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TarGene: A Nextflow pipeline for the estimation of genetic effects on human traits via semi-parametric methods.

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Software

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Summary

Genetic variants are the foundation of biological diversity, they play a crucial role in the adaptability, survival, and evolution of populations. Discovering which and how genetic variants affect human traits is an ongoing challenge with applications in healthcare and medicine. In some cases, genetic variants have an obvious effect because they change the coding sequence of a gene and thus its function. In the vast majority of cases however, variants occur in sequences of unknown function and could impact human traits or disease mechanisms in complex ways. TarGene is a Nextflow pipeline leveraging highly flexible machine-learning methods and semi-parametric estimation theory to capture these complex genetic dependencies including higher-order interactions.

Statement of Need

All currently existing software for the estimation of genetic effects are based on parametric distributions, additionally assuming linearity of the relationship between variants and traits (Loh et al., 2018; Purcell et al., 2007; Yang et al., 2011; Zhou et al., 2018). If these assumptions are violated, the reported effect sizes will be biased and error rates inflated. In particular, this can lead to inflated false discovery rates and suboptimal allocation of computational resources and research funding. Some recently published software also account for more complex relationships but do not offer the full modelling flexibility provided by TarGene. REGENIE fits a two-stage whole-genome model for each phenotype of interest but still assumes linearity and normality (Mbatchou et al., 2021). DeepNull is a semi-parametric method which models non-linear covariate effects but also assumes genetic effects to be linear and does not allow complex interactions between covariates and genetic variants (McCaw et al., 2022). KnockoffGWAS (Sesia et al., 2021) is non-parametric but does not estimate effect sizes, instead it aims at controlling the false discovery rate of variant selection in a genome-wide manner. In comparison, TarGene is the only method able to model arbitrarily complex genetic effects while preserving the validity of statistical inference. It does so by leveraging Targeted Learning (Van der Laan et al., 2011), a framework combining methods from causal inference, machine learning, and semi-parametric statistical theory. The estimation process works as follows. In a first step, flexible machine-learning algorithms are fitted to the data. In the second, targeting step, TarGene regularises the estimate of the quantity of interest in a theoretically optimal way.

Features

TarGene is a Nextflow pipeline which can be run as follow:

```
nextflow run https://github.com/TARGENE/targene-pipeline/ \
  -r TARGENE_VERSION \
  -c CONFIG_FILE \
  -resume
```

where the `CONFIG_FILE` provides the list of problem-specific parameters (data, arguments, options). Below we list some important features of TarGene. For detailed explanations, please refer to the online documentation.

Scalability

Machine learning methods are computationally intensive, however statistical genetics analyses need to scale to hundreds of thousands of variants and thousands of traits. For this reason, TarGene leverages Nextflow (Di Tommaso et al., 2017), a pipeline management system that can parallelize independent estimation tasks across HPC platforms.

Databases

TarGene works with standard formats, plink `.bed` and `.bgen` formats for genotypes, `.csv` or `.arrow` format for human traits. Furthermore, TarGene has direct support for two large scale biomedical databases, the UK Biobank (Bycroft et al., 2018) and the All of Us cohort (All of Us Research Program Investigators, 2019). The example considers the UK Biobank for which genotypes and traits are provided via `BED_FILES` and `TRAITS_DATASET` respectively. Because the UK Biobank has a non-standard format, the `UKB_CONFIG` provides trait definition rules. The following is an illustration for the body mass index phenotype, but the default is to consider all 768 traits as defined by geneAtlas (Canela-Xandri et al., 2018).

```
traits:
  - fields:
      - "21001"
    phenotypes:
      - name: "Body mass index (BMI)"
```

Study Designs

TarGene supports traditional study designs in population genetics, that is, genome-wide association studies (GWAS) and phenome-wide association studies (PheWAS). Because TarGene has a focus on complex effects, interactions (e.g. gene-gene, gene-environment, gene-gene-environment) can also be investigated up to any order.

Estimators

In TarGene we default to using Targeted Maximum-Likelihood Estimation (Van der Laan & Rose, 2018) and XGBoost (Chen & Guestrin, 2016) as the machine-learning model but any estimators defined in TMLE.jl is valid (Labayle, Ponting, et al., 2025). These defaults provided the best performance in simulations across a variety of genetics tasks (Labayle, Roskams-Hieter, et al., 2025). In the presence of computational restrictions, tradeoffs can be made and lighter models can be used.

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References

All of Us Research Program Investigators. (2019). The "All of Us" research program. *New England Journal of Medicine*, *381*(7), 668–676. https://doi.org/10.1056/NEJMsr1809937

Bycroft, C., Freeman, C., Petkova, D., Band, G., Elliott, L. T., Sharp, K., Motyer, A., Vukcevic, D., Delaneau, O., O'Connell, J., & others. (2018). The UK Biobank resource with deep phenotyping and genomic data. *Nature*, *562*(7726), 203–209. https://doi.org/10.1038/s41586-018-0579-z

Canela-Xandri, O., Rawlik, K., & Tenesa, A. (2018). An atlas of genetic associations in UK Biobank. *Nature Genetics*, *50*(11), 1593–1599. https://doi.org/10.1038/s41588-018-0248-z

Chen, T., & Guestrin, C. (2016). XGBoost: A scalable tree boosting system. *Proceedings of the 22nd Acm Sigkdd International Conference on Knowledge Discovery and Data Mining*, 785–794. https://doi.org/10.1145/2939672.2939785

Di Tommaso, P., Chatzou, M., Floden, E. W., Barja, P. P., Palumbo, E., & Notredame, C. (2017). Nextflow enables reproducible computational workflows. *Nature Biotechnology*, *35*(4), 316–319. https://doi.org/10.1038/nbt.3820

Labayle, O., Ponting, C. P., Laan, M. J. van der, Khamseh, A., & Beentjes, S. V. (2025). TMLE.jl: Targeted Minimum Loss-Based Estimation in Julia. *Journal of Open Source Software*, *10*(112), 8446. https://doi.org/10.21105/joss.08446

Labayle, O., Roskams-Hieter, B., Slaughter, J., Tetley-Campbell, K., Laan, M. J. van der, Ponting, C. P., Beentjes, S. V., & Khamseh, A. (2025). Semiparametric efficient estimation of small genetic effects in large-scale population cohorts. *Biostatistics*, *26*(1), kxaf030. https://doi.org/10.1093/biostatistics/kxaf030

Loh, P.-R., Kichaev, G., Gazal, S., Schoech, A. P., & Price, A. L. (2018). Mixed-model association for biobank-scale datasets. *Nature Genetics*, *50*(7), 906–908. https://doi.org/10.1038/s41588-018-0144-6

Mbatchou, J., Barnard, L., Backman, J., Marcketta, A., Kosmicki, J. A., Ziyatdinov, A., Benner, C., O'Dushlaine, C., Barber, M., Boutkov, B., & others. (2021). Computationally efficient whole-genome regression for quantitative and binary traits. *Nature Genetics*, *53*(7), 1097–1103. https://doi.org/10.1038/s41588-021-00870-7

McCaw, Z. R., Colthurst, T., Yun, T., Furlotte, N. A., Carroll, A., Alipanahi, B., McLean, C. Y., & Hormozdiari, F. (2022). DeepNull models non-linear covariate effects to improve phenotypic prediction and association power. *Nature Communications*, *13*(1), 241. https://doi.org/10.1038/s41467-021-27930-0

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., Maller, J., Sklar, P., De Bakker, P. I., Daly, M. J., & others. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics*, *81*(3), 559–575. https://doi.org/10.1086/519795

Sesia, M., Bates, S., Candès, E., Marchini, J., & Sabatti, C. (2021). False discovery rate control in genome-wide association studies with population structure. *Proceedings of the National Academy of Sciences*, *118*(40), e2105841118. https://doi.org/10.1073/pnas.2105841118

Van der Laan, M. J., & Rose, S. (2018). *Targeted Learning in Data Science*. Springer. https://doi.org/10.1007/978-3-319-65304-4

Van der Laan, M. J., Rose, S., & others. (2011). *Targeted Learning: Causal Inference for Observational and Experimental Data* (Vol. 4). Springer. https://doi.org/10.1007/978-1-4419-9782-1

Yang, J., Lee, S. H., Goddard, M. E., & Visscher, P. M. (2011). GCTA: A tool for genome-wide complex trait analysis. *The American Journal of Human Genetics*, *88*(1), 76–82. https://doi.org/10.1016/j.ajhg.2010.11.011

Zhou, W., Nielsen, J. B., Fritsche, L. G., Dey, R., Gabrielsen, M. E., Wolford, B. N., LeFaive, J., VandeHaar, P., Gagliano, S. A., Gifford, A., & others. (2018). Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nature Genetics*, *50*(9), 1335–1341. https://doi.org/10.1038/s41588-018-0184-y