# Collection of technology

# Sequencing

## Shotgun sequencing

Base pairs --> many segments --> assemble\

## Positional cloning

- 结合了基因定位（marker）和DNA克隆。
- Identify the first marker to map the gene, then clone the genes in the mapping region and try to find the target gene, if not then try to find next SNP marker; repeat until find the target gene

## Sanger sequencing

- Four separate PCR reactions
  - each one has a specific ddNTP with fluorescent or some label
- Because ddNTP lack of hydroxy in 3' end, after combining with a ddNTP, these chain will can't continue to enlonger.
- Then using Gel electrophoresis to show the results

## Next-generation sequencing, SBS, sequencing by synthesis

- Prepare genomic DNA
- Attach DNA to surface
- Bridge amplification
- Denature
- Sequencing
  - ddNTP with fluorescent
    - Therefore, every run can only add one base
    - After sequencing this run, you need to cut the azide(叠氮基) and fluorescent
  - polymerase

### Application

#### Genome resequencing

#### Prenatal testing

#### Counting device

## De novo genome assembly

- assembly without the reference genome

## PacBio

- SMARTBELL(DNA with ow hairpin) binds to DNA polymerase
- a fluorescent nucleotide bind
- a light pulse is produced
- Recognize bases

## Oxford Nanopore

- Using the electricity
- DNA pass through a small channel, which is the so-called nanopore
- The four bases can have different voltage
- Te affects to the currents can be recognized as bases

## Summary

| Sequencing | Advantages | Disadvantages |
|---|---|---|
| Sanger | Long reads(~700 bp)/ High accuracy | Low throughput |
| Second generation(illumina) | High throughput/ Whole genome coverage/High sensitivity | Short reads(~150bp)/PCR amplification bias |
| Third generation | Long reads(several kb)/High throughput/No amplification/ Miniaturisation (Nanopore,便于携带) | High error rate |

# Sequencing stratedies

## Whole genome sequencing

## Whole exome sequencing

## Targeted sequencing

## summary

| 测序类型 | 测序深度 | 覆盖基因组的百分比 | 主要目标 |
|---|---|---|---|
| 全基因组测序 | > 30X | 100% | 用于识别疾病相关基因中的罕见变异 |
| 全外显子测序 | > 50-100X | 2% | 只覆盖编码蛋白质的基因，可能是治疗靶点 |
| target sequencing | > 500X | 取决于面板 | 无意外发现，主要用于识别疾病相关基因中的罕见变异(已经有已知信息) |

# Mitochondrial replacement therapy

## 1. Three-parent baby

Healthy cytoplasm(**Donor egg**) + mother's nucleus + father's sperm

- Pronuclear transfer, PNT
- Spindle transfer
- Polar body transfer

| Method | Description | Advantages | Limitations |
|---|---|---|---|
| Pronuclear Transfer (PNT) | Involves the fusion of the donor egg with sperm. The term "pronuclear" refers to the state during fertilization where the genetic material of the sperm and egg have not yet fused. | - | - Use of cytoskeleton disruptor<br>- May disturb the centromeres and cause heteroploidy<br>- Takes more mitochondria |

| Method | Description | Advantages | Limitations |
| --- | --- | --- | --- |
| Spindle Transfer | Involves taking the egg under the situation (metaphase) of spindle into the donor egg. | Less mitochondria | - Use of cytoskeleton disruptors<br>- May cause heteroploidy |
| Polar Body Transfer | - | Few mitochondria in polar body<br>Easy to separate the polar body | Use of cytoskeleton disruptor |

### 2. Gene replacement therapy

- Injection of AVV

### 3. Genome editing

- mtZFN
- mitoTALENs
- DdCBE

# Twin studies , the genetic and environment

- Two pairs of twins:
  - MZ twins
  - DZ twins
- Calculate the concordance of MZ and DZ

# Genome-wide association studies(GWAS)

- SNP
  - haplotype block with tag SNP
- LD-based association studies
  - Using tag SNP to verify the gene of interest
- Common disease-Common variant hypothesis
  - Most variation is evolutionarily neural
  - The variation can have addictive effect to contribute to a disease
- However, about the **Rare variants** , GWAS is not suitable

# Resequence gene

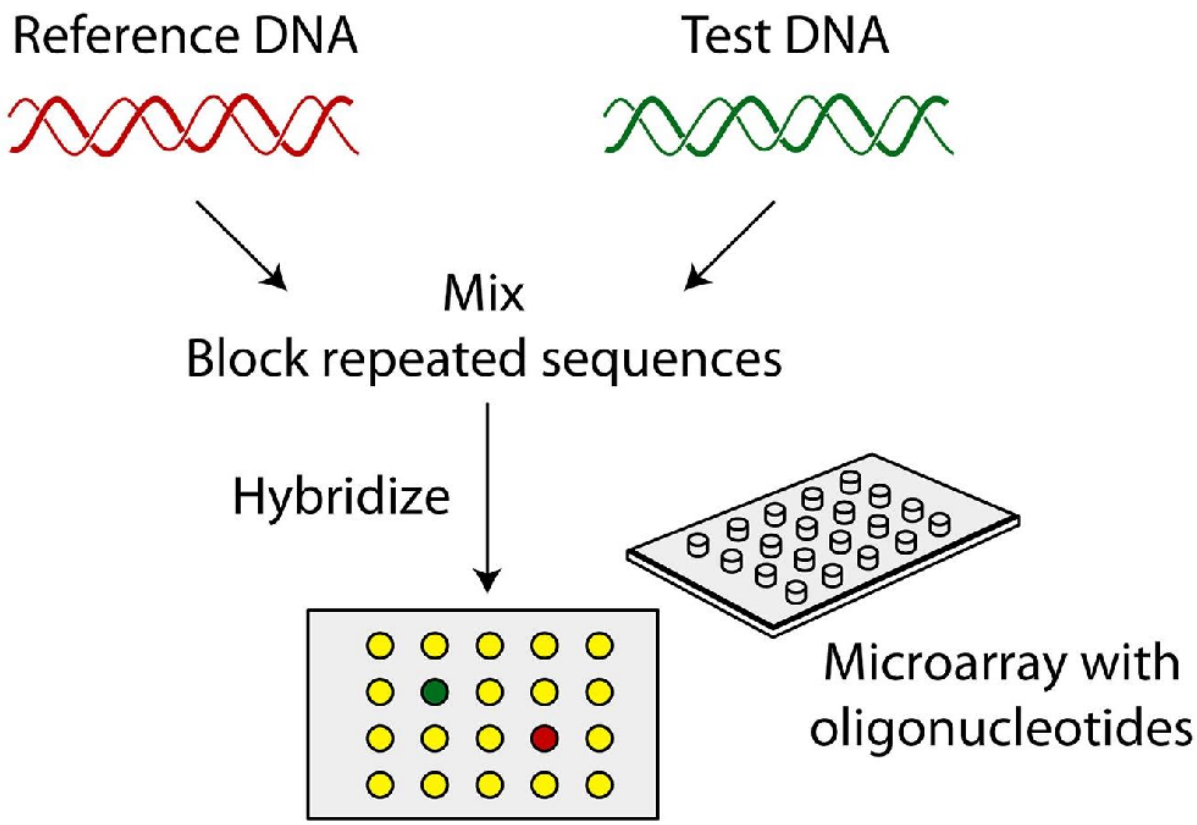
- Finding Rare variants
- 已知参考基因组，对特定的基因进行重测序，比对。

# Molecular tools

## Fluorescence in situ hybridization(FISH)

## Comparative genome hybridization(CGH)

- 比较被测试的DNA和标准的reference DNA的差异




# Gene clone with host cells

- Using vector to transfer gene in to host gene (Mostly bacteria)
- After introduce the gene segments, culture the bacteria under the environment with antibiotic
  - Because the gene we transfered has the antibiotic resistance gene for selection
  - 维持质粒，只有成功导入了质粒的细菌才能够存活下来
  - 确认克隆成功，如果进过抗生素筛选后细菌能活下来说明质粒倒入成功

# Gene ligation

- Process
  - Restriction enzyme cuts the DNA
  - Addition of a DNA fragment
  - DNA ligase
    - DAN ligase catalyzes the formation of a phosphodiester bond
    - Repair DNA strands

# Cancer gene detection

## GWAS for cancer

- Through GWS, to find the most frequency mutated genes identifies in selected cancer genome sequencing projects

## Liquid biopsy

### ctDNA

- check circulating tumor DNA

### CTCs

- Check circulating tumor cells

# CAR T

- Chimeric Antigen Receptor T cell therapy
- 体外编辑，培养T细胞，然后注射回体内
- 编辑患者自己的T细胞，让T细胞能够识别并且攻击cancer cell

# PD/PDL1 immunotherapy

- PD 1 in the T cell
- PDL1 in the surface of tumor cell
- PD 1 can bind to PDL 1 thus to stop the killing of tumor cell
- PD1/PDL1 immunotherapy can inhibit the binding of PD1 and PDL1
  - then the T cell $Ca^{2+}$n kill the tumor cells

# In situ hybridization(Mostly FISH, fluorescent)

- To localize a specific DNA or RNA, to detect chromosomal abnormalities or gene mutation
  - by labeled complimentary DAN, RNA, fluorescent probe
  The steps:
- Fixation of the cell
- Design of probes
  - should be complimentary with the fragment of interest
  - Also need to use fluorescent label to label the probe
- Denaturation
  - probe and Target DAN denaturate
- Hybridization
  - cool down and the probe will bind with the targeted DNA
- Visualize under fluorescence microscopy
  - if there are mutations, then it can't have fluorescent signal

# transgenic reporters

- Fluorescence add to the interest gene, to verify the result if the gene is transferred(reporter gene)

# Random mutagenesis

- Cells or organisms are exposed to mutagens
- Steps
  - make random mutations
  - screen for phenotypic change
  - identify the gene underlying mutant phenotype

# Target knock-out or knock-down

# Genome-wide RNAi Screening

- can produce siRNA(small interference RNA) which can bind to mRNA and then stop it to translate into protein
- Steps
  - Making dsRNA RNAi library(can be genome wide, can do many RNA i at once)
  - Introduce the RNAi to the cell
  - dsRNA will be cleaved into small fragments, which is siRNA, small interference RNA
  - siRNA bind with other proteins, to form the RISC complex
  - RISC bind with the targeted mRNA, now it can't express

# CRISPR-based screening

# Transcriptomics

## Microarray analysis (gene chip analysis)

- Can compare two sets of cells expression

## RAN sequencing

## single RNA sequencing

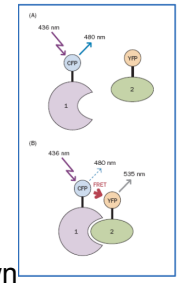
- RNA --> cDNA --> Amplified RNA/cDNA --> Sequencing library

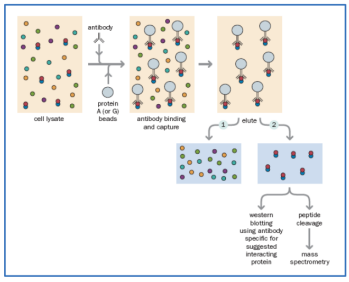
## Protein interactomes

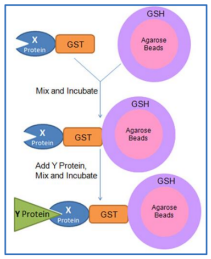
### Yeast two-hybrid screening

- Transcription factors bind to the upstream sequence
- then activate the down stream reporter gene

### validate protein-protein interaction:

1. FRET
2. Co-IP
3. Pull-Down 

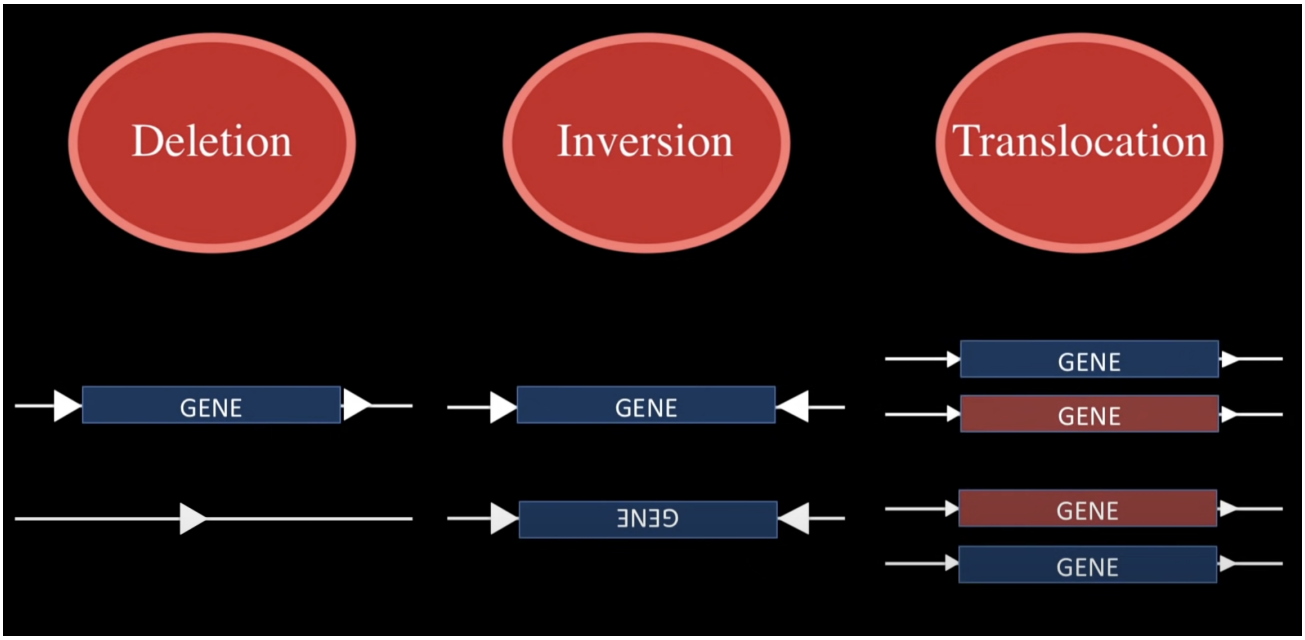




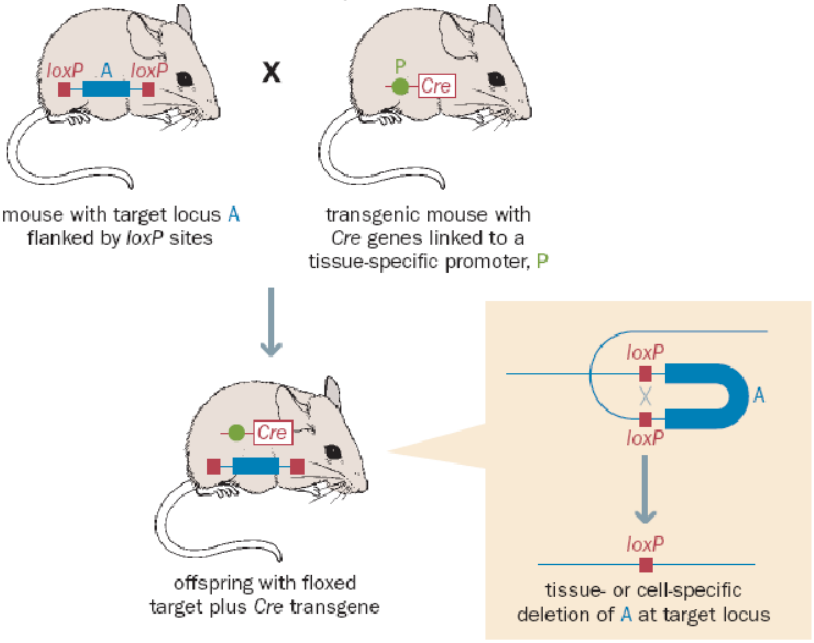
4. identify protein-DNA interaction:
   1. ChiP-Seq

# Cre-Lox system

- Two components
  - Cre recombinase, can bind with the substrate DNA
  - LoxP recognition site




- can be used to produce transgenic mice, or conditional knock out animals




# Standardized staining procedure

- can be used to view the structural feature about karyotypes
- Need to let cells stay in metaphase for better searching
  - Using mitogen, force the cells into metaphase
    - PHA, phhytohemagglutinin
    - Colchicine can be added to stop them in metaphase

# Carrier screening

- identify if a people is a carrier of a disease
- Carrier screening offers an opportunity to identify couples at risk before having the baby
- Options for a couple in which both partners are carriers include the following:
  - Choosing not to have children
  - egg donation
  - termination of an affected pregnancy

- prenatal diagnosis and planning for care of an affected child

# Prenatal screening

- Predict if a baby can have defects, before the birth

## Amniocentesis

- 羊水穿刺术

## Non-invasion prenatal testing

- test the blood of the mother
  - because there can be the dead flowing cell of the baby, then you can check it

# Newborn screening

## Heel stick

- 在脚踝刺一下，滴血测试

## hearing screening

- 确定新生儿是否存在听力损伤

## Congenital heart screen

## Bacterial inhibition assay(check PKU)

- 在培养皿上滴血
- The size of the halo related to the concentration of phenylalanine
- 血液样本中的会影响细菌生长，通过细菌来反映是否有PKU

# Direct to consumer(DTC)

# Treatment of genetic disease

## Diseases

- Phenylketonuria
  - Genetic deficiency of phenylalanine hydroxylase
- Congenital hypothyroidism
  - defect in thyroid development caused by gene mutation
- **Hemochromatosis**
  - hyperactivity to absorb too much iron from the diet
- Type 1 Tyrosinemia(Tyrosine的代谢收到影响)
  - deficiency of fumarylacetoacetate hydrolyase（FAH)
  - cause accumulation of Fumarylacetoacetate, which is toxic to liver

## Genetic drugs

### Small molecule drugs

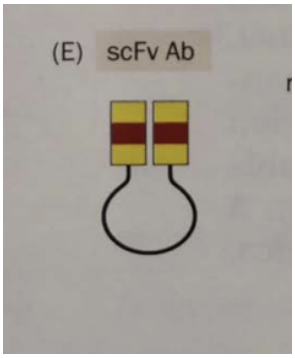
- Example: **PARP inhibitors**
  - PARP can fix the damage of the cancer cell

- SO PARP inhibitor can stop the cell to repair

## Application of genomic in drug discovery

- Genome-wide screen
  - identify new targets for drug development
  - to identify new small molecule **inhibitors** or **agonist**
  - new vaccines

#### Therapeutic proteins

- Examples
  - Insulin produced by recombinant DNA in **bacteria**
  - Therapeutic protein produced in the **milk of transgenic livestock**
  - **therapeutic antibodies**
    - hybridoma
    - genetic engineering
      - chimeric Ab
      - Humanized Ab
      - scFv Ab,没有constant region
      - 

        - single chain antibody
          - not contain the constant region of human
  - Making transgenic mice to express human monoclonal antibody
    - Mouse variable regions of the heavy and k light chains are placed by the human's
    - retain the mouse constant region for normal deveploment
  - Antibody–drug conjugates (ADCs)
    - 可以靶向杀死癌细胞
    - 抗体与细胞毒性(cellular toxin)药物连接而成
  - Aptamer

### Cell therapy

#### 1. Stem cell therapies

- Source
  - Pluripotent embryonic stem cell
  - Tissue stem cells
  - iPSC
  - Nuclear reprogramming
    - Somatic nuclear transfer
      - patient somatic cell nuclear + egg plasma

        - produce Patient-specific and disease- specific pluripotent stem cells
    - cell fusion
    - direct reprogramming

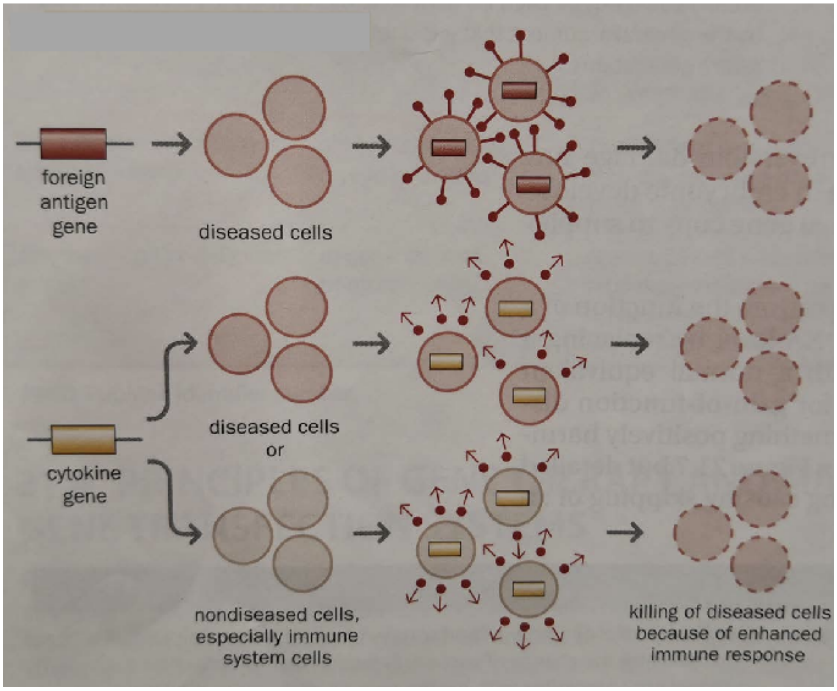
**2. Immunotherapies**

- Humoral immunity
    - Antibody-mediated
- Cellular immunity
    - T cell-mediated
- Chimeric antigen receptor T cells(CAR T)
    - CAR genes inserted into T cell via vector
        - CAR expressing T cell
        - Use of CIPDEL technology in CAR T construction
    - can recognize cancer cells
    - Single-chain antibody fragment

## Gene therapy

- Direct Genetic modification of cells
- In vivo and Ex vivo gene therapy
    - In vivo:直接对体内基因进行编辑
    - ex vivo: remove cells out of the body, and edit them and amplify . Return cells to body
        - The first gene therapy success was ex vivo, treat SCID
- Germ-line and somatic gene therapy
  - Germ line 目的是修正生殖细胞，为下一代提供正常的配子贡献
  - Somatic 是为了修正体细胞的DNA缺陷
- Gene augmentation therapy
    - disease cells lack of A --> transfer gene to make it can product
- Gene mutation correction
    - Disease cell can produce harmful products --> cut this gene, can't produce the harmful products again
- Gene expression inhibition
    - Using something to inhibit the produce of some kind of gene expression (but not gene change)
        - siRNA, antisense oligonucleotide
- Direct killing of disease cells
    - Using DTX, diphtheria toxin to kill the cells
- Assisted killing of disease cells
  - foreign antigen gene (可以被免疫系统识别成外来的抗原并杀死)
  - cytokine gene（加强免疫系统的能力）

- kill the disease cells because of enhanced immune response




- Delivery
  - Viral vector
  - Cationic liposomes
    - 外面包裹着正电荷（阳离子），穿过细胞膜

**RNA therapy**

- Ribozyme
- Antisense oligonucleotide(ASO)
- siRNA

## Example: Duchenne muscular dystrophy

# Gene editing in gene therapy

## Zinc finger nuclease(ZFN)

## TALEN

## CRISPR

- Exon deletion
- Exon skipping
- Exon reframing
- Exon knock-in