Development of Novel Microsatellite DNA Markers by Cross-Amplification and Analysis of Genetic Variation in Gerbils

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Abstract

The objectives of this study are to establish microsatellite loci for the Mongolian gerbil based on mouse microsatellite DNA sequences and to investigate genetic variation in the laboratory gerbil (Capital Medical University, CMU) and 2 wild gerbil populations (from Yin Chuan city [YIN] and the Hohehot Municipality [HOH]). In total, 536 mouse microsatellite markers were chosen to identify polymorphic dinucleotide repeat loci in the gerbil by cross-amplification. Of these markers, 313 (58.39%) have been discretely amplified from the CMU laboratory gerbil and been sequenced. Of the 313 sequenced markers, 130 were confirmed as simple sequence repeat (SSR) loci in the gerbil. In total, 6 of those newly identified loci plus 6 identified in previous reports were used to estimate the genetic polymorphism for 30 laboratory gerbils and 54 wild gerbils (27 each of the HOH and YIN groups). A total of 29 alleles were observed in the 3 populations, and 11 of 12 loci (91.67%) are polymorphic markers. Nei’s standard genetic distances of 0.0592 (CMU vs. HOH) and 0.1033 (CMU vs. YIN) were observed. The averages of observed versus expected heterozygosity are 0.5231/0.4008, 0.5051/0.3882, and 0.4825/0.3665 for the YIN, HOH, and CMU populations, respectively. These results show that cross-amplification using mouse microsatellite primers is an efficient way to identify gerbil SSR loci. By using these 12 selected markers, we have demonstrated that genetic variation level within the CMU population is higher than that has been reported previously and are comparable with the levels found in 2 wild populations.

Key words: cross-amplification, genetic variation, microsatellite DNA, Mongolian gerbil

The Mongolian gerbil (Meriones unguiculatus) is an important laboratory animal and has been widely studied as an animal model of several human diseases (Levine and Sohn 1969; Garcia 1984; Ginsberg and Busto 1989; Hossmann 1998). Its history as a laboratory animal can be traced back to 1935 when 20 pairs of gerbils were captured in eastern Mongolia and imported to Japan. From there, some descendant pairs were imported in the United State in 1954 and formed the breeding stock for laboratories. In China, there are 2 stocks of laboratory gerbils, located at the Zhejiang Laboratory Animal Centre (Ding et al. 2008) and in Capital Medical University (CMU). The CMU colony used in present study originated from approximately 400 pairs of wild gerbils captured in 1986 from the district of Hohehot Municipality, China, and has been cultivated for 23 years. Currently, the gerbil is particularly useful for studying strokes due to the lack of communicating arteries between the posterior vertebral arteries (through the basilar artery) and the 2 internal carotid arteries anteriorly. Without significant collateral flow from the vertebral blood supply to the forebrain, a transient bilateral common carotid artery occlusion induces consistent ischemic injury to selective regions of the hippocampus (Kirino 1982; Kirino and Sano 1984; Mitani and Kataoka 1991). The relative simplicity of carotid occlusion allows this model to be useful in the study of brain ischemic mechanisms (Hoffman and Boast 1995; Kuhmonen et al. 1997; Dowden and Corbett 1999; Tanaka et al. 2002; Shughrue and Merchenthaler 2003; Colangelo
et al. 2004; Li et al. 2004; Beck et al. 2007). Since 1996, *Helicobacter pylori*-infected Mongolian gerbils (Hirayama et al. 1996) have proved very useful in mimicking of human *Helicobacter pylori* infection, for analyses of underlying processes, and for studying gastritis and gastric cancer (Shibata et al. 2006; Chiba et al. 2008). The gerbil has also been used to study nutrition (Deming et al. 2000; Molldrem and Tanumihardjo 2004), aging (Chael 1986; Floyd and Carney 1991), epilepsy (Fujisawa et al. 2003), parasites (Arguello-Garcia and Ortega-Pieres 1997; Kato et al. 2005; Conchedda et al. 2006; Tantawi wattananon et al. 2006), and viral infections (Song 1999; Watanabe et al. 2001). Most recently, scientists have found that the gerbil is also a suitable model for studies on neoplastic prostate lesions. The gerbil prostate has compact lobes, somewhat similar to the human prostate, unlike rats and mice, which have distinct lobes. Therefore, it has been used as a prostate disease model (Cordeiro et al. 2008; Pereira et al. 2009). However, genetic studies of biomedical development using this model have been hampered due to the lack of an efficient gene mapping system in the gerbil.

Microsatellites, also known as simple sequence repeats (SSRs), are polymorphic loci that consist of short tandem repeated motifs of 1–6 bases and are widely used as genetic markers for genome mapping, population genetic studies, genetic dissection of complex traits, analysis of related species, identification of genotypes within pedigrees, and determination of paternity within a single population. They are especially useful in the study of genetic causes of inherited diseases due to their ubiquitous existence in the genome, frequent association with specific loci, codominance in inheritance, and high polymorphism and reproducibility (Jarne and Lagoda 1996). Microsatellites can be identified by polymerase chain reaction (PCR) amplification of the repeated alleles, using unique primer sequences from the flanking regions. Many genomic studies have shown that SSR primer sequences are often conserved across related species, which make them especially useful in coamplification of cross species genetic markers (deGortari et al. 1997; Navani et al. 2002).

More than 6000 microsatellite markers have been reported for the mouse, a laboratory animal closely related to the gerbil. However, few microsatellites have been isolated in the gerbil, and little genomic mapping effort has been devoted to this important laboratory animal (Neumann et al. 2001). The development of genetic markers for this species is urgently required to evaluate the prognostics of genetic mapping experiments and parental studies. The development of microsatellite markers for genetic mapping of the gerbil genome will be helpful for our studies of human ischemic brain damage using this model animal and will make these studies more practical and meaningful. We have isolated 130 microsatellite loci in this study from CMU laboratory gerbils that have been bred for 23 years in the animal facility of the CMU, Beijing, China. We have used 6 of these 98 as well as 6 previously reported loci to evaluate genetic variation within and between 3 different gerbil populations.

Materials and Methods

Gerbils

A total of 55 laboratory Mongolian gerbils, 4 months of age and weighing from 49 to 85 g, and 54 wild gerbils were used in this study. All laboratory animals were from the breeding colony of the Department of Laboratory Animal Science (Capital Medical University, Beijing, China). Only 25 of them were used to select microsatellite loci, and 30 of them were used to evaluate the genetic variation within the laboratory gerbil population. The 54 wild gerbils were captured in 2006 from the districts of Hohehot Municipality (HOH) and Yin Chuan city (YIN), China. Of these animals, 27 were trapped at a location approximately 50 km north of Hohehot Municipality, and 27 were trapped at a location approximately 30 km south of Yin Chuan city. The body weights of these wild animals range from 20 to 75 g, and their ages are unknown.

DNA Extraction and Mouse Microsatellite Markers Selection

In order to develop microsatellite loci in the Mongolian gerbil, about 536 mouse microsatellite molecular markers were manually selected from 20 chromosomes avoiding the Y chromosome. Genomic DNA was extracted from the frozen kidneys of 25 unrelated gerbils (males and females) following the method described in *Molecular Cloning: A Laboratory Manual, 3rd*. In brief, 100 mg of tissue was cut into small pieces that were then placed in 1 ml of sodium chloride Tris EDTA buffer (10 mM Tris–HCl, pH 8.0; 50 mM ethylenediaminetetraacetic acid, pH 8.0; 200 mM NaCl and 0.5% sodium dodecyl sulfate) and digested with 0.02 mg/ml proteinase K at 55 °C for 12 h. The digestion was then phenolchloroform extracted. The DNA was precipitated with ethanol and dissolved in TE buffer. The resulting DNA was quantitated with a Microplate Absorbance Reader (Bio-Rad 680, USA) and further evaluated by agarose gel electrophoresis.

Polymerase Chain Reaction

PCR was performed according to the following steps: 10 pmoles of each primer and 0.05 μg of genomic DNA were added to a total volume of 35 μl. After an initial denaturation step of 3 min at 94 °C, amplification proceeded for 35 cycles of 1 min at 94 °C, 1 min of annealing at a gradient temperature of 50–60 °C or a primer-specific temperature, and extension at 72 °C for 2 min. A final elongation step of 7 min was carried out at 72 °C. Next, 10 μl of the PCR product was loaded on a 2% agarose gel, electrophoresed, and visualized over UV light after ethidium bromide staining. Distinct PCR bands between 80 and 1000 bp were selected and purified for further determination of their sequences. A pair of microsatellite primers was scored positive if the sequence of their amplification product met the definition of microsatellite DNA (Chambers and MacAvoy 2000). Every pair of primers has been tested for the optimal Mg²⁺ concentration and annealing temperature for the amplification conditions.
Tab) and were confirmed as SSR loci (Figure 2). We next (41.53%) contained microsatellite DNA sequences (SupMat total 313 of these PCR products were sequenced and 130 (58.39%) successfully amplified discrete PCR products, of laboratory gerbils. In these reactions, 313 oligo pairs amplification of genomic DNA from a panel of 25 CMU Mouse Genome Informatics (MGI) were tested for Genetic Diversity Selection of Gerbil Microsatellite Loci and Measurement of Results

Genetic Polymorphism

To estimate the level of genetic polymorphism within and between the 3 gerbil populations, 84 samples comprising 30 CMU laboratory gerbils, 27 Hohehot, and 27 Yin Chuan wild gerbils were analyzed using 12 pairs of primers (6 pairs developed in this study and 6 pairs of primers developed by Neumann (see References). The PCR reactions were performed according to the optimal conditions, and the results were monitored by agarose gel electrophoresis. Statistics of allele frequencies, average number of alleles per locus, observed heterozygosity, and expected heterozygosity per population were calculated using Popgene 3.2 software. Deviations from linkage equilibrium between all pairs of loci across the 3 populations and conformation to Hardy–Weinberg equilibrium on a locus-by-locus basis within the populations were also tested using Popgene 3.2 software. The genetic distance between the 3 populations was measured according to the method of Nei’s standard distance and also calculated by Popgene 3.2.

Results

Selection of Gerbil Microsatellite Loci and Measurement of Genetic Diversity

In total, 536 mouse microsatellite markers chosen from the Mouse Genome Informatics (MGI) were tested for amplification of genomic DNA from a panel of 25 CMU laboratory gerbils. In these reactions, 313 oligo pairs (58.39%) successfully amplified discrete PCR products, of which 2 representatives are shown (Figure 1). Subsequently, total 313 of these PCR products were sequenced and 130 (41.53%) contained microsatellite DNA sequences (SupMat Tab) and were confirmed as SSR loci (Figure 2). We next used 12 molecular markers, including 6 of our newly identified SSR loci and 6 that had been previously reported (Neumann et al. 2001), to evaluate genetic polymorphisms in 84 gerbils, including 30 CMU laboratory gerbils, 27 HOH, and 27 YIN wild gerbils. These 84 samples displayed a wide range in the number of alleles for specific loci, ranging from 1 allele (locus CMU2009109) to 4 alleles (loci CMU2009107, CMU2009125, AF200941, and AF200945) with observed heterozygosity values ranging from 1.00 (locus CMU2009109) to 4.00 (locus AF200941). The effective number of alleles ranged from 1.00 (locus CMU2009109) to 2.8383 (locus AF200946). The Shannon’s information index and percentage of polymorphic loci were highest for the Yin Chuan population (0.6239 and 91.37%, respectively), whereas those of the Hohehot wild gerbil population were lowest (0.5962 and 83.33%, respectively). To compare the number of alleles per marker between the gerbil and the mouse, a set of 24 markers from our CMU laboratory gerbil database and their mouse counterparts (available at the website http://www.informatics.jax.org/) were studied, and the results are shown in SupMat Table. The number of alleles per locus in the laboratory gerbil is less than that in the mouse.

To investigate whether the loci amplified from the gerbil are orthologous to those of the mouse, we compared 24 loci from the gerbil genome with the counterpart of mouse. To our surprise, unlike what had been previously reported (Navani et al. 2002), none of the 24 gerbil loci sequenced shared the same repeats with their mouse counterparts (Figure 3).

Relationships and Genetic Distances among Populations

Allele frequencies represent the genetic diversity within a population. These results show that some differences in allele frequencies were present among the different populations of gerbils. The AF200941-A and AF200943-A alleles did not exist in the Hohehot wild gerbils and the CMU laboratory populations, respectively. Only the Yin Chuan gerbils carried the CMU2009128-A allele. However, one-way analysis of variance (ANOVA) reveals that there were no significant differences in allele frequencies between the 3 populations (P value > 0.05). These results indicate that, compared with the wild gerbil populations, CMU gerbil still maintain the same genetic variation after 23 years of breeding.

As shown in Table 1, the total expected heterozygosity was 0.4123, whereas the observed heterozygosity was 0.5025. The one-way ANOVA results show that the observed and expected heterozygositizes and the observed and expected homozygosities show no significant differences between the 3 gerbil populations. The Nei’s genetic variation value of the YIN population, at 0.3933, was larger than that of the HOH and CMU populations (0.3809 and 0.3592, respectively). This result suggests that the YIN group has higher variation than the other 2 groups.

Pairwise Genetic Distances

The results of structure clustering can indicate the kinships between populations. The values of the pairwise genetic distances were observed between each pair of the 3 gerbil populations (see Table 2). The value of Nei’s genetic distance between the CMU and HOH populations was highest (0.1033), whereas the value between the CMU and YIN populations was lowest (0.0592). Analysis of the structure clustering indicated that the HOH population first clustered together with the CMU population and then with the YIN population. These results showed no significant genetic differences between the CMU, HOH, and YIN gerbil populations.
Discussion

Isolation of SSRs has traditionally relied on the screening of genomic libraries using repetitive probes and sequencing positive clones in order to develop locus-specific primers. This process has been implemented in many organisms but is normally time consuming and labor intensive. Cross-amplification is generally possible among species within the same genus, but the success rate can be low (Dutech et al. 2007). In addition, cross-amplification often generates null alleles, which could bias genetic analyses (Hardy et al. 2003). Despite these caveats, for a species for which there are no microsatellite markers available, cross-amplification is still a valuable method to use. The gerbil is genetically related to mouse, albeit not in the same family. We therefore developed primers for gerbil microsatellite markers based on the available information for mouse microsatellite markers. In total, 536 mouse markers have been used to amplify gerbil DNA, and 98 markers gave positive results. The cross-amplification rate is 58.39%. Of the PCR products amplified by the markers, 313 have been sequenced, and 130 of these markers are confirmed as SSR sequences. The positive amplification rate (24.25%) is relatively low in comparison with other cross-amplification reports: 75% (81/108) amplification of buffalo markers by cattle primers (Navani et al. 2002), 58% (605/1036) of bovine primer pairs amplified a locus in sheep (deGortari et al. 1997), and 50% (14/28) of duck primer pairs amplified a locus in goose (Huang et al. 2005). This indicates that the closer the 2 species are in the phylogenetic tree, the higher the rate of successful cross-amplification will be. Nevertheless, our approach undoubtedly generated more than 24% of the gerbil's microsatellite loci. In fact, 130 loci are comparatively enough to study various diseases related to the gerbil model. We would conduct genetic mapping of gerbils using 130 loci in future. These loci have been successfully used to analyze the variation of laboratory gerbils and evaluate the prognostics of genetic mapping. We also used microsatellite markers derived from rat to amplify gerbil sequences and the positive amplification rate (less than 15%, 6/46) is lower than for mouse-derived markers. Owned to the abundant loci in GenBank and clearly genetic

Figure 2. Discrete amplification products of the gerbil genome generated at the loci D3MIT268 (up) and D7MIT190 (down) by mouse-derived primers. These 2 loci are on mouse chromosome 3 and 7, respectively. The underlined section indicates the repeat region.
background and higher positive cross-amplification rate, we preferred mouse marker as the coamplification of Mongolian gerbil.

To correlate the sequence identity between the 2 species, we compared 24 sequenced loci of 130 positive short tandem repeat from the gerbil genome and found that unlike Navani’s report that the repeats are almost the same in cattle and buffalos when the same primer was used, no marker contained exactly the same flanking or core repeat sequence between the gerbil and the mouse. Probably this is due to the fact that microsatellite primer pair sequences are often conserved across related species and can be used for the development of markers in related species (Navani et al. 2002). The results of the present study suggest that, although the same primers were used, the core repeat region and flanking sequences are different between the mouse and the gerbil. However, the fundamental reason for this question is still for us to look for.

Figure 3. Nucleotide sequence alignment of a mouse microsatellite marker (D3Mit268) and its gerbil ortholog. The top row shows the mouse sequence, and the bottom row shows the consensus sequence in gerbil. The core sequence of the mouse SSR is CA but that of the gerbil SSR is TG. The mouse sequence is a perfect microsatellite, and the gerbil sequence is an imperfect microsatellite.

Table 1  Observed number of alleles (Obs-alle), effective number of alleles (Eff-alle), observed heterozygosity (Obs-het), expected heterozygosity (Exp-het), Nei’s genetic variation (Nei’s-), percentage of polymorphic loci (Poly-loci%), and Shannon’s information index (Shan-) analyzed for 12 loci, including 6 newly identified and 6 previously identified markers

<table>
<thead>
<tr>
<th></th>
<th>Obs-alle</th>
<th>Eff-alle</th>
<th>Shan-</th>
<th>Poly-loci%</th>
<th>Obs-Het</th>
<th>Exp-Het</th>
<th>Nei’s-</th>
</tr>
</thead>
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<tr>
<td>AF200941</td>
<td>4.000</td>
<td>1.8749</td>
<td>0.8274</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AF200942</td>
<td>3.000</td>
<td>1.6215</td>
<td>0.7021</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AF200943</td>
<td>3.000</td>
<td>1.9403</td>
<td>0.7366</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AF200945</td>
<td>2.000</td>
<td>1.7121</td>
<td>0.6098</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AF200946</td>
<td>3.000</td>
<td>2.8383</td>
<td>1.0687</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AF200947</td>
<td>3.000</td>
<td>2.4235</td>
<td>0.9855</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CMU2009114</td>
<td>2.000</td>
<td>1.9989</td>
<td>0.6929</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CMU2009116</td>
<td>2.000</td>
<td>1.9655</td>
<td>0.6843</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CMU2009109</td>
<td>1.000</td>
<td>1.0000</td>
<td>0.0000</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CMU2009107</td>
<td>2.000</td>
<td>1.7212</td>
<td>0.6098</td>
<td>—</td>
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<tr>
<td>CMU2009125</td>
<td>2.000</td>
<td>1.9391</td>
<td>0.6774</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>CNU2009128</td>
<td>2.000</td>
<td>1.0364</td>
<td>0.0896</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CMU</td>
<td>2.2500</td>
<td>1.6968</td>
<td>0.5591</td>
<td>83.33</td>
<td>0.4825</td>
<td>0.3655</td>
<td>0.3592</td>
</tr>
<tr>
<td>HOH</td>
<td>2.2500</td>
<td>1.7195</td>
<td>0.5962</td>
<td>83.33</td>
<td>0.5051</td>
<td>0.3882</td>
<td>0.3809</td>
</tr>
<tr>
<td>YIN</td>
<td>2.4167</td>
<td>1.7505</td>
<td>0.6239</td>
<td>91.67</td>
<td>0.5231</td>
<td>0.4008</td>
<td>0.3933</td>
</tr>
<tr>
<td>total</td>
<td>2.4167</td>
<td>1.8401</td>
<td>0.6403</td>
<td>91.67</td>
<td>0.5025</td>
<td>0.4123</td>
<td>0.4098</td>
</tr>
</tbody>
</table>

CMU, the gerbil population domesticated in CMU; YIN, the wild gerbil population captured from Yin Chuan city; HOH, the wild gerbil population captured from Hohehot Municipality.
We next analyzed 12 markers, including 6 from our newly identified set and 6 that had been previously reported (Neumann et al. 2001), to evaluate genetic variation in the laboratory animals. The polymorphic and microsatellite variations in the CMU laboratory gerbil, as studied by this method, show no significant differences when compared with wild gerbils. Ding et al. (2008) also reported the laboratory gerbil (Z:ZCLA) and wild gerbil (captured from Shan Xi province, China) have no significant differences in genetic variation analyzed by Microsatellite DNA markers. However, the results reported by Neumann et al. (2001) are that laboratory gerbils have a lower level of genetic variation than wild animals. Another report also highlighted the low genetic variability present in laboratory gerbils (Razzoli et al. 2003). These different conclusions may be the result of the breeding history of the animals. The CMU colony was established and cultivated from approximately 400 pairs of wild gerbils captured in 1986 from the district of Hohehot Municipality, China. In contrast, the strain of laboratory gerbils used by Neumann and colleagues was established from 20 pairs of wild gerbils captured from Manchuria (Inner Mongolia, China) in 1935, from which almost all the laboratory gerbils used in United States and European laboratories have been derived. To maintain genetic variation, the CMU population has been outbred, which is believed to contribute significantly to its higher level of genetic variability. In addition, our results demonstrated that there were no differences between laboratory and wild gerbils in genetic variation. However, Neumann reported that the allele number of wild gerbils was higher than that of laboratory gerbils. The discrepancy might be related to the different resource of wild animal. The wild gerbils we used in this paper were captured from districts of Hohehot Municipality (HOH) and Yin Chuan City (YIN), China. The wild gerbils in Neumann’ report were “trapped at six different locations about 130–140 km southwest and 100 km west of Ulaanbaatar.” Despite the lower heterozygosity in the CMU strain and the fact that it has lost some alleles (e.g., AF200943-A and CMU2009128-A) in comparison with the 2 wild gerbil populations, there are no significant differences (P > 0.05) in allele frequencies between the 3 populations. These results show that under Hardy–Weinberg equilibrium, the CMU population has not displayed reduced genetic variation and has not lost heterozygosity when compared with the 2 wild populations. The Nei’s standard genetic distance value between the CMU and HOH populations is higher than that between the CMU and YIN populations, suggesting that the genetic linkage between the CMU and HOH populations is closer than that of the CMU and YIN populations. These data may simply reflect the fact that the CMU strain was originally captured from Hohehot district. Just as the smaller genetic distance between the CMU and the HOH populations reflects their common area of origin, so the larger genetic distance between the CMU population and the YIN population may reflect the geographic distance between the Hohehot district and Yin Chuan city, their respective areas of origin.

Because mapping and linkage studies in gerbils require a large set of informative markers and a high level of genetic variation, our present study makes it possible to conduct such studies in the laboratory gerbil. In addition to the 9 loci previously identified (Neumann et al. 2001), our 130 newly identified gerbil microsatellite markers will be prominently useful in genetic analysis of the gerbil genome, especially in the construction of a linkage map, analysis of biodiversity and gene flow, and parentage determination. These microsatellite loci have proved useful in our ongoing projects investigating genetic variations and their correlation to the onset of ischemia-related cerebrovascular diseases in the gerbil model of human disease.

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**References**


**Table 2** Nei’s original measures of genetic identity (above diagonal) and genetic distance (below diagonal) between the 3 gerbil populations

<table>
<thead>
<tr>
<th>Population</th>
<th>YIN</th>
<th>HOH</th>
<th>CMU</th>
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</thead>
<tbody>
<tr>
<td>YIN</td>
<td>—</td>
<td>0.9182</td>
<td>0.9019</td>
</tr>
<tr>
<td>HOH</td>
<td>0.0854</td>
<td>—</td>
<td>0.9425</td>
</tr>
<tr>
<td>CMU</td>
<td>0.1033</td>
<td>0.0592</td>
<td>—</td>
</tr>
</tbody>
</table>

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