**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.