Module1：实时荧光定量PCR技术原理

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什么是实时荧光定量PCR?

- 一种实时监控核酸扩增的技术

  在PCR反应体系中加入荧光基团，利用荧光信号的变化对PCR过程进行实时监控，以此实现对初始模板的定量分析。

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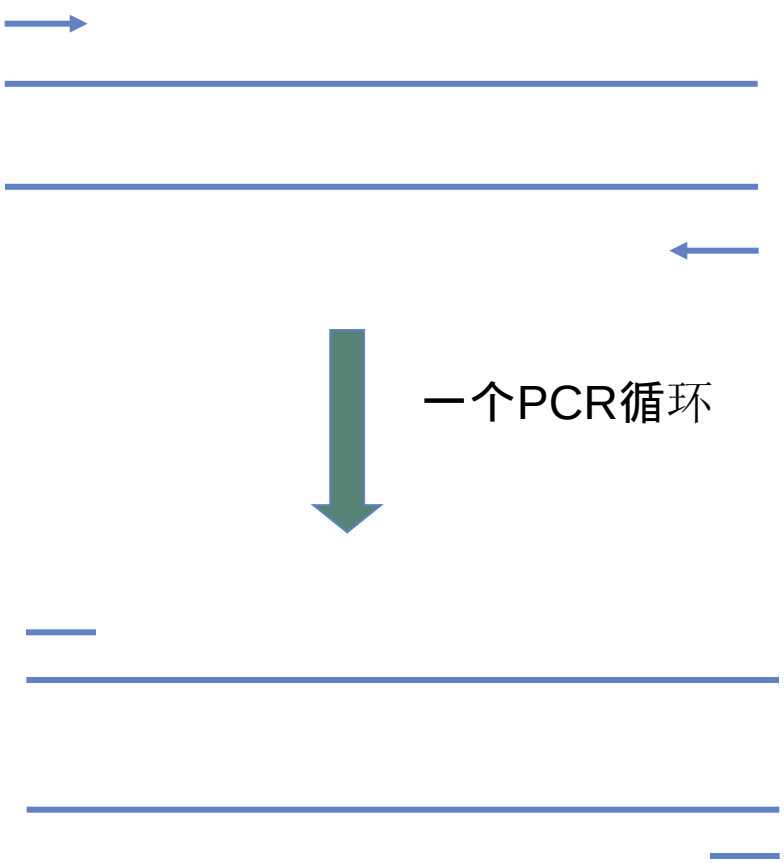
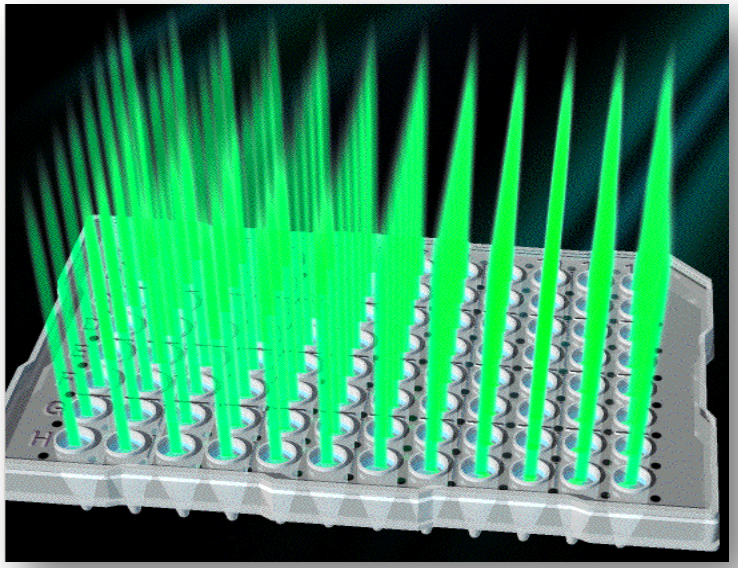
定量PCR如何工作?



- PCR反应液加入了

  各种类型的荧光标记物



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定量PCR如何工作?

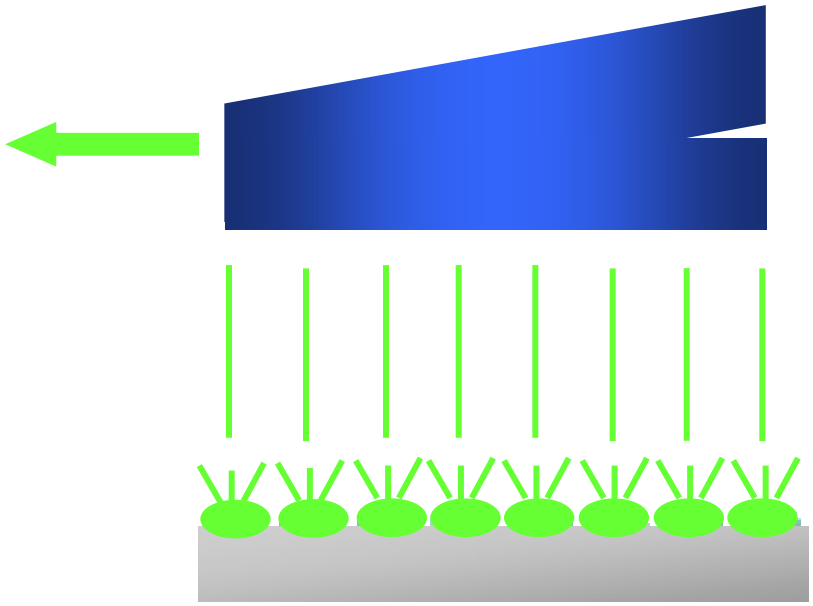
- 在扩增过程中,荧光信号随着PCR产物的增加而增强

定量PCR仪如何工作?

- 所有的定量PCR仪器都有三个共同的组成部分

2. 光路系统

3. 检测器



1. 热循环模块

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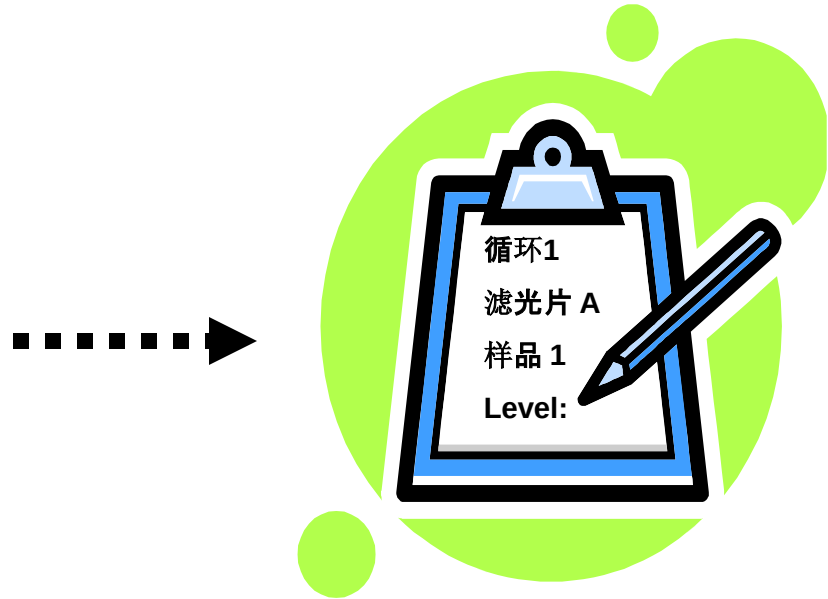
定量PCR如何工作?

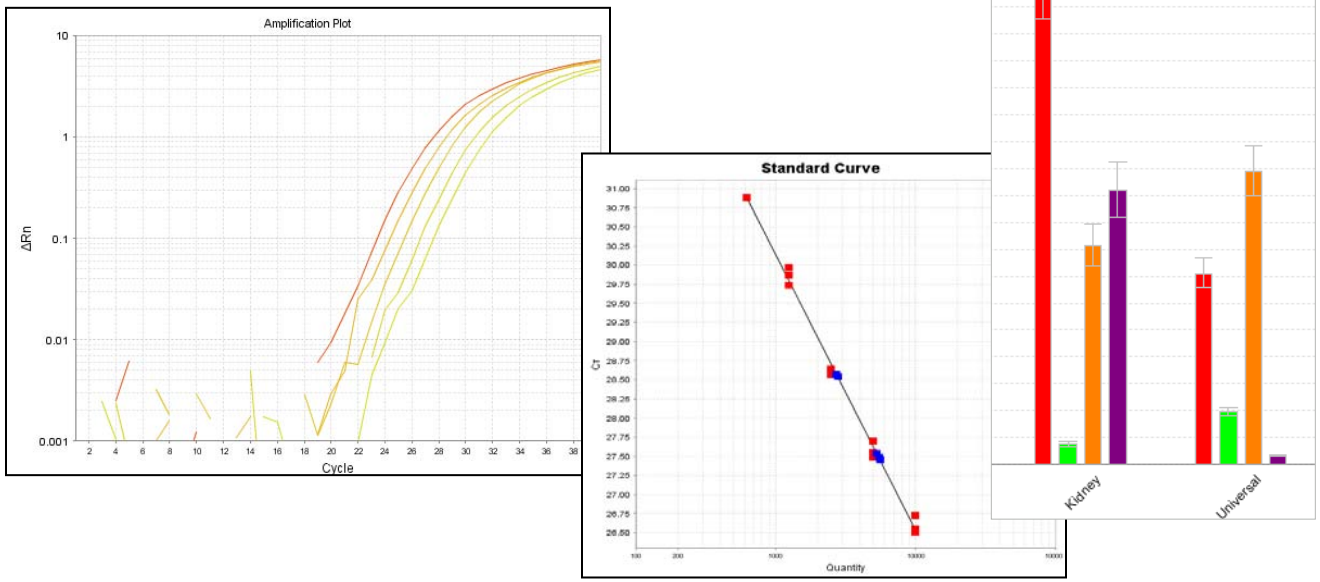
- 每个循环结束后, 定量PCR仪器通过光学系统记录荧光信号的增加



定量PCR如何工作?

- 最后, 定量PCR软件计算出数据，用于实验结果的分析

A7	☐	Liver	c-myc	UNKNOWN	...	20.6095	20.532211	...	7.386	1.057	86.438	-3.696	1.726
A8	☐	Liver	c-myc	UNKNOWN	...	20.454922	20.532211	...	8.133	1.057	86.438	-3.696	1.726
A9	☑	Liver	c-myc	UNKNOWN	...								
A...	☐	Liver	GAPDH	UNKNOWN	...	28.380135	28.455734	...	0.469		92.853	-3.506	
A...	☐	Liver	GAPDH	UNKNOWN	...	28.464178	28.455734	...	0.444		92.853	-3.506	
A...	☐	Liver	GAPDH	UNKNOWN	...	28.522894	28.455734	...	0.427		92.853	-3.506	
B1	☐	Kidney	c-myc	UNKNOWN	...	21.135685	21.461496	...	5.322	1.111	86.438	-3.696	1
B2	☐	Kidney	c-myc	UNKNOWN	...	21.555302	21.461496	...	4.098	1.111	86.438	-3.696	1
B3	☐	Kidney	c-myc	UNKNOWN	...	21.693499	21.461496	...	3.76	1.111	86.438	-3.696	1
B4	☐	Kidney	GAPDH	UNKNOWN	...	28.479626	28.505972	...	0.439		92.853	-3.506	
B5	☐	Kidney	GAPDH	UNKNOWN	...	28.518414	28.505972	...	0.428		92.853	-3.506	
B6	☐	Kidney	GAPDH	UNKNOWN	...	28.51987	28.505972	...	0.428		92.853	-3.506	



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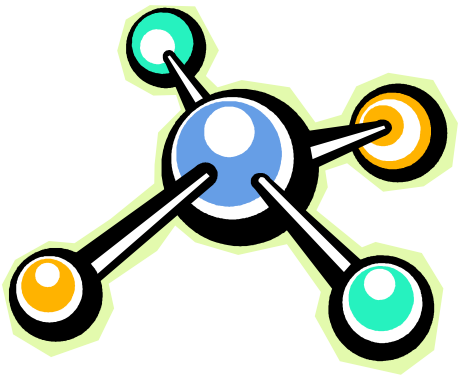
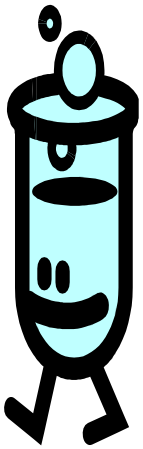
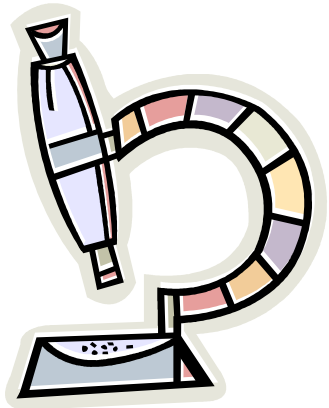


定量PCR的数学原理





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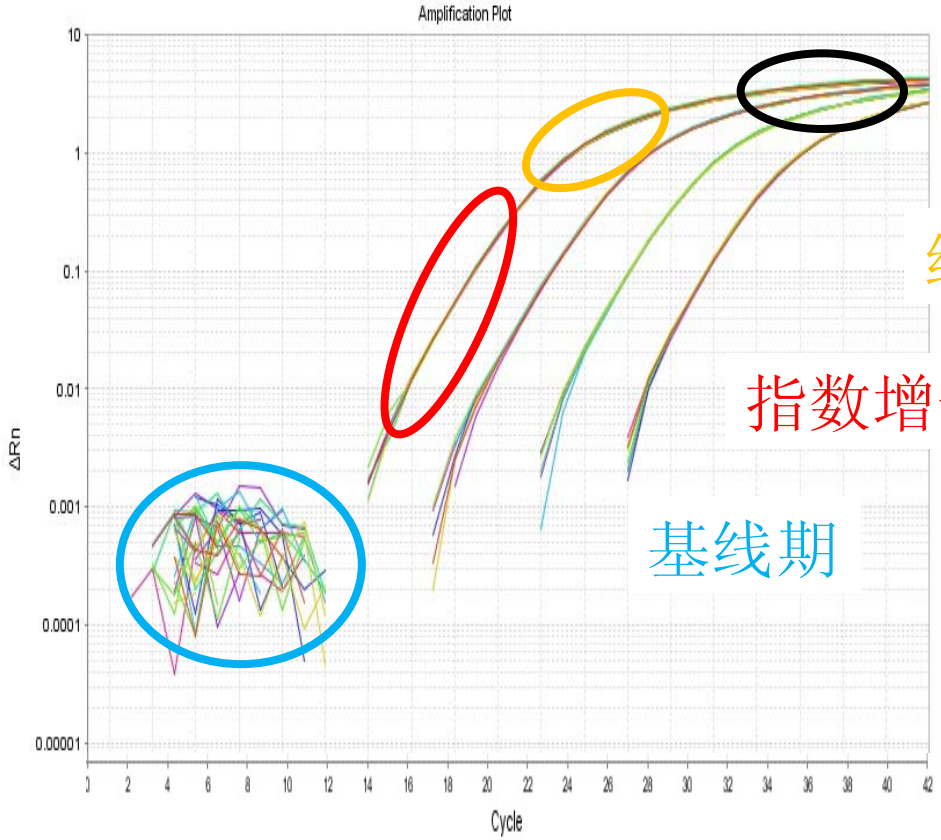
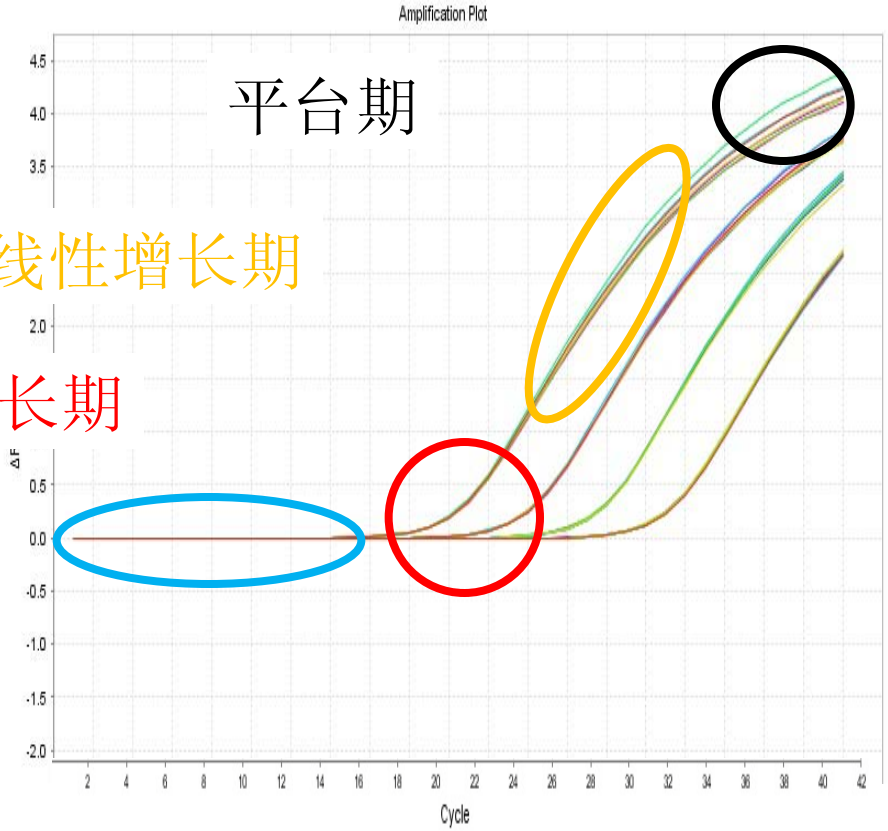
PCR动力学曲线和四个阶段





对数图谱

线性图谱

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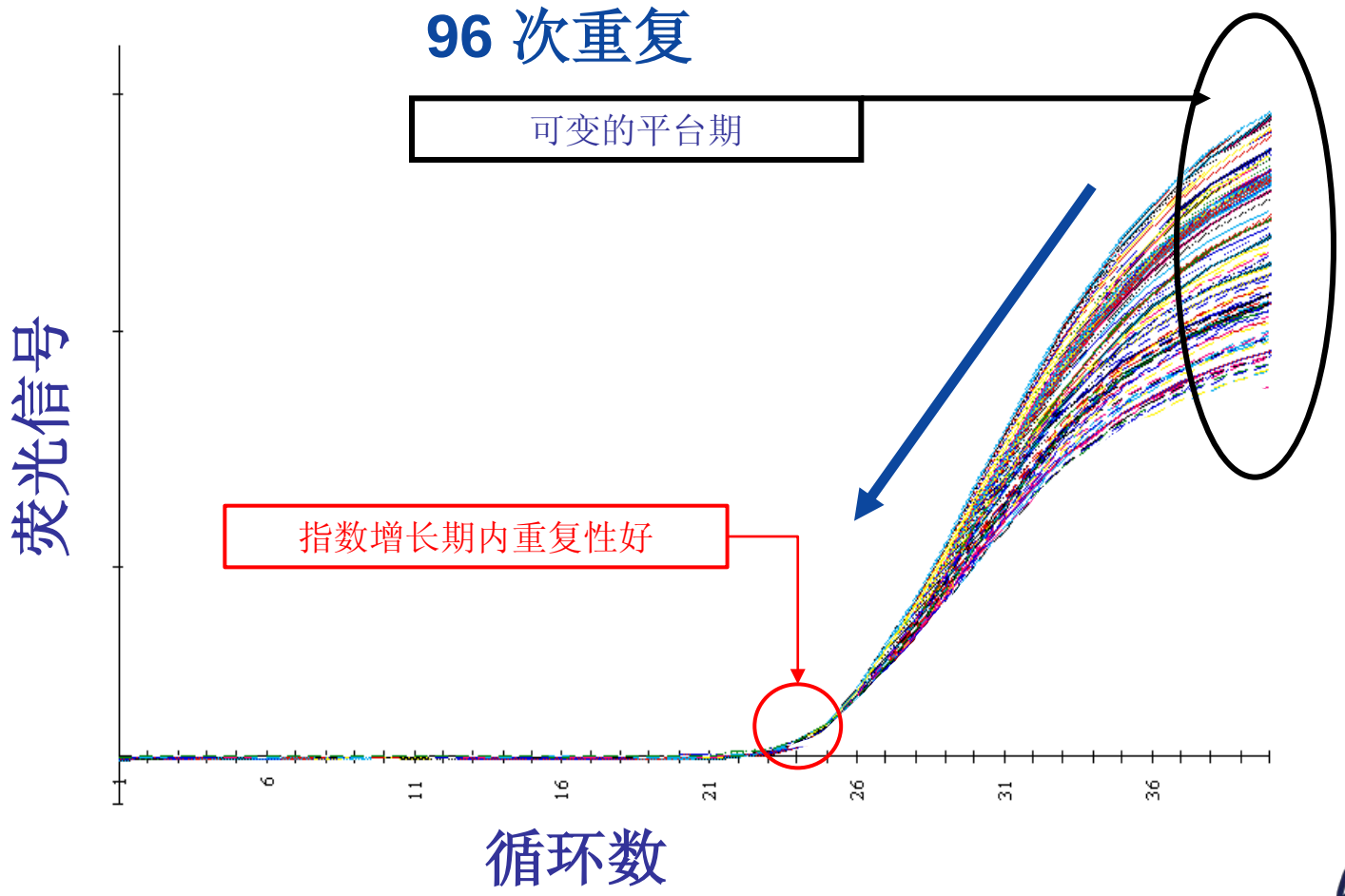
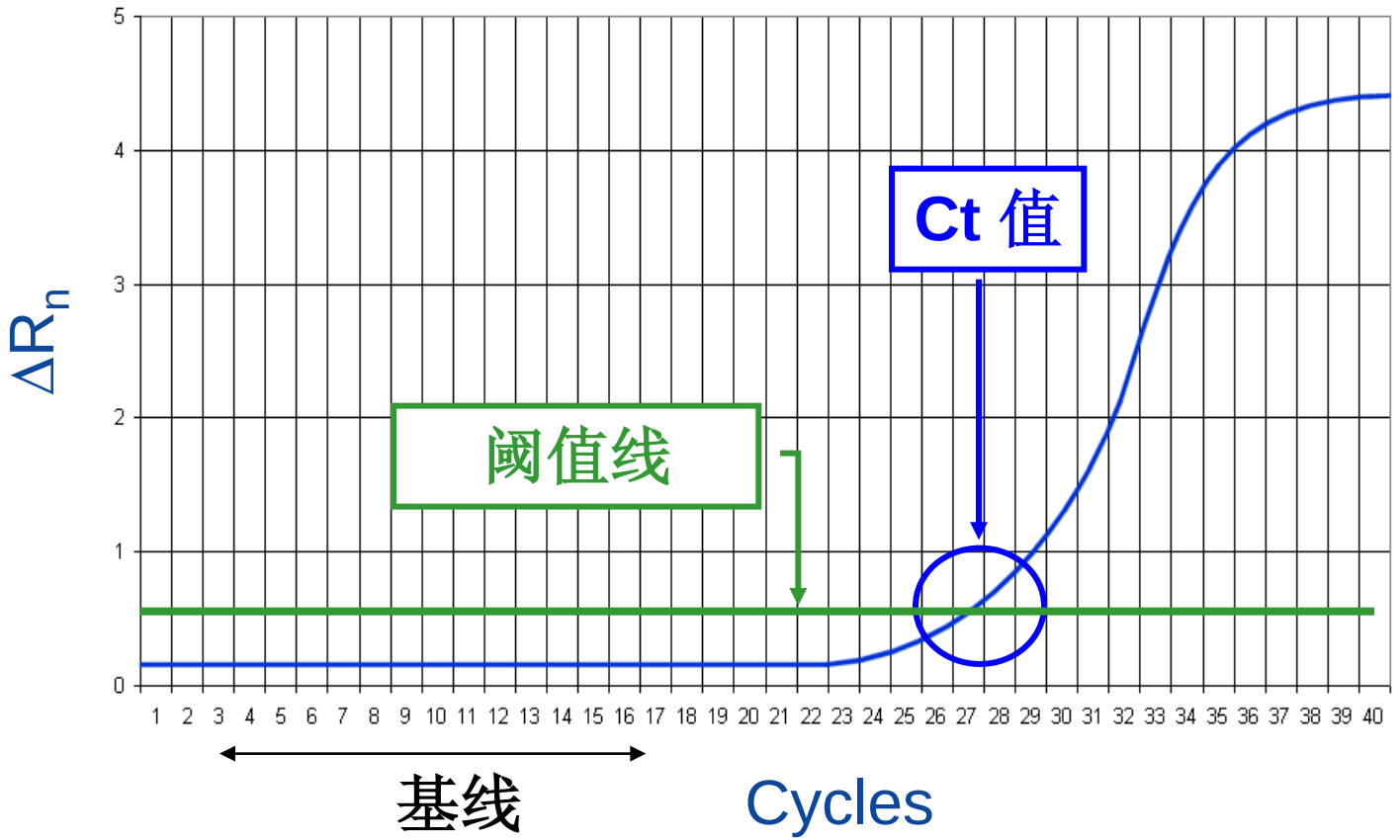
指数增长期内扩增曲线具有高度重复性



定量PCR扩增曲线的各种概念



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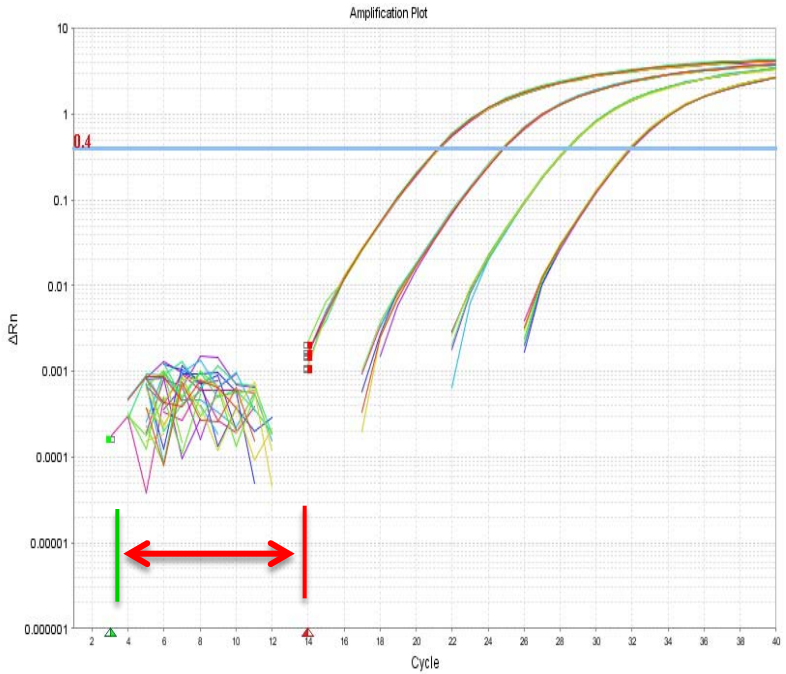
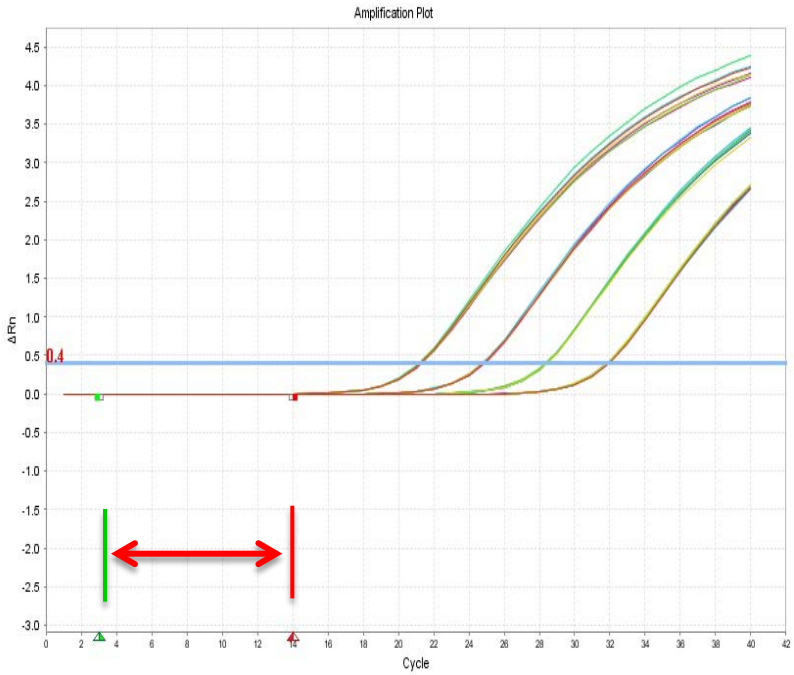
什么是基线？

- 基线是扩增曲线的水平部分



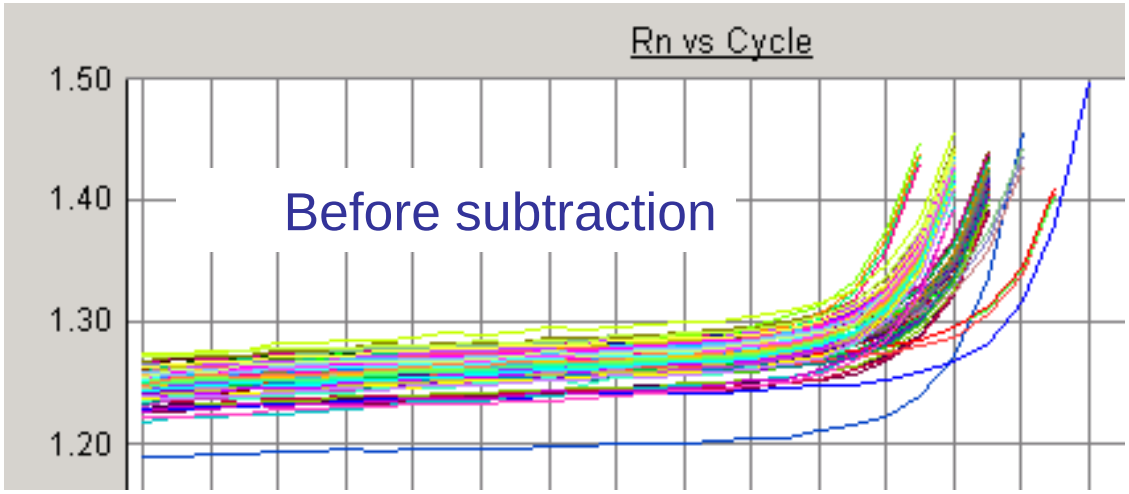
基线 对数图谱



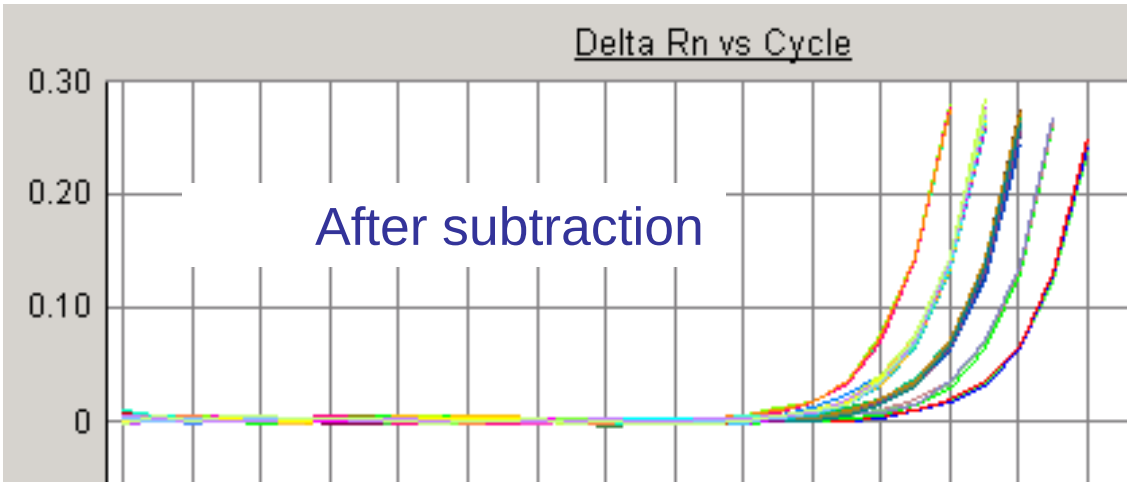
基线 线性图谱

基线扣除

Rn



ΔRn



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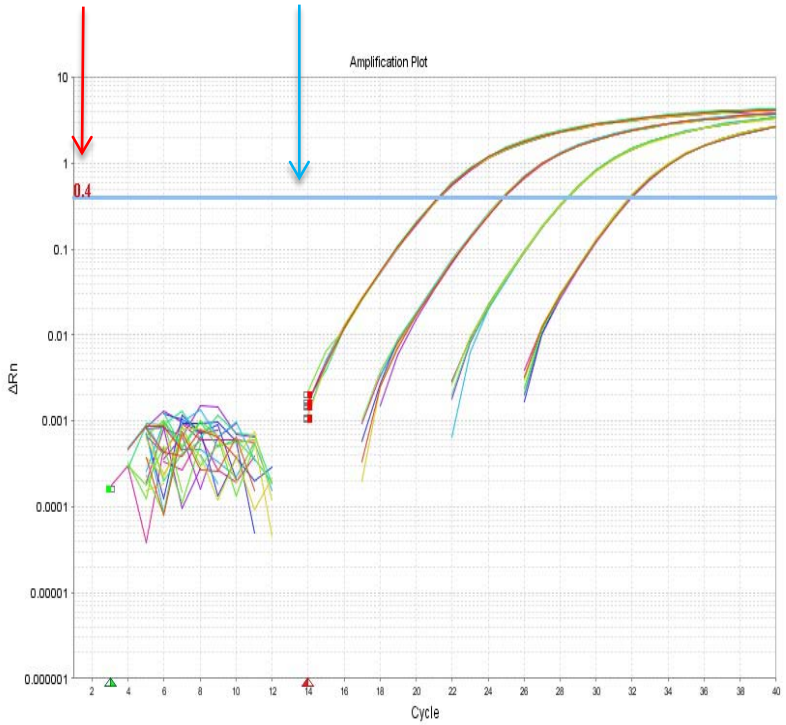
什么是阈值？

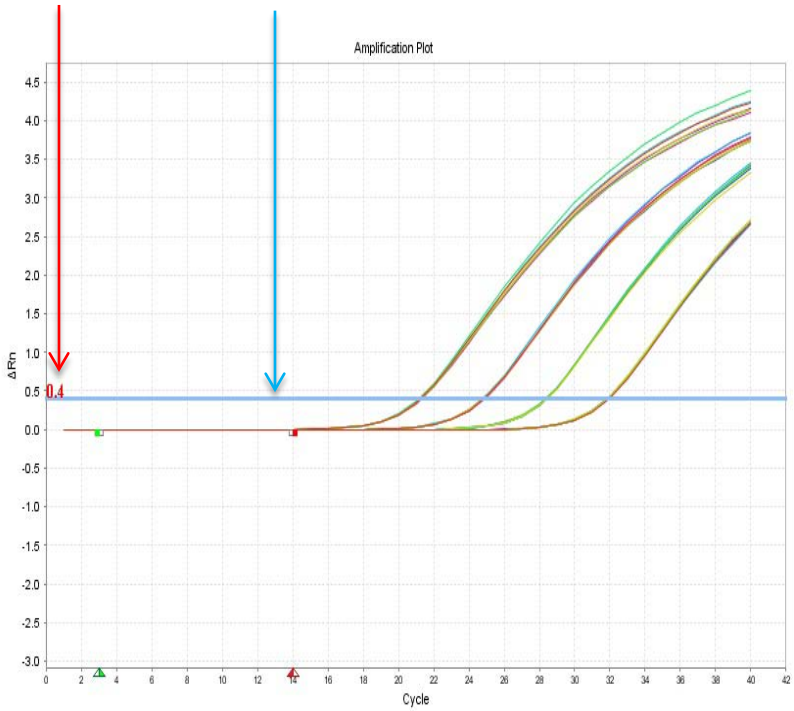
- 阈值是一个荧光强度值，穿过阈值与X轴平行的直线称为阈值线

阈值　　阈值线



对数图谱

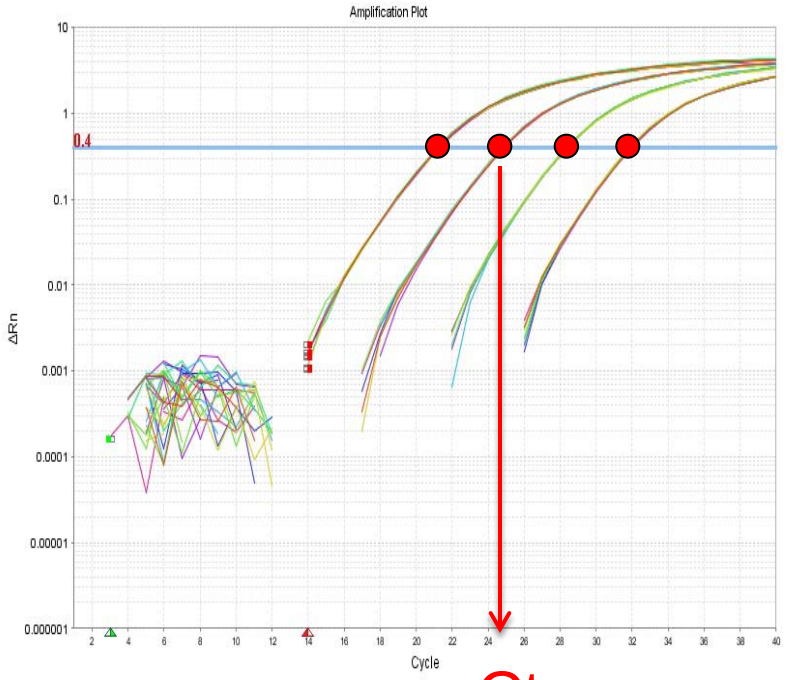
阈值　　阈值线



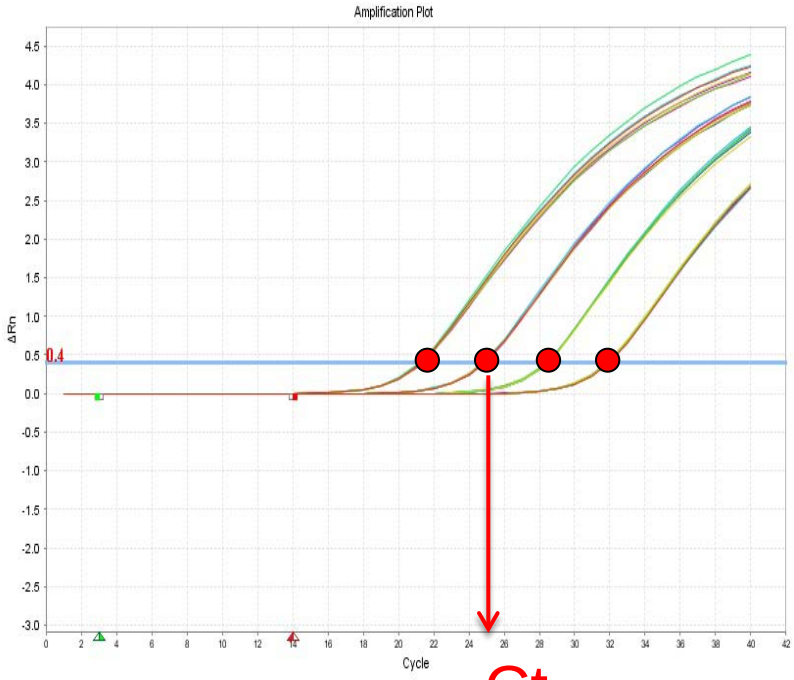
线性图谱

什么是Ct值？

- Ct值是阈值线与扩增曲线的交点对应在X轴上的值



对数图谱

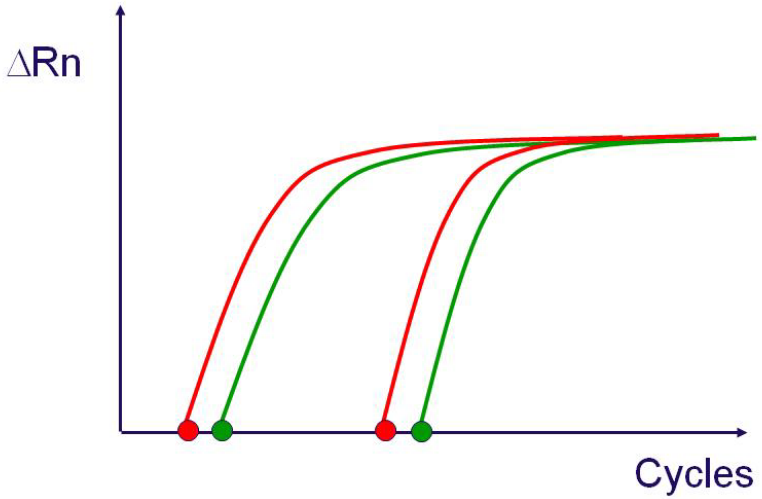


线性图谱

如何使用Ct值计算结果?

绝对定量

相对定量



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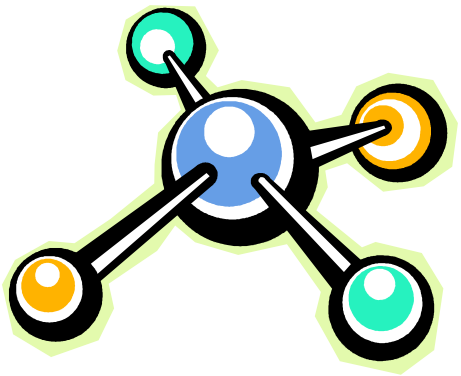
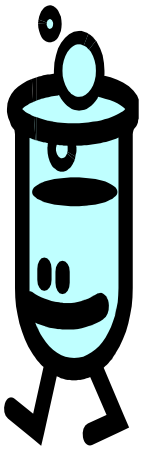
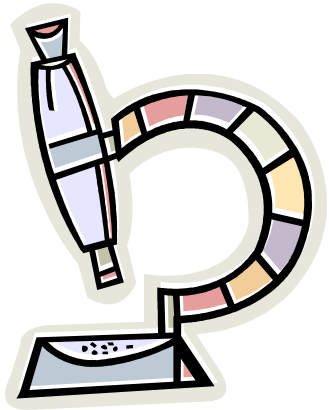


定量PCR的化学原理





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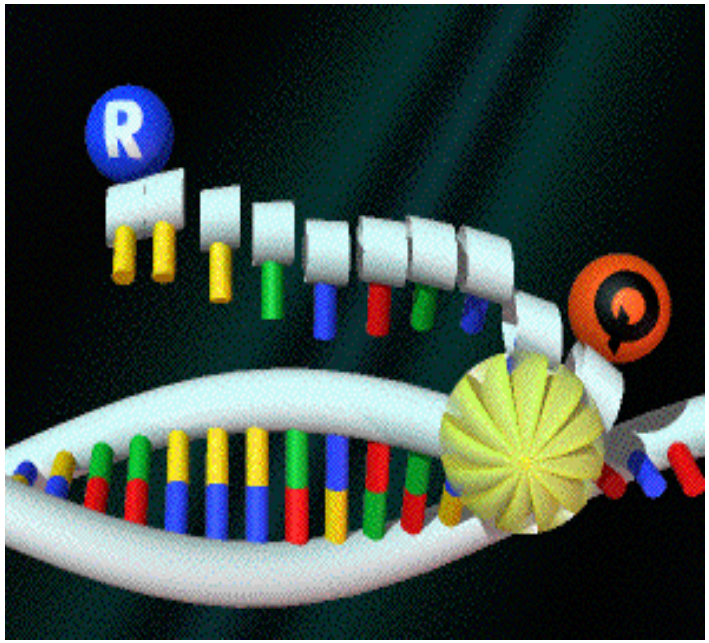
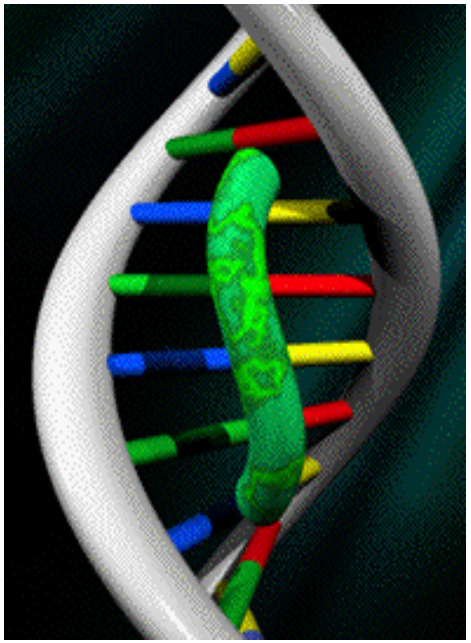
两种化学方法

TaqMan探针法



染料法



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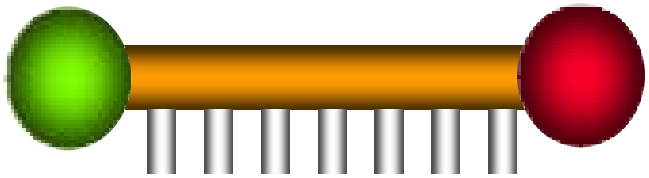
TaqMan探针法

- TaqMan水解探针：5′ 端 荧光报告基团

  与靶基因特异性结合的序列

  3′ 端 荧光淬灭基团

报告基团　　淬灭基团



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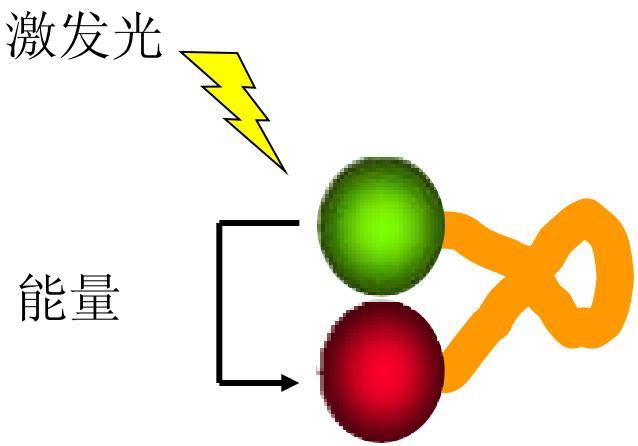
TaqMan水解探针作用机理

- 完整的探针：

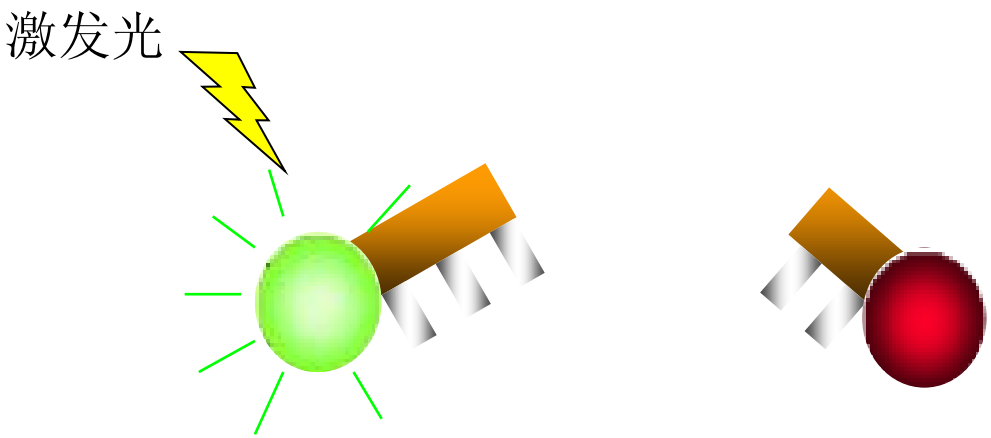
检测不到报告荧光



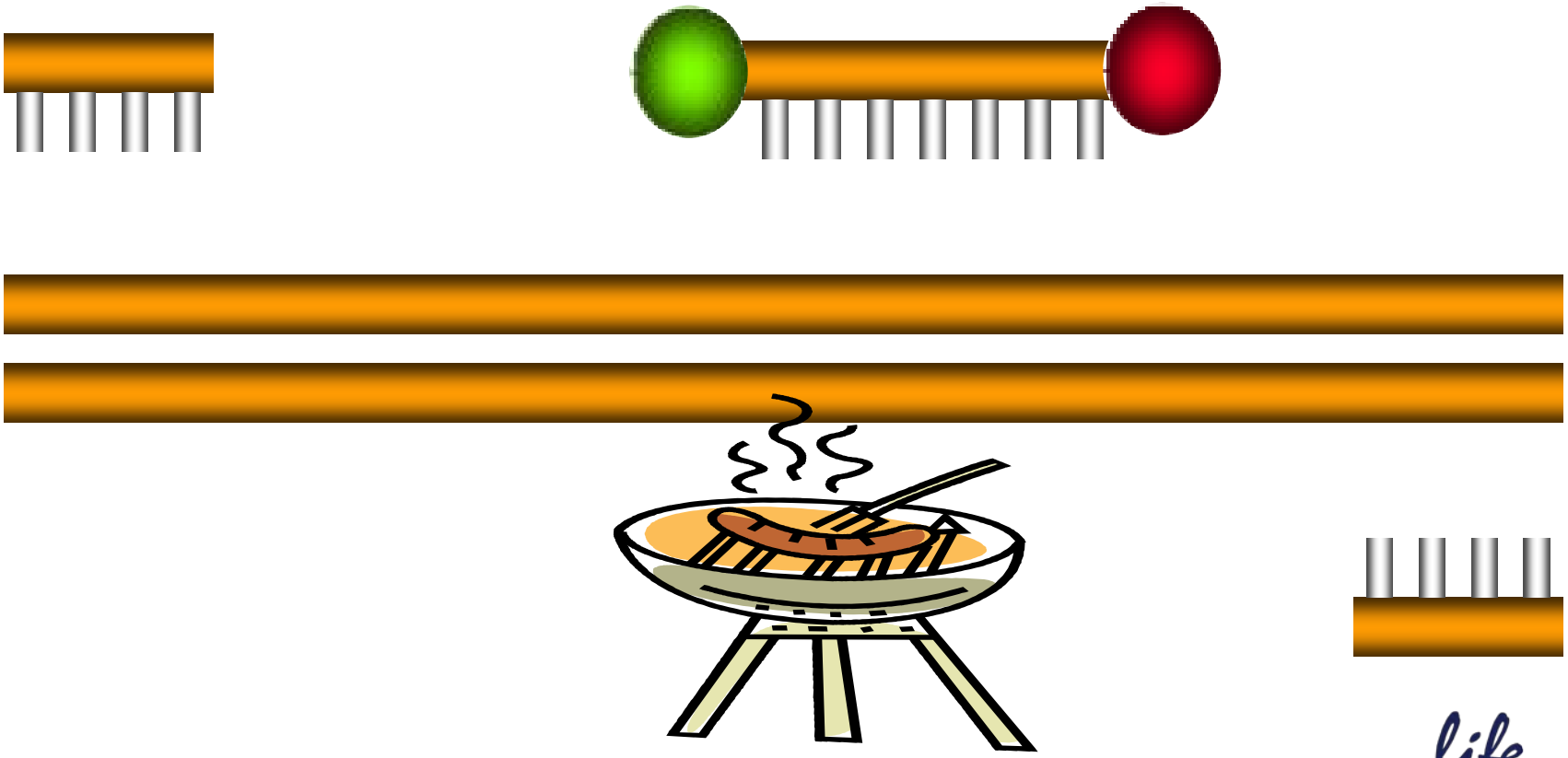
荧光共振能量转移，FRET

- 切断的探针：

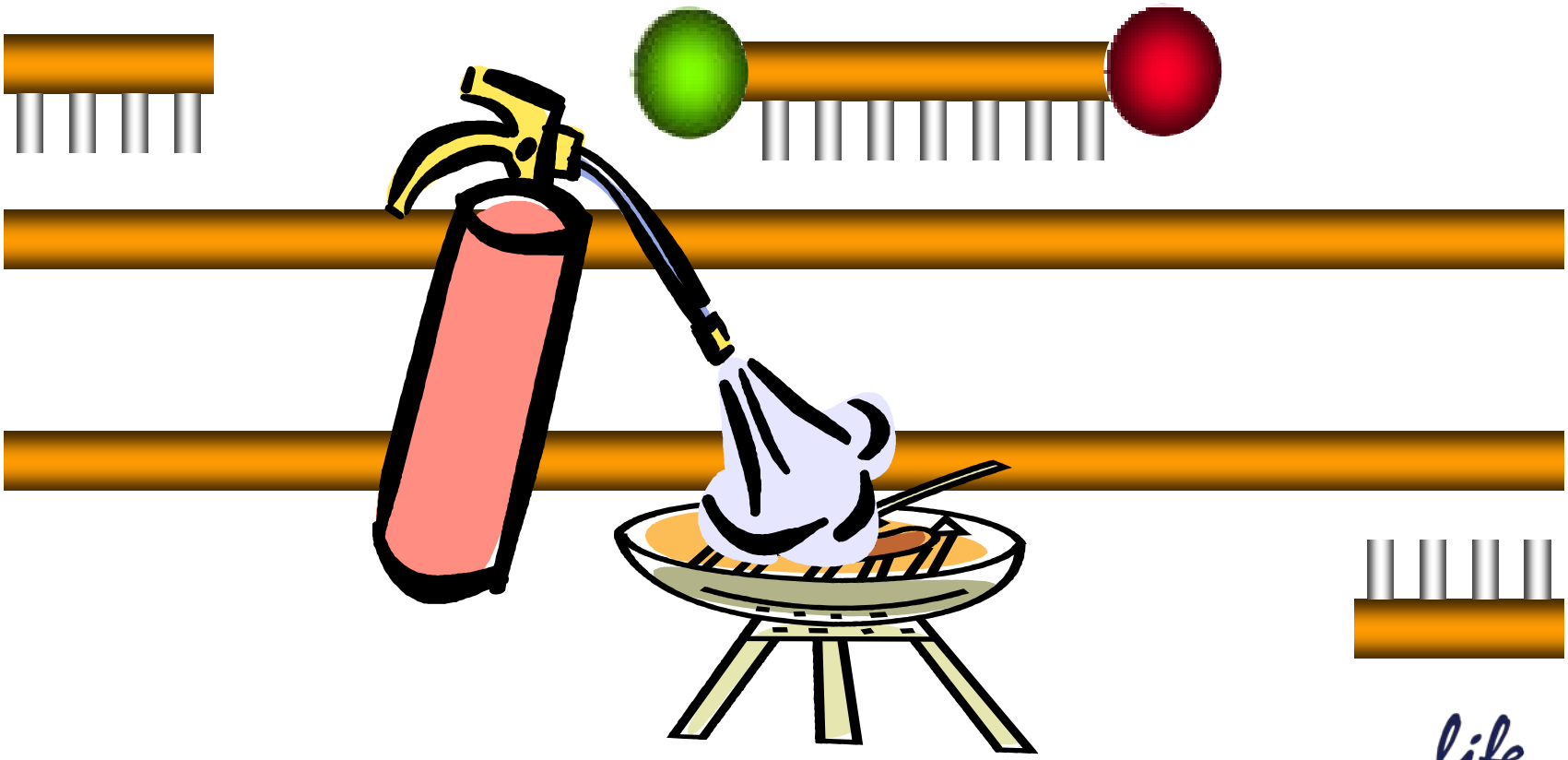
检测到报告荧光



变性 (95ºC)



退火 (60°C)



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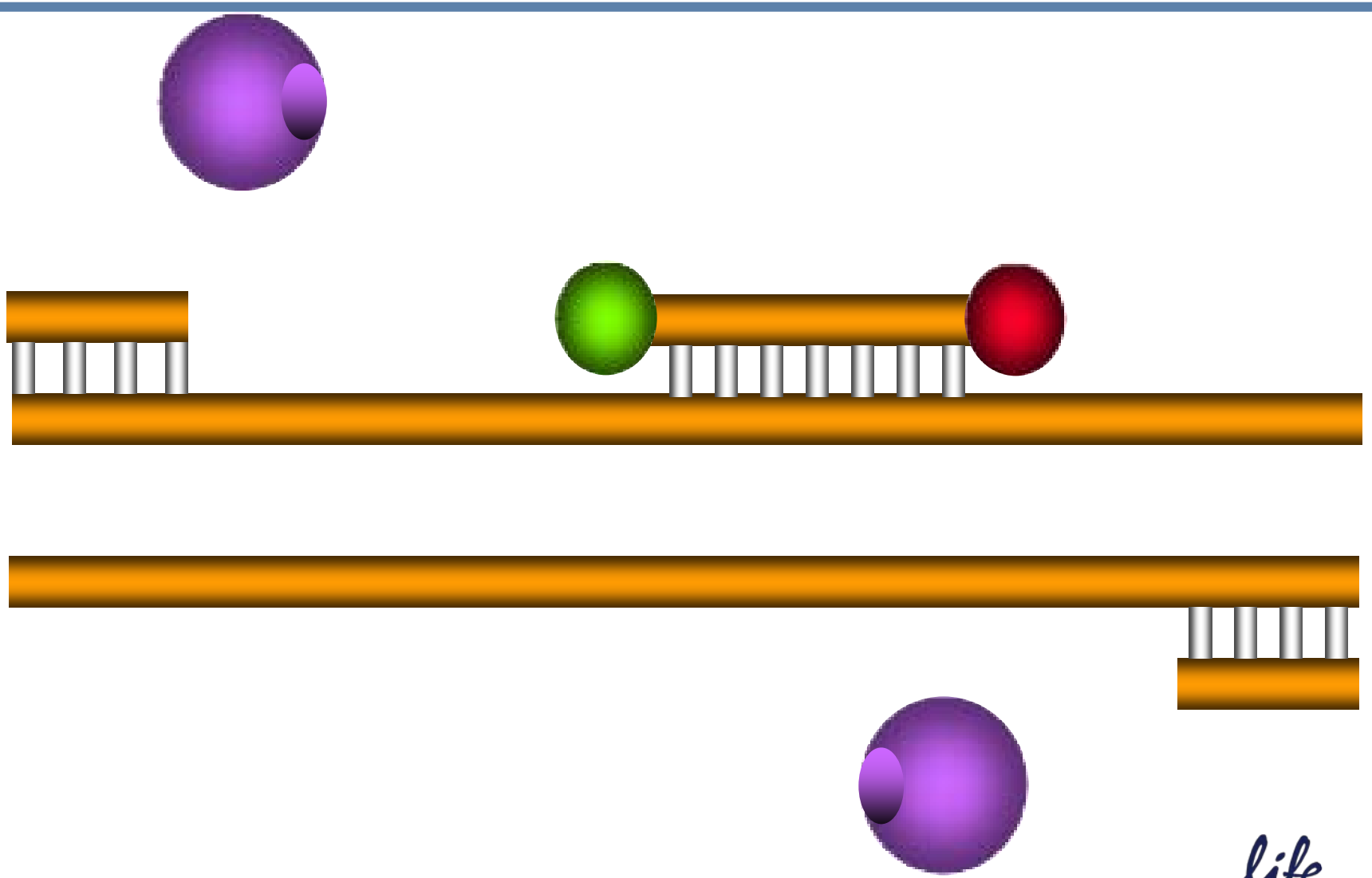

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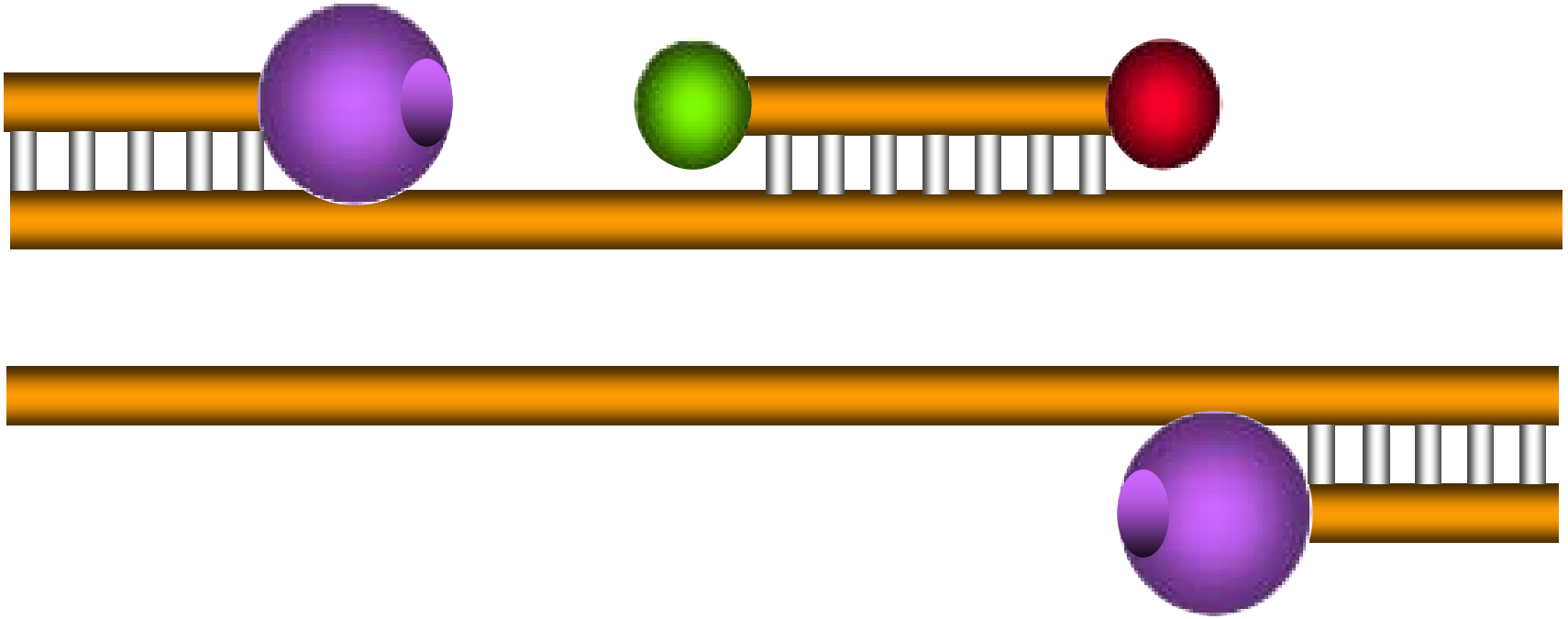

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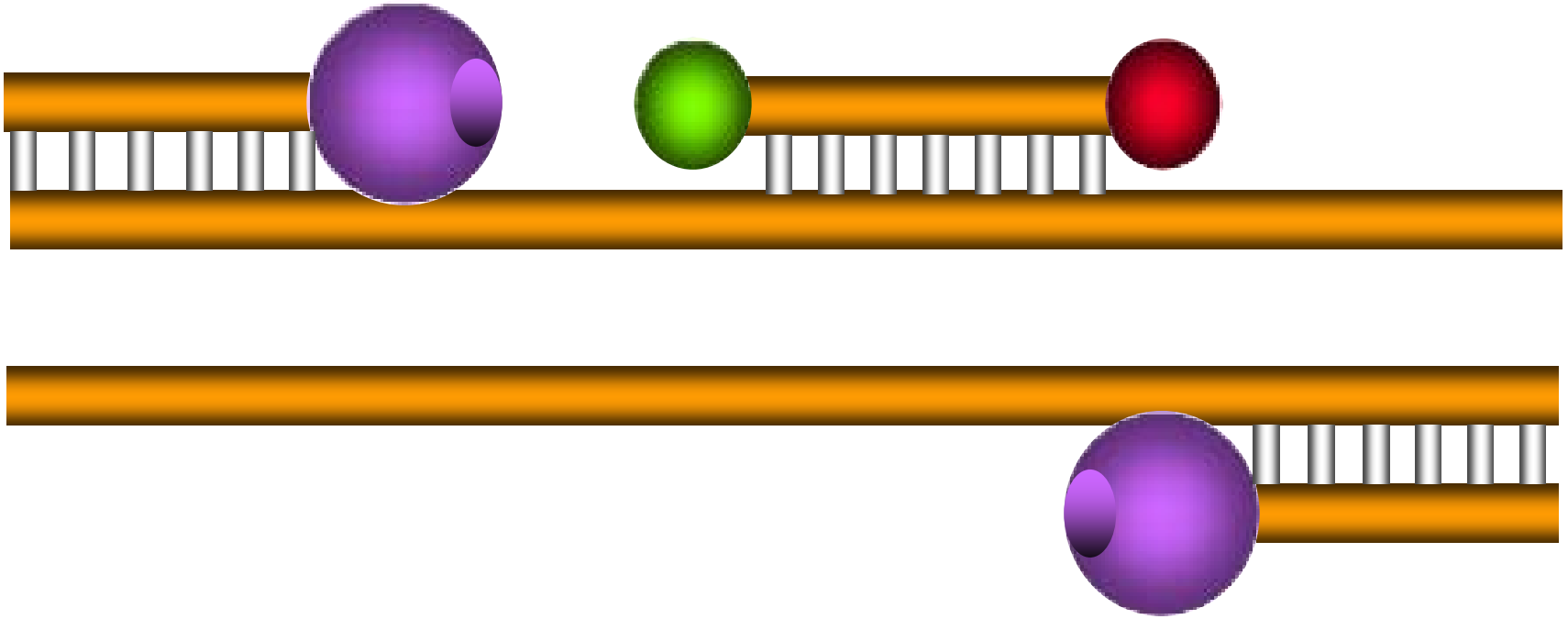

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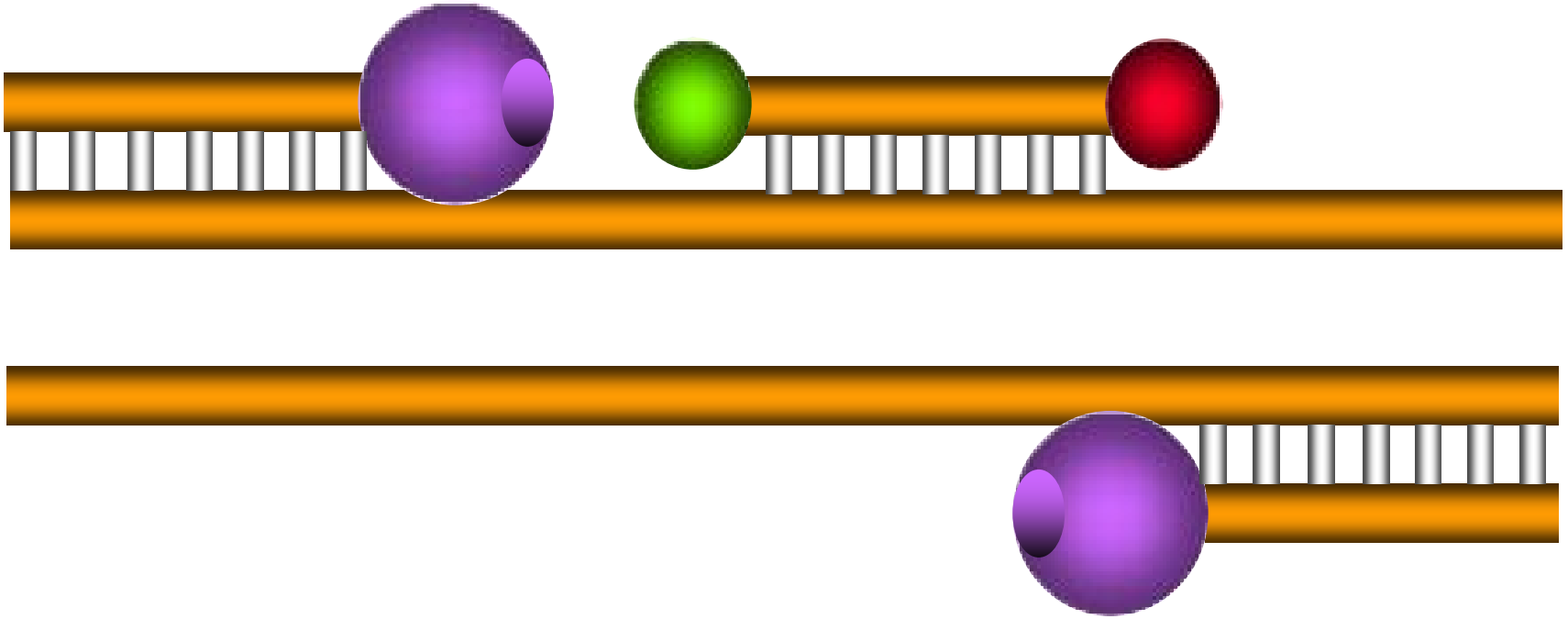

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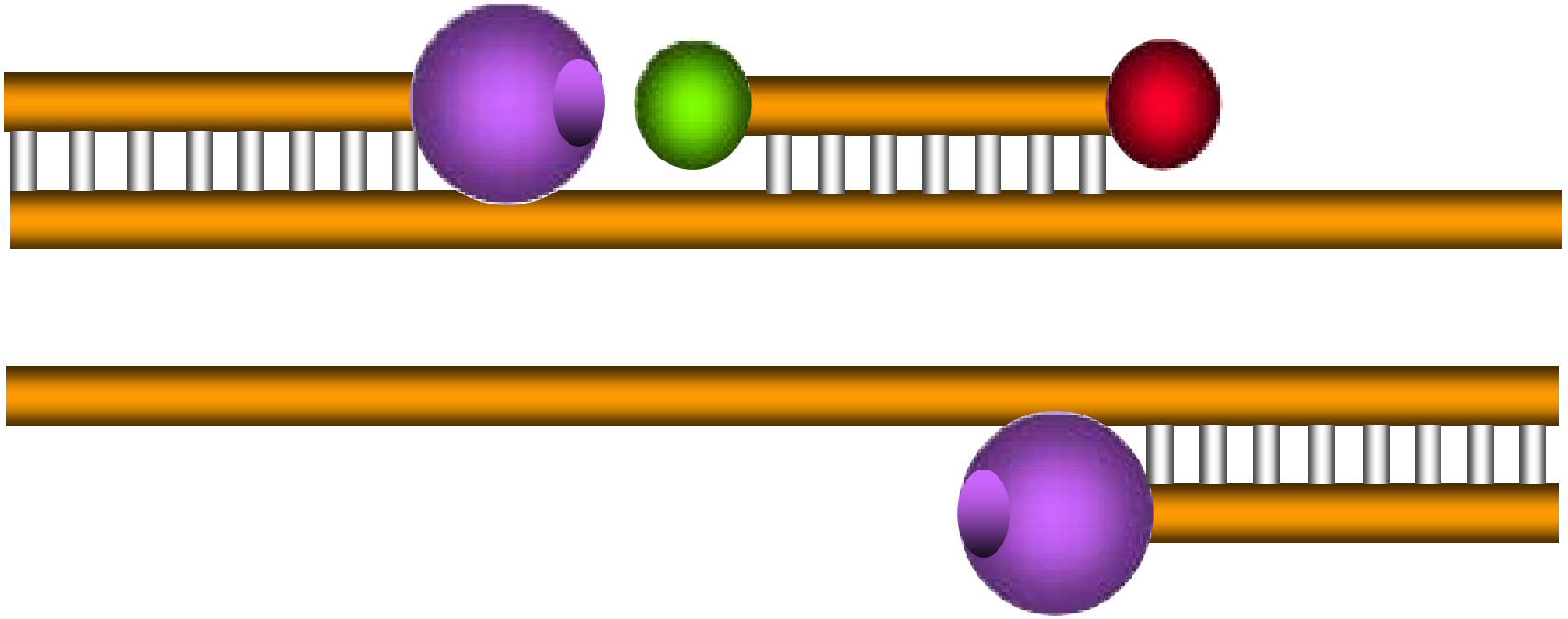

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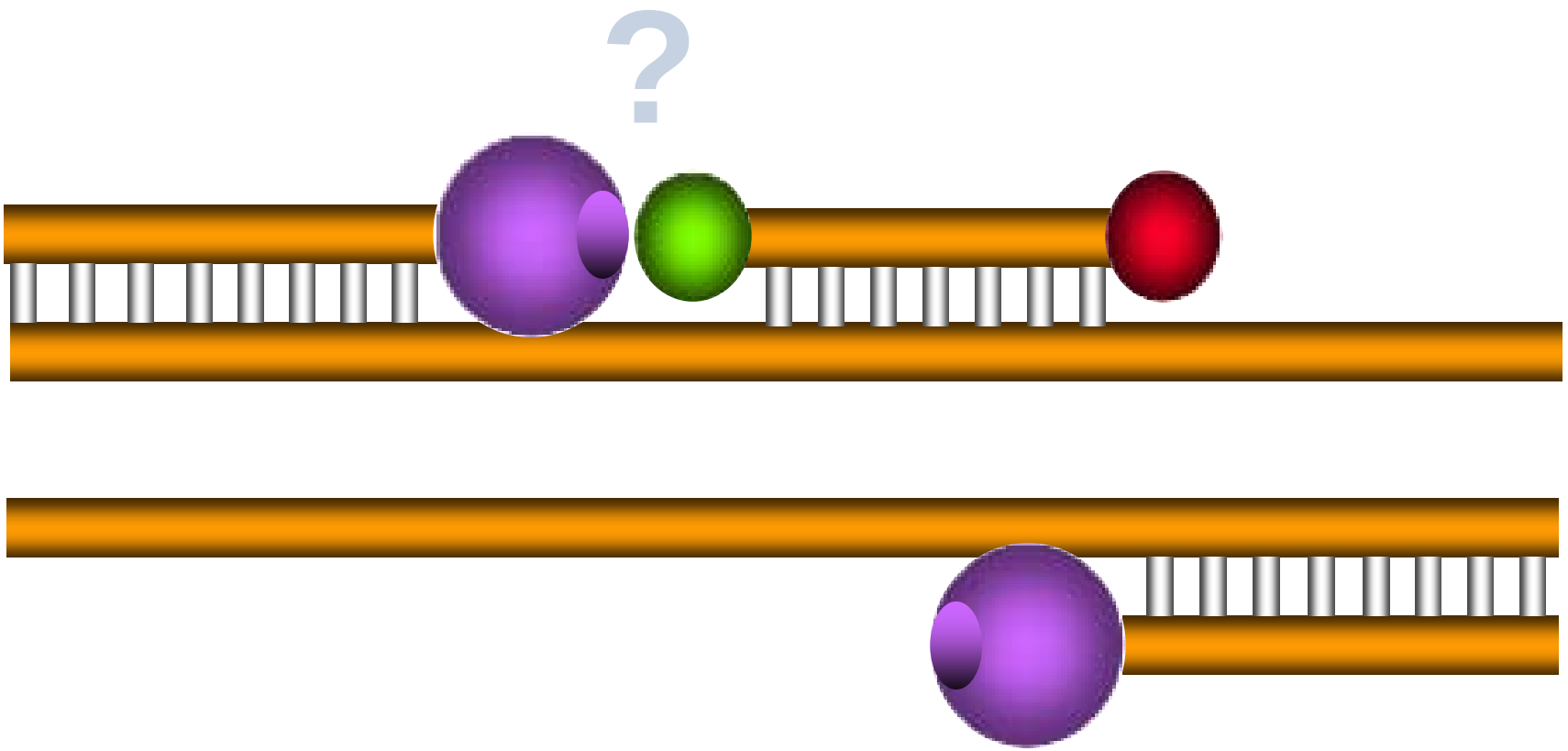

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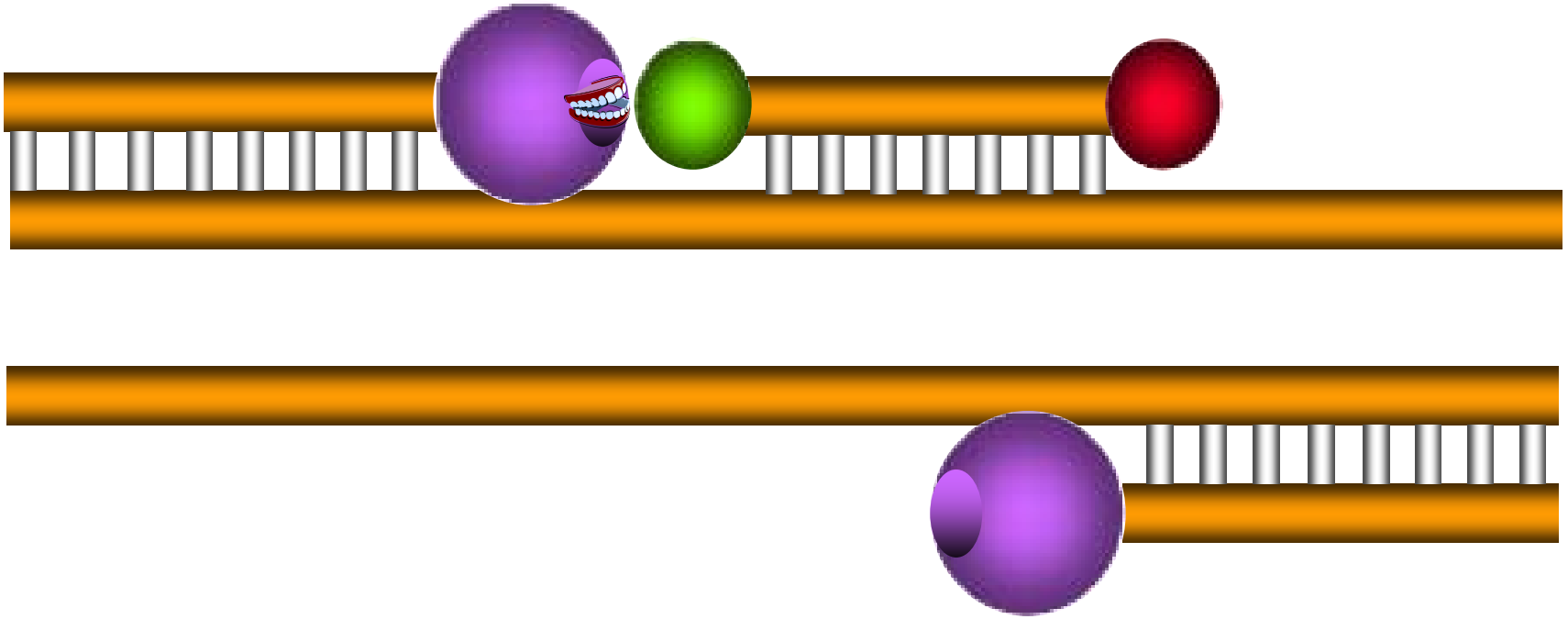
ThermoFisher SCIENTIFIC

- 5′-3′外切酶活性
- 水解探针，使报告基团和淬灭基团分离



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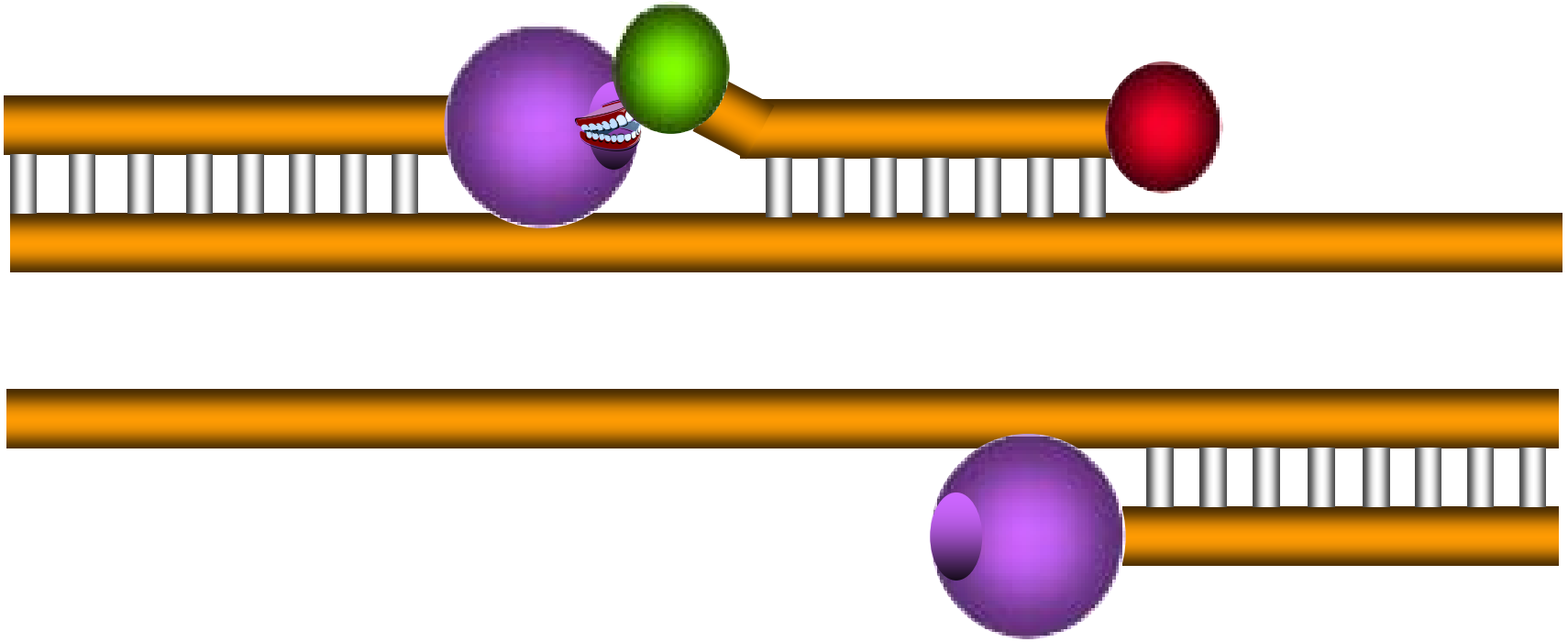

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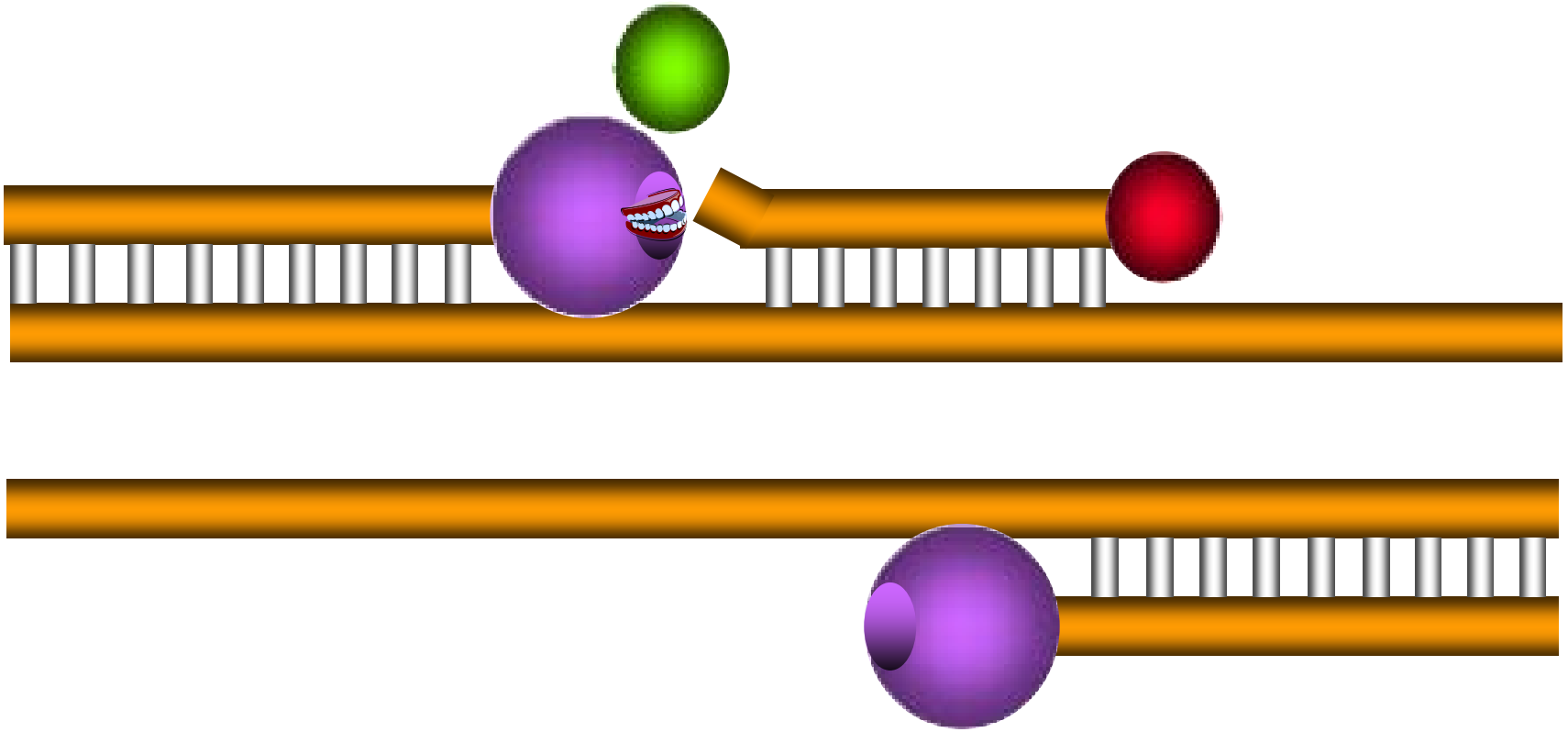

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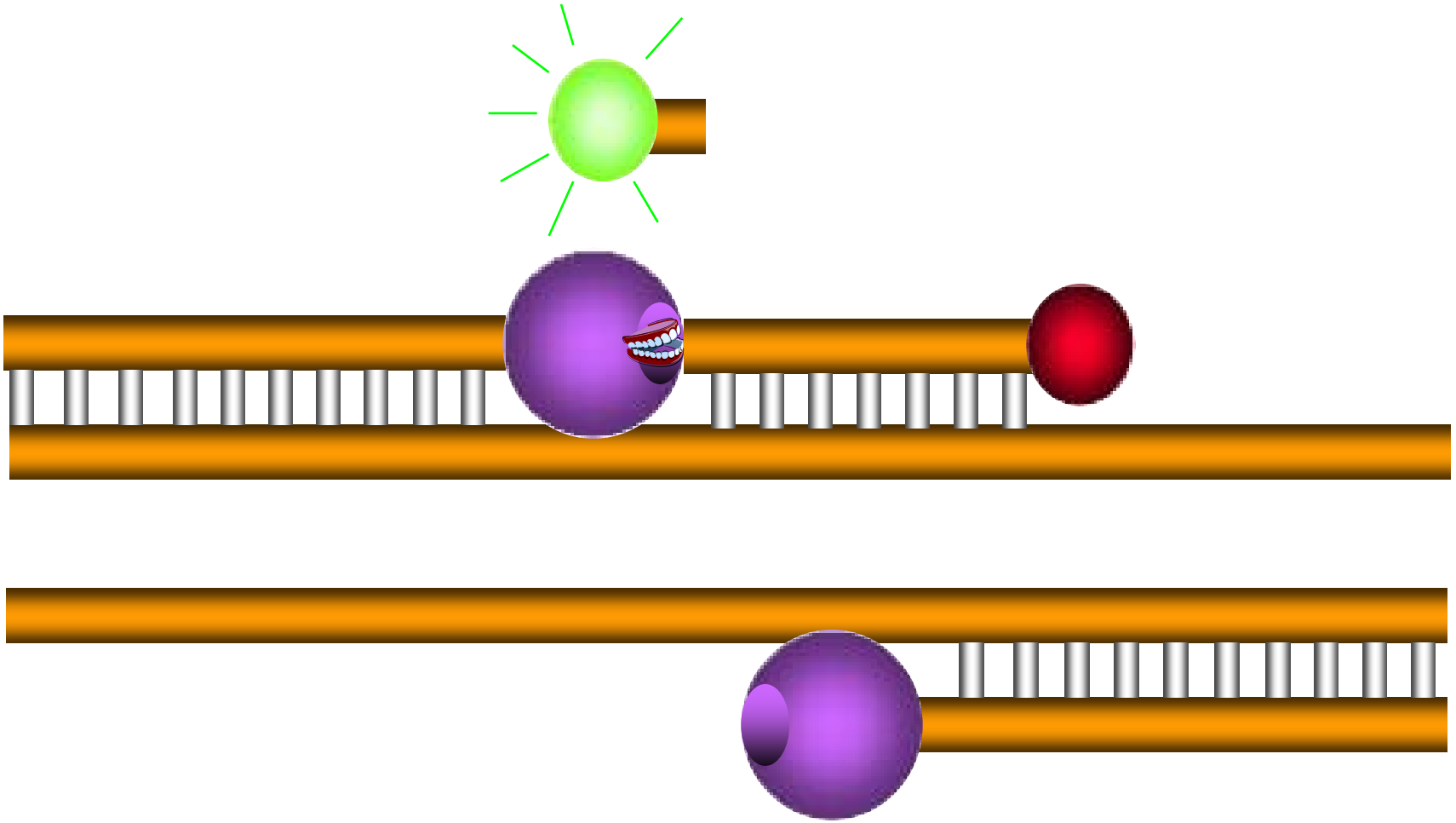

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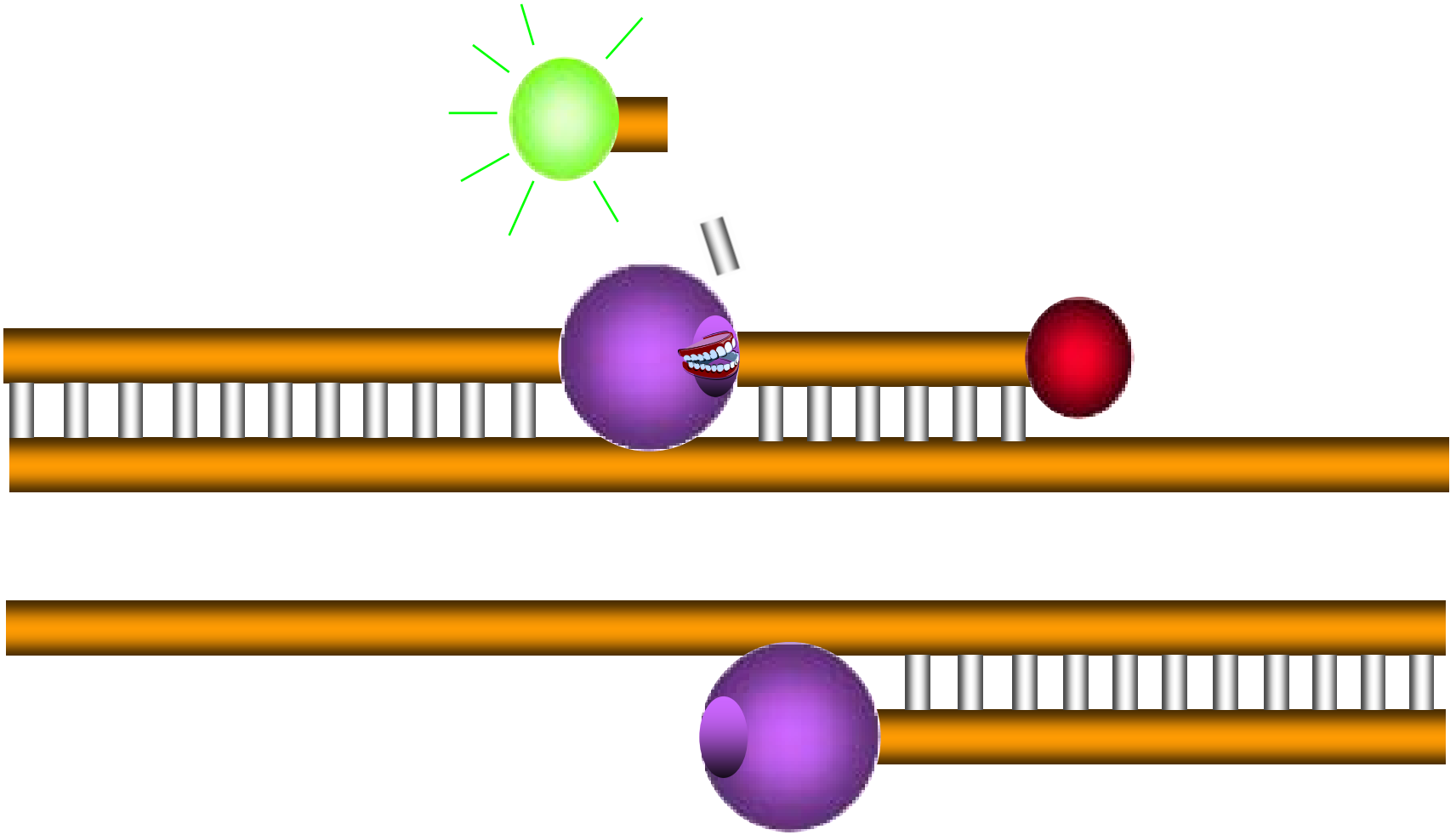

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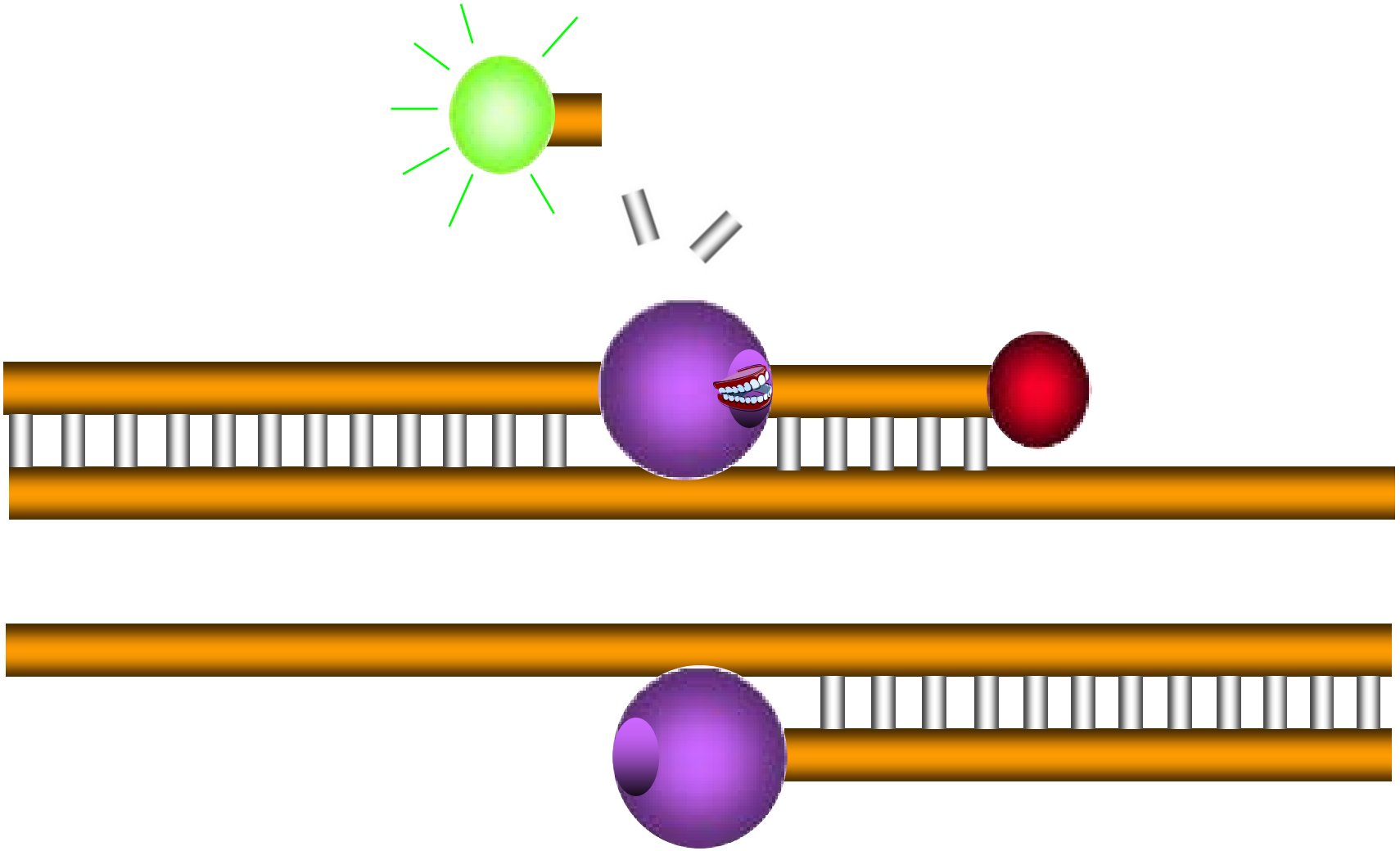

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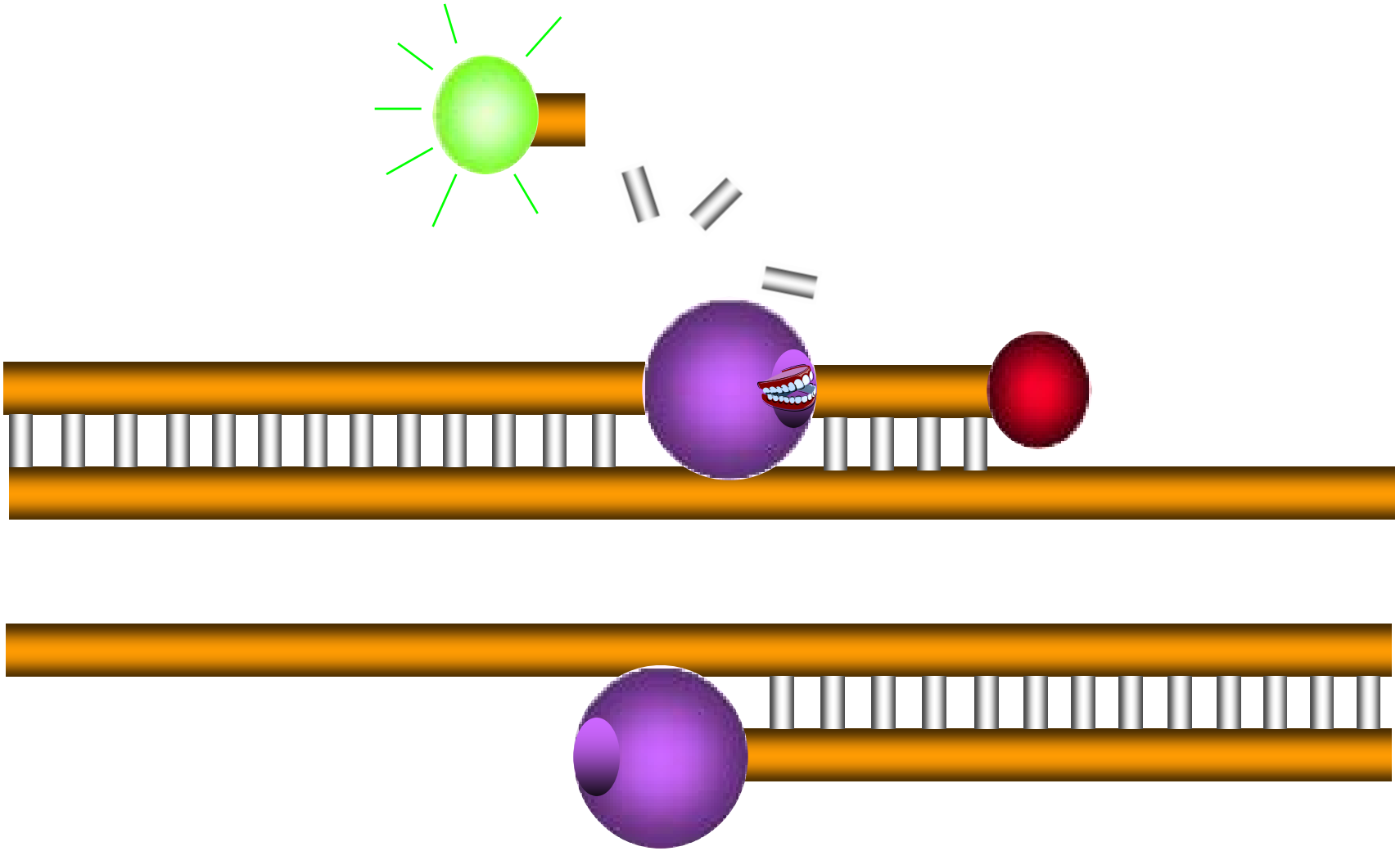

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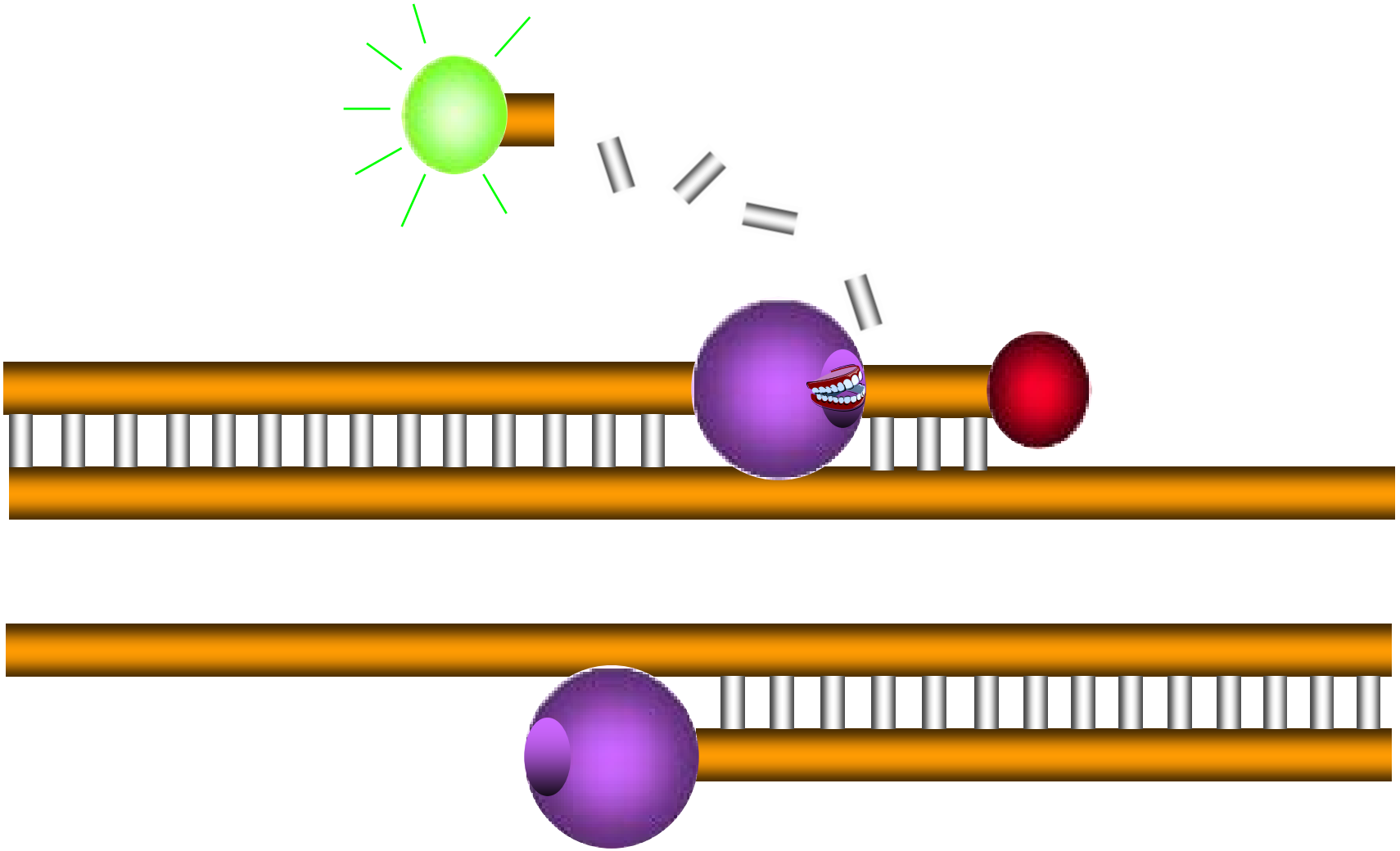

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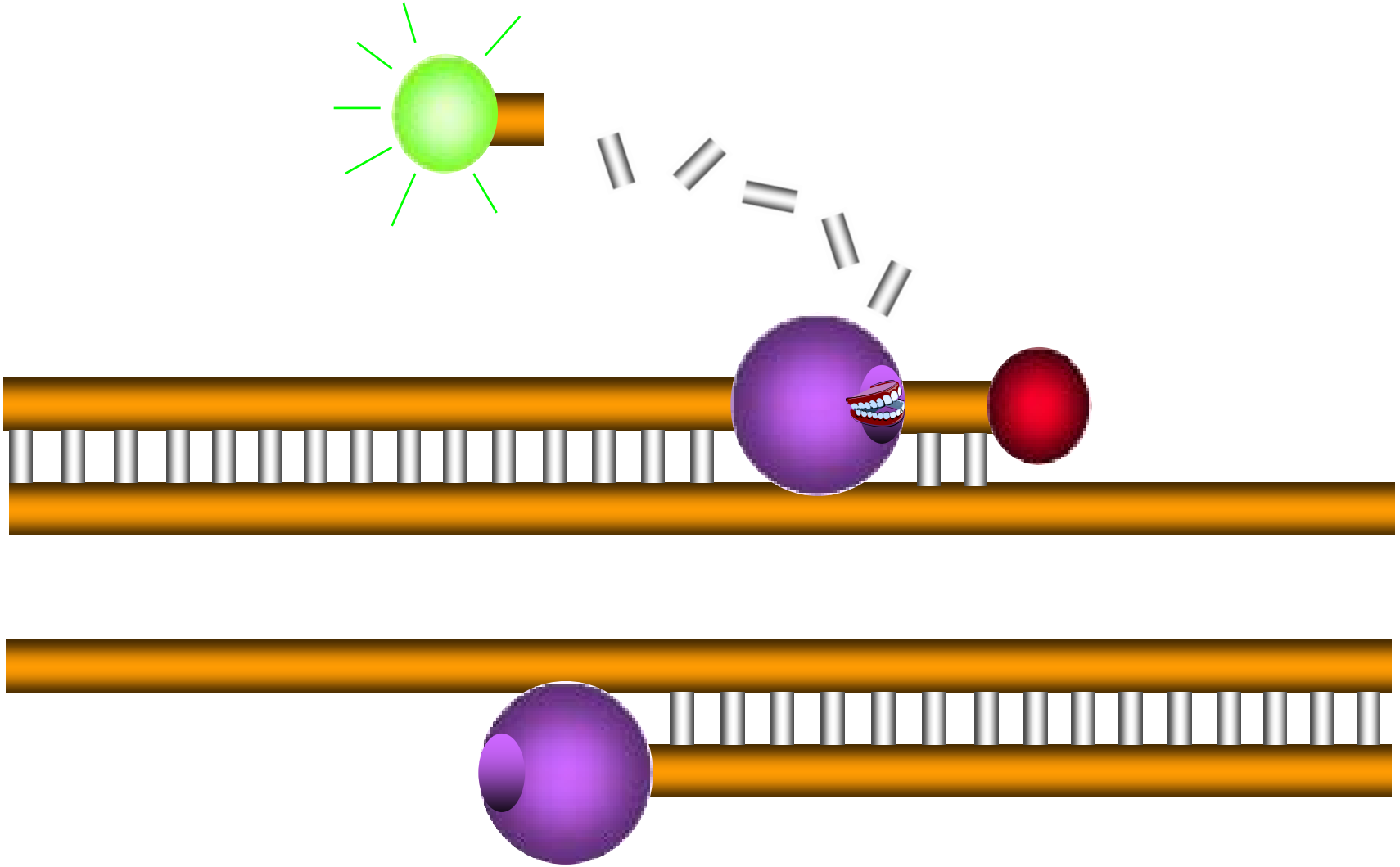

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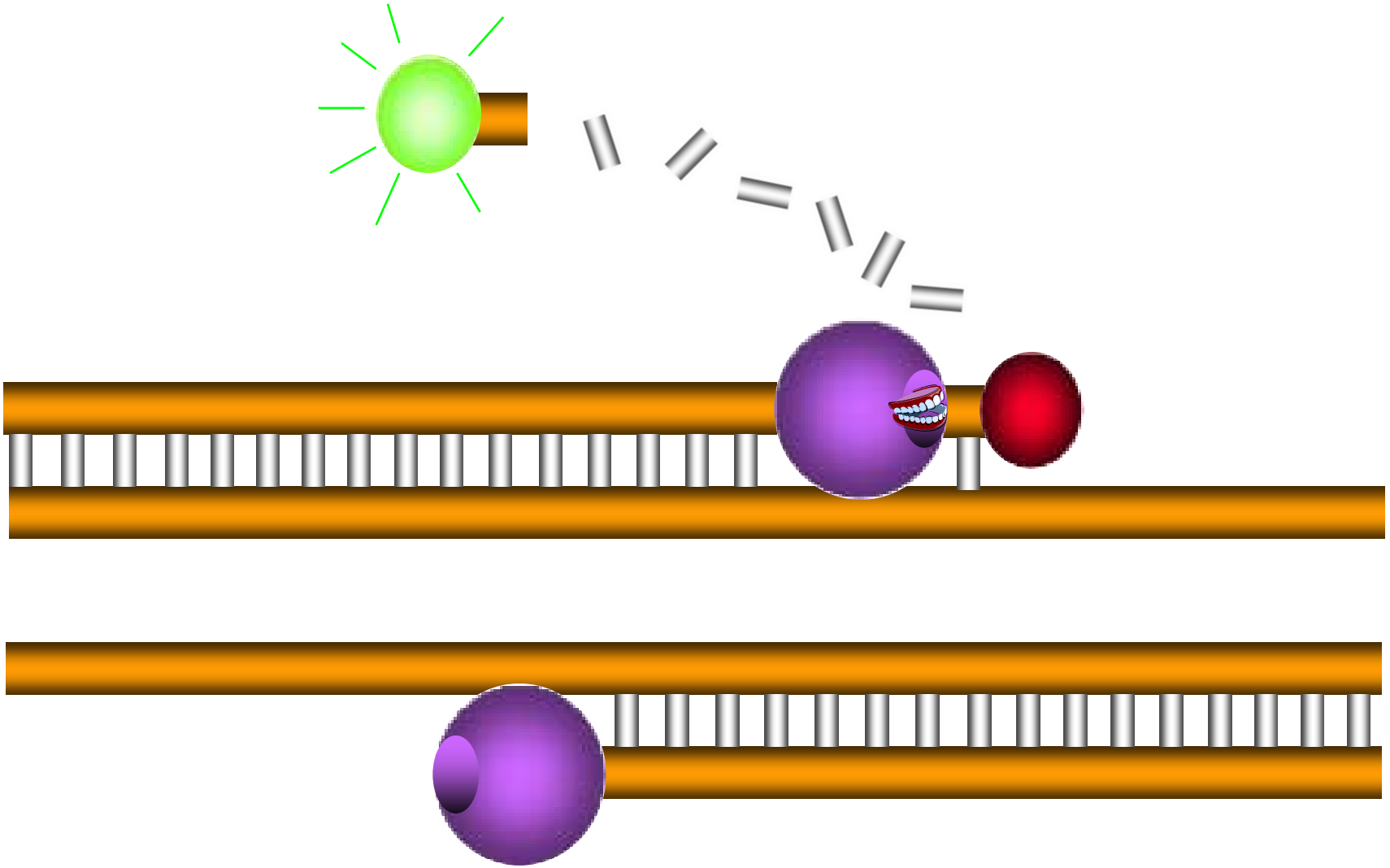

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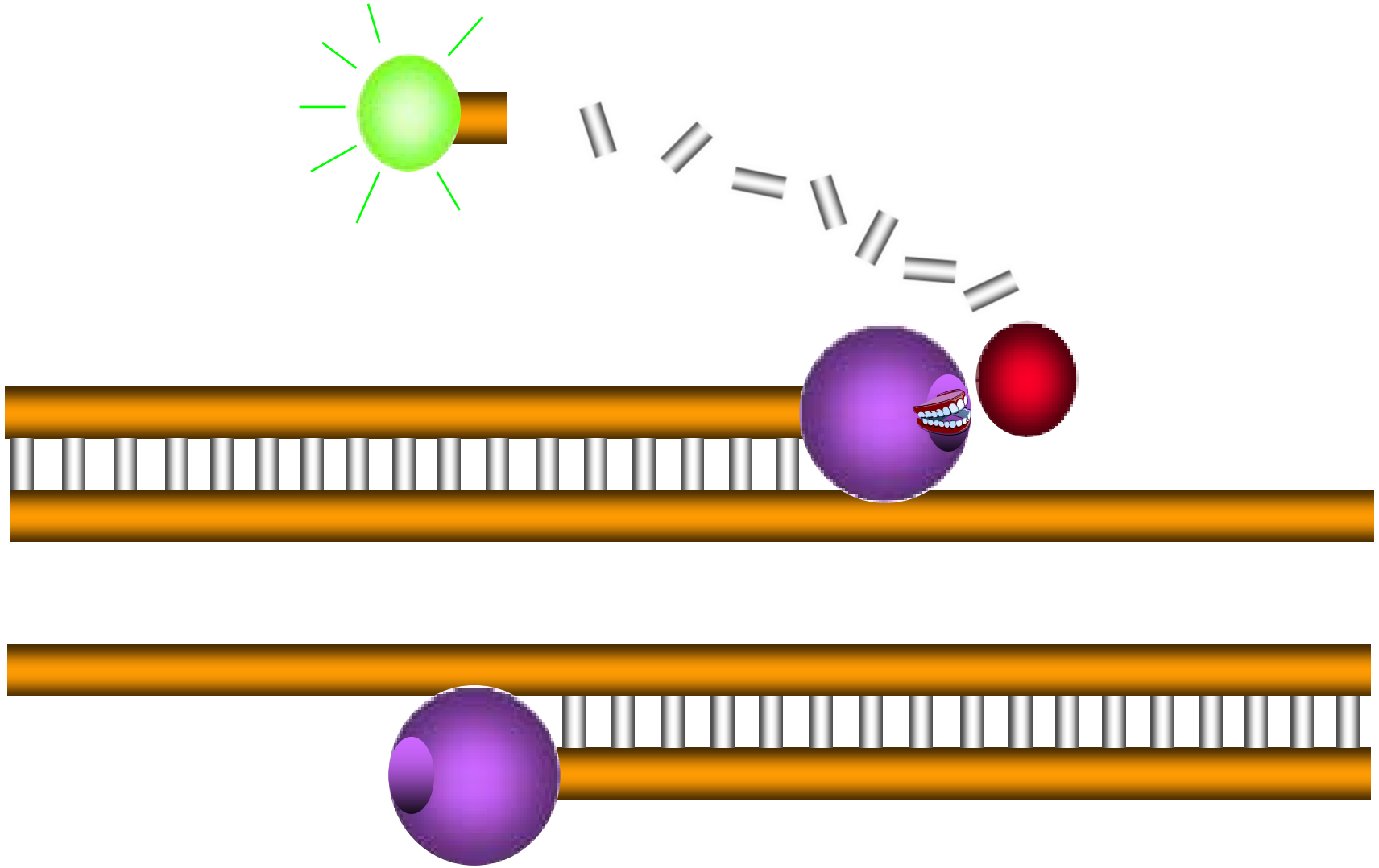

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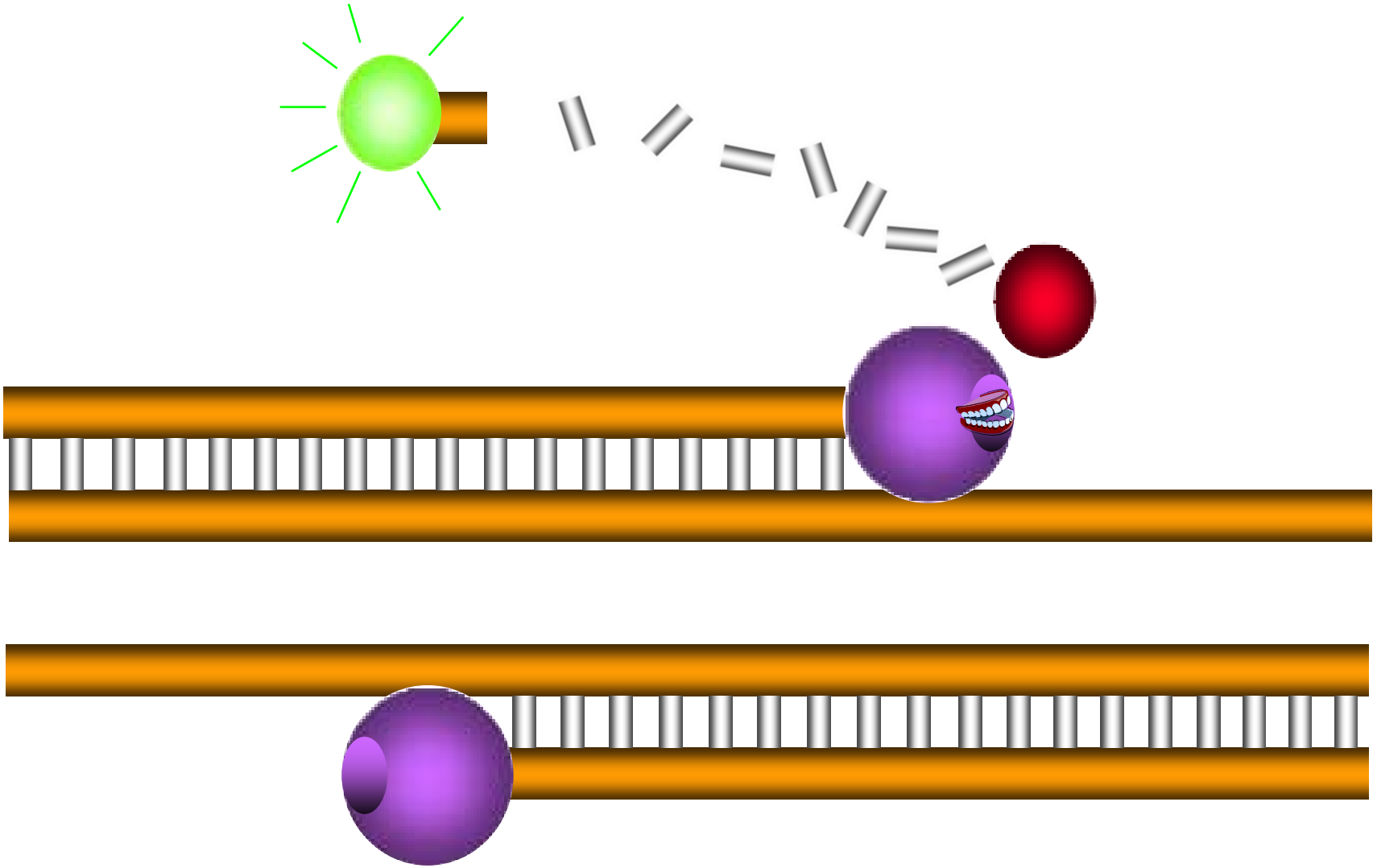

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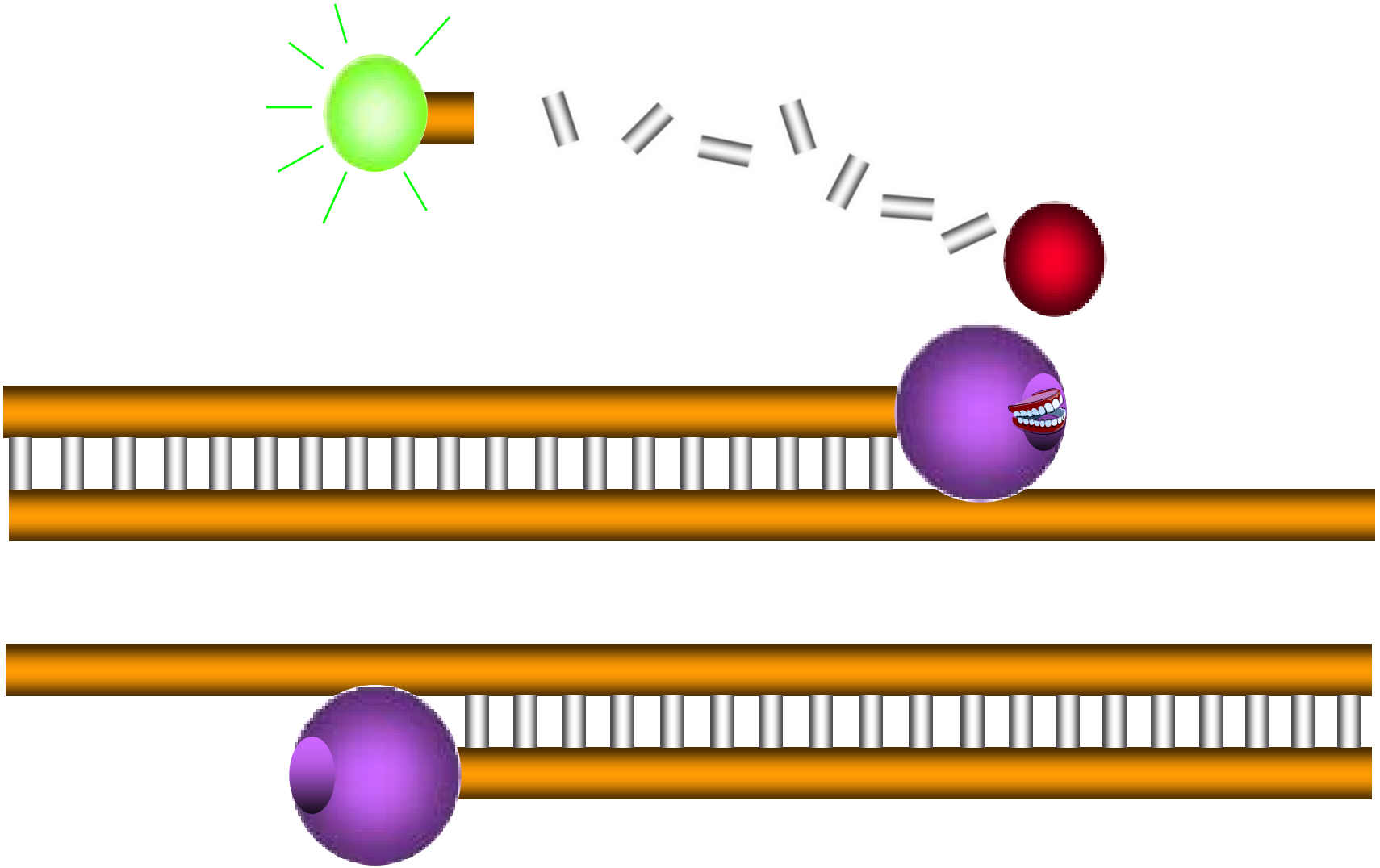

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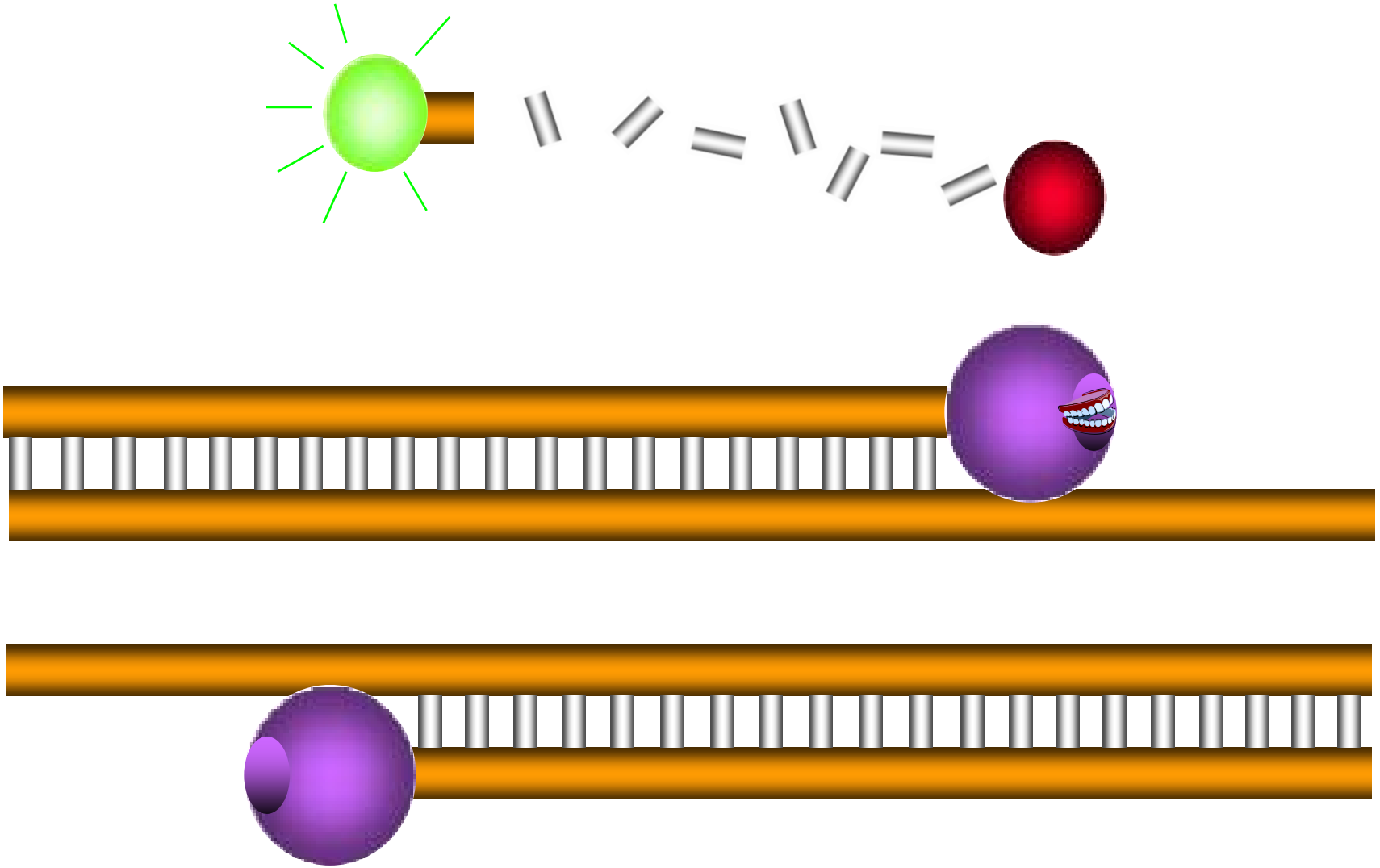

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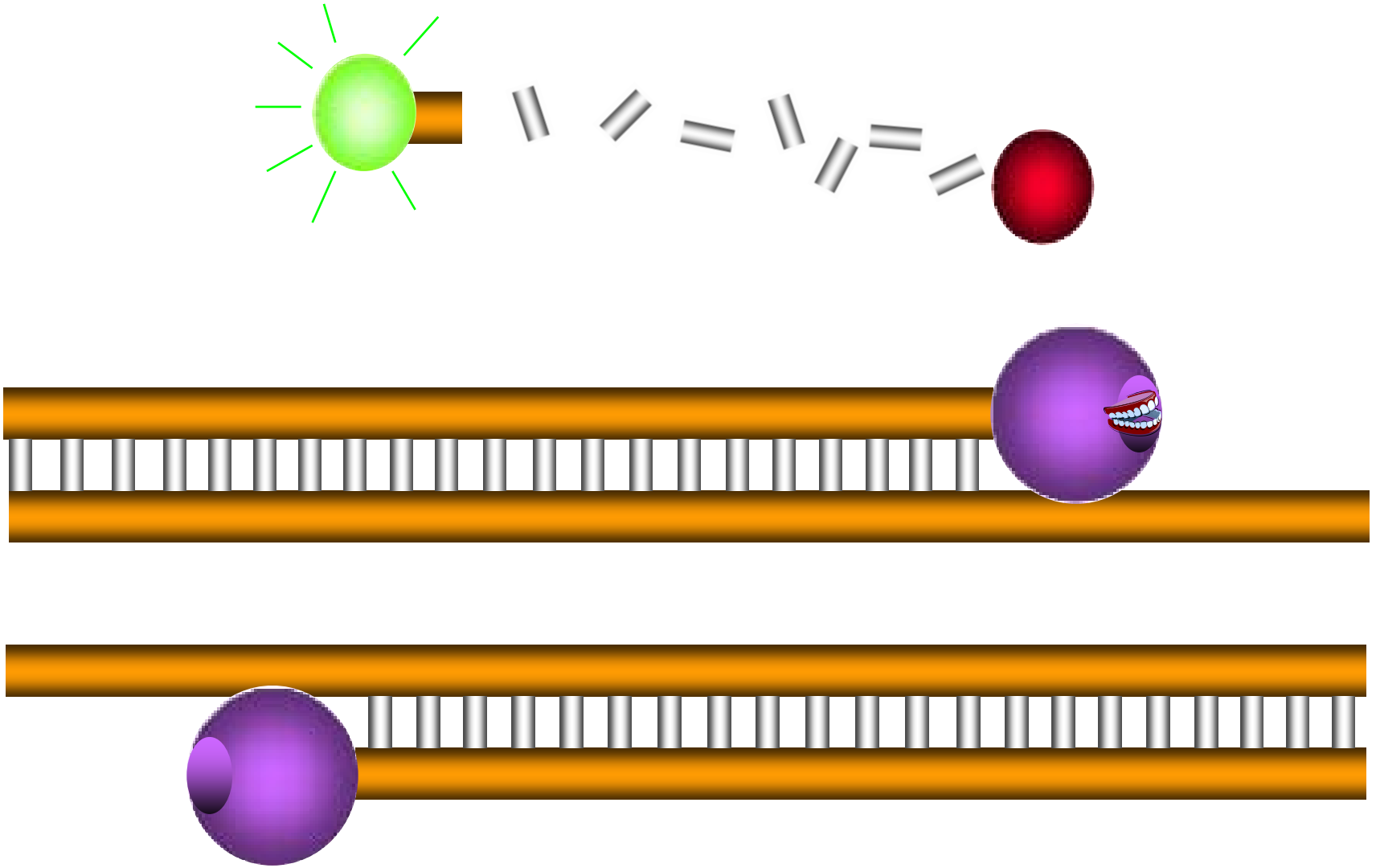

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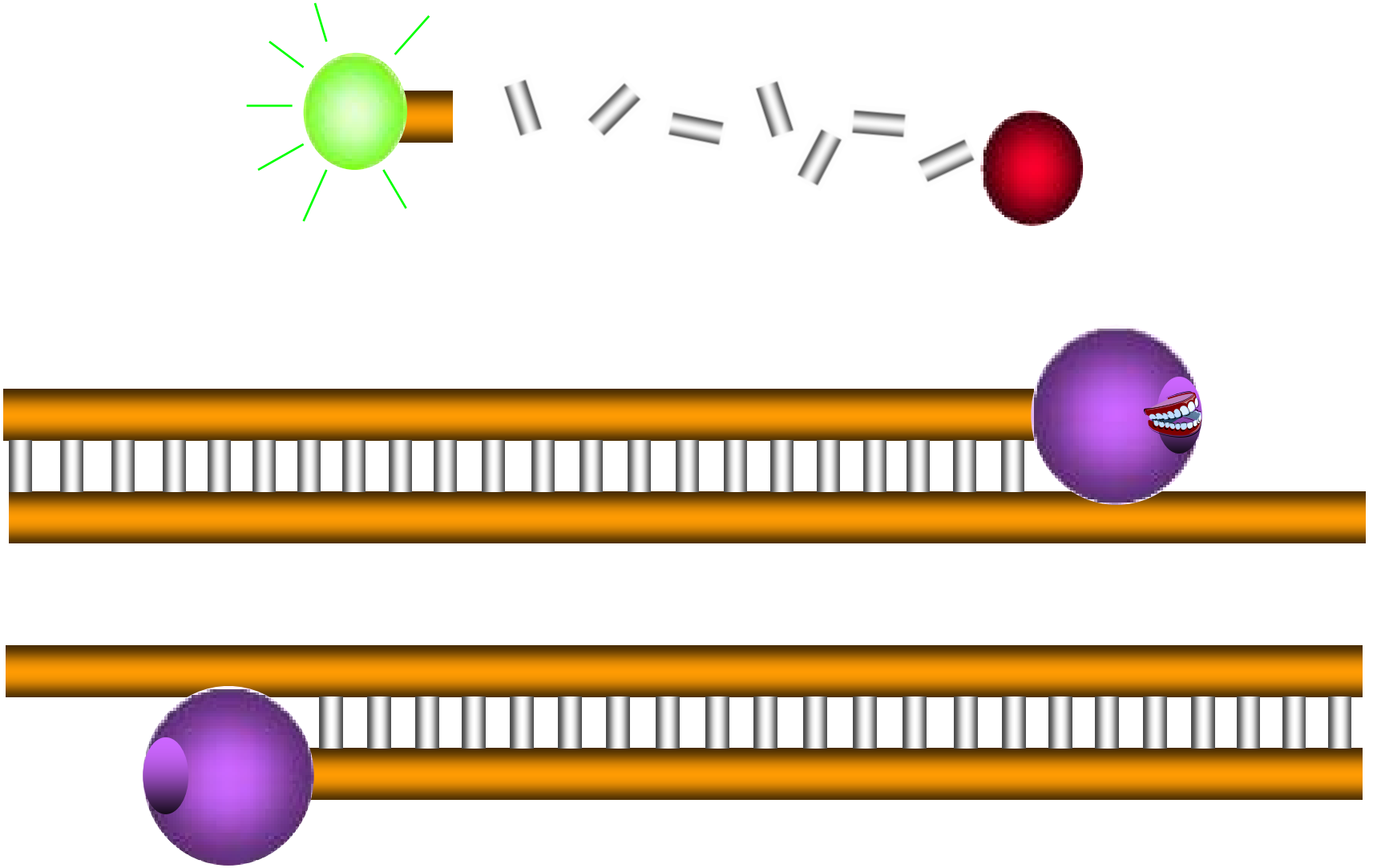

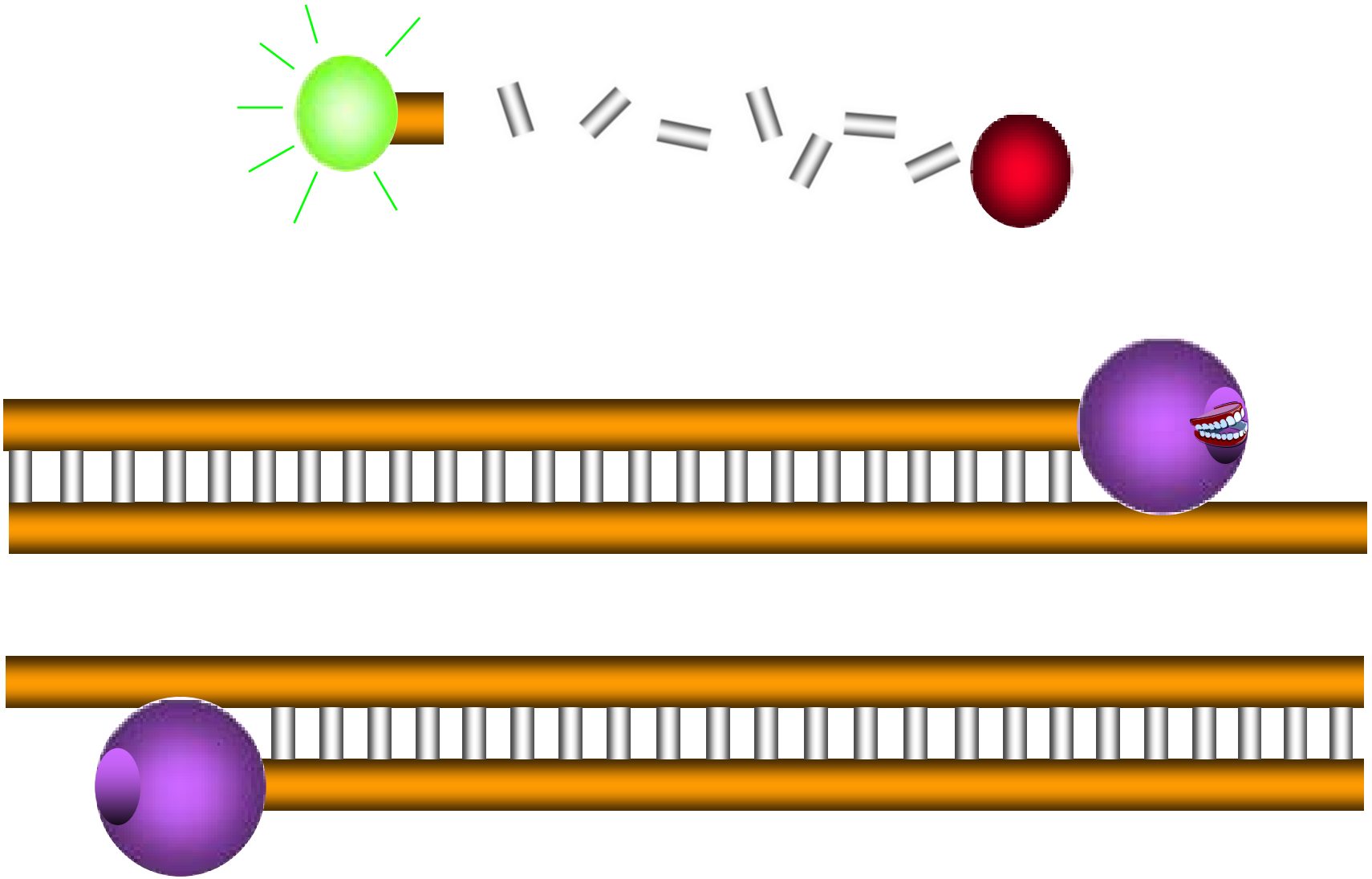

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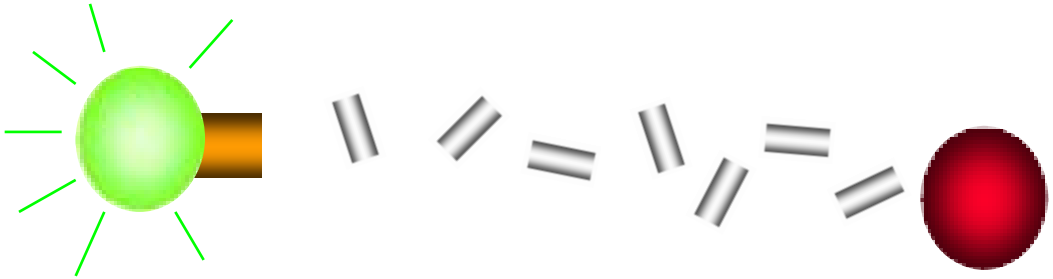

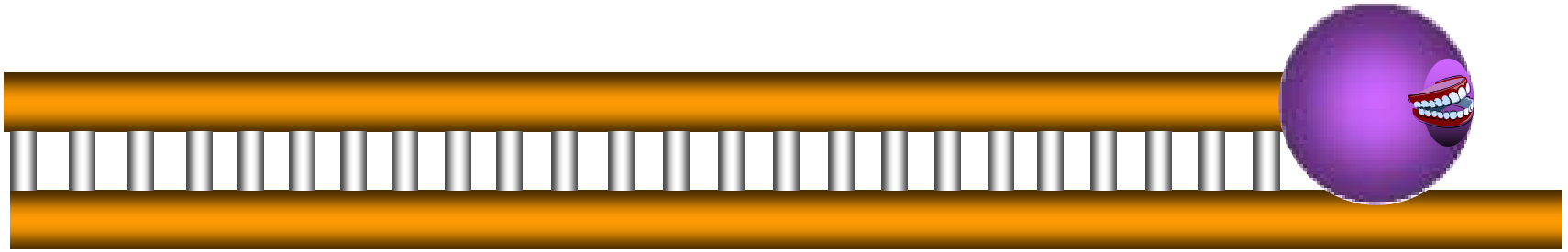

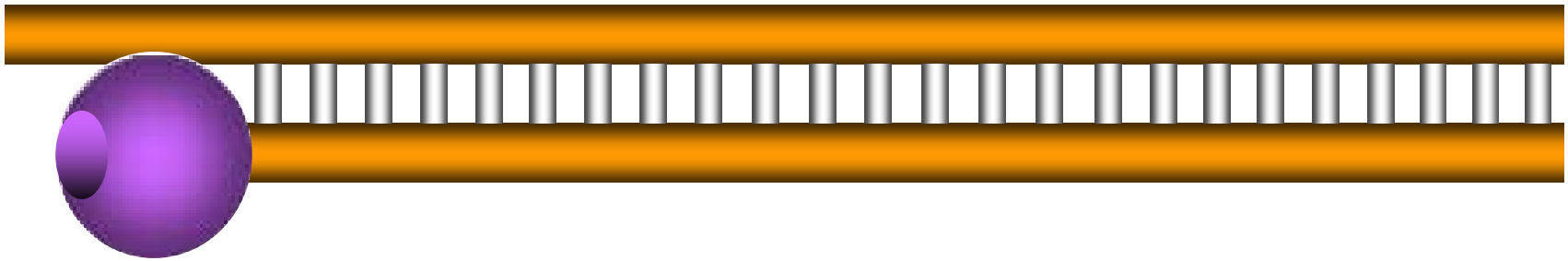

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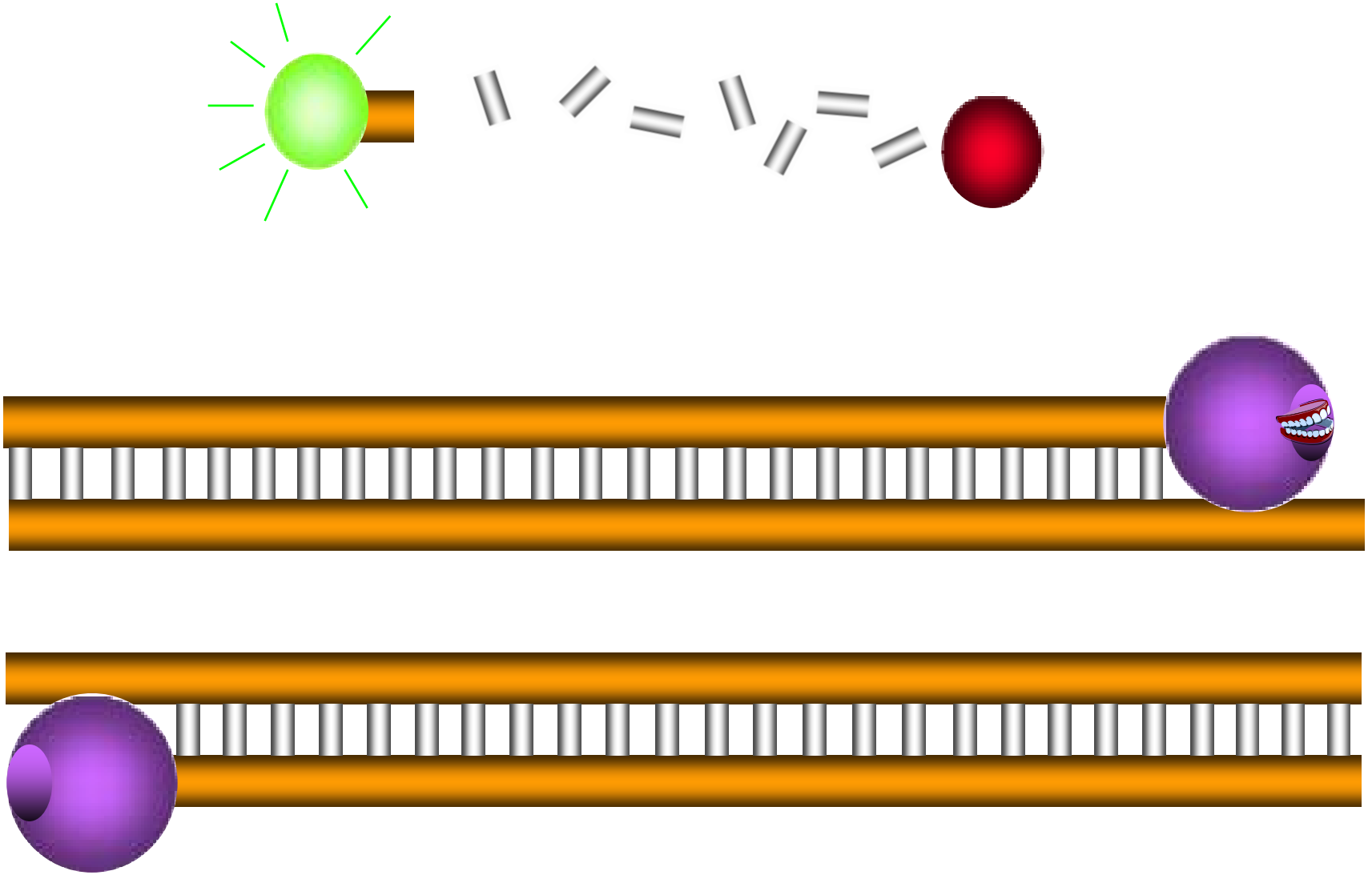

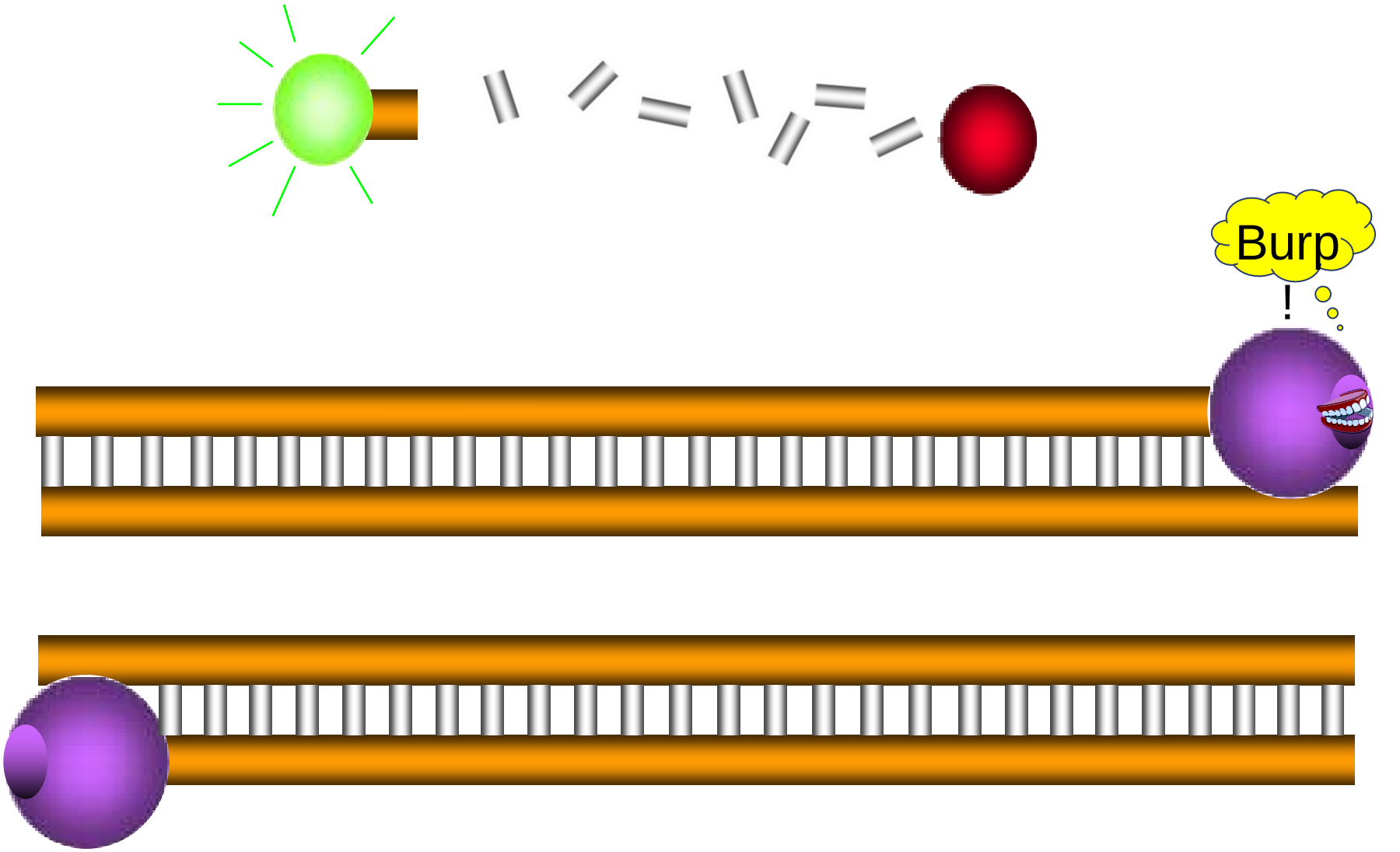
Burp
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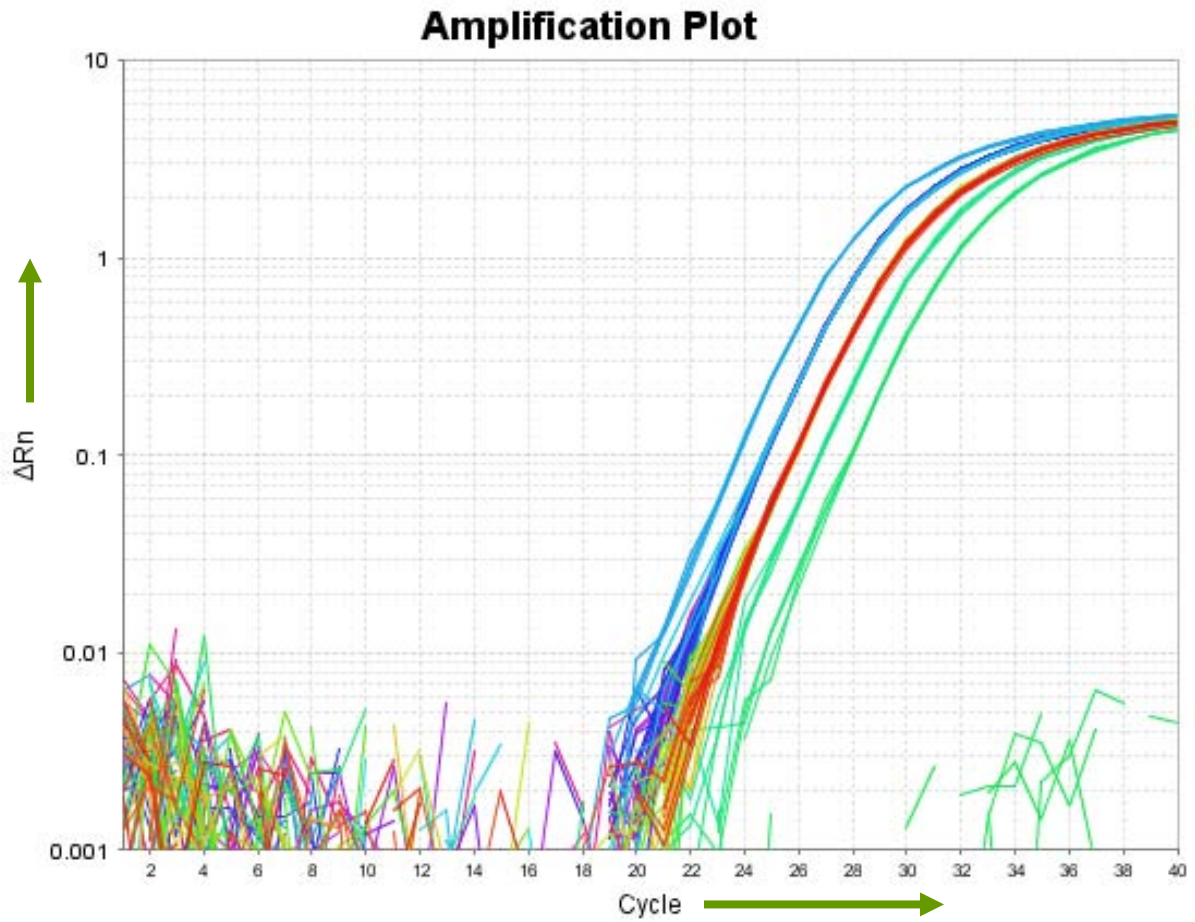
实时记录每个循环后荧光信号的变化



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荧光标记组合

报告荧光：6-FAM、JOE、VIC、NED、CY3、Texas Red、CY5

淬灭荧光：MGB-NFQ、TAMRA、BHQ

参比荧光：ROX

常用的荧光组合：

FAM	目的基因1
VIC	目的基因2/内对照
NED	目的基因3
ROX	参比荧光

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荧光染料组合

- StepOne™实时荧光定量PCR系统

Channel	Dye Examples	Excitation Wave Lenthgh	Emission Wave Lenthgh
1	FAM™, SYBR®	455~485nm	~520
2	VIC®, JOE™	510~530nm	~550
3	ROX™, Texas Red®	570~590nm	~620

- StepOnePlus™实时荧光定量PCR系统

Channel	Dye Examples	Excitation Wave Lenthgh	Emission Wave Lenthgh
1	FAM™, SYBR®	455~485nm	~520
2	VIC®, JOE™	510~530nm	~550
3	NED™, TAMRA™	540~550nm	~580
4	ROX™, Texas Red®	570~590nm	~620

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荧光染料组合

- 7500实时荧光定量PCR系统

Channel	Dye Examples	Excitation Wave Lenthgh	Emission Wave Lenthgh
1	FAM™, SYBR®	455~485nm	~520
2	VIC®, JOE™	510~530nm	~550
3	NED™, Cy3, TAMRA™	540~550nm	~580
4	ROX™, Texas Red®	570~590nm	~610
5	CY5 Dye™	630~650nm	~650

- ViiA™ 7实时荧光定量PCR系统

Channel	Dye Examples	Excitation Wave Lenthgh	Emission Wave Lenthgh
1	FAM™, SYBR®, SYTO®9 (MeltDoctor™), Fluorescein, SYPRO® Orange	455~485nm	~520nm
2	VIC®, JOE™, TET™, HEX™	510~530nm	~550nm
3	NED™, BODIPY® TMR-X, TAMRA™	540~550nm	~580nm
4	ROX™, Texas Red®	570~590nm	~620nm
5	LIZ™	630~650nm	~680nm
6	Alexa Fluor®, Joda-4	650~670nm	~710nm

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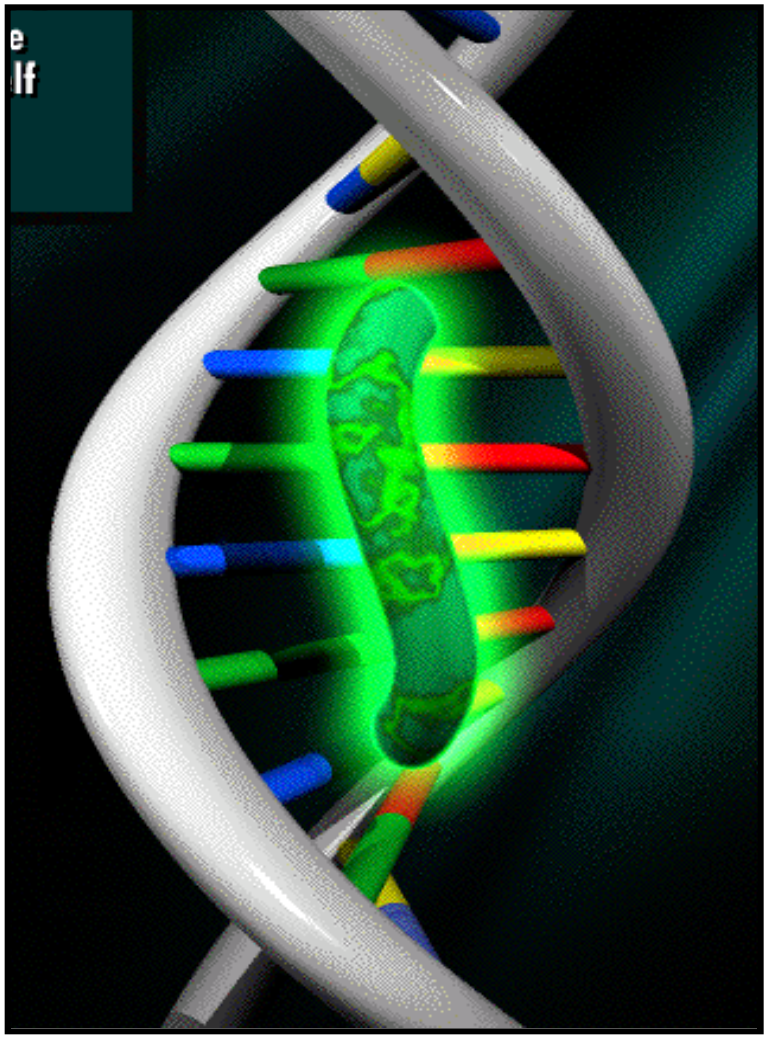
SYBR® Green I 染料



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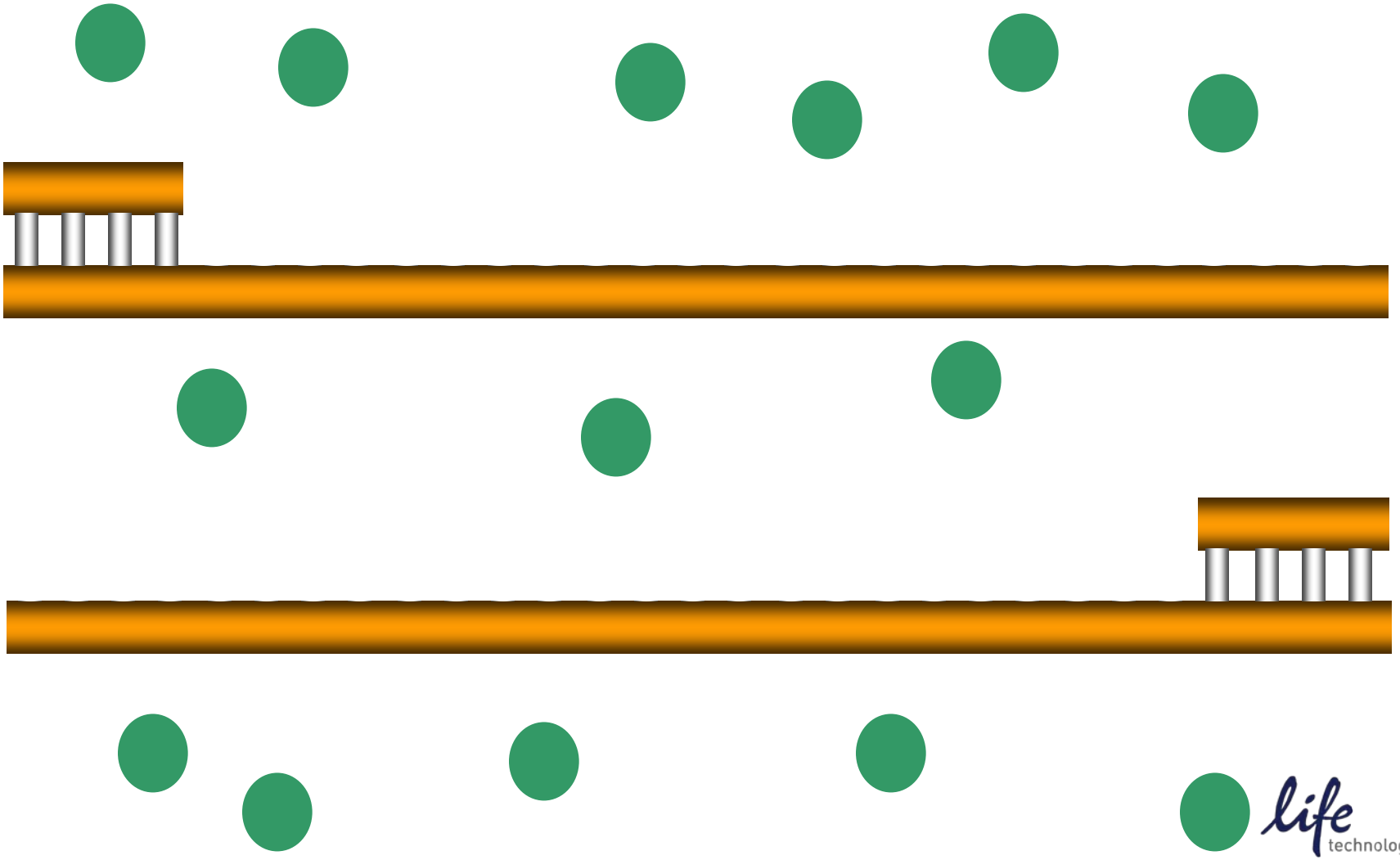
SYBR® Green I作用机理



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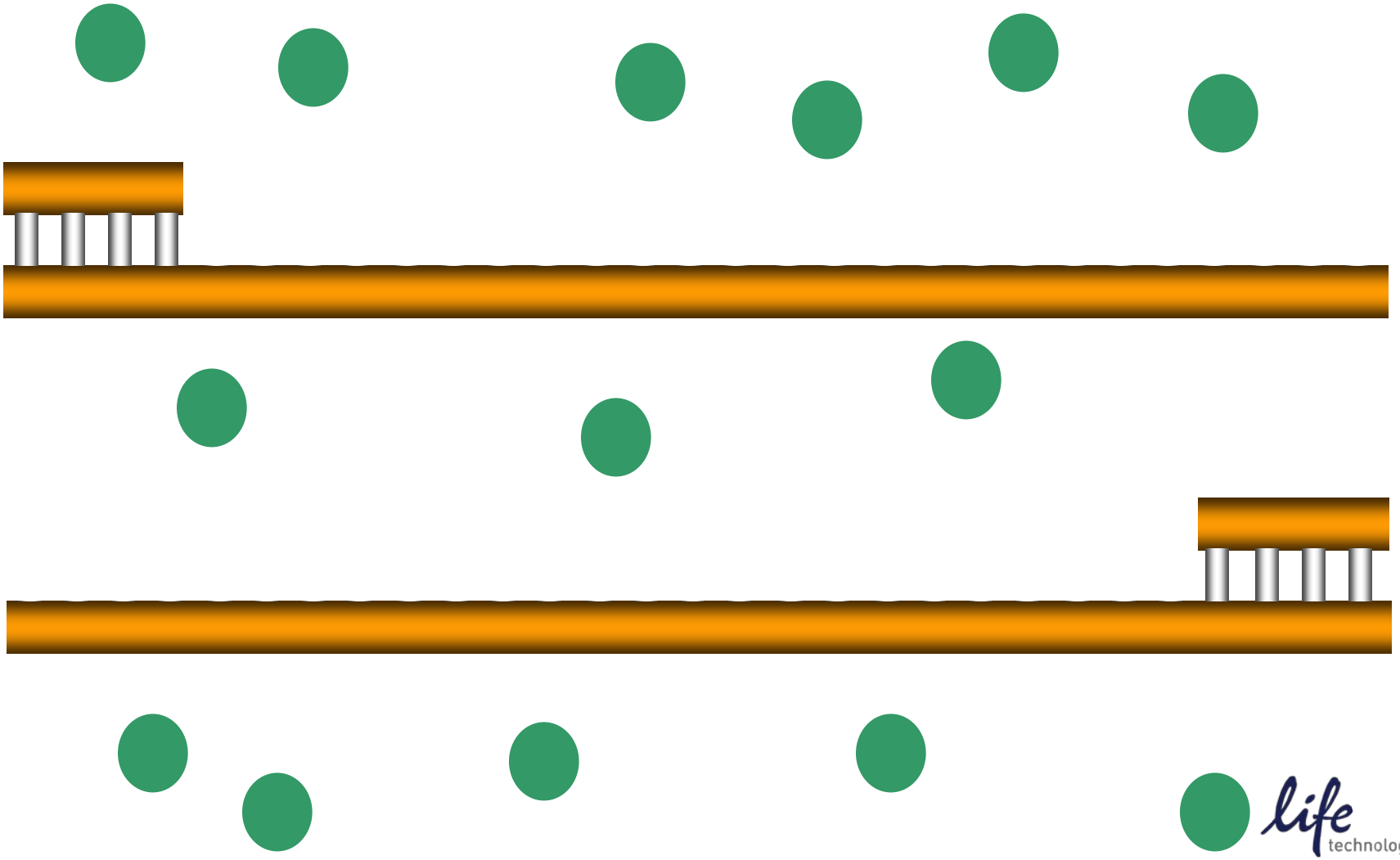
SYBR® Green I作用机理



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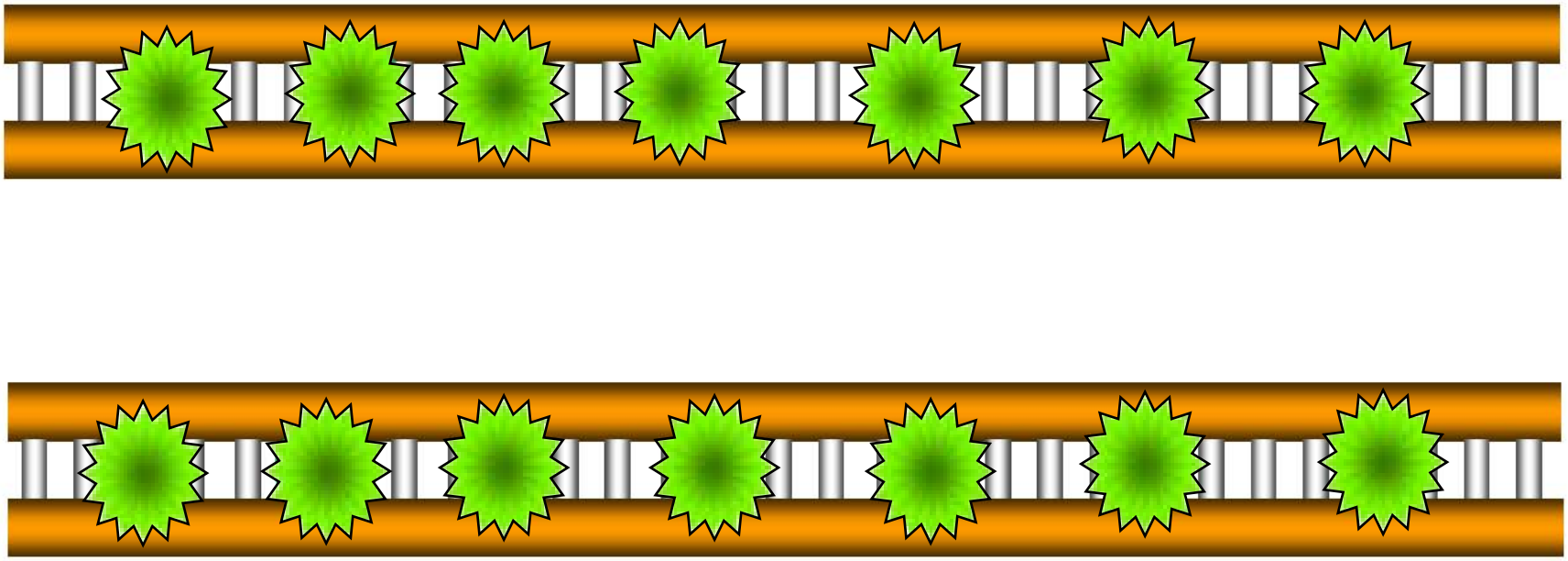
SYBR® Green I作用机理



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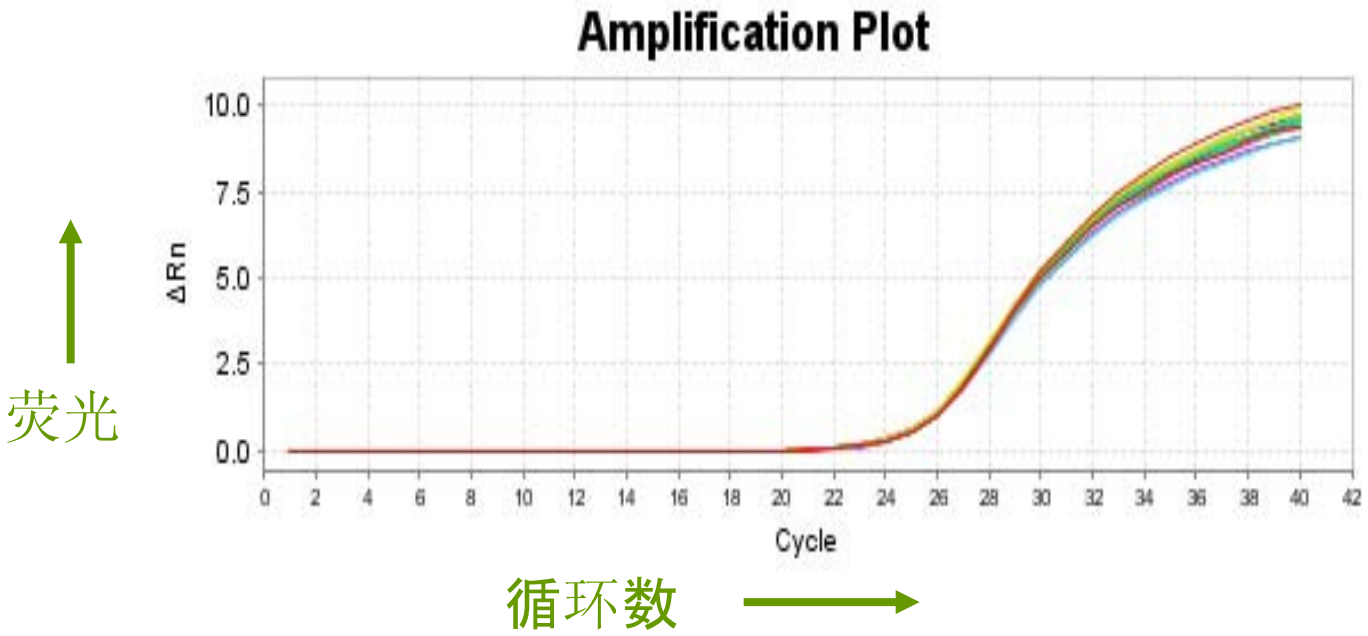
实时记录每个循环后荧光信号的变化



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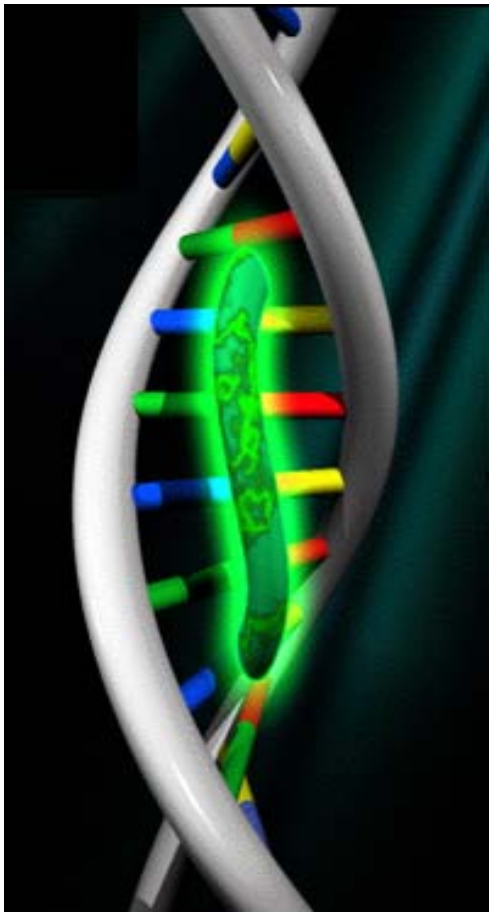
SYBR® Green I 方法的局限性



任何双链，非特异结合

↓

非特异产物信号

↓

不准确结果

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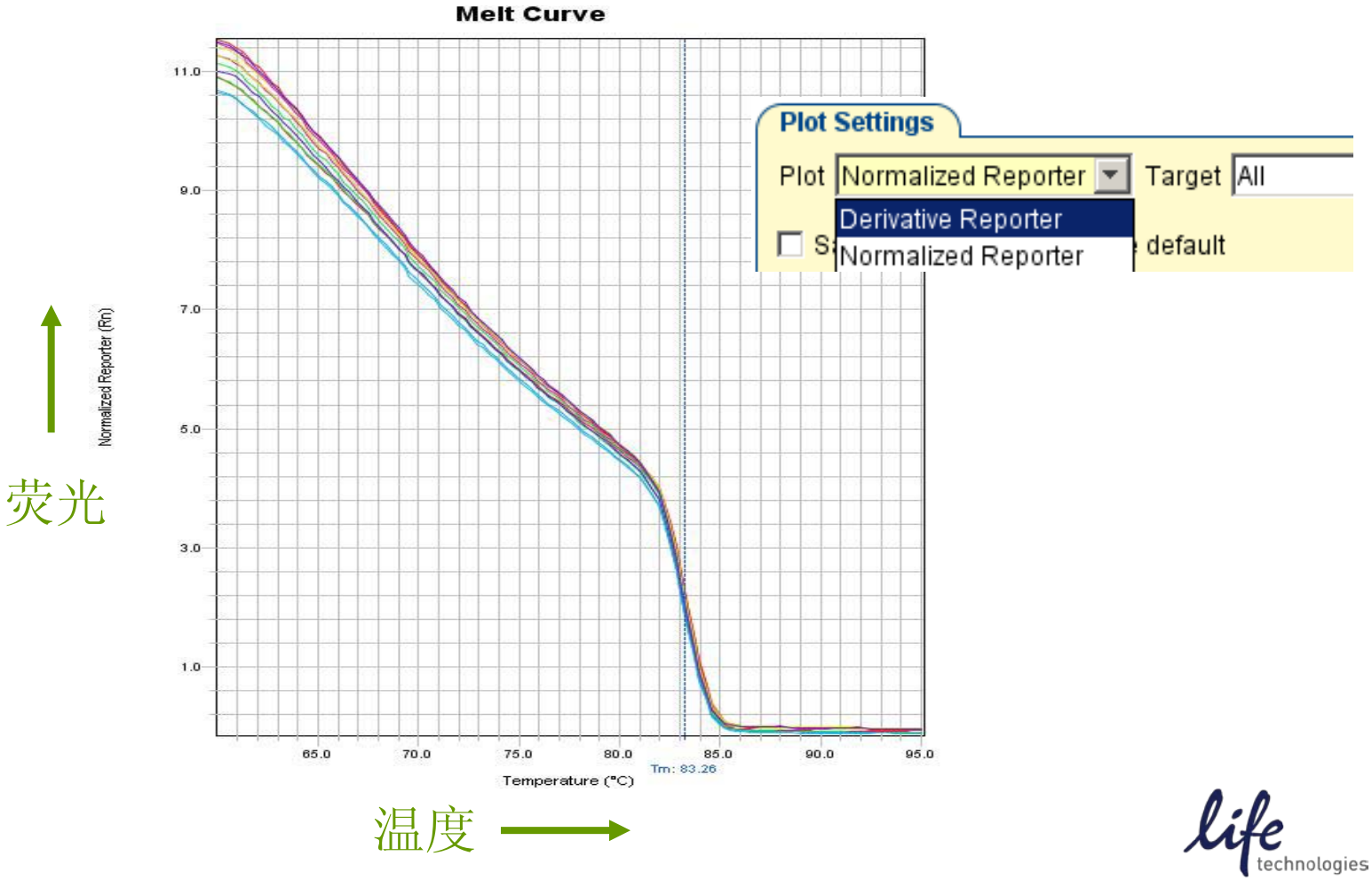
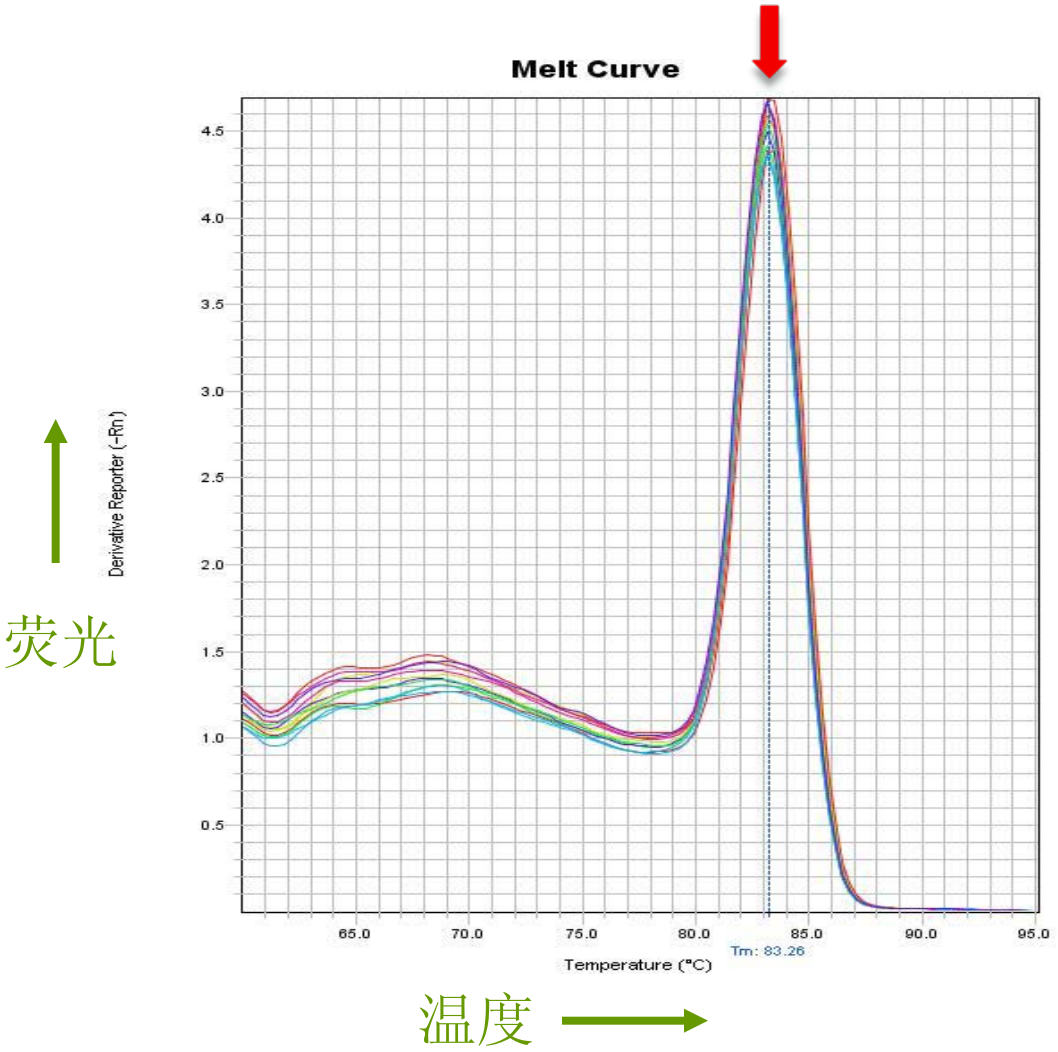
通过熔解曲线检查产物特异性

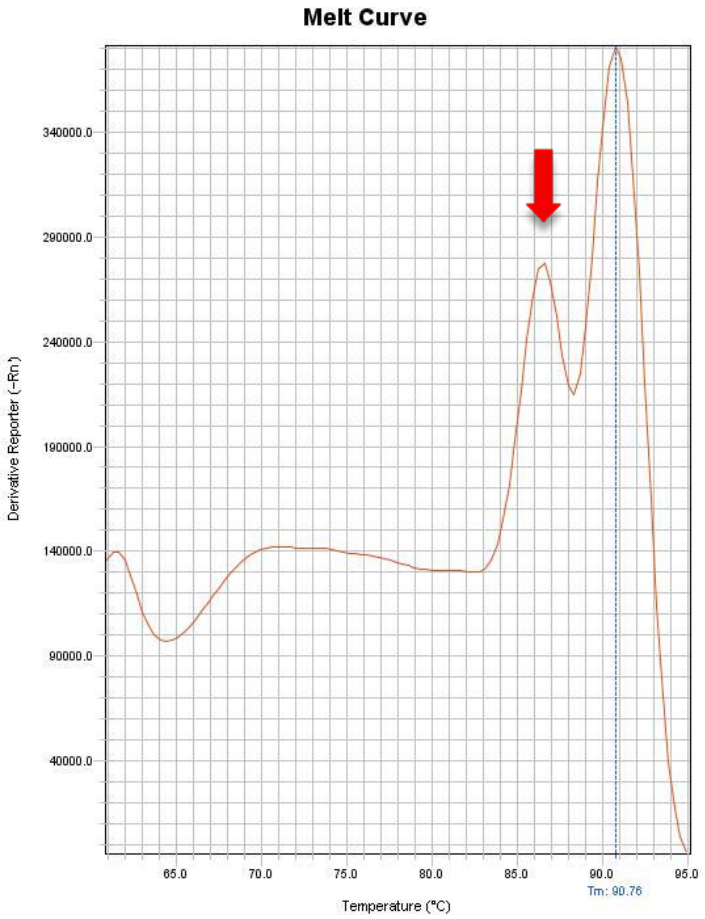
- Tm值：DNA的双螺旋结构打开一半时的温度。

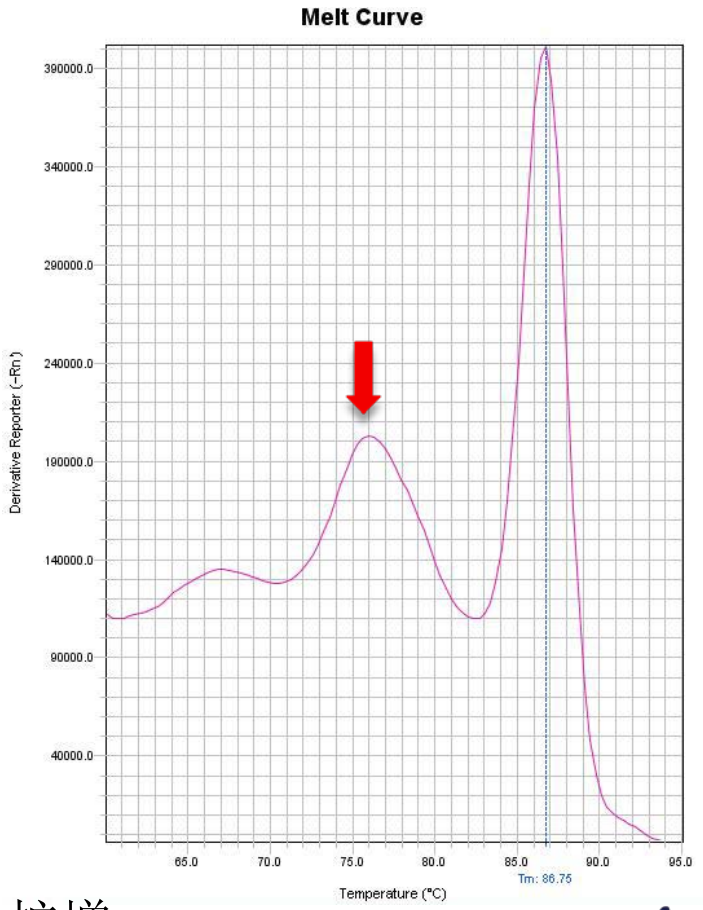


单一PCR产物的熔解曲线



多个PCR产物的熔解曲线





非特异扩增
引物二聚体

Questions ?