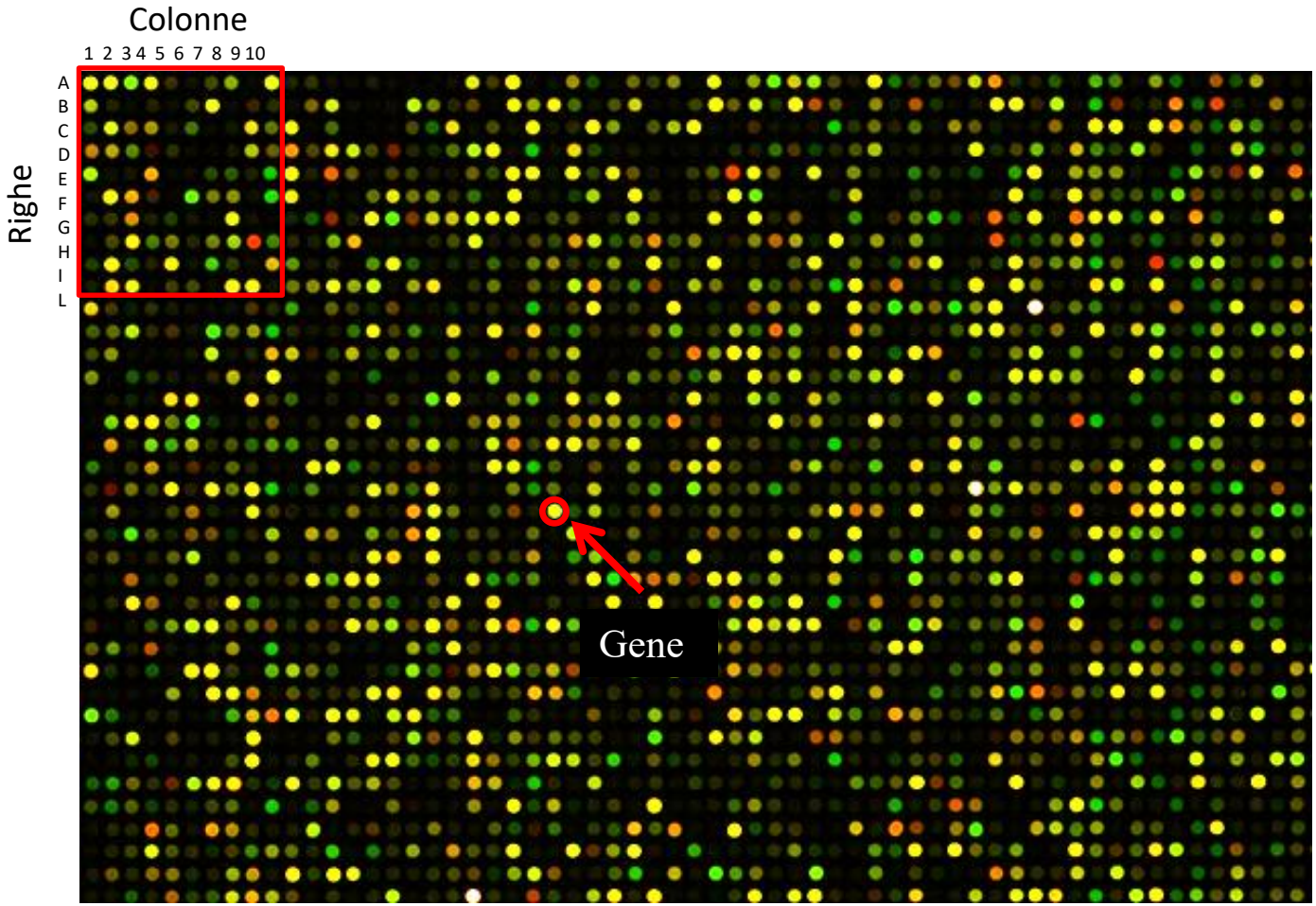
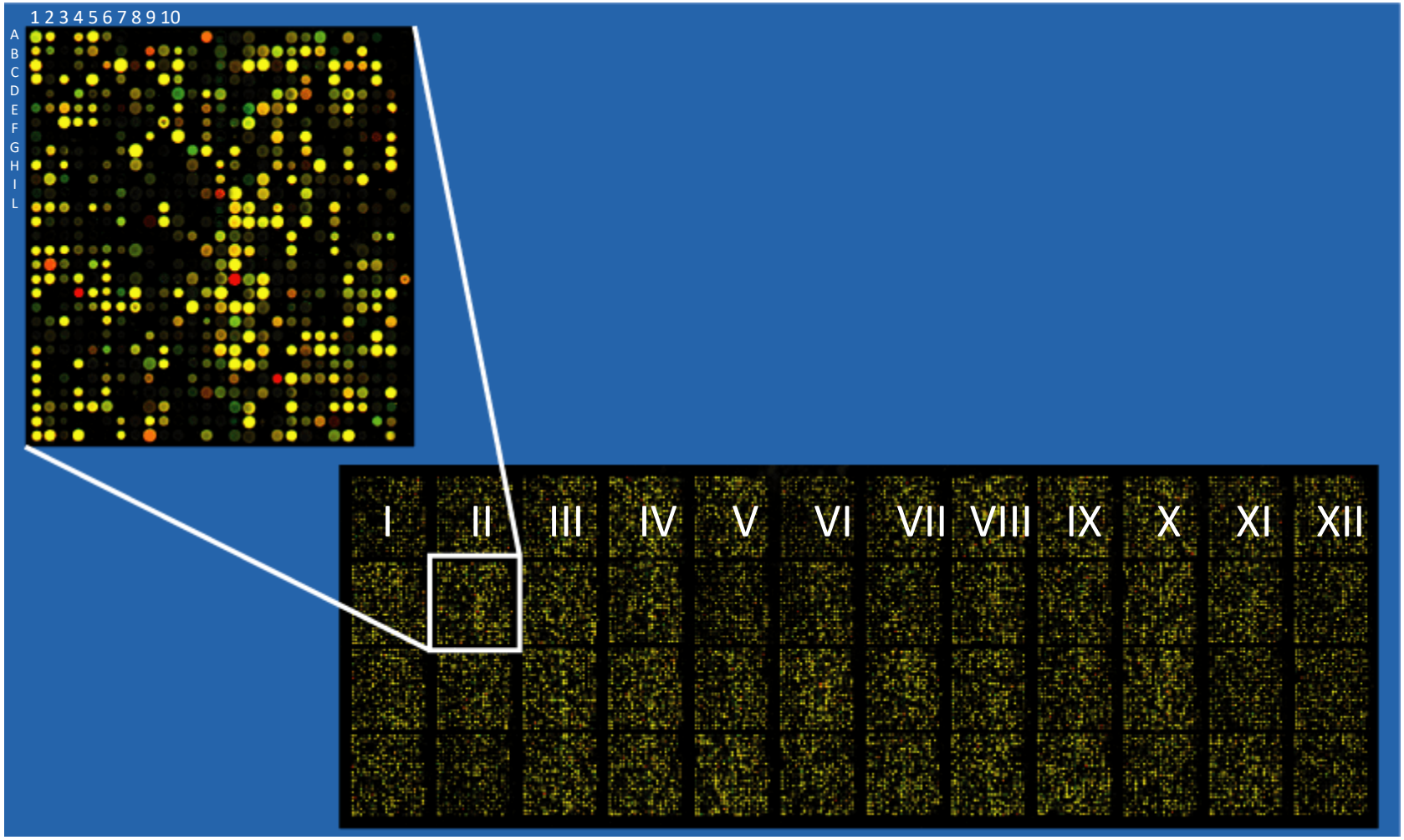

Microarrays

Cos'è un MICROARRAY?



Cos'è un MICROARRAY?



Cosa può essere inserito in un MICROARRAY?

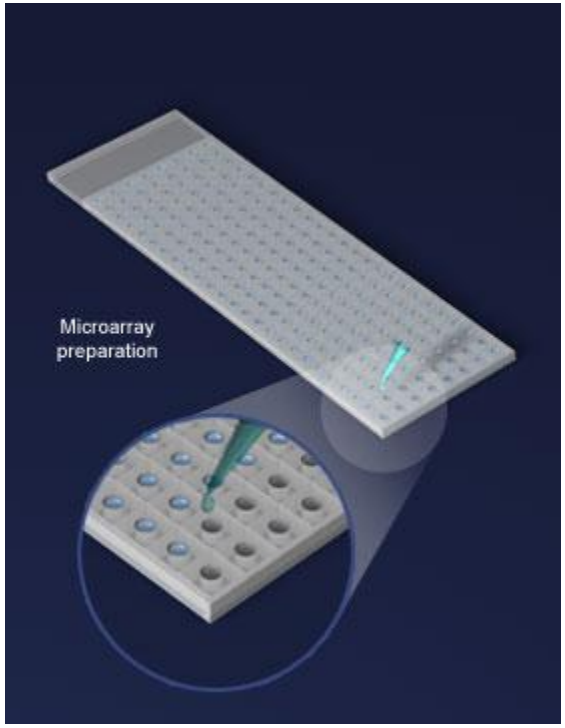
- Peptidi (anticorpi)
- Geni espressi
- DNA genomico

Come può essere costruito un MICROARRAY?

Chip a cDNA

1) Libreria a cDNA

2) Amplificazione per PCR



3) Deposizione sul Chip

4) Fissaggio tramite calore o raggi UV

5) Denaturazione tramite calore o agenti chimici

Metodo poco laborioso che ha però l'inconveniente di portare ad arrays a bassa densità

Come può essere costruito un MICROARRAY?

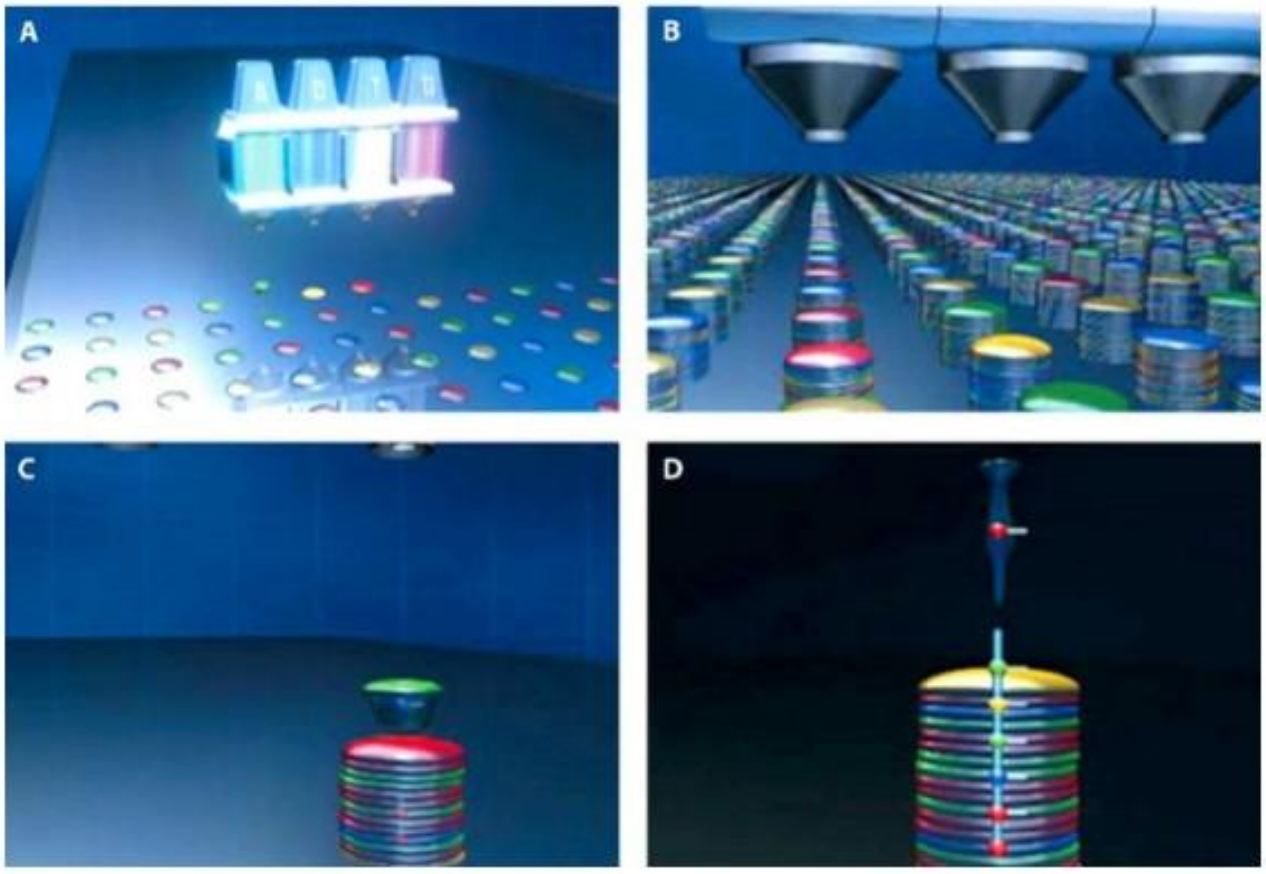
Chip a oligo

- **Metodo InkJet**
- **Metodo Affymetrix**

Tutti questi metodi prevedono la sintesi *in situ* di oligonucleotidi complementari alle sequenze dei geni che essi rappresentano

Portano alla realizzazione di chip ad alta densità

Agilent oligonucleotide microarray



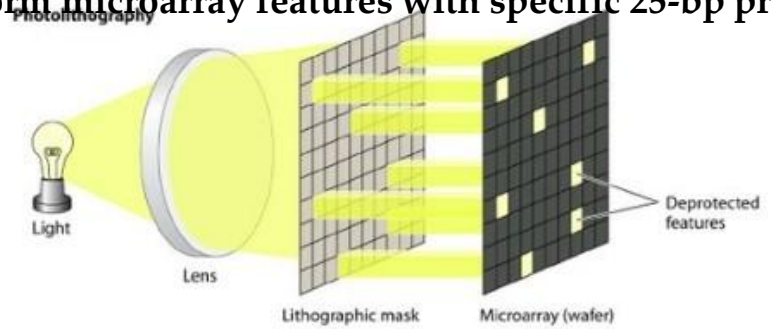
(A) Noncontact inkjet printing technology delivers a small and accurate volume (picoliters) of nucleotides to the first layer on the microarray surface. (B) Repeated rounds of base-by-base printing extend the length of specific oligonucleotide probes. (C) Close-up of growing oligonucleotide chain with a base being added. (D) The final product is a 60-mer in situ-synthesized probe as a feature on a microarray containing thousands of specifically synthesized probes

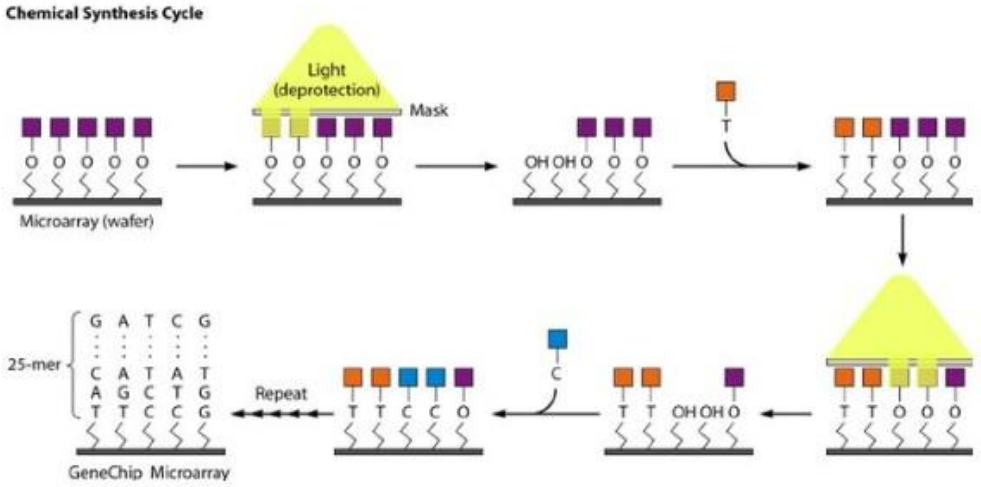
Affymetrix GeneChip oligonucleotide microarray

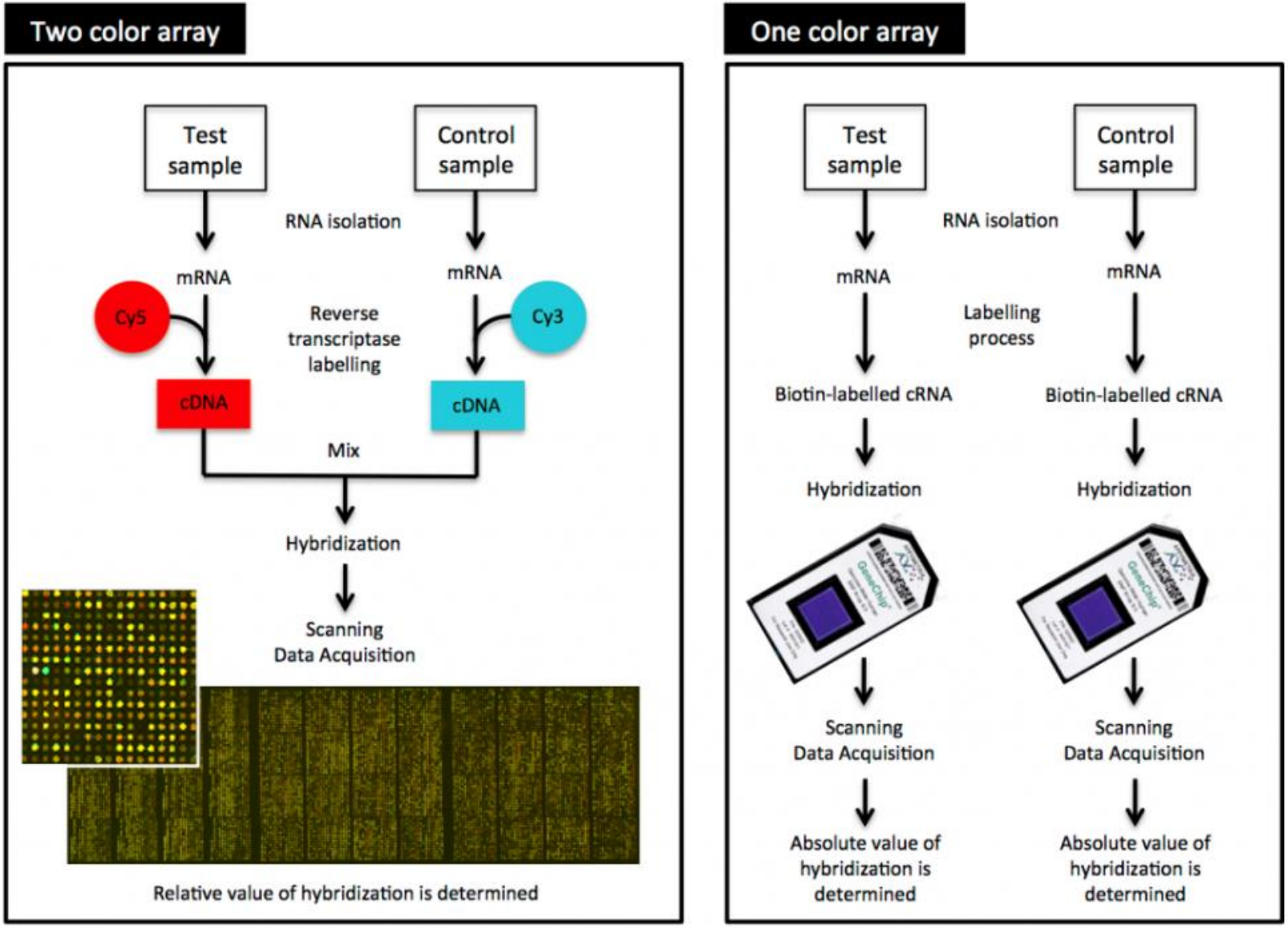
Photolithography.

Top: UV light is passed through a lithographic mask that acts as a filter to either transmit or block the light from the chemically protected microarray surface (wafer). The sequential application of specific lithographic masks determines the order of sequence synthesis on the wafer surface.

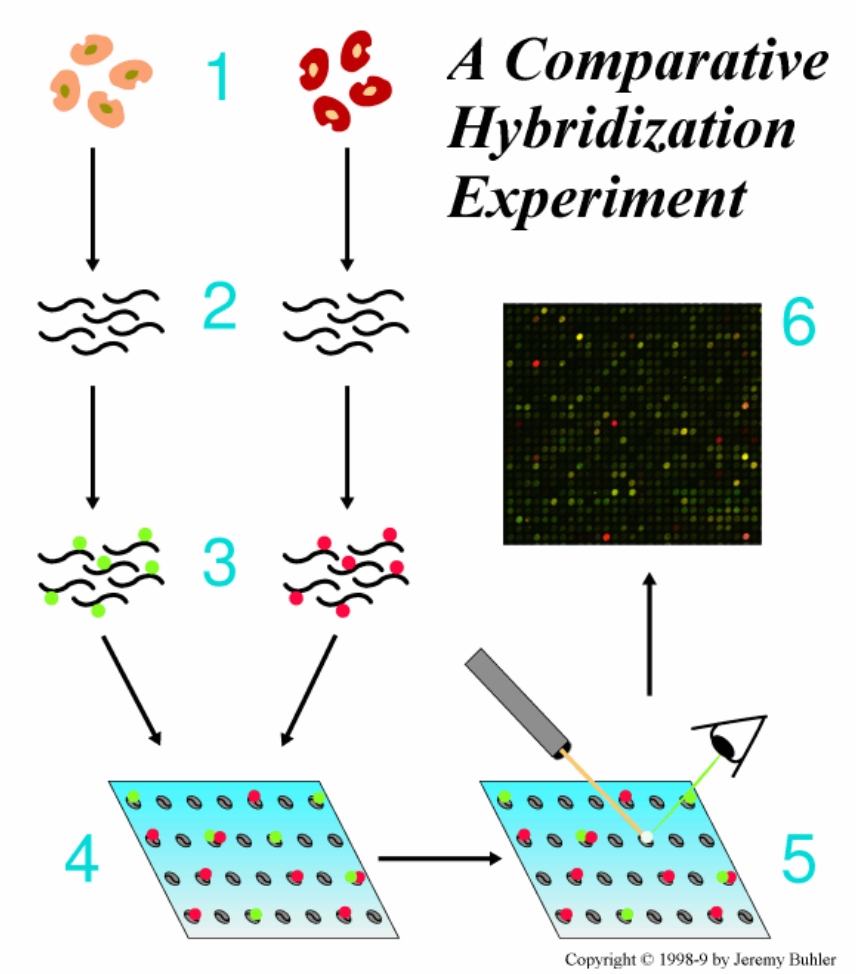
Bottom: Chemical synthesis cycle. UV light removes the protecting groups (squares) from the array surface, allowing the addition of a single protected nucleotide as it is washed over the microarray. Sequential rounds of light deprotection, changes in the filtering patterns of the masks, and single nucleotide additions form microarray features with specific 25-bp probes







In 2 colour microarrays, 2 biological samples (experimental/test sample and control sample) are labelled with different fluorescent dyes, usually Cy3 and Cy5. Equal amounts of labelled cDNA are then simultaneously hybridised to the same microarray chip. After this competitive hybridisation, the fluorescence measurements are made separately for each dye and represent the abundance of each gene in one sample (Cy5) relative to the control(Cy3).
The hybridisation data are reported as a ratio of the Cy5/Cy3 fluorescent signals at each probe. By contrast, in one colour microarrays, each sample is labelled and hybridised to a separate microarray and we get an absolute value of fluorescence for each probe.


A Comparative Hybridization Experiment
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Hybridisation-based approaches are high throughput and relatively inexpensive, but have several limitations which include:

- **reliance upon existing knowledge about the genome sequence**
- **high background levels owing to cross-hybridisation**
- **limited dynamic range of detection owing to both background and saturation signals**
- **Comparing expression levels across different experiments is often difficult and can require complicated normalisation methods**

Microarrays have been the standard for high-throughput gene expression studies before the introduction of RNA-sequencing, in the first half of the 2010s. While RNA-seq has replaced microarrays for most transcriptional profiling studies, DNA chips are still in use: an example is the Single Nucleotide Polymorphism (SNP) array used in diagnostics.