Using TraCeR to Reconstruct TCR Sequences from C1 Single-Cell mRNA Seq Data

Frequently Asked Questions

What is TraCeR analysis?

TraCeR is a tool developed by scientists at the EMBL-European Bioinformatics Institute and the Wellcome Trust Sanger Institute that reconstructs the sequences of rearranged and expressed T cell receptor (TCR) genes from single-cell mRNA sequencing data. It then uses the TCR sequences to identify cells that have the same receptor sequences and so derive from the same original clonally expanded cell. Find directions for setting up and using TraCeR on Script Hub™ or GitHub.

What tools does TraCeR analysis use?

TraCeR analysis uses several pre-existing, open-source toolsets to extract, reconstruct and assign T cell receptor sequence information and nomenclature to single-cell mRNA sequencing data. The pre-existing tool sets used include Bowtie 2, Trinity, IgBLAST and Kallisto. The single-cell TCR Seq application note provides details for downloading each component and integrating them into one cohesive analysis pipeline.

How can I be sure that I have successfully installed TraCeR?

The TraCeR installer includes a small (three cell) dataset for mouse α and β chain sequences in test_data/. This can be used to test the installation and config file and confirm that all the prerequisites are working.

What is the output of TraCeR analysis?

The last step of the TraCeR analysis generates clonotypic bar graphs, reconstructed α and β chain length distributions, and T cell receptor (TCR) sequence network graphs, via custom Python scripts. The clonotypic bar graphs provide a measure of the clonotype diversity and the number of cells detected for each clonotype. The network graphs show shared TCR sequences across the single-cell population measured with C1.

Do I need a special kit or IFC to run TCR sequencing on the C1?

No. The single-cell T cell receptor (TCR) sequencing application described on Script Hub™ uses data produced by the C1 Single-Cell mRNA Seq Protocol (PN 100-7168), which provides whole transcriptome amplification (WTA) with full-length mRNA transcripts. The protocol uses the existing C1 mRNA Seq IFC and reagent kits, and the resulting data is analyzed using the TraCeR analysis tool. Recommended sequencing depth is 1 million reads per T cell.

What types of T cells can be studied with the help of TraCeR analysis?

Stubbington et al. (*Nature Methods* 2016), who developed TraCeR, detected transcripts associated with a variety of mouse T cell subtypes, including activated, proliferating, central memory and effector memory T cells. Detecting transcripts in single, naïve T cells may be difficult because of their quiescent nature and low transcription rates. Tools are provided through TraCeR that enable sequencing of both mouse and human T cell receptors.

What cell preparation protocol is recommended to obtain optimal C1 data when using TraCeR analysis?

The successful detection of T cell receptor sequences will depend on the amount of the relevant transcript available for isolation, and this will vary depending on the subtypes of T cells captured by the C1 IFC and on the sample preparation protocol. Stubbington et al. (*Nature Methods* 2016), who developed TraCeR, used freshly isolated mouse splenic T cells and were able to detect a variety of subtypes, including activated proliferating T cells and central and effector memory T cells. Detecting transcripts in single, naïve T cells may be difficult due to their quiescent nature and low transcription rates. Recommended sequencing depth is 1 million reads per T cell.

The sample handling protocol will also influence the amount of transcript available for amplification. Take the following precautions to minimize cell stress:

- Avoid cryopreservation.
- If cryopreserved samples must be used, implement a protocol that has been optimized for cell recovery.
- Minimize the amount of time cells are held either post-harvest or post-thaw.
- Conduct antibody labeling or other enrichment steps at 4 °C.
- If you are pre-enriching by flow sorting, use a low-pressure and larger nozzle (100 µm) for reduced shear stress to the cells.

What sequencing depth is recommended for TraCeR analysis?

The data presented by Stubbington et al. (*Nature Methods* 2016), who developed TraCeR, was sequenced with paired-end 100 bp reads, with a sequencing depth analysis suggesting that 1 million reads per cell were found to be sufficient to detect most TCR α and β chain sequences in the demonstrated experimental model.

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