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Repetitive Elements May Comprise Over Two-Thirds of the Human Genome

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Repeats in eukaryotic genomes

- **Tandem repeats**
  - Microsatellites 1-6 nt
  - Minisatellites 10-60 nt
- **Transposable elements** (TE)
  - **Class-I: Retrotransposons**
    - SINEs: Alu, MIR
    - LINEs: L1, L2, CR1
    - LTRs
  - **Class-II: DNA transposons**

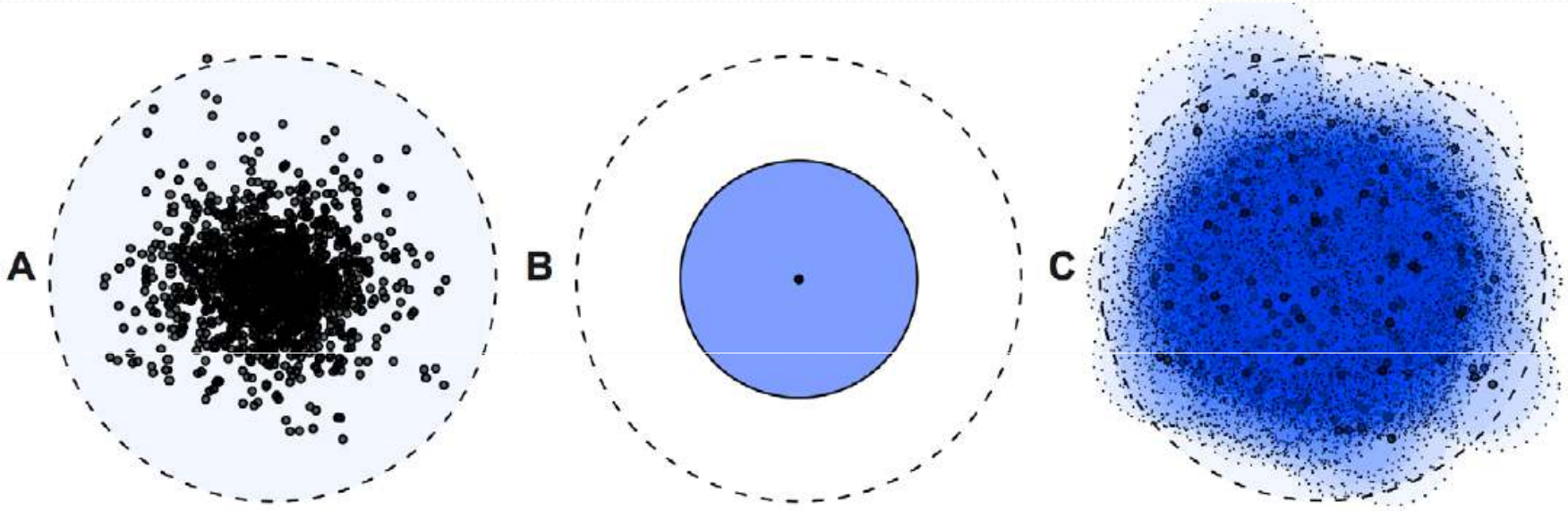


Figure 1. Principles of repeat identification using *P*-clouds. A) True data distribution representing divergence within a TE family from a master element sequence (center). B) Consensus sequence based search throws away information by collapsing observed data to a single sequence. C) *P-clouds* clusters related high-abundance oligos, thus providing better coverage of sequence space.

P-clouds

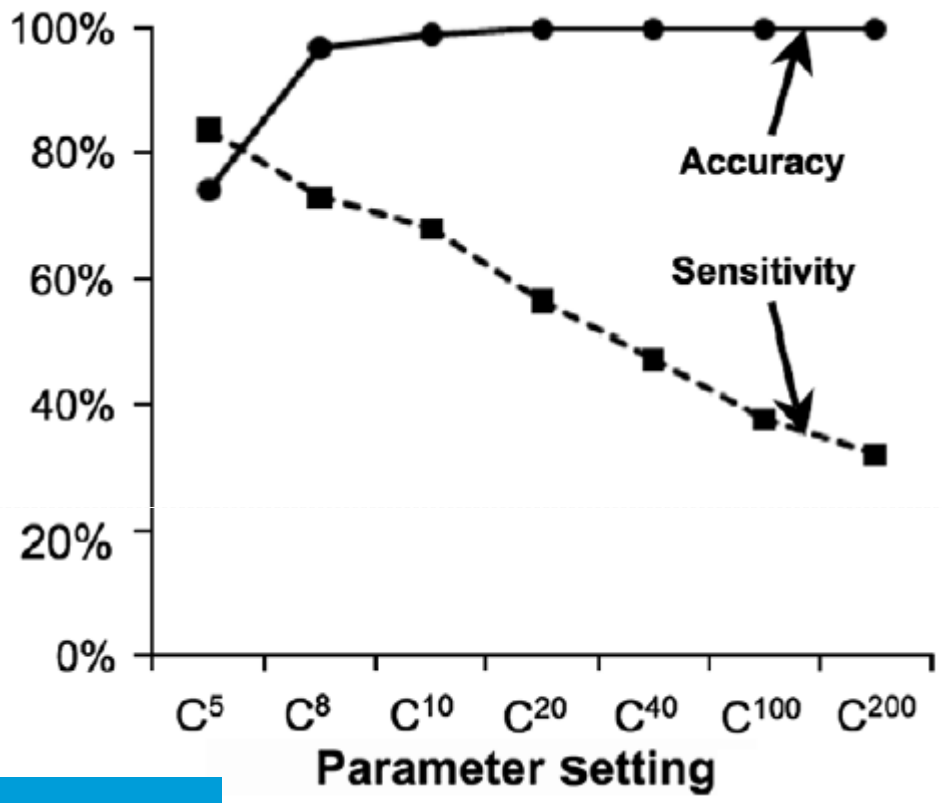
- Find the number of occurences of each specific oligo in a genome.
  - Oligo length $W = log_4(n)+1$
- The highest frequency oligo initiates a cloud.
- Similar high-frequency oligos are added to the cloud.
  - '*similar*': differences of up to 3 nt from core oligo (adjustable)
  - '*high-frequency*': adjustable

Repeat region annotation

- Oligos that were members of *P-clouds* were mapped back to the original genome sequence.
- Segments of the genome with high *P-cloud* oligo density were demarcated as “repeated regions”.
- 80% of every 10 consecutive oligos must be composed of *P-cloud* oligos (adjustable).

False positive assessment

- A simulated random non-repetitive genome sequence constrained to have the same dinucleotide frequencies in 1 Mbp windows as the original human genome.



C^5	(2, 5, 10, 100, 1000)
C^8	(2, 8, 16, 160, 1600)
C^{10}	(2, 10, 20, 200, 2000)
C^{20}	(2, 20, 40, 400, 4000)
C^{40}	(4, 40, 80, 800, 8000)
C^{100}	(10, 100, 200, 2000, 20 000)
C^{200}	(20, 200, 400, 4000, 40 000)

Gu *et al* (2008) Identification of repeat structure in large genomes using repeat probability clouds. *Analytical Biochemistry*, 380, 77-83

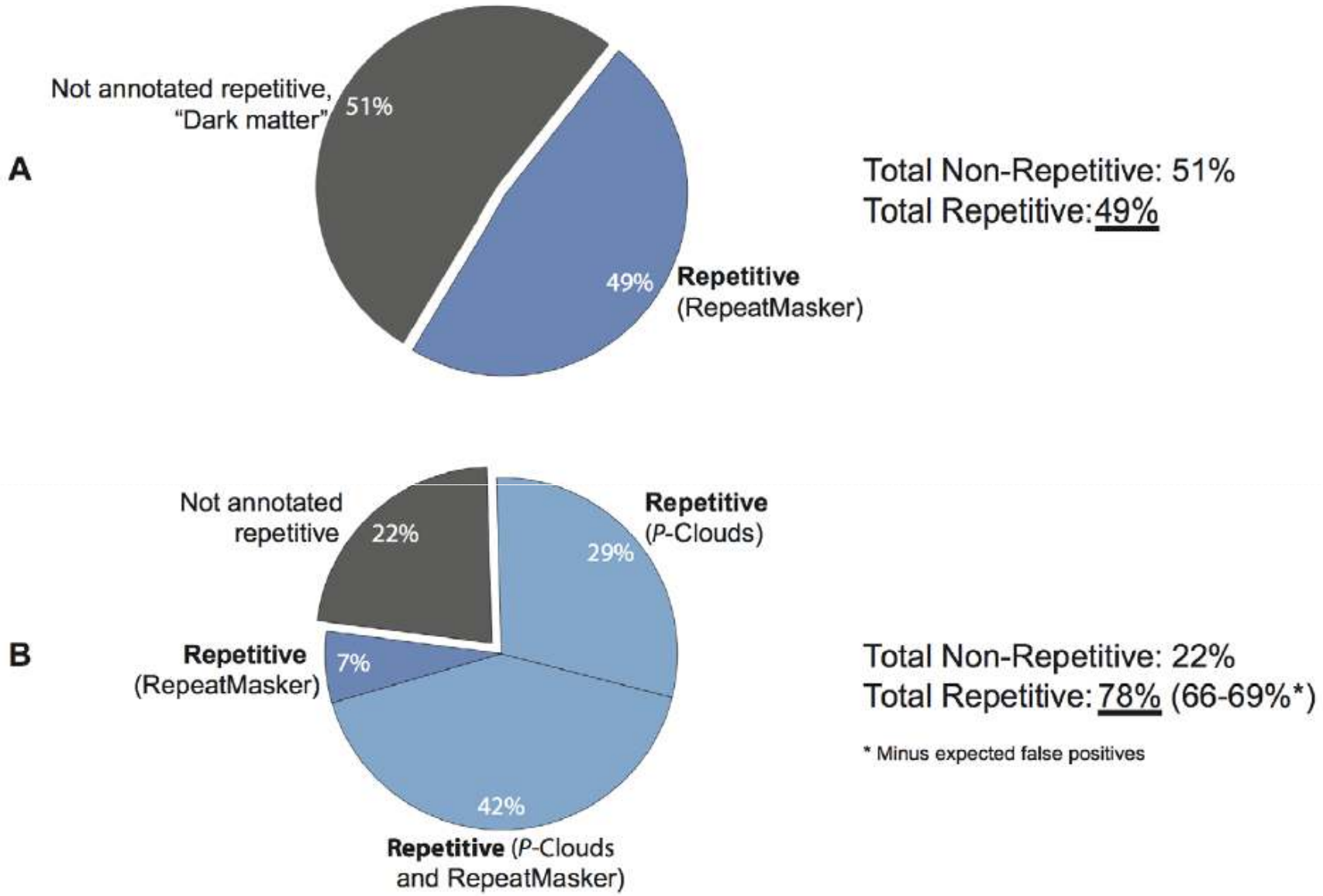


Figure 2. *P*-clouds and RepeatMasker annotation of the repeat structure of the human genome. Results are displayed as a percentage of the ungapped genome assembly length. A) Consensus results prior to this study indicate that <50% of the genome is repetitive (*RepeatMasker*). B) Analysis using *P-clouds* suggests more than two-thirds of the genome is repetitive or repeat-derived.

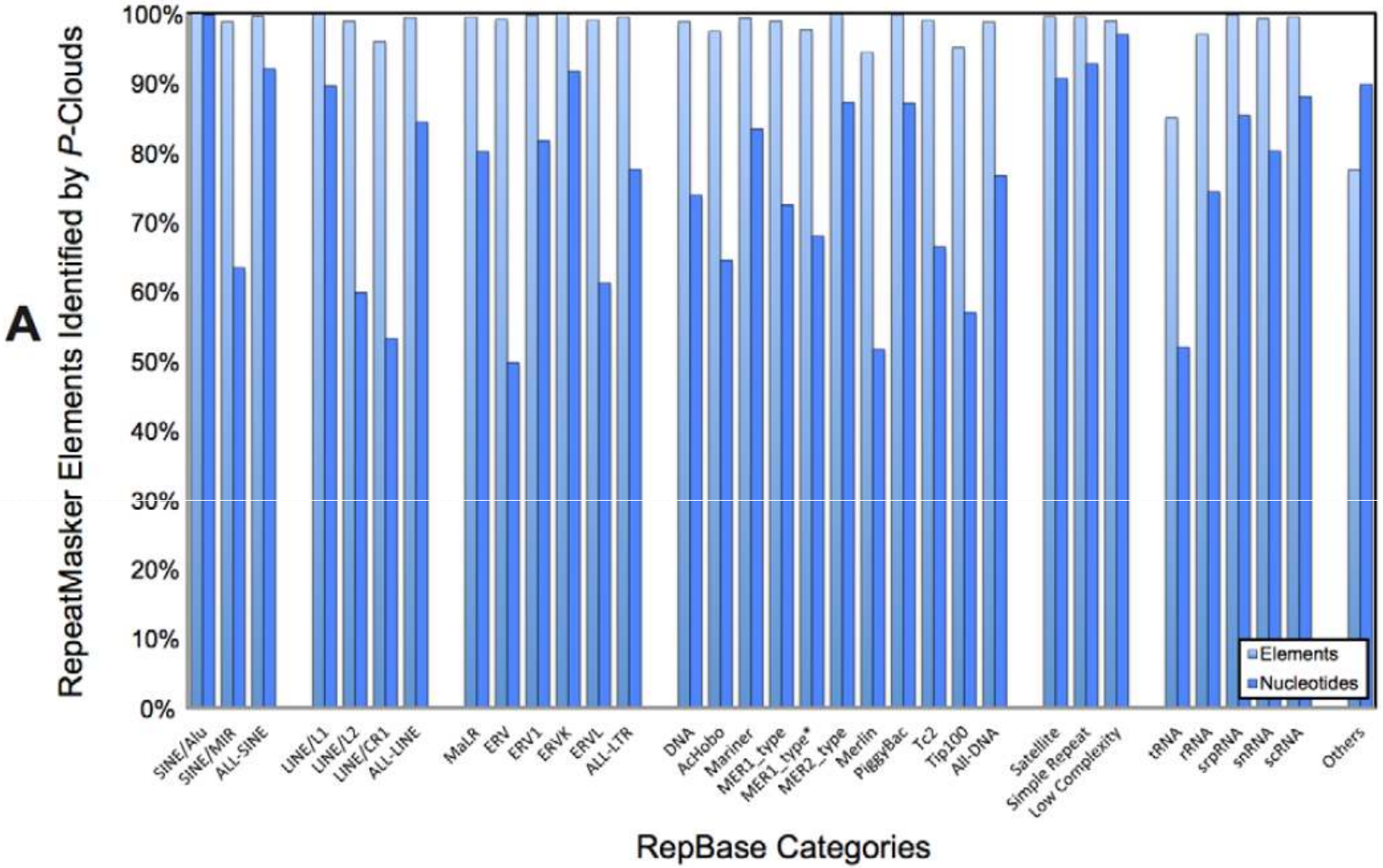
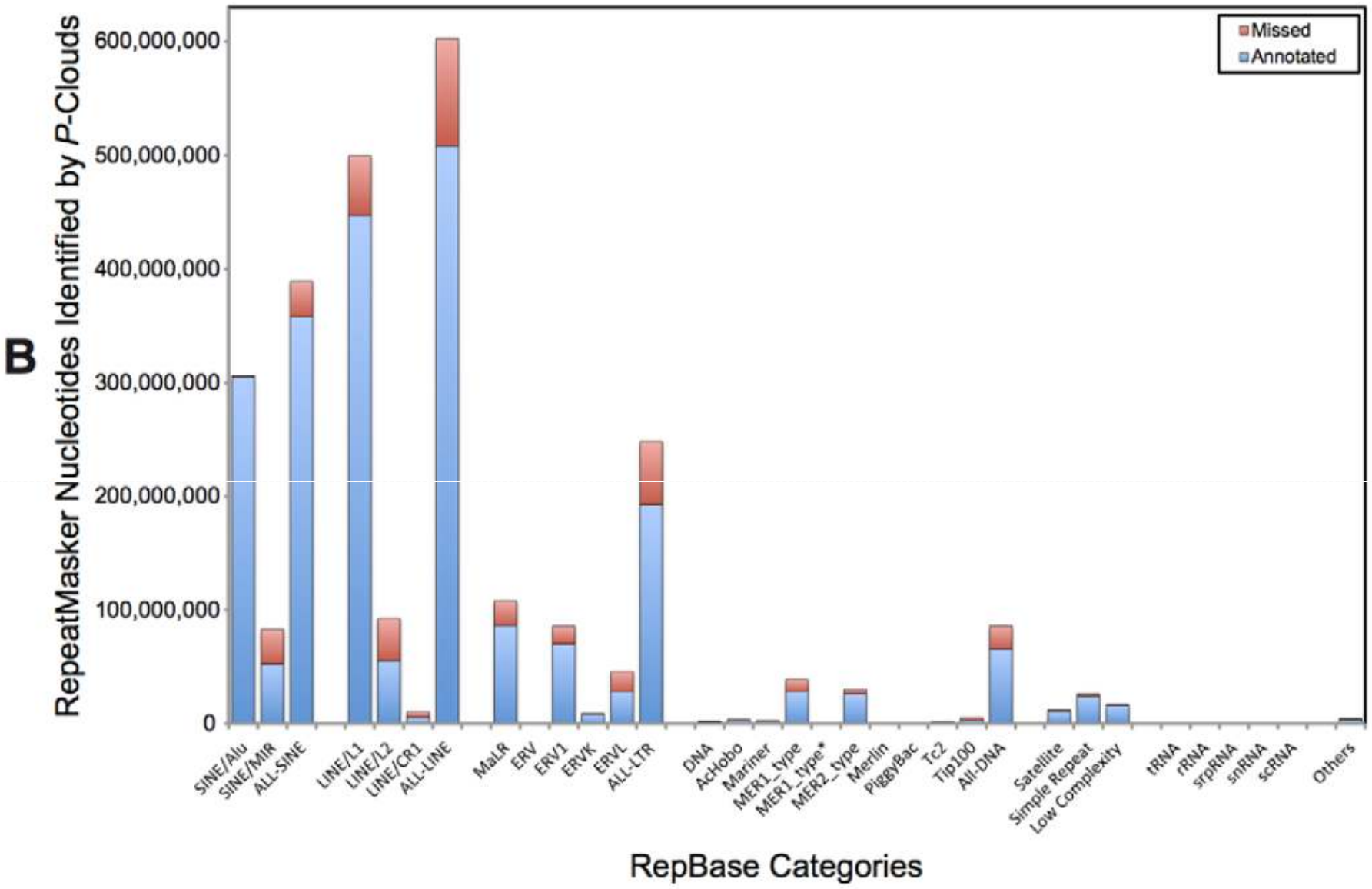


Figure 3. Percentage of previously-identified transposable elements annotated by *P-clouds*. A) The percentage of nucleotides and repeats for each family or repeat classification group. B) The number of nucleotides annotated or missed.



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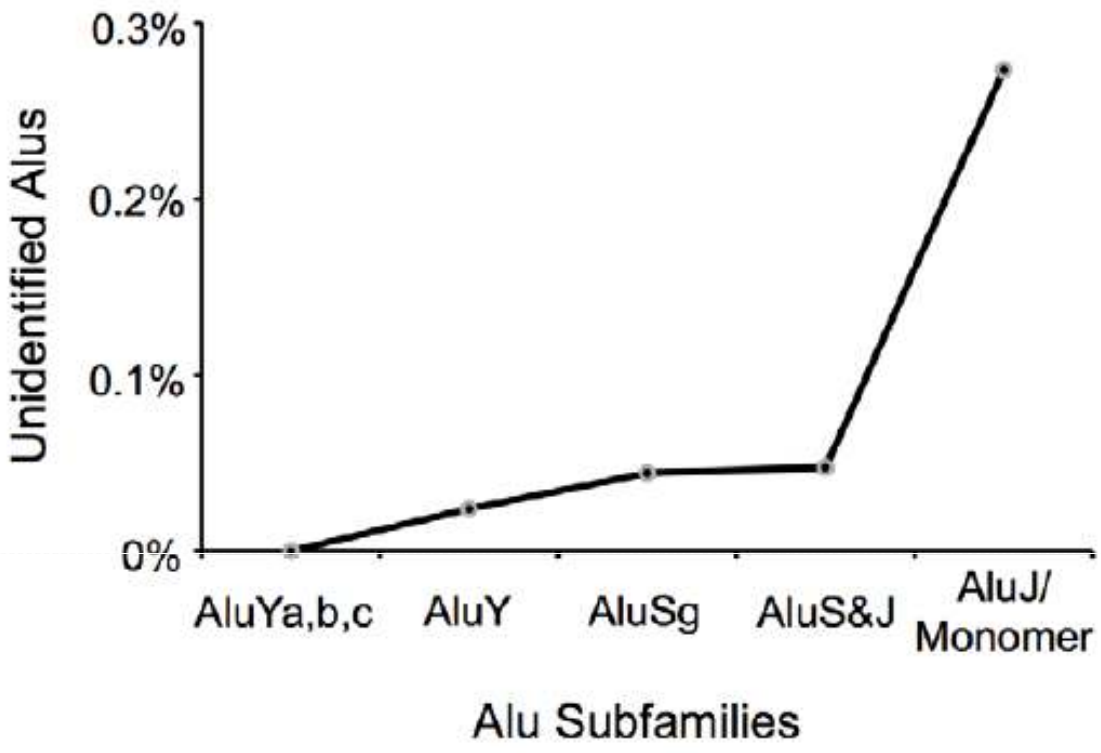

Figure 4. Percentage of Alu elements in different Alu subfamilies not annotated by *P-clouds* analysis. Displayed are elements for which no portion was annotated. The relative age of *Alu* subfamilies increases from left to right.

Table 1. Overlap between genome features and repetitive regions.

Genome Feature	Fraction of Genome	Fraction of RepeatMasker annotations	Fraction of *P-clouds* annotations	Fraction of Novel *P-clouds* annotations
Known Genes (transcribed unit)	37.48%	32.47%	36.02%	41.42%
Segmental Duplications	5.22%	5.33%	5.75%	6.02%
Duplicated Regions (WSSD)	3.53%	3.16%	3.87%	4.63%
Known Genes (exons)	1.12%	0.05%	0.56%	1.29%
Simple Repeats	1.91%	3.00%	2.36%	1.06%
CpG Islands	0.74%	0.07%	0.26%	0.56%
Pseudogenes	0.19%	0.07%	0.16%	0.28%
Total Size:	2.85 Gbp	1.39 Gbp	2.02 Gbp	0.84 Gbp

Total repetitive sequence detected by either RepeatMasker or *P-clouds* was 2.23 Gbp (out of a total 2.85 Gbp sequence in the ungapped assembly).
doi:10.1371/journal.pgen.1002384.t001

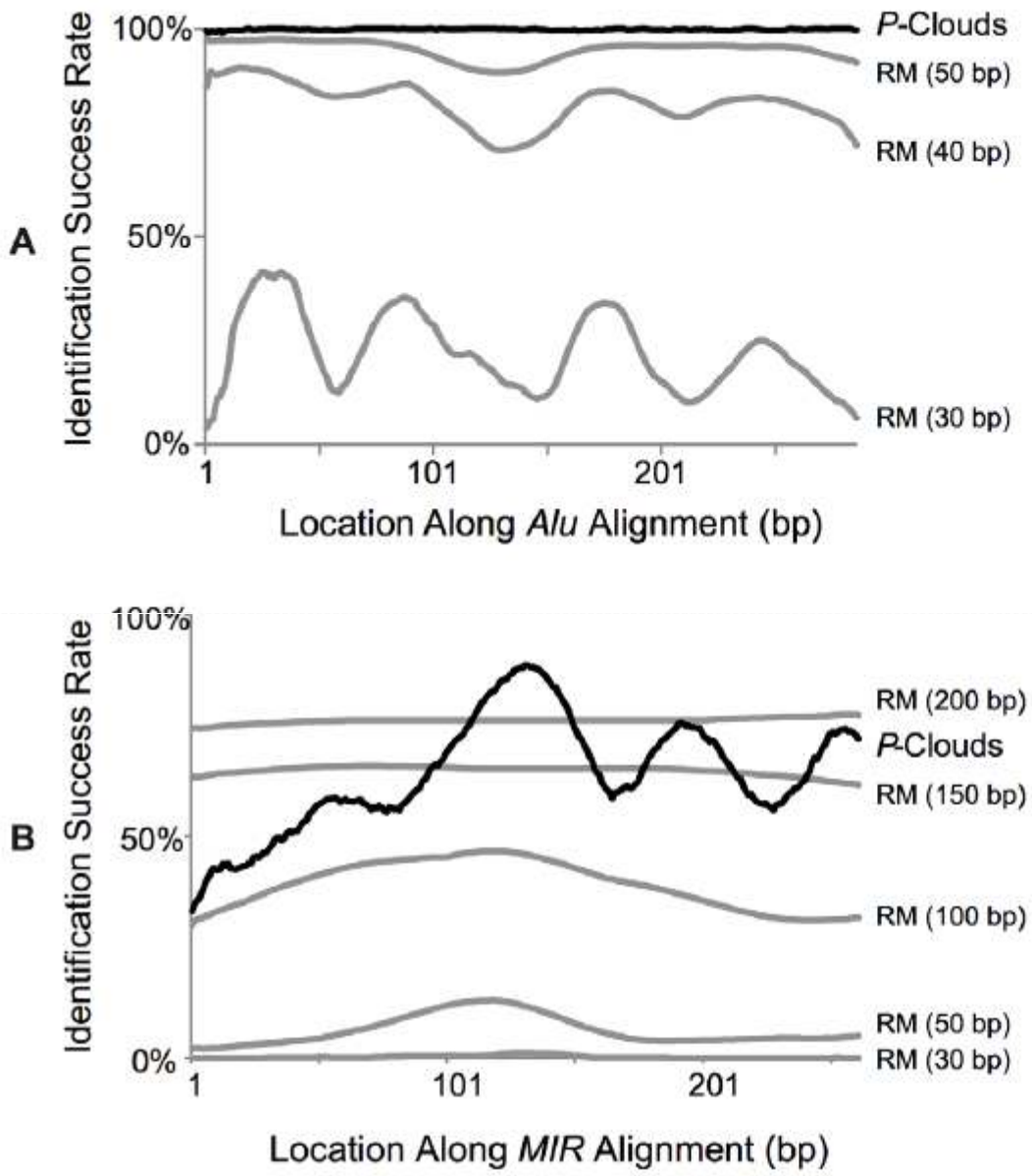

Figure 5. Percent detection success for fragments of known full-length *SINE* elements. A) *Alu* regions. B) *MIR* regions. Identification success is displayed as a running average of 10 bp starting positions.

Element specific *P-clouds* (*ESPs*)

Human genome regions that are not masked by RepeatMasker:

- 749,395 putative Alu regions
  - 20,919,291 bp (FP=22.17%)
- 7,518,362 putative MIR regions
  - 227,472,307 bp (FP=65.42%)

Relationship to other *de novo* estimates

Human chromosome 22

- RepeatMasker(RM) with RepBase 47.9%
- RepeatScout+RM 36.9%
- RepeatScout+RM with RepBase 52.5%
- RepSeek 56.2%

- P-clouds combined with RM+RepBase: 70.6%

Conclusions

- *De novo* search methods can be used to detect repeat-derived sequences that are too diverged or degraded to be easily detected by alignment to known transposable element consensus sequences.
- *P-clouds* predicts >840 Mbp of additional repetitive sequences in human genome.
- >66%-69% of the human genome sequence is repetitive or repeat-derived.
- ESPs identified ~100 Mb of previously unannotated human Alu and MIR elements.