Spotted DNA Array Design

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Making Your Own Array

- For Affymetrix-style GeneChips, most labs will use commercially available arrays, so no array design considerations
- Spotted DNA arrays can be home-made, so you *do* have to think about design

What Type of DNA to Spot?

- Do you want to use a PCR products or oligonucleotides (35-70 nt)?

- Oligonucleotides are much less work, no labor with PCR reaction & checking for correct products lengths
- Design considerations more important for oligos – where to place the oligos, how many, alternative splicing in euks, more spots on array per gene
- Not all oligos work (for poorly understood reasons) – so some trial and error to get oligos that get good hyb signal

PCR / Oligos

- For multiple oligos, analysis is also more complex – simple averaging of signal from spots covering different parts of gene may not be accurate
- For "intergenic" regions (regions between known genes), PCR product gives full coverage, oligos only partial (and on one strand)
- People may use a "mixed" array in the future, with both PCR products and oligos
- Verdict: No consensus yet which is generally better, but oligos are less labor intensive, so people are moving towards this approach more and more

PCR / Oligo Length

- What length do you want your DNA to be?
  - For PCR: full length genes (no brainer, easy design), or partial (for more uniform length, avoid “low-complexity” regions)
  - For oligos: short (35 nt), or longer (70 nt)
    - Shorter oligos may have greater variability in hybridization efficiency due to sequence-specific features, but are also much cheaper to synthesize

PCR Product Feature Design

- Want:

  Primers that have roughly uniform thermodynamic properties (similar annealing temperature)

  Standard Length, 18-22 nt: one forward primer, one reverse primer

  Avoid self- or cross-hybridization (lowers yield of final product)

  Avoid mis-priming at unintended sites in genome (causes multiple DNA fragments that you don't want in on your array – it's the wrong DNA!)

Primer Design Tools

- Some commercial programs available (i.e. Array Designer 2 from Premier Biosoft)
  - Very expensive, $4,885 !!
- Home-grown, publicly available
  - Primer3 from MIT, but not designed to pick thousands at once
  - Can write a Perl “wrapper” that runs Primer3 thousands of times, once for each feature
  - More work, but if you have programming skills, much cheaper and more control over precise design

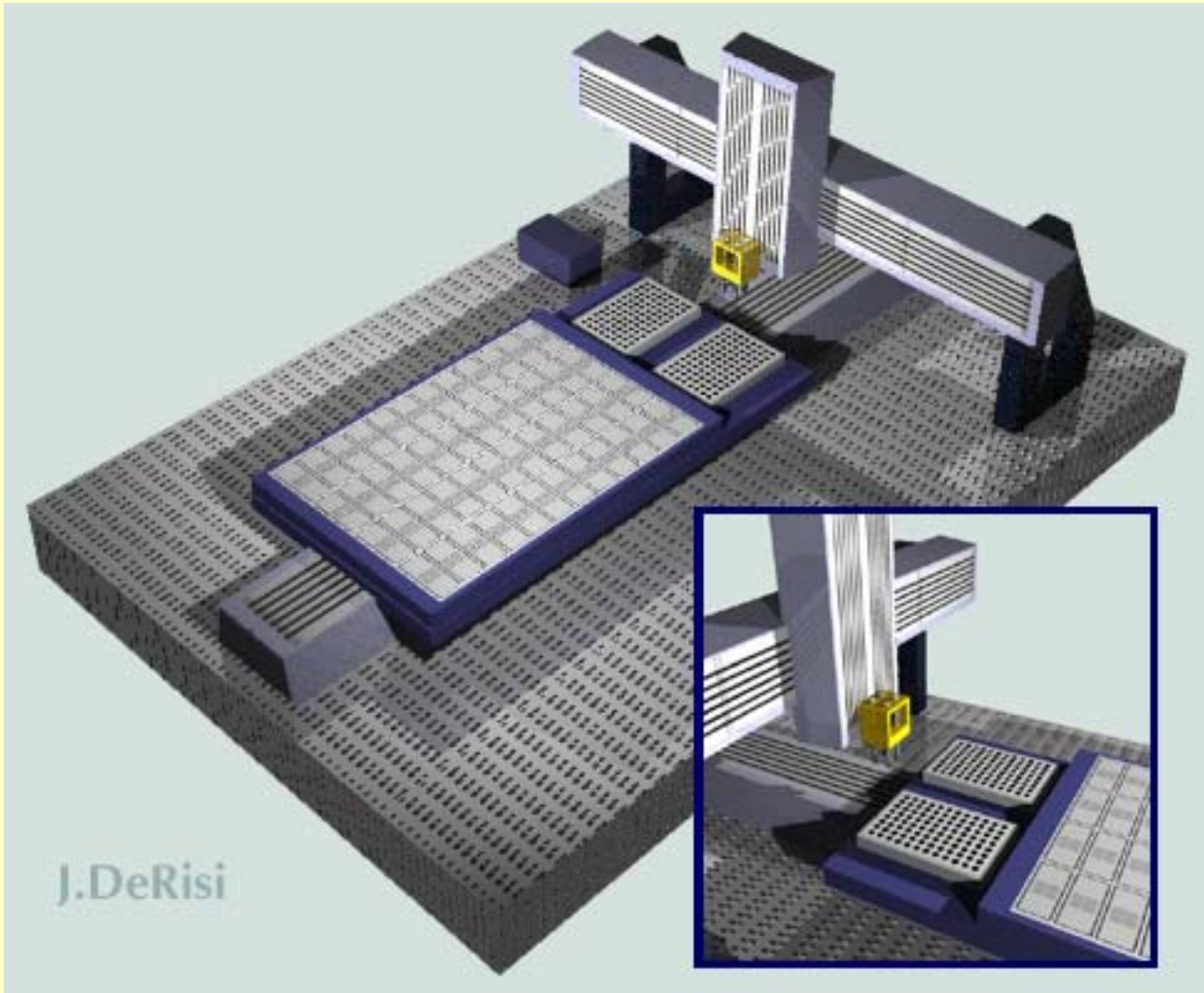
Building A Spotting Robot

- Go to Pat Brown's site for complete, detailed instructions:

http://cmgm.stanford.edu/pbrown/mguide/index.html

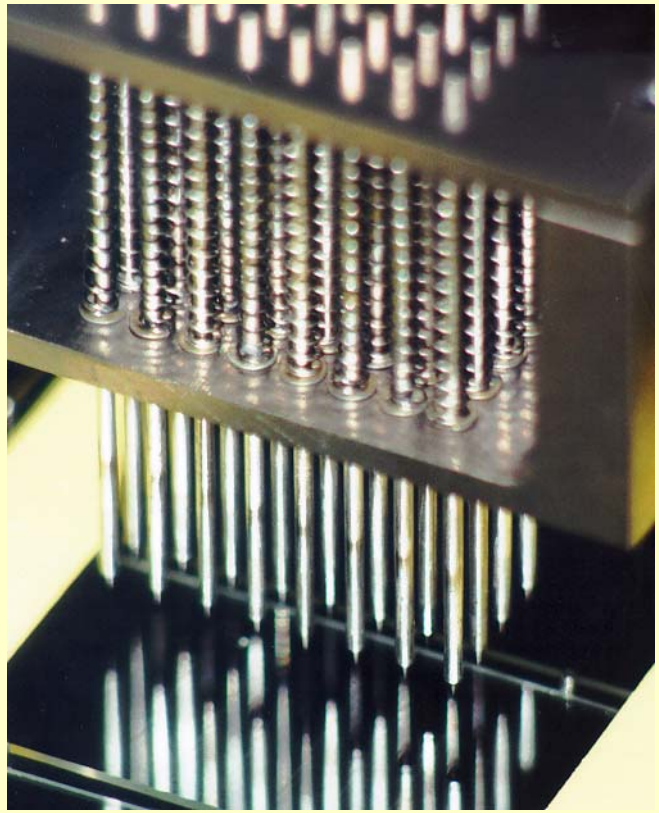
- Contains a parts list, ordering instructions, assembly instructions, free software (for academics) to download and run the spotting robot, protocols for preparing DNA for spotting, and more
- All information is freely available – cost is $20-$30,000 for materials, plus labor (grad students are cheap ;-)

Spotting Robot



Pins!

- Work like fountain pens
- Slit cut in steel pin
- When dipped into DNA solution, pick up small amount of liquid
- A light “tap” deposits a roughly uniform amount of DNA
- One tap for each slide being made



Array Geometry

- Each pin creates one “sector” of spots
- Total number of spots =

  (# spots per row) * (# spots / column) * (# pins)

  Typical (small) array

  24 spots/row * 24 spots/column * 16 pins = 9,216

  (uses 180 micron spot to spot spacing)

  Large Human array

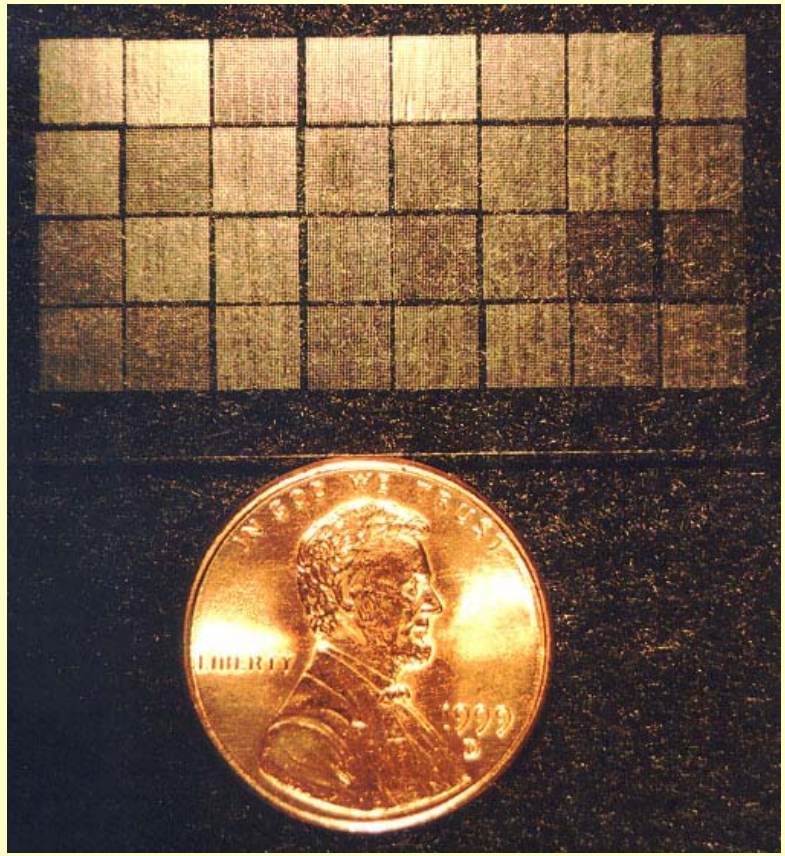
  32 * 32 * 48 pins = 49,152

Review

a)What kind of microarray is this?

b) Could you use it to measure single nucleotide changes between two Human DNA samples? How, or why not?

c) Knowing what kind of array this is, what is the maximum number of unique DNA elements you might be able to fit on one of these?



Preparing Slides

- Many complete protocols at "microarrays.org"
- Also, clear protocols given for making slides
- Basically:
  - Start with standard microscope glass slides
  - Inspect, discard slides with finger prints or scratches
  - Wash with sodium hydroxide
  - Rinse well with water
  - Soak in poly-L lysine solution
  - Dry in centrifuge, then vacuum
  - Inspect visually for uniform coating (blow lightly on slides, watch for uniform evaporation of moisture)

Preparing DNA Samples

- Oligos – ready to go
- PCR products
  - Run PCR reactions (template DNA + primers + enzyme) in 100 ul volume
  - Visualize PCR product sizes on agarose gels to make sure expected length (very labor intensive)
  - Ethanol precipitation to reduce volume to 15 ul (optional)

Spotting

- Load DNA samples into 96- or 384-well plate; generally reducing volume of original DNA solution (for PCR 100ul) down to 6 ul
- Tape slides to platter; allow for 1 “blotting slide”
- Check pins – make sure all are in good condition (replace if mashed)
- Calibrate spotter robot (correct plate, XYZ positions of plate, washer, dryer, slides)
- Print test slides
- If Ok, start run
- Swap plates ~ 30-50 minutes as many times as necessary

How Long?

- At 30 minutes / plate:
  - Full genome yeast array 6,200 spots: ~ 8 hours
  - Human arrays, 48,000 spots, takes > 2 *days*, so people go in shifts, all day, all night, non-stop

Post-processing of Slides

- After printing slides, must be post processed:
  - Re-hydrate slides & snap dry to get nice uniform, round spots
  - UV-cross link (optional)
  - Soak in blocking solution (keeps lysine from further reacting / binding other DNA)
  - Boil in water for 2 minutes (denatures double stranded DNA, making more available for hybridization)
  - Rinse in ethanol
  - Store for up to 6 months, or use immediately