TCRAFT vector amplification

Vector pools (1A, 1B, 2A, 2B)

Contents of vector pools:

- **1A**, containing **TRBV**-TRAC vectors for TRBV2 to 7-8 (24 vectors) (chloramphenicol)
- **1B**, containing **TRBV**-TRAC vectors for TRBV7-9 to 30 (24 vectors) (chloramphenicol)
- **2A**, containing TRBC-**TRAV** vectors for TRAV1-1 to 16 (23 vectors) (kanamycin)
- **2B**, containing TRBC-**TRAV** vectors for TRAV17 to 41 (22 vectors) (kanamycin)

The following is a protocol to amplify pools with electrocompetent cells.

Materials

1. 20 ng of each library (1A, 1B, 2A, 2B)
2. ElectroMAX DH10B *E. coli* (Thermo Fisher, 18290015) (includes S.O.C. medium)
3. Electroporation cuvettes (1 mm gap) (VWR, 89047-206)
4. LB media
5. LB plates:
    - chloramphenicol (Millipore Sigma, C0378)
    - kanamycin (RPI, 25389-94-0)
6. BTX® ECM® 630 electroporator
7. 30C incubator for plates
8. 30C shaking incubator

Protocol

1. Thaw one 100 μL aliquot of ElectroMax DH10B *E. coli* on ice for 15 minutes.
2. Pre-cool four Eppendorf tubes and four 1-mm cuvettes on ice.
3. Gently transfer 25 μL of DH10B bacteria to each Eppendorf tube. Add 20 ng of each library to the Eppendorf tubes (one each). Mix by tapping gently.
4. Incubate on ice for 10 minutes.
5. Transfer cell/DNA mixture into a chilled cuvette and put back on ice. Gently tap the cuvette to ensure complete contact of cells across the bottom of the chamber. Avoid bubbles.
6. Electroporate using the BTX® ECM® 630 electroporator with the settings:
    - 2000 V
    - 200 ohms
    - 25 μF
7. Immediately add 1000 μL of S.O.C. media right after shocking and transfer all to 15 mL Falcon tubes.
8. Incubate cells for 1h at 37C in a shaking incubator (225 rpm).
9. Transfer 1 μL to 100 μL of S.O.C. medium and plate (LB-Chlor for 1A/1B or LB-Kan for 2A/2B). Incubate overnight at 30C. This represents a 1:1000 dilution of the electroporation product.
10. Transfer the remainder to 200 mL of LB-Chlor or LB-Kan and incubate overnight at **30C**. Do not overgrow (avoids plasmid multimerization and recombination).
11. Next day: examine the plates for colonies. >25 colonies indicates >1000x coverage. An efficient transformation should generate far more than this. Continue with midiprep/maxiprep if electroporation successful (i.e. >25 colonies per plate).

pHIV-LacZ-SapI (destination vector)

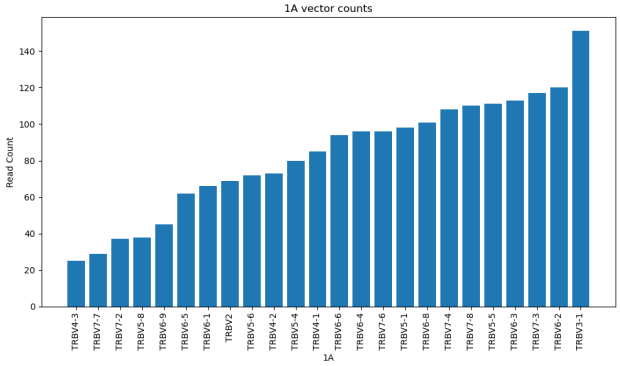
The pHIV-LacZ-SapI destination vector can be propagated with any standard heat shock transformation protocol. This plasmid is carbenicillin/ampicillin-resistant.

Distribution analysis of TCRAFT vector pools

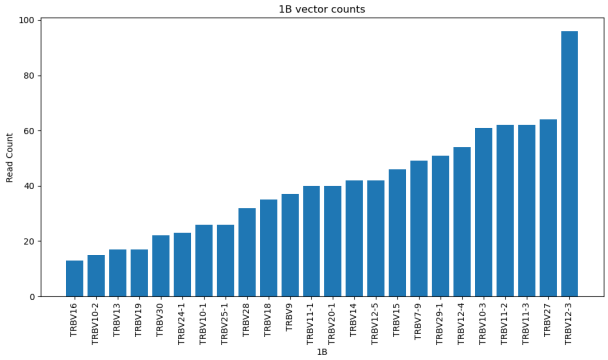
1. No prep: Submit vector pools to Plasmidsaurus or another whole-plasmid sequencing service.
2. Results:
    - **Download raw fastq data.**
    - Check gel/histogram of read length distribution.
3. Process raw fastq files to examine the distribution of plasmids in each pool using the provided code on Addgene or here: https://github.com/birnbaumlab/Gaglione-et-al-2025/tree/main/Vector_Pool_Scripts
    a. This code will output a table and plot (below) of read counts for each library member.
4. Check that all plasmids are present.

Sample data:

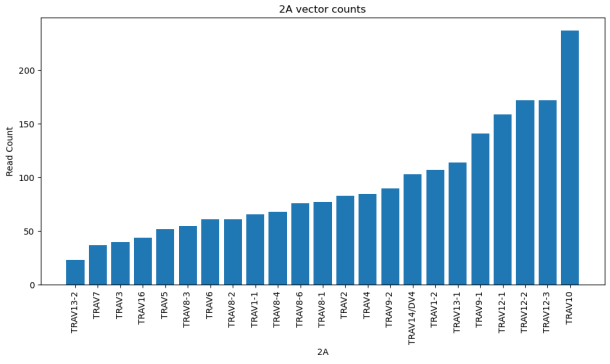
1A (TRBV2 to 7-8):



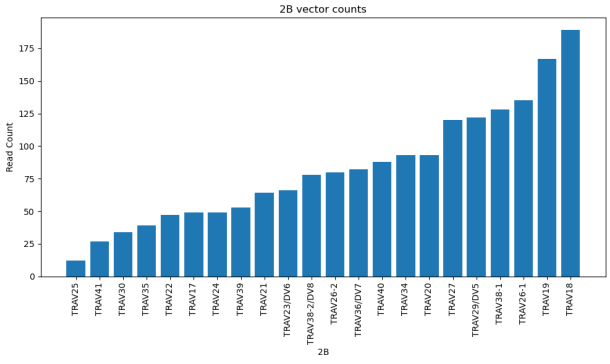
1B (TRBV7-9 to 30):



2A (TRAV1-1 to 16):



2B (TRAV17 to 41):



Alternatively, a conventional NGS-based approach can be used by PCR-amplifying each pool and sequencing. The following primers can be used to add partial Illumina adapters.

TRBV pools 1A and 1B:

TRBV_f	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNATCTAGAGCCACCGGCATGAG
TRBV_r	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNCAGACTTGTCACTGGATTTAGAGTCTCTCA

TRAV pools 2A and 2B:

TRAV_f	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGACGTTGAGGAAAACCCAGGAC
TRAV_r	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNCACTGAGCCTCCACCTAGCC