Genome sequencing in *Senecio squalidus*

Outline of project

- A new NERC funded grant, the genomic basis of adaptation and species divergence in *Senecio* in collaboration with Richard Abbott and Dmitry Filatov
- In Bristol we are focusing on the molecular basis of changes to gene expression in *Senecio*, previously identified in our lab by Matt Hegarty
- Particular focus will be on miRNA profiling and the use of ChIP-seq to identify both changes in promoter sequence and activity
- These approaches require a reference genome sequence, and it is the production of this which we are currently working on

Partial reference genome of *S. squalidus* OX6

- Next generation sequencing technology is extremely powerful, but larger and more complex genomes still present a capacity challenge for *de novo* assembly
- Use of 2 platforms. 454 sequencing to produce longer, more accurate reads and Illumina sequencing for greater yield
- Focus on the non-repetitive regions of the genome to maximise the yield of coding sequence and facilitate assembly
- Present funding will produce only a partial genome, but we intend to supplement this work with the longer term aim of producing a complete genome sequence

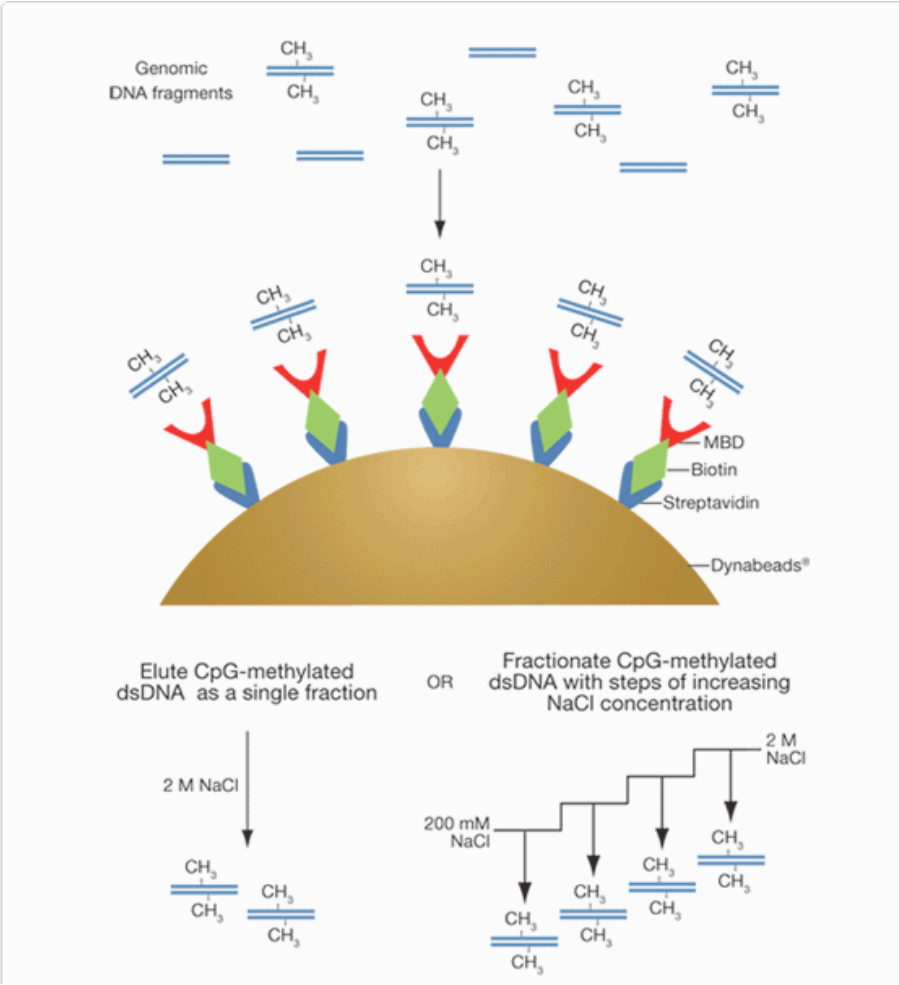
Sequencing strategy and approaches

- 454 sequencing of methyl-filtered libraries to produce a low coverage reference assembly
- Illumina sequencing of paired-end and mate-pair libraries to increase both genome coverage and sequencing depth
- Cot normalized Illumina libraries to complement the methyl-filtered data

Methyl-filtration

Repeat sequences in plant genomes have a tendancy to be heavily methylated, hence removal of the methylated fraction reduces their representation (Rabinowicz *et al.* 1999)

- Invitrogen MethylMiner kit
- Designed to purify sequences with a high proportion of CpG methylated sites
- Uses a methyl binding domain protein bound to magnetic beads to pull down target sequences
- Fractionation by variation of salt concentration during elution
- Filtered DNA used for preparation of libraries for 454 sequencing



Pilot 454 data overview

- Filtered and untreated libraries run on 1/16th of a 454 plate

	Methyl filtered	Untreated	Total
number of reads	107,138	107,842	214,980
Total sequence (Mbp)	32.97	39.84	72.81
Mean read length (bp)	311	379	-
Mode read length (bp)	412	500	-

Filtered vs untreated

- Filtered reads had approximately 10% more unique hits on SenecioDB
- Reads from each library were aligned using CD-Hit.
- Representative sequences from clusters comprising 30 or more sequences were Blasted against the Green Plants dataset at NCBI
- Of these larger clusters, all from the untreated library consisted of repeat elements, while all from the filtered data consisted of ribosomal sequence

Illumina sequencing

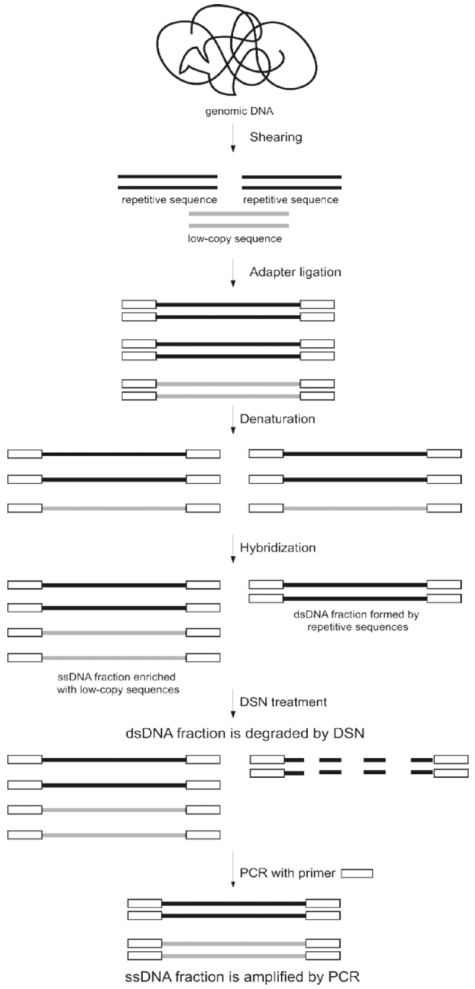
- Cost per base is ~40 fold less than 454 sequencing
- Use of paired-end and mate-pair libraries spanning a range of insert sizes will facilitate assembly
- 150bp read length is coming online now. We will pilot a run with this in the next couple of months
- Library preparation is carried out in-house, and sequencing is done by the transcriptomics facility in Bristol, so we have a greater degree of control over the process.

Illumina sequencing

- We have several libraries prepared with insert sizes ranging from 100 - 500bp
- A pilot run on a single lane using a 150bp insert paired end library with 76bp reads has yielded ~3.9Gbp of sequence that has passed quality filtering.
- This corresponds to ~2.3 fold genome coverage.

C_0t normalization of illumina libraries

- Method for enriching low copy number sequences
- Use of a thermostable DSN from crab rather than hydroxyapatite chromatography
- Used for normalization of plasmid and cDNA libraries
- Technique is easily adapted for normalization of illumina gDNA libraries



Shagina *et al.* (2010) Biotechniques **48**:455-459

Future genome development work

- 2 full 454 plates have been run at the NBAF Liverpool, and are currently in the data analysis pipeline.
- 8 remaining plates will be run over the next 2 months
- Optimisation of Cot filtration on illumina libraries and sequencing of these in Bristol.
- Sequencing of multiple insert size illumina libraries at the NBAF in Edinburgh
- Assembly and Annotation!

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