Supplementary Material

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TABLE OF CONTENT

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Supplementary S1: Summary of the methods

A summary of the methods behind all OpenProt features presented in the initial release was published in the OpenProt 2021 update [1]. We present below some minor modifications in this 2024 update resulting from new annotation releases and the use of the latest version for all software. We also present new developments.

1. Summary of previous methods with minor modifications

a. **OpenProt ORFeome, reference protein, novel isoform, alternative protein -** OpenProt retrieves the transcriptome from both NCBI RefSeq [2] (release 217) and Ensembl [3] (release 106) and predicts ORFs from a 3-frames in silico translation using EMBOSS Transeq (6.6.0) [4]. All ORFs with an ATG initiating codon and a minimal length of 30 codons are annotated and constitutes the OpenProt ORFeome. After in silico translation, three categories of proteins are annotated:

   i. A reference protein (or RefProt) is a protein matching a NCBI RefSeq [2], Ensembl [3] or UniProt [5] (release_2022_06) protein entry. A RefProt is identified with the accession number from NCBI RefSeq, Ensembl and/or UniProt.

   ii. A novel isoform is a protein encoded in the same gene as a RefProt with at least one of the two following features: (a) over 80 % of protein sequence identity with the RefProt over 50% of the length using Basic Local Alignment Search Tool (BLAST); (b) identical genomic coordinates of start or end codon with a sequence identity (EMBOSS Matcher PAM10 matrix score $\leq$ 100) over 20% of the length of the RefProt. A novel isoform is identified with a specific accession number starting with II_.

   iii. An alternative protein (or AltProt) does not have any significant similarity with a RefProt and is identified with a specific accession number starting with IP_.

b. **Ribosome profiling data reanalysis** - Ribo-seq datasets are downloaded from the Gene Omnibus platform [6] and reanalyzed using the PRICE workflow [7] run with default parameters, except for the FDR set at 1 % (instead of 10%). After filtering out ribosomal RNA reads, PRICE generates a list of ORF candidates based on likely codons. A stringent 1% FDR filter is applied for highly confident translation events.

c. **Conservation analysis -** Protein sequence homology is assessed using an InParanoid-like workflow [1], distinguishing orthologs (homologous sequences from different species) from paralogs (homologous sequences from the same species but different genes). Orthologs are identified through all-vs-all BLAST searches between species and within the same species for paralogs. Orthology relationships are established with a bit-score above 40 and an overlap exceeding

50% of the queried sequence.

d. **Protein functional domain -** All proteins annotated on OpenProt are scanned for the presence of known functional signatures using InterProScan (5.61-93.0) [8] with the default parameters. Domain predictions as well as gene ontology (GO) and pathway annotations are reported if significant (e-value < 10-3).

2. Updated mass spectrometry data analysis pipeline in the OpenProt

OpenProt re-analyzes mass spectrometry (MS) datasets to gather evidence of expression for every protein annotated in the OpenProt database, *i.e.* RefProts, AltProts and Isoforms. The updated pipeline is described in the main text. It is important to note that when a peptide matches both an AltProt or a novel isoform and a RefProt, it is assigned to the RefProt only.

3. Spectral viewer

A bespoke spectrum viewer was implemented in JavaScript to visualize all spectra that resulted from the mass spectrometry re-analysis. The peak annotations for each spectrum are from the PeptideShaker report of the corresponding analysis.

4. Integration of other predictions

a. Protein structure
MMseqs2 [9] was used to generate large-scale multiple alignment sequences (MSAs). These MSAs serve as inputs for the AlphaFold network, a feature provided by ColabFold with default settings [10]. The installer script was installed within a Singularity image and executed on supercomputers managed by Calcul Québec and the Digital Research Alliance of Canada. For proteins with multiple sequence alignments of less than 30, we utilized OmegaFold the first computational approach that achieved the successful prediction of high-resolution protein structures solely from a single primary sequence [11].

b. Subcellular localization
The DeepLoc 2.0 [12] program was obtained from https://services.healthtech.dtu.dk/cgi-bin/sw_request?software=deeploc&version=2.0&packageversion=2.0&platform=All, installed in an Apptainer container and executed. We used the high-quality (slow) and short output settings. The fasta file generated by OpenProt for all species was given as the input and the csv file of the output is displayed directly on the website.

c. Intrinsic disorder
A Docker image of the flDPnn disorder and disorder function predictor [13]

(https://gitlab.com/sina.ghadermarzi/fldpnn/-/tree/master) was utilized and executed within a Docker container. Input files in Fasta format containing the protein sequences were employed. The resulting output comprises a graph that illustrates the flDPnn score, which is a measure of the probability of disorder (with a threshold of 0.3), along with potential functions such as protein binding, DNA binding, RNA binding, and acting as a linker.

d. Short linear motifs (SLiMs)
We downloaded the tsv file containing all the ELM database Classes using the ELM API (http://elm.eu.org/elms/elms_index.tsv). SLiMs were predicted using the regular expression of the corresponding ELM class. Since this method tends to return a high amount of false positive and true SLiMs are mostly located in disordered regions of a protein [14], we used flDPnn disorder predictions to discard all predicted SLiMs containing at least one residue predicted as ordered.

e. Genotype-Tissue Expression (GTEx)
The data used for the analyses described in this manuscript were obtained from: the GTEx Portal on 07/20/2023 [15]. Expression data from all samples were kept structured at the transcript level rather than aggregated by genes in order to obtain transcript level distributions.

References

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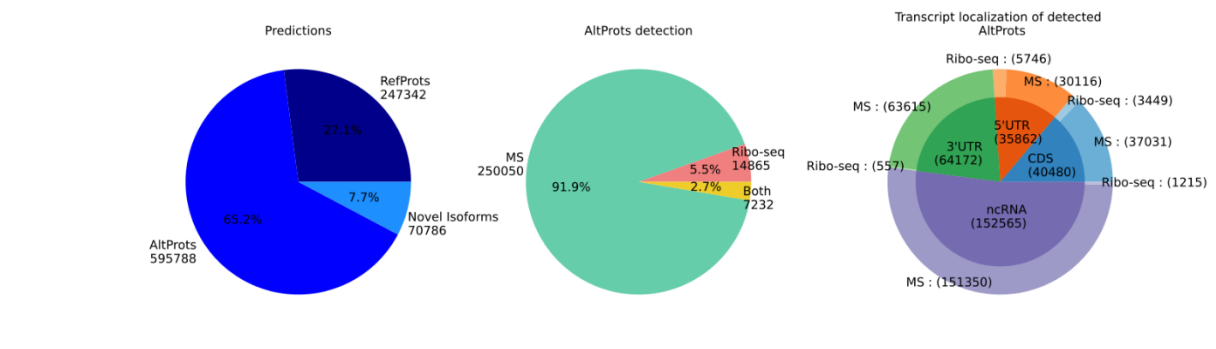
Supplementary S2: General statistics per species for OpenProt v2.0

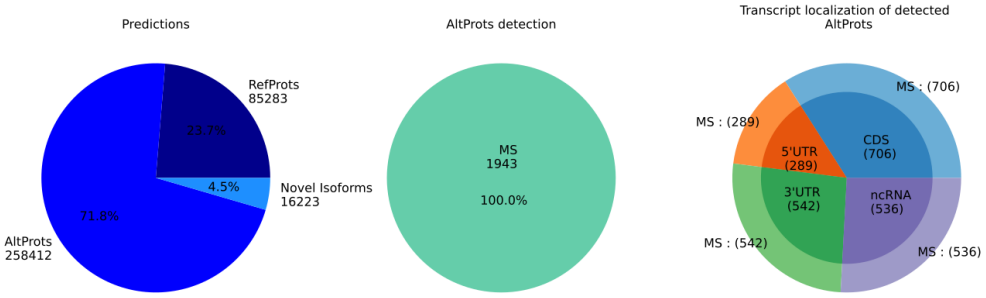
Here are the summary statistics for all species supported in OpenProt v2.0: *Homo sapiens*, *Pan troglodytes*, *Mus musculus*, *Rattus norvegicus*, *Bos taurus*, *Ovis aries*, *Danio rerio*, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Saccharomyces cerevisiae*.

Legend for the pie charts: ncRNA = non-coding RNA; UTR = untranslated region of an mRNA; MS
= mass spectrometry.

*** OpenProt v2.0 general statistics in ***Homo sapiens*** ***

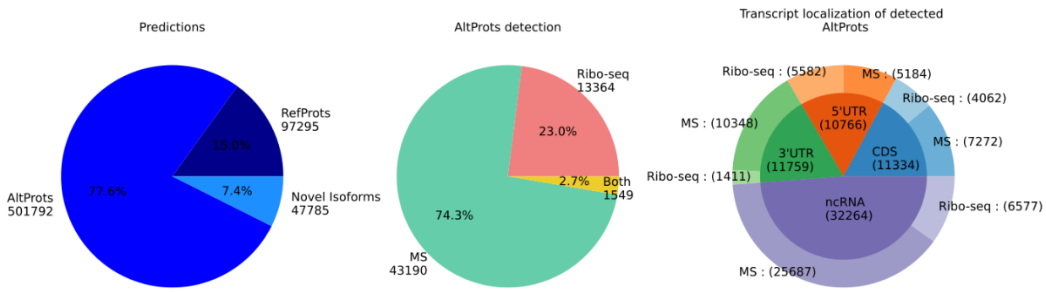


*** OpenProt v2.0 general statistics in ***Pan troglodytes*** ***

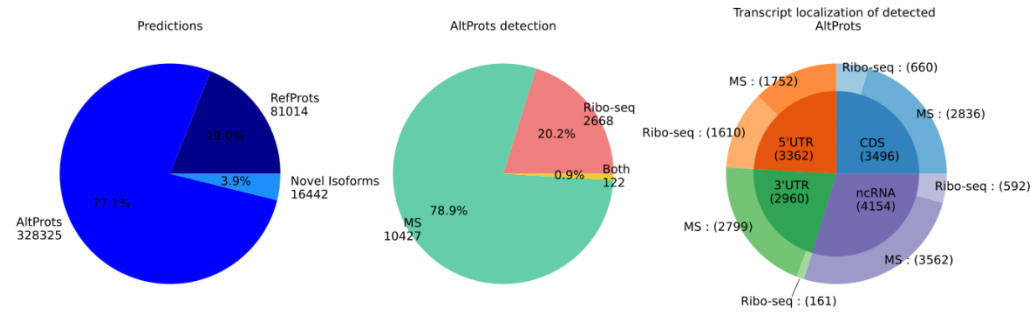


Please note that no ribosome profiling data was available for *Pan troglodytes*.

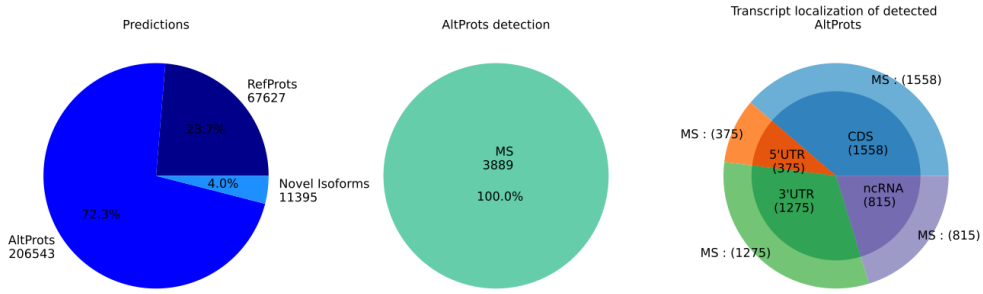
*** OpenProt v2.0 general statistics in ***Mus musculus*** ***



*** OpenProt v2.0 general statistics in ***Rattus norvegicus*** ***

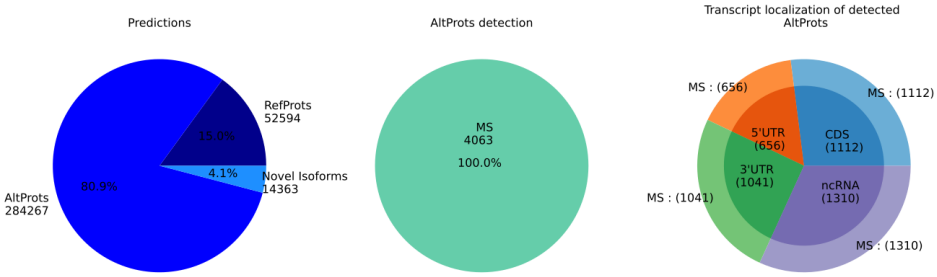


*** OpenProt v2.0 general statistics in ***Bos taurus*** ***



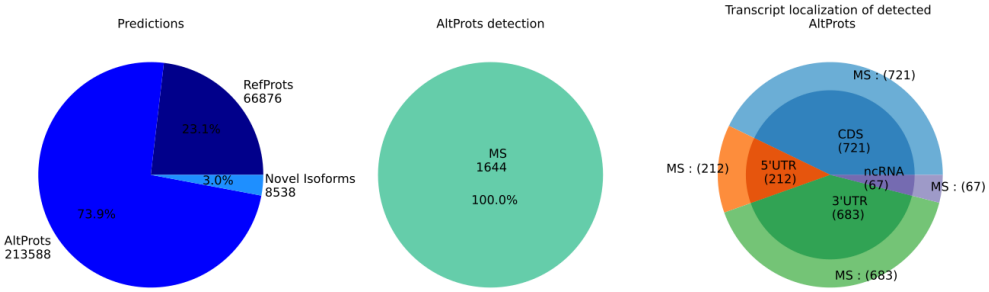
Please note that no ribosome profiling data was available for *Bos taurus*.

*** OpenProt v2.0 general statistics in ***Ovis aries*** ***



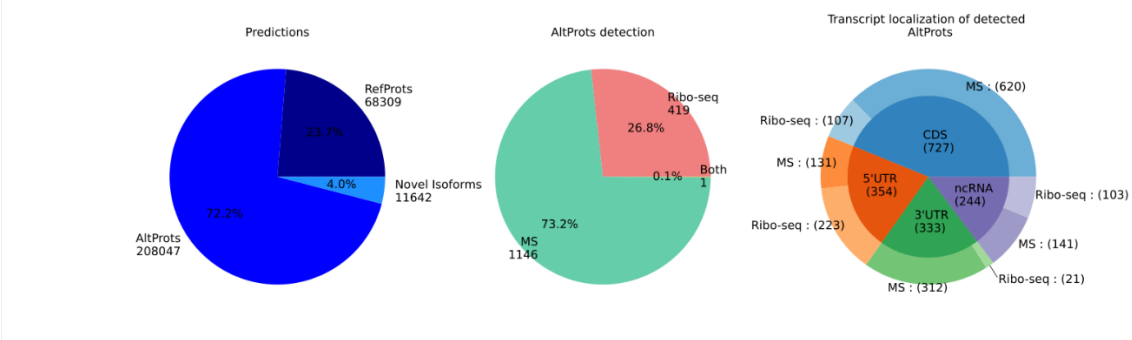
Please note that no ribosome profiling data was available for *Ovis aries*.

*** OpenProt v2.0 general statistics in ***Xenopus tropicalis*** ***

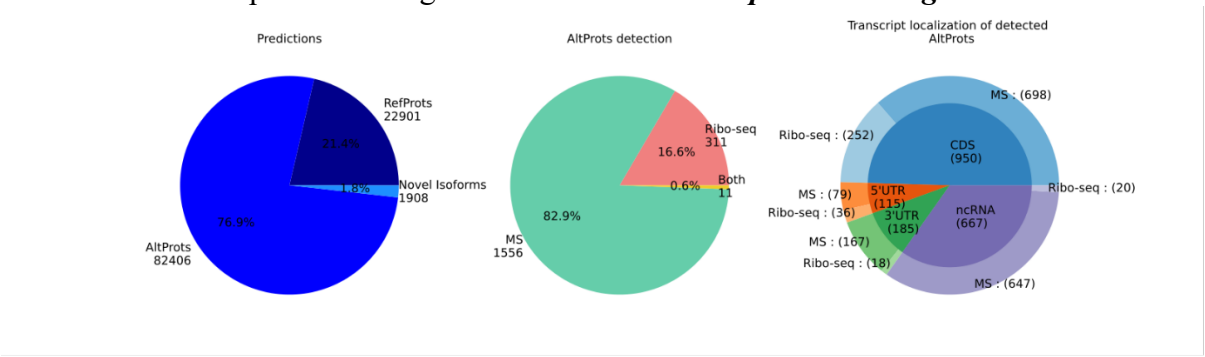


Please note that no ribosome profiling data was available for ***Xenopus tropicalis***.

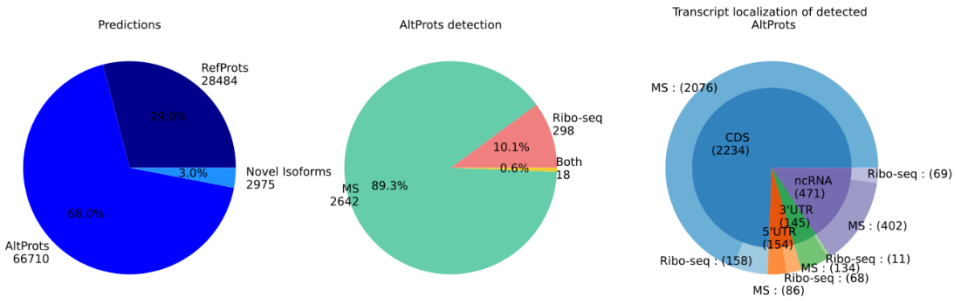
*** OpenProt v2.0 general statistics in ***Danio rerio*** ***



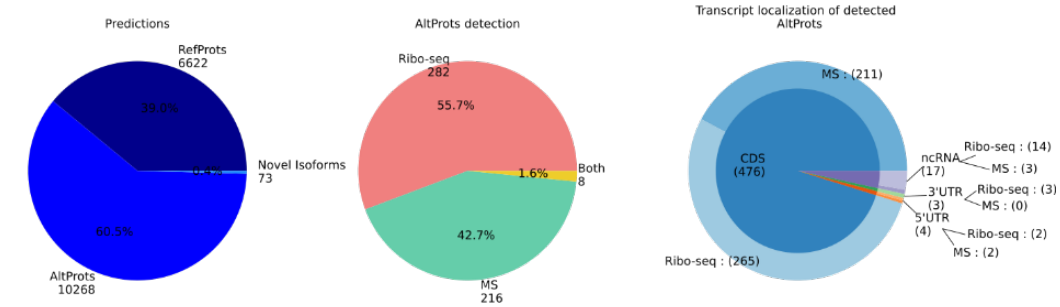
*** OpenProt v2.0 general statistics in ***Drosophila melanogaster*** ***



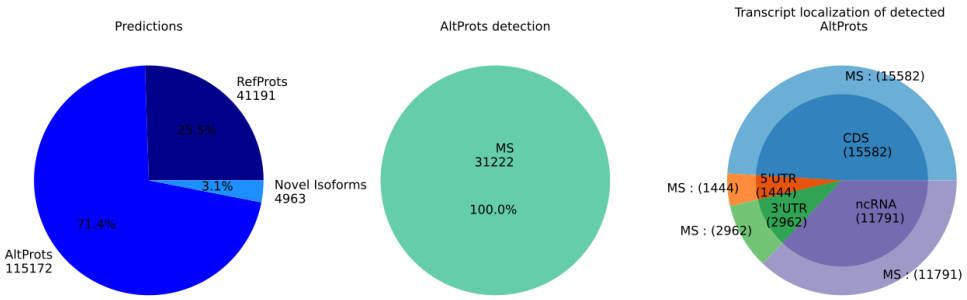
*** OpenProt v2.0 general statistics in ***Caenorhabditis elegans*** ***



*** OpenProt v2.0 general statistics in ***Saccharomyces cerevisiae S288c*** ***

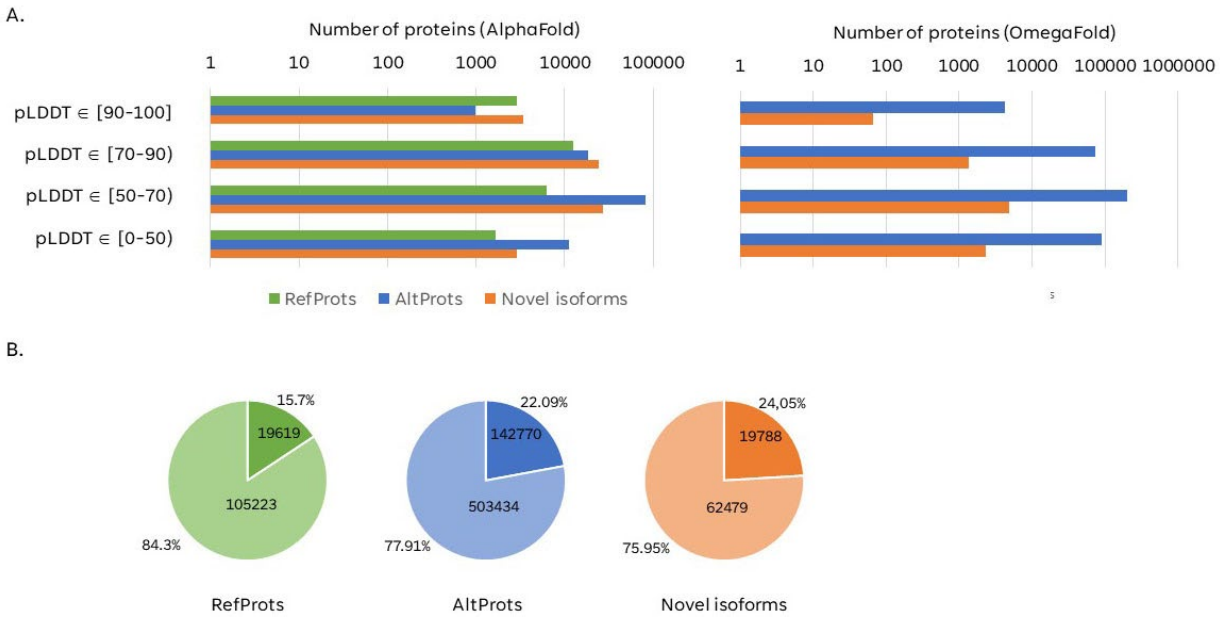


*** OpenProt v2.0 general statistics in ***Arabidopsis Thaliana*** ***



Please note that no ribosome profiling data was available for ***Arabidopsis Thaliana***.

Supplementary S3: protein structure and disorder predictions



(**A**) A significant number of human AltProts and novel isoforms display a high (90 > pLDDT > 70) to very high (pLDDT >90) confidence score. Proteins with more than 30 or less than 30 multiple sequence alignments were analyzed with AlphaFold or OmegaFold, respectively. For RefProts, pLDDT scores were retrieved from the AlphaFold database. (**B**) Charts represent the fraction of proteins with at least one IDR (plain colour) or with no IDR (pale colour).

Supplementary S4

List of mass spectrometry and ribosome profiling studies incorporated in OpenProt v2.0

List of the 101 **mass spectrometry** datasets incorporated in OpenProt v2.0 with the associated species and citation (PMID accession).

Species		Study ID	Data source	PMID
Arabidopsis	thaliana	PXD013868	https://www.ebi.ac.uk/pride/archive/projects/PXD013868	32188942
Bos	taurus	PXD001741	https://www.ebi.ac.uk/pride/archive/projects/PXD001741	25818294
Caenorhabditis	elegans	PXD015644	https://www.ebi.ac.uk/pride/archive/projects/PXD015644	31754102
Danio	rerio	PXD003455	https://www.ebi.ac.uk/pride/archive/projects/PXD003455	27696471
Danio	rerio	PXD004876	https://www.ebi.ac.uk/pride/archive/projects/PXD004876	27898262
Danio	rerio	PXD011929	https://www.ebi.ac.uk/pride/archive/projects/PXD011929	32284610
Danio	rerio	PXD006098	https://www.ebi.ac.uk/pride/archive/projects/PXD006098	28381614
Drosophila	melanogaster	PXD003944	https://www.ebi.ac.uk/pride/archive/projects/PXD003944	27956707
Drosophila	melanogaster	PXD001455	https://www.ebi.ac.uk/pride/archive/projects/PXD001455	25403936
Homo	Sapiens	PXD002815	https://www.ebi.ac.uk/pride/archive/projects/PXD002815	26496610
Homo	sapiens	PXD002516	https://www.ebi.ac.uk/pride/archive/projects/PXD002516	26892330
Homo	sapiens	PXD004710	https://www.ebi.ac.uk/pride/archive/projects/PXD004710	27801565
Homo	sapiens	PXD004242	https://www.ebi.ac.uk/pride/archive/projects/PXD004242	28007936
Homo	sapiens	TCGA_COCA	https://cptc-xfer.uis.georgetown.edu/publicData/Phase_II_Data/TCGA_Colorectal_Cancer_S_022/	25043054
Homo	sapiens	PXD003965	https://www.ebi.ac.uk/pride/archive/projects/PXD003965	28208246
Homo	sapiens	PXD002214	https://www.ebi.ac.uk/pride/archive/projects/PXD	26371159

			002214	
Homo	sapiens	CCLE_2020	https://portals.broadinstitute.org/ccle/about	31978347
Homo	sapiens	PXD004778	https://www.ebi.ac.uk/pride/archive/projects/PXD004778	28071820
Homo	sapiens	PXD004900	https://www.ebi.ac.uk/pride/archive/projects/PXD004900	27806443
Homo	sapiens	PXD004764	https://www.ebi.ac.uk/pride/archive/projects/PXD004764	28071820
Homo	sapiens	TCGA_BRCA	https://cptac-data-portal.georgetown.edu/cptac/s/S030	27251275
Homo	sapiens	TCGA_OVCA	https://cptac-data-portal.georgetown.edu/cptac/s/S026	27372738
Homo	sapiens	PXD000788	https://www.ebi.ac.uk/pride/archive/projects/PXD000788	24797263
Homo	sapiens	BioPlex_1	https://bioplex.hms.harvard.edu/download.php	26186194
Homo	sapiens	PXD003708	https://www.ebi.ac.uk/pride/archive/projects/PXD003708	27987026
Homo	sapiens	PXD003902	https://www.ebi.ac.uk/pride/archive/projects/PXD003902	28007913
Homo	sapiens	PXD002854	https://www.ebi.ac.uk/pride/archive/projects/PXD002854	27135364
Homo	sapiens	PXD005083	https://www.ebi.ac.uk/pride/archive/projects/PXD005083	28196878
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Homo	sapiens	PXD013647	https://www.ebi.ac.uk/pride/archive/projects/PXD013647	31513346
Homo	sapiens	PXD009208	https://www.ebi.ac.uk/pride/archive/projects/PXD009208	30157440
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Homo	sapiens	PXD004875	https://www.ebi.ac.uk/pride/archive/projects/PXD004875	27779380
Homo	sapiens	PXD004788	https://www.ebi.ac.uk/pride/archive/projects/PXD004788	28071820
Homo	sapiens	BioPlex_2	https://bioplex.hms.harvard.edu/download.php	28514442
Homo	sapiens	PXD004181	https://www.ebi.ac.uk/pride/archive/projects/PXD004181	28064214
Homo	sapiens	PXD002612	https://www.ebi.ac.uk/pride/archive/projects/PXD002612	27799870
Homo	sapiens	PXD004816	https://www.ebi.ac.uk/pride/archive/projects/PXD004816	28123004
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Homo	sapiens	PXD005210	https://www.ebi.ac.uk/pride/archive/projects/PXD005210	27794609
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Mus	musculus	PXD001597	https://www.ebi.ac.uk/pride/archive/projects/PXD001597	26227174
Mus	musculus	PXD003155	https://www.ebi.ac.uk/pride/archive/projects/PXD003155	26850065
Mus	musculus	PXD002156	https://www.ebi.ac.uk/pride/archive/projects/PXD002156	26080680
Mus	musculus	PXD001250	https://www.ebi.ac.uk/pride/archive/projects/PXD001250	26523646
Mus	musculus	PXD004072	https://www.ebi.ac.uk/pride/archive/projects/PXD004072	27188442
Mus	musculus	PXD002152	https://www.ebi.ac.uk/pride/archive/projects/PXD002152	27302655
Mus	musculus	PXD001792	https://www.ebi.ac.uk/pride/archive/projects/PXD001792	26280412
Mus	musculus	PXD005312	https://www.ebi.ac.uk/pride/archive/projects/PXD005312	28066266
Mus	musculus	PXD003860	https://www.ebi.ac.uk/pride/archive/projects/PXD003860	27432266
Mus	musculus	PXD004612	https://www.ebi.ac.uk/pride/archive/projects/PXD004612	28071813
Mus	musculus	PXD017284	https://www.ebi.ac.uk/pride/archive/projects/PXD	33257503

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Mus	musculus	PXD001293	https://www.ebi.ac.uk/pride/archive/projects/PXD001293	25504905
Mus	musculus	PXD000666	https://www.ebi.ac.uk/pride/archive/projects/PXD000666	25193168
Mus	musculus	PXD005635	https://www.ebi.ac.uk/pride/archive/projects/PXD005635	28345880
Mus	musculus	PXD011890	https://www.ebi.ac.uk/pride/archive/projects/PXD011890	30901149
Mus	musculus	PXD003442	https://www.ebi.ac.uk/pride/archive/projects/PXD003442	26900923
Mus	musculus	PXD003555	https://www.ebi.ac.uk/pride/archive/projects/PXD003555	27490109
Mus	musculus	PXD000747	https://www.ebi.ac.uk/pride/archive/projects/PXD000747	25205226
Mus	musculus	PXD004087	https://www.ebi.ac.uk/pride/archive/projects/PXD004087	27629805
Mus	musculus	PXD011334	https://www.ebi.ac.uk/pride/archive/projects/PXD011334	33216553
Mus	musculus	PXD000501	https://www.ebi.ac.uk/pride/archive/projects/PXD000501	24753479
Mus	musculus	PXD001514	https://www.ebi.ac.uk/pride/archive/projects/PXD001514	26139848
Mus	musculus	PXD001404	https://www.ebi.ac.uk/pride/archive/projects/PXD001404	25338131
Ovis	aries	PXD014050	https://www.ebi.ac.uk/pride/archive/projects/PXD014050	31882954
Ovis	aries	PXD013822	https://www.ebi.ac.uk/pride/archive/projects/PXD013822	31136077
Ovis	aries	PXD004989	https://www.ebi.ac.uk/pride/archive/projects/PXD004989	28615994
Ovis	aries	PXD004556	https://www.ebi.ac.uk/pride/archive/projects/PXD004556	27784645
Rattus	norvegicus	PXD004247	https://www.ebi.ac.uk/pride/archive/projects/PXD004247	27250205
Rattus	norvegicus	PXD001984	https://www.ebi.ac.uk/pride/archive/projects/PXD001984	26330543
Rattus	norvegicus	PXD001986	https://www.ebi.ac.uk/pride/archive/projects/PXD001986	26330543
Rattus	norvegicus	PXD004889	https://www.ebi.ac.uk/pride/archive/projects/PXD	27764671

			004889	
Rattus	norvegicus	PXD016447	https://www.ebi.ac.uk/pride/archive/projects/PXD016447	33453410
Rattus	norvegicus	PXD003520	https://www.ebi.ac.uk/pride/archive/projects/PXD003520	27358910
Rattus	norvegicus	PXD003375	https://www.ebi.ac.uk/pride/archive/projects/PXD003375	27250205
Xenopus	tropicalis	PXD009577	https://www.ebi.ac.uk/pride/archive/projects/PXD009577	31490923

List of the 136 **ribosome profiling** datasets incorporated in OpenProt v2.0 along the associated species, data source and citation (PMID accession).

Species	Study ID	Data source	PMID
CE	GSE52910	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52910	24440504
CE	GSE62859	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62859	25378320
CE	SRP014427	https://www.ncbi.nlm.nih.gov/sra/?term=SRP014427	22855835
DM	GSE49197	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE49197	24302569
DM	GSE52799	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52799	24882012
DM	GSE60384	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE60384	25144939
DM	GSE106697	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE106697	29548011
DR	GSE34743	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE34743	22422859
DR	GSE46512	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46512	23698349
DR	GSE47558	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE47558	24056933
DR	GSE52809	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52809	24476825
DR	GSE53693	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53693	24705786
HS	GSE102113	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102113	30449621
HS	GSE103719	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE103719	30257221
HS	GSE105082	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE105082	30591072
HS	GSE105172	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE105172	31160600
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