

Table S3. False positive rates (FPRs) calculated for four SNP calling approaches based on the simulation datasets of *Arabidopsis thaliana* and *Oryza sativa*.

| Read length | Cov <sup>a</sup> | <i>A. thaliana</i>      |                        |                        |                     | <i>O. sativa</i> |           |           |        |
|-------------|------------------|-------------------------|------------------------|------------------------|---------------------|------------------|-----------|-----------|--------|
|             |                  | iML_denovo <sup>b</sup> | ML_denovo <sup>c</sup> | TH_denovo <sup>d</sup> | ML_ref <sup>e</sup> | iML_denovo       | ML_denovo | TH_denovo | ML_ref |
| 35bp        | 4                | 0.5566                  | 0.6693                 | 0.4132                 | 0.1260              | 0.6271           | 0.7441    | 0.5519    | 0.1311 |
|             | 8                | 0.1774                  | 0.3026                 | 0.1871                 | 0.1301              | 0.2623           | 0.4300    | 0.2973    | 0.1258 |
|             | 12               | 0.0983                  | 0.2206                 | 0.1675                 | 0.0919              | 0.1446           | 0.3284    | 0.2518    | 0.0866 |
|             | 16               | 0.0745                  | 0.1997                 | 0.1563                 | 0.0333              | 0.1212           | 0.3079    | 0.2354    | 0.0350 |
|             | 20               | 0.0458                  | 0.1943                 | 0.1451                 | 0.0169              | 0.0815           | 0.3048    | 0.2215    | 0.0129 |
|             | 24               | 0.0415                  | 0.2024                 | 0.1427                 | 0.0062              | 0.0654           | 0.2992    | 0.2180    | 0.0063 |
|             | 28               | 0.0286                  | 0.1932                 | 0.1382                 | 0.0028              | 0.0536           | 0.2981    | 0.2135    | 0.0031 |
|             | 32               | 0.0225                  | 0.1901                 | 0.1377                 | 0.0010              | 0.0646           | 0.2971    | 0.2055    | 0.0030 |
|             | 36               | 0.0115                  | 0.1908                 | 0.1317                 | 0.0010              | 0.0584           | 0.2872    | 0.2070    | 0.0023 |
|             | 40               | 0.0112                  | 0.1963                 | 0.1293                 | 0.0003              | 0.0558           | 0.2865    | 0.2017    | 0.0016 |
| 50bp        | 4                | 0.4631                  | 0.6111                 | 0.2882                 | 0.1278              | 0.5856           | 0.7238    | 0.4255    | 1.0000 |
|             | 8                | 0.1298                  | 0.2247                 | 0.1178                 | 0.1224              | 0.2190           | 0.3562    | 0.2050    | 0.1307 |
|             | 12               | 0.0624                  | 0.1446                 | 0.0944                 | 0.0814              | 0.1099           | 0.2486    | 0.1705    | 0.0864 |
|             | 16               | 0.0457                  | 0.1266                 | 0.0863                 | 0.0363              | 0.0870           | 0.2221    | 0.1574    | 0.0370 |
|             | 20               | 0.0318                  | 0.1239                 | 0.0854                 | 0.0104              | 0.0608           | 0.2169    | 0.1511    | 0.0135 |
|             | 24               | 0.0240                  | 0.1209                 | 0.0804                 | 0.0056              | 0.0512           | 0.2188    | 0.1449    | 0.0079 |
|             | 28               | 0.0213                  | 0.1212                 | 0.0804                 | 0.0016              | 0.0425           | 0.2160    | 0.1442    | 0.0040 |
|             | 32               | 0.0156                  | 0.1223                 | 0.0770                 | 0.0009              | 0.0350           | 0.2206    | 0.1365    | 0.0018 |
|             | 36               | 0.0128                  | 0.1205                 | 0.0748                 | 0.0006              | 0.0296           | 0.2188    | 0.1346    | 0.0007 |
|             | 40               | 0.0104                  | 0.1213                 | 0.0724                 | 0.0004              | 0.0237           | 0.2195    | 0.1338    | 0.0011 |
| 100bp       | 4                | 0.3214                  | 0.4976                 | 0.1190                 | 0.1072              | 0.4402           | 0.6466    | 0.2167    | 0.1143 |
|             | 8                | 0.0967                  | 0.1623                 | 0.0436                 | 0.1110              | 0.1788           | 0.2879    | 0.0842    | 0.1065 |
|             | 12               | 0.0427                  | 0.0851                 | 0.0349                 | 0.0819              | 0.0915           | 0.1685    | 0.0709    | 0.0811 |
|             | 16               | 0.0350                  | 0.0678                 | 0.0317                 | 0.0394              | 0.0581           | 0.1211    | 0.0624    | 0.0411 |
|             | 20               | 0.0214                  | 0.0563                 | 0.0288                 | 0.0169              | 0.0375           | 0.1051    | 0.0600    | 0.0411 |
|             | 24               | 0.0143                  | 0.0498                 | 0.0281                 | 0.0070              | 0.0297           | 0.0972    | 0.0583    | 0.0070 |
|             | 28               | 0.0118                  | 0.0482                 | 0.0268                 | 0.0028              | 0.0258           | 0.0974    | 0.0544    | 0.0070 |
|             | 32               | 0.0102                  | 0.0492                 | 0.0270                 | 0.0016              | 0.0214           | 0.0977    | 0.0537    | 0.0020 |
|             | 36               | 0.0120                  | 0.0521                 | 0.0252                 | 0.0007              | 0.0199           | 0.0987    | 0.0522    | 0.0013 |
|             | 40               | 0.0085                  | 0.0509                 | 0.0250                 | 0.0007              | 0.0177           | 0.0991    | 0.0524    | 0.0011 |

<sup>a</sup> Coverage of the simulated sequencing;

<sup>b</sup> iML\_denovo, *de novo* SNP calling using the iML algorithm.

<sup>c</sup> ML\_denovo, *de novo* SNP calling using the ML algorithm;

<sup>d</sup> TH\_denovo, *de novo* SNP calling using the threshold approach;

<sup>e</sup> ML\_ref, reference-based SNP calling using the ML algorithm;

Table S4. False negative rates (FNRs) calculated for four SNP calling approaches based on the simulation datasets of *Arabidopsis thaliana* and *Oryza sativa*.

| Read length | Cov <sup>a</sup> | <i>A. thaliana</i>      |                        |                        |                     | <i>O. sativa</i> |           |           |        |
|-------------|------------------|-------------------------|------------------------|------------------------|---------------------|------------------|-----------|-----------|--------|
|             |                  | iML_denovo <sup>b</sup> | ML_denovo <sup>c</sup> | TH_denovo <sup>d</sup> | ML_ref <sup>e</sup> | iML_denovo       | ML_denovo | TH_denovo | ML_ref |
| 35bp        | 4                | 0.9172                  | 0.9140                 | 0.8324                 | 0.8148              | 0.9131           | 0.9070    | 0.8620    | 0.8001 |
|             | 8                | 0.5330                  | 0.5296                 | 0.5413                 | 0.3486              | 0.5753           | 0.5679    | 0.6226    | 0.3222 |
|             | 12               | 0.2663                  | 0.2606                 | 0.4093                 | 0.1348              | 0.3220           | 0.3128    | 0.5158    | 0.1033 |
|             | 16               | 0.1525                  | 0.1472                 | 0.3395                 | 0.0491              | 0.2380           | 0.2282    | 0.4588    | 0.0236 |
|             | 20               | 0.1203                  | 0.1119                 | 0.2991                 | 0.0304              | 0.1985           | 0.1847    | 0.4138    | 0.0026 |
|             | 24               | 0.1076                  | 0.0989                 | 0.2661                 | 0.0251              | 0.1877           | 0.1725    | 0.4013    | 0.0057 |
|             | 28               | 0.0929                  | 0.0840                 | 0.2450                 | 0.0220              | 0.1866           | 0.1699    | 0.3795    | 0.0015 |
|             | 32               | 0.0932                  | 0.0823                 | 0.2243                 | 0.0221              | 0.1831           | 0.1447    | 0.3586    | 0.0039 |
|             | 36               | 0.0956                  | 0.0843                 | 0.2048                 | 0.0235              | 0.1921           | 0.1419    | 0.3470    | 0.0020 |
|             | 40               | 0.0955                  | 0.0838                 | 0.1909                 | 0.0257              | 0.1839           | 0.1238    | 0.3343    | 0.0010 |
| 50bp        | 4                | 0.9391                  | 0.9375                 | 0.8275                 | 0.8406              | 0.9384           | 0.9349    | 0.8499    | 1.0000 |
|             | 8                | 0.5775                  | 0.5740                 | 0.5369                 | 0.3802              | 0.6105           | 0.6049    | 0.6022    | 0.3862 |
|             | 12               | 0.2820                  | 0.2783                 | 0.4047                 | 0.1459              | 0.3446           | 0.3378    | 0.4891    | 0.1636 |
|             | 16               | 0.1656                  | 0.1602                 | 0.3303                 | 0.0723              | 0.2349           | 0.2264    | 0.4274    | 0.0866 |
|             | 20               | 0.1205                  | 0.1145                 | 0.2882                 | 0.0449              | 0.1990           | 0.1887    | 0.3923    | 0.0473 |
|             | 24               | 0.1004                  | 0.0934                 | 0.2517                 | 0.0361              | 0.1866           | 0.1760    | 0.3670    | 0.0590 |
|             | 28               | 0.0970                  | 0.0883                 | 0.2296                 | 0.0339              | 0.1741           | 0.1622    | 0.3463    | 0.0229 |
|             | 32               | 0.0947                  | 0.0849                 | 0.2066                 | 0.0309              | 0.1820           | 0.1684    | 0.3240    | 0.0644 |
|             | 36               | 0.0899                  | 0.0820                 | 0.1874                 | 0.0287              | 0.1737           | 0.1602    | 0.3131    | 0.0535 |
|             | 40               | 0.0904                  | 0.0823                 | 0.1798                 | 0.0299              | 0.1751           | 0.1606    | 0.2994    | 0.0471 |
| 100bp       | 4                | 0.9805                  | 0.9796                 | 0.9719                 | 0.9069              | 0.9788           | 0.9776    | 0.9893    | 0.9030 |
|             | 8                | 0.7910                  | 0.7891                 | 0.7859                 | 0.5584              | 0.7972           | 0.7946    | 0.7773    | 0.5530 |
|             | 12               | 0.5238                  | 0.5206                 | 0.5354                 | 0.3129              | 0.5423           | 0.5380    | 0.5657    | 0.3019 |
|             | 16               | 0.3205                  | 0.3166                 | 0.3961                 | 0.2010              | 0.3528           | 0.3481    | 0.4279    | 0.2046 |
|             | 20               | 0.2073                  | 0.2024                 | 0.2973                 | 0.1608              | 0.2468           | 0.2403    | 0.3578    | 0.1959 |
|             | 24               | 0.1428                  | 0.1376                 | 0.2686                 | 0.1332              | 0.1996           | 0.1926    | 0.3368    | 0.1295 |
|             | 28               | 0.1278                  | 0.1220                 | 0.2434                 | 0.1235              | 0.1837           | 0.1762    | 0.3098    | 0.1340 |
|             | 32               | 0.1224                  | 0.1159                 | 0.2264                 | 0.1123              | 0.1772           | 0.1683    | 0.2928    | 0.1112 |
|             | 36               | 0.1277                  | 0.1213                 | 0.2059                 | 0.1114              | 0.1752           | 0.1666    | 0.2750    | 0.1067 |
|             | 40               | 0.1188                  | 0.1123                 | 0.1974                 | 0.1107              | 0.1738           | 0.1657    | 0.2640    | 0.1066 |

<sup>a</sup> Coverage of the simulated sequencing;

<sup>b</sup> iML\_denovo, *de novo* SNP calling using the iML algorithm.

<sup>c</sup> ML\_denovo, *de novo* SNP calling using the ML algorithm;

<sup>d</sup> TH\_denovo, *de novo* SNP calling using the threshold approach;

<sup>e</sup> ML\_ref, reference-based SNP calling using the ML algorithm;