Discovery of multiple uniquely transcribed genes in the electric organ of *Brienomyrus brachyistius* (Mormyridae: Teleostei)

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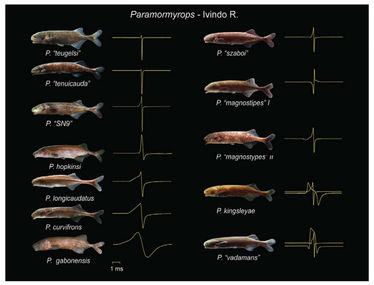
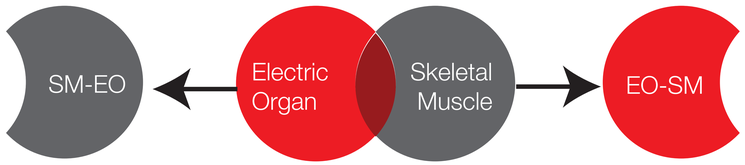
Introduction



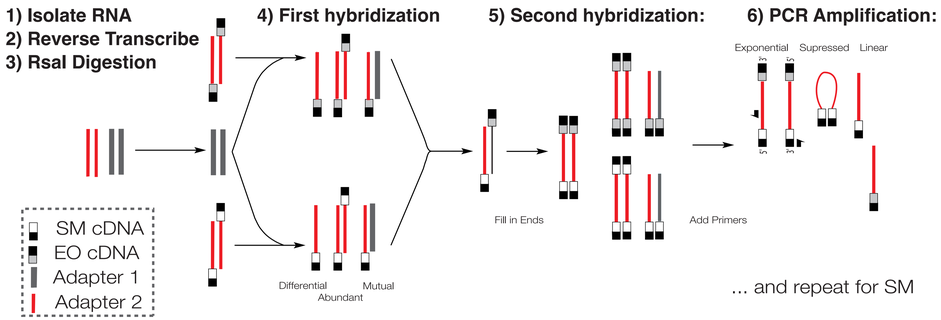
Paramormyrops of the Ivindo River exhibit a diversity of EOD waveform types. The molecular basis of EOD Diversity is presently unknown.

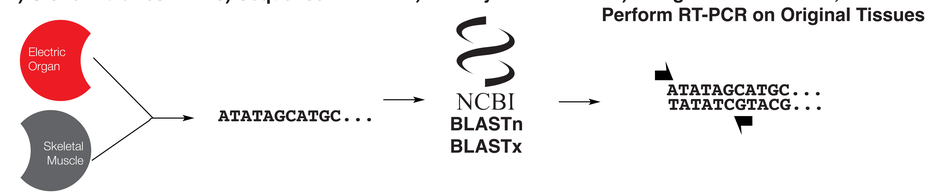
Bioelectrogenesis is especially diverse among the six families of fishes that produce Electric Organ Discharges (EODs) for defense, prey capture, electrolocation, and communication. The diversity illustrated here for eleven sympatric species from one genus in the family Mormyridae, includes differences in duration, number of peaks, polarity, and waveform fine structure. The identity of molecular components involved these differences in electric organs are currently unknown.

Suppressive Subtractive Hybridization as an approach to Gene Discovery



Since EO is developmentally derived from SM in several groups of fishes (Dahlgren 1914; Szabo 1960; Kirshbaum 1977; Denizot, Kirshbaum, Westby, and Tsuji, 1978), we decided to compare gene expression between thest two tissue types in the species *Brienomyrus brachyistius* . We utilized the technique of suppressive subtractive hybridization (SSH; Diatchenko, 1996) to identify genes that are diferentially expressed between SM and EO.



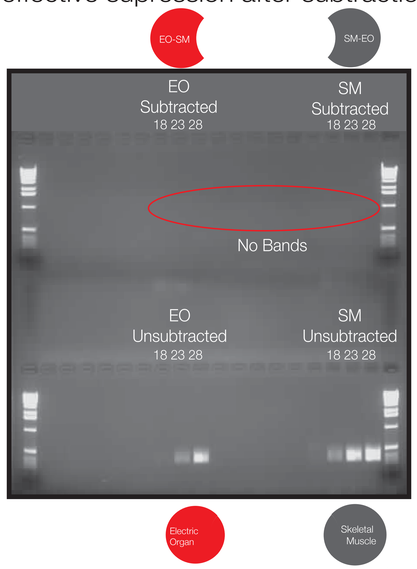


Detailed Methods are available as an accompanying handout to this poster.

Results

1. Proof of Supression

RT-PCR performed on actin reveals effective supression after subtraction.



2. Genes Identified

From a total of 181 Sequencing runs, we identified 7 genes differentially expressed in SM and 18 genes differential in EO.

EO-SM

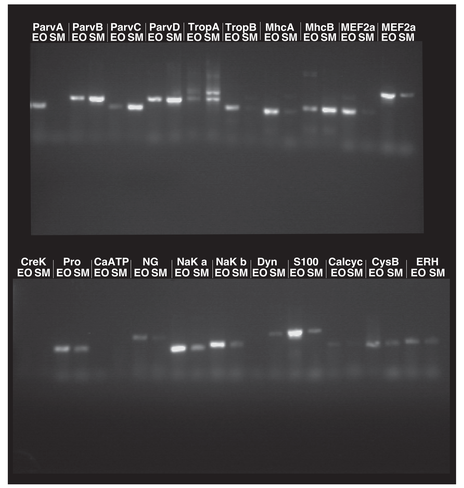
	# Hits	Size	Differential	Gel Name
1 Annexin a11	1	480	ND	N/A
2 Calcyclin Binding Protein	1	479	+	Calcyc
3 Cystatin B	2	480	-	CysB
4 Dynein Light Chain Roadblock-type 1	1	480	?	Dyn
5 Enhancer of Rudimentary	1	329	-	ERH
6 Heat Shock Protein Beta-1	1	480	ND	N/A
7 MEF2A	1	480	+	MEF2a (1)
8 Myoglobin	2	480	ND	N/A
9 Myosin Heavy Chain	1	480	+	MhcA
10a Na+/K+ transporting ATPase Alpha 1	2	480	ND	N/A
10b Na+/K+ transporting ATPase Alpha 3a	1	308	+	NaK a
11 Na+/K+ transporting ATPase Beta	3	389-480	+	NaK b
12 Neurogranin	1	480	+	Neurog
13 Parvalbumin 9	1	480	+	ParvA
14 Plasma Membrane Ca++ Transporting ATPase 4	2	265	+	CaATP
15 Programmed Cell Death Protein 6	1	327	ND	N/A
16 s100 Calcium Binding	3	480	+	S100
17 Prosaposin	1	323	+	Pro
18 Troponin 2b.1	1	468	+	TropB

SM-EO

	# Hits		Differential	Gel Name
1 Creatine Kinase-2	2	480	ND	CreK
2 MEF2A	1	480	?	MEF2a (2)
3 Myosin Heavy Chain			+	MhcB
Myosin Heavy Chain A	8	465	ND	N/A
Myosin Heavy Chain B	1	480	ND	N/A
Myosin Heavy Chain C	3	480	+	MHc B
Myosin Heavy Chain D	1	480	ND	N/A
4 Parvalbumin 2	3	480	-	ParvD
5 Parvalbumin Beta 2	5	480	+	ParvC
6 Parvalbumin Beta 1	8	480	+	ParvB
7 Troponin (tnnt3b)	1	480	+	TropA

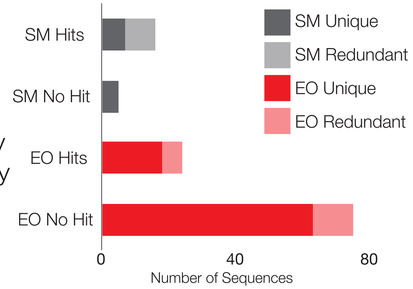
3. Confirmation of Differential Expression

RT-PCR confirmed 11 of the 18 EO genes and 4 of the 5 SM genes as differentially expressed. Shown are RT-PCR reactions after 25 cycles.



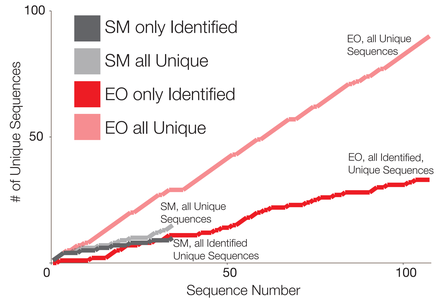
4. Distribution of Hits

The majority of genes sequenced were not identified using BLAST. We presume the idenitiy of these sequences may be UTR regions of known genes.



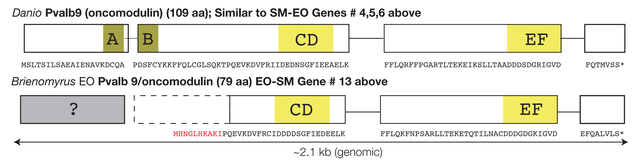
5. Rate of Gene Discovery

The number "unique" sequences (no significant alignment with other library sequences) is plotted by sequencing order for EO and SM clones, and for those that can be identified using BLAST.



6. Sequence Analysis of Parvalbumins

We sequenced entire open reading frames of four parvalbumins: three expressed in SM, (all 109 aa), and one unique to EO. Our analysis of the EO Pvalb sequence (Right) aligned to its homolouge in *Danio* suggests deletion of the vestigal A-B domain present in all other vertebrates (Arif, 2009).



Discussion

- After sequencing 180 clones we have identified 11 gene sequences which are differentially expressed in either SM or EO.
- Myocyte enhancing factor 2a (MEF2a) has an EO and SM specific form. MEF family transcription factors are important in early muscle development and differentiation.
- Ca++ transporting and binding molecules were the most abundant group of genes detected in this method: the deletion of 25% of the coding sequence for an EO-parvalbumin merits further investigation.

Acknowledgements:

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References are available on the accompanying handout.

Detailed Methods:

RNA was isolated from both SM and EO of 10 adult *Brienomyrus brachyistius* using the TRIZOL method, then PolyA+ purified (Invitrogen). Reverse Transcription was performed using SuperScript III reverse transcriptase, the remaining procedure was modified from SSH kit (Clontech). Libraries were cloned into chemically competent *E. coli* using a TA cloning system. DNA was isolated then sequenced by Cornell Sequencing Facilities. Sequences were identified using freely available BLAST2GO software (Götz et al, 2008); Searches were made against the NCBI BLAST nr database with blastx and blastn search types. Evalue cutoffs were 1.0E-5. Primers were designed to identified sequences; RT-PCR was performed using Invitrogen RT-PCR OneStep method on total RNA isolated from either EO or SM.

Sequence analysis and editing was carried out using the program lucy3 (for removing contaminating vector sequences), then edited in freely available CLC Sequence Viewer 6 or a simple text editor. Any alignments were made using the ClustalW algorithm using default parameters.

Sequences were identified as "unique" or "redundant" using the NCBI utility BLASTCLUST. Either EO or SM sequences were systematically clustered by the MEGAblast algorithm from DNA or translated Protein sequences. Default parameters were used: Matrix BLOSUM62; gap opening cost 11; gap extension cost 1; no low-complexity filtering. For DNA sequences: match reward 1, mismatch penalty -3, non-affine gapping costs wordsize 28. In both cases e-value threshold was set to 1e-6.

References:

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