# Annotation of Plasmid sequences

Plasmids are used in Molecular Biology and Genetics to introduce selected genetic information into cells and organisms. A typical plasmid contains at least one replication origin (to ensure autonomous replication in *E. coli*) and a selection marker (which enables to select cells which contain the plasmid). In addition the plasmid may contain additional genes, promoters and other features enabling them to be used for specific experiments.

Aim of this project is to analyse a nucleotide sequence of a plasmid (fasta or genbank file) and to annotate the plasmid by detecting all genetic features. To achieve this goal three approaches need to be combined:

> Common features: Based on a number of annotated plasmid sequences in genebank format common features of plasmids are extracted and a list of these features with annotations and sequences is build up ("learning"). The to be annotated plasmid sequences will then be analysed whether the feature is present.
>
> Protein coding genes: Protein coding genes not overlapping with the common features identified above will be identified through a BLAST request.
>
> Primer binding sites: Primers are used for sequencing and PCR amplification. A list of common primers and its sequences will be provided. Primer binding sites shall be detected on the plasmid sequences.
>
> Special translated features: For genetic engineering proteins may contain additional peptide sequences with special function, e.g. epitopes, tag etc. These peptides shall be recognized.

All annotations (common features, additional protein coding genes and primer binding sites) are then stored together with the sequence in a genbank file.

Hints:

Common features:
The extraction and categorization of the common features is the most demanding part of the project. You will be provided with a short and a long learning file. The short file vectors_100.gb contains 100 vectors and the file vectors.gb contains 3576 plasmids *records* with 33293 *features*. The short learning file is for development purposes only.

Filtering: Valid plasmid sequences have more than 1500 bases. *Records* with shorter sequences can be discarded as they only contain partial sequences. Only sequence *features* of the *types* ['promoter', 'CDS', 'polyA_signal','rep_origin', 'primer_bind', 'terminator', 'protein_bind', 'misc_binding', 'misc_recomb', 'oriT', 'LTR', 'misc_signal', 'enhancer', 'mobile_element', 'RBS', 'sig_peptide', '-10_signal', '-35_signal', 'mRNA', 'tRNA', 'rRNA'] need to be analysed.

Clustering: In a first step features with identical sequences can be grouped and identified. Manual inspection is required to give the correct "consensus" standard_name *feature.qualifier* and note *feature.qualifier*. Output generated by the script will facilitate this curation step.

In a second step features of the same *type* and similar annotation (to be assessed by the keys in *feature.qualifiers* note, product, db_xref, standard_name, gene) shall be sequence compared either using the multialignment server MUSCLE or by using a pairwise sequence alignment with *Bio.pairwise.*

For usage of MUSCLE, see this code:

```
from bioservices import *

sequences='>test_a\nAGAGAGAGAG\n\n>test_b\nAGAAAGAA\n\n>test_c\nAGAGGAGAG\n\n'

m=MUSCLE(verbose=False)
jobid=m.run(frmt="fasta", sequence=sequences, email="georg.lipps@fhnw.ch")
while m.getStatus(jobid)==u'RUNNING':
print "Status: ",m.getStatus(jobid)

result=m.getResult(jobid, "sequence")
print "sequence:"
print result
result=m.getResult(jobid, "aln-fasta")
print "aln-fasta:"
print result
result=m.getResult(jobid, "phylotree")
print "phylotree:"
print result
result=m.getResult(jobid, "pim")
print "pim:"
print result
result=m.getResult(jobid, "out")
print "out:"
print result
```

The near identical features can then be merged (PSSM !) and further manual inspection is required to give the correct “consensus” standard_name *feature.qualifier* and note *feature.qualifier*.

Hauptaufgabe

And the end of this process there will be a manually curated list of sequences and PSSMs describing common features found on plasmids. For every feature the number of instances in the learning file is also known (i.e. their importance).

Protein coding genes:
Via blastx it is possible to detect coding regions for known proteins of the plasmid. A single blastx request will probably only yield hits to the longest coding region (giving the highest scores). Therefore it has to be assured that the complete circular sequence is investigated and including the sequences around the beginning and end of the sequence file. Potential coding regions shall be checked whether they are actually part of an open reading frame and thus would give rise to a protein.

Primer binding sites:
For sequencing and PCR it is required that 15 bases of the 3’ end of the primer match perfectly with one of the strands of the plasmid. A list of common primers is found in file common_primer.mfasta. The annotation should indicate when there is only a partial match to a primer.

Special translated features:
A list of peptide sequences tags_epitopes.mfasta is provided. It has to checked whether the six frame translation of the plasmid sequence contains any instances of these peptides sequences.

Suggested partition of work:

member 1:
Extraction of sequence identical features. Assemble the annotation for every sequence by analysing the *feature.qualifiers* dictionary. Setup a statistic how often a term is used.

Check whether there is overlap among the set of extracted identical sequences (pairwise sequence alignment).

Blast the set of sequences against nucleotide database and collect top hits.

➔ The assembled output will help to curate the list of entries.

member 2:
Identification of features with similar annotation (look in to *feature.qualifiers* dictionary). Multiple alignment of these sequences. Merging of the nearly identical sequences into a multiple alignment and a PSSM.

Annotation statistics for every cluster via *feature.qualifiers* dictionary.

Blast of typical sequence against nucleotide database.

➔ The assembled output will help to curate the list of entries.

member 3:
Finding matches of primer binding sites in provided plasmid sequence.

BLAST plasmid sequence against protein database and collect possible protein coding regions. Verify they are open reading frames.

Finding instances of special translated features in plasmid sequences.

Write a genbank file with all annotations in feature fields.

Agreeing with member 1 and 2 how the sequence features are provided (for storing objects in files see cPickle). Performing annotation based on identical sequences or nearly identical sequences based on work of member 1 and 2.

Write a genbank file with all annotations in feature fields.

optional:
Output of a graphic of the plasmid with the annotated features.

## Example of a plasmid record in genbank:

```
LOCUS       AB669567                5056 bp    DNA     circular SYN 16-JUN-2012
DEFINITION  Cloning vector pUC-TTrepT DNA, complete sequence.
ACCESSION   AB669567
VERSION     AB669567.1  GI:379698694
KEYWORDS    .
SOURCE      Cloning vector pUC-TTrepT
  ORGANISM  Cloning vector pUC-TTrepT
            other sequences; artificial sequences; vectors.
REFERENCE   1
  AUTHORS   Vieira,J. and Messing,J.
  TITLE     The pUC plasmids, an M13mp7-derived system for insertion
            mutagenesis and sequencing with synthetic universal primers
  JOURNAL   Gene 19 (3), 259-268 (1982)
   PUBMED   6295879
REFERENCE   2
  AUTHORS   Fujita,A., Misumi,Y. and Koyama,Y.
  TITLE     Two versatile shuttle vectors for Thermus thermophilus-Escherichia
            coli containing multiple cloning sites, lacZalpha gene and
            kanamycin or hygromycin resistance marker
  JOURNAL   Plasmid 67 (3), 272-275 (2012)
   PUBMED   22252135
REFERENCE   3  (bases 1 to 5056)
  AUTHORS   Fujita,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2011) Contact:Atsushi Fujita National Institute
            of Advanced Science and Technology, Biomedical Research Institute;
            1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan URL
            :http://www.aist.go.jp/
FEATURES             Location/Qualifiers
     source          1..5056
                     /organism="Cloning vector pUC-TTrepT"
                     /mol_type="other DNA"
                     /db_xref="taxon:1085938"
                     /note="derivative of pUC13 containing a new MCS and T.
                     thermophilus repA gene; constructed for the E. coli-T.
                     thremophilus shuttle vector"
     misc_feature    216..533
                     /note="E. coli beta-galactosidase gene (LacZ) alpha
                     peptide"
     misc_feature    233..283
                     /note="polylinker
                     HindIII-NotI-SalI-NruI-AflII-EcoRV-Acc65I-EcoRI"
     misc_feature    690..3062
                     /note="derived from pYK225 (derivative of pTT8)"
     gene            complement(1197..2360)
                     /gene="repA"
     CDS             complement(1197..2360)
                     /gene="repA"
                     /codon_start=1
                     /transl_table=11
                     /product="putative RepA protein"
                     /protein_id="BAL70402.1"
                     /db_xref="GI:379698695"
                     /translation="MVLRAYAALRGLSPEALRAHLLAPPLRPERAREAFQRPYLAHFA
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                     NALFDRLRAEAYAHVALFRGVPGGEEAFRAWVEQRAHALNQSLFRDHPKGPLDPREVH
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                     RRGSRRRQEAEEKLTEALKRLQARGERVTARALAREAGVKPHTASKWLKRMRE"
     gene            3255..4115
                     /gene="Amp-R"
     CDS             3255..4115
                     /gene="Amp-R"
                     /codon_start=1
                     /transl_table=11
                     /product="beta-lactamase"
                     /protein_id="BAL70403.1"
                     /db_xref="GI:379698696"
                     /translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
                     IELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
                     YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRL
                     DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
                     LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
                     EIGASLIKHW"
     misc_feature    4276..4861
                     /note="origin of replication (ori) in E. coli"
ORIGIN
        1 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
       61 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaatg tgagttagct
      121 cactcattag gcaccccagg ctttacactt tatgcttccg gctcgtatgt tgtgtggaat
      181 tgtgagcgga taacaatttc acacaggaaa cagctatgac catgattacg ccaagcttgg
      241 cggccgcgtc gactcgcgac ttaagatatc cggtaccgaa ttcactggcc gtcgttttac
      301 aacgtcgtga ctgggaaaac cctggcgtta cccaacttaa tcgccttgca gcacatcccc
      361 ctttcgccag ctggcgtaat agcgaagagg cccgcaccga tcgcccttcc caacagttgc
      421 gcagcctgaa tggcgaatgg cgcctgatgc ggtattttct ccttacgcat ctgtgcggta
      481 tttcacaccg catatggtgc actctcagta caatctgctc tgatgccgca tagttaagcc
      541 agccccgaca cccgccaaca cccgctgacg cgccctgacg ggcttgtctg ctcccggcat
      601 ccgcttacag acaagctgtg accgtctccg ggagctgcat gtgtcagagg ttttcaccgt
      661 catcaccgaa acgcgcgaga cgaaagggcg gctatggagg ggttctccct gtctacgctt
      721 gagcgcccga gggccggtcc agcccccggg ccagagcggg ctccggaccc tgcccccaga
      781 aggaccctct cacccgtcct aaggccccta gaaggccggg gggcgggggg aggggtggga
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      901 aaaatcagtg tttttgccgt cctggacgat gaaaattgcg tgcgggcggg gtaaatatgt
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     1621 cggaaggcct cctccccccc ggggacgccc cggaagaggg ccacgtgggc gtaggcctcc
     1681 gcccgcaggc ggtcaaacag ggcgttgttc cgcccgtagg aggccaggcc cgggtccacc
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     3121 cgtcaggtgg cacttttcgg ggaaatgtgc gcggaacccc tatttgttta tttttctaaa
     3181 tacattcaaa tatgtatccg ctcatgagac aataaccctg ataaatgctt caataatatt
     3241 gaaaaaggaa gagtatgagt attcaacatt tccgtgtcgc ccttattccc ttttttgcgg
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     3481 gcgcggtatt atcccgtatt gacgccgggc aagagcaact cggtcgccgc atacactatt
     3541 ctcagaatga cttggttgag tactcaccag tcacagaaaa gcatcttacg gatggcatga
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     3961 gtgagcgtgg gtctcgcggt atcattgcag cactggggcc agatggtaag ccctcccgta
     4021 tcgtagttat ctacacgacg gggagtcagg caactatgga tgaacgaaat agacagatcg
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     4141 tactttagat tgatttaaaa cttcattttt aatttaaaag gatctaggtg aagatccttt
     4201 ttgataatct catgaccaaa atcccttaac gtgagttttc gttccactga gcgtcagacc
     4261 ccgtagaaaa gatcaaagga tcttcttgag atcctttttt tctgcgcgta atctgctgct
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```

```
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4621 cacagcccag cttggagcga acgacctaca ccgaactgag atacctacag cgtgagctat
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4741 tcggaacagg agagcgcacg agggagcttc caggggaaa cgcctggtat ctttatagtc
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4921 cttttgctca catgttcttt cctgcgttat cccctgattc tgtggataac cgtattaccg
4981 cctttgagtg agctgatacc gctcgccgca gccgaacgac cgagcgcagc gagtcagtga
5041 gcgaggaagc ggaaga
```
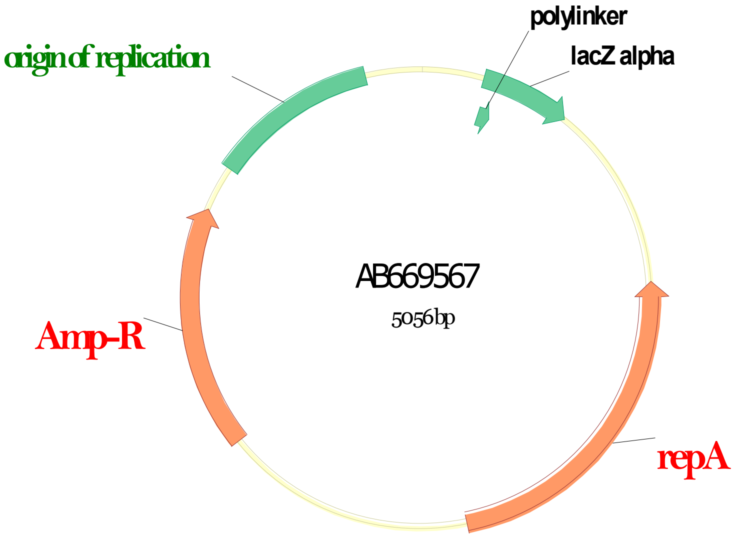

Simulated output of a *de novo* annotation of the plasmid sequence:


polylinker
lacZ alpha
origin of replication
AB669567
5056bp
Amp-R
repA