Supplementary Material

1. **Supplementary Figures and Tables**

## Supplementary Figures

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**Figure S1.** The relative percentages of each of the 611 *TRB* templates before amplification in pool 1 (A) and pool 2 (B).

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**Figure S2.** The *TRBV* (A, C) and *TRBJ* segments (B, D) usage frequency before amplification of pool 1 (A, B) and pool 2 (C, D).



**Figure S3.** Amplification bias of the multiplex PCR reaction system during primer mix optimization for pool 2. (A) The *TRBV* amplification bias before optimization. (B, C) The *TRBV* amplification bias during optimization. (D) The *TRBV* amplification bias after optimization. (E) The *TRBJ* amplification bias before optimization. (F, G) The *TRBJ* amplification bias during optimization. (H) The *TRBJ* amplification bias after optimization.

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**Figure S4.** Amplification biases for the first (A) and second (B) gradient mixture of the TRB templates

## Supplementary tables

**Table S1.** Primers used in multiplex PCR for amplifying the CDR3 of rearranged *TRB* gene.

|  |  |
| --- | --- |
| TRBV Primers | Primer Sequence |
| zeTRBV1 | GACCGCTTGGCCTCCGACTTATTTCACTCTGAAGATCCGGTCCAC  |
| zeTRBV2-new | GACCGCTTGGCCTCCGACTTGCTTGGTGACTCTGCTGTGTATTTC |
| zeTRBV3 | GACCGCTTGGCCTCCGACTTCAAGTCGCTTCTCACCTGAATG |
| zeTRBV4 | GACCGCTTGGCCTCCGACTTGCCAGTTCTCTAACTCTCGCTCT |
| zeTRBV5 | GACCGCTTGGCCTCCGACTTTCAGGTCGCCAGTTCCCTAAYTAT |
| zeTRBV6 | GACCGCTTGGCCTCCGACTTCACGTTGGCGTCTGCTGTACCCT |
| zeTRBV7 | GACCGCTTGGCCTCCGACTTCAGGCTGGTGTCGGCTGCTCCCT |
| zeTRBV10 | GACCGCTTGGCCTCCGACTTGGGATCCGTCTCCACTCTGAMGAT |
| zeTRBV11 | GACCGCTTGGCCTCCGACTTGGGATCCGTCTCTACTCTGAAGAT |
| zeTRBV12 | GACCGCTTGGCCTCCGACTTGGGATCTTTCTCCACCTTGGAGAT |
| zeTRBV13 | GACCGCTTGGCCTCCGACTTCCTGACTTGCACTCTGAACTAAACCT  |
| zeTRBV14 | GACCGCTTGGCCTCCGACTTCCTCACTCTGGAGTCTGCTGCC |
| zeTRBV15 | GACCGCTTGGCCTCCGACTTCCTCACTCTGGAGTCMGCTACC |
| zeTRBV16 | GACCGCTTGGCCTCCGACTTGCAGAGAGGCTCAAAGGAGTAGACT |
| zeTRBV17 | GACCGCTTGGCCTCCGACTTGAAGATCCAGCCCTCAGAACCCAG |
| zeTRBV18 | GACCGCTTGGCCTCCGACTTTCGATTCTCAGCTCAACAGTTC |
| zeTRBV19 | GACCGCTTGGCCTCCGACTTGGAGGGACGTATTCTACTCTGAAGG  |
| zeTRBV20 | GACCGCTTGGCCTCCGACTTTTCTTGACATCCGCTCACCAGG  |
| zeTRBV21 | GACCGCTTGGCCTCCGACTTCTGTAGCCTTGAGATCCAGGCTACGA |
| zeTRBV22 | GACCGCTTGGCCTCCGACTTTAGATGAGTCAGGAATGCCAAAG |
| zeTRBV23 | GACCGCTTGGCCTCCGACTTCTGTGACATCGGCCCAAAAGAAC |
| zeTRBV24 | GACCGCTTGGCCTCCGACTTAACCATGCAAGCCTGACCTT |
| zeTRBV25 | GACCGCTTGGCCTCCGACTTCTCCCTGTCCCTAGAGTCTGCCAT |
| zeTRBV26 | GACCGCTTGGCCTCCGACTTGCCCTCACATACCTCTCAGTACCTC  |
| zeTRBV27 | GACCGCTTGGCCTCCGACTTGATCCTGGAGTCGCCCAGC |
| zeTRBV28 | GACCGCTTGGCCTCCGACTTATTCTGGAGTCCGCCAGC |
| zeTRBV29 | GACCGCTTGGCCTCCGACTTAACTCTGACTGTGAGCAACATGAG |
| zeTRBV30-new | GACCGCTTGGCCTCCGACTTTCCTTCTCAGTGACTCTGGCTTCTATC |
| TRBJ primers | Primer Sequence |
| zeTRBJ1 | ACATGGCTACGATCCGACTTCTTACCTACAACTGTGAGTCTGGTG  |
| zeTRBJ2 | ACATGGCTACGATCCGACTTACCCCCAGCCTTACCTACA |
| zeTRBJ3 | ACATGGCTACGATCCGACTTCTTACCTACAACAGTGAGCCAACTT  |
| zeTRBJ4 | ACATGGCTACGATCCGACTTAAGACAGAGAGCTGGGTTCCACT |
| zeTRBJ5 | ACATGGCTACGATCCGACTTCTTACCTAGGATGGAGAGTCGAGTC  |
| zeTRBJ6 | ACATGGCTACGATCCGACTTCGAGTCAAGAGTGGAGCCC |
| zeTRBJ7 | ACATGGCTACGATCCGACTTCCTTCTTACCTAGCACGGTGA |
| zeTRBJ8 | ACATGGCTACGATCCGACTTCTTACCCAGTACGGTCAGCCT  |
| zeTRBJ9 | ACATGGCTACGATCCGACTTCCGCTTACCGAGCACTGTCAG  |
| zeTRBJ10 | ACATGGCTACGATCCGACTTAGCACTGAGAGCCGGGTCC |
| zeTRBJ11 | ACATGGCTACGATCCGACTTCGAGCACCAGGAGCCGCGT |
| zeTRBJ12 | ACATGGCTACGATCCGACTTCTCGCCCAGCACGGTCAGCCT  |
| zeTRBJ13 | ACATGGCTACGATCCGACTTCTTACCTGTGACCGTGAGCCTG |

**Table S2. The clinical information of the leukemia patients**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patients ID | Disease | Age | Gender | Risk group | Fusion gene | MRD on day 33 | MRD on day 64 | Clinical outcome |
| ALL-1 | B-ALL | 2 | M | SR | Normal | 0 | 0 | CR |
| ALL-2 | B-ALL | 5 | F | SR | TEL-AML1 (+) | 0 | 0 | CR |
| ALL-3 | B-ALL | 8 | M | IR |  Normal | 0 |  | CR |
| ALL-4 | B-ALL | 10 | F | IR | TEL-AML1 (+) | 0 |  | CR |
| ALL-5 | B-ALL | 1 | M | HR | MLL (+) | 0 |  | CR |
| ALL-6 | B-ALL | 2 | F | SR | Normal | 0 | 0 | CR |
| ALL-7 | B-ALL | 4 | M | SR | TEL-AML1 (+) | 0 | 0 | CR |
| ALL-8 | B-ALL | 2 | F | SR | TEL-AML1 (+) | 0 |  | CR |
| ALL-9 | B-ALL | 10 | M | HR | TEL-AML1 (+) | 0.6% |  | Unknown |
| ALL-10 | B-ALL | 3 | M | IR | TEL-AML1 (+) | 0.3% |  | CR |
| CYY | T-ALL | 11 | M | IR | Normal  | 3.8% |  | Deathafter bone marrow transplantation |
| TDJ | T-ALL | 4 | M | IR | Not done | 0 |  | CR after bone marrow transplantation |

R = Standard Risk; IR = Intermediate Risk; HR = high risk; CR = complete remission

**Table S3.** The expected, pre-amplification and post-amplification values for each TRBV segment of the first pool of 47 TRBV segments with different concentrations. The red color indicated the TRBV segments with gradient pooling.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TRBV segments | expected | pre-amplification | post-amplification | bias |
| V11-2 | 20 | 19.4754  | 16.7904  | 0.862  |
| V11-3 | 0.1 | 0.0856  | 0.0906  | 1.059  |
| V12-4 | 10 | 8.8974  | 11.8737  | 1.335  |
| V18 | 5 | 3.2469  | 7.0525  | 2.172  |
| V19 | 0.01 | 0.0089  | 0.0077  | 0.866  |
| V20-1 | 0.1 | 0.0957  | 0.0922  | 0.963  |
| V25-1 | 0.01 | 0.0088  | 0.0044  | 0.503  |
| V29-1 | 1 | 1.1062  | 1.1572  | 1.046  |
| V30 | 1 | 1.2363  | 0.6039  | 0.488  |
| V9 | 0.05 | 0.0449  | 0.0277  | 0.616  |
| V10-1 | 1.695  | 2.0043  | 2.9619  | 1.478  |
| V10-2 | 1.695  | 2.0942  | 1.0390  | 0.496  |
| V10-3 | 1.695  | 1.5912  | 1.4641  | 0.920  |
| V11-1 | 1.695  | 1.6642  | 2.0053  | 1.205  |
| V12-3 | 1.695  | 1.6602  | 1.8728  | 1.128  |
| V12-5 | 1.695  | 1.3689  | 1.9119  | 1.397  |
| V13 | 1.695  | 2.3374  | 2.5496  | 1.091  |
| V14 | 1.695  | 2.1300  | 2.8583  | 1.342  |
| V15 | 1.695  | 1.6489  | 2.8491  | 1.728  |
| V16 | 1.695  | 1.9802  | 0.9207  | 0.465  |
| V2 | 1.695  | 2.1288  | 1.1575  | 0.544  |
| V24-1 | 1.695  | 1.4965  | 1.1986  | 0.801  |
| V27 | 1.695  | 2.4446  | 2.5119  | 1.028  |
| V28 | 1.695  | 1.8385  | 1.3498  | 0.734  |
| V3-1 | 1.695  | 1.8930  | 0.6653  | 0.351  |
| V4-1 | 1.695  | 2.4844  | 1.0772  | 0.434  |
| V4-2 | 1.695  | 1.6215  | 0.8815  | 0.544  |
| V4-3 | 1.695  | 1.4234  | 0.8602  | 0.604  |
| V5-1 | 1.695  | 2.1375  | 2.9725  | 1.391  |
| V5-4 | 1.695  | 1.4855  | 0.6134  | 0.413  |
| V5-5 | 1.695  | 1.1276  | 1.6230  | 1.439  |
| V5-6 | 1.695  | 1.4410  | 0.9922  | 0.689  |
| V5-8 | 1.695  | 1.6262  | 3.1391  | 1.930  |
| V6-1 | 1.695  | 1.7295  | 1.2677  | 0.733  |
| V6-2 | 1.695  | 1.7845  | 1.0453  | 0.586  |
| V6-4 | 1.695  | 1.5745  | 1.6246  | 1.032  |
| V6-5 | 1.695  | 1.4956  | 1.6371  | 1.095  |
| V6-6 | 1.695  | 1.7689  | 1.0540  | 0.596  |
| V6-8 | 1.695  | 1.7901  | 1.8489  | 1.033  |
| V6-9 | 1.695  | 1.7762  | 1.1731  | 0.660  |
| V7-2 | 1.695  | 2.1106  | 2.4326  | 1.153  |
| V7-3 | 1.695  | 1.7253  | 2.4855  | 1.441  |
| V7-4 | 1.695  | 1.2211  | 1.1583  | 0.949  |
| V7-6 | 1.695  | 1.4123  | 0.9038  | 0.640  |
| V7-7 | 1.695  | 1.3106  | 0.8203  | 0.626  |
| V7-8 | 1.695  | 1.6617  | 5.2251  | 3.144  |
| V7-9 | 1.695  | 2.8050  | 0.1486  | 0.053  |

**Table S4.** The expected, pre-amplification and post-amplification values for each TRBV segment of the second pool of 47 TRBV segments with different concentrations. The red color indicated the TRBV segments with gradient pooling.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TRBV segments | Expected  | Pre-amplification | Post-amplification | Bias |
| V10-3 | 0.05 | 0.0439  | 0.0365  | 0.831  |
| V11-2 | 0.1 | 0.0895  | 0.0517  | 0.578  |
| V11-3 | 1 | 0.8329  | 0.6689  | 0.803  |
| V12-3 | 10 | 8.7331  | 11.9051  | 1.363  |
| V12-5 | 0.01 | 0.0176  | 0.0108  | 0.613  |
| V18 | 0.001 | 0.0037  | 0.0033  | 0.896  |
| V20-1 | 0.1 | 0.0934  | 0.0592  | 0.634  |
| V27 | 5 | 7.1276  | 11.6379  | 1.633  |
| V28 | 0.5 | 0.6248  | 0.5704  | 0.913  |
| V29-1 | 0.5 | 0.5200  | 0.4837  | 0.930  |
| V10-1 | 2.24  | 2.5580  | 4.6907  | 1.834  |
| V10-2 | 2.24  | 2.5833  | 1.5103  | 0.585  |
| V11-1 | 2.24  | 2.0662  | 2.2348  | 1.082  |
| V12-4 | 2.24  | 2.2287  | 3.4767  | 1.560  |
| V13 | 2.24  | 3.1439  | 2.5423  | 0.809  |
| V14 | 2.24  | 2.6529  | 2.9335  | 1.106  |
| V15 | 2.24  | 2.1081  | 3.4987  | 1.660  |
| V16 | 2.24  | 2.1724  | 1.2626  | 0.581  |
| V19 | 2.24  | 1.7107  | 2.4636  | 1.440  |
| V2 | 2.24  | 2.5651  | 1.5428  | 0.601  |
| V24-1 | 2.24  | 1.8134  | 1.8990  | 1.047  |
| V25-1 | 2.24  | 2.2356  | 3.7341  | 1.670  |
| V30 | 2.24  | 2.7498  | 1.8580  | 0.676  |
| V3-1 | 2.24  | 2.4507  | 1.8248  | 0.745  |
| V4-1 | 2.24  | 2.7526  | 1.0485  | 0.381  |
| V4-2 | 2.24  | 1.9369  | 0.8907  | 0.460  |
| V4-3 | 2.24  | 1.7397  | 0.8525  | 0.490  |
| V5-1 | 2.24  | 2.7568  | 3.0594  | 1.110  |
| V5-4 | 2.24  | 1.8297  | 0.4944  | 0.270  |
| V5-5 | 2.24  | 1.3699  | 1.4156  | 1.033  |
| V5-6 | 2.24  | 1.5478  | 0.8830  | 0.570  |
| V5-8 | 2.24  | 2.0762  | 3.1049  | 1.496  |
| V6-1 | 2.24  | 2.1709  | 1.6522  | 0.761  |
| V6-2 | 2.24  | 2.2966  | 1.3727  | 0.598  |
| V6-4 | 2.24  | 1.9500  | 2.1216  | 1.088  |
| V6-5 | 2.24  | 1.8812  | 2.3763  | 1.263  |
| V6-6 | 2.24  | 2.1565  | 1.3804  | 0.640  |
| V6-8 | 2.24  | 2.2405  | 2.9296  | 1.308  |
| V6-9 | 2.24  | 2.2858  | 1.8737  | 0.820  |
| V7-2 | 2.24  | 2.7227  | 2.4297  | 0.892  |
| V7-3 | 2.24  | 2.2913  | 2.6220  | 1.144  |
| V7-4 | 2.24  | 1.5622  | 1.0400  | 0.666  |
| V7-6 | 2.24  | 1.9350  | 1.0986  | 0.568  |
| V7-7 | 2.24  | 1.7203  | 0.7716  | 0.449  |
| V7-8 | 2.24  | 2.0960  | 3.0638  | 1.462  |
| V7-9 | 2.24  | 3.5444  | 0.1704  | 0.048  |
| V9 | 2.24  | 2.0123  | 2.4489  | 1.217  |

**Table S5.** The number of CDR3 nucleotide clonotypes on day 0, day 33 and the consistent clonotypes on day 0 and 33.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Patients | No. of clonotypes on day 0 | No. of clonotypes on day 33 | No. of shared clonotypes on day 0 and 33 | Percentage of vanished pre-existing clonotypes after treatment (%) | Percentage of emerging clonotypes on day 33 (%) |
| ALL-1 | 17334 | 49265 | 374 | 97.84  | 99.24  |
| ALL-10 | 20450 | 53152 | 602 | 97.06  | 98.87  |
| ALL-2 | 3107 | 41337 | 53 | 98.29  | 99.87  |
| ALL-3 | 20635 | 12777 | 572 | 97.23  | 95.52  |
| ALL-4 | 19760 | 35898 | 1240 | 93.72  | 96.55  |
| ALL-5 | 14392 | 26429 | 1136 | 92.11  | 95.70  |
| ALL-6 | 33870 | 41303 | 466 | 98.62  | 98.87  |
| ALL-7 | 9425 | 39394 | 381 | 95.96  | 99.03  |
| ALL-8 | 23102 | 54891 | 622 | 97.31  | 98.87  |
| ALL-9 | 3025 | 28154 | 263 | 91.31  | 99.07  |
| **Average** |  |  |  | **95.94**  | **98.16**  |