Systems biology

Generation of ENSEMBL-based proteogenomics databases boosts the identification of non-canonical peptides

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Abstract

Summary: We have implemented the pypgatk package and the pgdb workflow to create proteogenomics databases based on ENSEMBL resources. The tools allow the generation of protein sequences from novel protein-coding transcripts by performing a three-frame translation of pseudogenes, IncRNAs and other non-canonical transcripts, such as those produced by alternative splicing events. It also includes exonic out-of-frame translation from otherwise canonical protein-coding mRNAs. Moreover, the tool enables the generation of variant protein sequences from multiple sources of genomic variants including COSMIC, cBioportal, gnomAD and mutations detected from sequencing of patient samples. pypgatk and pgdb provide multiple functionalities for database handling including optimized target/decoy generation by the algorithm DecoyPyrat. Finally, we have reanalyzed six public datasets in PRIDE by generating cell-type specific databases for 65 cell lines using the pypgatk and pgdb workflow, revealing a wealth of non-canonical or cryptic peptides amounting to >5% of the total number of peptides identified.

Availability and implementation: The software is freely available. pypgatk: https://github.com/bigbio/py-pgatk/ and pgdb: https://nf-co.re/pgdb.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Proteogenomics is a rapidly developing multiomics field that integrates genomics and transcriptomics information with proteomics to improve gene annotation, often uncovering novel or non-canonical protein-coding regions in the genome (Branca et al., 2014). One of the most important applications is in the study of cancer cells and tumors, where identifying cancer-specific proteins holds great potential in both elucidating cancer biology and in developing cancer therapies. However, the discovery of such proteins remains particularly challenging and is still largely linked to evidence from genome sequencing data, rather than directly from the protein data that have become abundant (Perez-Riverol et al., 2019). Recent applications of proteogenomics have enabled multiomics detection of novel peptide sequences that are not present in the canonical protein database. For instance, Ruiz Caesas et al. (2021) recently identified a large number of non-canonical proteins in B cell lymphomas. However, customized protein databases are needed to enable the identification of such peptides. Recently, tools for generating sample-specific protein databases have been implemented using genomic sequencing data (Ruggles et al., 2016) and transcriptomics data (Cesnik et al., 2021; Cifani et al., 2018). Since matching sequencing data is not available for a large fraction of the currently available proteomics datasets, resources have been developed to provide protein databases generated from cancer somatic mutations and genomic variants (Zhang et al., 2017).

To make progress in high throughput proteogenomics analysis, we present a Python application integrated into a Nextflow
workflow to facilitate the generation of proteogenomics databases from sample-specific and public resources under varying conditions (e.g. cancer type and transcript biotype). The aim is to enable the identification of variant proteins (derived from single nucleotide variant mutations) and non-canonical or cryptic proteins (from normally dormant regions of the genome).

2 Implementation
We implemented pypgatk, a Python package that provides tools to generate protein databases from non-canonical sequences as well as DNA variants and mutations from public resources and custom files (Fig. 1a).

2.1 Non-canonical protein databases
Non-canonical proteins are a product of translation of transcripts that are not reported as protein coding in the reference protein databases, or a product of out-of-frame translation of canonical transcripts (Ruiz Cuevas et al., 2021). While many of the non-canonical proteins could be attributed to the yet incomplete reference databases, they might also be attributed to the activation of those genes under certain conditions such as genetic and epigenetic misregulation in cancer (Zhu et al., 2018). We have developed the dnaseq-to-proteindb tool to generate protein sequences from non-canonical transcripts such as pseudogenes and IncRNAs by performing three-frame translation. It also extracts alternative reading frames from canonical protein-coding genes to enable the detection of out-of-frame cryptic proteins. Furthermore, the ensemble-downloader tool enables automatic download of the latest ENSEMBL resources including gene annotations, the reference genome and canonical proteins for the species of interest.

2.2 Variant protein databases
Detection of altered proteins from proteomics data requires the inclusion of the mutated sequences in the target databases. However, due to a large number of potential DNA variants, only potentially relevant variant sequences should be included to keep the database size under control. Here, we implemented methods to automate generation of variant proteins from publicly available cancer mutations datasets, cancer cell lines and custom Variant Calling Format (VCF) files obtained from genome sequencing. cosmic-to-proteindb and cbioportal-to-proteindb enable the generation of cancer-type specific protein databases by generating mutated protein sequences based on genomic mutations identified in cancer samples. cosmic-to-proteindb curates mutations from the Catalogue Of Somatic Mutations In Cancer (COSMIC). It allows filtering the mutations based on cancer type or tissue of origin. Alternatively, cbioportal-to-proteindb translates genomics mutations reported by thousands of cancer studies through cBioPortal. pypgatk enables downloading and processing mutations from ENSEMBL and gnomAD resources. vcf-to-proteindb translates the genomic variants into variant protein sequences. The variants can be filtered based on functional consequences as well as allele frequency to enable a special focus on common variants. The vcf-to-proteindb command accepts a custom VCF file from any species or sample of interest and generates a database of altered protein-coding sequences, which is valuable when whole-exome or whole-genome sequencing data are available, for instance to detect cancer neoantigens from passenger mutations.

3 ENSEMBL-based proteogenomic databases
To enable the generation of ENSEMBL-based proteogenomic databases, we have also built the Proteomics-Genomics Data Base
developments and community support. *pypgatk* ([https://pgatk.readthedocs.io/en/latest/pypgatk.html](https://pgatk.readthedocs.io/en/latest/pypgatk.html)) and *pgdb* ([https://nf-co.re/pgdb/1.0.0/usage](https://nf-co.re/pgdb/1.0.0/usage)) include extensive documentation to help researchers create their custom proteogenomics databases.

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### Conflict of Interest

None declared.

### Data availability

We here explored proteomics datasets PXD005946, PXD019263, PXD004452 and PXD014145, which are from the public domain PRIDE database, at [https://www.ebi.ac.uk/pride/](https://www.ebi.ac.uk/pride/). Further data underlying this article are available in its online supplementary material.

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