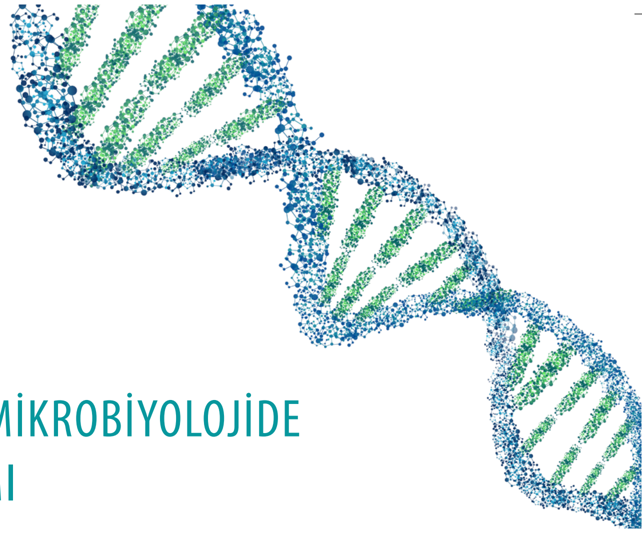

BÖLÜM 5

SENTETİK NÜKLEİK ASİTLERİN TANISAL MİKROBİYOLOJİDE KULLANIMI - OLİGONÜKLEOTİT TASARIMI

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Geçen yüzyılın ortalarında, oligonükleotitlerin ve ardından fonksiyonel genlerin laboratuvarda sentezi, kimyasal biyoloji alanındaki en önemli gelişmeler arasında sayılmaktadır [1,2]. Yöntemin geliştirilmeye başlandığı yıllarda, polinükleotitlerin kimyasal yöntem ile sentezi, çift sarmal DNA yapısının aydınlatılması, genetik şifrenin çözülmesi gibi temel sorulara yanıt bulunmasında kilit rol oynamıştır.

Günümüzde sentetik nükleik asitler, moleküler biyolojik araştırma, sentetik biyoloji, tanı ve son zamanlarda tedavi alanlarında yoğun şekilde kullanılmaktadır. Oligonükleotitlerin polimeraz zincirleme tepkimesi ve rekombinant DNA gibi diğer teknolojiler ile birlikte kullanımı ve giderek genişlemekte olan kullanım alanlarından bazıları aşağıda verilmiştir (Şekil 1):

- Canlı hücrelerin gen ifadesinin ya da hücresel fonksiyonlarının modülasyonunda kullanılacak nükleik asitlerin üretimi[3]
    - RNA interferansı
    - RNAaz-H aracılı transkript degradasyonu
    - Uçbirleştirme (splicing) modülasyonu
    - Programlanmış gen düzenlenmesi (gene editing) [4]
- Gen ve genomların üretimi
    - İfade vektörlerine klonlanarak aktarılan genlerin in-vivo ifadesi ile hücre fabrika teknolojileri
    - In-vitro transkripsiyon ve translasyon teknolojileri ile protein üretimi [5].
    - Bakteriyel ve viral genomların *in-vitro* sentezi [6].
- Yüksek afiniteli nükleik asit ligand kütüphanelerinin oluşturulması ile aptamer üretimi ve seçilimi [7].
- In-vitro ya da ex-vivo nükleik asit birleştirme yöntemleri ile uzun gen segmentlerinin oluşturulması "Sequence and Ligase Independent Cloning" (SLIC)" [8].
- "Seamless Ligation Cloning Extract (SLICE)", Gibson birleştirme tepkimesi [9].

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Tablo 6: Polimeraz zincirleme tepkimesinde kullanılan termostabil enzimler ve ekzonükleaz özellikleri

DNA polimeraz	Biyolojik kaynağı	3' → 5' ekzonükleaz (sağlamalı okuma)	5' → 3' ekzonükleaz
Pfu	*Pyrococcus furiosus*	+	-
Pfu + Sso7d (kimerik)		+	-
Pfu (exo-)		-	-
Psp	*Pyrococcus spp.*	+	-
Psp (exo-)		-	-
Pwo	*Pyrococcus wpesei*	+	-
Taq	*Thermus aquaticus*	-	+
Taq (N-terminal delesyonlu, Stoffel fragmanı)		-	-
Taq Phe667 → Tyr		-	+
Tbr	*Thermus brocianus*	?	+
Tfl	*Thermus flavus*	?	-
Tli	*Thermococcus litoralis*	+	-
Tli (exo-)		-	-
Tma	*Thermotoga maritima*	+	-
Tth	*Thermus thermophilus*	-	+

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