Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Material

Transcriptomic study of ciprofloxacin resistance in *Streptomyces coelicolor* A3(2)

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Supplementary files

SF1: List of all upregulated and down regulated genes

SF2: Microarray Protocol (details)

SF3: Learning set for computational analysis

Gene	Annotation	Fold Change	P value
Genes upregulated in response to ciprofloxacin			
DNA Replication/Repair			
SCO0760	putative methyltransferase	1.7	1.88E-04
SCO1343	uracil-DNA glycosylase	2.8	9.12E-04
SCO1520	crossover junction endodeoxyribonuclease	2.8	0.03
SCO1958	ABC excision nuclease subunit A	2.9	1.29E-05
SCO1966	ABC excision nuclease subunit B	7.3	1.11E-03
SCO2003	DNA polymerase I	3.7	7.24E-07
SCO2863	putative helicase	7.7	3.35E-02
SCO3351	putative DNA repair protein	2.1	2.89E-03
SCO3541	putative DNA polymerase	2.2	2.29E-03
SCO3873	DNA gyrase subunit A	1.6	0.04
SCO4577	putative helicase	2.0	0.04
SCO5143	DNA-3-methyladenine glycosylase I	1.7	3.66E-03
SCO5494	putative DNA ligase	4.6	2.33E-05
SCO5566	putative ATP-dependent DNA helicase	2.7	1.11E-04
SCO5761	putative ATP-dependent DNA helicase	2.8	2.78E-03
SCO5815	putative ATP-dependent DNA helicase	4.1	0.02
SCO6084	putative DNA polymerase	4.0	9.36E-05
SCO7522	putative DNA ligase	3.6	6.66E-04
SCO6341	putative exonuclease	3.0	0.02
SCO4814	bifunctional purine biosynthesis protein	1.6	0.01
SCO4647	transcription antitermination protein	1.6	0.01
SCO4729	DNA-directed RNA polymerase alpha chain	1.6	2.23E-03
SCO6743	putative transcriptional accessory protein	2.9	9.75E-05
SCO3542	integral membrane protein with kinase activity	2.3	3.53E-03
SCO2064	DNA polymerase III alpha chain	2.4	2.06E-03
SCO3878	DNA polymerase III, beta chain	1.5	0.01
SCO4067	DNA polymerase III subunit gamma	2.0	0.01
SCO5769	recombinase A	4.6	2.37E-04
Transport			
SCO1964	putative export associated protein	1.7	1.11E-03
SCO1965	putative export associated protein	2.0	6.63E-05
SCO2257	probable ABC transporter, NBD	1.5	0.01
SCO2258	probable ABC-transporter, MSD	1.8	0.01
SCO4963	putative ABC transporter ATP-binding protein	2.8	2.04E-04
SCO4964	putative integral membrane transport protein	3.6	0.04
Translation and ribosomes			
SCO1600	putative translation initiation factor IF-3	1.5	1.44E-03
SCO4661	elongation factor G	1.6	2.23E-04

SCO4725	translational initiation factor IF1	1.7	2.71E-03
SCO4981	putative bifunctional protein	1.7	0.03
SCO3906	putative 30S ribosomal protein S6	1.5	2.78E-04
SCO3908	putative 30S ribosomal protein S18	1.8	7.53E-04
SCO3909	50S ribosomal protein L9	1.5	3.92E-06
SCO4649	50S ribosomal protein L1	1.5	0.02
SCO4652	50S ribosomal protein L10	1.5	2.22E-04
SCO4659	30S ribosomal protein S12	1.6	1.17E-03
SCO4701	30S ribosomal protein S10	1.7	8.52E-04
SCO4702	50S ribosomal protein L3	1.8	1.58E-03
SCO4704	50S ribosomal protein L23	1.6	1.86E-02
SCO4706	30S ribosomal protein S19	1.5	2.38E-02
SCO4709	50S ribosomal protein L16	1.6	8.33E-05
SCO4712	50S ribosomal protein L14	1.5	0.02
SCO4714	50S ribosomal protein L5	1.7	4.18E-05
SCO4721	50S ribosomal protein L15	1.5	2.51E-03
SCO4727	30S ribosomal protein S13	1.6	1.45E-06
SCO4728	30S ribosomal protein S11	1.6	6.78E-04
SCO4734	50S ribosomal protein L13	1.7	9.93E-06
SCO4735	30S ribosomal protein S9	1.8	7.55E-07
SCO5591	30S ribosomal protein S16	1.5	0.02
SCO5624	30S ribosomal protein S2	1.5	3.03E-05
Secondary metabolism			
SCO5071	hydroxylacyl-CoA dehydrogenase	1.7	5.37E-04
SCO5073	putative oxidoreductase	1.5	0.05
SCO5074	putative dehydratase	1.5	1.41E-03
SCO5075	putative oxidoreductase	1.9	9.61E-04
SCO5079	conserved hypothetical protein	1.7	4.52E-03
SCO5087	actinorhodin polyketide beta-ketoacyl synthase	1.8	1.12E-04
SCO5088	actinorhodin polyketide beta-ketoacyl synthase	2.0	1.06E-03
SCO7147	putative ketoreductase	3.3	8.51E-05
Regulation			
SCO2105	putative transcriptional regulatory protein	2.8	1.17E-07
SCO2564	putative DNA-binding protein	3.8	6.62E-05
SCO4122	putative MarR-family transcriptional regulator	4.2	1.17E-07
SCO2950	DNA-binding protein Hu (hs1)	1.7	0.01
SCO5770	RecX, putative regulatory protein	6.0	6.62E-05
SCO4895	putative ECF sigma factor	3.9	1.21E-06
SCO3013	putative two-component system response regulator	1.5	0.04
SCO5749	two-component regulator	1.5	0.05
Not classified			
SCO4945	putative dehydrogenase	1.7	0.03
SCO5051	putative glycosyltransferase	1.7	1.06E-03

SCO5227	putative redoxin	1.5	0.01
SCO6687	putative DNA-binding protein	2.0	0.02
SCO6984	putative oxidoreductase.	7.6	4.90E-06
Hypothetical protein			
SCO1291	hypothetical protein SCBAC36F5.02	2.6	0.01
SCO1342	hypothetical protein	7.1	2.35E-05
SCO1404	hypothetical protein	1.7	0.05
SCO2211	hypothetical protein SC10B7.06	4.2	4.18E-04
SCO2353	hypothetical protein	4.3	1.22E-05
SCO2862	hypothetical protein SCE20.36c.	1.8	0.01
SCO2912	hypothetical protein	1.5	0.01
SCO3022	hypothetical protein SCE34.03c	2.3	4.44E-03
SCO3050	hypothetical protein	7.7	1.89E-05
SCO4226	hypothetical protein	1.5	0.05
SCO4346	hypothetical protein SCD19.01c	2.7	2.63E-03
SCO4611	hypothetical protein SCD39.11	4.4	0.01
SCO4802	hypothetical protein SCD63A.13c	2.3	1.64E-03
SCO4894	hypothetical protein 2SCK8.20c	2.6	5.63E-04
SCO7843	hypothetical protein SC8E7.40c.	1.9	0.04
Biosynthesis of cofactors			
SCO5859	ferrochelatase	2.0	0.01
SCO6041	putative protoporphyrinogen oxidase	2.1	0.02
SCO2104	putative thiamin phosphate pyrophosphorylase	3.4	3.67E-04
Membrane Proteins			
SCO4643	UDP-N-acetylenoylpyruvoylglucosamine reductase	2.6	9.02E-04
SCO1409	putative membrane protein.	1.6	2.67E-03
SCO2809	putative membrane protein	1.7	0.02
SCO3570	putative membrane protein	1.5	0.02
SCO5099	putative membrane protein	1.5	0.02
SCO5664	putative integral membrane protein	2.0	0.01
SCO0628	putative secreted protein	1.7	0.02
SCO1292	putative secreted protein	4.0	3.93E-05
SCO2116	putative secreted protein	1.9	0.04
SCO2591	putative secreted protein	1.5	0.04
SCO2725	putative lipoprotein	3.0	2.56E-04
SCO2808	putative secreted protein	1.5	0.02
SCO5029	putative secreted protein	1.5	5.69E-04
SCO6096	putative lipoprotein	2.2	2.17E-07
Conserved Hypothetical Proteins			
SCO0162	conserved hypothetical protein SCJ1.11	1.8	0.02

SCO0563	conserved hypothetical protein SCF73.10c	5.5	2.20E-07
SCO1181	conserved hypothetical protein SCG11A.12	3.2	1.58E-05
SCO1183	conserved hypothetical protein SCG11A.14	3.5	7.58E-04
SCO1184	conserved hypothetical protein SCG11A.15	1.6	0.05
SCO1406	hypothetical protein	1.6	0.03
SCO1925	conserved hypothetical protein	1.6	0.05
SCO1653	conserved hypothetical protein SCI41.36	4.0	0.03
SCO1729	hypothetical protein	1.6	0.05
SCO1950	hypothetical protein	2.0	0.01
SCO1952	conserved hypothetical protein	1.7	4.40E-04
SCO2204	hypothetical protein SC3H12.12	2.2	4.76E-04
SCO2340	hypothetical protein	1.6	0.03
SCO2901	hypothetical protein	1.7	0.02
SCO2986	conserved hypothetical protein SCE50.14c	1.5	1.82E-03
SCO3568	conserved hypothetical protein	2.1	0.02
SCO3858	conserved hypothetical protein	1.5	0.04
SCO3900	conserved hypothetical protein	2.1	3.78E-03
SCO4113	conserved hypothetical protein	3.2	7.60E-06
SCO4631	hypothetical protein SCD82.01c	2.7	4.83E-04
SCO4803	conserved hypothetical protein	7.1	3.44E-05
SCO5047	conserved hypothetical protein GlpX	1.6	8.39E-03
SCO5240	hypothetical protein	2.1	6.37E-04
SCO5570	hypothetical protein	1.8	0.04
SCO5645	conserved hypothetical protein SC6A9.22c	2.1	0.05
SCO6085	conserved hypothetical protein	2.0	7.87E-04
SCO6120	hypothetical protein SC9B2.07	3.6	6.23E-04
SCO6510	conserved hypothetical protein SC1E6.19c	1.8	1.16E-03
SCO6686	conserved hypothetical protein	5.2	3.91E-04
SCO6953	conserved hypothetical protein SC6F7.06c	1.9	5.14E-05
SCO6983	conserved hypothetical protein SC8F11.09.	3.5	4.36E-06
Others			
SCO0869	putative anti-sigma factor antagonist	1.5	0.01
SCO3795	aspartyl-tRNA synthetase	1.6	3.29E-03
SCO4612	putative amino acid transporter	1.8	0.03
SCO5059	polyphosphate glucokinase	2.3	0.01
SCO2026	putative glutamate synthase large subunit	1.6	0.03
SCO2532	PhoH-like protein	1.7	1.65E-03
SCO4145	polyphosphate kinase	1.9	1.88E-04
SCO3049	putative acyl-CoA hydrolase	2.7	1.09E-04
SCO5374	ATP synthase epsilon chain	1.5	0.02
SCO7417	putative cytochrome P450-family protein.	2.6	2.87E-05
SCO3731	cold-shock protein	1.6	0.01
SCO4684	cold shock protein	1.5	2.84E-04
SCO1405	putative heat shock protein (hsp90-family)	4.7	4.19E-03

Gene	Annotation	Fold change	P value
Down Regulated genes in response to ciprofloxacin			
Chaperons			
SCO3670	heat chock protein	2.4	5.33E-03
SCO3671	heat shock protein 70 (fragment)	1.9	0.05
SCO4296	chaperonin 2	1.8	0.01
SCO4761	10 kD chaperonin cpn10	2.1	9.23E-05
SCO4762	60 kD chaperonin cpn60	2.3	6.53E-04
Conserved hypothetical protein			
SCO0910	conserved hypothetical protein SCM1.43	1.6	0.03
SCO0921	conserved hypothetical protein SCM10.10c	2.0	6.46E-05
SCO1222	conserved hypothetical protein	1.5	0.02
SCO1375	conserved hypothetical protein SC10A9.17	2.3	0.01
SCO1566	putative acyltransferase	1.6	1.14E-03
SCO1640	conserved hypothetical protein SCI41.23c	1.7	6.38E-06
SCO2065	conserved hypothetical protein	1.6	0.02
SCO3790	conserved hypothetical protein	1.8	1.96E-03
SCO4201	conserved hypothetical protein	1.5	0.04
SCO4675	conserved hypothetical protein SCD40A.21c	1.5	0.01
SCO5581	conserved hypothetical protein	1.5	0.02
SCO5746	hypothetical protein SC7C7.01	1.5	3.67E-03
SCO6176	conserved hypothetical protein	1.5	0.01
SCO6192	conserved hypothetical protein SC2G5.13	1.7	0.03
SCO7251	conserved hypothetical protein	1.6	3.34E-03
SCO7617	conserved hypothetical protein	1.8	4.26E-03
SCO7748	conserved hypothetical protein	1.5	0.01
Metabolism			
SCO1661	putative glycerol-3-phosphate dehydrogenase	1.7	0.03
SCO6199	secreted esterase	2.1	1.13E-03
SCO2766	putative secreted ribonuclease	2.1	2.45E-04
SCO1969	putative DNA-methyltransferase	1.6	0.05
SCO6717	putative acyl-[acyl-carrier protein] desaturase	2.0	6.67E-04
SCO5144	putative acyl CoA isomerase	1.5	3.82E-03
SCO6691	putative phospholipase C	1.9	2.60E-03
Secreted proteins			
SCO0131	putative secreted protein	1.6	4.38E-03
SCO0297	putative secreted protein	1.7	1.24E-04
SCO1048	putative secreted protein	1.9	3.60E-04
SCO1196	putative secreted protein	2.3	4.28E-04
SCO1230	putative secreted tripeptidylaminopeptidase	1.5	0.05
SCO1565	putative glycerophosphoryl diester	1.6	3.17E-05

	phosphodiester		
SCO1860	putative secreted protein	1.7	0.01
SCO1906	putative secreted protein	1.6	6.47E-05
SCO1908	putative large secreted protein	1.8	2.63E-03
SCO2068	hypothetical protein "putative secreted alkaline p	1.7	1.72E-04
SCO2348	putative secreted protein	3.2	1.49E-05
SCO2383	putative secreted protein	1.5	1.24E-04
SCO4428	putative secreted protein	1.5	1.02E-04
SCO5013	putative secreted protein	1.6	2.14E-04
SCO5014	putative secreted protein	1.6	0.03
SCO5015	putative secreted protein	1.6	1.62E-03
SCO6198	putative secreted protein	2.2	1.08E-03
SCO7550	putative secreted hydrolase	1.8	3.19E-03
SCO7631	putative secreted protein	1.8	1.45E-03
Membrane proteins			
SCO6005	putative lipoprotein	1.5	0.02
SCO0644	putative membrane protein.	1.7	1.62E-03
SCO1160	putative membrane protein	1.5	3.86E-03
SCO1630	putative integral membrane protein	1.9	8.87E-04
SCO5650	putative membrane protein	1.6	1.61E-03
SCO7536	putative integral membrane protein.	1.9	0.01
SCO5998	putative bifunctional protein (fragment) "putative	1.7	0.01
SCO3375	putative Lsr2-like protein	1.9	1.55E-03
Regulator			
SCO5033	hydrogen peroxide sensing regulator	1.5	0.02
SCO6323	putative tetR-family regulatory protein	2.2	0.01
SCO3668	putative heat shock protein	2.1	0.04
hypothetical protein			
SCO0682	hypothetical protein SCF15.03c	2.2	1.50E-03
SCO1993	hypothetical protein	1.7	2.11E-03
SCO2384	hypothetical protein SC4A7.12	1.9	2.68E-04
SCO3350	alanine-rich hypothetical protein	1.7	2.93E-04
SCO3371	hypothetical protein	2.0	1.06E-03
SCO6145	hypothetical protein SC1A9.09	1.5	1.21E-03
Not classified			
SCO2286	putative alkaline phosphatase	2.2	7.79E-04
SCO5249	putative nucleotide-binding protein	2.4	7.99E-05
SCO5473	putative ATP/GTP binding protein	1.6	3.72E-03
SCO5679	putative aldehyde dehydrogenase	1.7	0.01
SCO7697	putative secreted hydrolase	1.5	4.08E-05
Secondary metabolism			
SCO5890	putative 8-amino-7-oxononanoate synthase	1.5	0.04

SCO5893	oxidoreductase	2.0	2.84E-06
SCO5894	thioesterase	2.5	7.51E-04
SCO5895	putative methyltransferase	2.7	9.36E-05
SCO5896	phosphoenolpyruvate-utilizing enzyme	2.4	4.72E-04
SCO5897	putative oxidase	3.8	2.37E-04
SCO5898	probable membrane protein	3.8	3.80E-05
SCO6273	putative type I polyketide synthase	2.1	1.26E-05
SCO6282	putative 3-oxoacyl-[acyl-carrier protein] reducta	2.2	0.05
SCO3232	CDA peptide synthetase III	2.9	5.31E-06
SCO3233	putative hydrolase	2.2	4.02E-05
SCO3234	putative phosphotransferase	3.6	2.46E-06
SCO6276	putative secreted protein	1.8	0.01
Transport			
SCO2164	putative integral membrane efflux protein "putativ	1.5	0.05
SCO2231	putative maltose-binding protein	1.9	0.03
SCO3507	putative integral membrane efflux protein	1.5	0.01
SCO5113	BldKB, putative ABC transport system lipoprotein	1.5	0.01
SCO5396	putative cellulose-binding protein	1.6	3.29E-04
Others			
SCO3672	putative cell surface biosynthesis associated protein	1.6	9.38E-07
SCO6284	putative decarboxylase	1.6	0.01
SCO6471	putative citratelyase	1.5	1.74E-03
SCO4152	putative secreted 5'-nucleotidase	2.3	3.37E-05
SCO2259	putative multi-domain regulatory protein.	1.8	0.01
SCO3023	adenosylhomocysteinase	1.6	7.67E-04
SCO1968	putative secreted hydrolase	2.3	4.73E-06
SCO5750	ftsK homolog	1.6	5.37E-04

SF2: Experimental details of Microarray Hybridization

Microarray hybridizations were optimized in our lab at IIT Bombay. The protocol was based on that developed at University of Minnesota in Prof. Wei-Shou Hu lab. In addition, the protocol developed by TIGR was also used. The detailed steps followed during the experiments are given below:

1. Aminoallyl-labelled cDNA synthesis

1. In an RNase-free tube, combine the following reagents:

component	
Total RNA	10 µg
Random Hexamers (3mg/mL)	9 µg
DEPC water	Up to 18.5 µl

2. Mix well and incubate at 70^{o}C for 10 minutes.
3. Chill on ice for 5 min. Centrifuge above 10,000 rpm briefly to bring down any condensation.
4. Mix the RNA/primers with reverse transcriptase and buffers:

RNA/primer mix	18.5µl
5x First Strand buffer	6 µl
0.1 M DTT	3 µl
dNTP/aa-UTP labeling mix	0.6µl
SuperscriptIII	2 µl
Total	30.1 µl

5. Mix and incubate at
   25^{o}C for 5 min
   37^{o}C 10 min
   50^{o}C 1 hr 45 min
6. Stop the first strand synthesis reaction
7. Hydrolyze the RNA in the cDNA/RNA mixture by adding:
   • 0.5 M EDTA 10 µL
   • 1 M NaOH 10 µL
8. Mix and incubate at 65^{o}C for 15 minutes.
9. Centrifuge above 10,000 rpm briefly to bring down any condensation.
10. Add 25 µL 1 M Tris (pH 7.0) to neutralize pH.

Removal of unincorporated aa-dUTP and free amines

1. Vigorously mix cDNA reaction with 400 µL (5X reaction volume) PB buffer (Qiagensupplied) before transferring to MinElute column.
2. Centrifuge at 13,000 rpm for 1 minute. Empty flow through.

3. To wash, add 750 µL phosphate wash buffer to the column and centrifuge at 13,000 rpm for 1 minute.
4. Empty the collection tube and centrifuge the column an additional 1 minute at ~13,000 rpm to remove residual wash buffer.
5. Transfer column to a new 1.5 mL microfuge tube and carefully add 30 µL phosphate elution buffer to the center of the column membrane.
6. Let sit for ~1 minute at room temperature.
7. Elute by centrifugation at 13,000 rpm for 1 minute.
8. Elute a second time into the same tube by repeating with another 30 µL of phosphate elution buffer, incubating an additional minute, and centrifuging sample.
9. The final elution volume should be ~60 µL.
10. Take readings of undiluted samples at wavelength of 260 to determine cDNA concentration.
11. Transfer the open tube to a speed vac and dry it down to completion (~30 minutes).

A230				
A260				
A280				
A320				
A260/A280				
A260/A230				
Conc.				
yeild				

2. Coupling aminoallyl-labelled cDNA to Alexa dyes.

1. Add 10 µl of dry DMSO to each vial of alexa dyes and mix well to dissolve the dye completely.
2. Resuspend aminoallyl-labeled cDNA in 4.5 µL 0.1 M sodium carbonate buffer pH 9.3 by pipetting up and down for several minutes making sure that the pellet is thoroughly resuspended.
3. Add 3 µL of the appropriate resuspended Alexa dye. Pipet up and down several times to thoroughly mix the sample.
4. Incubate the reaction at room temperature for at least 1 hour.
5. After coupling has finished, add 35 µL 100 mMNaOAc pH 5.2.

3. Pre-hybridization

Pre-hybridization solution

1. Set the waterbath at 42°C.
2. Prepare 50 ml prehybridization buffer for every 5 slides:
3. *(5x SSC, 0.1% SDS, 1% BSA)*

Recipe:	***Amount***
20x SSC	15 mL
10% SDS	600 µL
BSA powder	0.6 gm
MilliQ/DI water to	60 mL

4. Filter the prehybridizaton buffer with a 0.22 µm Mini-Miser (CA) Filter.
5. Transfer the solution to a clean Coplin jar (50ml centrifugation tube) and preheat the buffer at 42°C for approximately 10 minutes.
6. Place the printed slide(s) with their labels up in a Coplin jar containing preheated pre-hybridization buffer. Incubate at 42°C for at least an hour.

Washing Prehybridized Slides

7. Following the prehybridization, remove slides from 42°C waterbath. Pour out the prehybridization buffer, being careful not to pour the slides out as well.
8. Quickly fill the 50ml centrifugation tube with MilliQ water and replace the cap.
9. Carefully shake the Coplin jar for approximately 30 seconds to remove the prehybridization buffer from the slides.
10. Pour out the water and repeat the procedure approximately five times or until froth can no longer be seen in the water.
11. Fill a glass staining dish with MilliQ/DI water
12. Using forceps *carefully* grip the slides by the label and then remove the slides from the Coplin jar. Place them in the slide rack for a glass staining dish.
13. place the slide rack with the slides inside the staining dish.
14. Place the entire staining dish apparatus on top of a rotor shaker and let shake for about 2 minutes.
15. Change the water inside the staining dish every 2 minutes. Continue to wash until you have used ~2 liters total of wash water
16. Empty the staining dish and fill with isopropyl alcohol.
17. Wash in the isopropyl alcohol for two minutes on the rotary shaker. When done, leave the slides in the isopropyl alcohol and take them immediately to the centrifuge.

Drying Slides
Note: *DO NOT let the slides start to dry before putting them in the centrifuge.*
Allowing the slides to slowly air dry will cause background to appear on the slide.

18. Take the glass slide rack with the slides out of the isopropyl alcohol and remove the metal handle from the holder.
19. Put the slides into a centrifuge with a flat plate-holder adaptor lined with paper towels. Centrifuge the slides at ~1000 RPM's for at least 10 minutes at RT
20. Hold slides up to the light to check for any degree of streaking or spots. If any of these appear the slides must be re-washed and re-spun.

The slides should be used immediately

4. *Removal of free dye by using qiagen PCR purification kit*

1. Make 1.6 ml PE buffer [0.32mL PE concentrate (Qiagen supplied) +1.28 mL Ethanol]
2. Vigorously mix cDNA reaction with 250 µL (*5X reaction volume*) PB buffer (Qiagensupplied) before transferring to MinElute column.
3. Centrifuge at ~13,000 rpm for 1 minute. Empty flow through.
4. To wash, add 750 µL**PE Buffer** to the column and centrifuge at13,000 rpm for 1 minute.

5. Empty the collection tube and centrifuge the column an additional 1 minute at 13,000rpm to remove residual PE buffer.
6. Transfer column to a new 1.5 mL microfuge tube and carefully add 30 µL EB buffer(Qiagen supplied) to the center of the column membrane.
7. Let sit for ~1 minute at room temperature.
8. Elute by centrifugation at 13,000 rpm for 1 minute.
9. Elute a second time into the same tube by repeating with another 30 µL of EB buffer, incubating an additional minute, and centrifuging sample..
10. The final elution volume should be ~60 µL.
11. Take readings of undiluted samples at λ_{260} using lead factor 10

5.Dye Coupling statistics:

Absorbance				
A230				
A260				
A280				
A320				
A555				
A647				
Adye				
A260/A280				
A260/A230				
Conc.				
FOI				
Dye conc. Pmole/µl				
YEILD				

Dry the labeled cDNA in speedVac for about 30-45 minutes

6. Hybridization

Hybridization Mix

Component	
Formamide	
20X SSC	
10% SDS	
DTT	
MilliQ H_2O	

Add 60µl salmon sperm DNA/ml of Hyb Mix.
Add the hybridization mix to the pelleted probes (both cDNA)
Denature the cDNA probe for 5 minutes at 95°C,vortex thoroughly and again heat at 95°C for 5 min in the heat block

Array hybridization:

1.Preheat the waterbath at 50°C

2.Clean the Hybridization chamber and make it ready for putting slide
3.Drop the Lifterr slip on the array carefully entirely covering the printed area and so that some void space created on array and then slowly release the probe near to one edge of the array
4.Transfer the array in Hybridization chamber and wrap with AL foil without inverting it
5.Put the Hybridization chamber in water bath and allow to hybridize for 15 hrs at 50°C
6.Start Time_____ End Time_____

7. Washing

Preheat the low stringency buffer to 55°C before use. Prior to beginning the post-hybridization washes, add 1 mL of 100 mM DTT to 1 L of each of the wash buffers.

1. After the incubation at 50°C for 14-16h, remove foil and unseal hybridization chamber. Remove the slide from the chamber, taking care not to disturb the cover slip.
2. Fill a Pyrex glass dish about half full with low stringency buffer that has been warmed to 55°C.
3. To remove the coverslip grab the slide label with forceps and submerge it in the buffer. Shake the slide to loosen the coverslip. With time the coverslip will slide free of the slide surface.
4. Continue to wash the slide vigorously for about an additional minute once the cover slip has been removed.
5. Change the low stringency buffer in the Pyrex dish for every 5 slides washed. As the cover slips are removed, place every 5 slides in a glass slide holder. The slides should be spread out so they do not touch each other or the sides of the holder (as above).
6. Submerge the slide holder(s) into staining dish(s) containing **low stringency** wash buffer (preheated to 55°C). Agitate on a rotary shaker for 5 minutes.
7. After the 5 minutes, transfer the slides to a dish with new **low stringency** buffer (preheated to 55°C). Agitate an additional 5 minutes.
8. After the 5 minutes, transfer the slides to a dish with **medium stringency** buffer at room temperature and agitate 5 minutes.
9. After the 5 minutes, transfer the slides to a dish with new **medium stringency** buffer at room temperature. Agitate an additional 5 minutes.
10. After the 5 minutes, transfer the slides to a dish with **high stringency** buffer at room temperature and agitate 5 minutes.
11. After the 5 minutes, transfer the slides to a dish with new **high stringency** buffer at room temperature and agitate another 5 minutes.
12. After the 5 minutes, keep slides in the **high stringency buffer** (with DTT) until ready for scanning.
13. Dip one slide at a time several times in a dish containing clean MilliQ/DI water.
14. Dry the slide in a mini-slide centrifuge for approximately one minute. Ensure slide is completely dry before scanning.
15. Scan slide

SF3: Learning set for computational analysis

Gene	Annotation
SCO1343	uracil-DNA glycosylase
SCO1520	crossover junction endodeoxyribonuclease
SCO1958	ABC excision nuclease subunit A
SCO1966	ABC excision nuclease subunit B
SCO2003	DNA polymerase I
SCO2863	putative helicase
SCO3351	putative DNA repair protein
SCO3541	putative DNA polymerase
SCO3543	probable DNA topoisomerase I
SCO4577	putative helicase
SCO4797	putative ATP-dependent DNA helicase II
SCO5494	putative DNA ligase
SCO5566	putative ATP-dependent DNA helicase
SCO5761	putative ATP-dependent DNA helicase
SCO5769	recombinase A
SCO5770	RecX, putative regulatory protein
SCO5803	SOS regulatory protein
SCO5815	putative ATP-dependent DNA helicase
SCO6084	putative DNA polymerase
SCO7522	putative DNA ligase