“Optimizing Unbiased Degenerate PCR Primers for Comprehensive Detection of Human Papillomavirus Genotypes”

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Introduction

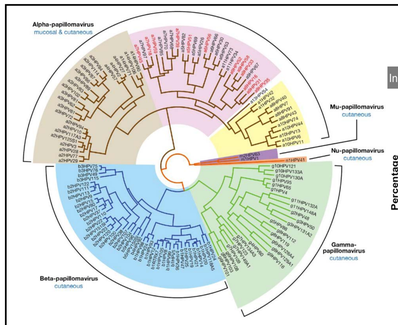


Figure 1 HPV Genotype Wheel

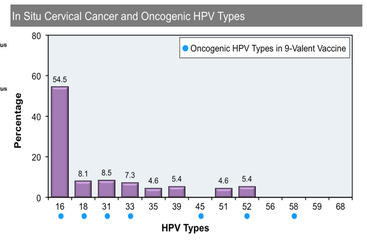
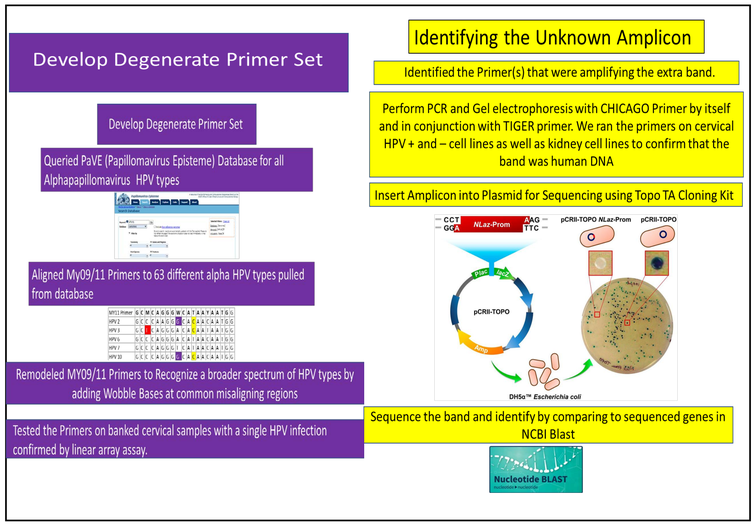


Figure 2 Bar graph depicting the most common oncogenic HPV types

- Human Papillomaviruses (HPV) are a family of small oncogenic viruses containing 200 distinct genotypes.
- Vaccination programs like Gardasil target a specific subset of HPV genotypes (6, 11, 16, 31, 33, 45, 52, and 58)
- Current clinical HPV tests are biased to detect these common vaccine types of HPV and can potentially be missing other clinically important HPV infections
- We are developing a high throughput DNA sequencing (MiSeq) assay that is comprehensive of all HPV types and unbiased to the most common types.
- Initial studies showed that the HPV-L1 gene specific degenerate primer pair MY09/MY11 preferentially amplified some HPV types but poorly amplified others.
- We redesigned the MY09/11 primers to improve amplification of known alpha human papillomaviruses.
- **Applications of this technology will give a more accurate depiction of the circulating HPV types in the population and show the emergence of new strains as others are eliminated by the vaccine.**

Methods



Results

MY11	G	C	M	C	A	G	G	G	W	C	A	T	A	A	Y	A	A	T	G	G
JABMY11 Chicago	G	C	I	C	A	G	G	G	I	C	A	Y	A	A	Y	A	A	T	G	G

MY09	C	G	T	C	C	M	A	R	R	G	G	A	W	A	C	T	G	A	T	C
JABMY09 Tiger	C	K	I	C	C	I	A	R	I	G	G	A	A	A	Y	T	G	R	T	C

MY09	C	G	T	C	C	M	A	R	R	G	G	A	W	A	C	T	G	A	T	C
JABMY09 Broadway	C	G	T	C	C	I	A	G	G	G	G	A	A	A	C	T	G	I	G	

Figure 3 Changes made to the MY09/11 primers

symbol	base mix
R	A,G
Y	C,T
M	A,C
K	G,T
S	C,G
W	A,T
H	A,C,T
B	C,G,T
V	A,C,G
D	A,G,T
N/I	A,C,G,T

Figure 4 Wobble Base Key

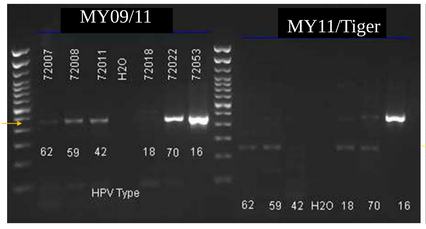


Figure 5 Gel Electrophoresis Tiger Trial that generated the 450bp band that correlates the L1 region on HPV.

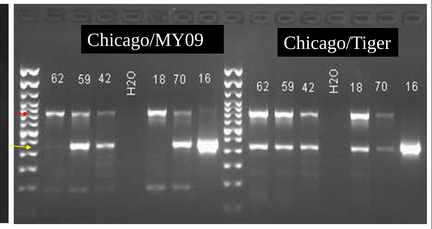


Figure 6 Chicago and Tiger Gel. Shows the 450bp band we were looking for but also shows an additional 800bp band.

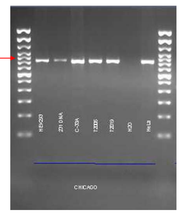


Figure 7 Testing Chicago alone and with Tiger with HPV + and – cell lines to determine which is generating the 800bp band

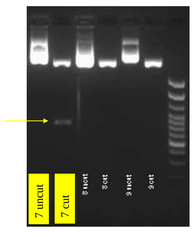


Figure 8 EcoR1 restriction digest of Chicago plasmid

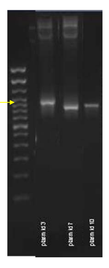


Figure 9 Gel of the different plasmids that were generated with the Topo TA cloning kit. We chose to sequence 3,7, and 10 because they contained the 800bp band we were questioning

Homo sapiens chromosome 3 clone RP11-470E10 map 3p

146871..147689bp

cCctcaggtgtcacaataatggaacttcaatgatagctttgaaacagaaagataaacttaaatgacatattccattt
gagagaagagagggacatccttgattttctagtaagagtctttcatcctacattttaacatataattcatgacactc
ctgaatgtcttcttaatttttataccaatttggctttacaggtgctagctgacatttattaggcagtattagttag
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caccagtcaaactatcttggccagatttttgtttccattgttgtcccccctgcCgctccttcac

Figure 10 The ~800bp section of the Rp 11-470E10 that the Chicago Primer amplified.

Conclusions

- **The redesigned degenerate primers have successfully amplified the 450 base pair band corresponding to the L1 region of HPV**
- **The Chicago Primer acted as a forward and reverse primer**
- **The Chicago Primer amplified the human cellular Rp 11-470E10 gene**

Future Directions

- **Redesign the Chicago Primer to avoid cross-amplification of human genomic DNA**
- **Test samples that are infected with more than one type of HPV in MiSeq platform using the Chicago-Tiger primer pair**
- **Analyze the efficiency of amplification of known HPV genotypes**

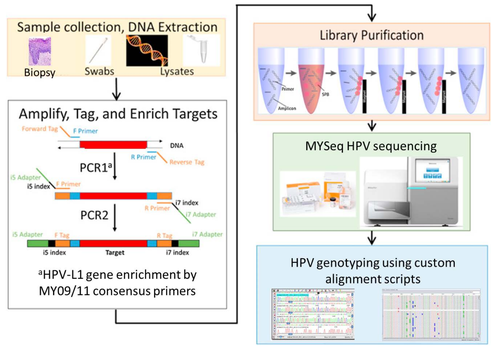

Figure 11 MySeq Protocol

References

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