

ESTIMATING THE OCCURRENCE RATE OF DNA PALINDROMES

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A DNA palindrome is a segment of letters along a DNA sequence with inversion symmetry that one strand is identical to its complementary one running in the opposite direction. Searching nonrandom clusters of DNA palindromes, an interesting bioinformatic problem, relies on the estimation of the null palindrome occurrence rate. The most commonly used approach for estimating this number is the average rate method. However, we observed that the average rate could exceed the actual rate by 50% when inserting 5000 bp hot-spot regions with 15-fold rate in a simulated 150,000 bp genome sequence. Here, we propose a Markov based estimator to avoid counting the number of palindromes directly, and thus to reduce the impact from the hot-spots. Our simulation shows that this method is more robust against the hot-spot effect than the average rate method. Furthermore, this method can be generalized to either a higher order Markov model or a segmented Markov model, and extended to calculate the occurrence rate for palindromes with gaps. We also provide a p -value approximation for various scan statistics to test nonrandom palindrome clusters under a Markov model.

1. Introduction. A chromosome is a long double-stranded helix of DNA that consists of adenine–thymine (A–T) pairs or cytosine–guanine (C–G) pairs. Thus, one DNA strand decides the sequence of its complementary strand. A DNA palindrome with minimum half length L is defined as a segment of DNA letters with half length greater than or equal to L that one strand is identical to its complementary one running in the opposite direction. This inversion symmetry increases the probability to form secondary structures conferring significant biological functions ranging from RNA transcription to DNA replication [Leach (1994)].

It has been observed that DNA palindromes are common candidates for searching genetic motifs involved in different cellular processes, including gene transcriptions, gene replications, and gene deletions. For example, among nine octamers (segments with 8 bp) suggested to be transcription factor binding sites, three are palindromes [FitzGerald et al. (2004)]. Many studies have focused on investigating the occurrence rates of palindromes in suspicious regions against random sequences. For example, Lisnic et al. (2005) investigated the frequencies of palindromes in the yeast *Saccharomyces cerevisiae* genome. Chew, Cho and Leung (2005) proposed various score schemes to quantify palindromes and found

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an association between high score regions and the replication origins. Lu et al. (2007) compared the scores of the suspicious regions, including introns, exons, and upstream of transcription start sites, against simulated random sequences, and reported that meaningful sites tend to have higher palindrome scores.

The analysis of these comparisons strongly depends on the null occurrence rate. This rate usually is estimated by the genome-wide average or the i.i.d. model-based method using the DNA letter frequencies [Chew, Cho and Leung (2005)]. We tested these two methods on a herpes virus sequence *bohV1* (sequence ID “BHV1CGEN”). Its average rate is 0.00178 and the i.i.d. model-based estimate is 0.00073. The large discrepancy between these two alerted us, and further studies indicated that the average rate might be biased due to hot-spots, and the i.i.d. model might be too naive to describe the DNA sequence. Therefore, we propose a Markov based estimator using the DNA pairs’ frequencies in addition to the letters’ and get the estimate 0.00109. Compared to the i.i.d. model, the Markov model is more close to real sequences but yet not too complicated to estimate its parameters. The simulation shows that our method performs better than the average rate in estimating the null occurrence rate against hot-spots under a variety of model settings. We also show that this method can be generalized for either a higher order Markov model or a segmented Markov model. Furthermore, we demonstrate that this method can be extended to calculate the occurrence rate when the DNA palindrome contains a gap.

Many related p -value approximations have been developed based on the assumption that the events can be modeled as a Poisson process. This assumption has been justified in many cases including DNA palindromes [Reinert and Schbath (1998); Leung et al. (2005); Hansen (2009)]. Chan and Zhang (2007) developed a method to approximate the p -value for a scan of score statistics over a Poisson process, when the score can be modeled through an exponential family. To apply their method, the analytic formula for the moment generating function (MGF) of the score is required. However, the distribution of the palindrome scores has not been well studied except the length score under the i.i.d. assumption. Thus, we develop a method to derive the analytic formulae for the MGF of various scores under a Markov model. Another challenge in calculating the p -value approximations of scan statistics is to calculate an overshoot function [Woodroffe (1979); Siegmund (1985); Tu (2009)]. This function relates to the characteristic function of a random variable defined by the difference between the statistic and the threshold given the condition that the statistic exceeds the threshold. We provide a more general formula to calculate the overshoot functions on various scores under a Markov model.

In this paper we first show that three scores for detecting DNA palindrome clusters proposed by Chew, Cho and Leung (2005) can be formulated as likelihood ratio statistics. Second, we show that the occurrence rates can be calculated accurately under a Markov model. Third, we derive the moment generating function

for various scores under a Markov model. Fourth, we present a p -value approximation method for those statistics to detect DNA palindrome clusters. In Section 3 we show the results on both real data and simulated data. This paper ends with a brief discussion. The related derivations are collected in online supplementary materials [Tu, Wang and Huang (2013a)].

2. Method.

2.1. *Notation and the log-likelihood ratio statistics.* Let $N(t)$ be the counting process for the palindrome events and let $N_w(t) = N(t + w) - N(t)$ denote the number of palindromes whose starting positions fall in the interval $(t, t + w]$. Leung et al. (2005) proved that $N(t)$ can be approximated by a Poisson process under a Markov model. We let X_i be the score for the i th palindrome (event) along the genome sequence, and $S_{N_w(t)}$ is the summation of the palindrome scores in $(t; t + w]$:

$$(1) \quad S_{N_w(t)} = \sum_{i=N(t)+1}^{N(t+w)} X_i.$$

To search the clusters of palindromes, Chew, Cho and Leung (2005) proposed three schemes to quantify palindromes, including the palindrome count score (PCS), the palindrome length score (PLS), and the base-pair weighted score of order m (BWS_m). PCS gives the same score for each DNA palindrome; PLS gives the score as the palindrome length divided by its minimum required length; and BWS_m gives the score as the minus log-likelihood under Markov order m assumption.

We would like to show that both $N_w(t)$ and $S_{N_w(t)}$ are equivalent to the log-likelihood ratio statistics when the alternative hypotheses are properly constructed. This equivalence is useful for developing the p -value approximations. Under the Poisson process model, we also assume that X_i 's can be treated as i.i.d. with a density function $f_\theta(x) = f_0(x) \exp(\theta x - \phi(\theta))$, where $f_0(x)$ is an unknown distribution and $\phi(\theta) = \log \int e^{\theta x} f_0(x) dx$ is its log of the MGF. For events that occur outside of the interval $(t_a, t_a + w]$, the parameters are (λ_0, θ_0) . For events that occur in the interval $(t_a, t_a + w]$, the parameters for $N(t)$ and X_i are (λ_a, θ_a) . The null hypothesis is that $\lambda_a = \lambda_0$ and $\theta_a = \theta_0$. When t_a is known, the likelihood ratio is $f_{\lambda_a, \theta_a}(N_w(t_a), S_{N_w(t_a)}) / f_{\lambda_0, \theta_0}(N_w(t_a), S_{N_w(t_a)})$, and the likelihood is as follows:

$$\begin{aligned} & f_{\lambda, \theta}(N_w(t), S_{N_w(t)}) \\ &= f_\lambda(N_w(t)) f_\theta(S_{N_w(t)} | N_w(t)) \\ &= \frac{(\lambda w)^{N_w(t)} e^{-\lambda w}}{N_w(t)!} \left(\prod_{i=N(t)+1}^{N(t+w)} f_0(x_i) \right) \exp(\theta S_{N_w(t)} - N_w(t)\phi(\theta)). \end{aligned}$$

Because t_a is usually unknown, we search for the maximum of the statistic over all possible t .

Case 1. If the alternative hypothesis is constructed as $H_a : \lambda_a = \lambda_1 > \lambda_0$ and $\theta_a = \theta_0$, then the log-likelihood ratio statistic is equivalent to PCS in Chew, Cho and Leung (2005):

$$\begin{aligned}
 \max_t l_t(\lambda_1, \theta_0) &= \max_t \log \left(\frac{f_{\lambda_1, \theta_0}(N_w(t), S_{N_w(t)})}{f_{\lambda_0, \theta_0}(N_w(t), S_{N_w(t)})} \right) \\
 &= \max_t N_w(t) \log \left(\frac{\lambda_1}{\lambda_0} \right) - (\lambda_1 - \lambda_0)w.
 \end{aligned}
 \tag{2}$$

Case 2. If the alternative hypothesis is constructed as $H_a : \lambda_a = \lambda_1 > \lambda_0$ and $\theta_a = \theta_1 > \theta_0$, where λ_1 and θ_1 are constrained to satisfy

$$\log \left(\frac{\lambda_1}{\lambda_0} \right) - (\phi(\theta_1) - \phi(\theta_0)) = 0,
 \tag{3}$$

the log-likelihood ratio statistic in formula (4) can be equivalent to PLS or BWS_m proposed by Chew, Cho and Leung (2005), depending on the definition of X_i 's:

$$\begin{aligned}
 \max_t l_t(\lambda_1, \theta_1) &= \max_t \log \left(\frac{f_{\lambda_1, \theta_1}(N_w(t), S_{N_w(t)})}{f_{\lambda_0, \theta_0}(N_w(t), S_{N_w(t)})} \right) \\
 &= \max_t \left\{ -(\lambda_1 - \lambda_0)w + (\theta_1 - \theta_0)S_{N_w(t)} \right\}.
 \end{aligned}
 \tag{4}$$

It can be observed that (2) is equivalent to $\max_t N_w(t)$ and (4) is equivalent to $\max_t S_{N_w(t)}$. While (2) tests only the Poisson parameter λ , (4) tests both the Poisson parameter λ and the score parameter θ with the constraint (3). $N_w(t)$ can be treated as a special case of $S_{N_w(t)}$ with $X_i = 1$ for each i .

We applied the method developed by Chan and Zhang (2007) to derive the threshold value of $\max_t S_{N_w(t)}$. Let $N(t)$ be a Poisson process with mean λ_0 and the log of the MGF of X_i is $\phi(\theta)$. X_i 's are i.i.d. with mean μ_0 , then

$$\begin{aligned}
 P_0 \left(\max_{0 < t < W} S_{N_w(t)} \geq b \right) \\
 \sim 1 - \exp \left\{ -(W - w) \nu_{\lambda_1, \theta_1} (b - \lambda_0 \mu_0) e^{-[b\theta_1 - w(\lambda_1 - \lambda_0)]} \right. \\
 \left. \times (2\pi w \lambda_1 [\ddot{\phi}(\theta_1) + \dot{\phi}^2(\theta_1)])^{-1/2} \right\},
 \end{aligned}
 \tag{5}$$

where W is the total length of the sequence, $\dot{\phi}(\theta_1)$ and $\ddot{\phi}(\theta_1)$ are the first and the second derivative of $\phi(\theta_1)$ representing the mean and the variance of X_i with density $f_{\theta_1}(x)$, and $\nu_{\lambda_1, \theta_1}$ is an overshoot function indexed with (θ_1, λ_1) satisfying the equations:

$$\begin{aligned}
 w \lambda_1 \phi'(\theta_1) &= b, \\
 \log(\lambda_1 / \lambda_0) &= \phi(\theta_1) - \phi(\theta_0).
 \end{aligned}$$

It is obvious from (5) that λ_0 always plays a crucial role in deciding the critical value for the tests. The most challenging part in applying (5) to calculate the p -value is to calculate the overshoot function $\nu_{\lambda_1, \theta_1}$. As a pioneer, Woodroffe (1979)

derived a computable formula to calculate the overshoot function for i.i.d. nonarithmetic random variables given the characteristic functions. Tu (2009) generalized this formula for i.i.d. arithmetic random variables. Incorporating their results, we develop Theorem 4 for more general cases.

2.2. *Occurrence rate of DNA palindromes under Markov model.* Let T be a 4×4 matrix with $T_{ij} = P_{b_i b_j} P_{\tilde{b}_j \tilde{b}_i}$ which groups together the transition probabilities of symmetric complementary pairs, where $(b_1, b_2, b_3, b_4) = (A, C, G, T)$ and \tilde{b}_j refers to the complementary letter of b_j . T is considered as a quasi transition matrix because the sum of its rows do not equal one.

THEOREM 1. *Assume that DNA letters along the genome sequence follow a Markov model with transition probability $\{P_{a,b} \mid a, b \in \{A, C, G, T\}\}$ and letter frequency $P'_0 = (\pi_A \ \pi_C \ \pi_G \ \pi_T)$. The occurrence probability of a palindrome I_i (given a starting position i) with minimum half length L is*

$$(6) \quad \lambda_M \equiv P(\|I_i\| \geq L) = P'_0 T^{L-1} P_1,$$

where $\|I_i\|$ denotes the corresponding maximum length, and

$$P'_1 = (P_{AT} \ P_{CG} \ P_{GC} \ P_{TA}),$$

and

$$T = \begin{pmatrix} P_{AA}P_{TT} & P_{AC}P_{GT} & P_{AG}P_{CT} & P_{AT}P_{AT} \\ P_{CA}P_{TG} & P_{CC}P_{GG} & P_{CG}P_{CG} & P_{CT}P_{AG} \\ P_{GA}P_{TC} & P_{GC}P_{GC} & P_{GG}P_{CC} & P_{GT}P_{AC} \\ P_{TA}P_{TA} & P_{TC}P_{GA} & P_{TG}P_{CA} & P_{TT}P_{AA} \end{pmatrix}.$$

REMARK 1. Theorem 1 gives an exact formula to calculate the palindrome occurrence rate under a Markov model. To apply this formula, one needs to estimate the Markov parameters including the stationary probabilities $\{\pi_a \mid a \in \{A, C, G, T\}\}$ and the transition matrix $\{P_{a,b} \mid a, b \in \{A, C, G, T\}\}$ which usually are estimated by the letter frequencies of W letters and the pair frequencies of $W - 1$ letter pairs, where W is the total sequence length. Because the size of the hot-spot regions is much smaller than W these Markov parameter estimations are not heavily influenced by hot-spots and neither is λ_M to estimate the null occurrence rate. On the other side, for the rare events like the DNA palindromes, the average rate counts the total number of palindromes of which a nonnegligible portion is potentially contributed from the hot-spots in real sequences and, hence, the average rate is easily inflated when estimating the null occurrence rate.

REMARK 2 (i.i.d. model). When the Markov model is reduced to the i.i.d. model, P'_1 becomes

$$P'_2 = (\pi_T \ \pi_G \ \pi_C \ \pi_A),$$

and T becomes $P_2 P'_0$. Thus,

$$(7) \quad \lambda_{i.i.d.} \equiv P(\|I_i\| \geq L) = P'_0(P_2 P'_0)^{L-1} P_2 = (P'_0 P_2)^L = \gamma^L,$$

where $\gamma = 2(\pi_A \pi_T + \pi_C \pi_G)$. Equality (7) has been shown in Leung et al. (2005).

REMARK 3 (Higher order Markov models). When the DNA sequence does not follow a first-order Markov model, a higher order Markov model may be considered. Theorem 1 can also be applied to higher order Markov models. For example, a four-state second-order Markov model can be described as a first-order Markov model with sixteen states, in which each state represents one adjacent letter pair, like $a_1 a_2$ where $a_i \in \{A, T, G, C\}$ and the probability model becomes $P(a_1 a_2 a_3) = P(a_1 a_2) P(a_2 a_3 | a_1 a_2)$. Under this setting, only four elements in each row or each column of the 16×16 transition matrix will be nonzero, because $P_{a_1 a_2, a_3 a_4} = 0$ if $a_2 \neq a_3$. The elements in the corresponding quasi transition matrix will be like $P_{a_1 a_2, a_3 a_4} P_{\tilde{a}_4 \tilde{a}_3, \tilde{a}_2 \tilde{a}_1}$. The corresponding P_1 in (6) is a column vector with length sixteen, in which the elements are like $P_{a_1 a_2, a_2 \tilde{a}_2} P_{\tilde{a}_2 \tilde{a}_2, \tilde{a}_2 \tilde{a}_1}$.

REMARK 4 (Segmented Markov models). Another alternative model is the segmented Markov model which relaxes the stationary condition [Chen and Zhou (2010)]. Considering a three-state segmented Markov model $\{\xi_1, \xi_2, \xi_3\}$, each state contributes total length W_1, W_2 and W_3 and has its own p -values p_1, p_2 and p_3 . Two constraints on these p -values are $1 - (1 - p_1)(1 - p_2)(1 - p_3) = 0.05$ by the Bonferroni approximation on the overall 0.05 significance level and $\frac{p_1}{W_1} = \frac{p_2}{W_2} = \frac{p_3}{W_3}$ that the p -values are proportional to their contributed length. Given change points that separate different hidden states ξ 's, each parameter set can be estimated, respectively. As such, Theorems 1 and 4 can be applied to calculate the palindrome occurrence rate for each set and their corresponding thresholds.

REMARK 5 (Hairpin structures). It is of interest to consider DNA palindromes with gaps. When a gap exists at the center position of a DNA palindrome, the single strand segment may form a hairpin secondary structure [Leach (1994)]. Theorem 1 can be extended to calculate the occurrence rate for such patterns. Consider a palindrome with the half-length $\geq L$ and a gap with length β at the center position of the palindrome, then the probability to see such a pattern given a start position is $P'_0 T^{L-1} \text{diag}((P^\beta - P^{\beta-2}) \tilde{P})$ when $\beta \geq 2$ and $P'_0 T^{L-1} \text{diag}(P \tilde{P})$ when $\beta = 1$, where \tilde{P} is the transition matrix with the index order (A C G T) for row and (T G C A) for column. The technical derivation is in Appendix A.2.

THEOREM 2. Under the same assumption discussed in Theorem 1, the PLS score for the i th palindrome is defined as $X_i = \|I_i\|/L$ conditional on $\|I_i\| \geq L$. As such, the MGF for X_i is

$$(8) \quad K_{\text{PLS}}(t) \equiv E(e^{X_i t} | \|I_i\| \geq L) = \frac{e^t}{\lambda_M} P'_0 T^{L-1} (I - e^{t/L} T)^{-1} (I - T) P_1.$$

REMARK 6 (i.i.d. model). When the Markov model is reduced to the i.i.d. model,

$$K_{\text{PLS}}(t) = \sum_{k=L}^{\infty} e^{kt/L} (\gamma^k - \gamma^{k+1}) / \gamma^L = \frac{e^t(1-\gamma)}{1 - e^{t/L}\gamma}.$$

THEOREM 3. Under the same assumption discussed in Theorem 1, the BWS score is defined as $X_i = -\log(P(I_i))$ conditional on $\|I_i\| \geq L$. Then, the MGF for X_i is

$$(9) \quad K_{\text{BWS}}(t) \equiv E[e^{X_i t} \mid \|I_i\| \geq L] = \frac{1}{\lambda_M} \mathbf{v}'(t) (I - Q(t))^{-1} (Q(t))^{L-1} \mathbf{u}(t),$$

where $\mathbf{v}(t) = (v_1(t) \ v_2(t) \ v_3(t) \ v_4(t))'$ is defined as $v_i(t) = ((I - T)P_0)_i^{1-t}$; $Q(t)$ is defined as $Q_{ij}(t) = (T_{ij})^{(1-t)}$; and $\mathbf{u}(t) = (u_1(t) \ u_2(t) \ u_3(t) \ u_4(t))'$ is defined as $u_i(t) = (P_1)_i^{1-t}$ with $i = 1, \dots, 4$.

REMARK 7 (i.i.d. model). When the Markov model is reduced to the i.i.d. model,

$$(10) \quad K_{\text{BWS}}(t) = \frac{(1-\gamma)^{1-t}}{1-\gamma_t} \left(\frac{\gamma_t}{\gamma} \right)^L,$$

where $\gamma_t = P'_0(t)P_2(t) = 2[(\pi_A\pi_T)^{1-t} + (\pi_C\pi_G)^{1-t}]$. To provide a more general approximation for the p -value of maximum of (1), we develop Theorem 4.

THEOREM 4. Let N be a Poisson process with constant rate $\lambda_0 > 0$ and let random variables $X_1, \dots, X_n \stackrel{\text{i.i.d.}}{\sim} f_{\theta_0}(\cdot)$ with the MGF $\exp(\phi(\theta))$. Let λ_1 and θ_1 satisfy two conditions: (a) $w\lambda_1\phi'(\theta_1) = b$. (b) $\log(\lambda_1/\lambda_0) - (\phi(\theta_1) - \phi(\theta_0)) = 0$. Let $W \rightarrow \infty$ as $w \rightarrow \infty$ such that $W - w \rightarrow \infty$. Then

$$\begin{aligned} & P_0 \left(\max_{0 < s < W} S_{N_w(s)} \geq b \right) \\ & \approx 1 - \exp\{-(W-w)v_{\lambda_1, \theta_1}(b - \lambda_0\mu_0)e^{-I(b)w} \\ & \quad \times (2\pi w\lambda_1[\ddot{\phi}(\theta_1) + \dot{\phi}^2(\theta_1)])^{-1/2}\}, \end{aligned}$$

where $v_{\lambda_1, \theta_1} = \frac{1 - E_0 e^{-S_{\tau_+}(\theta_1 - \theta_0)}}{(1 - e^{-(\theta_1 - \theta_0)})E_0 S_{\tau_+}}$ and $I(b) = b(\theta_1 - \theta_0)/w - (\lambda_1 - \lambda_0)$. The definition of S_{τ_+} and the proof of Theorem 4 are put online.

REMARK 8. Theorem 4 follows Theorem 1 of Chan and Zhang (2007) but extends their result to allow more varieties of scores on the events. The assumptions of Theorem 4 include that the event can be modeled as a Poisson process and the scores for those events are i.i.d. It has been reported that the approximation error percentage (compared to the Monte Carlo simulation) is less than 5%

when $W = 20w$ and $w = 9$ unit length in Tu (2013). However, in such an application to search nonrandom palindrome clusters, the window size 9 bp cannot cover even one palindrome. Thus, the issue of choosing w is addressed by efficiently searching the clusters. The criterion should be that w needs to be large enough to cover the clusters but not too large to dilute its density. In our experience, 500 to 1000 bp is a reasonable size of w to search the palindrome clusters of the herpes virus genomes. Another example appeared in Chan and Zhang (2007) that they used the window size 245 to search GATC clusters as DAM sites in an *E. coli* genome sequence.

3. Real data analysis and simulations. We studied 27 herpes virus genome sequences among which bohv1 with total length 135,301 bp is chosen as our model sequence. Two replication origins of bohv1 have been reported in the literature [Leung et al. (2005)] and there exist distinct deviations among its average rate (0.00178), Markov model estimate (0.00109), and the i.i.d. estimate (0.00073). Its transition matrix and stationary probabilities are estimated as¹

$$(11) \quad P_{\text{bohv1}} = \begin{pmatrix} & \text{A} & \text{C} & \text{G} & \text{T} \\ \text{A} & 0.1854 & 0.3288 & 0.3556 & 0.1303 \\ \text{C} & 0.1258 & 0.2932 & 0.4347 & 0.1463 \\ \text{G} & 0.1343 & 0.4512 & 0.2994 & 0.1151 \\ \text{T} & 0.1141 & 0.3151 & 0.3695 & 0.2012 \end{pmatrix},$$

$$\pi_{\text{bohv1}} = (0.1354 \quad 0.3588 \quad 0.3654 \quad 0.1405).$$

We also employed a second-order Markov model on bohv1, and estimated the occurrence rate as 0.00113. The closeness of this value to that from a first-order Markov model (0.00109) suggests the appropriateness of a first-order Markov model for this sequence. We followed the criterion that minimum half length times the square of the occurrence rate is most close to 0.16 proposed in Leung et al. (2005). Thus, among the 27 herpes virus sequences, we use $L \geq 6$ as the palindrome criterion for 5 sequences including bohv1, cehv1, hsv2, muhv4, and thv, and $L \geq 5$ for the remaining 22 sequences.

3.1. Real data analysis. We downloaded from the EBI Nucleotide Sequences database 27 herpes virus genome sequences. For each sequence, we estimated its own transition matrix and stationary probabilities. Theorem 1 was then applied to estimate the null occurrence rate for each sequence. These results are compared with the average rate estimates in Figure 1. As shown, the average rate estimates have higher values except for the sequence athv3. Based on these two occurrence

¹Standard errors of the estimated transition probabilities are roughly $\sqrt{0.4 \cdot 0.6 / 135,301} \approx 0.0013$.

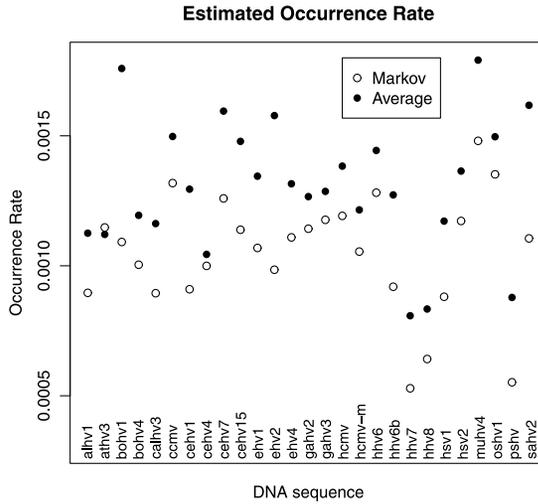


FIG. 1. 27 herpes virus genomic sequences were downloaded from the EBI Nucleotide Sequences database. Two methods for estimating the null palindrome rates are presented, including the average rate and the Markov model based estimator. We used the abbreviation for naming the genome sequences that was used in Leung et al. (2005).

rate estimates and given the total length for each sequence, Theorem 4 is applied to derive the 0.05 significance thresholds for the scan statistics of PLS and BWS, shown in Figure 2. Both the PLS and the BWS scan scores of bohv1, hhv6, and hhv8 become significant at the 0.05 thresholds when the average rate estimator is replaced by the Markov rate estimate. These results suggest that the Markov rate estimate is potentially more powerful in detecting nonrandom clusters.

Figure 3 presents a more detailed analysis for bohv1. For both PLS and BWS plots, two peaks occur at positions 113,488 and 124,582, and they are close to

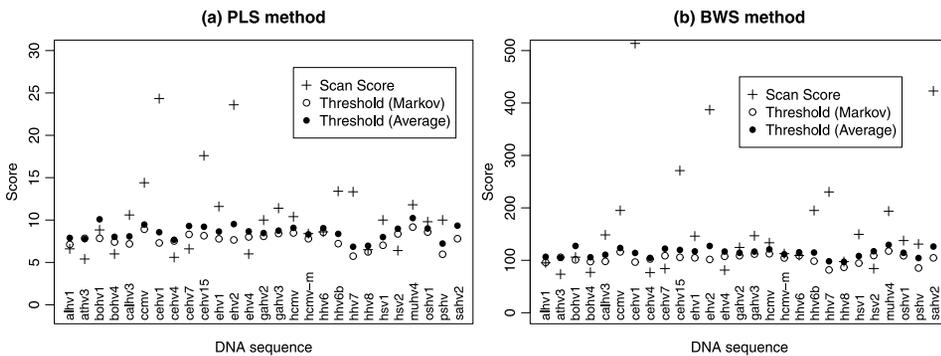


FIG. 2. The thresholds based on the two occurrence rate estimates for the 27 herpes virus genomic sequences are compared. The solid circles label the thresholds derived by the average rates and the empty circles by the Markov model estimates. The crosses are the maximum scores for each sequence.

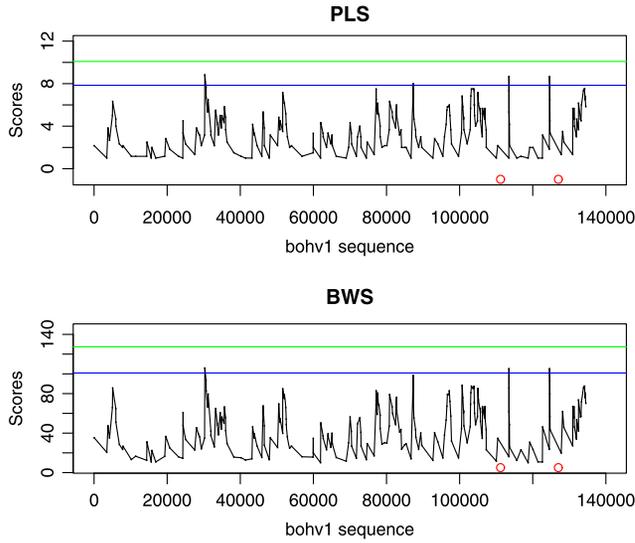


FIG. 3. The PLS and BWS scan scores are shown verse their genomic positions of bohv1. The blue horizontal lines are the thresholds by our occurrence rate estimate and the green lines are by the average rates. The red circles label the position of the replication origins of bohv1. Two peaks of PLS and BWS occur at positions 113,488 and 124,582, and they are close to two replication origins, respectively, at positions 111,080–111,300 (OriS) and 126,918–127,138 (OriS) [Leung et al. (2005)].

two replication origins, respectively, at positions 111,080–111,300 (OriS) and 126,918–127,138 (OriS) [Leung et al. (2005)]. The scores and the threshold values by both the Markov rate estimate and the average rate are summarized in Table 1. Matlab scripts for calculating thresholds are provided [Tu, Wang and Huang (2013b)]. The scores are above the thresholds by the Markov rate and below that by the average rate. The analysis based on the Markov rate estimate is consistent with the hypothesis that nonrandom palindrome clusters may play a role to search the replication origins, proposed by Chew, Cho and Leung (2005). The discrepancies between these two methods could be due to the nonrandom palindrome clusters of bohv1 which cause the inflation of the average rate. The Markov rate estimates are

TABLE 1

Two peaks which are close to two replication origins of bohv1 happen to share the same scores for both PLS and BWS. A Markov model estimate provides a more robust occurrence rate estimate against nonrandom clusters which leads to more appropriate threshold values and gains power

bohv1 sequence	Scores of two peaks	Thresholds by Markov model	Thresholds by average method
PLS	8.67	7.84	10.08
BWS	105.3	100.9	127.4

TABLE 2

Three methods for estimating the palindrome occurrence rate are compared on both i.i.d. and Markov random sequences. If the model is correctly specified, both model calculation estimators are consistent with the mean rate. If the model is not correct, $\hat{\lambda}_M$ still performs well but $\hat{\lambda}_i$ does not

No hot-spots	$\hat{\lambda}_i$	$\hat{\lambda}_M$	$\bar{\lambda}$
i.i.d. random sequences	0.00073	0.00073	0.00071
Markov random sequences	0.00073	0.00109	0.00101

based on the letter frequencies and the pair frequencies of the whole genome in which the effect from those clusters becomes negligible.

3.2. *Simulation study.* When there exists no nonrandom clusters, the palindrome events along these random sequences could be well approximated by a homogeneous Poisson process, for either a Markovian sequence or an i.i.d. sequence [Leung et al. (2005)]. In this case, the average rate is the maximum likelihood estimator (MLE) for the occurrence rate and can be treated as a target reference. Table 2 shows that when i.i.d. random sequences are generated, all the three methods perform equally well. However, when Markov random sequences are generated, the i.i.d. model-based estimator 0.00073 falls below 27.7% of the target 0.00101. The reason is that the i.i.d. model is a submodel of the Markov model while the reverse is not true. Thus, the Markov model is a better choice than the i.i.d. model.

We designed a simulation experiment to investigate the power performance of the estimates when hot-spot regions exist for a first-order Markov model. We used P_{bohV1} and π_{bohV1} to generate a stationary random sequence of length 150,000 and then simulated the hot-spots by inserting various intensity of palindromes that are resampled from the bohV1 palindrome bank. The length distribution of this bank is shown in Table 3. We set five 1000 bp regions with palindrome occurrence rates $(r_1, r_1, r_1, r_2, r_2) \times \hat{\lambda}_M$, where $\hat{\lambda}_M = 0.00109$ is the Markov rate estimate of the bohV1 sequence.

We chose $r_1 = 20$ to make the average rate close to that of bohV1 and let $r_2 \in \{6, 7, 8, 9, 10\}$ to check their power performance. The palindrome insertion for each hot-spot includes three steps: (a) to generate a Poisson random number \hat{M} with mean $1000\hat{\lambda}_M r_i$; (b) to resample \hat{M} palindromes from the bohV1 palindrome

TABLE 3

The length distribution of the palindromes collected from bohV1. The genome length is 135,301 bp and the total number of palindromes (half length ≥ 6) is 241

Half length	6	7	8	9	10	11	12	13	14	15	16	17	18	>19
Counts	132	54	22	10	12	4	4	1	0	0	1	0	1	0

TABLE 4

Powers are compared for using $\hat{\lambda}_M$ and $\bar{\lambda}$ to estimate the null occurrence rates of DNA palindromes when five 1000 bp hot-spots are inserted with relative intensity $(r_1, r_1, r_1, r_2, r_2)$ into a 150,000 bp DNA sequence generated by a Markov model. $r_1 = r_2 = 0$ is presented as the control group in which no palindromes are inserted. Powers are defined as the frequencies of detecting the hot-spot based on 250 replicates. For the hot-spot region with $r_1 = 20$, the power reaches 1 and hence is not shown in the table. Only the powers for the two sites with r_2 intensity are shown. $\bar{\lambda}$ tends to overestimate the occurrence rates and constructs an overly conservative test, leading to power loss

r_1	r_2	Markov model			Average method				
		$\hat{\lambda}_M$	Threshold	Powers	$\bar{\lambda}$	Threshold	Powers		
PLS									
0	0	0.00109	7.90	0.000	0.000	0.00109	7.88	0.000	0.000
20	0	0.00111	7.97	0.000	0.000	0.00151	9.21	0.000	0.000
20	6	0.00111	7.98	0.644	0.616	0.00161	9.53	0.480	0.464
20	7	0.00111	7.97	0.756	0.776	0.00164	9.63	0.636	0.628
20	8	0.00112	7.99	0.844	0.836	0.00165	9.67	0.708	0.716
20	9	0.00111	7.99	0.920	0.900	0.00166	9.74	0.840	0.800
20	10	0.00111	7.99	0.972	0.964	0.00168	9.79	0.912	0.916
BWS									
0	0	0.00109	101.65	0.000	0.000	0.00109	101.45	0.000	0.000
20	0	0.00111	102.22	0.000	0.000	0.00151	118.62	0.000	0.000
20	6	0.00111	102.34	0.644	0.608	0.00161	122.40	0.452	0.460
20	7	0.00111	102.28	0.736	0.768	0.00164	123.41	0.640	0.572
20	8	0.00112	102.41	0.856	0.828	0.00165	123.78	0.696	0.708
20	9	0.00111	102.39	0.912	0.884	0.00166	124.41	0.812	0.788
20	10	0.00111	102.39	0.972	0.956	0.00168	124.91	0.892	0.908

bank; (c) to start at \hat{M} uniformly random positions inside the 1000 bp segments, the DNA letters are replaced with the resampled palindromes. For each generated sequence, both Markov rate and average rate are estimated and their corresponding threshold values are derived by Theorem 4. The comparisons between the two estimate methods on occurrence rates, threshold values, and powers based on 250 replicas are presented in Table 4. The powers for those hot-spots with intensity $r_1 = 20$ -fold intensity reach one for both two methods of all cases and thus are omitted from the table.

When there exists no hot-spot ($r_1 = r_2 = 0$), both methods match very well on estimating the occurrence rate and thus share similar thresholds. When the three hot-spots are generated ($r_1 = 20$ and $r_2 = 0$), the average rate becomes 0.00151 (39% increase) while the Markov rate is 0.00111 (2% increase), resulting in threshold values 9.21 and 7.97 for PLS, and 122.40 and 102.34 for BWS. When $r_1 = 20$ and r_2 increases up to 10, the Markov estimate increases no more than 3%, while that by the average rate goes up to 54%. The PLS and BWS threshold values for

TABLE 5

Three hidden states (ξ_1, ξ_2, ξ_3) are constructed to generate the segmented Markov model. The letter frequencies and the palindrome occurrence rates for the three states ξ_1, ξ_2 , and ξ_3 are presented. ξ_2 has a higher CG ratio and ξ_3 has a lower one. The stationary probability distribution for (ξ_1, ξ_2, ξ_3) is (0.8, 0.1, 0.1)

State	A	C	G	T	Occurrence rate
ξ_1	0.1354	0.3588	0.3654	0.1405	0.00109
ξ_2	0.0833	0.4138	0.4162	0.0867	0.00310
ξ_3	0.1874	0.3038	0.3146	0.1941	0.00049

our Markov method are virtually the same and that for the average method goes more than 23%. Evidently, our method gains more power.

We further applied a hidden Markov model (HMM) to generate segmented Markov DNA sequences. We first generated a three-state-Markov chain $S_t \in \{\xi_1, \xi_2, \xi_3\}$ whose stationary probabilities are (0.8, 0.1, 0.1) for $1 \leq t \leq 30$. Given each state $S_t = \xi_i$, we used its own transition matrix to generate the DNA letters of length 5000. Thus, we generated a segmented Markovian DNA sequence of length 150,000 [Chen and Zhou (2010)]. We used P_{bohv1} as the transition matrix for ξ_1 and added 0.05 on the second and the third column and deducted 0.05 from the first and the fourth columns of P_{bohv1} to generate the transition matrix for ξ_2 and then we exchanged the addition and deduction to generate that for ξ_3 . By doing so, ξ_2 has higher CG ratio and ξ_3 has lower CG ratio compared to that of bohv1 and the average transition matrix keeps the same as P_{bohv1} . The stationary probabilities and the occurrence rates for the three states are summarized in Table 5.

In Table 6 we compare the power performance of the average method and the Markov method when the underlying sequence follows a segmented model. Table 6 has similar results as that in Table 4. The two methods, by the Markov model and the average rate, match very well when no hot-spot exists. When hot-spots constitute a significant portion of the total counts, the average rate is inflated to result in power loss and, by contrast, our Markov method is robust against the hot-spot effect and gains more power in detecting the nonrandom clusters.

In summary, both simulations of the first-order Markov model and the segmented model show that the average rate can overestimate the occurrence rate seriously due to the hot-spots effect and lead to power loss eventually. On the other hand, the Markov rate estimate is robust against the hot-spots and can maintain the threshold values appropriately and thus gains more power than the average rate method.

4. Discussion. In scan statistics, the average rate method is popular for estimating the null occurrence rate. In this paper, however, we report that the average

TABLE 6

Powers are compared for using $\hat{\lambda}_M$ and $\bar{\lambda}$ to estimate the null occurrence rates of DNA palindromes when five 1000 bp hot-spots are inserted with relative intensity $(r_1, r_1, r_1, r_2, r_2)$ into a 150,000 bp DNA sequence generated by a three-state segmented Markov model. The parameters of the three states are in Table 5. $r_1 = r_2 = 0$ is presented as the control group in which no palindromes are inserted. Powers are defined as the frequencies of detecting the hot-spot based on 250 replicates. For the hot-spot region with $r_1 = 20$, the power reaches 1 and hence is not shown in the table. Only the powers for the two sites with r_2 intensity are shown. $\bar{\lambda}$ tends to overestimate the occurrence rates and constructs an overly conservative test, leading to power loss

r_1	r_2	Markov model			Average method				
		$\hat{\lambda}_M$	Threshold	Powers	$\bar{\lambda}$	Threshold	Powers		
PLS									
0	0	0.00109	7.91	0.000	0.000	0.00109	7.89	0.004	0.000
20	0	0.00111	7.96	0.000	0.000	0.0015	9.18	0.000	0.000
20	6	0.00111	7.97	0.632	0.656	0.00161	9.54	0.488	0.512
20	7	0.00111	7.98	0.768	0.764	0.00164	9.62	0.628	0.644
20	8	0.00111	7.98	0.896	0.868	0.00164	9.64	0.780	0.712
20	9	0.00111	7.99	0.940	0.936	0.00166	9.70	0.872	0.836
20	10	0.00112	7.99	0.948	0.924	0.00167	9.78	0.884	0.880
BWS									
0	0	0.00109	101.74	0.000	0.000	0.00109	101.54	0.000	0.000
20	0	0.00111	102.17	0.000	0.000	0.00150	118.34	0.000	0.000
20	6	0.00111	102.27	0.636	0.640	0.00161	122.57	0.464	0.488
20	7	0.00111	102.32	0.744	0.764	0.00164	123.36	0.584	0.600
20	8	0.00111	102.33	0.876	0.848	0.00164	123.53	0.776	0.728
20	9	0.00111	102.39	0.944	0.924	0.00166	124.18	0.860	0.804
20	10	0.00112	102.4	0.932	0.916	0.00167	124.72	0.888	0.872

rate method does not always work. The average rate can overestimate the null occurrence rate up to 50% above the actual number, because the hot-spots have the potential of contributing to a large portion of the number of events, especially when the null occurrence rate is very low. Thus, we propose an estimator based on a Markov model and define it as a function of the Markov parameters so we can estimate the Markov parameters by the letter frequencies and the adjacent pair frequencies without using the number of events. Therefore, as long as the size of the hot-spot regions is much smaller than the total length of the genomes, the estimated Markov parameters would have little influence on the presence of the hot-spots, rendering our method insensitive to the hot-spot effect. Our study suggests that a model-based estimator might be more appropriate than the average rate for null occurrence rate estimation, especially when the Poisson process involves rare events with hot-spots, which are quite common in epidemiology studies involving rare diseases.

SUPPLEMENTARY MATERIAL

Supplement A: Appendix for the paper “Estimating the occurrence rate of DNA palindromes” (DOI: [10.1214/12-AOAS622SUPPA](https://doi.org/10.1214/12-AOAS622SUPPA); .pdf). The technical proofs for the theorems and corollaries in this paper are put in Supplement A as the appendix.

Supplement B: Matlab scripts for the paper “Estimating the occurrence rate of DNA palindromes” (DOI: [10.1214/12-AOAS622SUPPB](https://doi.org/10.1214/12-AOAS622SUPPB); .zip). The matlab scripts to calculate the thresholds derived in Theorem 4 are provided. The instruction is in the file “README.txt.”

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