

# Unearthing natural products from a metagenomic fosmid library

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## Introduction

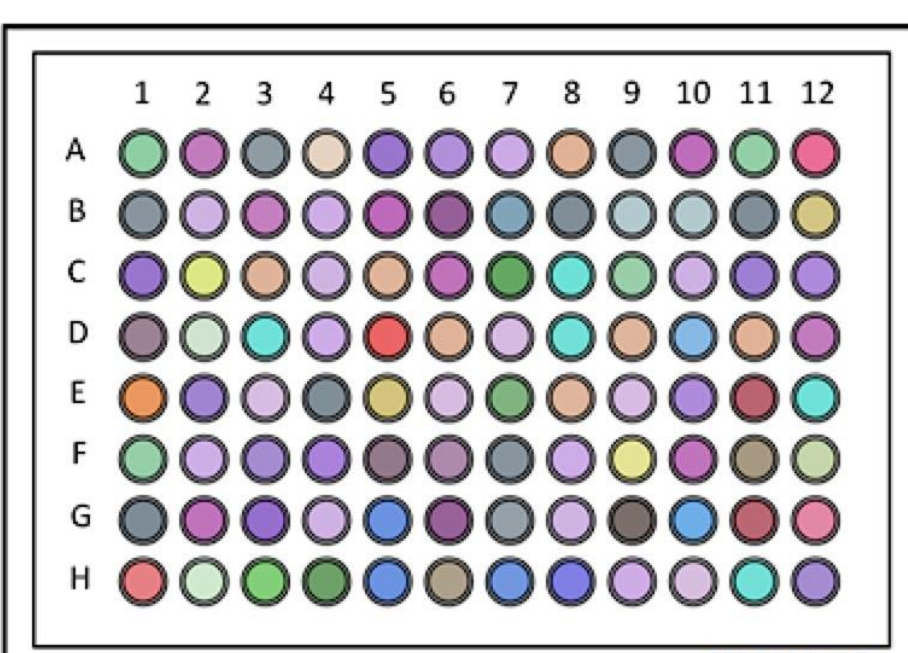
- Modern biotechnologies allow genomic information to be studied collectively to isolate genes and their products of interest
- The metagenome contains vast numbers of undiscovered natural products, with potential clinical and commercial applications
- A fosmid library containing genes for Type I and Type II polyketide synthases, and non-ribosomal peptide synthetases is analyzed in this study



Figure 1. Sampling locations, Ottawa ON (L,C), Syracuse NY (R)

## Methodology

- Colonies selected from previously screened fosmid library for further study

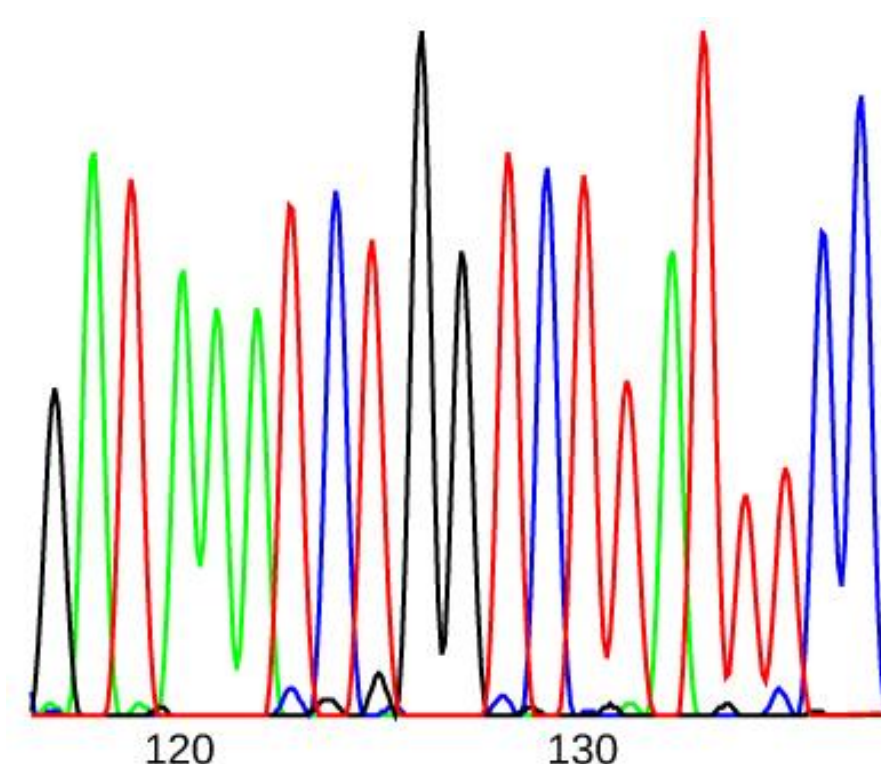


- Cultures grown in media with antibiotic, fosmids induced to higher copy number



- Fosmid DNA extracted, isolated, and purified using differential sedimentation method

- Samples digested, fragments separated via pulse field gel electrophoresis
- Samples sent for Sanger sequencing using the FP and T7 forward primers, and RP reverse primer



CopyControl™ Fosmid Library Production Kit, pCC1FOS™ Vector used in creation and isolation of fosmid DNA

## Results

### Verification of Insertions



Figure 2. Pulse field gel electrophoresis. 14 extracted samples were digested with HindIII and BamHI, gel was run for 24 hours at 145 V. Backbone 8.1 kb, insert up to 42 kb.

### Insert Identification and Characterization

Table 1. Summary of unique BLAST Results. Sequences obtained and run using the NCBI's BLASTn algorithm

Sample	Species	% Coverage	% Identity
1A T7	<i>Sulfurifustis variabilis</i> , strain skN76	39%	76%
1G T7	<i>Streptomyces sp.</i> PBH53	100%	99%
2E R	<i>Streptomyces iranensis</i>	90%	84%
3C F	<i>Streptomyces sp.</i> PBH53	96%	99%
3C R	<i>Streptomyces sp.</i> PBH53	94%	99%
3G T7	<i>Streptomyces sp.</i> PBH53	100%	100%

- PBH53 is currently draft genome, assembly of these pathways can help in completing genome structure

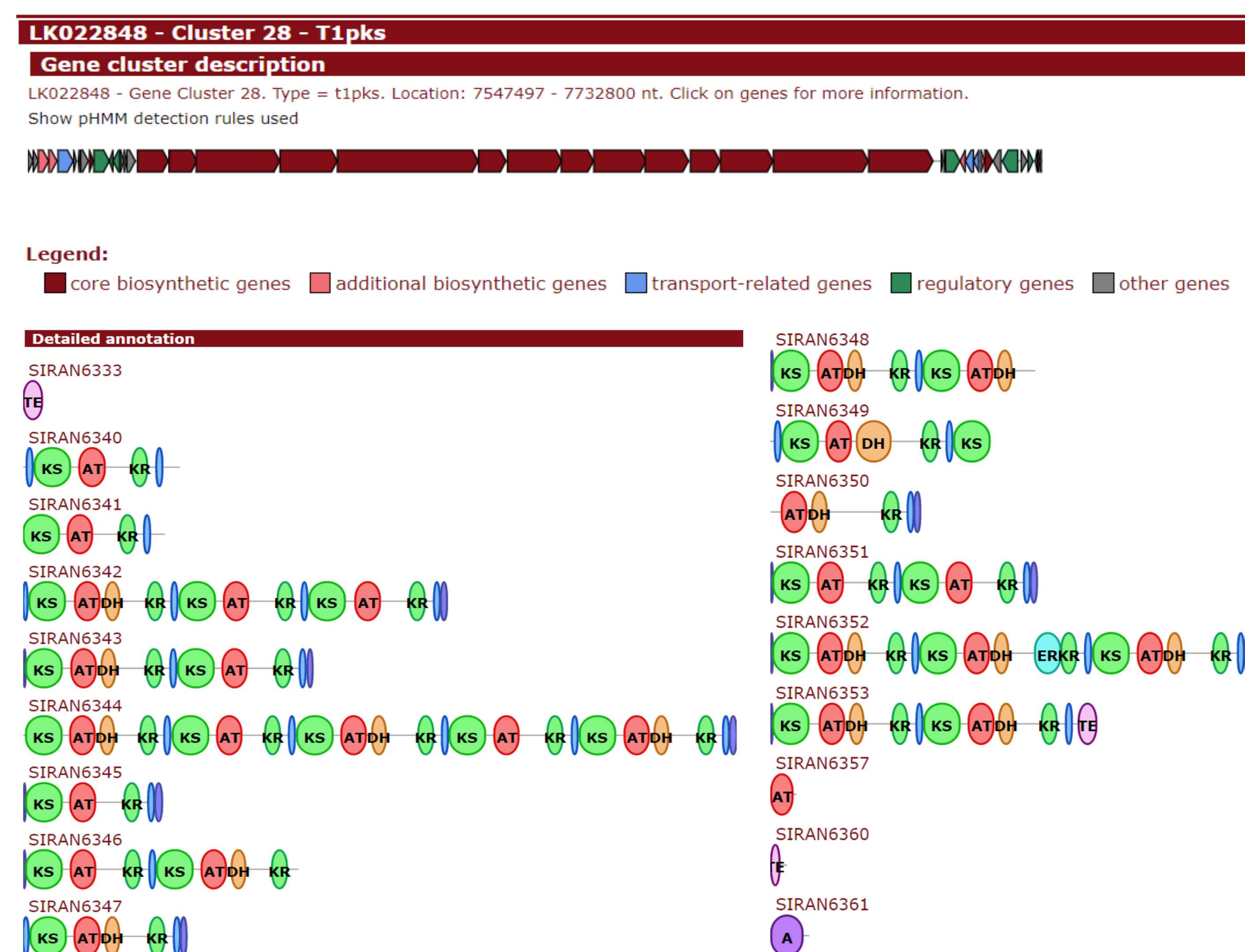
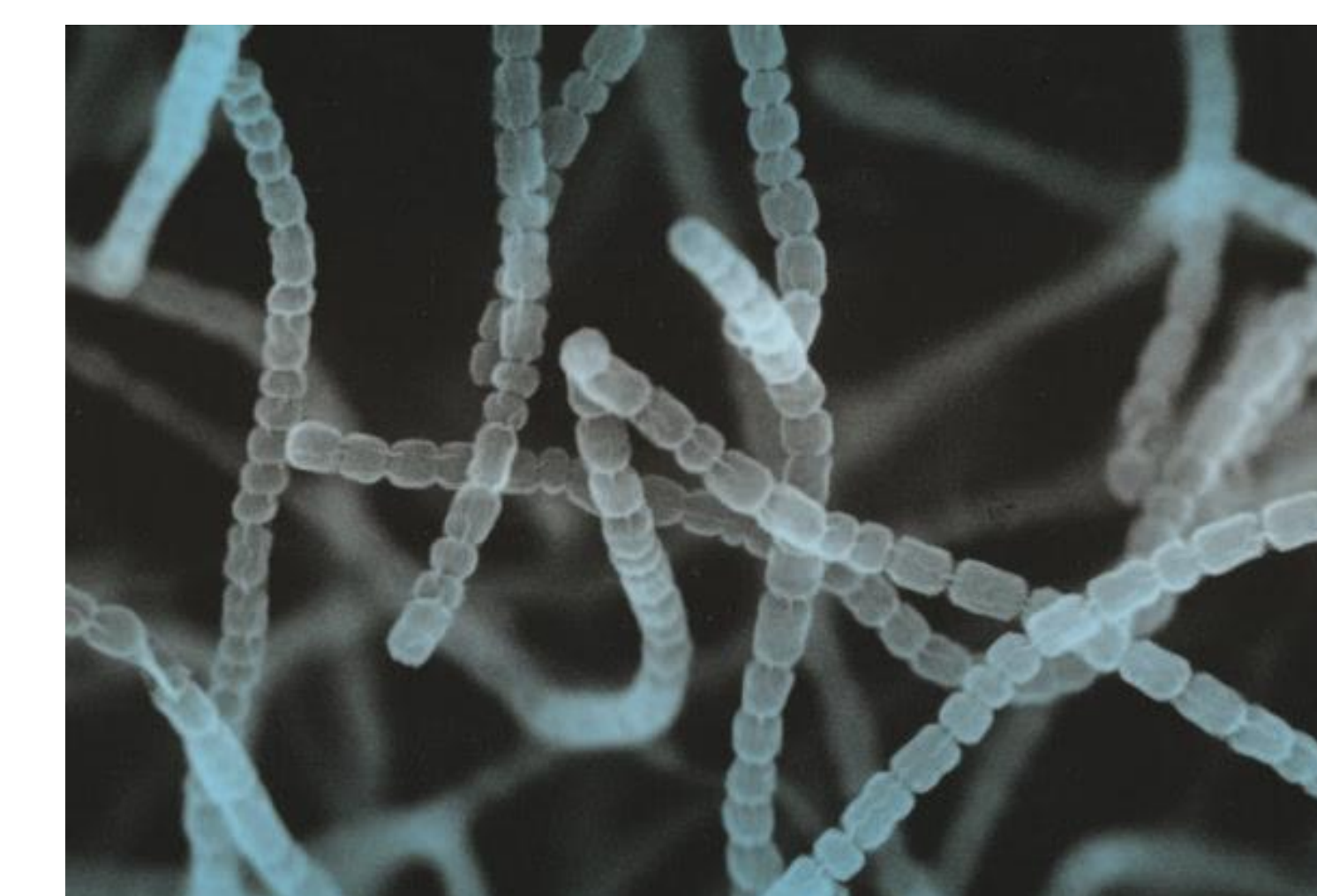


Figure 3. antiSMASH Type I PKS pathway identified. Cluster comes from *Streptomyces iranensis*, where 2E R aligns in BLAST. antiSMASH bacterial version used to identify secondary metabolite pathways in identified sequences.

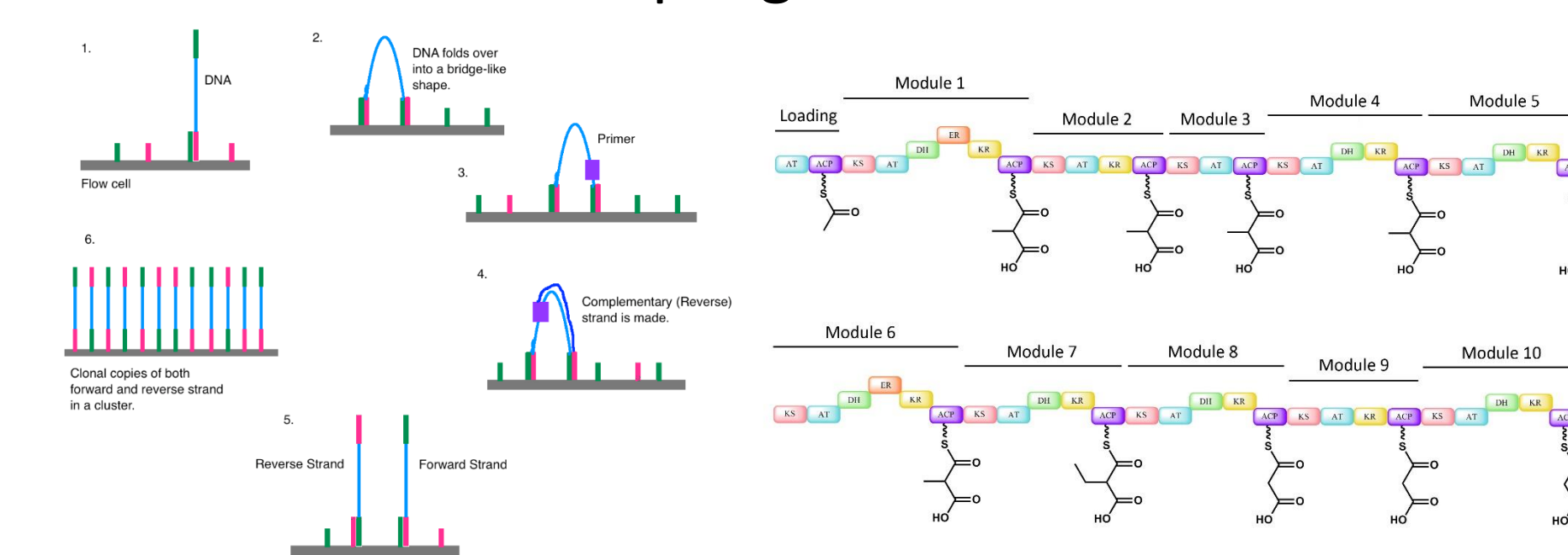
## Discussion

- Smearing of lanes is indicative of a large quantity of DNA present from extraction. Samples diluted in further study
- Samples contain bands corresponding to the 8.1 kb backbone of the pCC1 fosmid. Additional bands verify the presence of inserts, up to 42 kb
- Streptomyces sp.* PBH53 is a common hit on BLAST, Actinobacteria were prioritized in library construction, PBH53 known to contain compelling gene clusters
- Many promising leads on natural product pathways, especially Type I PKS pathways
- Some samples unrecognized in BLAST, further study on this sample required to determine its nature



## Conclusions

- Sequencing analysis is required using next generation sequencing methods to obtain a more complete depiction of the inserts
- Samples with key PKS pathway genes, or no matches within the BLAST database, should be prioritized for further investigation
- Streptomyces sp.* PBH53 contains many unique and intriguing pathways that should be further studied for potential drug uses
- Streptomyces sp.* PBH53 commonly found in soil sampling across Ottawa



## Acknowledgements

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