Decoding cell lineage from acquired mutations using arbitrary deep sequencing

Cheryl A Carlson, Arnold Kas, Robert Kirkwood, Laura E Hays, Bradley D Preston, Stephen J Salipante & Marshall S Horwitz

| Supplementary Figure 1 | Arbitrary PCR of samples, showing consistency of amplification |
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Note: Supplementary Table 2 and Supplementary Software are available on the Nature Methods website.
Supplementary Figure 1

Consistency of arbitrary PCR across samples. Samples 1-15 are separated on 1.5% agarose TBE gel with ethidium bromide staining following arbitrary PCR. Flanked by molecular weight markers (M).
Supplementary Figure 2

Arbitrary PCR under limiting template dilution. 50 ng of DNA (lane 2) was serially diluted by two-fold. The amount of DNA was calculated to correspond to the equivalent quantity expected to be present in the indicated number of cells and then used as template for arbitrary PCR. Products are resolved under the same conditions reported for Supplementary Figure 1.
Supplementary Figure 3

Genome browser view, whole chromosome level. Figure is equivalent to Fig. 3, except that tracks for all 15 samples are shown.
Supplementary Figure 4

Neighbor-joining phylogenetic reconstructions. (a) Reconstruction of 15-node tree. (b) Reconstruction of terminal-node only tree. Contrast trees with Bayesian phylogenetic reconstructions shown in Fig. 1b and 1d, respectively.
Distribution of mutations consistent with known tree. At each bifurcation, we report the number of mutations supporting the known phylogenetic relationship.
**Supplementary Figure 6**

| quality | consistent | not-consistent | total | false positive | true positive |
|---------|-------------|----------------|-------|----------------|---------------|
| 5       | 1036        | 859            | 1895  | 0.829150579    | 1             |
| 10      | 827         | 605            | 1432  | 0.583976834    | 0.798262548   |
| 15      | 695         | 449            | 1144  | 0.433397683    | 0.670849421   |
| 20      | 626         | 375            | 1001  | 0.361969112    | 0.604247104   |
| 25      | 556         | 313            | 869   | 0.302123552    | 0.536679537   |
| 30      | 491         | 262            | 753   | 0.252895753    | 0.473938224   |
| 35      | 443         | 224            | 667   | 0.216216216    | 0.427606178   |
| 40      | 400         | 197            | 597   | 0.19015444     | 0.386100386   |
| 45      | 367         | 175            | 542   | 0.168918919    | 0.354247104   |
| 50      | 333         | 152            | 485   | 0.146718147    | 0.321428571   |

**ROC analysis**

ROC analysis performed on 15-node dataset, with read depth cutoff ≥15×. Since the true positive rate is actually unknown, we assumed that it was equal to the value of consistent mutations (1036) identified at the lowest evaluated Phred quality score (5).

Receiver operating characteristic (ROC) analysis of mutational detection parameters. ROC analysis performed on 15-node dataset, with read depth cutoff ≥15×. Since the true positive rate is actually unknown, we assumed that it was equal to the value of consistent mutations (1036) identified at the lowest evaluated Phred quality score (5).
Supplementary Table 1 – Pearson Correlation Coefficients (pairwise comparison between each sample)

| Sample | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1      | 1.00| 0.98| 0.98| 0.98| 0.99| 0.98| 0.96| 0.98| 0.97| 0.95| 0.98| 0.95| 0.98| 0.95| 0.98|
| 2      | 0.98| 1.00| 0.99| 0.98| 0.98| 0.96| 0.98| 0.99| 0.97| 0.96| 0.97| 0.95| 0.97| 0.95| 0.97|
| 3      | 0.99| 0.98| 1.00| 0.98| 0.99| 0.98| 0.99| 0.99| 0.98| 0.97| 0.98| 0.96| 0.98| 0.95| 0.98|
| 4      | 0.98| 0.99| 0.98| 1.00| 0.98| 0.98| 0.99| 0.96| 0.98| 0.99| 0.98| 0.97| 0.97| 0.97| 0.97|
| 5      | 0.98| 0.98| 0.99| 0.97| 1.00| 0.98| 0.99| 0.99| 0.99| 0.97| 0.93| 0.96| 0.93| 0.96| 0.96|
| 6      | 0.99| 0.98| 0.99| 0.98| 0.98| 1.00| 0.98| 0.99| 0.98| 0.97| 0.96| 0.98| 0.94| 0.98| 0.98|
| 7      | 0.99| 0.98| 1.00| 0.98| 0.99| 0.99| 1.00| 0.97| 0.99| 0.99| 0.95| 0.96| 0.96| 0.96| 0.97|
| 8      | 0.98| 0.99| 0.99| 0.99| 0.99| 0.98| 0.96| 1.00| 0.99| 0.99| 0.95| 0.96| 0.94| 0.96| 0.97|
| 9      | 0.96| 0.96| 0.97| 0.96| 0.99| 0.95| 0.97| 0.97| 1.00| 0.98| 0.92| 0.93| 0.92| 0.94| 0.97|
| 10     | 0.98| 0.99| 0.99| 0.98| 0.99| 0.97| 0.99| 0.99| 0.98| 1.00| 0.99| 0.95| 0.96| 0.95| 0.97|
| 11     | 0.97| 0.99| 0.98| 0.99| 0.97| 0.97| 0.98| 0.99| 0.96| 0.99| 1.00| 0.97| 0.97| 0.96| 0.98|
| 12     | 0.95| 0.96| 0.95| 0.98| 0.93| 0.96| 0.95| 0.95| 0.92| 0.95| 0.97| 1.00| 0.98| 0.98| 0.98|
| 13     | 0.98| 0.97| 0.98| 0.98| 0.96| 0.98| 0.96| 0.98| 0.93| 0.96| 0.97| 0.98| 1.00| 0.97| 0.99|
| 14     | 0.95| 0.95| 0.95| 0.97| 0.93| 0.94| 0.95| 0.94| 0.92| 0.95| 0.96| 0.98| 0.97| 1.00| 0.96|
| 15     | 0.98| 0.97| 0.98| 0.99| 0.96| 0.98| 0.97| 0.94| 0.97| 0.98| 0.98| 0.99| 0.96| 1.00| 0.96|
**Supplementary Table 3 – Mutation Rate of Mlh1Δ/Δ Pold1Δ/+ Mouse Embryo Fibroblasts Determined by Fluctuation Analyses**

|                         | Experiment 1 | Experiment 2 | Combined  |
|-------------------------|--------------|--------------|-----------|
| Replicas                | 12           | 12           | 24        |
| Replicas with mutants   | 12           | 12           | 24        |
| Mutants per replica     |              |              |           |
| Mean ± SD               | 692 ± 807    | 144 ± 234    | 418 ± 645 |
| Range                   | 13 – 2076    | 12 – 706     | 12 – 2076 |
| Maximum likelihood analysis |             |              |           |
| Plating efficiency      | 10%          | 40%          | 25%       |
| Mutations (m)           | 66 (46 – 88) | 19 (13 – 25) | 29 (23 – 36) |
| Cells per replica (N_t) | 6.4 × 10^5   | 7.5 × 10^5   | 7.0 × 10^5 |
| Mutation rate           |              |              |           |
| per cell division (× 10^{-5}) | 10.3 (7.2 – 13.7) | 2.5 (1.8 – 3.3) | **4.2 (3.3 – 5.1)** |
| per base pair / cell division (× 10^{-6}) | 3.4 (2.4 – 4.6) | 0.8 (0.6 – 1.1) | **1.4 (1.1 – 1.7)** |
Supplementary Table 4 – Generational Resolution as a Function of Mutation Rate and Target Size

| target_size | Num_generations | target_size | Num_generations | target_size | Num_generations | target_size | Num_generations | target_size | Num_generations | target_size | Num_generations |
|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|
| 16000000    | 22.3            | 16000000    | 11.2            | 16000000    | 5.6            | 16000000    | 3.7            | 16000000    | 2.8            | 16000000    | 2.2            |
| 15000000    | 23.8            | 15000000    | 11.9            | 15000000    | 6.0            | 15000000    | 4.0            | 15000000    | 3.0            | 15000000    | 2.4            |
| 14000000    | 25.5            | 14000000    | 12.8            | 14000000    | 6.4            | 14000000    | 4.3            | 14000000    | 3.2            | 14000000    | 2.6            |
| 13000000    | 27.5            | 13000000    | 13.7            | 13000000    | 6.9            | 13000000    | 4.6            | 13000000    | 3.4            | 13000000    | 2.7            |
| 12000000    | 29.8            | 12000000    | 14.9            | 12000000    | 7.4            | 12000000    | 5.0            | 12000000    | 3.7            | 12000000    | 3.0            |
| 11000000    | 32.5            | 11000000    | 16.2            | 11000000    | 8.1            | 11000000    | 5.4            | 11000000    | 4.1            | 11000000    | 3.2            |
| 10000000    | 35.7            | 10000000    | 17.9            | 10000000    | 8.9            | 10000000    | 6.0            | 10000000    | 4.5            | 10000000    | 3.6            |
| 9000000     | 39.7            | 9000000     | 19.8            | 9000000     | 9.9            | 9000000     | 6.6            | 9000000     | 5.0            | 9000000     | 4.0            |
| 8000000     | 44.6            | 8000000     | 22.3            | 8000000     | 11.2           | 8000000     | 7.4            | 8000000     | 5.6            | 8000000     | 4.5            |
| 7000000     | 51.0            | 7000000     | 25.5            | 7000000     | 12.8           | 7000000     | 8.5            | 7000000     | 6.4            | 7000000     | 5.1            |
| 6000000     | 59.5            | 6000000     | 29.8            | 6000000     | 14.9           | 6000000     | 9.9            | 6000000     | 7.4            | 6000000     | 6.0            |
| 5000000     | 71.4            | 5000000     | 35.7            | 5000000     | 17.9           | 5000000     | 11.9           | 5000000     | 8.9            | 5000000     | 7.1            |
| 4000000     | 89.3            | 4000000     | 44.6            | 4000000     | 22.3           | 4000000     | 14.9           | 4000000     | 11.2           | 4000000     | 8.9            |
| 3000000     | 119.0           | 3000000     | 59.5            | 3000000     | 29.8           | 3000000     | 19.8           | 3000000     | 14.9           | 3000000     | 11.9           |
| 2000000     | 178.6           | 2000000     | 89.3            | 2000000     | 44.6           | 2000000     | 29.8           | 2000000     | 22.3           | 2000000     | 17.9           |