

## **Designed Armadillo Repeat Proteins as General Peptide Binding Scaffolds: Consensus Design and Computational Optimization of the Hydrophobic Core**

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### Residue Choices in Internal Repeats

Several residues present in the originally defined consensus sequences (Fig. S1) were replaced and some maintained despite their apparent unfavorable properties. The rationale behind these choices is illustrated here. The residues obtained from the consensus sequences were maintained in the positions not mentioned here.

Gln5, present in the consensus type I and C, can potentially form a hydrogen bond with Asp9 of the same repeat, stabilizing H1 (observed in structure 1EE5<sup>1</sup>). Gly11 is conserved for its crucial role in bending the polypeptide chain between H1 and H2, being compatible with a positive  $\phi$  angle required at this position. Pro14 is an unusual conserved feature of armadillo repeats, present at a frequency of 62% in the alignment of importin repeats, 35% for catenin/plakoglobin repeats and 50% in the overall alignment. It is located at the beginning of H2, at a position where it is still not necessary to involve the backbone nitrogen in a hydrogen bond; instead of disrupting the secondary structure, it adopts the  $\phi/\psi$  angles typical for  $\alpha$ -helices in the available crystal structures. Asn37 is a well conserved residue in all consensus sequences, due to its critical role in binding to the backbone of target peptides. Trp33 is also involved in binding, specifically in the recognition of target side chains in the case of importin- $\alpha$  proteins. Thus, it appears with high frequency in the importin subfamily and it is therefore present in the overall consensus as well. All the residues described above were maintained.

Further modifications were introduced in the original consensus sequences (Fig S1) to meet the requirements for protein production (e.g. lack of cysteines) or to avoid structural defects (e.g. presence of potential clashes) that could have arisen from a purely sequence-based alignment. Cys30 (18%) and Cys41 (21%) in the catenin consensus were replaced by the second most common amino acid (Ala 12% and His 17%, respectively), to avoid the formation of undesired disulfide bonds that might limit possible future applications. Pro2 (30%), in the importin consensus, was substituted with the approximately equally conserved Glu (26%), as Pro at such position would probably disrupt H1, as indicated by importin- $\alpha$  crystal structures. In the catenin consensus, position 9 shows a preference for long aliphatic side-chains, either non-polar or polar (Leu, Glu, Gln); however, this residue is solvent-exposed, and Leu9 (21%) was substituted by the second most common amino acid Glu (19%). In the importin consensus, Pro15 (19%) (Fig. S1) was derived

from sequences which do not possess a Pro in position 14. A double Pro14-Pro15 never occurs in the observed sequences and it is likely to be extremely destabilizing for H2. Position 15 is usually occupied by small hydrophobic residues in combination with Pro14. Arg also represents a relatively common choice (16%) but it occurs almost exclusively in the second repeat of natural importins. Val was therefore chosen as more general substitution, instead of Arg, due to the slightly higher frequency of occurrence (13%) compared to other residues. The catenin consensus has Gln as most frequent amino acid (27%) at position 18. However, both Arg and Lys are represented almost at the same frequency (25% and 20% respectively), indicating a preference for positively charged residues. Arg was thus chosen at this position due to its higher frequency. Positions 24 and 25 at the joint between loop H2-H3 and H3 show a clear preference for acidic residues in all consensus sequences. However, a pair of negatively charged residues never occurs in the observed sequences, and it could lead to charge repulsion or formation of a negatively charged belt along the whole protein. The most conserved residues in importin and catenin consensus sequences were preserved (Asp24 in the importin consensus (49%) and Glu25 in the catenin/plakoglobin consensus (43%), respectively). To reduce the local negative charge, Asn (11%) was chosen to replace Glu (19%) at position 25 in the importin consensus, because it is the second most frequent polar residue. Asp24 in the catenin/plakoglobin consensus (20%) was substituted by Asn (10%), a choice driven by the similarity to the original residue in a pool of candidates with almost the same frequency (Arg, Asn, Met, Ser, Tyr, Val). In the overall consensus, a preferred position for the negative charge is not pronounced (Asp24 36%, Glu25 27% in type C), and the alternative amino acids have all very low frequency (<10%). The residues selected for replacement were thus chosen to improve the H3 stability: Glu25 was kept in the overall consensus due to its higher helical propensity and Asn24 was introduced to keep structural similarity to the more frequent residue Asp and to take advantage of its propensity as an N-cap residue<sup>2; 3; 4; 5</sup>. Gly was introduced at position 42 for cloning purposes. It is noteworthy that, because of short H3-H1 loops (1-3 residues in general), one position of the loop is very often occupied by a Gly. Taking into account that position 41 is sometimes involved in binding and will then possibly be subjected to

mutation for applications, it is important to keep a constant glycine inside the loop to maintain the required flexibility.

### **Designed capping repeats**

Capping repeats were designed based on type C internal repeat. A detailed description of the residues introduced in the capping repeats is provided here. The original residues of the type C repeat were maintained in the positions which are not described.

The N-terminal designed capping repeat (Na) goes from position 12 to 42 and includes only H2 and H3. Positions 12, 19, 27, 34 are occupied by hydrophobic residues in the consensus and had to be replaced by hydrophilic residues based on structures and common residues obtained from alignment of N-terminal capping sequences. Ser12 provides the N-terminal helix cap of H2. Asn14 substitutes the more common proline, providing a polar residue with a relatively short side chain. Glu15 can interact with Ser12 in a helix and can additionally stabilize it<sup>6</sup>. Lys18 can form a salt bridge with Glu15, stabilizing the helix, and, in general, a long polar residue is required at this position. Gln19 provides a hydrophobic part for interaction with the neighboring internal repeat as well as a polar moiety for solvent exposure. Asn21 is common at this position and it has a good propensity as helix C-capping residue. Asp23 and Asp24 are conserved as a charged couple in several N-terminal capping repeats. Gln25 is well conserved, polar and with high helical propensity. Gln27 provides a hydrophobic part for interaction with the neighboring internal repeat as well as a polar moiety for solvent exposure. Gln33, well conserved among capping repeats, substitutes the Trp, present at high frequency in internal modules because involved in binding in importins. Lys34 is present at moderate frequency in N-terminal capping repeats, among other polar residues. Arg36 has a high frequency of occurrence and seems to be able to interact with Trp33 present in the importin and overall consensus. Gln37 has a long side chain typical for residues at this position, and, instead of the more common lysine, avoids the formation of a positively charged spot in combination with Arg36. Asp41 maintains the negative charge often present in this position and breaks the helix. Gly42 was introduced to add flexibility and for further module assembly.

The C-terminal designed capping repeat (Ca) includes all three helices. Positions 8, 13, 17, 20, 28, 32, 35, 38, 39 are occupied by hydrophobic residues in the consensus and had to be replaced by hydrophilic residues based on structures and common residues obtained from alignment of C-terminal capping sequences. Lys4 can potentially contribute to the hydrophobic core with the long aliphatic part of the side chain, while contacting the solvent with the positively charged amino-group. Lys8 is present to avoid the formation of a cluster of negative charges that would be formed if a conserved glutamate was used at this position, while keeping a high helical propensity and a long side chain. Glu9 is a highly conserved residue. Ala12 can potentially interact with the hydrophobic core. Glu14 is a common polar residue with high helical propensity as substitute for proline. Lys15 is also a common polar residue with relatively high helical propensity. Leu13 occupies a former hydrophobic core position, but it was retained for its high helical propensity and its ability to interact with Phe39. Glu17 corresponds to a core position in an internal repeat; the hydrophobic residue was substituted with this frequently occurring hydrophilic amino acid with high helical propensity. Gln20 is the more frequent polar residue used to substitute the conserved leucine present in the internal repeats. Positions from 21 to 23 are not clearly defined, showing strong conservation in the catenin/plakoglobin subfamily (maybe for functional reasons) and higher degree of variability in importins. The most conserved residues from the importin subfamily were thus chosen to occupy these positions. Gln28 can provide hydrophobic interactions and a polar side chain, and represents a better choice compared to a conserved tyrosine in the importin subfamily and an alanine in the catenin/plakoglobin subfamily. Gln32 provides high helical propensity and a polar side chain. The presence of frequent aromatic residues at this position does not seem to have a structural reason, judging from the crystal structures. Glu33 is one of the charged residues often found at this position and it has high helical propensity. Glu36 has high frequency in importins where this position is occupied by acidic residues, while in the catenin/plakoglobin subfamily phenylalanine and tyrosine are present. The aromatic residues have probably a functional role, but in our case a charged residue constitutes the better choice, because of the exposed position. Lys37 was chosen to replace the conserved asparagine in the internal repeats. Gln38 was chosen to replace hydrophobic residues, always present at

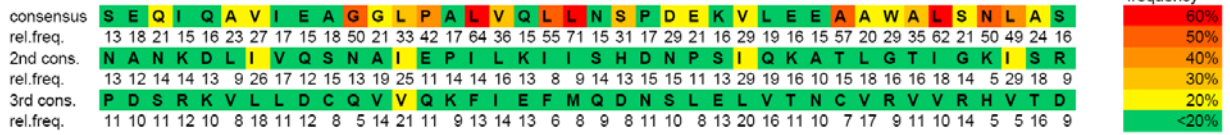
this position both in internal and capping repeats, while providing a polar moiety in contact with the solvent. Phe39 is conserved in several capping repeats. From the available structures, it seems to be important for sealing of the hydrophobic core of importins and for compactness of C-terminal capping repeat via interaction with Leu13. His41 has been added as capping residue to stabilize H3.

## References

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2. Serrano, L., Sancho, J., Hirshberg, M. & Fersht, A. R. (1992). Alpha-helix stability in proteins. I. Empirical correlations concerning substitution of side-chains at the N and C-caps and the replacement of alanine by glycine or serine at solvent-exposed surfaces. *J Mol Biol* **227**, 544-59.
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(a)

initial consensus from SMART



(b)

importin consensus



catenin/plakoglobin consensus



overall consensus



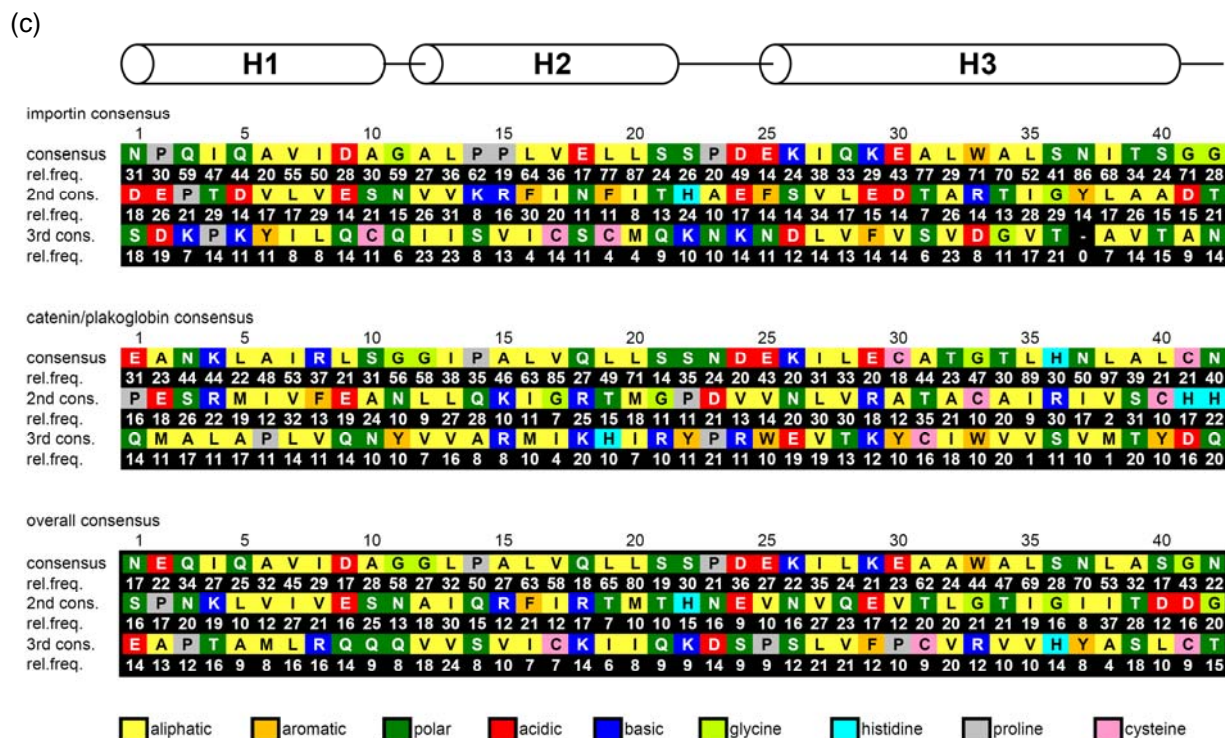


Fig. S1 Consensus sequences derived from multiple alignments. (a) Consensus sequence obtained from alignment of SMART armadillo seed sequences. (b) Consensus sequences of importin and catenin/plakoglobin subfamilies and overall consensus; sequences used for the alignment were retrieved using a profile based on SMART sequences. The sequences are limited to 40 residues and do not contain the loop between adjacent repeats. Amino acids are colored according to their relative frequency. (c) Consensus sequences of importin and catenin/plakoglobin subfamilies and overall consensus after manual refinement of the alignment. The cylinders indicate the putative  $\alpha$ -helices and the numbers denote the positions inside the single repeats according to the conventions introduced. The residues are colored according to amino acid type, as indicated at the bottom. For each position of these sequences, the most frequent, the second and the third most frequent type of residue are indicated, with the relative frequency expressed as percentage.



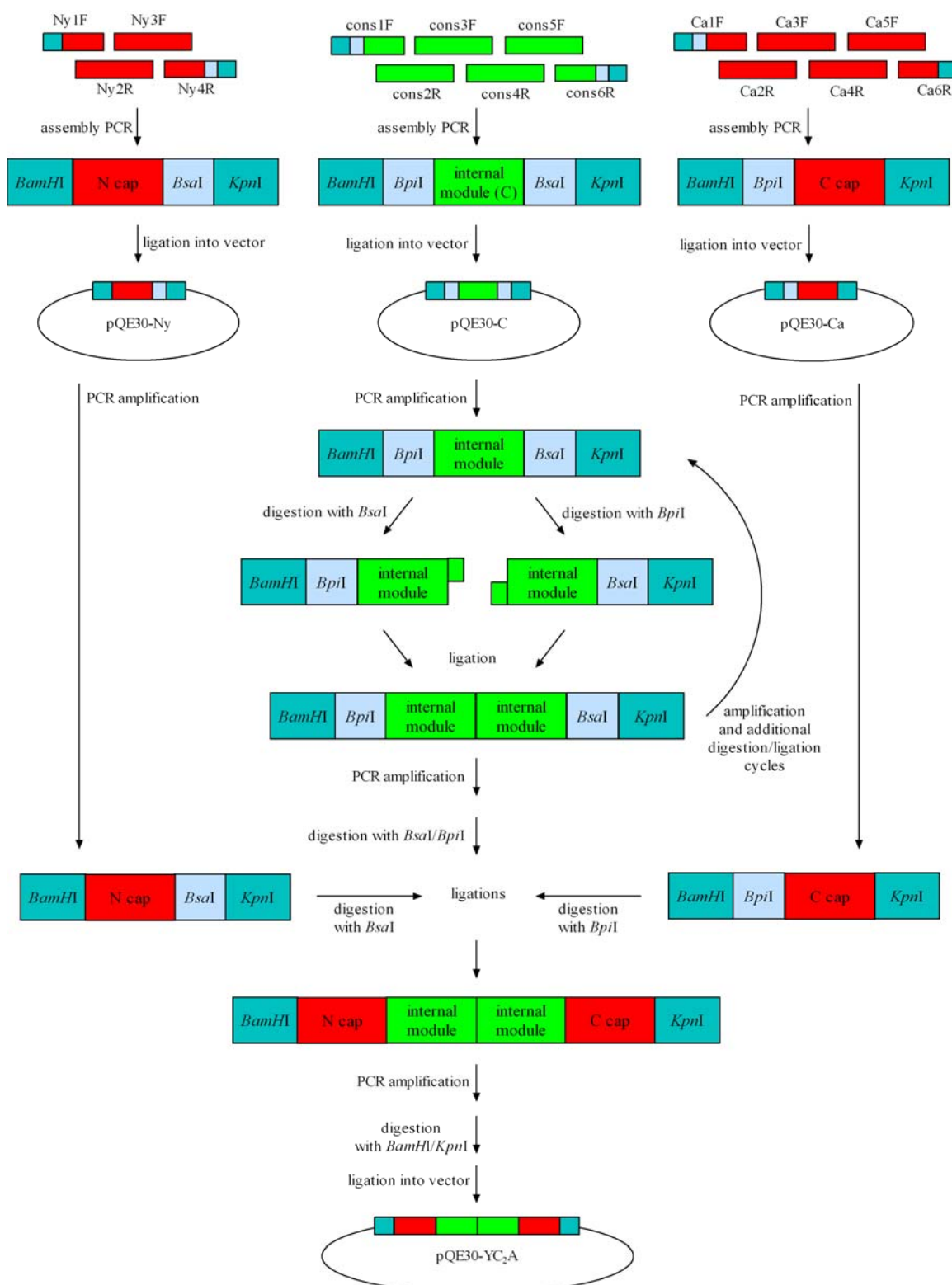


Fig. S2 Scheme of the assembly strategy for designed armadillo repeat protein constructs at the DNA level. Oligonucleotides are assembled to an internal or terminal capping module by PCR. The single modules contain external restriction sites for *Bam*HI and *Kpn*I for insertion in the vector and sites for the type IIS restriction enzymes *Bsa*I and *Bpi*I for ligation of the modules. The construction of YC<sub>2</sub>A from the internal module C, the N-terminal cap Ny and the C-terminal cap Ca is shown as an example.

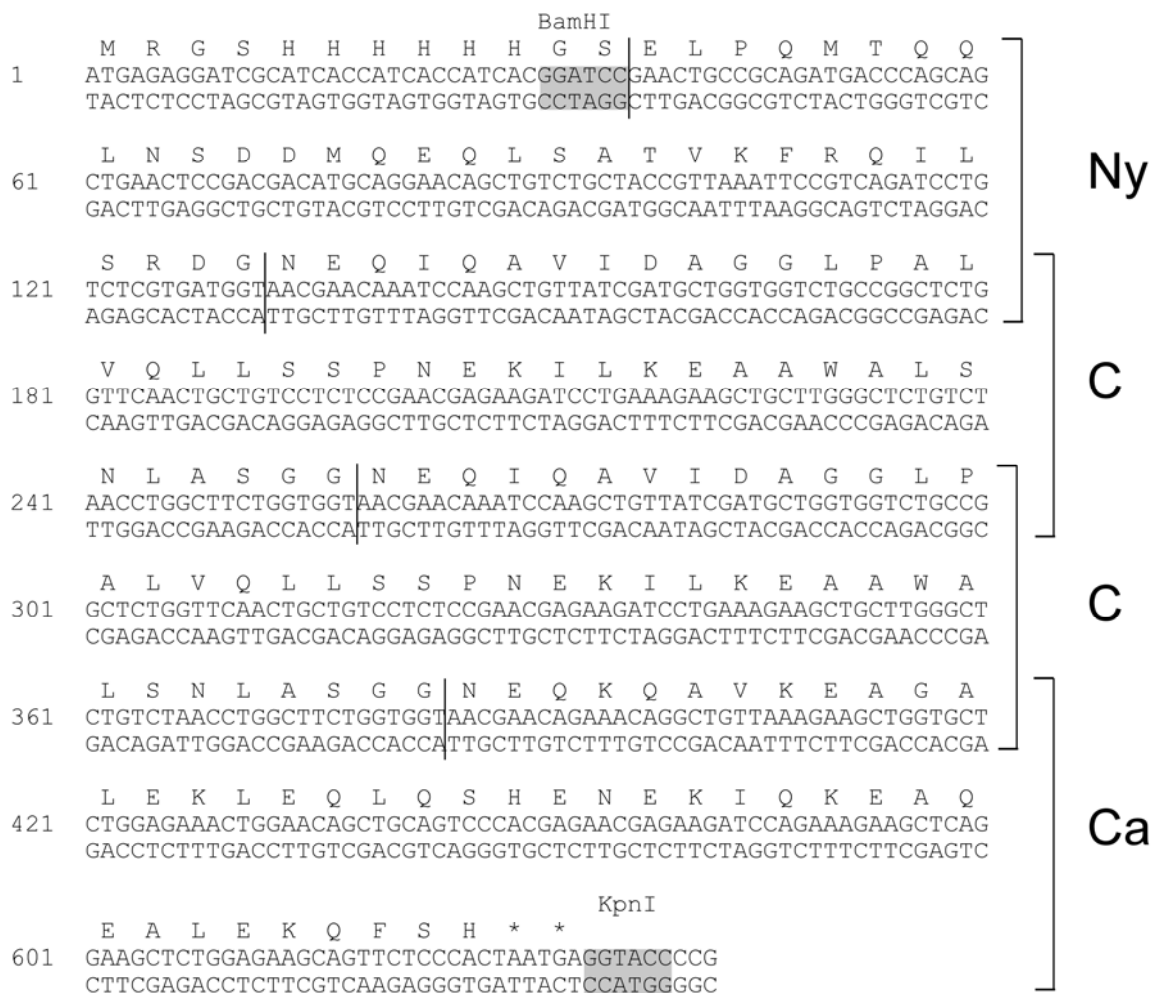


Fig. S3 Sequence of the designed armadillo repeat protein YC<sub>2</sub>A. The translated amino acid sequence is shown on the top of the DNA sequence as single letter code. The bars indicate the separations between the modules and between the MRGSHis<sub>6</sub> tag provided by the vector and the N-terminal capping module. The modules are indicated on the right. The restriction sites used for cloning are highlighted in gray. The star (\*) indicates the presence of a stop codon.

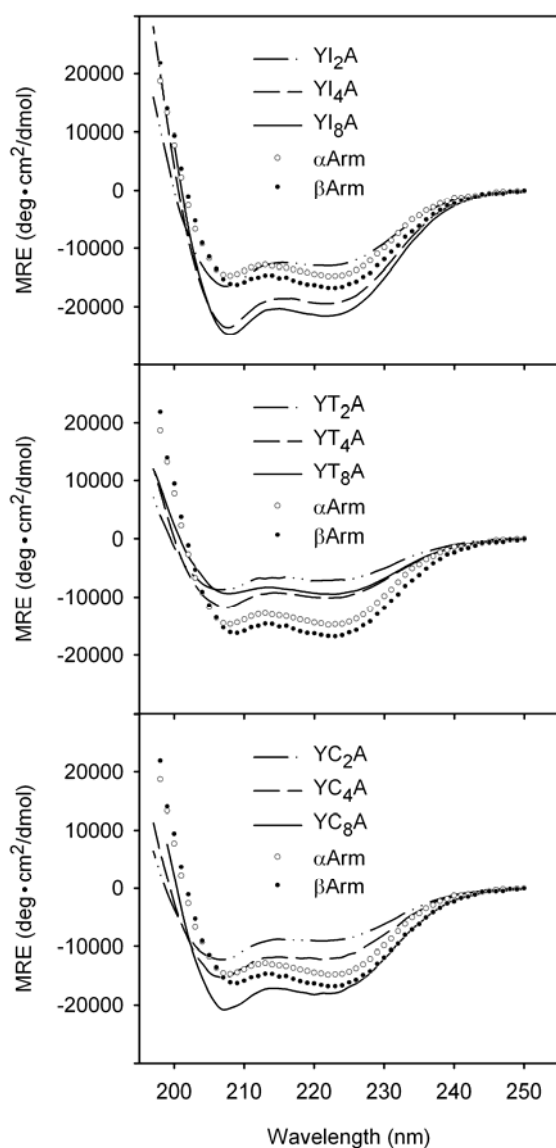


Fig. S4 Circular dichroism (CD) spectra of designed consensus armadillo repeat proteins. From the top, I-type, T-type and C-type proteins containing 2, 4 or 8 internal modules are shown. The CD spectra of the natural armadillo domains of human importin- $\alpha$ 1 ( $\alpha$  Arm) and mouse  $\beta$ -catenin ( $\beta$ Arm) are indicated by empty and filled circles, respectively. The values are reported as mean residue ellipticity (MRE).

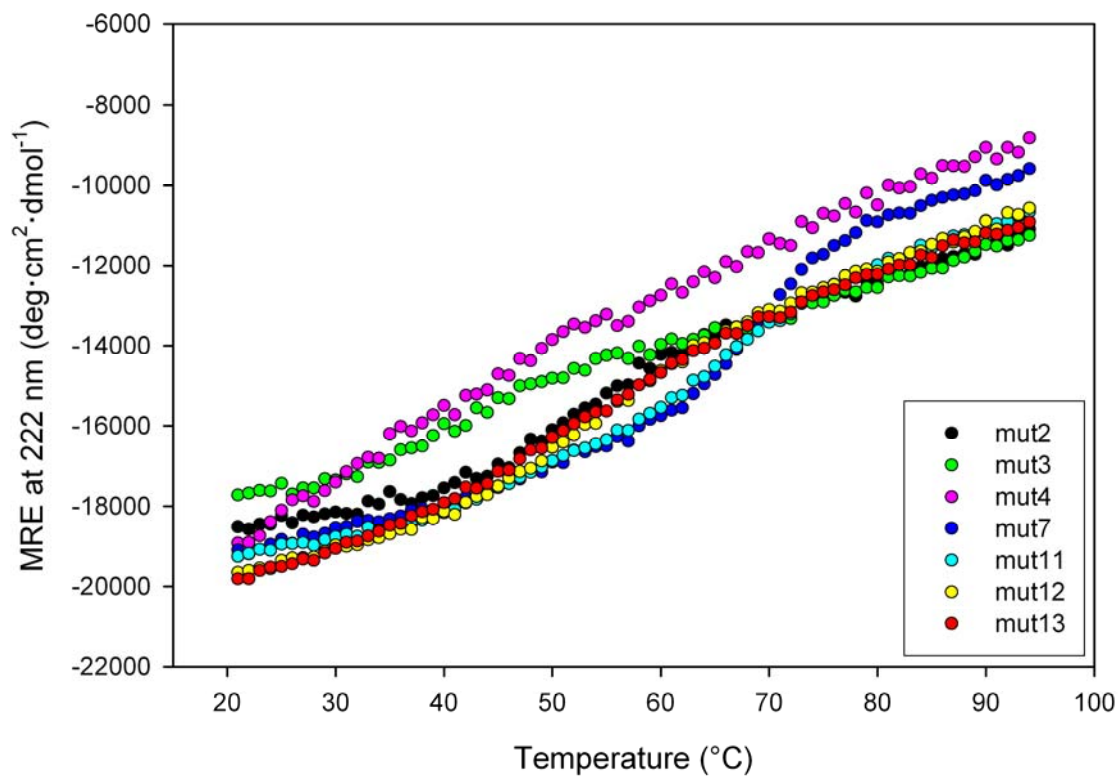


Fig. S5 Thermal denaturation of hydrophobic core mutants. The most promising mutants, based on size exclusion chromatography and ANS binding experiments, are shown. The CD signal at 222 nm is reported as mean residue ellipticity (MRE) as a function of temperature. Remarkably, all the mutants have similar ellipticity at 20°C. mut7 is characterized by the steepest and largest transition.

**Table S1: Oligonucleotides used for the assembly and cloning of designed and natural armadillo repeat protein genes**

name	sequence 5'-3' direction	description (for=forward, rev=reverse)
AcatFOR	CGGGATCCACACGTGCAATTCCTG	for $\beta$ -catenin mouse
AcatREV	GCGGTACCATTAGTCCTCAGACATTCGG	rev $\beta$ -catenin mouse
IMAF5	CGGGATCCCATCACTTCTGACATGATTGAG	for importin- $\alpha$ 1 human
IMAR5	GCGGTACCATTACCCGAAGTAATGCTCAATAAG	rev importin- $\alpha$ 1 human
pQE_f_1	CGGATAACAATTTACACAG	forward primer for pQE vectors
pQE_r_1	GTTCTGAGGTCATTACTG	reverse primer for pQE vectors
Ny1F	CCAGGGATCCGAACTGCCGCAGATGACCCAGCAGCTGAACTCTG	for assembly Ny module and amplification
Ny2R	CGGTAGCAGACAGCTGTTTCCTGCATGTCTCAGAGTTCAGCTGCTGGG	rev assembly Ny module
Ny3F	GAACAGCTGTCTGCTACCGTTAAATTCCTCAGATCCTGTCTCGTGATGG	for assembly Ny module
Ny4R	TTCCTGGTACCCTAAGGTCTCAACCATCA CGAGACAGGATCTG	rev assembly Ny module and amplification
Na1F	CCAGGGATCCTCTCTGAACGAACTGGTTAACAGCTGAACTCCG	for assembly Na module and amplification
Na2R	CTGAGCAGCTTCTTTTCAGCTGTTTCTGGTCGTCGGAGTTCAGCTGTTTAACCAG	rev assembly Na module
Na3F	CAGCTGAAAGAAGCTGAAAGAAGCTGCTCAGAACTGCGTCAGCTGGCTTCCGATGG	for assembly Na module
Na4R	TTCCTGGTACCCTAAGGTCTCAACCATCGGAAGCCAGCTG	rev assembly Na module and amplification
Cy1F	CCAGGGATCCTAGGAAGACCTTGGTGACAACATCAACG	for assembly Cy module and amplification
Cy2R	GCCACCAGCCTTCTCGATGAAGTCCGCGTTCTCGTTGATGTTGTCACCAAGG	rev assembly Cy module
Cy3F	CGAGAAGGCTGGTGGCATGGAGAAGATCTCAACTGCCAGCAGAACG	for assembly Cy module
Cy4R	GCTTTCTCGTAGATCTTGTCTGTTCTCGTTCTGCTGGCAGTTG	rev assembly Cy module
Cy5F	CGACAAGATCTACGAGAAAGCTTACAAGATCATCGAAACCTACTTCGGC	for assembly Cy module
Cy6R	TTCCTGGTACCTCATTAGCCGAAGTAGGTTTCGATG	rev assembly Cy module and amplification
CyM1F	CCAGGGATCCTAGGAAGACCTTGGTAACGAGAACGCGG	for assembly Cm module and amplification
CyM2R	GCCACCAGCCTTCTCGATGAAGTCCGCGTTCTCGTTACCAAGG	rev assembly Cm module
CyM3F	CGAGAAGGCTGGTGGCATGGAGAAGATCTCAACGCTCAGCAGAACG	for assembly Cm module
CyM4R	GCTTTCTCGTAGATCTTGTCTGTTCTCGTTCTGCTGAGCGTTG	rev assembly Cm module

Ca1F	CCAGGGATCCTAGGAAGACCTTGGTAACG AACAGAAACAGGC	for assembly Ca module and amplification
Ca2R	GTTTCTCCAGAGCACCAGCTTCTTTAACA GCCTGTTTCTGTTTCGTTACC	rev assembly Ca module
Ca3F	GCTGGTGCTCTGGAGAACTGGAACAGCT GCAGTCCCACGAG	for assembly Ca module
Ca4R	CCTGAGCTTCTTTCTGGATCTTCTCGTTC TCGTGGGACTGCAGC	rev assembly Ca module
Ca5F	GATCCAGAAAGAAGCTCAGGAAGCTCTGG AGAAGCAGTTCTCCC	for assembly Ca module
Ca6R	TTCCTGGTACCTCATTAGTGGGAGAACTG CTTCTCCAG	rev assembly Ca module and amplification
imp1F	CCAGGGATCCTAGGAAGACCTTGGTAACG AACAGATCC	for assembly importin module and amplification
imp2R	ACCGGCAGAGCACCAGCGTCGATAACAGC CTGGATCTGTTTCGTTACCAAGG	rev assembly importin module
imp3F	CTGGTGCTCTGCCGGTCTGGTTGAACTG CTGTCCTCTCCGGAC	for assembly importin module
imp4R	CCACAGAGCTTCTTTCTGGATCTTGTGT CCGGAGAGGACAGCAG	rev assembly importin module
imp5F	TCCAGAAAGAAGCTCTGTGGGCTCTGTCT AACATCACTTCTGGTGGTTGAGACC	for assembly importin module
imp6R	TTCCTGGTACCCTAAGGTCTCAACCACCA GAAGTG	rev assembly importin module and amplification
cat1F	CCAGGGATCCTAGGAAGACCTTGGTGAAG C	for assembly catenin module and amplification
cat2R	CCACCAGATTCACGGATAGCCAGTTTGTT AGCTTCACCAAGGTCTTCC	rev assembly catenin module
cat3F	CTATCCGTGAATCTGGTGGTATCCCGGCT CTGGTTCGTCTGCTGTCCTC	for assembly catenin module
cat4R	TAGCAGCTTCCAGGATCTTCTCGTTGTTA GAGGACAGCAGACGAACC	rev assembly catenin module
cat5F	AAGATCCTGGAAGCTGCTACTGGCACTCT GCACAACCTGGCTCTGCATGGTTGAG	for assembly catenin module
cat6R	TTCCTGGTACCCTAAGGTCTCAACCATGC AGAGCC	rev assembly catenin module and amplification
cons1F	CCAGGGATCCTAGGAAGACCTTGGTAACG AACAAATCC	for assembly consensus module and amplification
cons2R	AGCCGGCAGACCACCAGCATCGATAACAG CTTGGATTTGTTTCGTTACCAAGG	rev assembly consensus module
cons3F	GGTGGTCTGCCGGCTCTGGTTCAACTGCT GTCCTCTCCGAACG	for assembly consensus module
cons4R	CCAAGCAGCTTCTTTCAGGATCTTCTCGT TCGGAGAGGACAGC	rev assembly consensus module
cons5F	CCTGAAAGAAGCTGCTTGGGCTCTGTCTA ACCTGGCTTCTGGTGGTTGAG	for assembly consensus module
cons6R	TTCCTGGTACCCTAAGGTCTCAACCACCA GAAGCCAG	rev assembly consensus module and amplification
2A-rev	AGCCGGCAGAGCACCAGCATCGATAACAG CTTGGATTTGTTTCGTTACCAAGG	rev hydrophobic core mutants assembly
2AVrev	AGCCGGAACAGCACCAGCATCGATAACAG CTTGGATTTGTTTCGTTACCAAGG	rev hydrophobic core mutants assembly
2AIrev	AGCCGGGATAGCACCAGCATCGATAACAG CTTGGATTTGTTTCGTTACCAAGG	rev hydrophobic core mutants assembly

3A-for	GGTGCTCTGCCGGCTCTGGTTCAACTGCT GTCCTCTCCGAACG	for hydrophobic core mutants assembly
3AVfor	GGTGCTGTTCCGGCTCTGGTTCAACTGCT GTCCTCTCCGAACG	for hydrophobic core mutants assembly
3AIfor	GGTGCTATCCCGGCTCTGGTTCAACTGCT GTCCTCTCCGAACG	for hydrophobic core mutants assembly
4LRev	CCACAGAGCTTCTTTTCAGCAGCTTCTCGT TCGGAGAGGACAGC	rev hydrophobic core mutants assembly
4LVrev	CCAAACAGCTTCTTTTCAGCAGCTTCTCGT TCGGAGAGGACAGC	rev hydrophobic core mutants assembly
4VRev	CCACAGAGCTTCTTTTCAGAACCTTCTCGT TCGGAGAGGACAGC	rev hydrophobic core mutants assembly
4L-rev	CCAAGCAGCTTCTTTTCAGCAGCTTCTCGT TCGGAGAGGACAGC	rev hydrophobic core mutants assembly
4-Lrev	CCACAGAGCTTCTTTTCAGGATCTTCTCGT TCGGAGAGGACAGC	rev hydrophobic core mutants assembly
5L--for	CTGAAAGAAGCTCTGTGGGCTCTGTCTAA CCTGGCTTCTGGTGGTTGAG	for hydrophobic core mutants assembly
5V--for	CTGAAAGAAGCTGTTTGGGCTCTGTCTAA CCTGGCTTCTGGTGGTTGAG	for hydrophobic core mutants assembly
5-V-for	CTGAAAGAAGCTGCTTGGGTTCTGTCTAA CCTGGCTTCTGGTGGTTGAG	for hydrophobic core mutants assembly
5LV-for	CTGAAAGAAGCTCTGTGGGTTCTGTCTAA CCTGGCTTCTGGTGGTTGAG	for hydrophobic core mutants assembly
5VV-for	CTGAAAGAAGCTGTTTGGGTTCTGTCTAA CCTGGCTTCTGGTGGTTGAG	for hydrophobic core mutants assembly
5--Ifor	CTGAAAGAAGCTGCTTGGGCTCTGTCTAA CATCGCTTCTGGTGGTTGAG	for hydrophobic core mutants assembly
5L-Ifor	CTGAAAGAAGCTCTGTGGGCTCTGTCTAA CATCGCTTCTGGTGGTTGAG	for hydrophobic core mutants assembly
5LVIfor	CTGAAAGAAGCTCTGTGGGTTCTGTCTAA CATCGCTTCTGGTGGTTGAG	for hydrophobic core mutants assembly
6Irev	TTCTGGTACCCTAAGGTCTCAACCACCA GAAGCGAT	rev hydrophobic core mutants assembly and amplification

**Table S2 : oligonucleotides used in assembly of single internal modules**

<b>Mutant</b>	<b>oligo #1</b>	<b>oligo #2</b>	<b>oligo #3</b>	<b>oligo #4</b>	<b>oligo #5</b>	<b>oligo #6</b>
mut1	cons1F	cons2R	cons3F	4LLrev	5L--for	cons6R
mut2	cons1F	2A-rev	3A-for	4VLrev	5L--for	cons6R
mut3	cons1F	2A-rev	3A-for	4LLrev	5L--for	cons6R
mut4	cons1F	2A-rev	3A-for	4L-rev	5--Ifor	6Irev
mut5	cons1F	2A-rev	3A-for	4L-rev	5-V-for	cons6R
mut6	cons1F	2A-rev	3A-for	4L-rev	cons5F	cons6R
mut7	cons1F	2A-rev	3A-for	4-Lrev	5L-Ifor	6Irev
mut8	cons1F	2AVrev	3AVfor	4LLrev	5L--for	cons6R
mut9	cons1F	2AIrev	3AIfor	4LLrev	5L--for	cons6R
mut10	cons1F	2A-rev	3A-for	4LVrev	5V--for	cons6R
mut11	cons1F	2A-rev	3A-for	4VLrev	5LV-for	cons6R
mut12	cons1F	2A-rev	3A-for	4LLrev	5LV-for	cons6R
mut13	cons1F	2AIrev	3AIfor	4LLrev	5LV-for	cons6R
mut14	cons1F	2A-rev	3A-for	4LVrev	5VV-for	cons6R
mut15	cons1F	2A-rev	3A-for	cons4R	cons5F	cons6R
mut16	cons1F	cons2R	cons3F	4LLrev	5L-Ifor	6Irev
mut17	cons1F	2AVrev	3AVfor	4LLrev	5LVIfor	6Irev
mut18	cons1F	2AIrev	3AIfor	cons4R	cons5F	cons6R
mut19	cons1F	cons2R	cons3F	4L-rev	cons5F	cons6R



### Summary of Table S3

Table S3 shows the summary of the results from the simulated annealing approach for the 432 mutants (left column) and the original structures (in the upper part).

In the top rows, energy values for the original crystal structures after simulated annealing are indicated. 2bct, 1q1t, 1ee4 are the original structures of mouse  $\beta$ -catenin, mouse importin- $\alpha$  and yeast importin- $\alpha$ , respectively. Catm, impm, impy are the starting models derived from the original structures where the capping repeats have been replaced by the designed capping repeats Ncap and Ccap.

In the leftmost column (#), each hydrophobic core mutant is identified by a number, from 0 to 431 and the mutants are ordered according to the total rank (second leftmost column). These values do not take into account electrostatic contributions. The value used for the ranking (m1m) is the sum of the ranks of median (me), first percentile (1) and minimum (mi) in all the three starting structures. A second value (m1) is given, which denotes the sum of median and first percentile among the three structures. Individual ranks are also reported for median (me), first percentile (1), minimum (mi) referring to each starting structure. On the right of the ranks, the corresponding potential energy values (expressed as kcal/mol) are indicated for median, first percentile (1<sup>st</sup> perc.) and minimum in each structure.

For each mutant the hydrophobic core residues are indicated as a change, compared to the original C-type consensus: the dashes indicate no change. The amino acids are indicated in single letter code. The positions not mutated are indicated in gray.

The average volume for core residues of internal repeats is indicated for mouse  $\beta$ -catenin, mouse importin- $\alpha$ , yeast importin- $\alpha$  (corresponding PDB ID are indicated) and for the C-type consensus. In the case of mutants, the core volume is expressed as difference to the core volume of C-type consensus sequence:  $V(\text{mut})-V(\text{cons})$ . Volumes were calculated according to Chothia<sup>1</sup> in units of  $\text{\AA}^3$ .

The mutants selected for expression and characterization are indicated left of the first column with the corresponding name. The first eight mutants, with a ranking value better than the C-type consensus (#264) were selected. Other high ranking mutants, even if not present in the very top group, were selected because of particularly interesting sequences or results in the ranking process:

mut5 (#2): moderate volume increase, comparison with #8, same composition, only position 27 and 28 exchanged.

mut6 (#0): equivalent to consensus among the first 50 top mutants. Always good rank, only high median in importin mouse structure; it was thus considered to be a good candidate

mut7 (#53): most similar to importin consensus sequence

mut10 (#8): moderate volume increase, comparison with #8, same composition, only position 27 and 28 exchanged.

mut13 (#78): high volume and several  $\beta$ -branched residues

mut14 (#10): high volume and several  $\beta$ -branched residues

mut15 (#48): simplest mutation with volume gain (G->A) compared to consensus

mut16 (#221): good rank, only high median in catenin mouse structure; it was thus considered to be a good candidate

mut17 (#151): high volume and several  $\beta$ -branched residues

mut18 (#120): good rank, only high median in catenin mouse structure; it was thus considered to be a good candidate

mut19 (#216): highest ranking mutant with low core volume

## References

1. Chothia, C. (1975). Structural invariants in protein folding. *Nature* **254**, 304-8.

sequentially tested mutants	catenin			catenin mouse			importin mouse			potential energy			importin yeast			potential energy		
	mutant number	total rank	rank	median	1st perc.	minimum	median	1st perc.	minimum	median	1st perc.	minimum	median	1st perc.	minimum	median	1st perc.	minimum
mut1	220	144	65	-1080.38	-1115.28	-1208.05	-808.08	-821.4	-848.27	-833.52	-852.25	-901.68	-413.6	-442.15	-495.27	-833.52	-852.25	-901.68
mut2	28	146	75	-1036.43	-1058.17	-1090.76	-807.4	-821.89	-870.77	-815.47	-855.78	-877.98	-831.47	-855.78	-877.98	-815.47	-855.78	-877.98
mut3	4	156	75	-1036.43	-1058.17	-1090.76	-807.4	-821.89	-870.77	-815.47	-855.78	-877.98	-831.47	-855.78	-877.98	-815.47	-855.78	-877.98
mut4	148	237	136	-1033.73	-1050.59	-1081.51	-803.98	-816.56	-840.53	-811.12	-836.12	-862.62	-833.36	-852.47	-888.01	-811.12	-836.12	-862.62
mut5	6	295	196	-1050.42	-1070.24	-1104.47	-805.66	-816.12	-837.15	-812.32	-837.15	-862.62	-812.32	-837.15	-862.62	-812.32	-837.15	-862.62
mut6	30	319	211	-1026.21	-1037.11	-1091.37	-804.86	-816.32	-845.83	-804.86	-816.32	-845.83	-804.86	-816.32	-845.83	-804.86	-816.32	-845.83
mut7	3	333	195	-1021.69	-1036.49	-1087.25	-795.15	-806.48	-831.27	-795.15	-806.48	-831.27	-795.15	-806.48	-831.27	-795.15	-806.48	-831.27
mut8	264	347	227	-1014.51	-1033.29	-1085.75	-791.55	-801.25	-837.55	-791.55	-801.25	-837.55	-791.55	-801.25	-837.55	-791.55	-801.25	-837.55
mut9	2	380	223	-1021.11	-1034.98	-1070.13	-799.12	-809.69	-830.89	-799.12	-809.69	-830.89	-799.12	-809.69	-830.89	-799.12	-809.69	-830.89
mut10	416	277	171	-1044.99	-1058.77	-1091.21	-787.51	-795.01	-831.18	-787.51	-795.01	-831.18	-787.51	-795.01	-831.18	-787.51	-795.01	-831.18
mut11	53	449	320	-1000.91	-1022.83	-1082.14	-805.86	-816.56	-838.18	-805.86	-816.56	-838.18	-805.86	-816.56	-838.18	-805.86	-816.56	-838.18
mut12	144	449	270	-1001.91	-1022.83	-1082.14	-805.86	-816.56	-838.18	-805.86	-816.56	-838.18	-805.86	-816.56	-838.18	-805.86	-816.56	-838.18
mut13	24	480	345	-1006.55	-1027.26	-1076.27	-801.59	-814.46	-843.5	-801.59	-814.46	-843.5	-801.59	-814.46	-843.5	-801.59	-814.46	-843.5
mut14	222	487	332	-1006.55	-1027.26	-1076.27	-801.59	-814.46	-843.5	-801.59	-814.46	-843.5	-801.59	-814.46	-843.5	-801.59	-814.46	-843.5
mut15	18	500	246	-1004.4	-1024.12	-1078.65	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut16	42	525	252	-1009.34	-1032.34	-1090.94	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut17	60	548	319	-1017.68	-1031.78	-1086.23	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut18	151	554	391	-1018.88	-1031.62	-1086.39	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut19	218	559	391	-1018.88	-1031.62	-1086.39	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut20	25	585	332	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut21	62	625	445	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut22	21	637	516	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut23	31	650	459	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut24	150	661	464	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut25	72	667	424	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut26	20	668	325	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut27	292	676	357	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut28	232	688	352	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut29	74	712	451	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut30	256	739	467	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut31	64	740	498	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut32	124	754	467	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut33	312	754	467	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut34	100	766	468	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut35	100	766	468	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut36	69	817	476	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut37	44	824	566	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut38	40	826	608	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut39	102	832	537	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut40	188	836	515	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut41	127	840	535	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut42	27	840	535	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut43	104	849	591	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut44	104	849	591	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut45	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut46	224	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut47	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut48	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut49	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut50	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut51	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut52	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut53	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut54	108	925	569	-1004.34	-1024.04	-1074.56	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5	-802.49	-814.46	-843.5
mut55	108	9																

1079	668	174	224	257	-1000.67	-1011.64	-1046.23	-793.06	-815.42	-842.85	97	115	122	-819.63	-830.59	965.1
1082	629	40	55	123	-1003.42	-1037.44	-1066.62	-784.02	-820.91	-850.44	71	14	15	-803.08	-837.65	973.16
388	333	1107	759	45	72	-1023.43	-1038.15	-1078.44	-784.02	-820.91	28	24	32	-803.37	-816.59	959.96
393	1108	703	236	247	213	-991.45	-1004.57	-1045.64	-787.95	-802.91	28	24	32	-833.26	-847.65	975.2
293	1121	687	84	138	-1015.75	-1042.04	-1063.58	-787.95	-802.91	-843.03	108	60	66	-817.63	-834.88	952.15
296	1133	713	180	44	-1015.75	-1042.04	-1063.58	-787.95	-802.91	-843.03	108	60	66	-817.63	-834.88	952.15
295	1138	710	250	250	-988.25	-1019.52	-1035.89	-788.35	-800.15	-822.42	98	29	35	-835.45	-849.61	964.81
366	1138	717	62	69	93	-1019.76	-1036.02	-1072.27	-788.35	-800.15	230	189	275	-802.14	-820.17	946.25
61	1159	741	201	194	237	-996.29	-1016.13	-1050.07	-788.35	-800.15	162	148	103	-810.36	-826.98	987.65
266	1165	779	24	21	41	-1028.12	-1047.88	-1085.81	-788.35	-800.15	21	38	31	-835.29	-844.12	979.29
336	1167	715	115	113	94	-1009.75	-1026.49	-1072.14	-788.35	-800.15	74	61	153	-823.24	-837.07	960.95
30	1177	715	115	113	94	-1009.75	-1026.49	-1072.14	-788.35	-800.15	74	61	153	-823.24	-837.07	960.95
197	1176	590	86	150	188	-1015.06	-1021.42	-1056.99	-788.35	-800.15	14	18	88	-838.12	-851.3	971.37
362	1182	590	73	37	74	-1016.18	-1042.97	-1077.2	-788.35	-800.15	253	241	115	-815.34	-866.25	966.25
217	1187	765	54	83	88	-1021.44	-1032.88	-1073.28	-788.35	-800.15	252	263	232	-799.82	-812.61	950.81
289	1187	765	54	83	88	-1021.44	-1032.88	-1073.28	-788.35	-800.15	252	263	232	-799.82	-812.61	950.81
412	1214	715	10	12	60	-1037.25	-1054.3	-1081.21	-788.35	-800.15	210	247	191	-804.07	-814.49	955.35
192	1225	805	277	193	144	-985.6	-1000.79	-1054.2	-788.35	-800.15	210	247	191	-804.07	-814.49	955.35
389	1232	877	117	98	18	-1008.82	-1030.62	-1087.49	-788.35	-800.15	232	225	206	-801.78	-816.77	955.08
122	1240	775	253	220	264	-986.64	-1012.31	-1065.85	-788.35	-800.15	232	225	206	-801.78	-816.77	955.08
249	1254	775	253	220	264	-986.64	-1012.31	-1065.85	-788.35	-800.15	232	225	206	-801.78	-816.77	955.08
197	1266	957	220	191	61	-993.35	-1016.47	-1081.11	-788.35	-800.15	109	139	128	-816.04	-834.06	964.36
132	1276	957	220	191	61	-993.35	-1016.47	-1081.11	-788.35	-800.15	109	139	128	-816.04	-834.06	964.36
41	1304	1003	145	131	177	-1004.44	-1023.7	-1058.58	-788.35	-800.15	146	134	61	-777.85	-792.81	951.11
361	1304	842	199	176	256	-996.61	-1018.54	-1046.72	-788.35	-800.15	71	87	106	-788.49	-796.45	951.11
35	1311	871	229	210	253	-1005.47	-1039.89	-1056.27	-788.35	-800.15	63	121	161	-776.95	-786.56	951.11
26	1312	871	167	214	207	-1001.11	-1033.46	-1045.33	-788.35	-800.15	63	121	161	-776.95	-786.56	951.11
305	1312	871	167	214	207	-1001.11	-1033.46	-1045.33	-788.35	-800.15	63	121	161	-776.95	-786.56	951.11
84	1314	793	82	96	177	-1016.17	-1031.53	-1058.65	-788.35	-800.15	125	116	187	-781.16	-789.13	951.11
304	1330	843	5	6	4	-1043.4	-1058.92	-1115.4	-788.35	-800.15	244	239	295	-765.36	-781.3	905.85
230	1330	843	5	6	4	-1043.4	-1058.92	-1115.4	-788.35	-800.15	244	239	295	-765.36	-781.3	905.85
316	1330	843	5	6	4	-1043.4	-1058.92	-1115.4	-788.35	-800.15	244	239	295	-765.36	-781.3	905.85
30	1331	877	100	108	173	-1010.41	-1032.8	-1062.3	-788.35	-800.15	175	159	138	-774.27	-789.05	953.47
100	1351	877	100	108	173	-1010.41	-1032.8	-1062.3	-788.35	-800.15	175	159	138	-774.27	-789.05	953.47
164	1361	820	97	97	52	-1016.53	-1032.72	-1063.06	-788.35	-800.15	126	132	262	-780.6	-792.83	951.11
88	1363	820	97	97	52	-1016.53	-1032.72	-1063.06	-788.35	-800.15	126	132	262	-780.6	-792.83	951.11
244	1368	944	107	115	75	-1010.97	-1026.15	-1076.77	-788.35	-800.15	262	264	307	-782.33	-791.44	951.11
244	1368	944	107	115	75	-1010.97	-1026.15	-1076.77	-788.35	-800.15	262	264	307	-782.33	-791.44	951.11
296	1373	1151	108	99	91	-1010.63	-1030.38	-1072.66	-788.35	-800.15	412	425	98	-796.11	-805.33	951.11
50	1385	889	139	80	226	-1005.44	-1018.26	-1051.64	-788.35	-800.15	185	161	251	-773.14	-789.54	951.11
145	1413	1047	306	300	171	-991.3	-999.65	-1059.66	-788.35	-800.15	208	160	175	-780.68	-792.83	951.11
315	1411	1047	306	300	171	-991.3	-999.65	-1059.66	-788.35	-800.15	208	160	175	-780.68	-792.83	951.11
298	1417	988	56	102	-1021.18	-1036.21	-1070.81	-788.35	-800.15	241	271	242	-765.78	-777.66	951.11	
315	1417	988	56	102	-1021.18	-1036.21	-1070.81	-788.35	-800.15	241	271	242	-765.78	-777.66	951.11	
295	1442	988	159	157	72	-1020.02	-1021.23	-1059.43	-788.35	-800.15	224	176	181	-767.94	-788.23	951.11
305	1442	988	159	157	72	-1020.02	-1021.23	-1059.43	-788.35	-800.15	224	176	181	-767.94	-788.23	951.11
372	1447	938	118	63	87	-1008.8	-1036.5	-1073.32	-788.35	-800.15	48	56	34	-792.09	-803.84	951.11
66	1447	938	118	63	87	-1008.8	-1036.5	-1073.32	-788.35	-800.15	48	56	34	-792.09	-803.84	951.11
276	1459	957	93	102	126	-1013.37	-1029.51	-1066.15	-788.35	-800.15	366	190	266	-747.05	-786.3	951.11
276	1459	957	93	102	126	-1013.37	-1029.51	-1066.15	-788.35	-800.15	366	190	266	-747.05	-786.3	951.11
241	1462	940	472	416	216	-1000.8	-1021.58	-1052.93	-788.35	-800.15	209	168	123	-771.62	-786.25	951.11
241	1462	940	472	416	216	-1000.8	-1021.58	-1052.93	-788.35	-800.15	209	168	123	-771.62	-786.25	951.11
70	1469	1004	19	53	103	-1013.03	-1025.85	-1073.61	-788.35	-800.15	16	9	8	-766.97	-785.79	951.11
70	1469	1004	19	53	103	-1013.03	-1025.85	-1073.61	-788.35	-800.15	16	9	8	-766.97	-785.79	951.11
112	1480	894	168	213	218	-1001.1	-1013.59	-1052.75	-788.35	-800.15	139	188	244	-778.94	-786.3	951.11
98	1485	957	93	102	126	-1013.37	-1029.51	-1066.15	-788.35	-800.15	366	190	266	-747.05	-786.3	951.11
276	1506	1248	46	48	19	-1023.4	-1040.89	-1096.03	-788.35	-800.15	421	421	37	-286.85	-299.1	951.11
276	1506	1248	46	48	19	-1023.4	-1040.89	-1096.03	-788.35	-800.15	421	421	37	-286.85	-299.1	951.11
103	1517	1074	344	372	341	-974.08	-984.97	-1030.79	-788.35	-800.15	66	91	48	-789.62	-799.1	951.11
103	1517	1074	344	372	341	-974.08	-984.97	-1030.79	-788.35	-800.15	66	91	48	-789.62	-799.1	951.11
226	1523	851	65	78	190	-1019.21	-1034.78	-1056.93	-788.35	-800.15	287	260	345	-759.77	-775.12	951.11
116	1529	1322	142	138	186	-1005.01	-1023.01	-1057.49	-788.35	-800.15	82	58	80	-776.14	-786.68	951.11
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126	1540	928	77	70	47	-1017.17	-1035.94	-1064.86	-788.35	-800.15	238	297	355	-766.17	-774.33	951.11
126	1540	928	77	70	47	-1017.17	-1035.94	-1064.86	-788.35	-800.15	238	297	355	-766.17	-774.33	951.11
226	1552	955	95	114	90	-1012.88	-1026.39	-1072.97	-788.35	-800.15	147	171	241	-775.18	-789.06	951.11
296	1558	989	15	16	43	-1009.47	-1050									

62	1715	1211	143	103	20	-1004.7	-1023.19	-1055.6	189	187	246	-772.76	-768.75	-812.21	291	258	238	-795.2	-809.41	545.71	62
63	1716	1212	144	104	21	-1005.6	-1024.08	-1056.5	190	188	247	-773.65	-769.64	-813.10	292	259	239	-796.1	-810.3	546.6	63
64	1717	1213	145	105	22	-1006.5	-1024.97	-1057.4	191	189	248	-774.54	-770.53	-813.99	293	260	240	-797.0	-811.2	547.5	64
65	1718	1214	146	106	23	-1007.4	-1025.86	-1058.3	192	190	249	-775.43	-771.42	-814.88	294	261	241	-797.9	-812.1	548.4	65
66	1719	1215	147	107	24	-1008.3	-1026.75	-1059.2	193	191	250	-776.32	-772.31	-815.77	295	262	242	-798.8	-813.0	549.3	66
67	1720	1216	148	108	25	-1009.2	-1027.64	-1060.1	194	192	251	-777.21	-773.20	-816.66	296	263	243	-799.7	-813.9	550.2	67
68	1721	1217	149	109	26	-1010.1	-1028.53	-1061.0	195	193	252	-778.10	-774.09	-817.55	297	264	244	-800.6	-814.8	551.1	68
69	1722	1218	150	110	27	-1011.0	-1029.42	-1061.9	196	194	253	-778.99	-774.98	-818.44	298	265	245	-801.5	-815.7	552.0	69
70	1723	1219	151	111	28	-1011.9	-1030.31	-1062.8	197	195	254	-779.88	-775.87	-819.33	299	266	246	-802.4	-816.6	552.9	70
71	1724	1220	152	112	29	-1012.8	-1031.20	-1063.7	198	196	255	-780.77	-776.76	-820.22	300	267	247	-803.3	-817.5	553.8	71
72	1725	1221	153	113	30	-1013.7	-1032.09	-1064.6	199	197	256	-781.66	-777.65	-821.11	301	268	248	-804.2	-818.4	554.7	72
73	1726	1222	154	114	31	-1014.6	-1032.98	-1065.5	200	198	257	-782.55	-778.54	-822.00	302	269	249	-805.1	-819.3	555.6	73
74	1727	1223	155	115	32	-1015.5	-1033.87	-1066.4	201	199	258	-783.44	-779.43	-822.89	303	270	250	-806.0	-820.2	556.5	74
75	1728	1224	156	116	33	-1016.4	-1034.76	-1067.3	202	200	259	-784.33	-780.32	-823.78	304	271	251	-806.9	-821.1	557.4	75
76	1729	1225	157	117	34	-1017.3	-1035.65	-1068.2	203	201	260	-785.22	-781.21	-824.67	305	272	252	-807.8	-822.0	558.3	76
77	1730	1226	158	118	35	-1018.2	-1036.54	-1069.1	204	202	261	-786.11	-782.10	-825.56	306	273	253	-808.7	-822.9	559.2	77
78	1731	1227	159	119	36	-1019.1	-1037.43	-1070.0	205	203	262	-787.00	-783.00	-826.45	307	274	254	-809.6	-823.8	560.1	78
79	1732	1228	160	120	37	-1020.0	-1038.32	-1070.9	206	204	263	-787.89	-783.89	-827.34	308	275	255	-810.5	-824.7	561.0	79
80	1733	1229	161	121	38	-1020.9	-1039.21	-1071.8	207	205	264	-788.78	-784.78	-828.23	309	276	256	-811.4	-825.6	561.9	80
81	1734	1230	162	122	39	-1021.8	-1040.10	-1072.7	208	206	265	-789.67	-785.67	-829.12	310	277	257	-812.3	-826.5	562.8	81
82	1735	1231	163	123	40	-1022.7	-1040.99	-1073.6	209	207	266	-790.56	-786.56	-830.01	311	278	258	-813.2	-827.4	563.7	82
83	1736	1232	164	124	41	-1023.6	-1041.88	-1074.5	210	208	267	-791.45	-787.45	-830.90	312	279	259	-814.1	-828.3	564.6	83
84	1737	1233	165	125	42	-1024.5	-1042.77	-1075.4	211	209	268	-792.34	-788.34	-831.79	313	280	260	-815.0	-829.2	565.5	84
85	1738	1234	166	126	43	-1025.4	-1043.66	-1076.3	212	210	269	-793.23	-789.23	-832.68	314	281	261	-815.9	-830.1	566.4	85
86	1739	1235	167	127	44	-1026.3	-1044.55	-1077.2	213	211	270	-794.12	-790.12	-833.57	315	282	262	-816.8	-831.0	567.3	86
87	1740	1236	168	128	45	-1027.2	-1045.44	-1078.1	214	212	271	-795.01	-791.01	-834.46	316	283	263	-817.7	-831.9	568.2	87
88	1741	1237	169	129	46	-1028.1	-1046.33	-1079.0	215	213	272	-795.90	-791.90	-835.35	317	284	264	-818.6	-832.8	569.1	88
89	1742	1238	170	130	47	-1029.0	-1047.22	-1079.9	216	214	273	-796.79	-792.79	-836.24	318	285	265	-819.5	-833.7	570.0	89
90	1743	1239	171	131	48	-1029.9	-1048.11	-1080.8	217	215	274	-797.68	-793.68	-837.13	319	286	266	-820.4	-834.6	570.9	90
91	1744	1240	172	132	49	-1030.8	-1049.00	-1081.7	218	216	275	-798.57	-794.57	-838.02	320	287	267	-821.3	-835.5	571.8	91
92	1745	1241	173	133	50	-1031.7	-1049.89	-1082.6	219	217	276	-799.46	-795.46	-838.91	321	288	268	-822.2	-836.4	572.7	92
93	1746	1242	174	134	51	-1032.6	-1050.78	-1083.5	220	218	277	-800.35	-796.35	-839.80	322	289	269	-823.1	-837.3	573.6	93
94	1747	1243	175	135	52	-1033.5	-1051.67	-1084.4	221	219	278	-801.24	-797.24	-840.69	323	290	270	-824.0	-838.2	574.5	94
95	1748	1244	176	136	53	-1034.4	-1052.56	-1085.3	222	220	279	-802.13	-798.13	-841.58	324	291	271	-824.9	-839.1	575.4	95
96	1749	1245	177	137	54	-1035.3	-1053.45	-1086.2	223	221	280	-803.02	-799.02	-842.47	325	292	272	-825.8	-840.0	576.3	96
97	1750	1246	178	138	55	-1036.2	-1054.34	-1087.1	224	222	281	-803.91	-799.91	-843.36	326	293	273	-826.7	-840.9	577.2	97
98	1751	1247	179	139	56	-1037.1	-1055.23	-1088.0	225	223	282	-804.80	-800.80	-844.25	327	294	274	-827.6	-841.8	578.1	98
99	1752	1248	180	140	57	-1038.0	-1056.12	-1088.9	226	224	283	-805.69	-801.69	-845.14	328	295	275	-828.5	-842.7	579.0	99
100	1753	1249	181	141	58	-1038.9	-1057.01	-1089.8	227	225	284	-806.58	-802.58	-846.03	329	296	276	-829.4	-843.6	579.9	100
101	1754	1250	182	142	59	-1039.8	-1057.90	-1090.7	228	226	285	-807.47	-803.47	-846.92	330	297	277	-830.3	-844.5	580.8	101
102	1755	1251	183	143	60	-1040.7	-1058.79	-1091.6	229	227	286	-808.36	-804.36	-847.81	331	298	278	-831.2	-845.4	581.7	102
103	1756	1252	184	144	61	-1041.6	-1059.68	-1092.5	230	228	287	-809.25	-805.25	-848.70	332	299	279	-832.1	-846.3	582.6	103
104	1757	1253	185	145	62	-1042.5	-1060.57	-1093.4	231	229	288	-810.14	-806.14	-849.59	333	300	280	-833.0	-847.2	583.5	104
105	1758	1254	186	146	63	-1043.4	-1061.46	-1094.3	232	230	289	-811.03	-807.03	-850.48	334	301	281	-833.9	-848.1	584.4	105
106	1759	1255	187	147	64	-1044.3	-1062.35	-1095.2	233	231	290	-811.92	-807.92	-851.37	335	302	282	-834.8	-849.0	585.3	106
107	1760	1256	188	148	65	-1045.2	-1063.24	-1096.1	234	232	291	-812.81	-808.81	-852.26	336	303	283	-835.7	-849.9	586.2	107
108	1761	1257	189	149	66	-1046.1	-1064.13	-1097.0	235	233	292	-813.70	-809.70	-853.15	337	304	284	-836.6	-850.8	587.1	108
109	1762	1258	190	150	67	-1047.0	-1065.02	-1097.9	236	234	293	-814.59	-810.59	-854.04	338	305	285	-837.5	-851.7	588.0	109
110	1763	1259	191	151	68	-1047.9	-1065.91	-1098.8	237	235	294	-815.48	-811.48	-854.93	339	306	286	-838.4	-852.6	588.9	110
111	1764	1260	192	152	69	-1048.8	-1066.80	-1099.7	238	236	295	-816.37	-812.37	-855.82	340	307	287	-839.3	-853.5	589.8	111
112	1765	1261	193	153	70	-1049.7	-1067.69	-1100.6	239	237	296	-817.26	-813.26	-856.71	341	308	288	-840.2	-854.4	590.7	112
113	1766	1262	194	154	71	-1050.6	-1068.58	-1101.5	240	238	297	-818.15	-814.15	-857.60	342	309	289	-841.1	-855.3	591.6	113
114	1767	1263	195	155	72	-1051.5	-1069.47	-1102.4	241	239	298	-819.04	-815.04	-858.49	343	310	290	-842.0	-856.2	592.5	114
115	1768	1264	196	156	73	-1052.4	-1070.36	-1103.3	242	240	299	-819.93	-815.93	-859.38	344	311	291	-842.9	-857.1	593.4	115
116	1769	1265	197	157	74	-1053.3	-1071.25	-1104.2	243	241	300	-820.82	-816.82	-860.27	345	312	292	-843.8	-858.0	594.3	116
117	1770	1266	198	158	75	-1054.2	-1072.14	-1105.1	244	242	301	-821.71	-817.71	-861.16	346	313	293	-844.7	-858.9	595.2	117
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262	2649	1965	264	269	317	494	78	1005	22	1036	25	308	321	234	757	48	771	63	813	16	405	377	333	774	56	797	09	837	09	47	
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33	3356	2204	301	330	419	848	23	984	23	1008	66	322	310	10	762	05	762	05	777	59	33	320	320	769	73	787	26	815	26	101	
327	3463	2317	340	379	337	874	8	983	82	1031	44	375	390	411	743	92	758	27	780	52	327	415	418	788	74	792	25	816	36	-1	
191	3502	2360	403	408	366	853	58	973	65	1027	42	395	413	430	730	26	745	42	769	52	191	375	366	796	34	781	41	836	01	48	
403	3503	2302	392	405	424	860	44	974	32	1000	05	335	366	388	752	16	764	22	786	08	403	396	408	776	29	787	4	818	84	48	
431	3528	2340	395	398	371	897	45	976	63	1026	18	383	368	397	751	85	764	18	792	73	431	421	422	795	39	777	04	803	6	48	
163	3532	2380	402	413	427	895	93	971	96	1006	38	383	396	394	739	81	754	51	791	66	163	402	394	321	775	24	792	79	838	82	102
384	3532	2380	402	413	427	895	93	971	96	1006	38	383	396	394	739	81	754	51	791	66	384	381	392	786	39	792	79	838	82	102	
397	3574	2345	381	361	362	869	95	988	27	1021	06	424	426	431	757	27	765	33	768	53	397	381	392	777	65	795	04	829	26	-2	
311	3574	2440	428	429	352	854	07	955	98	1029	66	381	395	388	739	86	755	33	792	71	311	405	402	789	34	774	95	789	34	75	
335	3584	2378	347	377	401	872	95	984	05	1014	39	401	417	422	721	91	738	17	773	24	335	417	419	788	01	781	97	826	27	49	
351	3627	2403	399	403	362	895	74	975	32	1022	73	389	405	423	735	9	749	35	773	02	351	407	400	773	83	790	44	815	95	26	
359	3640	2381	409	420	428	948	81	964	35	1008	42	346	376	393	750	45	762	52	791	98	359	418	420	768	04	780	74	806	85	26	
416	3640	2381	409	420	428																										