Supplementary Materials

Supplementary Table 1  Details of samples used in single family-multigeneration genome analysis.

| Sample Number | Birthdate (YY-MM-DD) | Genotype<sup>a</sup> | Sex<sup>b</sup> |
|---------------|----------------------|-----------------------|---------------|
| 1             | 13-02-16             | g/g                   | ♂             |
| 2             | 13-03-26             | +/+                   | ♀             |
| 3             | 13-03-26             | +/+                   | ♀             |
| 4             | 13-06-06             | +/g                   | ♀             |
| 5             | 13-06-06             | +/g                   | ♀             |
| 6             | 13-06-06             | +/g                   | ♀             |
| 7             | 13-06-06             | +/g                   | ♂             |
| 8             | 13-06-06             | +/g                   | ♂             |
| 9             | 13-06-06             | +/g                   | ♂             |
| 10            | 13-06-29             | +/g                   | ♂             |
| 11            | 13-06-29             | +/g                   | ♂             |
| 12            | 13-06-29             | +/g                   | ♂             |
| 13            | 13-08-19             | +/g                   | ♀             |
| 14            | 13-08-05             | +/g                   | ♀             |
| 15            | 13-08-05             | +/g                   | ♂             |
| 16            | 13-08-26             | +/g                   | ♀             |
| 17            | 13-08-05             | +/+                   | ♂             |
| 18            | 13-08-27             | +/g                   | ♀             |
| 19            | 13-08-27             | +/+                   | ♀             |
| 20            | 13-10-07             | +/g                   | ♀             |
| 21            | 13-10-07             | +/+                   | ♀             |
| 22            | 13-10-07             | +/g                   | ♀             |
| 23            | 13-12-04             | +/?                   | ♀             |
| 24            | 13-12-04             | +/?                   | ♀             |
| 25            | 13-12-10             | +/?                   | ♀             |
| 26            | 13-12-10             | +/?                   | ♀             |
| 27            | 13-12-10             | +/?                   | ♀             |
| 28            | 13-12-19             | +/+                   | ♀             |
| 29            | 13-12-19             | +/+                   | ♀             |
| 30            | 13-12-19             | +/+                   | ♀             |
| 31            | 13-12-19             | +/+                   | ♀             |

<sup>a</sup> Here, g/g (grt/grt) represents missense mutation at the grt locus of chromosome 5 in the tyrosylprotein sulfotransferase 2 (Tpst2) gene and results in dwarf phenotype; +/+ is wild type; +/g (+/grt) is heterologous state of mutation; +/? means either +/g or +/+ (in either case the phenotype is same). As this mutation is autosomal recessive, only sample 1 is different phenotypically i.e. dwarf.

<sup>b</sup> ♂ refers to male and ♀ refers to female.
**Supplementary Table 2** Genome distance ($d_G$) matrix obtained from pfM 3-amplified genome profiles corresponding to the Figure 1B tree.

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | 0   | 0.025 | 0.022 | 0.016 | 0.017 | 0.014 | 0.017 | 0.018 | 0.043 | 0.027 | 0.030 | 0.025 | 0.028 | 0.040 | 0.023 | 0.031 |
| 2  | 0   | 0.017 | 0.025 | 0.021 | 0.016 | 0.019 | 0.018 | 0.044 | 0.034 | 0.038 | 0.023 | 0.019 | 0.030 | 0.018 | 0.022 |
| 3  | 0   | 0.017 | 0.018 | 0.017 | 0.019 | 0.017 | 0.017 | 0.051 | 0.040 | 0.042 | 0.025 | 0.024 | 0.031 | 0.019 | 0.021 |
| 4  | 0   | 0.019 | 0.016 | 0.016 | 0.019 | 0.060 | 0.048 | 0.049 | 0.019 | 0.026 | 0.040 | 0.025 | 0.033 |
| 5  | 0   | 0.013 | 0.016 | 0.019 | 0.038 | 0.029 | 0.03 | 0.020 | 0.019 | 0.035 | 0.018 | 0.026 |
| 6  | 0   | 0.010 | 0.019 | 0.046 | 0.034 | 0.036 | 0.017 | 0.016 | 0.030 | 0.017 | 0.024 |
| 7  | 0   | 0.023 | 0.05 | 0.039 | 0.039 | 0.020 | 0.019 | 0.030 | 0.019 | 0.026 |
| 8  | 0   | 0.041 | 0.032 | 0.029 | 0.020 | 0.026 | 0.036 | 0.023 | 0.027 |
| 9  | 0   | 0.015 | 0.025 | 0.047 | 0.047 | 0.038 | 0.045 | 0.044 |
| 10 | 0   | 0.020 | 0.042 | 0.039 | 0.037 | 0.036 | 0.037 |
| 11 | 0   | 0.043 | 0.040 | 0.04 | 0.043 | 0.038 |
| 12 | 0   | 0.014 | 0.030 | 0.017 | 0.022 |
| 13 | 0   | 0.020 | 0.010 | 0.014 |
| 14 | 0   | 0.022 | 0.012 |
| 15 | 0   | 0.012 |
| 16 | 0   |       |

| 0.020 ≥ | 0.020–0.025 | 0.025–0.030 | 0.030–0.035 | 0.035–0.040 | 0.040 ≤ |

Sharma et al.: Genome profiling-based familial clustering of mice
**Supplementary Table 3**  Genome distance \( (d_G) \) matrix obtained from pfM 12-amplified genome profiles corresponding to the Figure 1C tree.

|    | 1   | 2     | 3     | 4     | 5     | 6     | 7     | 8    | 9    | 10   | 11   | 12    | 13    | 14     | 15    | 16    |
|----|-----|-------|-------|-------|-------|-------|-------|------|------|------|------|-------|-------|--------|-------|-------|
| 1  | 0   | 0.024 | 0.021 | 0.030 | 0.044 | 0.019 | 0.024 | 0.032 | 0.034 | 0.038 | 0.047 | 0.03   | 0.029  | 0.024  | 0.030 | 0.036 |
| 2  | 0   | 0.018 | 0.022 | 0.049 | 0.021 | 0.021 | 0.028 | 0.026 | 0.031 | 0.036 | 0.043 | 0.032  | 0.029  | 0.028  | 0.032 |       |
| 3  | 0   | 0.019 | 0.044 | 0.021 | 0.016 | 0.027 | 0.028 | 0.027 | 0.037 | 0.029 | 0.026 | 0.021  | 0.023  | 0.026  |       |       |
| 4  | 0   | 0.041 | 0.023 | 0.024 | 0.024 | 0.033 | 0.032 | 0.040 | 0.039 | 0.032 | 0.032 | 0.024  | 0.024  | 0.030  |       |       |
| 5  | 0   | 0.051 | 0.030 | 0.040 | 0.054 | 0.054 | 0.062 | 0.052 | 0.041 | 0.038 | 0.040 | 0.044  |       |       |       |       |
| 6  | 0   | 0.025 | 0.025 | 0.029 | 0.028 | 0.033 | 0.040 | 0.034 | 0.029 | 0.026 | 0.030 |       |       |       |       |       |
| 7  | 0   | 0.026 | 0.030 | 0.029 | 0.038 | 0.028 | 0.022 | 0.019 | 0.022 | 0.030 |       |       |       |       |       |       |
| 8  | 0   | 0.026 | 0.028 | 0.033 | 0.037 | 0.031 | 0.029 | 0.022 | 0.024 |       |       |       |       |       |       |       |
| 9  | 0   | 0.014 | 0.017 | 0.026 | 0.033 | 0.029 | 0.023 | 0.030 |       |       |       |       |       |       |       |       |
| 10 | 0   | 0.016 | 0.023 | 0.031 | 0.027 | 0.021 | 0.027 |       |       |       |       |       |       |       |       |       |
| 11 | 0   | 0.036 | 0.037 | 0.040 | 0.023 | 0.032 |       |       |       |       |       |       |       |       |       |       |
| 12 | 0   | 0.025 | 0.016 | 0.027 | 0.025 |       |       |       |       |       |       |       |       |       |       |       |
| 13 | 0   | 0.017 | 0.019 | 0.023 |       |       |       |       |       |       |       |       |       |       |       |       |
| 14 | 0   | 0.020 | 0.02 |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 15 | 0   | 0.019 |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 16 | 0   |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |

Color key:
- **0.020 ≥**
- **0.020–0.025**
- **0.025–0.030**
- **0.030–0.035**
- **0.035–0.040**
- **0.040 ≤**
**Supplementary Table 4**  Genome distance ($d_G$) matrix obtained from pfM 19-amplified genome profiles corresponding to the Figure 1D tree.

|    | 1     | 2   | 3   | 4 | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  |
|----|-------|-----|-----|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | 0     | 0.036 | 0.02 | 0.028 | 0.023 | 0.022 | 0.018 | 0.032 | 0.050 | 0.045 | 0.030 | 0.027 | 0.032 | 0.039 | 0.027 |
| 2  | 0     | 0.030 | 0.025 | 0.038 | 0.041 | 0.04 | 0.037 | 0.054 | 0.04 | 0.054 | 0.036 | 0.037 | 0.045 | 0.049 | 0.041 |
| 3  | 0     | 0.019 | 0.029 | 0.025 | 0.024 | 0.029 | 0.049 | 0.045 | 0.051 | 0.028 | 0.027 | 0.031 | 0.035 | 0.029 |
| 4  | 0     | 0.026 | 0.028 | 0.023 | 0.033 | 0.053 | 0.05 | 0.058 | 0.04 | 0.031 | 0.033 | 0.045 | 0.038 |
| 5  | 0     | 0.024 | 0.027 | 0.033 | 0.053 | 0.054 | 0.049 | 0.04 | 0.039 | 0.039 | 0.044 | 0.035 |
| 6  | 0     | 0.025 | 0.031 | 0.048 | 0.05 | 0.05 | 0.034 | 0.032 | 0.029 | 0.04 | 0.024 |
| 7  | 0     | 0.026 | 0.041 | 0.049 | 0.048 | 0.036 | 0.025 | 0.032 | 0.041 | 0.032 |
| 8  | 0     | 0.055 | 0.039 | 0.05 | 0.04 | 0.028 | 0.038 | 0.051 | 0.034 |
| 9  | 0     | 0.028 | 0.022 | 0.037 | 0.043 | 0.037 | 0.04 | 0.04 |
| 10 | 0     | 0.039 | 0.038 | 0.037 | 0.04 | 0.038 | 0.044 |
| 11 | 0     | 0.046 | 0.047 | 0.049 | 0.05 | 0.048 |
| 12 | 0     | 0.026 | 0.022 | 0.026 | 0.024 |
| 13 | 0     | 0.032 | 0.034 | 0.029 |
| 14 | 0     | 0.024 | 0.027 |
| 15 | 0     | 0.033 |
| 16 | 0     |

| 0.020 ≥ | 0.020–0.025 | 0.025–0.030 | 0.030–0.035 | 0.035–0.040 | 0.040 ≤ |
Supplementary Table 5  Average genome distance \((d_G)\) matrix obtained from combined results of three different probes experiments corresponding to the Figure 1E tree. ± Standard deviation values of 3 independent experiments.

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | 0   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 2  | 0.028 ± 0.007 | 0   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3  | 0.021 ± 0.001 | 0.022 ± 0.007 | 0   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4  | 0.025 ± 0.007 | 0.024 ± 0.002 | 0.018 ± 0.006 | 0   |     |     |     |     |     |     |     |     |     |     |     |     |
| 5  | 0.028 ± 0.014 | 0.036 ± 0.014 | 0.031 ± 0.013 | 0.029 ± 0.011 | 0   |     |     |     |     |     |     |     |     |     |     |     |
| 6  | 0.018 ± 0.004 | 0.026 ± 0.013 | 0.021 ± 0.004 | 0.022 ± 0.006 | 0.029 ± 0.002 | 0   |     |     |     |     |     |     |     |     |     |     |
| 7  | 0.020 ± 0.004 | 0.027 ± 0.011 | 0.020 ± 0.004 | 0.021 ± 0.004 | 0.024 ± 0.007 | 0.020 ± 0.007 | 0   |     |     |     |     |     |     |     |     |     |
| 8  | 0.028 ± 0.008 | 0.027 ± 0.01 | 0.025 ± 0.007 | 0.030 ± 0.01 | 0.025 ± 0.006 | 0.025 ± 0.001 | 0.025 ± 0.001 | 0   |     |     |     |     |     |     |     |     |
| 9  | 0.042 ± 0.014 | 0.043 ± 0.01 | 0.049 ± 0.014 | 0.049 ± 0.014 | 0.041 ± 0.004 | 0.040 ± 0.007 | 0.041 ± 0.007 | 0.014 | 0   |     |     |     |     |     |     |     |
| 10 | 0.037 ± 0.009 | 0.038 ± 0.009 | 0.043 ± 0.01 | 0.046 ± 0.014 | 0.037 ± 0.011 | 0.039 ± 0.010 | 0.033 ± 0.010 | 0.019 ± 0.005 | 0.008 | 0   |     |     |     |     |     |     |
| 11 | 0.040 ± 0.009 | 0.043 ± 0.007 | 0.049 ± 0.009 | 0.047 ± 0.016 | 0.040 ± 0.009 | 0.042 ± 0.006 | 0.037 ± 0.011 | 0.021 ± 0.004 | 0.025 ± 0.012 | 0   |     |     |     |     |     |     |
| 12 | 0.029 ± 0.003 | 0.034 ± 0.010 | 0.028 ± 0.002 | 0.033 ± 0.011 | 0.037 ± 0.016 | 0.030 ± 0.011 | 0.028 ± 0.008 | 0.033 ± 0.010 | 0.037 ± 0.010 | 0.034 ± 0.001 | 0.041 ± 0.005 | 0   |     |     |     |
| 13 | 0.028 ± 0.009 | 0.029 ± 0.009 | 0.026 ± 0.001 | 0.030 ± 0.003 | 0.033 ± 0.012 | 0.027 ± 0.010 | 0.022 ± 0.003 | 0.029 ± 0.002 | 0.041 ± 0.007 | 0.036 ± 0.004 | 0.041 ± 0.005 | 0.022 ± 0.007 | 0   |     |     |
| 14 | 0.031 ± 0.008 | 0.035 ± 0.009 | 0.027 ± 0.006 | 0.036 ± 0.004 | 0.037 ± 0.001 | 0.029 ± 0.007 | 0.027 ± 0.007 | 0.034 ± 0.004 | 0.035 ± 0.005 | 0.035 ± 0.008 | 0.043 ± 0.005 | 0.023 ± 0.007 | 0.023 ± 0.008 | 0   |     |
| 15 | 0.031 ± 0.008 | 0.032 ± 0.016 | 0.026 ± 0.008 | 0.031 ± 0.013 | 0.034 ± 0.012 | 0.028 ± 0.011 | 0.027 ± 0.011 | 0.032 ± 0.016 | 0.036 ± 0.009 | 0.039 ± 0.014 | 0.039 ± 0.005 | 0.021 ± 0.012 | 0.022 ± 0.002 | 0   |     |
| 16 | 0.031 ± 0.004 | 0.032 ± 0.009 | 0.025 ± 0.004 | 0.034 ± 0.004 | 0.035 ± 0.009 | 0.026 ± 0.003 | 0.029 ± 0.003 | 0.029 ± 0.005 | 0.040 ± 0.001 | 0.036 ± 0.008 | 0.039 ± 0.001 | 0.024 ± 0.007 | 0.022 ± 0.007 | 0.020 ± 0.010 | 0   |     |

0.020 ≥ 0.020–0.025 0.025–0.030 0.030–0.035 0.035–0.040 0.040 ≤
Supplementary Figure 1  Steps involved in genome profiling (GP). The first step of GP is amplification of DNA samples using a single universal primer by random PCR mode. It involves relaxed-mode binding (bulge or mismatch containing) of one primer and template DNA at a low annealing temperature. Acquired random PCR products are subjected to micro-temperature gradient gel electrophoresis (µTGGE) and each transition point in DNA bands (featuring point) is further processed for normalization using computer and converted to spiddos. Spiddos of two genome profiles are used for obtaining pattern similarity score (PaSS). Genome distance ($d_G$) value is defined to be $1-PaSS$. 

\[
P_{i}^{(1)} - P_{i}^{(2)}
\]
Supplementary Figure 2  Types of spiddos pairing. (A) Overlapped spiddos maps showing close placement of corresponding spiddos pairs by deterministic or absolutely accurate spiddos assignment. When two genomes are close enough, then both sets of spiddos become overlapping for each pair of corresponding spiddos. (B) The stochastic spiddos assignment usually results in non-overlapping spiddos in the superimposed map of two genomes which are distantly related (for details see Supplementary Protocol).
Supplementary Figure 3  Basic data for samples used for ccgf analysis. Genome profiles of 16 mouse samples amplified by the probe pfM 19. Red arrow indicates the band chosen as commonly conserved genetic fragment (ccgf). Faint ccgf bands of samples 10, 11, and 14 are shown in magnified form (red frame).
Supplementary Figure 4  18S rDNA sequencing analysis of three mouse families. Referring to the *Mus musculus* 18S ribosomal RNA (Rn18s) (NR_003278.3) sequence, specific primers 18SF (forward primer; 5′-GGAACTGAGGCCATGATTAAGA-3′) and 18SR (reverse primer; 5′-ATCGCTCCACCAACTAAGAAC-3′) were designed. PCR and sequencing of 18S rDNAs were performed similarly to the case of ccgf. (A) 18S rDNA sequences for 16 mouse samples were aligned by MUSCLE. (B) Phylogenetic tree for 18S rDNA sequence data was drawn by neighbor-joining method using MEGA 5.1 software.
**Supplementary Figure 5** Allelic bands in genome profiles. In case of heterozygous alleles, a single DNA band in GP shows two different transitions. Here, four examples of such case are shown. Magnified images of regions of allelic bands are shown in insets with their transitions indicated by red arrow. Inversely, the other bands without red arrow show the presence of homozygous alleles, and are composed of only a single band with a single transition. For both cases of homo- and heterozygosity, only a single *spiddos* (*i.e.*, the transition occurring at the higher temperature) is allotted for those alleles in case of heterozygosity and in case of homozygosity, naturally, the overlapped unique one.
**Supplementary Protocol:**

The whole process of featuring points assignment, normalization, and PaSS (pattern similarity score) calculation was performed following the algorithm realized in the microTGGE software:

1. First of all, the initial melting point of internal references are selected and then sample featuring points are extracted by selecting double-stranded to single-stranded transitioning points of sample bands. Generally, only darker bands (~10 bands) are selected and faint bands can be avoided due to obscurity of their transition points. The numbering of featuring points in each profile is done in the descending order of their intensity (from darker to lighter bands). If allelic bands (visible as two transitions from one band) are appearing, then only the transition occurring at higher temperature (rightmost one) should be selected (Supplementary Figure 4).

2. The position of each featuring point is converted to coordinates X (temperature) and Y (mobility) normalized with the guide of internal references, of which coordinates are known in advance. After normalization, each featuring point is called as “spiddos”.

3. The algorithm for assigning the corresponding spiddos in Eq. 1 is as follows:

   A computer software arbitrarily chooses a pair of spiddos from genome 1 and genome 2 spiddos. Then, another pair of spiddos is assigned from the remaining ones. This process is repeated until all of spiddos for Genome 1 [which has a less or equal number of spiddos than genome 2 (i.e., genomes 1 and 2 have n and m (n ≤ m) spiddos)] can be paired (Supplementary Figure 2). Then PaSS value is obtained following Eq. [1].

   A set of spiddos of two genomes are compared and reduced to PaSS (pattern similarity score) as follows

   \[
   PaSS = 1 - \frac{1}{n} \sum_{i=1}^{n} \frac{\left| \hat{P}_i^{(1)} - \hat{P}_i^{(2)} \right|}{\hat{P}_i^{(1)} + \hat{P}_i^{(2)}} \tag{1}
   \]

   Here \( \hat{P}_i^{(1)} \) and \( \hat{P}_i^{(2)} \) represent the normalized positional vectors (function of temperature and mobility) for spiddos \( P_i^{(1)} \) and \( P_i^{(2)} \) from genome (1) and genome (2) respectively, while i
designates serial number of spiddos. The point at zero mobility and zero Kelvin temperature acts as the origin of vector space for the positional vectors. PaSS will be 1 for a complete match in two sets of spiddos. In general,

\[ 0 \leq PaSS \leq 1. \]  \hspace{1cm} [2]

A measure of genome distance \( d_G \) can be derived from PaSS:

\[ d_G = 1 - PaSS \quad (0 \leq d_G \leq 1) \]  \hspace{1cm} [3]

Higher the \( d_G \) value, higher the distance between two genomes. In other words, \( d_G \) value is 0 for a perfect match and near 0 for members of the same species.

In the next step, the initial pair is changed and then the similar pairing process is iterated to the final pairing, again, PaSS value is obtained for this set of pairing. After exhaustive generation of a set of pairing and calculation of PaSS, the set of pairs which provides the maximum PaSS value is defined to the representative set of pairs for the relevant genomes. The maximum PaSS value is assigned to be PaSS for these genomes. This process is termed to be stochastic one while there is a non-stochastic one which manually pairs by experimenters (usually for genomes within the same species or so). In this study the stochastic process for pairing spiddos was employed. When two genomes are sufficiently close and have the same and close proximity of spiddos in an overlapping spiddos map, then corresponding spiddos can be assigned deterministically without a probabilistic nature (Supplementary Figure 2).