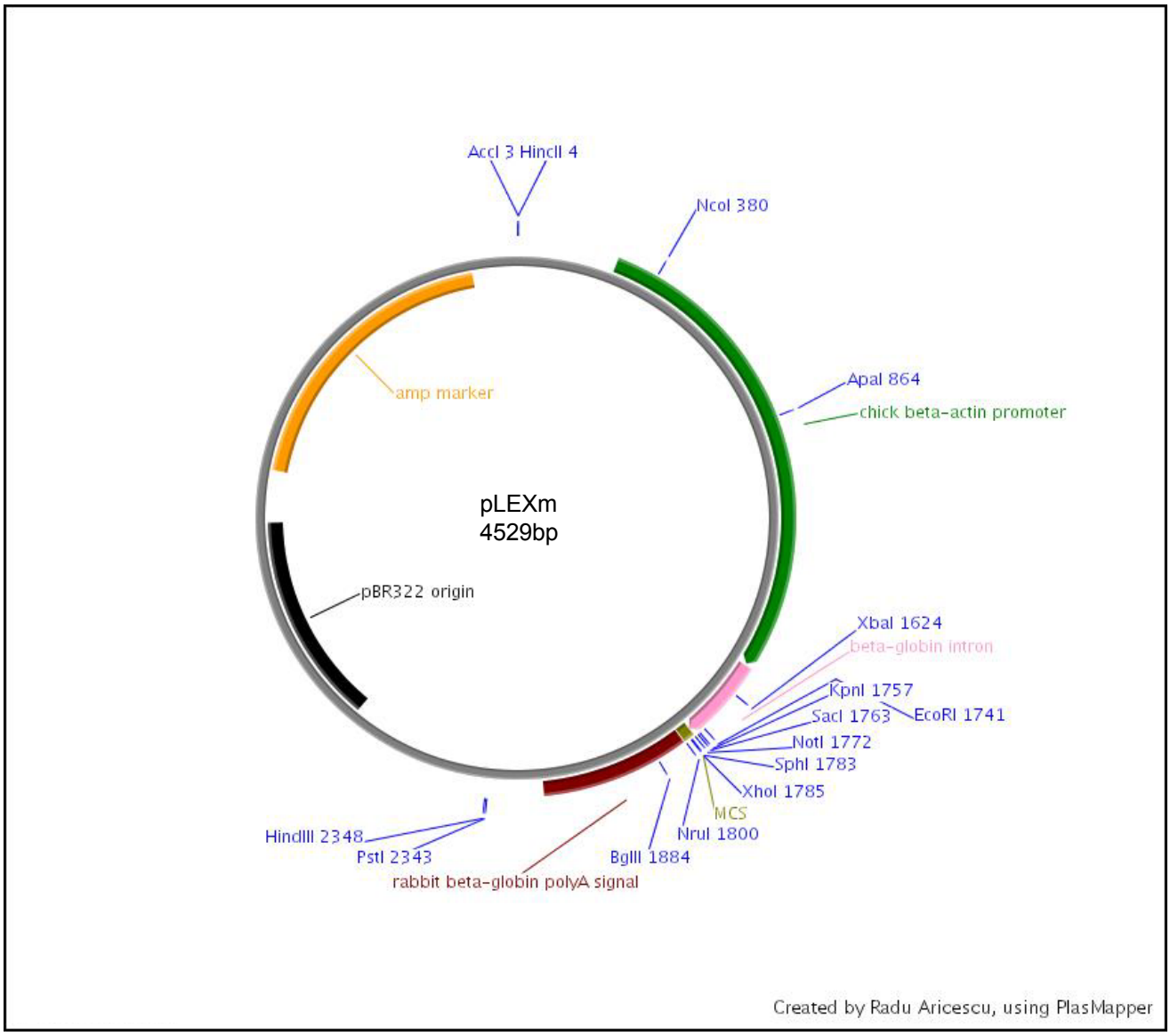
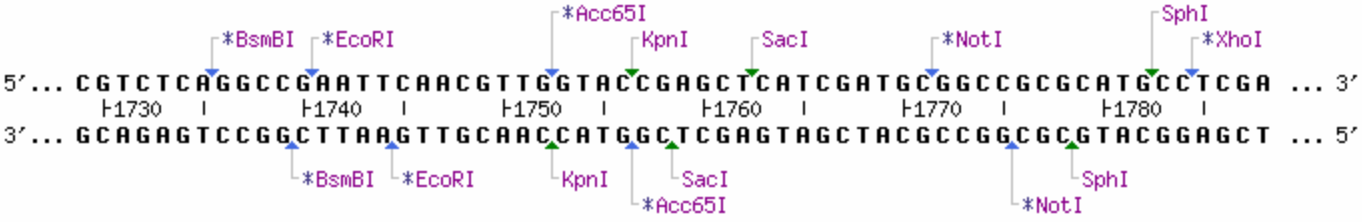
Aricescu, A.R., Lu, W. and Jones, E.Y. “A time and cost efficient system for high level protein production in mammalian cells”

Supplementary information: vector maps



MCS:



For **secreted constructs**, a vector termed **pHLsec** is available. It is based on the pLEXm backbone, the MCS however was reduced when several new features were introduced:

-a **Kozak sequence**;
-a **secretion signal sequence**;
-a **C-terminal K_His6 tag**.

Constructs can be cloned into this vector, preserving and making use of the features listed above, by using the **AgeI** (compatible with XmaI, BspEI, NgoM IV) and **KpnI** (isoschizomer Acc65I is compatible with BsiWI and BsrGI) sites.

```
EcoRI HindIIIKozak M  G  I  L  P  S  P  G  M  P  A  L  L  S
GAATTCAAGCTTGCCACCATGGGGATCCTTCCCAGCCCTGGGATGCCTGCGCTGCTCTCC

 L  V  S  L  L  S  V  L  L  M  G  C  V  A  E  T  G
CTCGTGAGCCTTCTCTCCGTGCTGCTGATGGGTTGCGTAGCTGAAACCGGT...insert...

 G  T  K  H  H  H  H  H  H  *  *    XhoI
GGTACCAAGCACCACCATCACCATCACTAATGATCACTCGAG
```

Fc-tagged constructs, allowing purification of fusion proteins on Protein A affinity columns, can be expressed using the **pHL-FcHis** vector.
The vector is based on the pLEXm backbone and it contains a **3C protease cleavage site** followed by the **human IgGγ1 hinge and Fc** regions and finally a **KHis6** tag, all cloned between the **KpnI** site and the **XhoI** site.

Constructs can be cloned into this vector, preserving and making use of the features listed above, by using **EcoRI** (compatible with MfeI) and **KpnI** (isoschizomer Acc65I is compatible with BsiWI and BsrGI) sites or by ligation-independent cloning.

```
EcoRI
GAATTC .....................insert.............................

KpnI
 G  T  L  E  V  L  F  Q  G  P  K  S  C  D  K  T  H  T  C  P
GGTACCCTGGAGGTGCTGTTCCAGGGCCCCAAATCTTGTGACAAAACTCACACATGCCCA
 P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCC
 K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S
AAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC
 H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A
CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC
 K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T
AAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
 V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCC
 L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q
CTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
 V  Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C
GTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGC
 L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P
CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG
 E  N  N  Y  K  A  T  P  P  V  L  D  S  D  G  S  F  F  L  Y
GAGAACAACTACAAGGCCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTAC
 S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V
AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
 M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
 H  H  H  H  H  H  *  *    XhoI
CACCACCATCACCATCACTAATGATCACTCGAG
```

Biotinylated constructs (for surface plasmon resonance binding experiments for example, see Aricescu *et al.*, 2006) can be expressed using the vector termed **pHL-Avitag3**.
The vector is based on the pLEXm backbone and it contains a **GGS linker region** followed by the **biotin ligase [BirA] recognition site** and finally a **KHis6** tag, all cloned between the **KpnI** site and the **XhoI** site.

Constructs can be cloned into this vector, preserving and making use of the features listed above, by using **EcoRI** (compatible with MfeI) and **KpnI** (isoschizomer Acc65I is compatible with BsiWI and BsrGI) sites or by ligation-independent cloning.

```
EcoRI
GAATTC .....................insert.............................

KpnI
 G  T  G  G  S  G  G  S  G  L  N  D  I  F  E  A  Q  K  I  E
GGTACCGGAGGTTCCGGTGGTTCCGGTCTGAATGATATCTTTGAAGCTCAGAAGATTGAA

 W  H  E  G  R  T  K  H  H  H  H  H  H  *  *    XhoI
TGGCATGAAGGACGTACCAAGCACCACCATCACCATCACTAATGATCACTCGAG
```

Reference

Aricescu, A. R., Hon, W. C., Siebold, C., Lu, W., van der Merwe, P. A. & Jones, E. Y. (2006). *EMBO J* **25**, 701-712.