession in column 1.
- In situations of hierarchical containments, the TPF should always display the nearest contained relationship for any contained sequences.
 - Example:
 - C
 - TPF should report:
 - B contained in A
 - C contained in B
- In situations of hierarchical containments the order of the contained sequences on the TPF does not need to reflect the desired sequence order on the AGP.
- In situations where multiple sequences are contained within the same sequences (but are not hierarchical), the order of the contained sequences on the TPF does not need to reflect the desired sequence order on the AGP.

Specifying Orientation for TPF Components

Component orientation may be optionally specified in TPF column 4 (PLUS or MINUS).

- If the TPF component with specified orientation is a singleton, the specified orientation will be used in the AGP.
- If no orientation is specified for singleton TPF components, they will be placed in "plus" orientation in the AGP by default.

Although there is no need to specify orientation for non-singleton TPF components (their orientation is defined automatically via the alignments with neighboring components), this is allowed. This can be useful for cases where a TPF component is flanked by phase 1 or "?" components and will thus appear as a singleton in the AGP.

- If the TPF component with specified orientation appears as a singleton in the AGP, the specified orientation will be used in the AGP.
- If the TPF component with specified orientation does not appear as a singleton in the AGP, the orientation derived from its alignments will still be used for the purposes of contig building. However, if the component orientation in the AGP conflicts with the orientation specified on the TPF, a warning/error message will be reported.

Orientations cannot be specified on the TPF for contained components. Their orientation will always be derived from the alignment with the container clone.

Specific TPF examples:

1. A TPF may contain one or more partial contigs. For example:

```
##ORGANISM: Mus musculus
##CHROMOSOME: 1
##ASSEMBLY NAME: NOD/MrkTac
##STRAIN/HAPLOTYPE/CULTIVAR: NOD/MrkTac
##TYPE: Contig
##VERSION: 1
##COMMENT: EXAMPLE TPF ONLY
##SUBMITTER: Schneider, NCBI
##CREATE DATE: Nov 3 2006 12:16PM
##UPDATE DATE: Nov 3 2006 12:16PM
##=== Beginning of TPF Data ===
AL645951 DN-29B18 NCBI_Mmchr1_ctg163678
AL596283 DN-257N2 NCBI_Mmchr1_ctg163678
CR936839 DN-120A16 NCBI_Mmchr1_ctg37199
CR936842 DN-189K17 NCBI_Mmchr1_ctg37199
AL671997 DN-396020 NCBI_Mmchr1_ctg163206
AL672024 DN-378G17 NCBI_Mmchr1_ctg163206
##=== End of TPF Data ===
```

The absence of gap lines implies there is no known order for these contigs. Note that contigs may be separated by "#". This is not required and is only done to facilitate reading of the TPF by individuals.

2. A TPF may specify an entire chromosome. For example:

```
##ORGANISM: Mus musculus
##CHROMOSOME: 2
##ASSEMBLY NAME: Reference
##STRAIN/HAPLOTYPE/CULTIVAR: C57BL/6J
##TYPE: Complete Chromosome
##VERSION: 2
##COMMENT: EXAMPLE TPF ONLY
##SUBMITTER: Schneider, NCBI
##CREATE DATE: Oct 29 2007 04:39PM
##UPDATE DATE: Oct 29 2007 04:39PM
##=== Beginning of TPF Data ===
GAP CENTROMERE 300000
       WI1-1974M19 Mmchr2 ctg1
? WI1-923N3 Mmchr2 ctg1
CU207330 WI1-2764C4 Mmchr2 ctg1
AL928883 RP23-60E18 Mmchr2_ctg1
AL732620 RP23-167G19 Mmchr2_ctg1
AL929080 RP24-129J15 Mmchr2_ctg1
AL732328 RP23-106P4 Mmchr2_ctg1
AL844530 RP23-248L2 Mmchr2_ctg1
```

```
WI1-2132D7 Mmchr2 ctg1 CONTAINED AL845441 RP23-32715
   CU181741
 AL845441 RP23-32715 Mmchr2_ctg1
AL935139 RP23-74F20 Mmchr2_ctg1
AL954325 RP23-416H10 Mmchr2_ctg1
AL928978 RP23-95M7 Mmchr2_ctg1 CONTAINED_TURNOUT AL954325 RP23-416H10
AL928947 RP23-69N1 Mmchr2_ctg1 CONTAINED_TURNOUT AL954325 RP23-416H10
AL807832 RP23-198D21 Mmchr2_ctg1
AL807832 RP23-198D21 Mmchr2_ctg1
AL928550 RP23-272I15 Mmchr2_ctg1
AL807778 RP23-198G1 Mmchr2_ctg1
BX682541 RP23-349P20 Mmchr2_ctg1
AL928662 RP23-379M5 Mmchr2_ctg1
AL732403 RP23-119L20 Mmchr2_ctg1
BX323054 RP23-329P15 Mmchr2_ctg1
AL928940 RP23-94J17 Mmchr2_ctg1
AL929142 RP23-340A13 Mmchr2_ctg1
AL928958 RP23-344N23 Mmchr2_ctg1
AL928924 RP23-307E15 Mmchr2_ctg1
CR388026 RP23-39L16 Mmchr2_ctg1
AL928735 RP23-413M3 Mmchr2_ctg1
  AL928735 RP23-413M3 Mmchr2_ctg1
AL928735 RP23-413M3 Mmchr2_ctg1
BX842658 RP23-86F17 Mmchr2_ctg1
AL845275 RP23-112F5 Mmchr2_ctg1
AL845515 RP23-256D19 Mmchr2_ctg1
AL845492 RP23-222P7 Mmchr2_ctg1
AL929240 RP23-245A10 Mmchr2_ctg1
AL772190 RP23-124P7 Mmchr2_ctg1
CR936244 RP24-562B12 Mmchr2_ctg1
CU207293 WI1-632G16 Mmchr2_ctg1
 AL845485 RP23-237D23 Mmchr2_ctg1
BX649227 RP23-114C18 Mmchr2_ctg1
AL840637 RP23-113H3 Mmchr2_ctg1
AL845488 RP23-215P8 Mmchr2_ctg1
AL928600 RP23-348019 Mmchr2_ctg1
AL929143 RP23-339G18 Mmchr2_ctg1
AL773590 RP23-181A13 Mmchr2_ctg1
BX005023 RP24-316N23 Mmchr2_ctg1
AL928713 RP23-399M5 Mmchr2_ctg1
AL929149 RP23-93B24 Mmchr2_ctg1
BX293551 RP23-107021 Mmchr2_ctg1
AL929043 RP23-58H6 Mmchr2_ctg1 CONTAINED BX293551 RP23-107021
AL929187 RP23-8G6 Mmchr2_ctg1 CONTAINED AL929043 RP23-58H6
BX294115 RP24-465I11 Mmchr2_ctg1
AL929440 RP23-292F11 Mmchr2_ctg1
AL928665 RP23-294023 Mmchr2_ctg1
AL928832 RP23-261N18 Mmchr2_ctg1
AL929194 RP23-385L14 Mmchr2_ctg1
AL929194 RP23-385L14 Mmchr2_ctg1
AL772377 RP23-141B15 Mmchr2_ctg1
AL845529 RP23-276D17 Mmchr2_ctg1
AL928704 RP23-393G10 Mmchr2_ctg1
  AL773590 RP23-181A13 Mmchr2 ctg1
 AL845329 RP23-276D17 Mmchr2_ctg1
AL928704 RP23-393G10 Mmchr2_ctg1
AL772367 RP23-119N4 Mmchr2_ctg1
AL953853 RP23-6M16 Mmchr2_ctg1
AL772216 RP23-153M22 Mmchr2_ctg1
AL844485 RP23-436D21 Mmchr2_ctg1
AL845264 RP23-116F18 Mmchr2_ctg1
AL772352 RP23-147F6 Mmchr2_ctg1
 AL928715 RP23-373D16 Mmchr2_ctg1
AL929020 RP23-291L24 Mmchr2_ctg1
BX679665 RP23-247J17 Mmchr2_ctg1
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AL929179 RP23-428M4 Mmchr2_ctg1 AL845548 RP23-3114B13 Mmchr2_ctg1 AL928995 RP23-307N14 Mmchr2_ctg1 AL928909 RP23-320118 Mmchr2_ctg1 AL845313 RP23-202C2 Mmchr2_ctg1 AL845533 RP23-202C2 Mmchr2_ctg1 AL845533 RP23-272N6 Mmchr2_ctg1 AL928641 RP23-272N6 Mmchr2_ctg1 AL928560 RP23-353N23 Mmchr2_ctg1 AL929209 RP23-415C3 Mmchr2_ctg1 AL928807 RP23-97D20 Mmchr2_ctg1 AL928807 RP23-97D20 Mmchr2_ctg1 AL773538 RP23-141C4 Mmchr2_ctg1 AL772303 RP23-141C2 Mmchr2_ctg1 AL84560 RP23-334M9 Mmchr2_ctg1 AL84560 RP23-334M9 Mmchr2_ctg1 AL845434 RP23-201A19 Mmchr2_ctg1 AL845290 RP23-21103 Mmchr2_ctg1 AL929158 RP23-33812 Mmchr2_ctg1 AL928632 RP23-303M15 Mmchr2_ctg1			
AL845548 RP23-307N14 Mmchr2_ctg1 AL928909 RP23-38B1 Mmchr2_ctg1 AL928909 RP23-320I18 Mmchr2_ctg1 AL845313 RP23-202C2 Mmchr2_ctg1 AL845533 RP23-232K16 Mmchr2_ctg1 AL928641 RP23-272N6 Mmchr2_ctg1 AL928560 RP23-353N23 Mmchr2_ctg1 AL929209 RP23-415C3 Mmchr2_ctg1 AL928807 RP23-336A16 Mmchr2_ctg1 AL928807 RP23-97D20 Mmchr2_ctg1 AL772303 RP23-14124 Mmchr2_ctg1 AL772303 RP23-145P20 Mmchr2_ctg1 AL928918 RP23-403G13 Mmchr2_ctg1 AL845434 RP23-21103 Mmchr2_ctg1 AL845434 RP23-21103 Mmchr2_ctg1 AL845290 RP23-338M9 Mmchr2_ctg1 AL845290 RP23-337812 Mmchr2_ctg1 AL845290 RP23-337812 Mmchr2_ctg1 AL84583 RP23-157J23 Mmchr2_ctg1 AL928632 RP23-303M15 Mmchr2_ctg1 AL928632 RP23-303M15 Mmchr2_ctg1 AL928632 RP23-303M15 Mmchr2_ctg1 AL84466 RP23-107K21 Mmchr2_ctg1 AL928632 RP23-303M15 Mmchr2_ctg1 AL928632 RP23-303M15 Mmchr2_ctg1 AL928634 RP23-31915 Mmchr2_ctg1 AL845417 RP23-92015 Mmchr2_ctg1 AL845434 RP23-919 Mmchr2_ctg1 AL845434 RP23-95M4 Mmchr2_ctg1 AL845417 RP23-95M9 Mmchr2_ctg1 AL84584 RP23-3191F2 Mmchr2_ctg1 AL92888 RP23-3281Z0 Mmchr2_ctg1 AL92888 RP23-3281Z0 Mmchr2_ctg1 AL92888 RP23-3281Z0 Mmchr2_ctg1 AL92888 RP23-3193F1 Mmchr2_ctg1 AL92888 RP23-3194F0 Mmchr2_ctg1 AL92888 RP23-3194F0 Mmchr2_ctg1 AL92889 RP23-3194F0 Mmchr2_ctg1 AL92865 RP23-3194F0 Mmchr2_ctg1 AL92865 RP23-3194F0 Mmchr2_ctg1 AL92865 RP23-3194F0 Mmchr2_ctg1 AL92865 RP23-3194F0 M		RP23-428M4	Mmchr2_ctg1
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