# DNA Technology in the 21st Century

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# Programme

- 10:00-10:15 Welcome and overview
- 10:15-10:45 Introduction to DNA manipulation
- 10:45-12:00 Preparation of agarose gels
- 12:00-13.00 Lunch
- 13:00-14:00 Extraction of DNA from bacteria
- 14:00-15:00 Analysis of the DNA by agarose gel electrophoresis
- 15:00-15:30 Questions and review

This programme fits in with Science learning objectives 1.2 (*How science works* – Practical and enquiry skills) and 2:1 (Life Processes) of the Framework for secondary science http://www.standards.dcsf.gov.uk/secondary/framework/science/fwss/o5sop.

# Outline

- DNA and personalized genomics
- Bacterial plasmids and other vectors
- Extracting plasmid DNA from bacteria
- Analysis of DNA using agarose gel electrophoresis

# Nucleotides: the building blocks of DNA

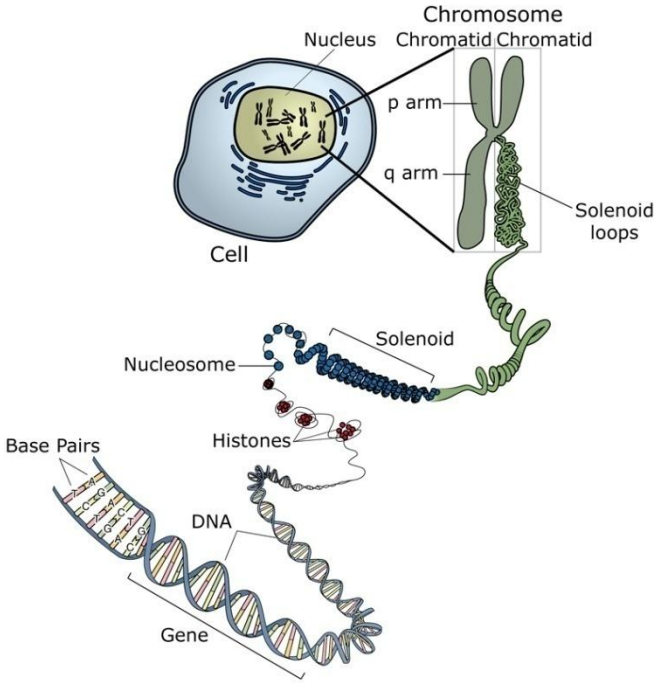
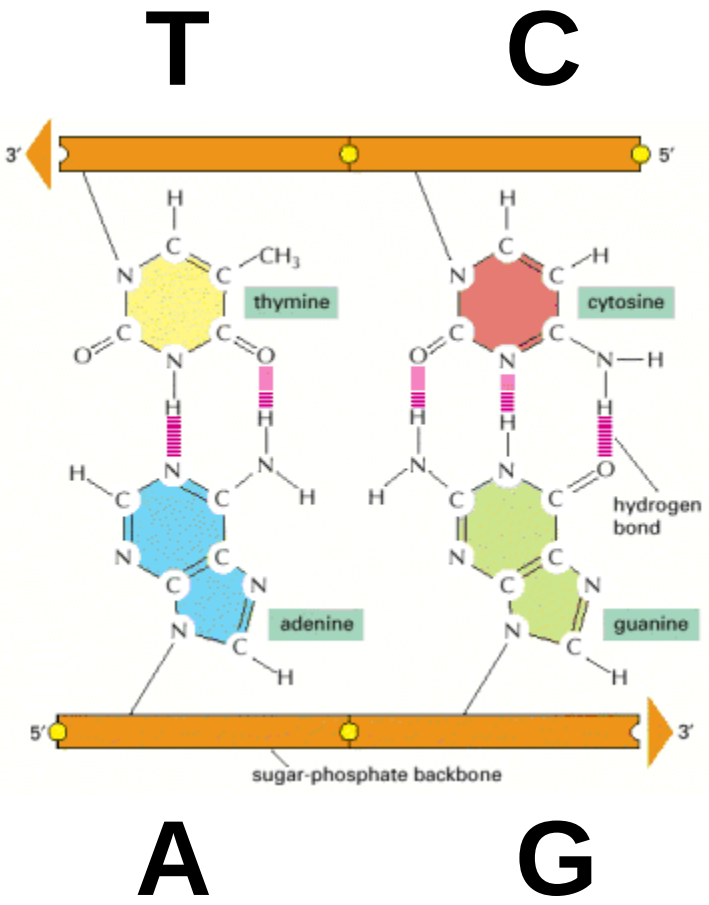


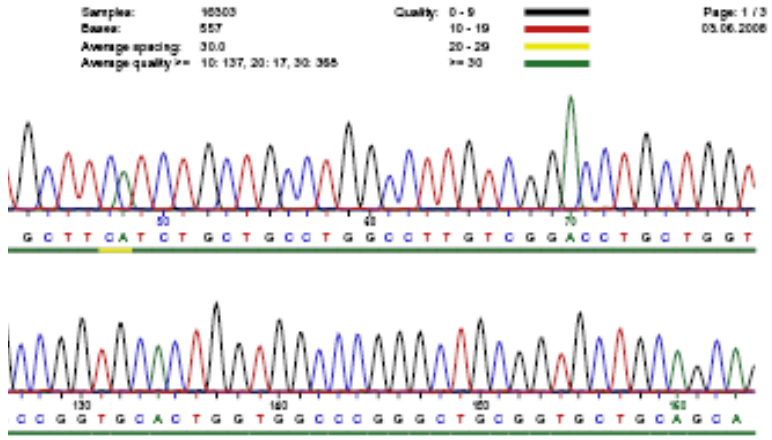

Image adapted from: National Human Genome Research Institute.

http://images1.clinicaltools.com/images/gene/chromosomebreakdown_large.jpg




http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.figgrp.599

# DNA sequencing

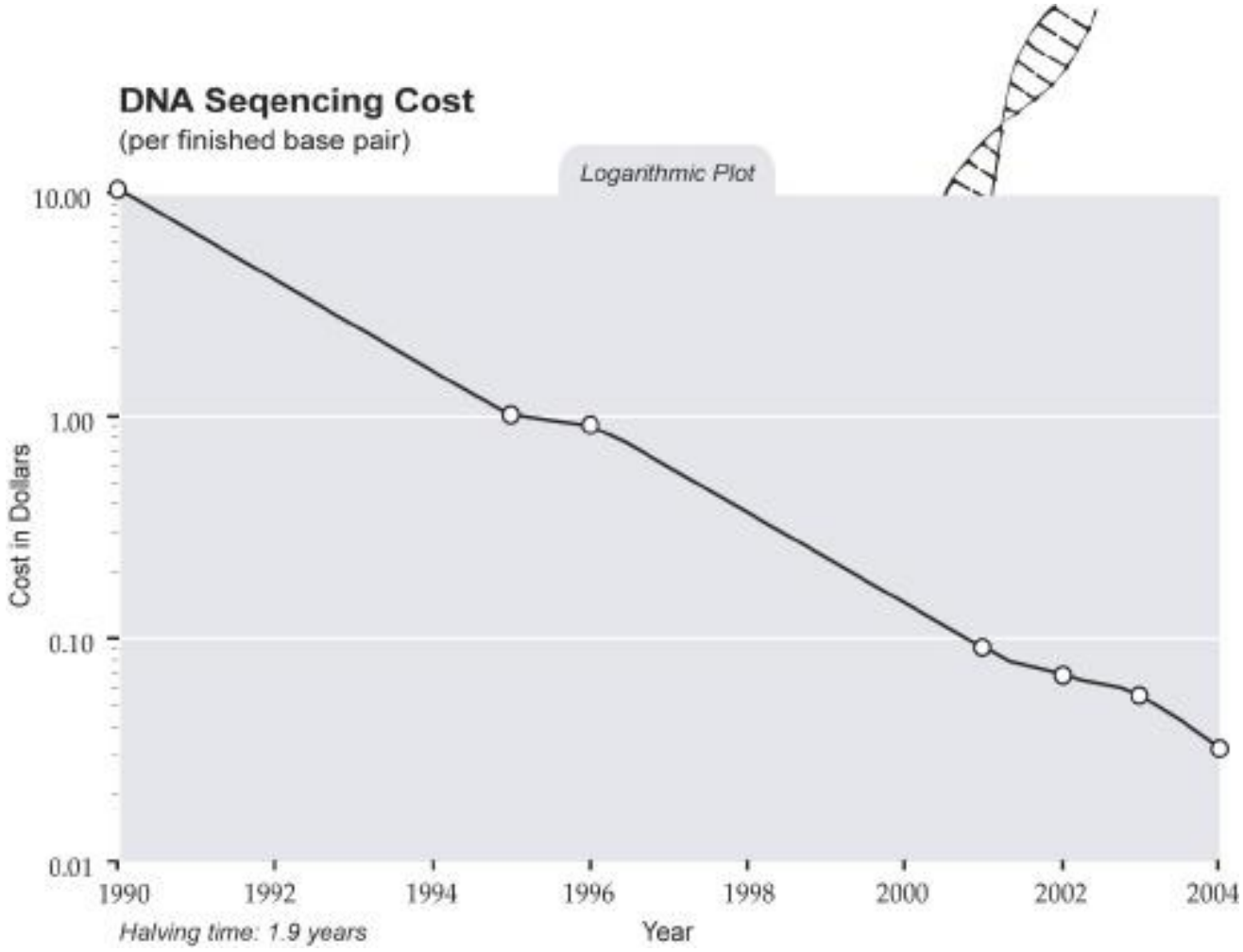
GATTACAGATTACA

http://www.brisbanetimes.com.au/entertainment/dannii-minogue-speaks-of-baby-joy-20100120-mjme.html

# Falling cost of genome sequencing

$30 billion

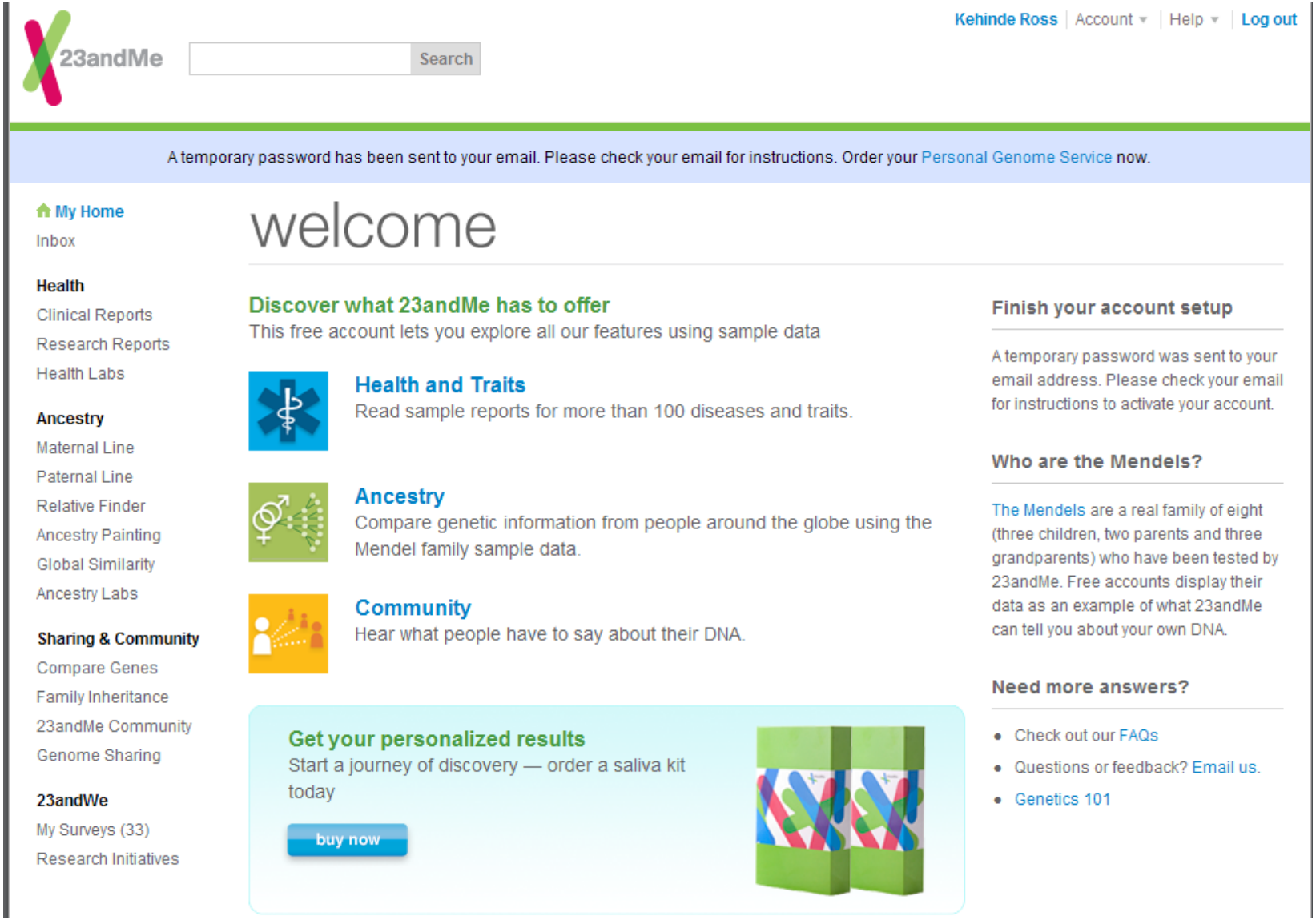
$300 million




http://singularity.com/images/charts/DNAsequencingCost.jpg

# Grand Challenge

- Genome sequencing for $1000
- Current: $10,000
- Equipment: .$500,000
- http://www.genomicslawreport.com/index.php/2010/01/12/another-stop-on-the-road-to-the-1000-genome/




Co-founded by Mrs Google

Anna Wojcicki, wife of Sergey Brin

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Research Reports
Health Labs

**Ancestry**
Maternal Line
Paternal Line
Relative Finder
Ancestry Painting
Global Similarity
Ancestry Labs

**Sharing & Community**
Compare Genes
Family Inheritance
23andMe Community
Genome Sharing

**23andWe**
My Surveys (33)
Research Initiatives

# clinical reports

**Show results for** Lilly Mendel (Mom)

Print summa

## Disease Risks (12)

- Psoriasis
- Type 2 Diabetes
- Breast Cancer
- Rheumatoid Arthritis
- Age-related Macular Degeneration
- 1 locked report

See all 12 risk reports...

## Carrier Status (24)

- Familial Hypercholesterolemia Type B new
- Phenylketonuria new
- Alpha-1 Antitrypsin Deficiency
- Bloom's Syndrome
- Cystic Fibrosis
- 1 locked report

See al

## Traits (10)

| | |
|---|---|
| Alcohol Flush Reaction | Does Not Flush |
| Bitter Taste Perception | Can Taste |
| Earwax Type | Wet |
| Eye Color | Likely Blue |
| Lactose Intolerance | Likely Tolerant |

See all 10 traits...

## Drug Response (8)

- Oral Contraceptives, Hormone Replacement T and Risk of Venous Thromboembolism
- Abacavir Hypersensitivity
- Alcohol Consumption, Smoking and Risk of Esophageal Cancer
- Clopidogrel (Plavix®) Efficacy
- Fluorouracil Toxicity

# Disease

septic shock

Asthma

Psoriasis

INFLAMMATORY DISEASES

Inflammatory Bowel Disease

Rheumatoid Arthritis

http://www.lib.uiowa.edu/hardin/MD/pictures22/dermnet/psoriasis_04pb.jpg

http://www.bio.davidson.edu/courses/immunology/Students/spring2006/Dresser/RA.html

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Martha Stewart and her team find out what lies within their DNA - deCODEme featured on Martha Stewart

Dr. Stefansson on the Martha Stewart Show

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**Scientific leadership**
deCODE leads the field in the discovery of genetic variants for common diseases

deCODE's scientific discoveries

**Compare with competitors**
We capture more medically relevant genetic variants than our competitors

compare our genetic tests

RSS News from our blog > Find Common Ancestors by Comparing Genomes in deCODEme

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# Is ignorance bliss?


http://talkabouthealth.wordpress.com/2009/02/27/254/

# Personalized Genomics

- McBride *et al.,* (2008). **Putting science over supposition in the arena of personalized genomics**
  - http://www.ncbi.nlm.nih.gov/pubmed/18665132

- Coriell Personalized Medicine Collaborative™
  - http://www.coriell.org/index.php/content/view/92/257/

- The Biotechnology Institute
  - http://forums.biotechinstitute.org/showthread.php?p=1772

# Outline

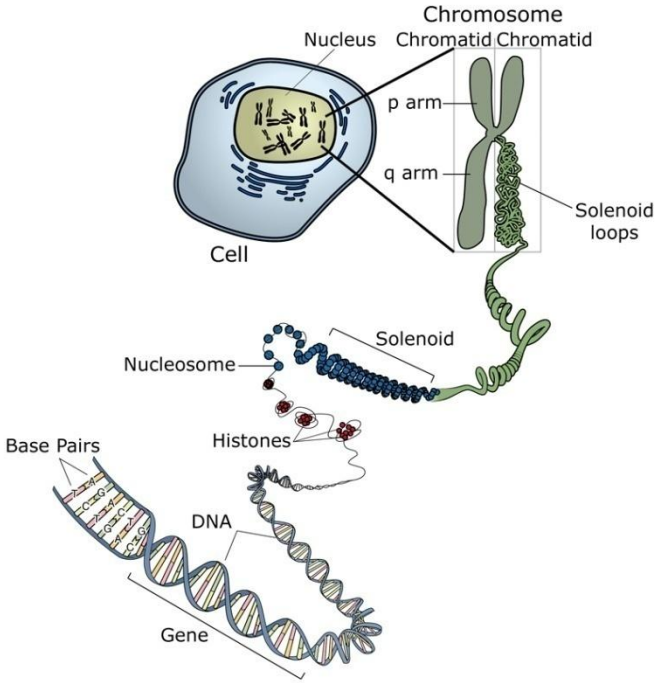
- DNA and personalized genomics
- Bacterial plasmids and other vectors
- Extracting plasmid DNA from bacteria
- Analysis of DNA using agarose gel electrophoresis

# Odd one out?




http://oceanexplorer.noaa.gov/explorations/03bio/logs/sept10/media/lasonolide3.html

# Nucleotides: the building blocks of DNA

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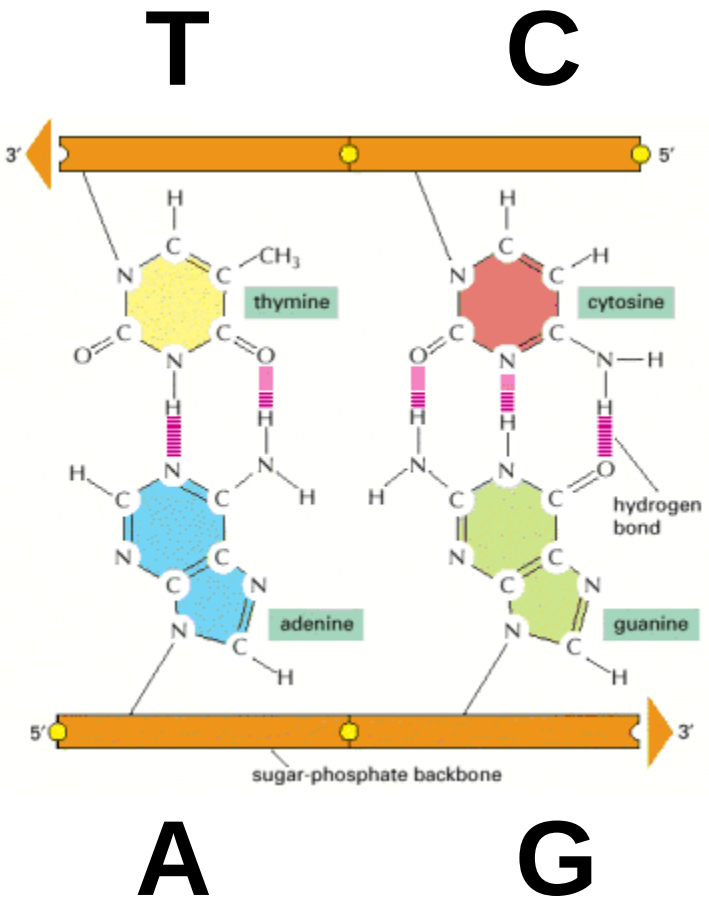


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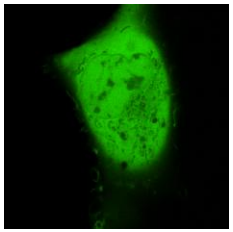
# Introduction to DNA manipulation



Extract DNA
from cell

**<u>Clone</u> gene of interest into plasmid vector**

**<u>Sequence gene directly</u>**

AGTTGACGCCA

Identify **mutations** that cause diseases

Identify **single nucleotide polymorphisms**

# Cloning (in this context)

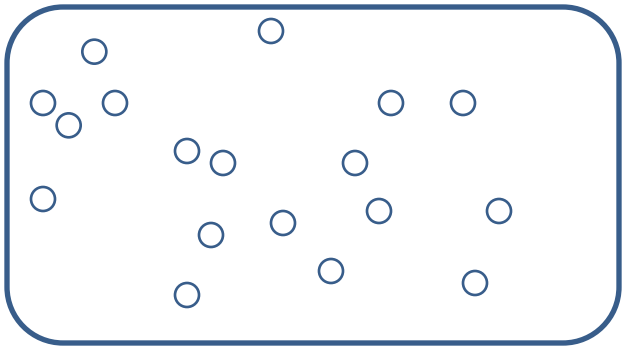
- **make identical copies of** genes of interest
- **Cloned** genes can used to study gene function in cells
- **Cloned** genes can used for gene therapy
- Can be used to make biologically important proteins
  - insulin
  - growth hormone

# Plasmids

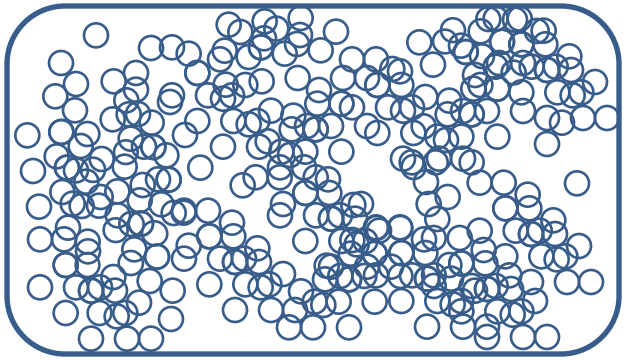
- A vector is a DNA molecule into which a fragment of foreign DNA can be inserted. The vector can then be propagated in a host cell
- Plasmids are one type of vector:
  - Circular double-stranded DNA molecules that can replicate themselves in bacteria
  - Generally circular, but can be linear

# Copy number

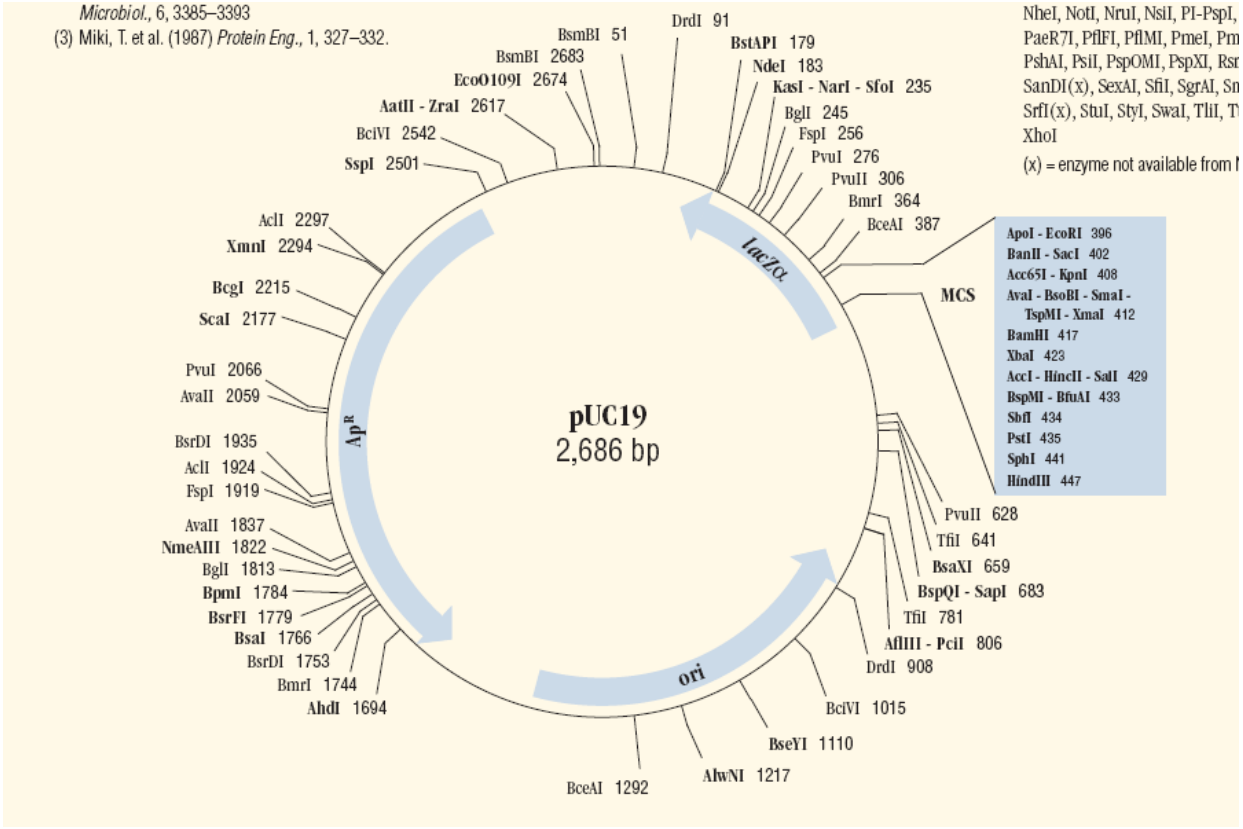
- Some plasmids only generate a few copies per bacterial cells: pBR322



- Others produce >500 copies per cell
  - e.g. pUC19, pBluescript II



# pUC18/pUC19:classic vectors




- http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/maps/pUC19_map.pdf

# Other vectors

- Plasmids
  - Limited by the size of the foreign DNA insert
- Viral vectors
  - Bacteriophage (viruses that infect bacteria)
  - Adenoviral, retroviral: used in gene therapy
- Artificial chromosomes

# Outline

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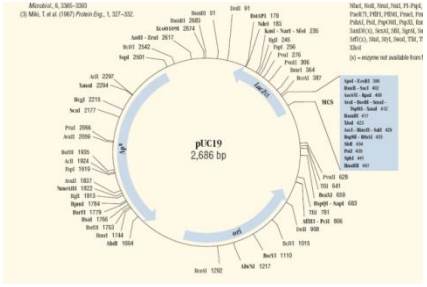
# Natural versus man-made plasmids

- Many bacteria naturally contain plasmids
  - 25 of 65 members of the *Lactobacillus* genus

    Wang and Lee (1997). Crit. Rev. Biotech.
  - The plasmids can help the bacteria
    - Antibiotic resistance
    - Protein degradation
    - Metabolism of carbohydrates, amino acids


- In molecular biology, we normally put man-made or **recombinant** plasmids into bacteria

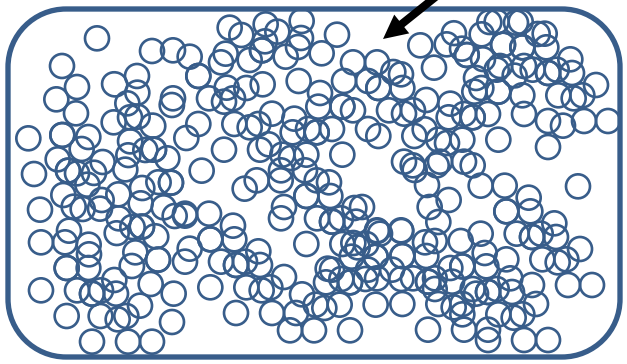
# Recombinant plasmids




http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/maps/pUC19_map.pdf

DNA of interest e.g. insulin gene

Plasmid cut open by **restriction digestion**



# A very short guide to cloning

- Cut/digest/linearize the plasmid with the **restriction enzyme**
- Treat the foreign DNA **– the insert –** with the same enzyme
- **Ligate** the insert into the plasmid using **DNA ligase**
- Put the plasmid into bacteria **(*E.coli*)** for growth and propagation**: transformation**

# Getting plasmids into *E.coli*

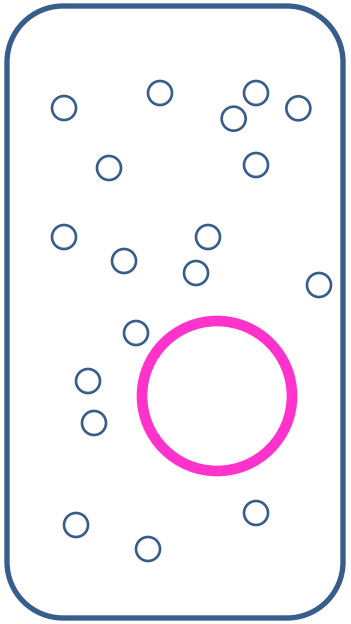
- Most strains of *E. coli* are harmless
- Plasmid propagation with *E. coli* K567
  - Harmful *E coli:* 0157:H7
- First, the cells have to be made *competent*
  - Capable of taking up the DNA
  - Then they are exposed so a sudden change in temperature - *Heat shock*

# Growing bacteria in the lab

- LB (Luria-Bertani) medium/agar
  - Tryptone (or peptone)
  - Yeast extract
  - NaCl
  - Add agar (seaweed extract) for agar plates
  - Autoclave: heat to 121°C for 15 min
- Grow bacteria overnight in LB medium
  - Medium must contain antibiotic *so that only those bacteria containing plasmids will grow*

# Purifying plasmid DNA

- Challenge is to separate the plasmid DNA from
  - Chromosomal DNA
  - Other cellular molecules (proteins, RNA etc)
- ALKALINE LYSIS
  - Birnboim and Doly (1979)
  - http://nar.oxfordjournals.org/cgi/reprint/7/6/1513



# Commercial suppliers of mini-prep kits

- Invitrogen http://www.invitrogen.com/site/us/en/home.html
- Promega http://www.promega.com/uk/
- Qiagen http://www1.qiagen.com/
- Sigma-Aldrich http://www.sigmaaldrich.com/united-kingdom.html
- Stratagene http://www.stratagene.com

# Plasmid purification

- Solution 1: resuspension solution
  - Tris-HCl + EDTA
- Solution 2: lysis solution
  - sodium dodecyl sulphate and NaOH
- Solution 3: neutralization solution
  - Potassium acetate and acetic acid

- All commercial kits have these solution but you can make them yourself in a standard molecular biology lab

- pp44,45 of Qiagen plasmid purification handbook
- http://www1.qiagen.com/Plasmid/handbooks.aspx

The key innovation of is the *anion-exchange column* for binding the plasmid DNA: http://www1.qiagen.com/Plasmid/AnionExchangeResin.aspx

# Summary

# Outline

- DNA and personalized genomics
- Bacterial plasmids and other vectors
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# Agarose gel preparation

http://www.youtube.com/watch?v=wXiiTW3pflM

# Agarose gel electrophoresis

- Purified from agar, a seaweed (marine algae) extract
  - the same agar used for agar plates
- Agar is widely used as a gelling agent in the food industry
- Agarose is a polysaccharide consisting of alternating galactose

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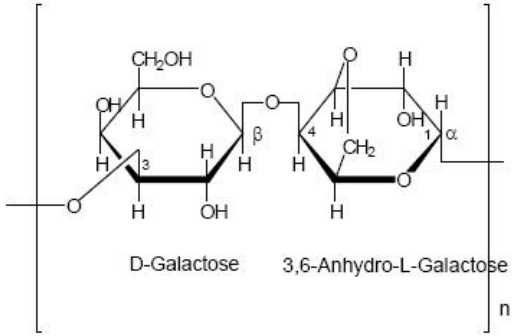
**ProductInformation**

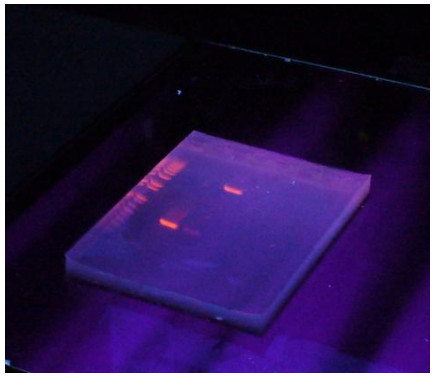
**AGAROSE**

**CAS NUMBER:** 9012-36-6
**CAS NUMBER:** 39346-81-1 (products A9414, A4018, A6560, A9045, A0701)
**SYNONYMS:** 3,6-Anhydro-α-L-galacto-β-D-galactan; FastLane agarose; Indubiose A4; NuSieve GTG; Odigose; Seakem; Sepharose



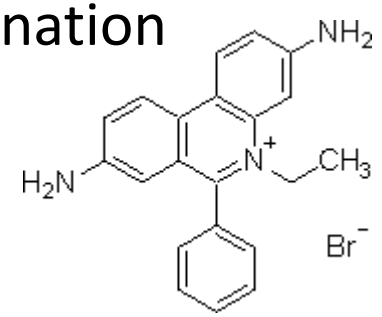
# Agarose gel electrophoresis

- Agarose prepared in a **buffer**
  - TAE (Tris-acetate-EDTA) buffer
  - TBE (Tris-borate-EDTA) buffer



*Wikipedia*

- We will prepare a 1% gel for analysis of DNA
  - 1 gram of agarose powder in 100 ml of buffer
- **Ethidium bromide** is added to the agarose to enable visualization by UV transillumination



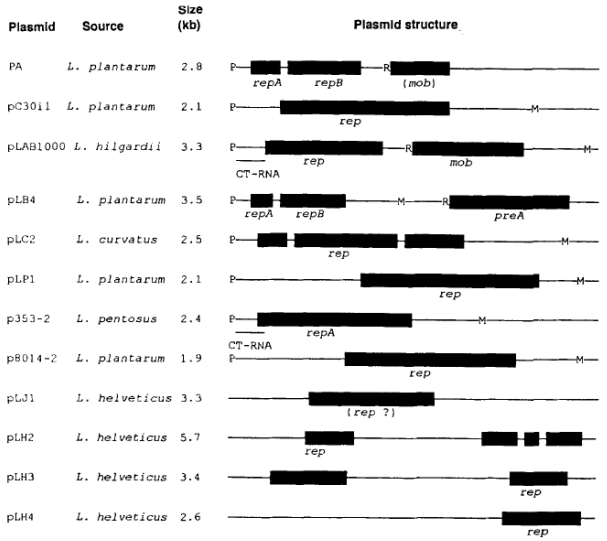
# Research question

- Do the “friendly” bacteria in Yakult contain plasmids?
- *Lactobacillus casei*

*Critical Reviews in Biotechnology*, 17(3):227–272 (1997)

**Plasmids in *Lactobacillus***

*Tsung-Tsan Wang and Byong H. Lee**
Department of Food Science and Agricultural Chemistry, McGill University, Macdonald Campus, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9




Wang and Lee (1997). Critical Reviews in Biotechnology 17:227-272

# Recommended reading

- Christopher Howe, Gene Cloning and Manipulation, Cambridge University Press, 2007.
- Qiagen Plasmid Purification Handbook.
  - http://www1.qiagen.com/Plasmid/handbooks.aspx

- Wellcome Trust website
  - http://genome.wellcome.ac.uk/resources/glossary/#G

# PubMed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Newest 2009 H1N1 Flu Outbreak Sequences
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