RNA dynamics in the developing mouse face

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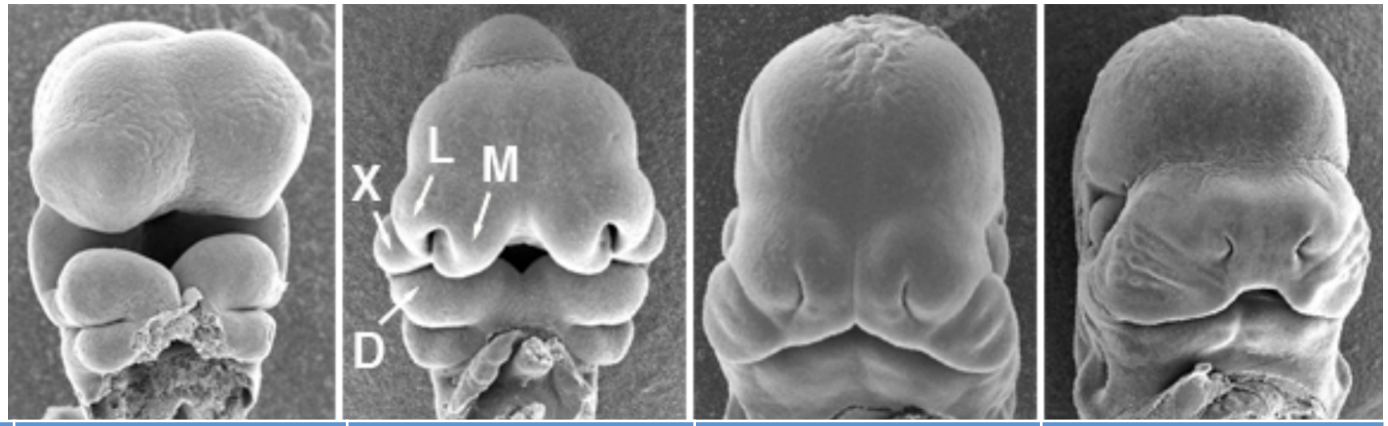
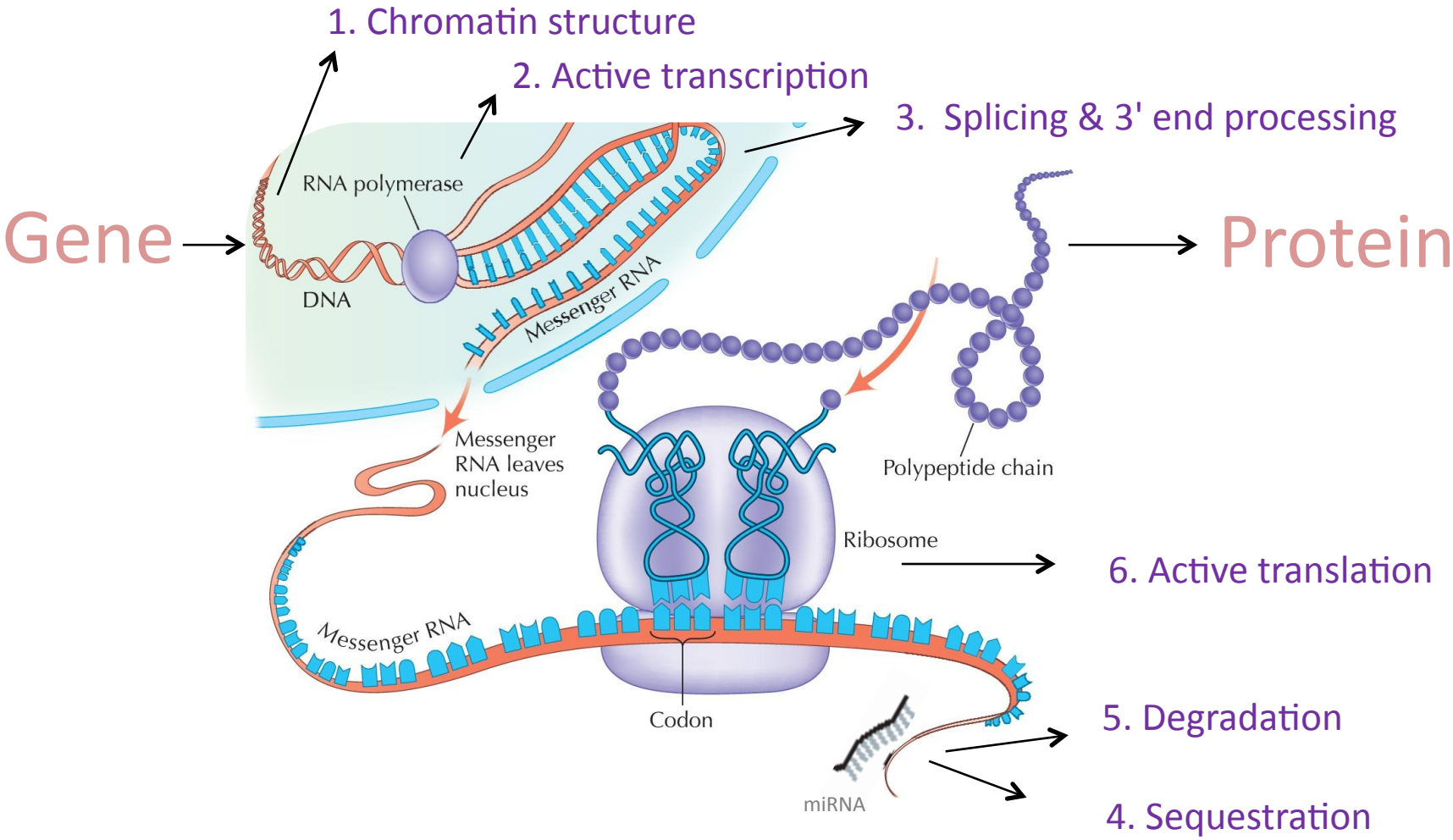
FaceBase

Mouse mid-face morphogenesis



	E9.5	E10.5	E11.5	E12.5
Face width (growth)	0.7 mm	1.25 mm	1.6 mm	5 mm
Morphogenesis	arches distinct, filled with NCCs	olfactory pit, maxillary bulge	Nares, midline contact, primary palate contact	Fusion completed
Vascularization	Pharyngeal arch arteries	Primitive Mx, Md and olfactory arteries	Well developed branched system	
Innervation (V)	Neurogenesis	Axon growth begins	Synaptogenesis begins	
Olfactory epithelium	placode	invagination, neurogenesis	Axon growth begins, Epithelial folding	
Rathke's pouch	initiation	invagination	invagination	scission
Salivary glands			sub-mandibular placode	sub-lingual & parotid placodes
Teeth		Dental lamina (MdP)	incisor placode	molar placodes
Tongue		lateral lingual swellings	Fusion Into tongue	Taste papillae placodes
Vibrissae			placodes	innervation

From Genome to Protein to Morphology: regulatory dynamics

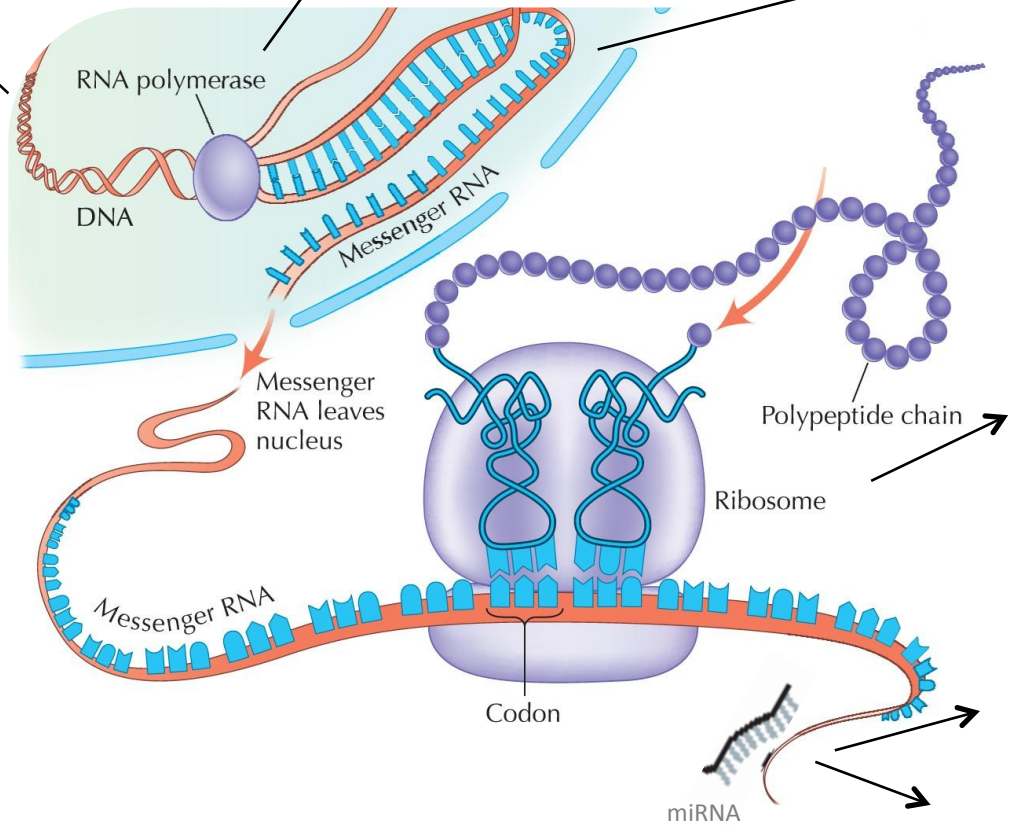


Experimental approach

1. Chromatin structure:
histone ChIP-Seq

2. Active transcription:
RNApol2 ChIP-Seq

3. Splicing & 3' end processing:
mRNA-seq

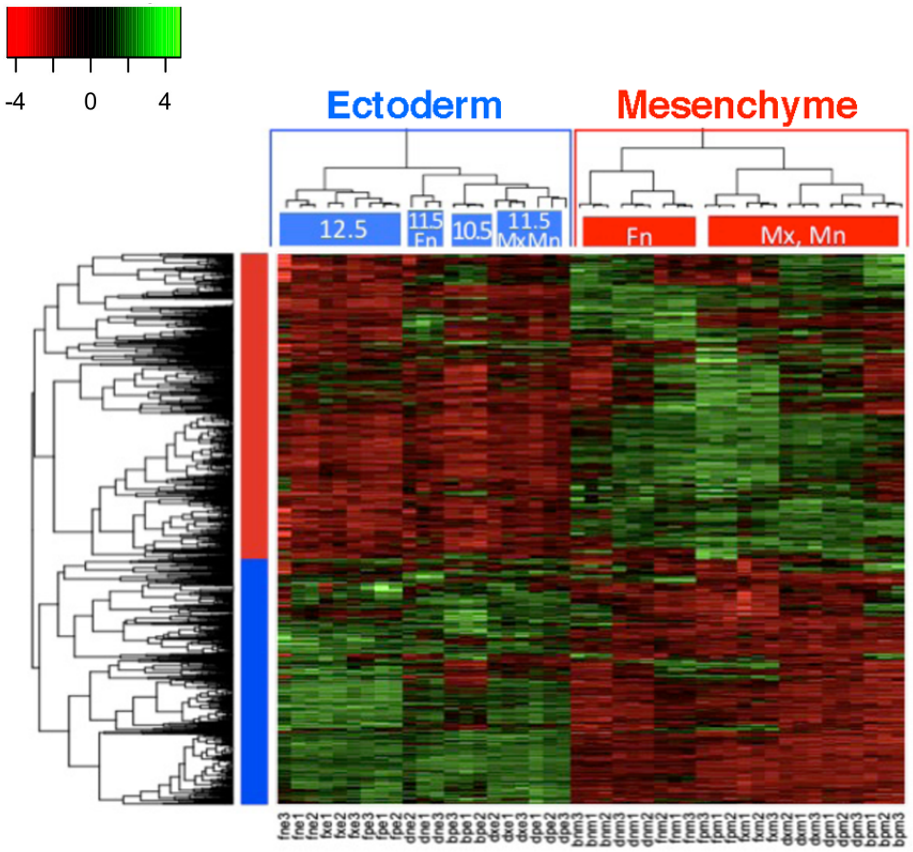


6. Active translation:
ribosomal profiling

5. Degradation:
Ago2 ClIP-Seq
& miRNA-Seq

4. Sequestration:
deduced from
mRNA vs. ribosome

Ectoderm genetic programs are distinct & important

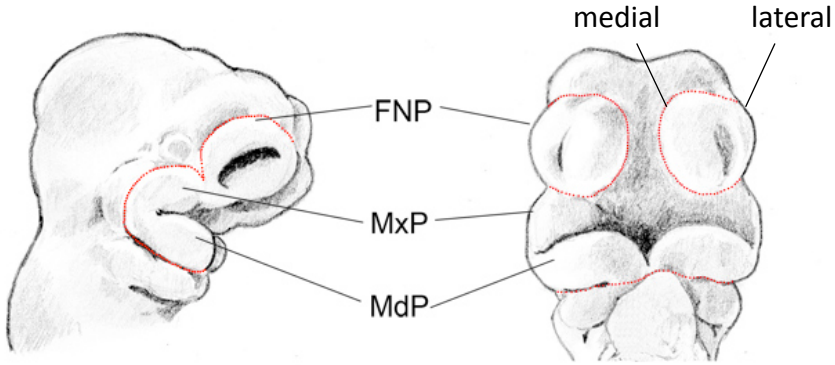


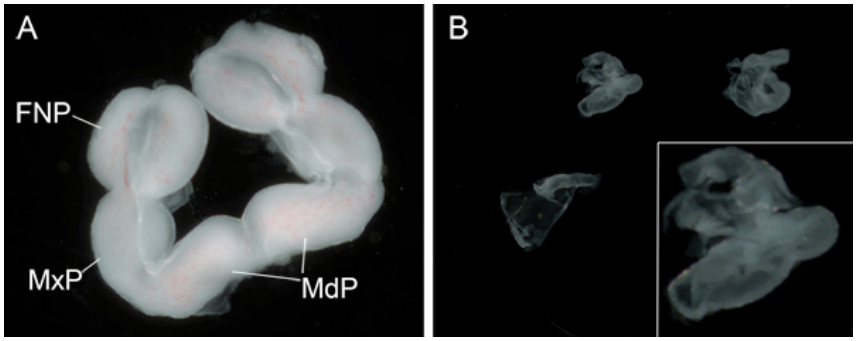
Hierarchical Clustering of 5545 Differentially Expressed Genes

Crect; Ctnnb1

E18.5 mutant embryo

3 facial prominences x 3 ages, ectoderm & mesenchyme





dissected prominences

peeled ectoderm

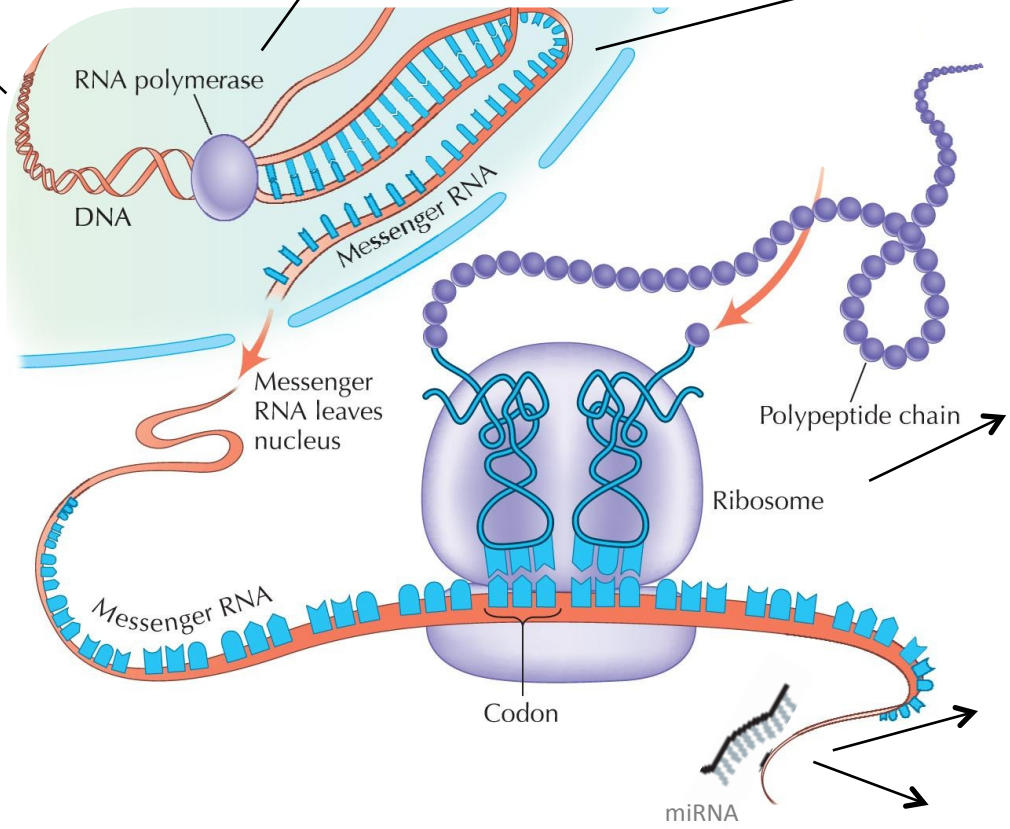
	FNP ect	medial FNP mes	lateral FNP mes	MdP ect	MdP mes	MxP ect	MxP mes
E10.5							
E11.5							
E12.5							

Experimental approach/Progress

1. Chromatin structure:
histone ChIP-Seq
Visel & Wysocka/Selleri

2. Active transcription:
RNApol2 ChIP-Seq

3. Splicing & 3' end processing:
mRNA-seq



6. Active translation:
ribosomal profiling

5. Degradation:
Ago2 ClIP-Seq
& miRNA-Seq

4. Sequestration:
deduced from
mRNA vs. ribosome

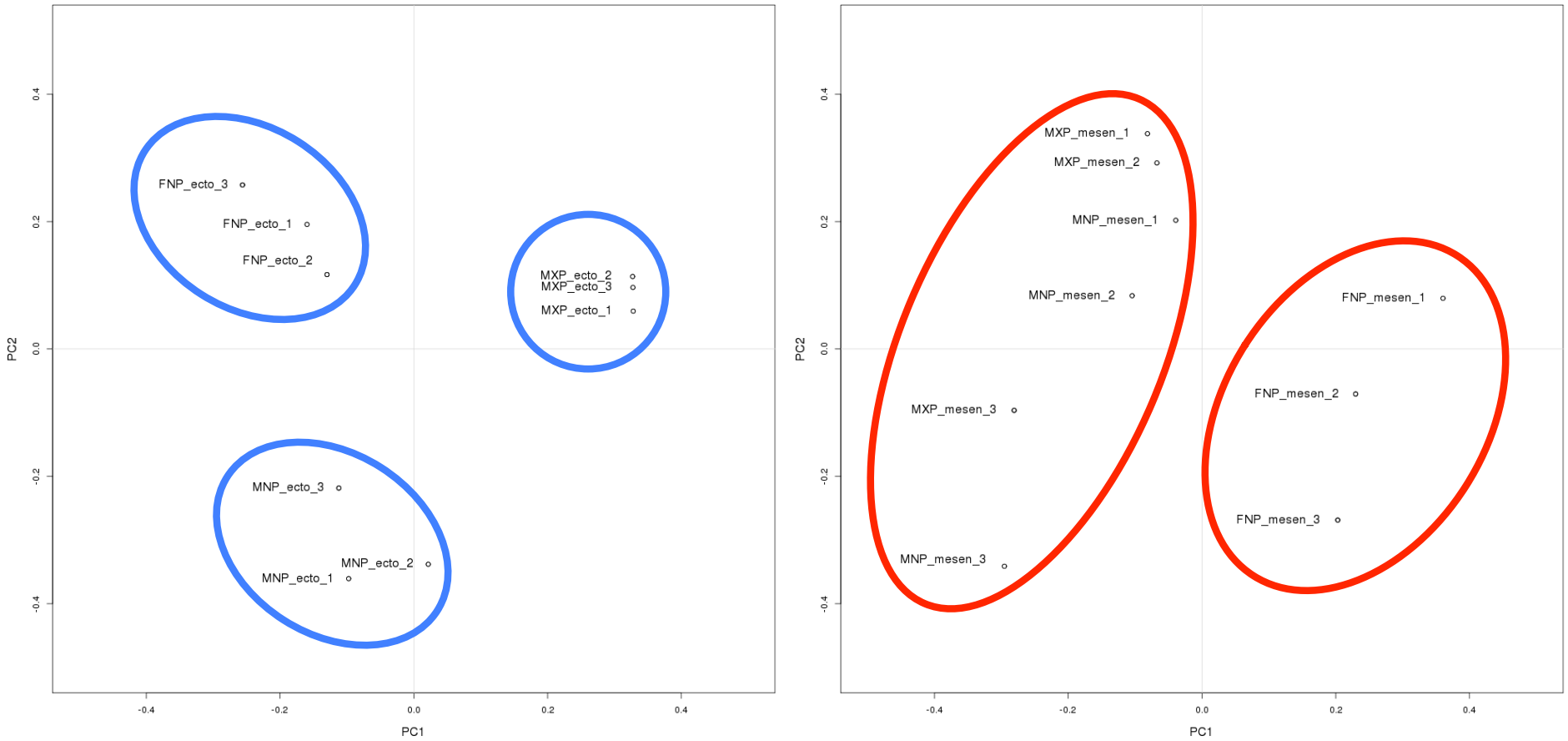
RNAseq for transcriptome/isoforms: E12.5 data QC

Sample	Raw reads	Passing QC		Mapped properly	
FNP Ect 1	189,058,630	186,537,238	98.7%	161,348,697	86.5%
FNP Ect 2	190,129,088	187,199,286	98.5%	162,156,735	86.6%
FNP Ect 3	204,383,454	200,724,662	98.2%	174,809,322	87.1%
MxP Ect 1	188,181,940	184,428,374	98.0%	158,998,846	86.2%
MxP Ect 2	191,744,178	189,233,228	98.7%	163,712,615	86.5%
MxP Ect 3	179,480,398	176,327,384	98.2%	153,187,528	86.9%
MdP Ect 1	175,687,048	173,241,268	98.6%	149,817,002	86.5%
MdP Ect 2	189,174,468	185,285,462	97.9%	159,538,983	86.1%
MdP Ect 3	163,289,440	160,837,994	98.5%	139,918,299	87.0%
FNP Mes 1	197,849,346	194,212,590	98.2%	171,517,756	88.3%
FNP Mes 2	181,087,508	178,055,728	98.3%	157,733,515	88.6%
FNP Mes 3	224,298,960	219,735,842	98.0%	193,359,007	88.0%
MxP Mes 1	209,511,878	206,885,502	98.7%	182,395,655	88.2%
MxP Mes 2	199,369,018	195,821,806	98.2%	162,440,851	83.0%
MxP Mes 3	204,185,952	201,128,068	98.5%	177,133,366	88.1%
MdP Mes 1	250,162,700	247,033,396	98.7%	218,593,681	88.5%
MdP Mes 2	214,944,406	210,890,682	98.1%	184,742,153	87.6%
MdP Mes 3	191,647,144	188,646,336	98.4%	165,244,756	87.6%
Nasal Epi 1	416,705,698	367,015,522	88.1%		
Nasal Epi 2	178,157,962	174,971,698	98.2%	153,125,865	87.5%
Nasal Epi 3	178,956,848	176,128,482	98.4%	156,401,232	88.8%

21 samples (3 prominences x 2 tissues + nasal epithelum x triplicates)

RNAseq for transcriptome/isoforms:

Principal components on E12.5 data



Ectoderm, mesenchyme and nasal epithelium clusters

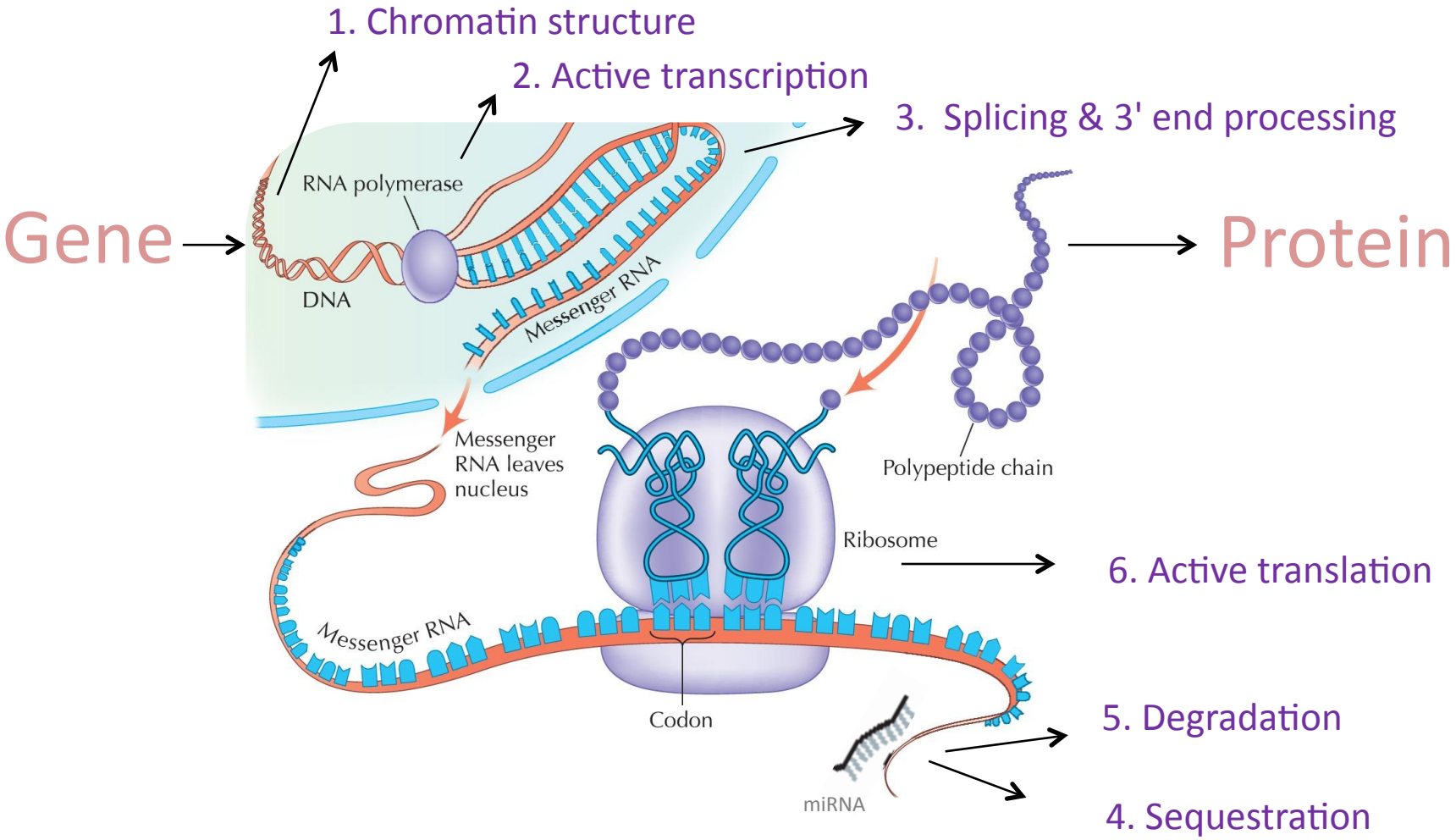
RNAseq for transcriptome/isoforms: progress

- E12.5 bam files (full dataset of 21 samples) available for upload
- Isoform and splicing analysis pipeline under development - E12.5 MxP Ect/Mes pilot data
- Isoform and splicing verification by microarray (Affy MTA2.0 Array) – E12.5 MxP Ect/Mes pilot analysis very promising

Small RNAs

	Size	Number of genes	Cannonical structure	Cannonical function	Other functions
miRNA	~22 nt	> 1500	Stem-loop precursor, RISC complex	mRNA sequestering, degrading	lincRNA sequestering, degrading
piRNA	~27 nt	~1000	PIWI complex	Germline transposon silencing, etc.	Neuronal/ memory?
snoRNA, scaRNA	72-95 nt	~ 200-500	HAcaBox or CDBox Stem-loops	t/rRNA modifications	Alt splicing, RNA editing, RNA cleavage
snRNA	~150 nt	10 genes, Tandem repeats	Spliceosome (SM of SML)	splicing	mRNA sequestering
tRNA	76-90 nt	~600	'Cloverleaf'	translation	
5S-RNA	~120 nt	tandem repeats	ribosome	translation	

From Genome to Protein: regulatory dynamics

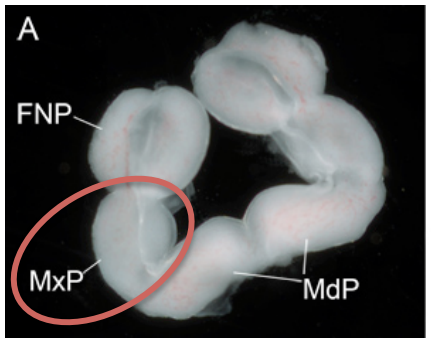


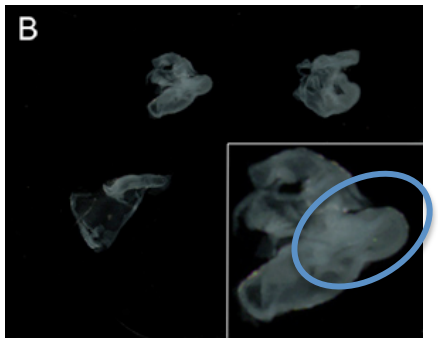
miRNAs & other small RNAs

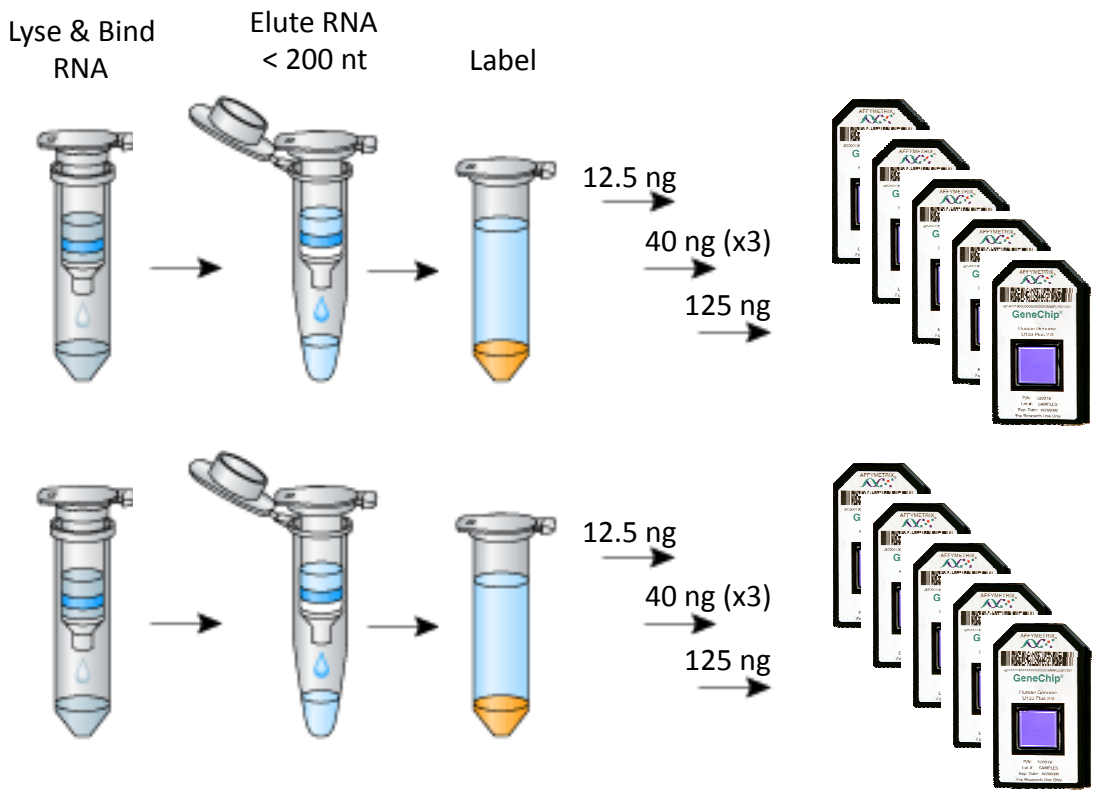
- Objective: unbiased quantitation of all classes
  - miRNAs (~22 nts), piRNAs (~27 nts), snoRNAs (~90 nts), snRNAs (~150 nts)
- Technical challenges for RNAseq:
  - Size diversity
  - Exclude VERY ABUNDANT rRNAs (~120 nts) and tRNAs (~90 nts)
  - 5' end modifications
  - Quantitation across multiple samples
- RNAseq alternatives
  - Small RNA chip (Affymetrix miRNA4.0)?

miRNA4.0	total	miRNA	stem-loop	snoRNA	CDBox	HAcaBox	scaRNA
# genes on array	36190	30424	3770	1491	319	155	31

pilot on miRNA4.0 chip



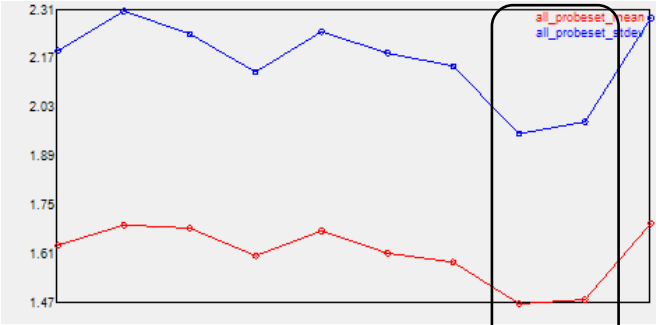




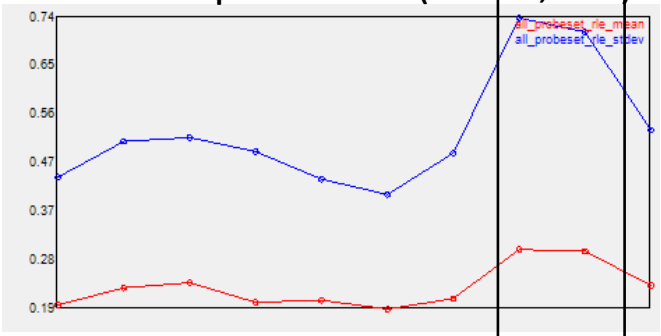
- Sensitivity: What is detected? With varying input?
- Specificity: Usual suspects?
- Statistical properties: mean/variance? number DE? @2-fold?

sensitivity : 40 ng is enough

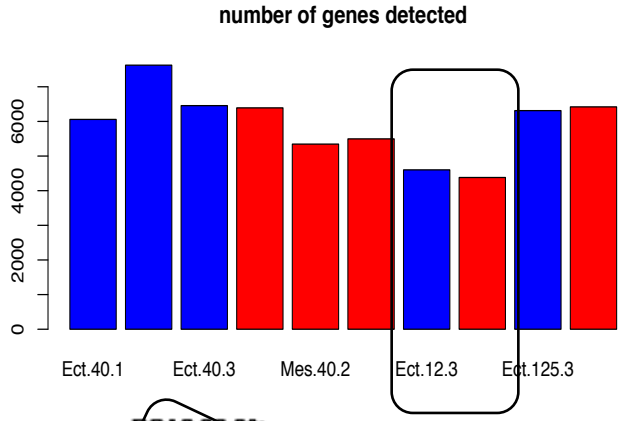
Signal: all probesets (mean, std)

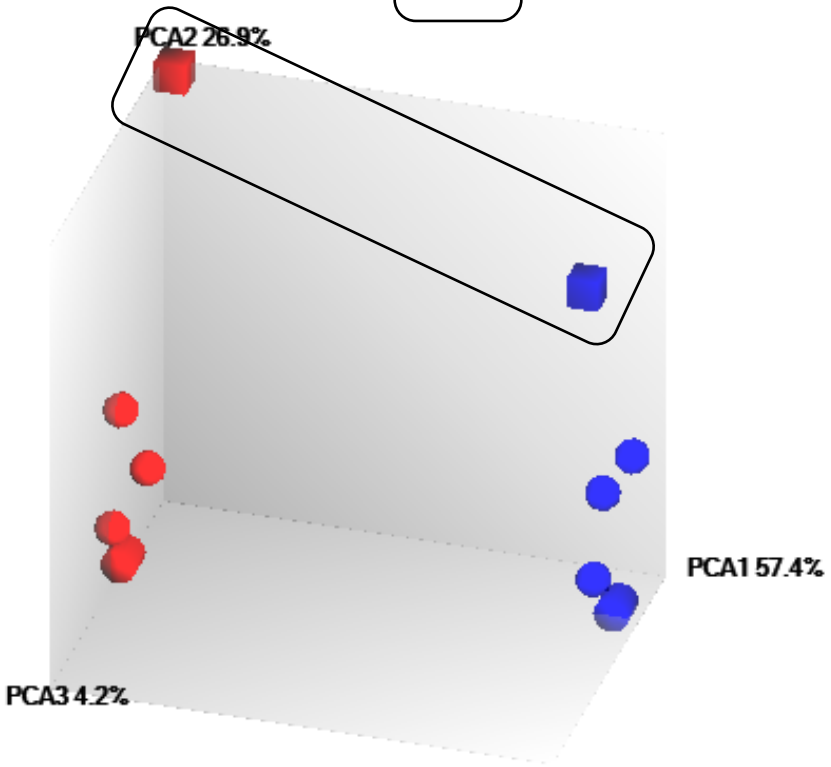


Noise: all probesets (mean, std)



12.5 ng





Usual Suspects:

Small RNAs in E12.5 MxP by RNAseq

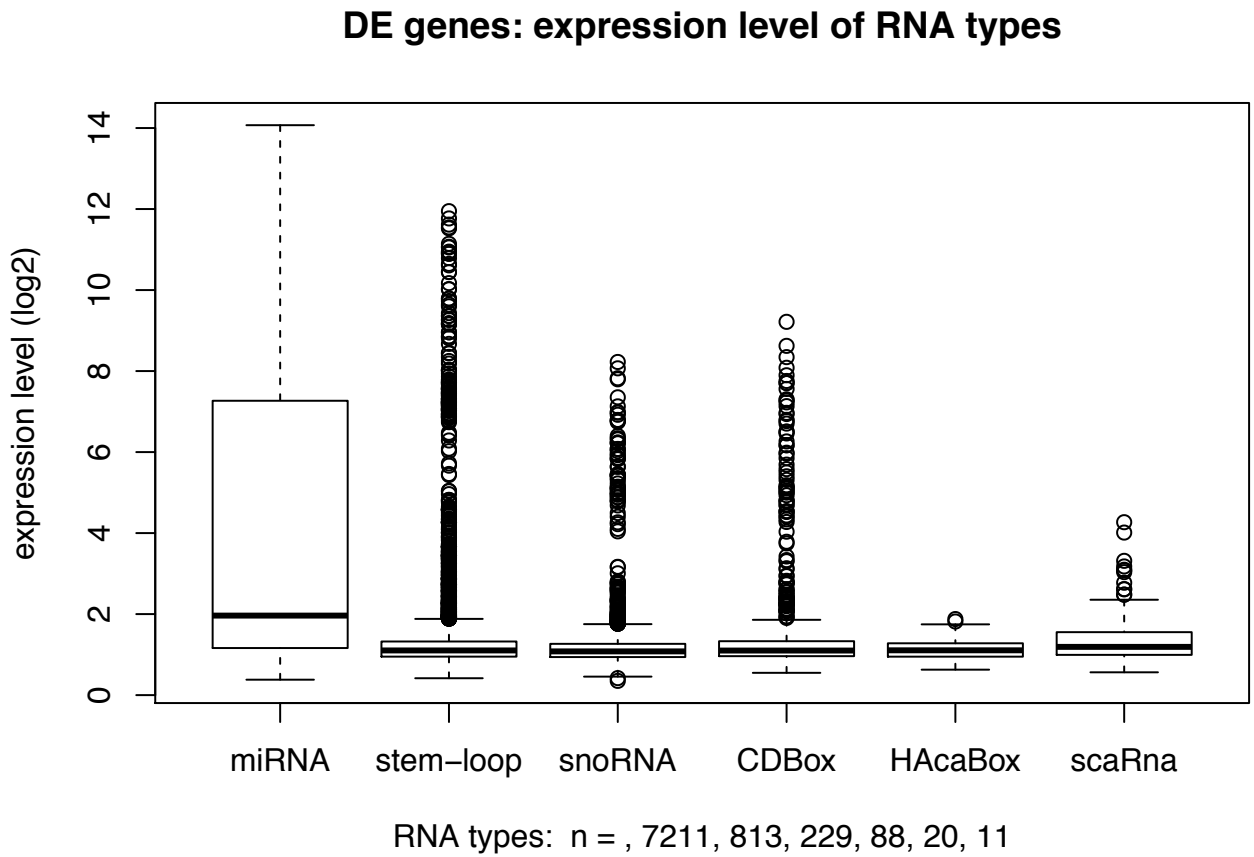
(Clouthier et *al.*)

	total	miRNA, stem-loop	*(Mouse mir)*	*(Other mir)*	snoRNA	lincRNA	snRNA	t/rRNA, other
# of genes detected	1465	1152	*(813)*	*(339)*	187	45	27	54
% of total		78.6%	*(55.5%)*	*(23.1%)*	12.8%	3.1%	1.8%	3.3%

	total	miRNA, stem-loop	*(Mouse mir)*	*(Other mir)*	snoRNA, CDBox, HAcaBox	lincRNA	snRNA	t/rRNA, other
# genes detected	3314	3025	913	2112	289	---	---	---
% of detected			86%	9.7%	2.7%	1.1%	0.2%	0.1%

Small RNAs in E12.5 MxP by microarray

Non-mir detection is not good



- snRNAs are not on the chip
- snoRNA, scaRNA signals are too weak to use
  - Why: probes derived from human genes with ~70-98% identity with mouse genes
  - Solution: develop software to call them based on only conserved probes (with Affy?)
  - OR custom chip for mouse miRNAs, snoRNAs, scaRNAs & snRNAs

miRNAs: usual suspects are detected

- *miR-17-92 (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-92a-1)* promotes palate fusion via BMP signaling. (Wang:2013) and chondrogenesis via Noggin & BMP signaling (Ning:2013)
  - All are detected, but not different in mesenchyme *vs.* ectoderm
- *miR-140* promotes chondrogenesis via *Pdgfa* (Eberhart:2008) and *Dnpep* (Nakamura:2011)
  - *miR-140-3p* is 5.3-fold enriched in mesenchyme vs. ectoderm
- *mirR-200b* promotes epithelial morphology/suppresses EMT
  - *miR-200-5p & miR-200-3p* are highly enriched in ectoderm (54-fold and 115-fold)
- 68 miRNAs detected in E12.5 primary and secondary palate microarray (Mukhopadhyay, 2010)
  - 67 of 68 detected here (6 Ect enriched, 11 Mes enriched)
- 813 miRs, 187 snoRNAs and 27 snRNAs detected in E12.5 whole MxP RNAseq (Clouthier et *al*.)
  - 589 of those miRs and 20 of those snoRNAs were detected here

Small RNAs going forward

- RNAseq is not appropriate for snoRNAs or snRNAs
- miRNAs are efficiently detected with miRNA4.0 chip
- information for some snoRNAs might be extracted from conserved probes on chip - custom software
- Naming is not standardized - a BIG deal for secondary data users

- Custom chip with mouse snRNA and snoRNA probes would be best; cost estimates forthcoming

Specific Aims

Aim 1. Describe the transcriptional dynamics of mouse facial development.

RNA-seq analysis of facial prominence ectoderm and mesenchyme during development to examine steady state levels of miRNA, mRNA, and other RNAs

Aim 2. Experimental and bioinformatics analysis of differential splicing.

Assess tissue-specific differential splicing from studies in Aim 1 using in silico methods, and validate using alternative technology (capillary high-throughput qRT-PCR)

Aim 3. Describe the post-transcriptional RNA dynamics of mouse facial development.

Clip-Seq and Ribosomal Profiling to study miRNA targets and mRNA usage.

Graphic Interface will be developed for ease of user analysis – in collaboration with hub - and datasets will be uploaded to FaceBase2 Hub.

credits

- Trevor Williams
  - Hong (Helen) Li
  - ‘Rene Choi
- Ken Jones
  - Phil O’Neil
- Joan Hooper