

Immunoglobulin humanization

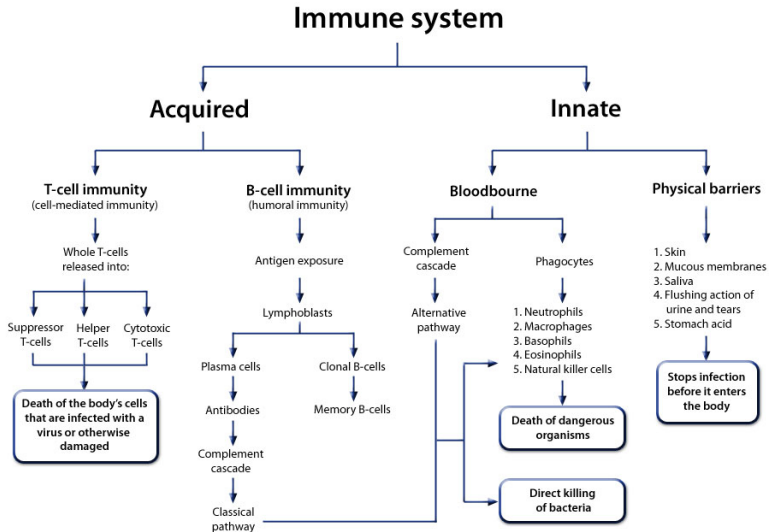
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Immune system

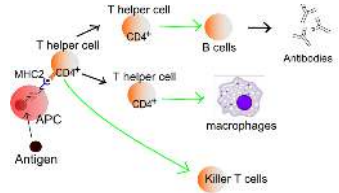
Overview



Adaptive immunity

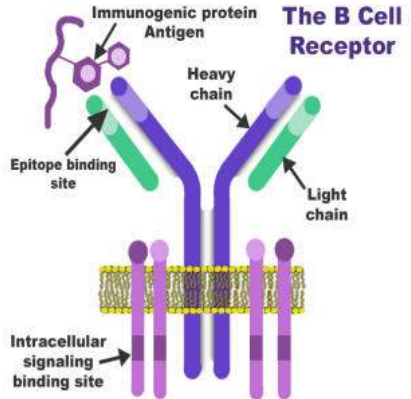
Immune response

- Pathogen gets into the organism.
- Macrophages in mucosa absorb some antigens and take them to T-helpers
- T-helpers make stimulation signal to B-cells and make T-killers and T-suppressors active
- B-cells create immunoglobulins (Ig) and memory cells
- Ig defends free antigens
- Macrophages catch and kill antibody-antigen complexes
- T-killers destroy the cells that were infected by pathogen
- T-suppressors stop the immune response
- Infected cells create interferons to protect other cells

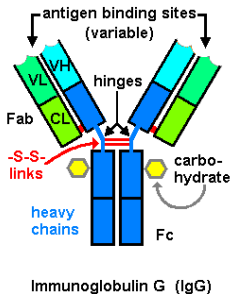


Adaptive immunity

B-Cells



IgG



- 4-chain structure
- Chains linked by disulfide bonds
- Chain are coiled into “domains”
 - ~110 amino acids
- Fc regions formed from H chains and determine isotype
- Fab regions formed from H and L chains and determine binding mechanism
- Biological function IgG
 - Neutralizes toxins
 - Activate complement
 - Opsonization
 - Able to cross placenta
 - Agglutination

Humanization steps:

- Detect regions
- Align immunoglobulin to human antibodies
- Use one of humanization methods, get a set of immunoglobulins.

IgBLAST was developed at NCBI to facilitate analysis of immunoglobulin V region sequences. It uses **BLAST** search algorithm. In addition to performing a regular BLAST search, IgBLAST has several additional functions:

- Reports the germline V, D and J gene matches to the query sequence.
- Annotates the immunoglobulin regions (FWR1 through FWR3).
- Reveals the V(D)J junction details such as nucleotide homology between the ends of V(D)J segments and N nucleotide insertions.
- Indicates the whether the rearrangement is in-frame or out-of-frame.

Problems:

- Paths problems in usage
- Remote usage of NCBI databases broken
- Works good only with nucleotide sequences
- Changes source sequence during aligning

- **install.sh** - installs NCBI IgBLAST, additional databases and Humanization Tools with correct paths and configs
- **bin/**
 - **igblastp.py** - runs NCBI IgBLASTP from any place, using local and network databases
 - **region_finder.py** - region detection by alignment methods
 - **homologs.py** - finding murine/human homologs of Ig
 - **humanizator.py** - humanizing Ig using one of possible algorithms
- **methods/** - humanization methods algorithms
- **scripts/** - common functions, classes and globals

Humanization

Consensus carcass regions

NP-class: $O(M^N)$

- **M** - length of chain V-domain
- **N** - number of homologs, using in humanization

Changing only not-CDR regions

Should be carefully choose the coefficients:

- **up threshold** - amino acid certainly changes to homologs consensus one
- **mid threshold** - make some humanized Ig with different amino acid in current position

Humanization

Humanize

```
$ ./bin/humanizator.py ~/BIO/Ig/Fd138-80.fasta con
SPECIE: human
DOMAIN SYSTEM: imgt
```

Fd138-80

VL:

```
DIVMTQSHKFMSTSVGDRVSITCKASQDVGSVAVVWHQQKSGQSPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTITNVQSEDLADYFCQQYSIFPLTF$
DIVMTQSHKFMSTSVGDRVSITCKASQDVGSVAVVWHQQKSGQSPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTITNVQSEDLADYFCQQYSIFPLTF$
#####cccccc#####ccc#####ccccccc...$
DIQMTQSPSFLASVSGDRVITITCKASQDVGSALVWVYQQKPGKAPKLLIYWASTLHTGVPSRFSGSGSGTDFTLTISLQPEDLATYYCQQYSIFPLTF$
#####cccccc#####ccc#####ccccccc...$
..QL...PS.L.A.....T...R...GIS.YLA.Y...P.KA.....A...LQS...S.S.....E...SSL.P..F.T.Y...LNSY...$
..Q...PSSL.A.....T...Q...ISNYLN.Y...P.KA.....D.NLE...S.S.....F...SSL.P..I.T.Y...DNL...$
..Q...PSSL.A.....T...Q...ISNYLN.Y...P.KA.....D.NLE...S.S.....F...SSL.P..I.T.Y...DNL...$
```

VH:

```
QVQLQQSDAELVKPGASVKISCKVSGYTFDHTIHWMKQRPEQGLEWFGYIYPRDGHTRYSEKFKGKATLTADKSASTAYMHLNLSLTSEDSAVYFCAR$
QVQLQQSDAELVKPGASVKISCKVSGYTFDHTIHWMKQRPEQGLEWFGYIYPRDGHTRYSEKFKGKATLTADKSASTAYMHLNLSLTSEDSAVYFCAR$
#####cccccc#####cccccc#####cccccc#####c...$
QVQLVQSGAEVVKPGASVKISCKVSGYTFDHTMHVVKAPGQGLEWMGYIYPRDGHTRYSEKFRVTLTADTSASTAYMELSSLRSEDTAVYFCAR$
#####cccccc#####cccccc#####cccccc#####c...$
E...V.G.VK...T.....YYM..VQ.A.GK...M.LVD.E..E.I.A...Q.RV.I...T.TD...E.S..R...T...Y...$
...V.G.VK.....V..A.....SYAM.VR.A.G.R...M.W.NAGN.N.K..Q..Q.RV.I.R.T.....E.S..R...T...Y...$
...V.G.VK.....V..A.....SYAM.VR.A.G...M.I.N.SG.S.S.AQ..Q.RV.M.R.T.T..V..E.S..R...T...Y...$
```

≥ 90% congruence with US Patent 5,530,101 (June 25, 1996)

Humanization

Humanize

```
$ ./bin/humanizator.py ~/BIO/Ig/mik-betta1.fasta con -db ncbi-db/nr -n 10 -t 4
SPECIE: human
DOMAIN SYSTEM: imgt
```

mik-betta1

VL:

```
QIVLTQSPAIMASASPGEKVTMTCSGSSSVSFMYWYQQRPGSSPRLLIYDTSNLASGVPVRFSGSGGTSYSLTISRMEAEDAATYYCQQWSTYPLTFGA$
QIVLTQSPAIMASASPGEKVTMTCSGSSSVS-FMY--WYQQRPGSSPRLLIYDTSNLASGVPVRFSGSGGTSYSLTISRMEAEDAATYYCQQWSTYP--$
#####c#####c#####c...$
QIVLTQSPAIMASASPGEKVTMTCSGSSSVS-FMY--WYQQRPGSSPRLLIYDTSNLASGVPVRFSGSGGTSYSLTISRMEAEDAATYYCQQWSTYP--$
QIVLTQSPAIMASASPGEKVTMTCSA#####S-FMY--WYQQRPGSSPRLLIYDTSNLASGVPVRFSGSGGTSYSLTISRMEAEDAATYYCQQWSTYP--$
QIVLTQSPAIMASASPGEKVTMTCSGSSSVS-FMY--WYQQRPGSSPRLLIYDTSNLASGVPVRFSGSGGTSYSLTISRMEAEDAATYYCQQWSTYP--$
QIVLTQSPAIMASASPGEKVTMTCSA#####S-FMY--WYQQRPGSSPRLLIYDTSNLASGVPVRFSGSGGTSYSLTISRMEAEDAATYYCQQWSTYP--$
#####c#####c#####c...$
E.....TL.L...RA.LS.RA.Q...SYLA.....K..QA.....A..R.T.I.A.....DFT...SL.P..F.V.....R.NW...$
E.....TL.L...RA.LS.GA.Q...SS.LA...K..LA.....A.SR.T.I.D.....DFT...L.P..F.V.....YGSS...$
E.....TL.L...RA.LS.RA.Q...SYLA.....K..QA.....A..R.T.I.A.....RDFT...SL.P..F.V.....R.NW...$
.....$
.....$
.....A.....Y.....K.....P..A.....S...$
.....A.....Y.....C..K.....D.....I...$
.....F.....YI.....K.....F.....S...$
.....A..G..Y.....K.....S...$
.....A..IN.Y.....K.....SF...$
.....Q.....A.....Y.....K.....A.....S..PM$
ELQM.....A.....Y.....K.....S...$
.....T.....A.....Y.....F..K.....S...$
```

Humanization

What is next?

- Release more alignment-based humanization methods
- Create region detecting algorithm using three-dimensional Ig model
- Try to use hypothesis about changing Fab position in case of changing amino acids

Thank you for your attention!
Q&A

hg clone <https://zmactep@bitbucket.org/zmactep/ighumanizer>