**Online Supplementary Material**

Table S1. List of 39 blood group-related genes included in the NGS panel

|  |  |  |  |
| --- | --- | --- | --- |
| **Blood group system** | **Gene** | **Mean depth of coverage\* (×)** | **Mean percentage of target bases covered\*** |
| ≥ **5×** | ≥ **30×** | ≥ **100×** |
| ABO | *ABO* | 623.6  | 100.0 | 97.0  | 95.0  |
| MNS | *GYPA* | 337.0  | 100.0  | 100.0  | 87.6  |
| *GYPB* | 392.0  | 100.0  | 100.0  | 95.9  |
| P1PK  | *A4GALT* | 911.0  | 100.0  | 100.0  | 100.0  |
| Rh | *RHD* | 691.0  | 93.9  | 93.9  | 92.6  |
| *RHCE* | 753.9  | 93.4  | 93.1  | 91.7  |
| Lutheran | *BCAM*  | 850.8  | 100.0  | 99.9  | 99.5  |
| Kell  | *KEL*  | 723.6  | 100.0  | 100.0  | 100.0  |
| Lewis | *FUT3*  | 782.4  | 100.0  | 100.0  | 100.0  |
| Duffy  | *ACKR1* | 536.4  | 100.0  | 100.0  | 98.6  |
| Kidd  | *SLC14A1* | 575.9  | 100.0  | 100.0  | 99.1  |
| Diego  | *SLC4A1* | 967.5  | 100.0  | 100.0  | 100.0  |
| Yt  | *ACHE* | 905.9  | 100.0  | 100.0  | 100.0  |
| Scianna  | *ERMAP* | 638.5  | 100.0  | 100.0  | 100.0 |
| Dombrock  | *ART4*  | 600.5  | 100.0  | 100.0  | 99.6 |
| Colton | *AQP1*  | 681.9  | 100.0  | 100.0  | 99.8 |
| Landsteiner-Wiener  | *ICAM4*  | 681.6  | 100.0  | 100.0  | 99.7 |
| H  | *FUT1*  | 1108.0  | 100.0  | 100.0  | 100.0  |
| *FUT2* | 772.3  | 100.0  | 100.0  | 100.0  |
| Kx  | *XK* | 406.7  | 100.0  | 100.0  | 97.1 |
| Gerbich  | *GYPC* | 549.3  | 100.0  | 100.0  | 99.5 |
| Cromer | *CD55*  | 413.6  | 100.0  | 99.4  | 96.6  |
| Knops  | *CR1*  | 390.8  | 70.4  | 66.4  | 63.5  |
| Indian  | *CD44*  | 538.1  | 100.0  | 100.0  | 100.0  |
| Ok  | *BSG*  | 698.5  | 100.0  | 100.0  | 99.4 |
| Raph  | *CD151* | 794.1  | 100.0  | 100.0  | 100.0  |
| John Milton Hagen | *SEMA7A*  | 807.2  | 100.0  | 99.9  | 98.4  |
| I  | *GCNT2*  | 600.6  | 100.0  | 100.0  | 99.9 |
| Globoside  | *B3GALNT1* | 532.3  | 100.0  | 100.0  | 98.4 |
| Gill | *AQP3*  | 1100.5  | 100.0  | 100.0  | 100.0  |
| Rh-associated glycoprotein  | *RHAG*  | 598.0  | 100.0  | 100.0  | 100.0  |
| FORS | *GBGT1*  | 758.9  | 100.0  | 100.0  | 100.0  |
| JR  | *ABCG2*  | 494.6  | 100.0  | 100.0  | 99.8 |
| LAN | *ABCB6*  | 728.3  | 100.0  | 100.0  | 99.6 |
| Vel  | *SMIM1* | 845.1  | 100.0  | 100.0  | 100.0  |
| CD59 | *CD59* | 693.2  | 100.0  | 100.0  | 100.0  |
| Augustine | *SLC29A1* | 623.2  | 100.0  | 100.0  | 99.7 |
| Erythroid-specific transcription factor genes | *GATA1* | 510.9  | 100.0  | 100.0  | 97.0 |
| *KLF1* | 588.7  | 100.0  | 100.0  | 97.7 |

\* Only uniquely mapped reads were used for calculating coverage.

Fig. S1. Detection of two distinct RBC populations in dizygotic twins (A: patient 2; B: patient 3) using the gel method and FC analysis. In the gel method, RBCs of the patients showed MF agglutination with anti-A and anti-B reagents, and agglutination with anti-B was stronger than that with anti-A. FC analysis confirmed that group B RBCs were the major RBC population, whereas group A RBCs were the minor RBC population. In histograms obtained from flow cytometry analysis, the x‐axis represents fluorescence intensity, and the y‐axis represents the number of cells.