

Epitope Mapping report

Date: 08.03.2015

Target: 10D2 (AJ Roboscreen, Leipzig, Germany)
 Antigen: alpha-synuclein (human)
 Panning conditions: Immobilized on M270 carboxy beads
 NGS library statistics:

Dataset:	10D2 1pr (1 st panning round)
Number of sequences:	433917
Number of valid sequences:	280835
Pattern/Library:	ENTE1
3-mer motifs:	7253
4-mer motifs:	131481

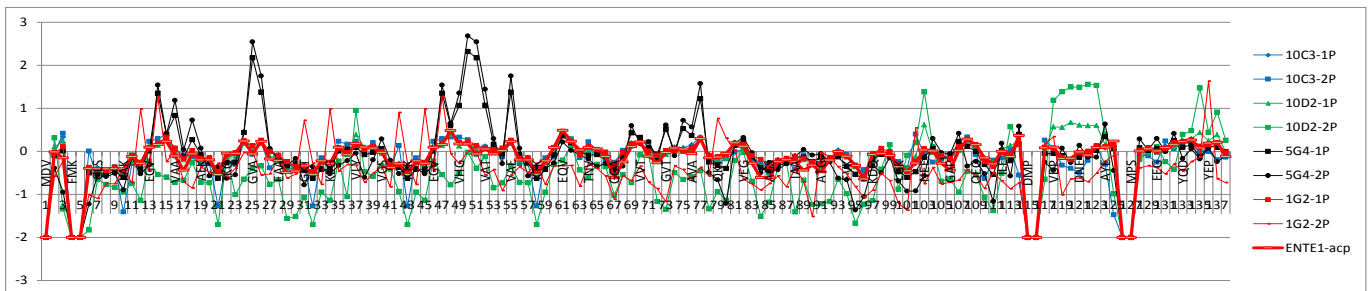
Number of sequences is the output from NGS
Valid sequences are those finally fitting all QC standards and used for the analysis

Dataset:	10D2 2pr (2 nd panning round)
Number of sequences:	599923
Number of valid sequences:	395307
Pattern/Library:	ENTE1
3-mer motifs:	7242
4-mer motifs:	101860

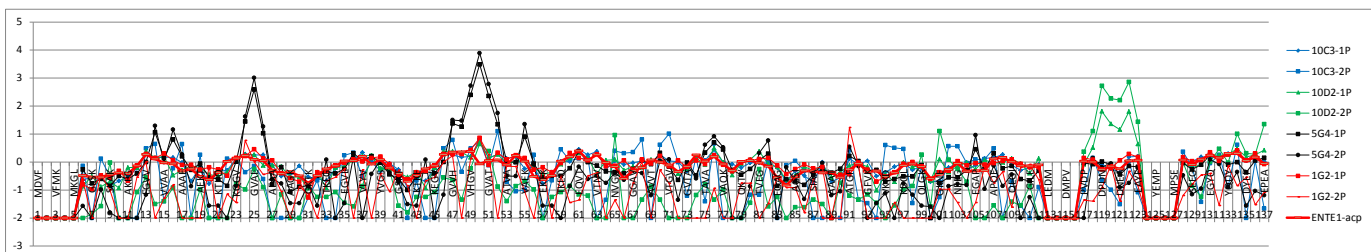
The number of *motifs* in the first round should be close to theory, i.e. ca 7,200 resp. 132,000 for medium size data sets of up to 500,000 sequences

Motif enrichment statistics (green curve for 10D2)

3mers



4mers



Data based on all selection rounds, 3mer and 4mer motifs in the data sets plotted against the alpha synuclein sequence. The reference data set (red) contains almost 2 Mio Sequences from the naïve library. Black = 5G4, another synuclein antibody, blue = unrelated antibody.

X-axis: All possible 4mer fragments of the antigen starting from 1-4, 2-5 ...to... end
 Y-axis: log of enrichment vs. calculated probability in the data set, e.g. 3 is 10³ resp. 1000-fold enrichment. Control ENTE1 naïve starting library has max 0.5 deviation from 0. After the first panning round the library is depleted of motifs, many approach a cut off of <-2, which should not be taken as a save value with respect to the number of observed sequences. Because this would mean that the motif is less than 1/100 of the expected average, which usually is less than 1 sequence in a million.

Potential Epitope:

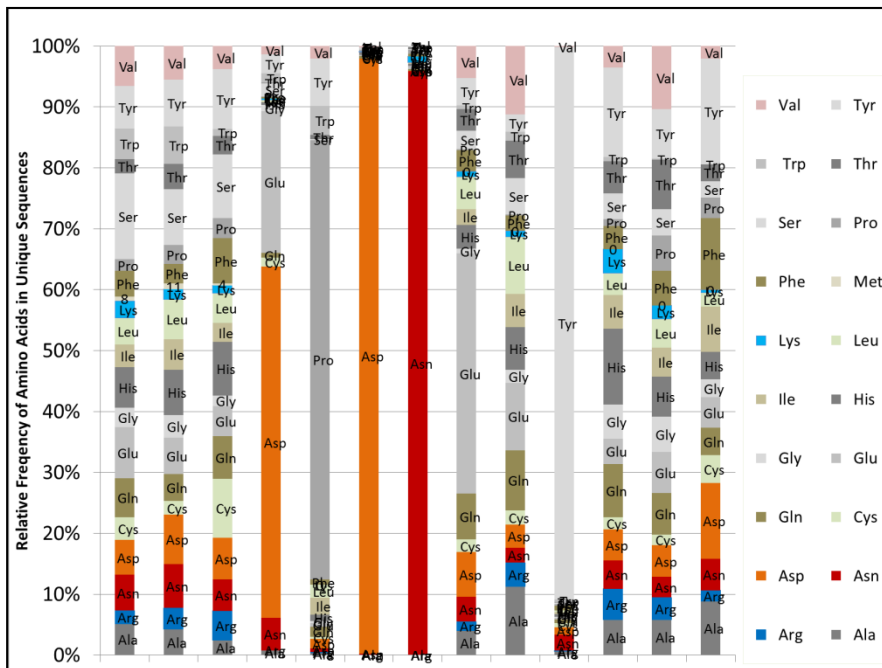
119 - DPDNEAY

potential epitope variations:

vDPDNEaY

Fingerprint

Here a broader analyses of all **1149 individual Sequences with either DPD, PDN or DNE motif**. Sequences were aligned and the frequency of the amino acids is listed. These are corresponding to **> 15,000 observed sequences** from the second selection round.



This epitope is based on the statistical analysis based on the protein sequence below. Genetic variants may lead to other results.

The web-logo is available online:
<http://weblogo.berkeley.edu/>

Fingerprint is the absolute or relative frequency of amino acids observed surrounding the epitope core sequence. Fingerprints may vary with the search sequence. Usually repeated runs with the ENTE-1 library result in similar fingerprints. The motif fingerprint of an antibody is therefore as reliable as a human fingerprint.

Epitope in Sequence

MDVFMKGLSKAKEGVVAAAETKQGVAAEAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGV
 TAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPV**DPDNEAY**EMPSEEGYQDYEP EA

List of found sequence examples

constant regions of the library:

FATVVQAG ... SSG

1st round

DPD**AY

1	FATVVQAGHWRCAFNDKDPDNTAYSSG
1	FATVVQAGTQPCDPDQRAYKFYLCSSG
4	FATVVQAGVNNSITWAVDPDNCAYSSG
6	FATVVQAGHNMCDPDNSAYVPDRFSSG
1	FATVVQAGVRVSNFYTDPDNTAYSSG
1	FATVVQAGTEMCNHQVNDPDNDAYSSG
3	FATVVQAGWNTCWAFFVSDPDNTAYSSG
1	FATVVQAGWVWSTISHYDPDNTAYSSG
8	FATVVQAGRQACWVGYNPDNEAYSSG
16	FATVVQAGDHMCWDYTQDPDNCAYSSG
1	FATVVQAGHHPCSVFKFDPDETAYSSG
1	FATVVQAGWLPFSYWIQDPDNRAYSSG
2	FATVVQAGRWWCFHDVQDPDNIAYSSG
5	FATVVQAGNITCEIWPDPDNCAYSSG
9	FATVVQAGQTFCREFOFDPDNCAYSSG
1	FATVVQAGYENSLDDPDNCAYNTFSSG
10	FATVVQAGVIGCFESTADPDNCAYSSG
2	FATVVQAGQDSCDPDNCAYIQQGDSSG
2	FATVVQAGEKGCPEEFDPDNCAYSSG

DPD***Y

1	FATVVQAGLHQSFESLNDPDNCAYSSG
2	FATVVQAGYFSPIFDPDNCAYSSG
1	FATVVQAGKQCFDFRDDPDNCAYSSG
2	FATVVQAGYQVCYDDPDNCAYQCSSG
1	FATVVQAGRHHCYLDEHDPDNTLYSSG
1	FATVVQAGELFSNNRVDPDNCAYSSG
3	FATVVQAGDWASDPDNCAYIRHQYSSG
3	FATVVQAGYGLSVVWQEDPDNCAYSSG
11	FATVVQAGHEFCDPDNCAYTDIHFSSG
4	FATVVQAGAEVCFDPDNCAYQEDSSG
1	FATVVQAGHPQCGNHQDPDNCAYSSG
21	FATVVQAGLQCDPDNCAYSYNGERFSSG
36	FATVVQAGHNHSDPDNCAYHYTYQDSSG
2	FATVVQAGTDHSYHYWYDPDNCAYSSG
1	FATVVQAGYENSLDDPDNCAYNTFSSG
1	FATVVQAGPVSVCTVPIKDPDNCAYSSG
2	FATVVQAGYKRCFSWDNDPDNCAYSSG

18 FATVVQAGHYNSDPDNEYKGYI I S S G
 9 FATVVQAGNAFCDELAHDPDNVYSSG
 3 FATVVQAGASQCTSSTHDPDYHVYSSG
 5 FATVVQAGEHMSGVSERDPDNIFYSSG
 8 FATVVQAGHNHSDPDNDHYCNTDISSG
 5 FATVVQAGEVHSWPRYTDPDNFQYSSG
 1 FATVVQAGQHSSPTQDPDEPIFYSSG
 3 FATVVQAGDQPCLGWWCDDPNVGYSSG
 1 FATVVQAGPTMSQDHSRDPDNFQYSSG
 6 FATVVQAGQKTSDDPNECYFQACSSG
 4 FATVVQAGNPFCKIQQADPDNQLYSSG
 1 FATVVQAGVRSNNFYTDPDNTAYSSG
 2 FATVVQAGRHGSLHVWQDPDNQSYSSG
 4 FATVVQAGQFWCEDHFQDPDVAFYSSG
 1 FATVVQAGTRLCPIDQLDPDNAVYSSG
 1 FATVVQAGTLTSSHLDCDDPDNDEYSSG
 3 FATVVQAGSNPSPDNNHYIVPTYSSG
 4 FATVVQAGIANSDDPNNLQLELASSG
 1 FATVVQAGYDVCFRTVSDPDNQEYSSG
 1 FATVVQAGWHMCHLVPEDPDNEQYSSG
 4 FATVVQAGQRSPTQDPDEPIFYSSG
 4 FATVVQAGNERSGDDPNQQYQCAFSSG
 1 FATVVQAGDGNSDPDNLEYQSSAISSG
 3 FATVVQAGVNMSLQIYVDPDNQSYSSG
 39 FATVVQAGVWQSWANLHDPDNVQYSSG
 2 FATVVQAGEWMSLPEYFDPDNFSYSSG
 4 FATVVQAGHYRSDPDNVCYTATSASSG
 1 FATVVQAGQHQCYEKTADPDNHTYSSG
 2 FATVVQAGEKGCPEEFDPDNAAYSSG
 10 FATVVQAGVIGCFESTADPDNHAYSSG
 3 FATVVQAGPQFCAQDDPNEIYSVASSG
 1 FATVVQAGSNTSLGNWVDPDNYLYSSG
 3 FATVVQAGDKNCDPDNTLYREELASSG
 16 FATVVQAGDHMCWDYTQDDPNSAYSSG
 32 FATVVQAGWHMCHLFPDDPDNEQYSSG
 11 FATVVQAGEYFSQNIDLDPDNQFYSSG
 2 FATVVQAGQHRCDDPDNLEYHKNHISSG
 1 FATVVQAGIWASVLQNHDPDNYCYSSG
 3 FATVVQAGYTVSYSILVDPDNKRYSSG
 1 FATVVQAGHNHSDPDNDHYTYQNSSG
 1 FATVVQAGRQTSNAQWIDPDNARYSSG
 2 FATVVQAGRDPVCSFTQDDPDNIEYSSG
 4 FATVVQAGHSVSLQQLNDPDNHIYSSG
 6 FATVVQAGNVFSYHHEVDDPNEKYSSG
 1 FATVVQAGVPHSIVLQDDPDNVNYSSG
 4 FATVVQAGAWLSDDPDNDHYRCQHFSSG
 1 FATVVQAGDINSNFVPRDDPDNDGYSSG
 2 FATVVQAGRWWCFHDVQDDPDNIAYSSG
 1 FATVVQAGNVQSKQRPRDPDTFSYSSG
 5 FATVVQAGSWQSGRPHIDPDNRLYSSG
 9 FATVVQAGQPRSVDDPDNYWYLTDYSSG
 2 FATVVQAGTPFCDDPDNDTYNGDFASSG

3 FATVVQAGFEHCFWQDQDPDNIYSSG
1 FATVVQAGQWQCDDPNHFYSHIPISSG
1 FATVVQAGVPQSSIWFDDPDNFNYS
1 FATVVQAGHNWCARRQVDPDNVTYSSG
2 FATVVQAGHTACVADVADPDNKIYSSG
3 FATVVQAGPTMSQDHSHPDNFQYSSG
9 FATVVQAGQTFCREFQFDPDNHAYSSG
8 FATVVQAGPTGCDPDNELYHYDQFSSG
4 FATVVQAGIVMCEVDPDNERYVKDSSG
3 FATVVQAGEYGSIAFPDGLDQYSSG
1 FATVVQAGQHVCKADPDNERYILFSSG
5 FATVVQAGNGVCWSDPDPQYHNCSSG
35 FATVVQAGDDHSLQDRLDPDNQRYSSG
40 FATVVQAGHPRCYLDEHDPDNTLYSSG
2 FATVVQAGPVRCTGTQYDPDNRLYSSG
2 FATVVQAGGENSLWDPDQCCYKSCSSG
1 FATVVQAGHHPCSVFKFDPDETAYSSG
3 FATVVQAGQGACRIDACDPDNDGYSSG
1 FATVVQAGFQHSSQHPTDPDNHLYSSG
4 FATVVQAGKEHSDPDNSHYKYFPCSSG
1 FATVVQAGHWRCAFNDKDPDNTAYSSG
2 FATVVQAGEWSCQGELRDPDNAVYSSG
2 FATVVQAGQTASTIDPDNQIYEQYSSG
42 FATVVQAGEHWCDFKVCDDPNLYSSG
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3 FATVVQAGSEACNNVRTDPDNTRYSSG
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1 FATVVQAGAQQSDPDNCTYIDPPDSSG
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6 FATVVQAGNENSTNFSVDPDNIYSSG
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1 FATVVQAGIWQCYDPDNLEYDRFYSSG
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9 FATVVQAGTVLSHEESCDPDNFLYSSG
1 FATVVQAGGPQSSIWFYDPPDNFNYS
1 FATVVQAGHGFSSIDYYDPDNVSYSSG
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 1 FATVVQAGISVCYTWTGDPDNARYSSG
 6 FATVVQAGFQGCIFYWKQDPDNDYSSG
 1 FATVVQAGDEHSYSFHHDPDNCQYSSG
 2 FATVVQAGFEFSFNDEFDPDNQVYSSG
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 1 FATVVQAGFVHSITDIRDPDNNYSSG
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 2 FATVVQAGQHRCDPDNLEYQKRLDSSG
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 2 FATVVQAGERHCEHQSDPDPHYSSG
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 1 FATVVQAGPTLCNTAAYDPLHTYSSG
 1 FATVVQAGPTMSQDHSHPDNFHYSSG
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 6 FATVVQAGHNMCDPDNSAYVPDRFSSG
 1 FATVVQAGFQGCIFYWKQDPDNDYSSG
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 4 FATVVQAGPANSTWHNYDPDNYQYSSG
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 1 FATVVQAGQQFSWFDPDNRYSSASSG
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 20 FATVVQAGEAQSVQGDYDPDNYTYSSG
 1 FATVVQAGEDLCQGFDDPDNNLYSSG
 8 FATVVQAGHQNSVI IWSDPDNLQYSSG
 3 FATVVQAGVTNCNLDPDNIHYTDYSSG

DPD**A

1 FATVVQAGHWRCANFNDKDPDNTAYSSG
 1 FATVVQAGTQPCDPDQRAYKFYLCSSG
 4 FATVVQAGVNNSITWAVDPDNCAYSSG
 1 FATVVQAGYTPSVDSVDPDQIAASSG

6 FATVVQAGHNMCDPDNSAYVPDRFSSG
 1 FATVVQAGYAVCDPDVNAWEQLDCSSG
 1 FATVVQAGVRVSNFYTDPDNTAYSSG
 1 FATVVQAGSQSDPDVYAWFFAQISSG
 1 FATVVQAGTEMCNHQVNDPDNDAYSSG
 3 FATVVQAGWNTCWAFFVSDPDNTAYSSG
 1 FATVVQAGWVWSTISHYDPDNTAYSSG
 8 FATVVQAGRQACWVGYNPDNEAYSSG
 16 FATVVQAGDHMCWDYTQDPDNSAYSSG
 1 FATVVQAGGPMCYLEVNDPDHQADSSG
 1 FATVVQAGHHPCSVFKFDPDETAYSSG
 1 FATVVQAGWLPFSYWIQDPDNRAYSSG
 2 FATVVQAGRWWCFHDVQDPDNIAYSSG
 5 FATVVQAGNITCEIWPPDPDNQAYSSG
 9 FATVVQAGQTFCREFQFDPDNHAYSSG
 1 FATVVQAGYENSLDDPDNCAYNTFSSG
 10 FATVVQAGVIGCFESTADPDNHAYSSG
 2 FATVVQAGQDSCDPDNYAYIQGDSSG
 2 FATVVQAGEKGPCHEEFDPDAAAYSSG

2nd round

DPD**AY

21 FATVVQAGHWRCAFNDKDPDNTAYSSG
 1 FATVVQAGYVHSHSIAEDPDHIAAYSSG
 15 FATVVQAGVNNSITWAVDPDNCAYSSG
 1 FATVVQAGPGACHWHVHDPDNTAYSSG
 2 FATVVQAGRQSCWVGYNPDNEAYSSG
 26 FATVVQAGHNMCDPDNSAYVPDRFSSG
 31 FATVVQAGRIQCPHHPQDPDNSAYSSG
 56 FATVVQAGVRVSNFYTDPDNTAYSSG
 1 FATVVQAGNITCEIWPPDPDDQAYSSG
 2 FATVVQAGRQTCWVGYNPDNEAYSSG
 1 FATVVQAGDHMCWDYTHDPDNSAYSSG
 17 FATVVQAGTEMCNHQVNDPDNDAYSSG
 50 FATVVQAGWNTCWAFFVSDPDNTAYSSG
 12 FATVVQAGWVWSTISHYDPDNTAYSSG
 91 FATVVQAGRQACWVGYNPDNEAYSSG
 2 FATVVQAGDHMCWDYNQDPDNSAYSSG
 117 FATVVQAGDHMCWDYTQDPDNSAYSSG
 1 FATVVQAGRQACWVGYNPDNNAYSSG
 1 FATVVQAGFEFSFNDEFDPDNQAYSSG
 2 FATVVQAGRWWCFHDVQDPDNIAYSSG
 52 FATVVQAGNITCEIWPPDPDNQAYSSG
 19 FATVVQAGQTFCREFQFDPDNHAYSSG
 21 FATVVQAGYENSLDDPDNCAYNTFSSG
 73 FATVVQAGVIGCFESTADPDNHAYSSG
 1 FATVVQAGRHACWVGYNPDNEAYSSG
 16 FATVVQAGQDSCDPDNYAYIQGDSSG

15 FATVVQAGEKGCPCHEEFDPDAAAYSSG
 7 FATVVQAGYNNSAEFRTPDPDNFAYSSGDPD**A

 1 FATVVQAGHWRCAFNDKDPDNTAYSSG
 1 FATVVQAGTQPCDPDQRAYKFYLCSSG
 4 FATVVQAGVNSITWAVDPDNCAAYSSG
 1 FATVVQAGYTSPVDSVDPDQIAASSG
 6 FATVVQAGHNMCDPNSAYVPDRFSSG
 1 FATVVQAGYAVCDPDVNAWEQLDCSSG
 1 FATVVQAGVRVSNFYTDPDNTAYSSG
 1 FATVVQAGSQSDPDVYAWFFAQISSG
 1 FATVVQAGTEMCNHQVNDPDNDAYSSG
 3 FATVVQAGWNTCWAFFVSDPDNTAYSSG
 1 FATVVQAGWVWSTISHYDPDNTAYSSG
 8 FATVVQAGRQACWVGYNPDNEAYSSG
 16 FATVVQAGDHMCWDYTQDPDNSAYSSG
 1 FATVVQAGGPMCYLEVNDPDHQADSSG
 1 FATVVQAGHHPCSVFKFDPDETAYSSG
 1 FATVVQAGWLPCTSYWIQDPDNRAYSSG
 2 FATVVQAGRWWCFHDVQDPDNIAYSSG
 5 FATVVQAGNITCEIWPDPDNQAYSSG
 9 FATVVQAGQTFCREFOFDPDNHAYSSG
 1 FATVVQAGYENSLDDPDNCAYNTFSSG
 10 FATVVQAGVIGCFESTADPDNHAYSSG
 2 FATVVQAGQDSCDPDNYAYIQQGDSSG
 2 FATVVQAGEKGCPCHEEFDPDAAAYSSG