Microarray methods for the detection of pathogenic bacteria and viruses in plants.

José E. Pérez-Ortín

Laboratory of DNA-Chips. *Universitat de València*

In my lab we are conducting a project in collaboration with the group of Dr. Mariano Cambra (Instituto Valenciano de Investigaciones Agrarias, Spain) on the developing of microarrays for the detection of viruses and pathogenic bacteria in potato plants.

Complications when developing microarrays for pathogens detection

Microarrays are capable of analyzing hundreds of different loci simultaneously in a short period of time. However, most microchip arrays require large amounts of template DNA, or RNA, for efficient rapid, passive hybridization.

The oligonucleotide capture probes should be designed to maintain uniform stringency conditions for each hybridization reaction.

Multiplex amplification is a possible method of obtaining high concentration of input DNA but often leads to large decreases in amplification efficiency.

Microelectronic chips are potentially able to circumvent these problems.

Microelectronic Chips

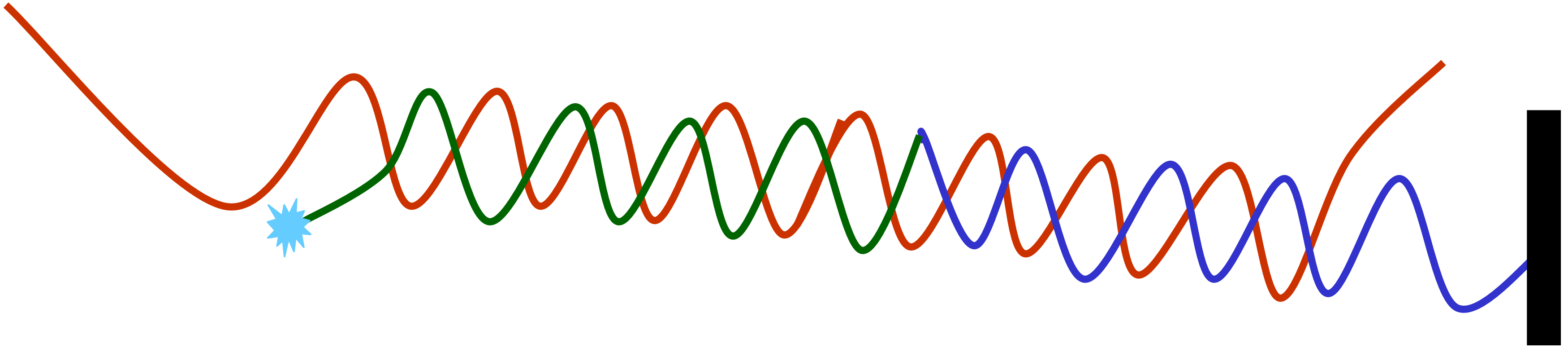
Electronically controlled microelectrode arrays are an interesting option for the DNA analysis as a diagnostic tool.

In contrast to the passive hybridization environment of other methods, these devices offer the ability to actively transport DNA to, and to hybridize at, discreet locations on the microelectrode array surface.

In addition to this multiple probes can be hybridized to to one or multiple samples independently.

Tripartite hybridization: a sandwich

The sample molecule can hybridize simultaneously with two complementary probes, provided that they do not overlap. One probe is bound to the solid surface: capture. The other one, reporter, is labeled with a fluorophor. This eliminates the requirement for sample labeling.



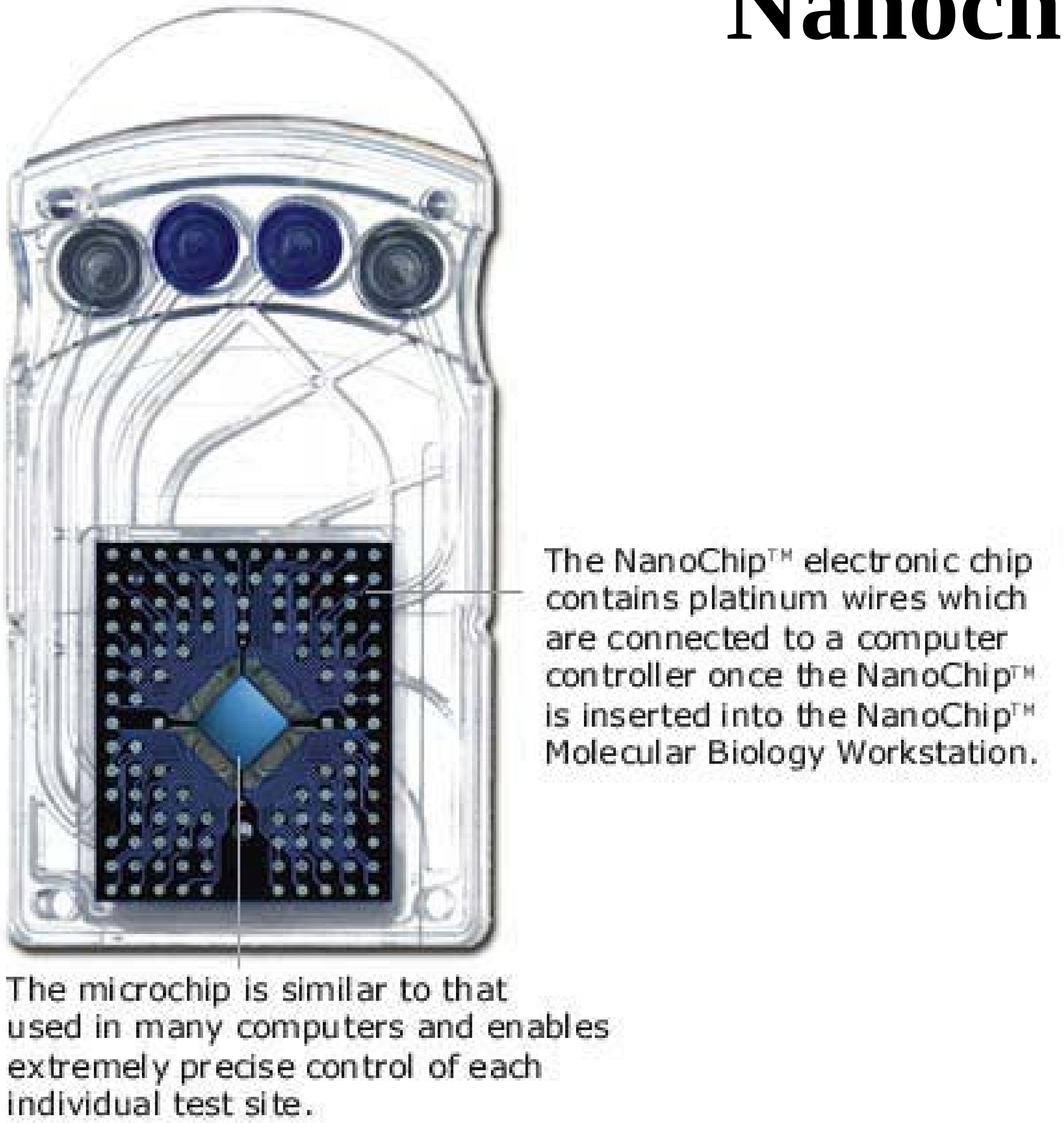
Making and using an electronic chip

The negative charge of DNA is used to address the capture probes to specific test sites on the chip surface.

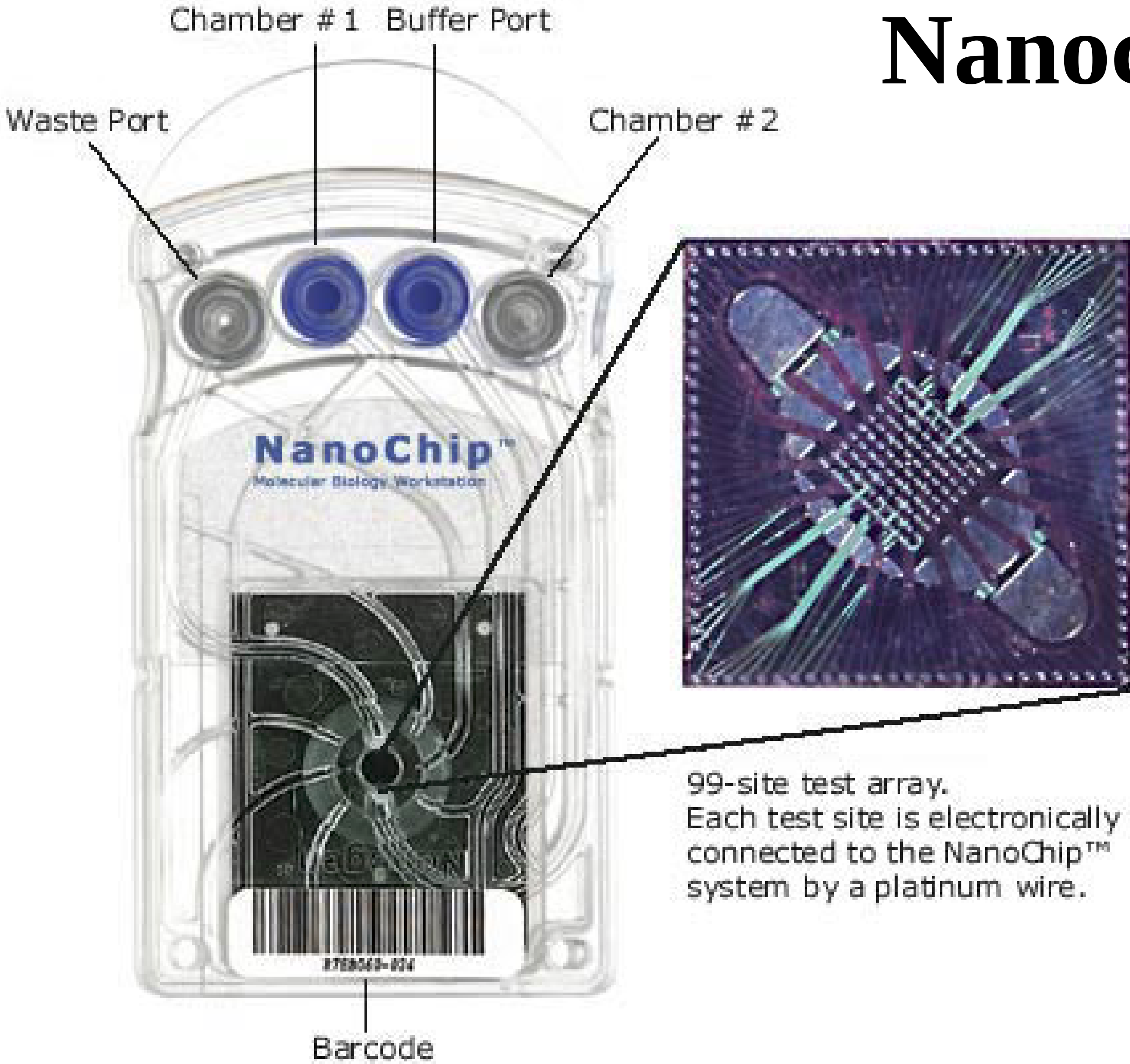
Different samples can be addressed to specific chip test sites, therefore, to specific capture probes.

Hybridization on the test sites can be controlled either by salt stringency or by temperature in the whole array or independently for each test site electronically.

Nanochip


The NanoChip™ electronic chip contains platinum wires which are connected to a computer controller once the NanoChip™ is inserted into the NanoChip™ Molecular Biology Workstation.
The microchip is similar to that used in many computers and enables extremely precise control of each individual test site.

Nanochip


Chamber #1
Buffer Port
Waste Port
Chamber #2
NanoChip™
Molecular Biology Workstation
Barcode
99-site test array.
Each test site is electronically connected to the NanoChip™ system by a platinum wire.

Nanogen Workstation

Loader: to make the chips

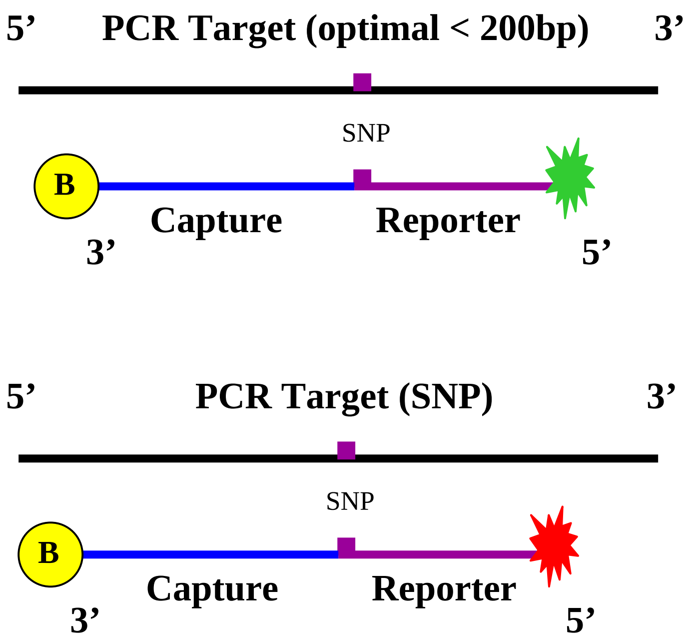
Hybridization chamber and reader

Hybridization occurs in about 3 min

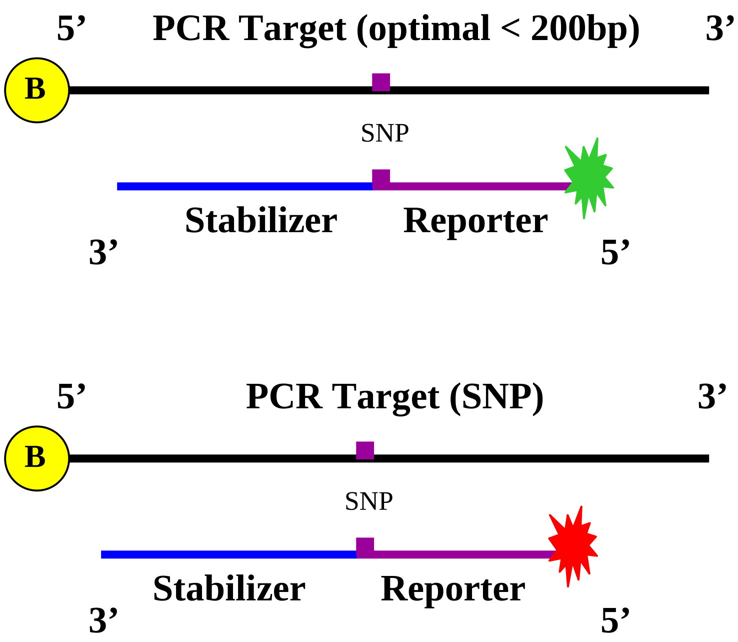
The whole process of analysis is completed in less than 30 minutes

SNP GENOTYPING SYSTEM

Capture Down



Amplicon Down

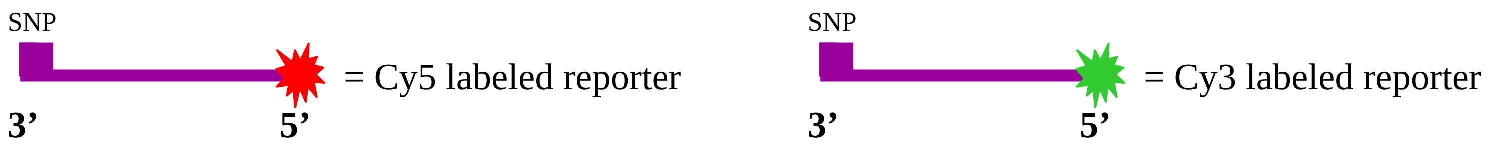


= reporter: Tm ~ 30C, 9-12nt

=capture/stabilizer: Tm >60C, 25-35nt

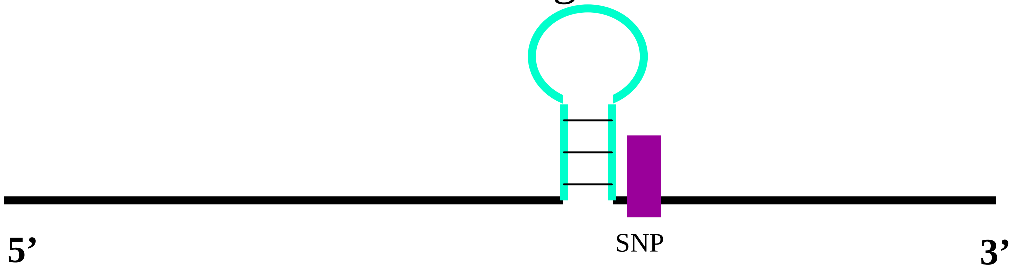
PROBE DESIGN GUIDELINES - SNP

Reporter oligos must have Cy3 or Cy5 fluorophore at the 5’ end, and SNP mutation at the 3’ end. Keep melting temperature around 30^{o}C. Reporter should be about 9-12nt.



Stabilizer and capture oligos should be immediately downstream from reporter oligos. Capture oligos should be biotinylated at the 3’ end. They should about 25-35nt in length with melting temperature above 60^{o}C.

Once assay format (capture vs. amplicon down) is determined, locate potential secondary structure relative to the SNP on the target DNA.



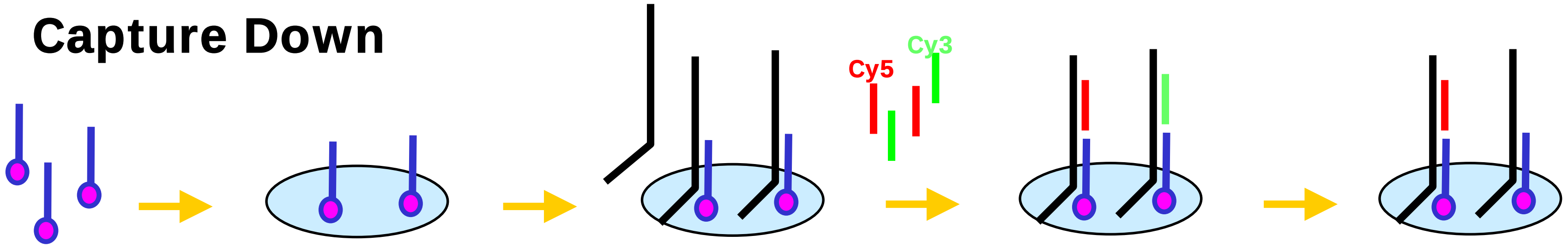
BASE-STACKING ENERGIES

5’ - 3’	Energy
GC	-14.59
GT	-10.51
AC	-10.51
GA	-9.81
TC	-9.81
CG	-9.69
GG	-8.26
CC	-8.26

5’ - 3’	Energy
AG	-6.78
CT	-6.78
AT	-6.57
CA	-6.57
TG	-6.57
AA	-5.37
TT	-5.37
TA	-3.82

SNP ASSAY FORMATS

Capture Down



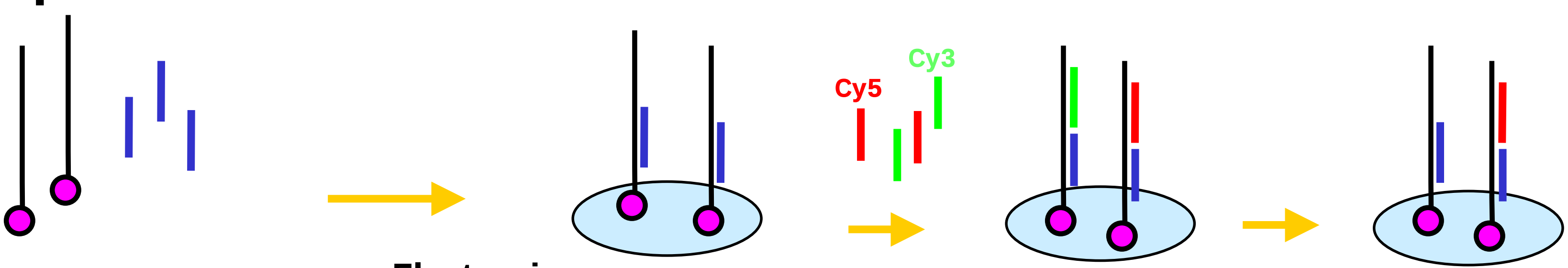
Electronic Deposition of Biotinylated Capture Probes

Electronic Hyb. of Denatured PCR Product

Passive Hyb. Of Two Different Reporter Oligos

Thermal Denaturation of Reporter Oligos

Amplicon Down



Biotinylated PCR product and stabilizer oligo together in solution

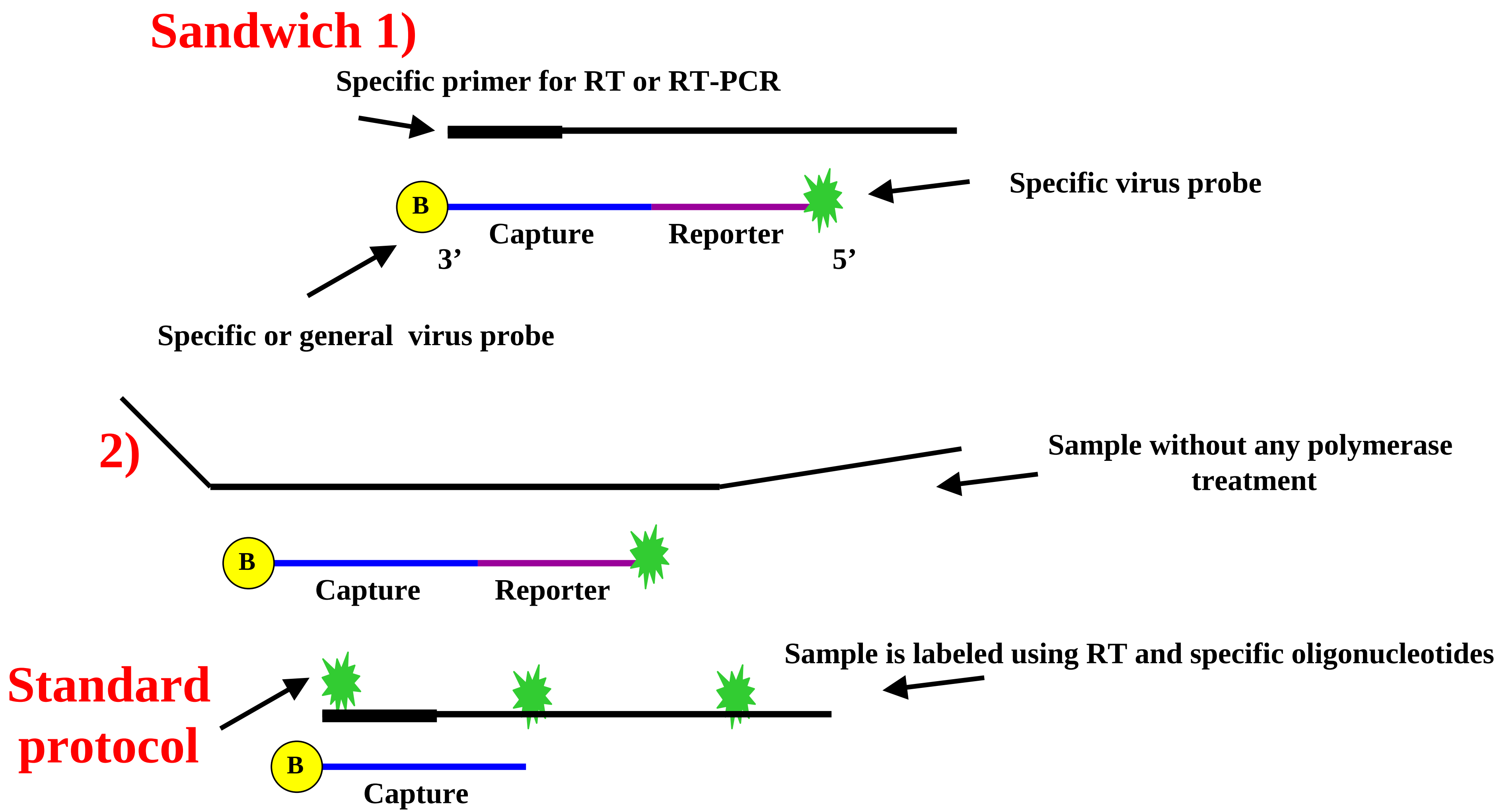
Electronic Deposition of Biotinylated denatured PCR product and stabilizer oligo

Passive Hyb. Of Two Different Reporter Oligos

Thermal Denaturation of Reporter Oligos

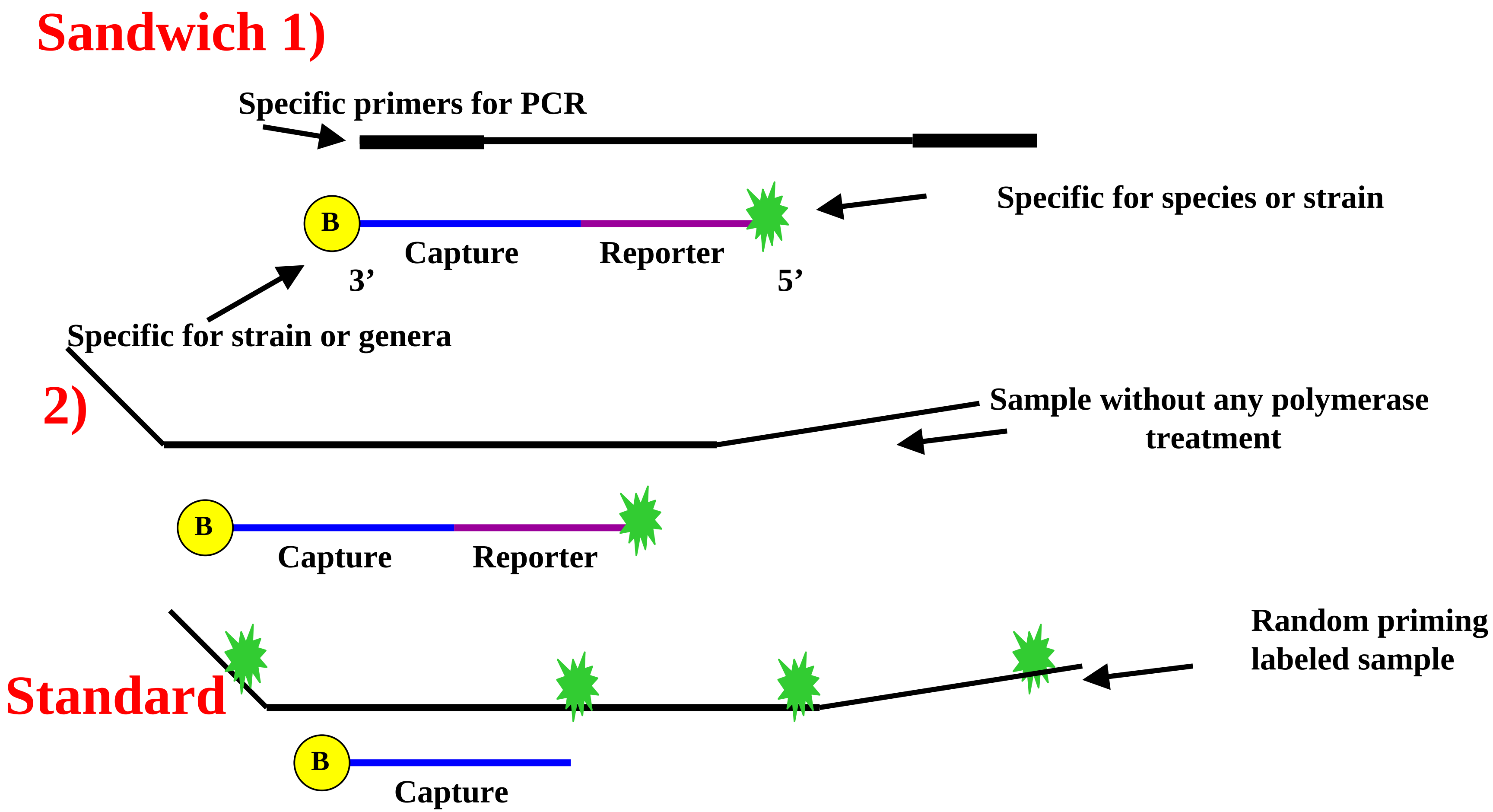
ANALYSIS OF VIRUSES

PVY, PVX and PLRV



ANALYSIS OF PATHOGENIC BACTERIA

Erwinia carotovora sub. atroseptica, E. carotovora sub. carotovora, E. chrysanthemi, Clavibacter michiganensis sub. sepedonicus, Ralstonia solanacearum



Preliminary results