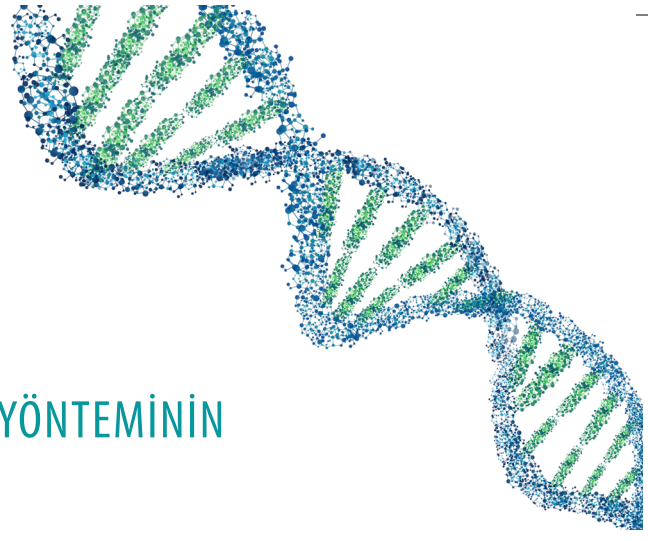


BÖLÜM 10

DİJİTAL POLİMERAZ ZİNCİR REAKSİYON YÖNTEMİNİN PRENSİPLERİ VE AVANTAJLARI



Pınar YURDAKUL MESUTOĞLU *

Çağatay ACUNER **

Kantitatif polimeraz zincir reaksiyonunun (qPCR), birçok bilim insanınca sanıldığından daha karmaşık olduğu bildirilmiştir¹. Bu bölümde, geleneksel PCR ve gerçek zamanlı PCR'dan (Rt-PCR) sonraki üçüncü nesil ve en güncel qPCR yöntemi olarak, dijital (sayısal) polimeraz zincir reaksiyonu (tepkimesi) (dPCR) yönteminin temel prensipleri ve göreceli avantajları açıklanacaktır. Dijital polimeraz zincir reaksiyonu yönteminin^a temel olarak dayandığı üç prensip şunlardır: 1- klinik örnekteki sayımı yapılmak istenen molekülün, sınırlayıcı seyreltme ("limiting dilution", bölümlenme, bölüntü, "partition") tekniğiyle birim bölüntü hacmindeki sayısının azaltılması (açk. dPCR için seyreltme birim bölüntü hacmi öylesine küçük olmalıdır ki, ideal olarak her bir birim hacimde ya tek molekül olmalı ya da hiç olmamalıdır; böylelikle her bir birim hacimde "var"

^a Laboratuvar yöntemi, tekniği, teknolojisi, deney, test, teknolojik platform (aygıt sistemi) tanımlarının ayırımı için bkz. Acuner İ.Ç. *Laboratuvarda teknoloji seçimi. 4. Ulusal Viroloji Kongresi, 23-26 Haziran, Türk Mikrobiyoloji Cemiyeti ve Klinik Mikrobiyoloji Uzmanları Derneği, İstanbul. Kongre Kitabı, s: (2011).*

ya da "yok" gözlemi yapılabilmelidir; tarihsel olarak dijital teknolojiler, Claude Shannon'un 1948'de geliştirdiği enformasyon teorisi çerçevesinde, iki değerli bilişi birimi "bit" [0, 1; ilet var veya yok] temelinde kurgulanmıştır; sayısal/dijital adlandırmasının rasyoneli^b),

^b Bir laboratuvar testinde çıktı ("output") olarak (açk. aslında herhangi bir bilimsel gözlem/deney sonucunda) elde edilebilecek veri ("data") (açk. ham/işlenmemiş çıktılar/sonuçlar) türleri*:

- Kategorik Veri (Niteliksel) ("Categorical/Qualitative Data")
 - Binomial Kategorik Veri (Dikotomik veri; var/yok, pozitif/negatif)
 - Multinomial (>2 Kategori; örn. Antimikrobiyal Duyarlılık Testi Sonuçları; Duyarlı, Arada -artmış maruziyetle duyarlı-, Dirençli)
- Sayısal Veri (Niceliksel) ("Numerical/Quantitative Data")
 - Ayrışık Sayısal Veri ("Discrete Numerical Data"; örn. 1/10, 1/20, ...; 1+, 2+, ...; 0, 1, 2, ...)
 - Sürekli Sayısal Veri ("Continuous Numerical Data"; örn. 60 - 500 mg/dL arasında herhangi bir değer; 110,50 mg/dL)
- Dijital PCR yönteminde, her bir bölüntüde amplifikasyon var/yok biçimindeki "binomial kategorik veri", "ayrışık sayısal veri" ("binary discrete numerical data"; 0/1) biçiminde dönüştürülerek sayılaştırılmakta ve sayısallaştırılmaktadır (açk. "digitization", "digitalization"; bu iki kavram/terim farklıdır; sırasıyla, kategorik verinin sayılaştırılması, sayılaştırılmış ham verinin sayısal işlenmesi; dPCR yönteminde her iki süreç de gereklidir ve uygulanmaktadır).

*Acuner İ.Ç., Gürol Y., Çelik G. "Klinik laboratuvarın klinik karar vermeye katkısı ve klinik mikrobiyoloji laboratuvarında yorumlayıcı raporlama." *Yeditepe Hastanesi Mikrobiyoloji Laboratuvarı, Bülten-2, İstanbul. (2011).*

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Sağlık hizmetindeki gereksinimler ve moleküler biyoloji alanındaki araştırma yönelimleri nedeniyle; bu alandaki rekabetin artması, maliyetlerin daha uygun olması, kullanıcı kolaylığı bakımından tam otomasyona uyarlanmış cihaz sistemlerinin yaygınlaşması, aynı anda çalışabilen numune sayısının ve saatteki çıktı sayısının artması, taşınabilir hasta başı test cihaz sistemlerinin ("Point of Care Testing, POCT") geliştirilmesi ve kullanım alanlarının genişlemesi beklenmektedir^{24,106}. Dijital PCR teknolojisindeki gelişmelerle, yine tek molekül analizi duyarlılığına sahip, yüksek çıktı sayılı, yeni nesil dizileme teknolojisindeki gelişmelerin, birbirini desteklediği düşünülmektedir¹⁰⁶.

Deney tasarımı, optimizasyonu, validasyonu, verifikasyonu, araştırma sonuçlarının bildirim ve test sonucu raporlama bakımlarından standardizasyonu yapıldığında, dPCR, nükleik asitler için yüksek kesinlik ve doğrulukta bir "mutlak niceliksel değer" saptama yöntemi olarak, birçok uygulama alanında kullanılabilme potansiyeline sahiptir⁵⁹.

Kaynaklar

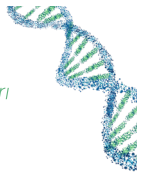
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