Detecting DNA modifications from SMRT sequencing data by modeling sequence context dependence of polymerase kinetic

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Hyperparameters estimation for alternative model

In this section, we explain how to estimate hyperparameters by assuming control sample is available. As hierarchical model without control data is basically a special case of hierarchical model with control data, one can simply remove $y_0$, $\mu_0$ and $\sigma_0^2$ to get hyperparameter estimation when control sample is not available, and algorithm is unchanged. When alternative model is true (see section Hierarchical model with control data and Hierarchical model without control data in the main text), posterior distribution of $\mu_i$ and $\sigma_i^2$, $i = 0, 1, ..., m$, are[1]

$$p(\mu_i | y_i, \sigma_i^2, \theta, \kappa) = N(\frac{\kappa}{\kappa + n_i} \theta + \frac{n_i}{\kappa + n_i} y_i, \frac{\sigma_i^2}{\kappa + n_i})$$

$$p(\sigma_i^2 | y_i, v, \tau^2) = scaled\;inverse - \chi^2(v + n_i, \tilde{\sigma}_i^2)$$

where

$$\tilde{\sigma}_i^2 = \frac{1}{v + n_i} \left( v\tau^2 + (n_i - 1)s_i^2 + \frac{\kappa n_i}{\kappa + n_i} (y_i - \theta)^2 \right)$$ (1)
\[ \bar{y}_i = \frac{1}{n_i} \sum_{j=1}^{n_i} y_{ij} \text{ and } s_i = \frac{1}{n_i-1} \sum_{j=1}^{n_i}(y_{ij} - \bar{y}_i)^2. \] Posterior distribution of \( \sigma_c^2 \) is\(^1\)

\[ p(\sigma_c^2|\mathbf{y}_c, \nu, \tau^2) = \text{scaled inverse } - \chi^2(\nu + n_c, \tilde{\sigma}_c^2) \]

where

\[ \tilde{\sigma}_c^2 = \frac{1}{\nu + n_c} \left( \nu \tau^2 + (n_i - 1)s_c^2 \right) \]

We used posterior expectations of \( \mu_i \) and \( \sigma_i^2 \) as their estimations, which are

\[ \hat{\mu}_i = E(\mu_i|\mathbf{y}_i, \theta, \kappa) = \frac{\kappa}{\kappa + n_i} \theta + \frac{n_i}{\kappa + n_i} \bar{y}_i \] (2)

where \( i = 0, 1, \ldots, m \).

\[ \hat{\sigma}_i^2 = E(\sigma_i^2|\mathbf{y}_i, \nu, \tau^2) = \frac{n_i + \nu}{n_i + \nu - 2 \hat{\sigma}_i^2} \]

where \( i = c, 0, 1, \ldots, m \). We estimate hyperparameters \((\theta, \kappa, \nu, \tau^2, \mu_c)\) from the data by maximizing the marginal log-likelihood function, which is
\[ L(\mathbf{y}_c, \mathbf{y}_0, \mathbf{y}_1, \ldots, \mathbf{y}_m; \theta, \kappa, \tau^2, \mu_c) = \log(p(\mathbf{y}_c, \mathbf{y}_0, \mathbf{y}_1, \ldots, \mathbf{y}_m|\theta, \kappa, \tau^2, \mu_c)) \]

\[ = \log(p(\mathbf{y}_c|\theta, \kappa, \tau^2, \mu_c)) \]

\[ + \sum_{i=0}^{m} \log(p(\mathbf{y}_i|\theta, \kappa, \tau^2)) \]

where

\[ \log(p(\mathbf{y}_c|\theta, \kappa, \tau^2, \mu_c)) = \frac{v}{2} \log(v \tau^2) + \log(\Gamma(\frac{v + n_i}{2})) \]

\[ - \frac{v + n_i}{2} \log(\sum_{j=1}^{n_c} (y_{cj} - \mu_c)^2 + \nu \tau^2) - \log(\Gamma(\frac{v}{2})) \]

\[ - \frac{n_c}{2} \log(\pi) \]

\[ \sum_{i=0}^{m} \log(p(\mathbf{y}_i|\theta, \kappa, \tau^2)) = \sum_{i=0}^{m} \left[ \frac{1}{2} \log(\kappa) + \log(\Gamma(\frac{\nu + n_i}{2})) + \frac{\nu}{2} \log(v \tau^2) \right. \]

\[ - \frac{1}{2} \log(\kappa + n_i) - \log(\Gamma(\frac{\nu}{2})) - \frac{\nu + n_i}{2} \log((\nu + n_i) \tilde{\sigma}_i^2) \]

\[ - \frac{n_i}{2} \log(\pi) \]

It is obvious that \( L(\mathbf{y}_c, \mathbf{y}_0, \mathbf{y}_1, \ldots, \mathbf{y}_m; \theta, \kappa, \tau^2, \mu_c) \) can be maximized by setting \( \mu_c = \frac{1}{n_c} \sum_{j=1}^{n_c} y_{cj} \). However, it is difficult to get a close form of \((\theta, \kappa, \nu, \tau^2)\) and we therefore adopted a EM algorithm (Algorithm 1) to esti-
mate them numerically.

In the EM procedure, $\mu_i$ and $\sigma_i^2$ were regarded as missing data, and the log-likelihood function with the complete data was

$$l(y, \mu, \sigma^2; \theta, \kappa, \tau^2, v)$$

$$= \log(p(y|\mu, \sigma^2)) + \log(p(\mu|\sigma^2, \theta, \kappa)) + \log(p(\sigma^2|v, \tau^2))$$

$$= \log(p(y|\mu, \sigma^2)) + \sum_{i=0}^{m} \log(p(\mu_i|\sigma_i^2, \theta, \kappa)) + \sum_{i=c,0,1,...,m} \log(p(\sigma_i^2|\tau^2, v)) \quad (3)$$

where $y = (y_c, y_0, y_1, ..., y_m)$, $\mu = (\mu_0, \mu_1, ..., \mu_m)$ and $\sigma^2 = (\sigma_c^2, \sigma_0^2, \sigma_1^2, ..., \sigma_m^2)$. Initial values $(\theta_0, \kappa_0, \tau_0^2, v_0)$ were assigned to $(\theta, \kappa, \tau^2, v)$, and in the $t$th step $(t \geq 1)$ of EM algorithm, $(\theta, \kappa, \tau^2, v)$ were updated by the optimal value $(\theta_{opt}, \kappa_{opt})$ maximizing $E(l(y, \mu, \sigma^2; \theta, \kappa, \tau^2, v) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, v_{t-1})$, where $E(.)$ is expectation in term of $(\mu, \sigma^2)$, i.e. $\theta_t = \theta_{opt}$, $\kappa_t = \kappa_{opt}$, $\tau_t = \tau_{opt}$, $v_t = v_{opt}$. This procedure was repeated until convergence.

In the $t$th step of the EM algorithm, $(\theta_{opt}, \kappa_{opt})$ and $(\tau_{opt}^2, v_{opt})$ can be obtained by maximizing posterior expectation of the second term and the third term of equation (3), i.e. $\sum_{i=0}^{m} E\left( \log(p(\mu_i|\sigma_i^2, \theta, \kappa)) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, v_{t-1} \right)$ and $\sum_{i=c,0,1,...,m} E\left( \log(p(\sigma_i^2|\tau^2, v)) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, v_{t-1} \right)$, respectively.

$$\frac{1}{\Delta l} = \frac{E(l(y, \mu, \sigma^2; \theta_{opt}, \kappa_{opt}, \tau_{opt}^2, v_{opt}) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, v_{t-1}) - E(l(y, \mu, \sigma^2; \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, v_{t-1}) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, v_{t-1})}{E(l(y, \mu, \sigma^2; \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, v_{t-1}) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, v_{t-1})}$$
Algorithm 1 EM algorithm for hyperparameters estimation

Assign initial values to hyperparameters, $\theta = \theta_0$, $\kappa = \kappa_0$, $\nu = \nu_0$ and $\tau^2 = \tau^2_0$.

Set $t = 1$

while $\Delta l \leq 0.1$ do

E-step: Calculate conditional expectation of log likelihood function in terms of $\mu_i$ and $\sigma_i^2$, i.e. $E(l(y, \mu, \sigma^2; \theta, \kappa, \tau^2, \nu) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau^2_{t-1}, \nu_{t-1})$.

M-step: Update $(\theta, \kappa, \nu, \tau^2)$ by setting $\theta_t = \theta_{opt}$, $\kappa_t = \kappa_{opt}$, $\nu_t = \nu_{opt}$, $\tau_t = \tau_{opt}^2$, where $(\theta_{opt}, \kappa_{opt}, \nu_{opt}, \tau_{opt}^2)$ are hyperparameters maximizing $E(l(y, \mu, \sigma^2; \theta, \kappa, \tau^2, \nu) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau^2_{t-1}, \nu_{t-1})$.

Set $t = t + 1$

end while

Estimating $\theta$ and $\kappa$  

By taking posterior expectation of the second term of equation (3), we can get

$$
\sum_{i=0}^{m} E \left( \log(p(\mu_i | \sigma_i^2, \theta, \kappa)) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau^2_{t-1}, \nu_{t-1} \right) \\
= -\frac{\kappa}{2} \sum_{i=0}^{m} \left( \frac{1}{\kappa_{t-1} + n_i} + \frac{(\theta - \bar{\mu}_{i(t-1)})^2}{\bar{\sigma}_{i(t-1)}^2} \right) - \frac{m + 1}{2} \log(\kappa) + C
$$

where $\bar{\sigma}_{i(t-1)}^2$ and $\bar{\mu}_{i(t-1)}$ are estimated $\bar{\sigma}_i^2$ and $\mu_i$ given $(\theta_{t-1}, \kappa_{t-1}, \tau^2_{t-1}, \nu_{t-1})$ (equation (1) and (2)), and $C$ is a constant, which doesn’t contain any hyperparameters.  

$\sum_{i=0}^{m} E \left( \log(p(\mu_i | \sigma_i^2, \theta, \kappa)) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau^2_{t-1}, \nu_{t-1} \right)$ can be
maximized by $\theta_{opt}$ and $\kappa_{opt}$, which are

$$
\theta_{opt} = \sum_{i=0}^{m} \frac{\hat{\mu}_i(t-1)}{\tilde{\sigma}_i^2(t-1)} / \sum_{i=0}^{m} \frac{1}{\tilde{\sigma}_i^2(t-1)}
$$

$$
\kappa_{opt} = (m + 1) / \sum_{i=0}^{m} \left( \frac{1}{\kappa_{t-1} + n_i} + \frac{(\theta_{opt} - \hat{\mu}_i(t-1))^2}{\tilde{\sigma}_i^2(t-1)} \right)
$$

**Estimating $\tau^2$ and $\nu$** By taking posterior expectation of the third term of equation (3), we can get

$$
\sum_{i=c,0,1,...,m} E(\log(p(\sigma_i^2 | \nu, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \nu_{t-1})) | y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \nu_{t-1})
$$

$$
= \frac{(m + 2)\nu}{2} \log\left(\frac{\nu \tau^2}{2}\right) - \left(\frac{\nu}{2} + 1\right) \sum_{i=c,0,1,...,m} E(\log(\sigma_i^2) | y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \nu_{t-1}) - \frac{\tau^2 \nu}{2} \sum_{i=c,0,1,...,m} E\left(\frac{1}{\sigma_i^2} | y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \nu_{t-1}\right)
$$

$$
= \frac{(m + 2)\nu}{2} \log\left(\frac{\nu \tau^2}{2}\right) - \left(\frac{\nu}{2} + 1\right) \sum_{i=c,0,1,...,m} \left(\log\left(\frac{(\nu_{t-1} + n_i)\tilde{\sigma}_i^2}{2}\right) - \psi\left(\frac{\nu_{t-1} + n_i}{2}\right)\right) - \frac{\tau^2 \nu}{2} \sum_{i=c,0,1,...,m} \frac{1}{\tilde{\sigma}_i^2(t-1)} - (m + 2) \log(\Gamma\left(\frac{\nu}{2}\right))
$$

where $\Gamma(.)$ is gamma function and $\psi(.)$ is digamma function.

By setting
\[
\begin{align*}
\frac{\partial}{\partial \tau^2} \sum_{i=c,0,1,...,m} E \left( \log(p(\sigma_i^2|\tau^2, \nu)) \middle| \mathbf{y}, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \nu_{t-1} \right) &= 0 \\
\frac{\partial}{\partial \nu^2} \sum_{i=c,0,1,...,m} E \left( \log(p(\sigma_i^2|\tau^2, \nu)) \middle| \mathbf{y}, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \nu_{t-1} \right) &= 0
\end{align*}
\]
we got

\[
\begin{align*}
\sum_{i=c,0,1,...,m} \tau_{t-1}^2 - \frac{1}{\nu_{t-1}} &= 0 \\
(m + 2) \left( \log(\frac{\nu}{2}) + \log(\tau^2) - \psi(\frac{\nu}{2}) \right) - \sum_{i=c,0,1,...,m} \log\left( \frac{(\nu_{t-1}+n_i)\hat{\sigma}_{i(t-1)}^2}{2} \right) - \psi\left(\frac{\nu_{t-1}+n_i}{2}\right) &= 0
\end{align*}
\]
we got close form solution of the above equations by using approximation of digamma function, which is \( \psi\left(\frac{\nu}{2}\right) \approx \log(\frac{\nu}{2}) - \frac{1}{\nu} - \frac{1}{3\nu^2} \).

\[
\tau_{opt}^2 = \frac{m + 2}{\sum_{i=c,0,1,...,m} \frac{1}{\hat{\sigma}_{i(t-1)}^2}} \\
\nu_{opt} = \frac{2}{3\sqrt{1 + \frac{4}{3}T - 1}}
\]
where \( T = \frac{1}{m+2} \sum_{i=c,0,1,...,m} \left( \log\left( \frac{(\nu_{t-1}+n_i)\hat{\sigma}_{i(t-1)}^2}{2} \right) - \psi\left(\frac{\nu_{t-1}+n_i}{2}\right) \right) - \log(\tau_{opt}^2) \).

**Hyperparameters estimation for null model**

For hierarchical model with control data, we denote pooled Box-Cox transformed IPD of native and control data as \( \mathbf{y}_p \) (i.e. \((y_{c1}, y_{c2}, \ldots, y_{cn_c}, y_{01}, y_{02}, \ldots, y_{0m_0})\)).

For hierarchical model without control data, we simply let \( \mathbf{y}_c \), Box-Cox transformed IPD of native sample, equal to \( \mathbf{y}_p \), because it is a special case of
hierarchical model with control data, in which $y_0$ is empty. We assume $y_p$ follows a normal distribution

$$y_p \sim N(\mu_p, \sigma_p^2)$$

and $(\mu_p, \sigma_p^2), (\mu_1, \sigma_1^2), (\mu_2, \sigma_2^2), \ldots, (\mu_m, \sigma_m^2)$ have the same prior distribution, which is

$$p(\sigma_i^2 | v, \tau^2) = scaled \ inverse - \chi^2(v, \tau^2)$$

$$p(\mu_i | \sigma_i^2, \theta, \kappa) = N(\theta, \frac{\sigma_i^2}{\kappa})$$

where, $i = p, 1, \ldots, m$. posterior distribution of $\mu_i$ and $\sigma_i^2$, $i = p, 1, \ldots, m$, are

$$p(\mu_i | y_i, \sigma_i^2, \theta, \kappa) = N\left(\frac{\kappa}{\kappa + n_i} \theta + \frac{n_i}{\kappa + n_i} \bar{y}_i, \frac{\sigma_i^2}{\kappa + n_i}\right)$$

$$p(\sigma_i^2 | y_i, v, \tau^2) = scaled \ inverse - \chi^2(v + n_i, \tilde{\sigma}_i^2)$$

where

$$\tilde{\sigma}_i^2 = \frac{1}{v + n_i} \left(v \tau^2 + (n_i - 1)s_i^2 + \frac{\kappa n_i}{\kappa + n_i} (\bar{y}_i - \theta)^2 \right)$$
[1]. We used posterior expectations of $\mu_i$ and $\sigma^2_i$ as their estimations, which are

$$
\hat{\mu}_i = E(\mu_i | y_i, \theta, \kappa) = \frac{\kappa}{\kappa + n_i} \theta + \frac{n_i}{\kappa + n_i} \bar{y}_i
$$

$$
\hat{\sigma}^2_i = E(\sigma^2_i | y_i, \nu, \tau^2) = \frac{n_i + \nu}{n_i + \nu - 2} \tilde{\sigma}^2_i
$$

where $i = p, 1, ..., m$. Like the previous section, we adopt a EM algorithm (Algorithm 1) to maximize marginal log-likelihood function $L(y_p, y_1, ..., y_m; \theta, \kappa, \nu, \tau^2)$. In the EM procedure, $\mu_i$ and $\sigma^2_i$ were regarded as missing data, and the log-likelihood function with the complete data was

$$
l(y, \mu, \sigma^2; \theta, \kappa, \tau^2, \nu) = \log(p(y | \mu, \sigma^2)) + \sum_{i=p,1, ..., m} \log(p(\mu_i | \sigma^2_i, \theta, \kappa)) + \sum_{i=p,1, ..., m} \log(p(\sigma^2_i | \tau^2, \nu))$

(4)

where $y = (y_p, y_1, ..., y_m)$, $\mu = (\mu_p, \mu_1, ..., \mu_m)$ and $\sigma^2 = (\sigma^2_p, \sigma^2_1, ..., \sigma^2_m)$. 

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Estimating $\theta$ and $\kappa$  By taking posterior expectation of the second term of equation (4), we can get

$$\sum_{i=p,1,\ldots,m} E(\log(p(\mu_i|\sigma_i^2, \theta, \kappa)) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \upsilon_{t-1})$$

$$= -\frac{\kappa}{2} \sum_{i=p,1,\ldots,m} \left( \frac{1}{\kappa_{t-1} + n_i} + \frac{(\theta - \hat{\mu}_{i(t-1)})^2}{\tilde{\sigma}_{i(t-1)}^2} \right) - \frac{m+1}{2} \log(\kappa) + C$$

Thus, $\sum_{i=p,1,\ldots,m} E(\log(p(\mu_i|\sigma_i^2, \theta, \kappa)) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \upsilon_{t-1})$ can be maximized by

$$\theta_{opt} = \sum_{i=p,1,\ldots,m} \frac{\hat{\mu}_{i(t-1)}}{\tilde{\sigma}_{i(t-1)}^2} / \sum_{i=0}^m \frac{1}{\tilde{\sigma}_{i(t-1)}^2}$$

$$\kappa_{opt} = (m+1) / \sum_{i=p,1,\ldots,m} \left( \frac{1}{\kappa_{t-1} + n_i} + \frac{(\theta_{opt} - \hat{\mu}_{i(t-1)})^2}{\tilde{\sigma}_{i(t-1)}^2} \right)$$

Estimating $\tau^2$ and $\upsilon$  By taking posterior expectation of the third term of equation (4), we can get

$$\sum_{i=p,1,\ldots,m} E(\log(p(\sigma_i^2|\tau^2, \upsilon)) \mid y, \theta_{t-1}, \kappa_{t-1}, \tau_{t-1}^2, \upsilon_{t-1})$$

$$= \frac{(m+1)\upsilon}{2} \log\left(\frac{\upsilon\tau^2}{2}\right) - \left(\frac{\upsilon}{2} + 1\right) \sum_{i=p,1,\ldots,m} \left( \log\left(\frac{(\upsilon_{t-1} + n_i)\tilde{\sigma}_{i(t-1)}^2}{2}\right) - \psi\left(\frac{\upsilon_{t-1} + n_i}{2}\right) \right) - \frac{\tau^2\upsilon}{2} \sum_{i=p,1,\ldots,m} \frac{1}{\tilde{\sigma}_{i(t-1)}^2} - (m+1) \log(\Gamma\left(\frac{\upsilon}{2}\right))$$

(5)
By using approximation of digamma function, which is \( \psi\left(\frac{\nu}{2}\right) \approx \log\left(\frac{\nu}{2}\right) - \frac{1}{\nu} - \frac{1}{3\nu^2} \), (5) can be maximized by

\[
\tau_{\text{opt}}^2 = \frac{m + 1}{\sum_{i=p,1,\ldots,m} \frac{1}{\sigma_i^2(t-1)}}
\]

\[
\nu_{\text{opt}} = \frac{2}{3\left(\sqrt{1 + \frac{4}{3}T - 1}\right)}
\]

where \( T = \frac{1}{m+1} \sum_{i=p,1,\ldots,m} \left( \log\left(\frac{(v_{t-1}+n_i)^2}{2}\right) - \psi\left(\frac{v_{t-1}+n_i}{2}\right) \right) - \log(\tau_{\text{opt}}^2). \)

References