

miRNA expression analysis in HPV positive cervical scrapes as biomarkers for cervical disease detection

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Authors of abstract

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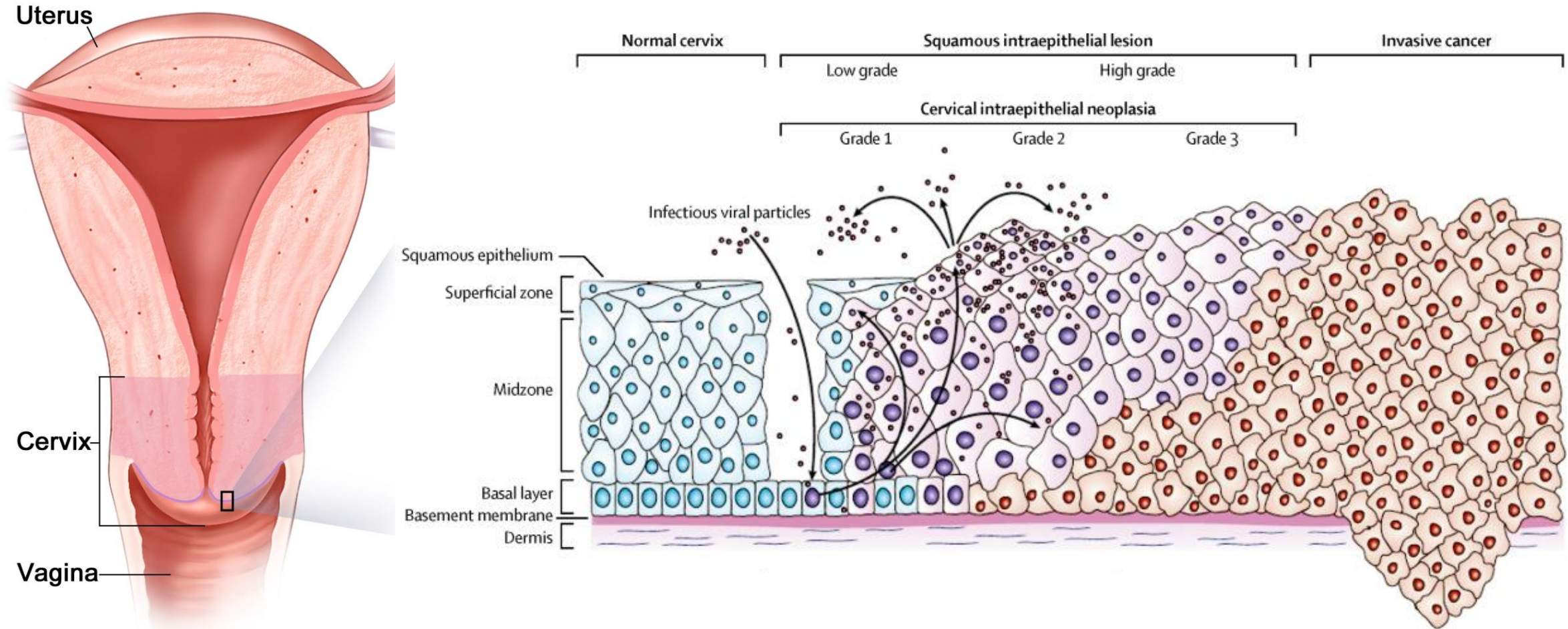
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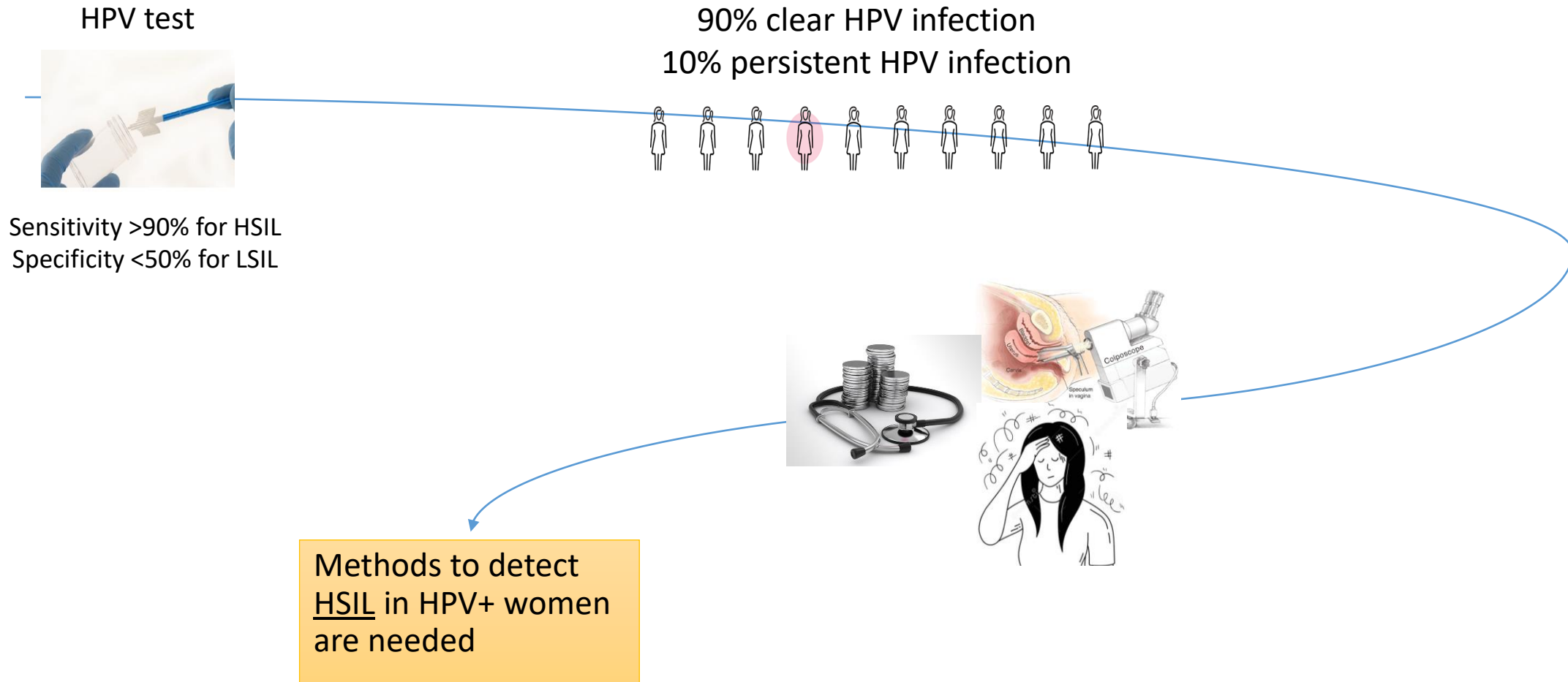
Cervical Cancer Burden



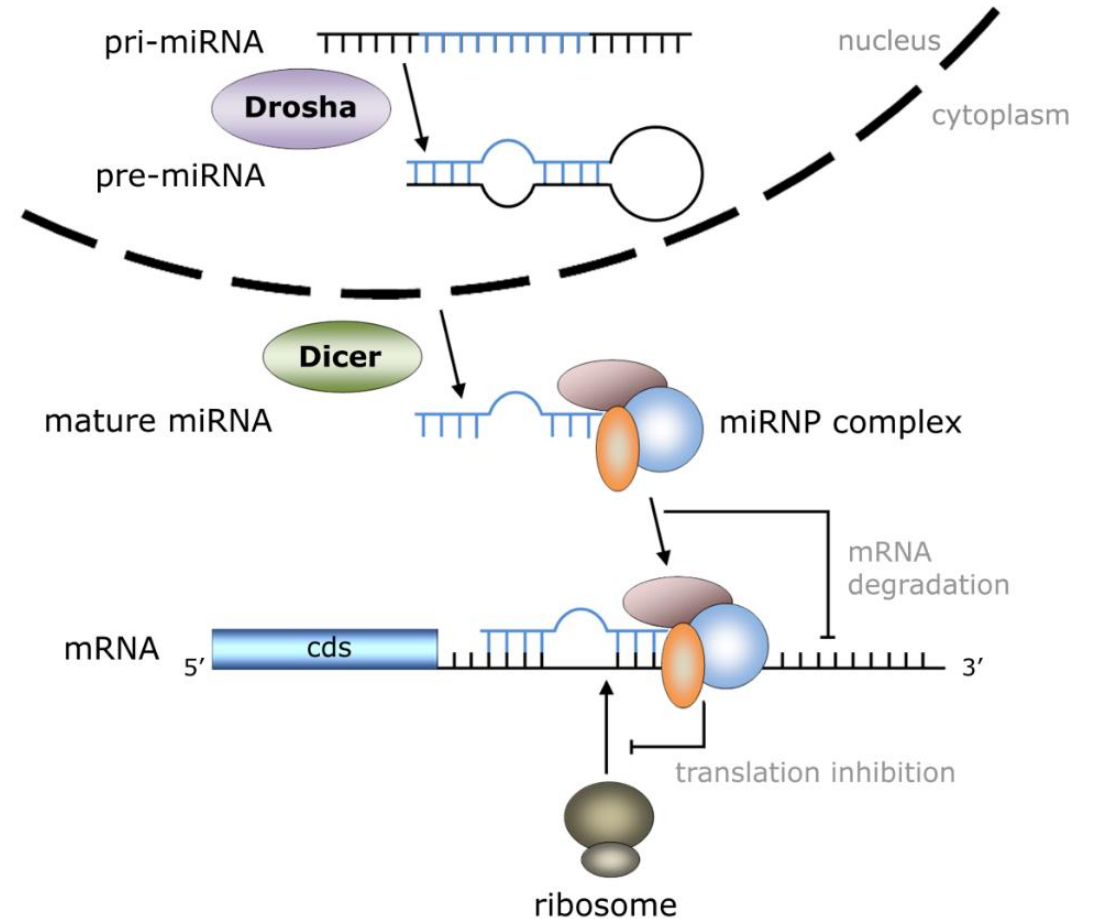
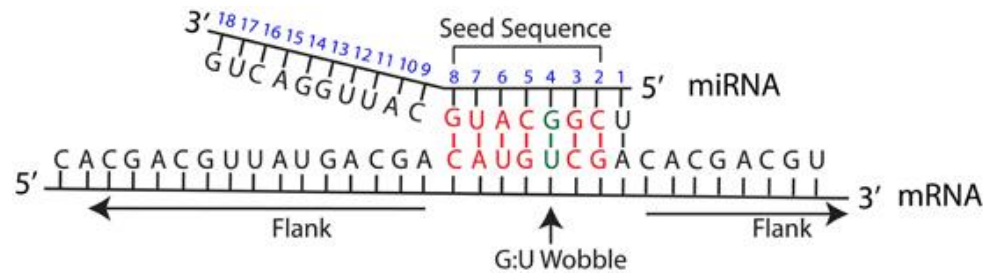
Cervical Cancer



Need of point-of-care tests to manage HPV+ women in low resource settings

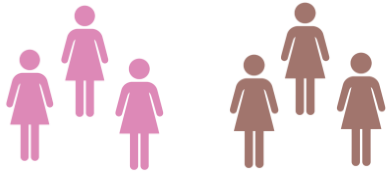


miRNAs are Promising biomarkers



AIM

To identify miRNAs differentially expressed between high-grade and low-grade cervical lesions as candidate biomarkers for early detection of cervical disease.

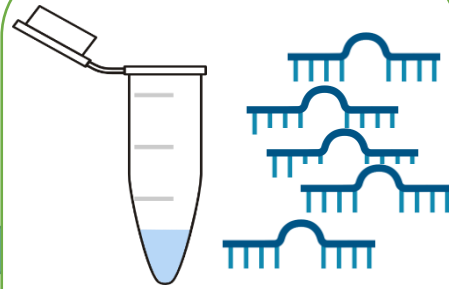


≤ CIN 1
n = 45

CIN 3+
n = 45

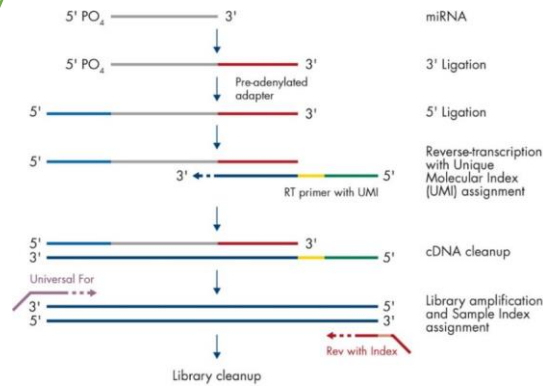
Cervical scrapes from hrHPV+ women, 20-62 years of age undergoing Colposcopy
ASCUS-COL-Trial
PMID:33006400

Samples included

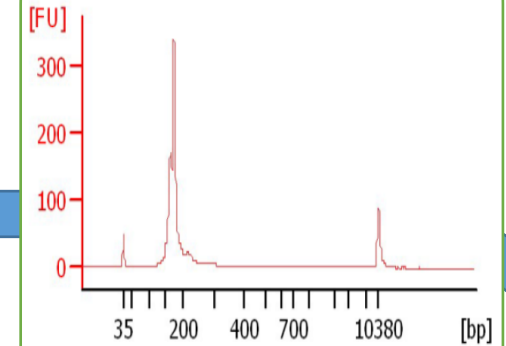


Total RNA
miRNAs included

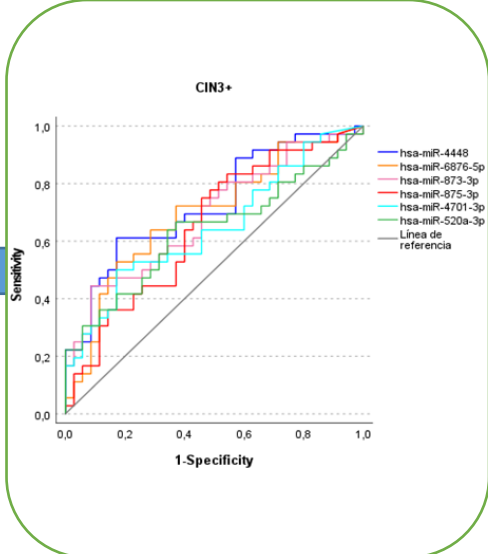
RNA isolation



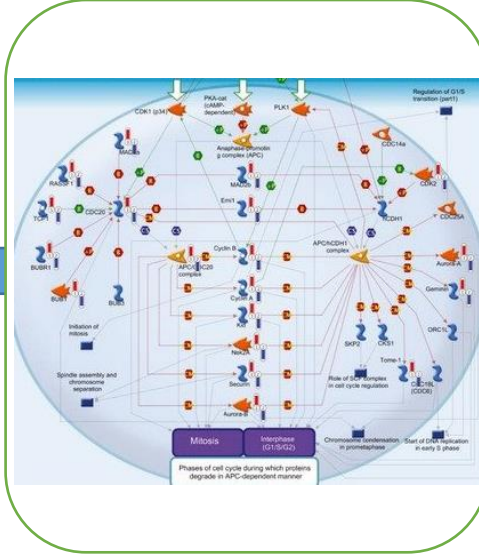
QIAseq® miRNA Library Kit



Quality Control
-> 180pb



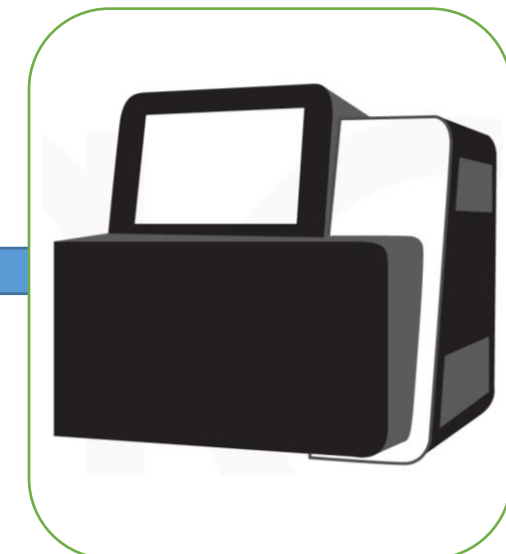
AUCs from miRNAs and predictive miRNAs combination to discriminate CIN3+



Metacore enrichment analysis from miRNAs

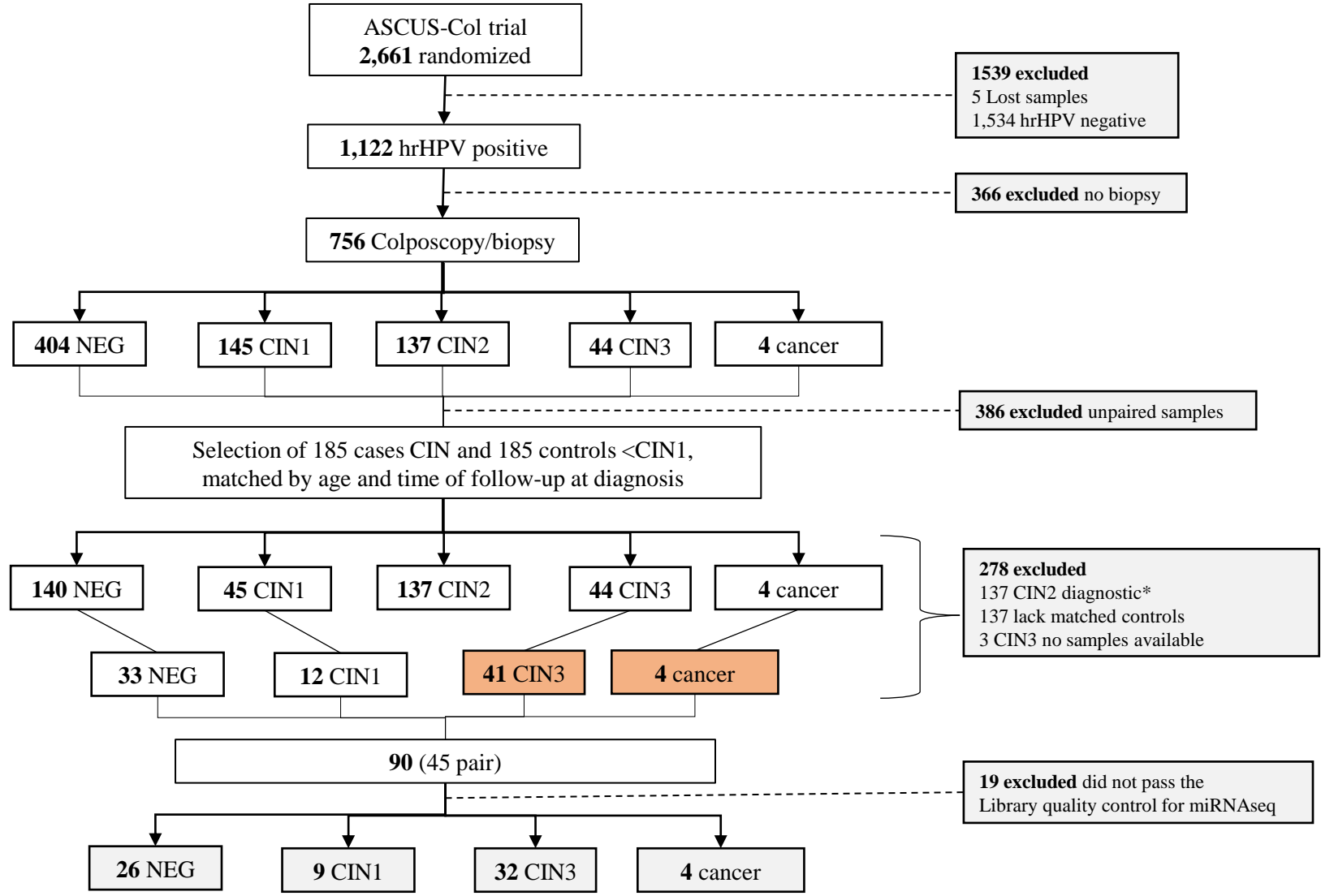


Depuration, Alignment, and differential miRNA expression



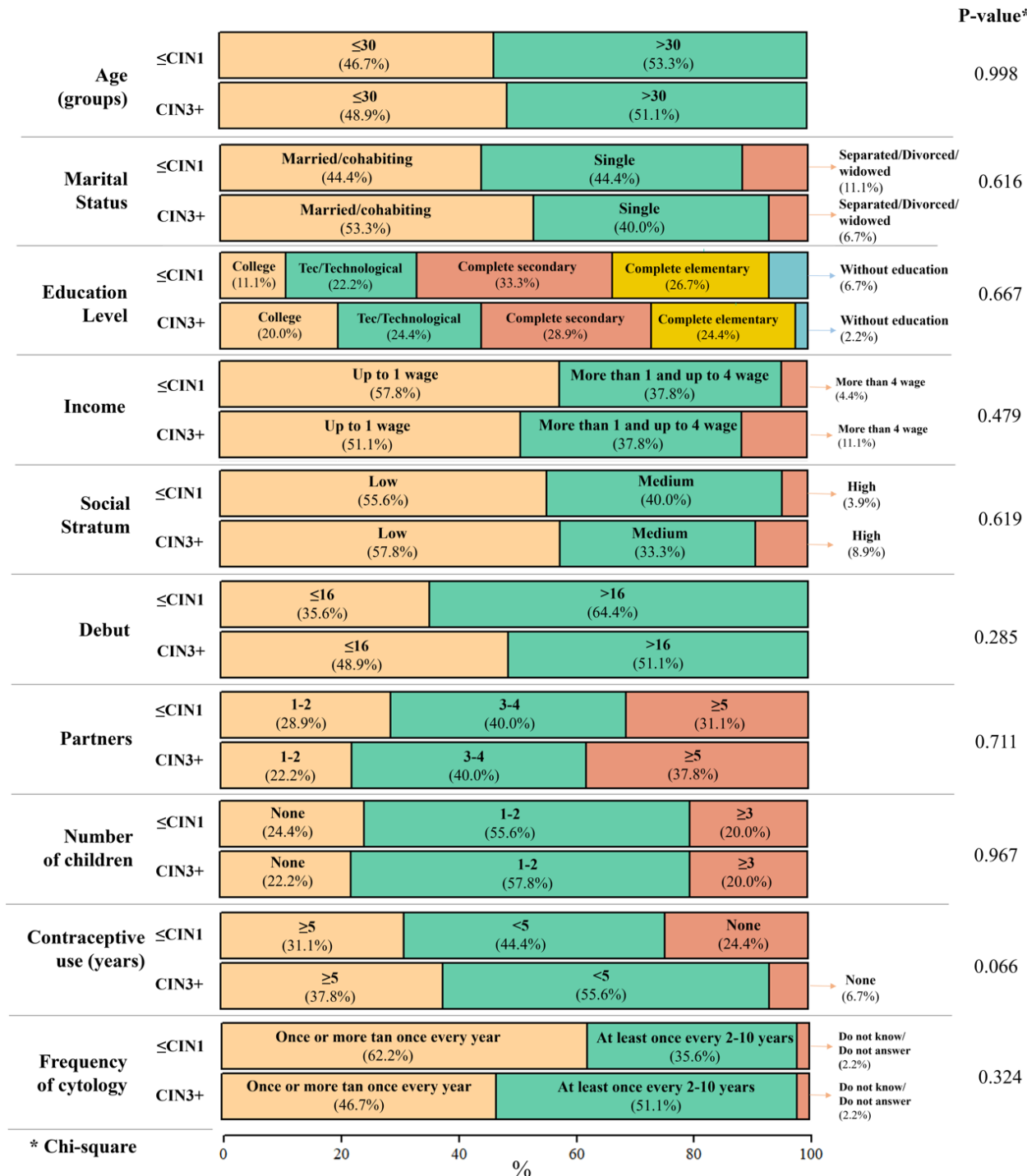
NextSeq 500/550 High Output Kit v2.5 (75 Cycles)

Flowchart of the selection of samples



Baena A,..Sanchez GI; ASCUS-COL Trial Group. Comparison of immediate colposcopy, repeat conventional cytology and hrHPV testing for the clinical management of ASC-US cytology in routine health services of Medellin, Colombia: The ASCUS-COL Trial. Int J Cancer. 2020 Oct 2

Description of the sociodemographic variables of the population



Average and distribution of reads in the sequenced samples

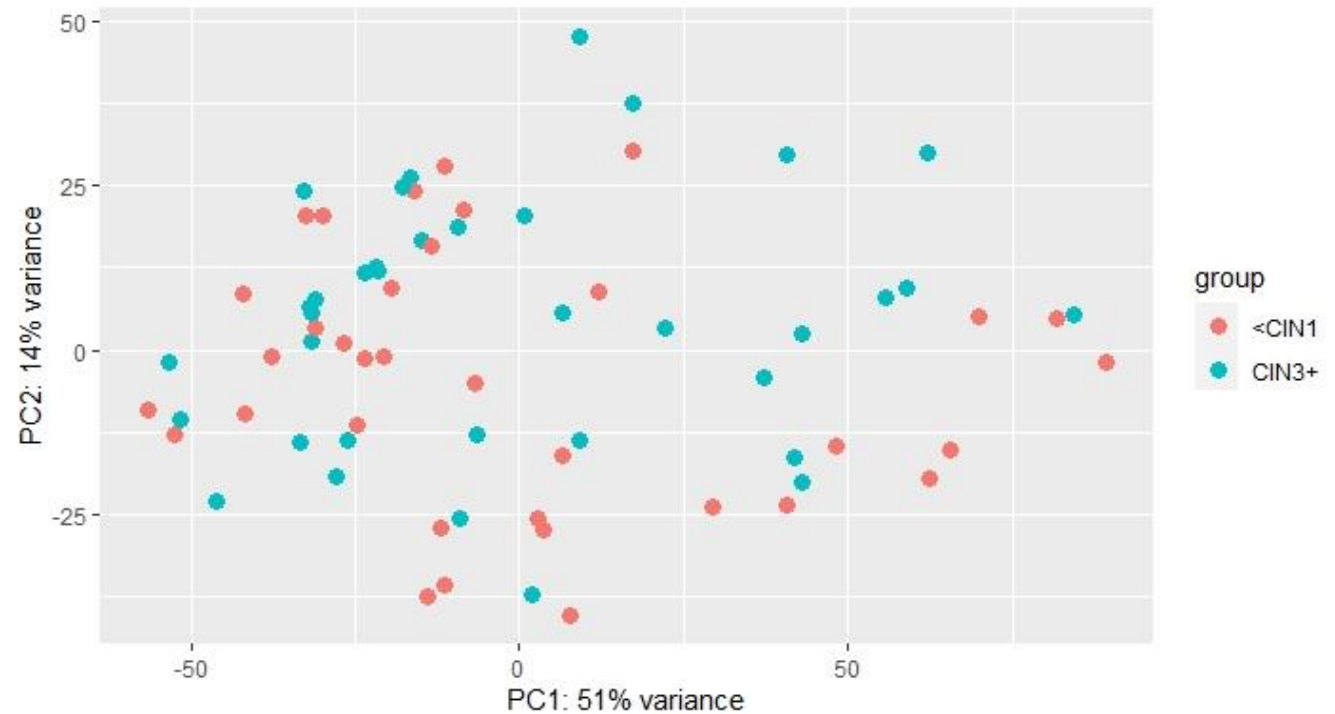
GeneGlobe-Qiagen:

-Cut-adapt

-Bowtie --> miRbaseV21 \approx 2.500 miRNAs

read set	Average per sample
no_adapter_reads	430.177
too_short_reads	2.032.263
UMI_defective_reads	216.515
miRNA_Reads	3.583.769
hairpin_Reads	3.386
piRNA_Reads	65.604
rRNA_Reads	345.495
tRNA_Reads	847.256
mRNA_Reads	64.729
otherRNA_Reads	96.689
notCharacterized_Mappable	464.056
notCharacterized_notMappable	967.217
total_reads	9.117.157

Average of Reads on samples



The principal component analysis showed homogeneous distribution of all samples.

Raw counts reads table

miRNA	94434- MU14851- CIN3- Case_S22- UMIs	91117- MU23459- CIN3- Case_S24- UMIs	80956- MU07413- NEG- Control1_ S14-UM	92717- MU09199- NEG- Control1_ S2-UM	78794- MU13067- NEG- Control1_ S8-UM	92807- MU08116- CIN1- Control1_ S12-UM
hsa-let-7a-2-3p	36	4	22	18	23	5
hsa-let-7a-3p	1450	706	1383	1110	678	669
hsa-let-7a-5p	33093	230636	374033	433174	88580	158780
hsa-let-7b-3p	557	444	619	677	400	393
hsa-let-7b-5p	191800	255191	220309	294439	312331	98752
hsa-let-7c-3p	52	29	58	110	63	24
hsa-let-7c-5p	11647	12616	28126	58473	25574	7370
hsa-let-7d-3p	305	253	812	660	223	606
hsa-let-7d-5p	2709	15528	16030	14913	7519	8294
hsa-let-7e-3p	37	21	93	91	43	38
hsa-let-7e-5p	1056	7030	16270	23049	1577	5225
hsa-let-7f-1-3p	175	70	81	101	75	78
hsa-let-7f-2-3p	170	64	224	85	96	68
hsa-let-7f-5p	34917	118966	206646	234641	87343	116184
hsa-let-7g-3p	65	97	367	122	127	293
hsa-let-7g-5p	32480	87243	72890	61851	53138	38429
hsa-let-7i-3p	359	362	740	282	178	502
hsa-let-7i-5p	259563	166439	79978	127260	203811	61761
hsa-miR-100-3p	292	2	33	20	24	6

Columns: 71 samples
Rows: 2558 miRNAs

Targets table

Sample	Group
94434-MU14851-CIN3-Case_S22-UMIs	CIN3+
91117-MU23459-CIN3-Case_S24-UMIs	CIN3+
80956-MU07413-NEG-Control1_S14-UMIs	≤CIN1
92717-MU09199-NEG-Control1_S2-UMIs	≤CIN1
78794-MU13067-NEG-Control1_S8-UMIs	≤CIN1
92807-MU08116-CIN1-Control1_S12-UMIs	≤CIN1
98162-MU00409-CIN1-Control1_S4-UMIs	≤CIN1
9351-MU26144-CIN3-Case_S10-UMIs	CIN3+
73283-MU09529-CIN3-Case_S36-UMIs	CIN3+
58363-MU14074-CIN3-Case_S6-UMIs	CIN3+
60766-MU07422-CIN3-Case_S16-UMIs	CIN3+
73002-MU22051-CIN3-Case_S27-UMIs	CIN3+
60166-MU00546-CIN3-Case_S5-UMIs	CIN3+
72217-MU22855-ADC-Case_S30-UMIs	CIN3+
78673-MU21337-CIN3-Case_S32-UMIs	CIN3+
76908-MU23321-CIN3-Case_S33-UMIs	CIN3+

Columns: 2
Rows: 71 samples

Filter-Normalized counts read table

	94434- MU14851- CIN3- Case_S22- UMIs	91117- MU23459- CIN3- Case_S24- UMIs	80956- MU07413- NEG- Control1_S1 4-UMIs	92717- MU09199- NEG- Control1_S2- UMIs	78794- MU13067- NEG- Control1_S8- UMIs	92807- MU08116- CIN1- Control1_S1 2-UMIs
hsa-let-7a-2-3p	3.94498971	1.51102047	3.53116655	3.81186079	3.59409396	0.80522095
hsa-let-7a-3p	10.4150784	10.9935345	10.8214918	10.9776325	9.69202115	9.89702384
hsa-let-7a-5p	15.086535	19.1271348	18.7680907	19.3216601	16.8885457	17.8557066
hsa-let-7b-3p	8.91813609	10.2822012	9.57671329	10.2185597	8.86045897	9.06203595
hsa-let-7b-5p	17.5287276	19.2451224	18.1121703	18.8679869	18.5270742	17.2307252
hsa-let-7c-3p	4.74420799	5.76280148	5.58784945	7.30245975	5.71219107	4.0216031
hsa-let-7c-5p	13.5511899	15.2788944	15.3025814	16.7853292	15.140208	13.5242741
hsa-let-7d-3p	7.94355585	9.40503494	10.0004087	10.1791428	7.91332902	9.7426936
hsa-let-7d-5p	11.3683896	15.5769981	14.4845918	14.8503939	13.3389186	13.6996267
hsa-let-7e-3p	4.00668564	5.13549419	6.45694979	6.98090608	4.96110635	5.00414718
hsa-let-7e-5p	9.92342892	14.4273302	14.5064034	15.4797033	10.9950667	13.0123161
hsa-let-7f-1-3p	7.01408361	7.32089003	6.20918287	7.15844438	6.03711698	6.37038582
hsa-let-7f-2-3p	6.96447331	7.16981197	7.94937431	6.86366407	6.48246983	6.12262255
hsa-let-7f-5p	15.1641736	18.3253104	18.030627	18.5930212	16.8693907	17.4471591
hsa-let-7g-3p	5.20034283	7.86408997	8.75089309	7.47593909	6.969981	8.59417243
hsa-let-7g-5p	15.059443	17.931933	16.6454527	16.8620186	16.1823353	15.9298354
hsa-let-7i-3p	8.21133504	9.96529072	9.85578427	8.84477765	7.53944137	9.44782087
hsa-let-7i-5p	17.9226998	18.7385591	16.7728088	17.8217328	17.9907483	16.5926059
hsa-miR-100-3p	7.87162883	0.48521942	4.44154561	4.05003428	3.69249599	1.07577057

Columns: 71 samples
Rows: 2420 miRNAs

Differential expression analysis between CIN3+ and ≤CIN1

DESeq2

```
library(DESeq2)

#Cargar tablas
countdata1 <- read.table("2558_rowcounts_GeneGlobe.txt")
countdata1 <- as.matrix(countdata1)
colnames(countdata1) <- NULL
storage.mode(countdata1) = "integer"
calldata <- read.table("Targets.txt")

# Se crea la matriz de datos
dds <- DESeqDataSetFromMatrix(countData = countdata1, colData = calldata,
                              design = ~ Group)

#Filtra los miRNAs que tengan conteos de lecturas mayores o iguales a 5,
#en al menos el 50% de las muestras para cada miRNA ( 36 muestras de 71)
idx <- rowSums(counts(dds) >=5) >= 36
dds <- dds[idx,]

#Estabilizacion varianzas y obtención de matriz de conteos normalizada
vsd <- varianceStabilizingTransformation(dds)
expr <- assay(vsd)
write.table(expr, file="2420_norm+filter_counts_Rstudio.txt")

#Análisis de Expresion Diferencial - Comparaciones multiples
resultsNames(dds)
res <- results(dds,alpha=0.05)

#los miRNAs con padj <0.05
res <- res[(res$padj< 0.05),]

#45 miRNAs|
```

Results table

	baseMean	log2FoldCl	lfcSE	stat	pvalue	padj
hsa-miR-424-5p	2632.24755	-1.9137997	0.39401025	-4.8572333	1.19E-06	0.00248907
hsa-miR-214-5p	112.74414	-1.4163251	0.33313823	-4.2514636	2.12E-05	0.00888165
hsa-miR-424-3p	108.964566	-1.3143787	0.30629714	-4.2911884	1.78E-05	0.00888165
hsa-miR-616-3p	99.6519347	-1.3990206	0.32468445	-4.3088622	1.64E-05	0.00888165
hsa-miR-6774-5p	17.1938571	2.23122709	0.5203156	4.28821868	1.80E-05	0.00888165
hsa-miR-26a-1-3p	217.088199	-1.4667926	0.35649013	-4.1145391	3.88E-05	0.01270016
hsa-miR-4448	19.6204248	0.76472002	0.18681948	4.0933633	4.25E-05	0.01270016
hsa-miR-5708	154.190791	-1.8933882	0.46964442	-4.0315357	5.54E-05	0.01448373
hsa-miR-557	77.2147658	-1.4687393	0.37453731	-3.9214766	8.80E-05	0.02044719
hsa-miR-4701-3p	18.7116626	1.21260105	0.31942143	3.79624201	0.00014691	0.02465003
hsa-miR-5009-3p	1902.5495	-2.1792876	0.57303199	-3.803082	0.00014291	0.02465003
hsa-miR-5585-3p	56.1231295	-1.2174313	0.3215835	-3.7857394	0.00015325	0.02465003
hsa-miR-875-3p	23.0868821	1.09240305	0.28634922	3.81493284	0.00013622	0.02465003
hsa-miR-6876-5p	19.9939756	1.05370798	0.28156818	3.74228355	0.00018236	0.02723611
hsa-miR-4690-3p	31.7451229	-1.0810115	0.29079499	-3.717435	0.00020126	0.02805505
hsa-miR-493-5p	58.3778251	-0.9915325	0.27115803	-3.6566591	0.00025552	0.02812107
hsa-miR-4999-3p	111.957552	-1.5796613	0.43117895	-3.6635864	0.00024871	0.02812107
hsa-miR-520d-3p	36.0166836	1.19771266	0.3237714	3.69925405	0.00021623	0.02812107
hsa-miR-889-5p	227.910525	-1.6921908	0.46227053	-3.6606071	0.00025162	0.02812107

Columns: 6
Rows: 2420 miRNAs

38 miRNAs differential expression between CIN3+ and \leq CIN1

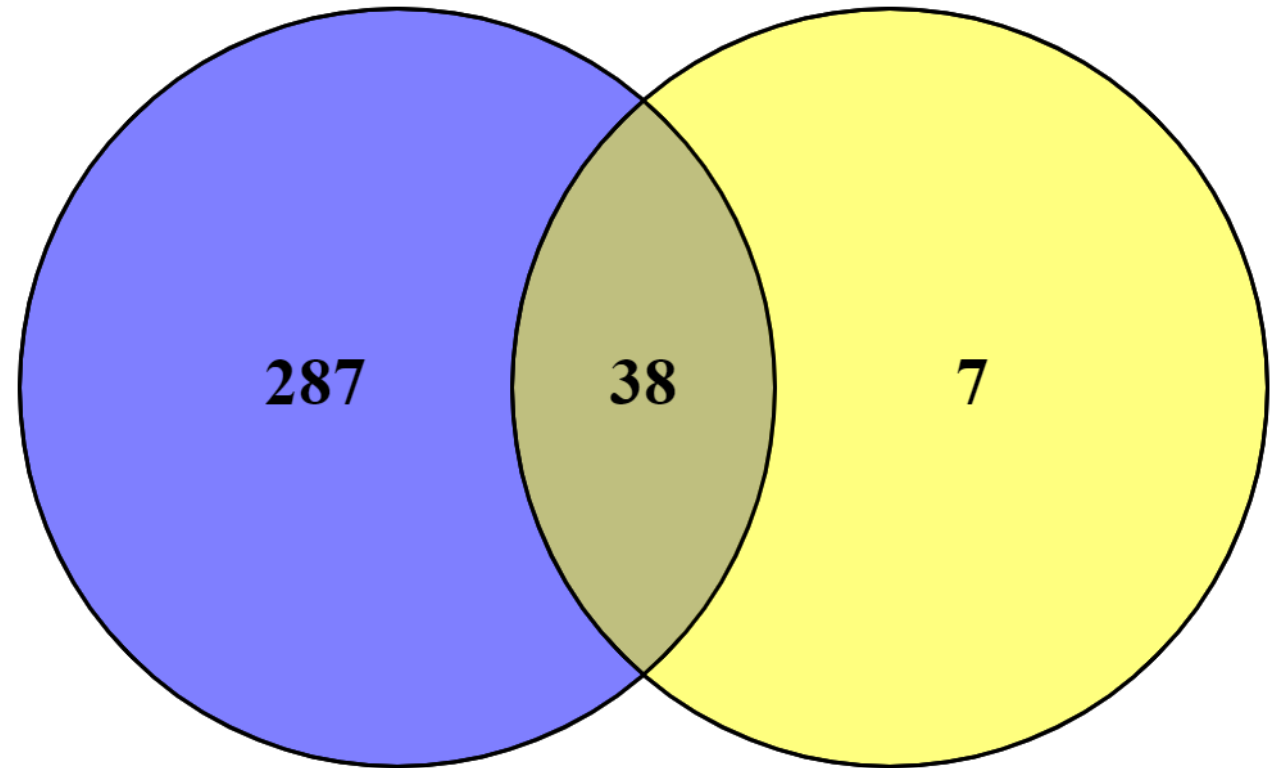
GeneGlobe 325

RStudio 45

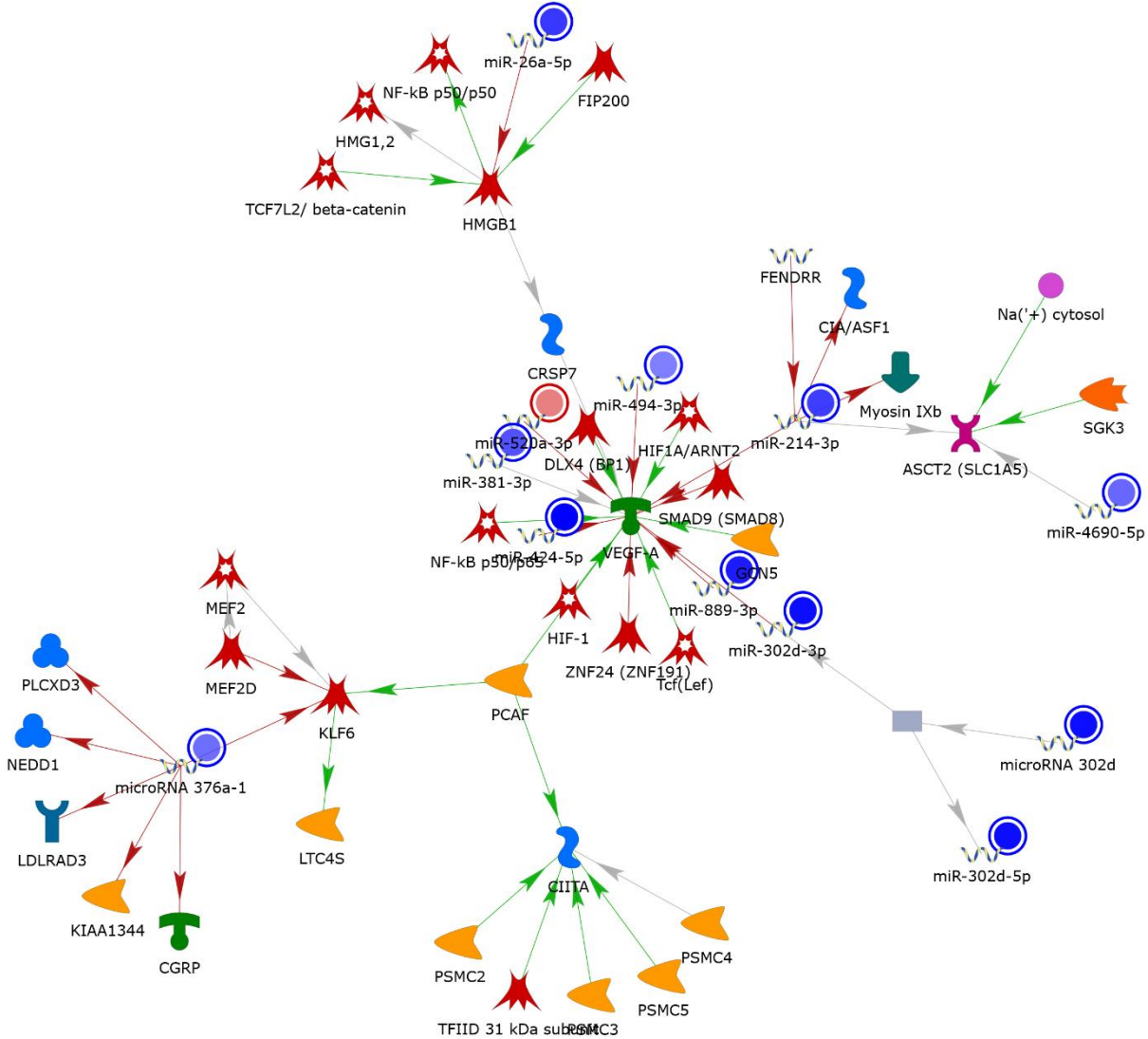
DESeq2

1. GeneGlobe Qiagen
(No padj FDR)

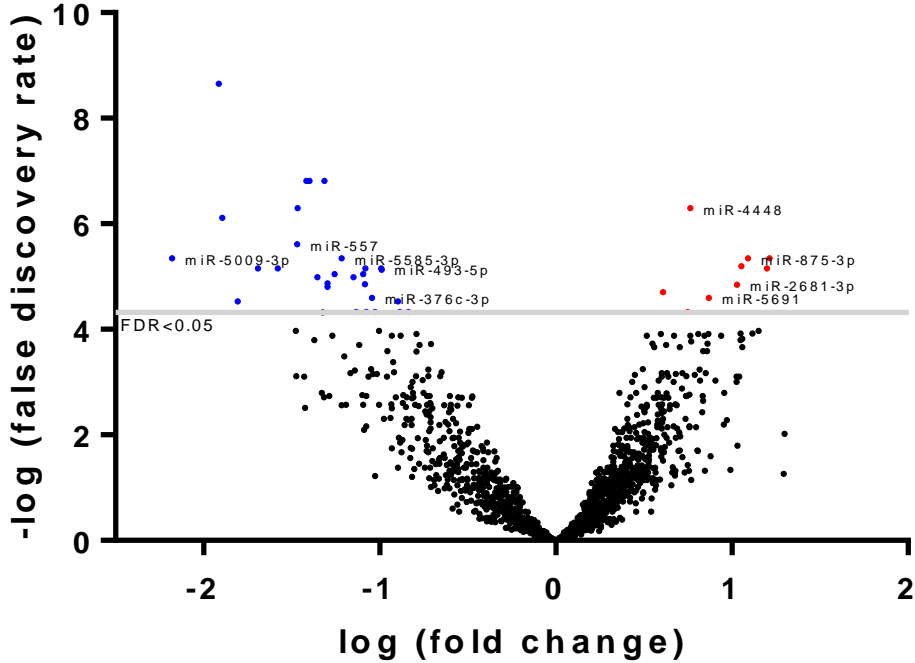
2. R.Studio
(Si padj FDR)



VEGF is target of five miRNAs differentially overexpressed in CIN3+ vs CIN2.



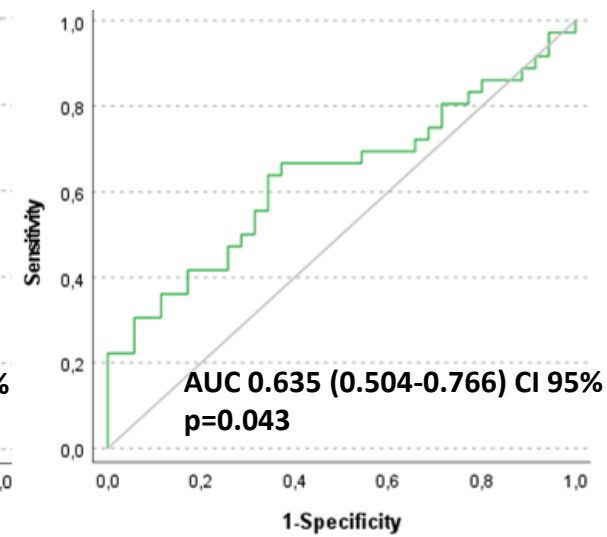
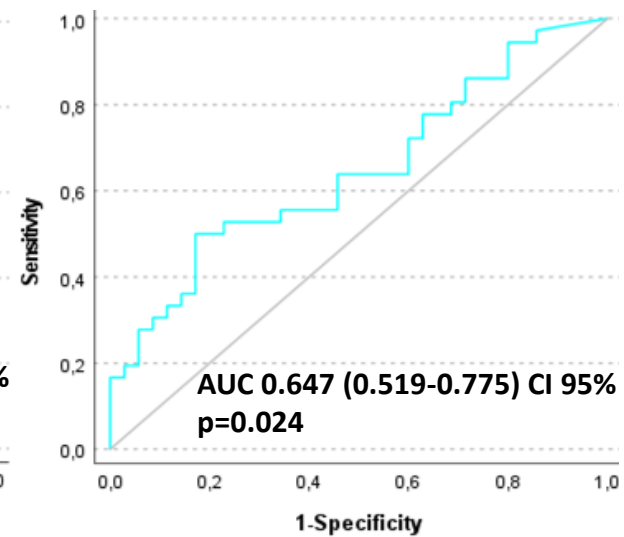
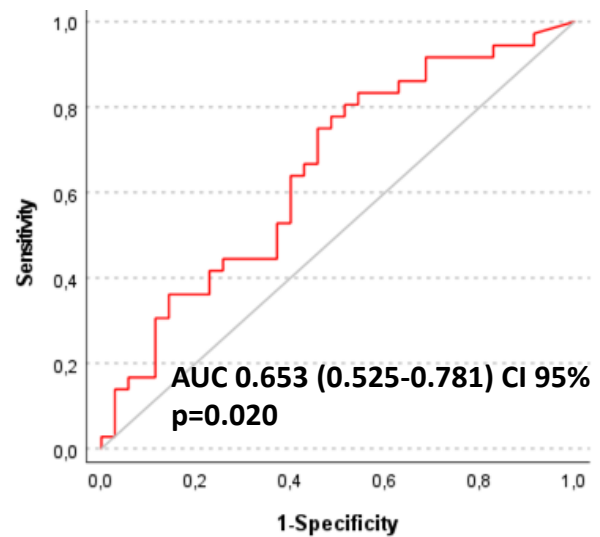
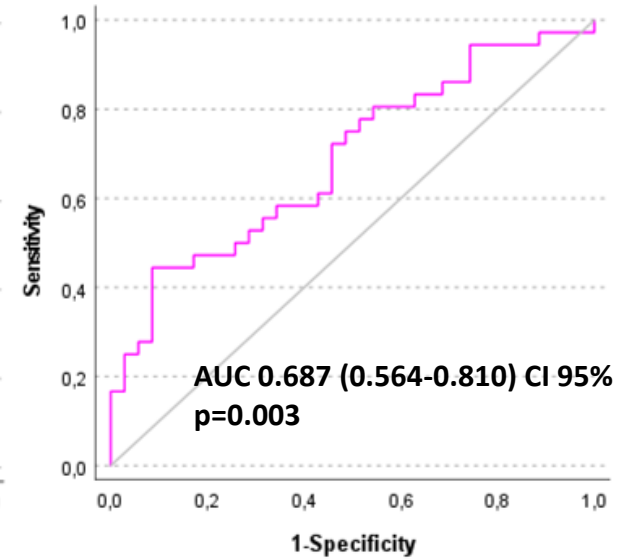
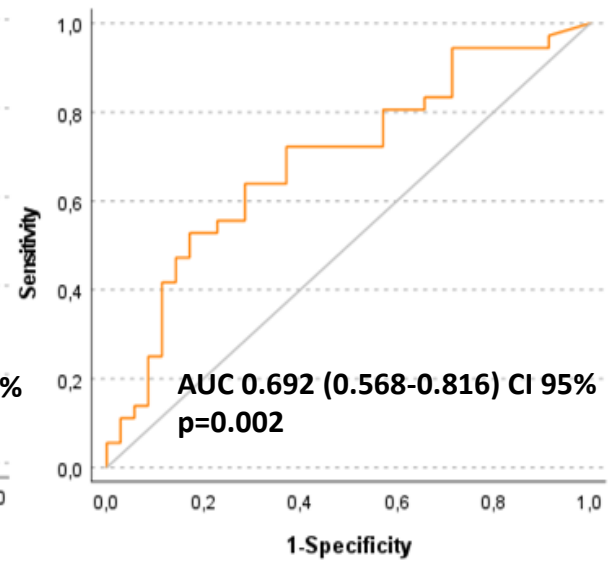
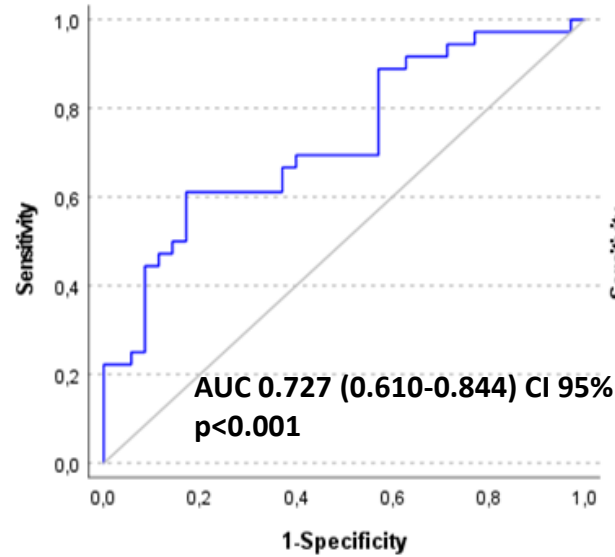
38 miRNAs differential expression between CIN3+ and ≤CIN1



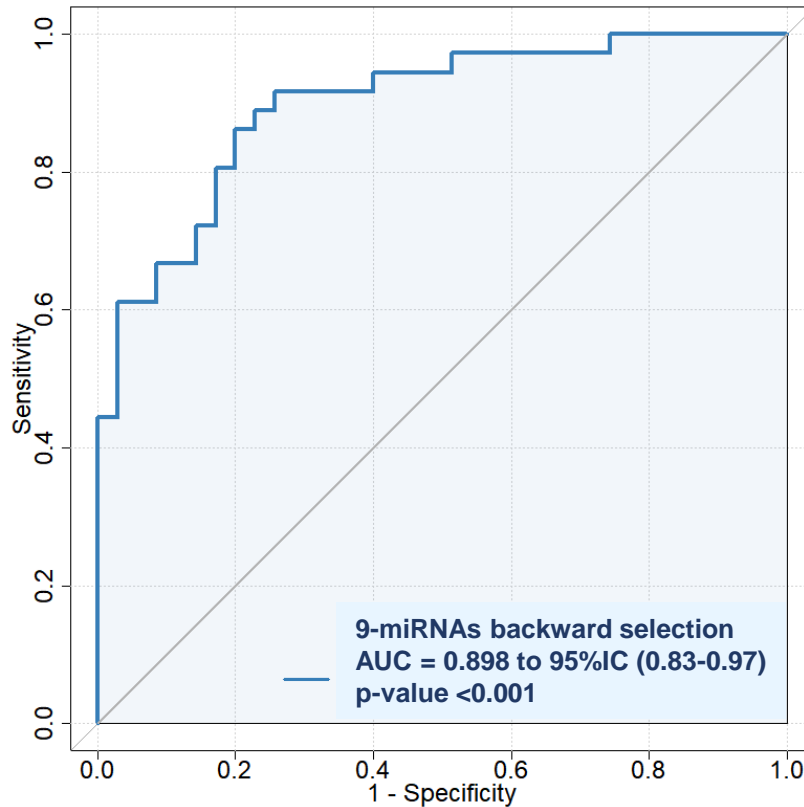
Volcano-plot, 38 miRNAs with $FDR \leq 0.05$

miRNAs	FC	padj
hsa-miR-	2,318	0,025
hsa-miR-	2,294	0,028
hsa-miR-	2,132	0,025
hsa-miR-	2,076	0,027
hsa-miR-	2,039	0,035
hsa-miR-	1,827	0,041
hsa-miR-	1,699	0,013
hsa-miR-	1,679	0,050
hsa-miR-	1,524	0,038
hsa-miR-	0,560	0,050
hsa-miR-	0,541	0,050
hsa-miR-	0,537	0,043
hsa-miR-	0,504	0,029
hsa-miR-	0,503	0,028
hsa-miR-	0,491	0,050
hsa-miR-	0,485	0,041
hsa-miR-	0,474	0,050
hsa-miR-	0,473	0,028
hsa-miR-	0,472	0,035
hsa-miR-	0,469	0,030
hsa-miR-	0,456	0,050
hsa-miR-	0,451	0,032
hsa-miR-	0,430	0,025
hsa-miR-	0,419	0,030
hsa-miR-	0,407	0,036
hsa-miR-	0,407	0,034
hsa-miR-	0,402	0,009
hsa-miR-	0,392	0,032
hsa-miR-	0,379	0,009
hsa-miR-	0,375	0,009
hsa-miR-	0,362	0,013
hsa-miR-	0,361	0,020
hsa-miR-	0,335	0,028
hsa-miR-	0,309	0,028
hsa-miR-	0,286	0,043
hsa-miR-	0,269	0,014
hsa-miR-	0,265	0,002
hsa-miR-	0,221	0,025

**Six miRNAs
show higher
AUCs 0.6 with
p-value <0.05
vs AUC 0.5**



Proposed multivariate model of 9 miRNAs to CIN3+ detection, AUC 0.898 to 95%IC (0.83-0.97), p-value <0.001 vs AUC 0.5



miRNAs	Beta	Pr	OR (IC 95%)
miR	1.64	<0.001	4.2 (1.9 - 11.4)
miR	0.70	0.046*	2.0 (1.1-4.3)
miR	0.95	0.010*	2.2 (1.1-4.7)
miR	0.61	0.032*	1.8 (1.1-3.2)
miR	0.99	0.002*	2.6 (1.6-5.2)
miR	-1.24	0.012*	0.4 (0.2-0.8)
miR	1.09	0.024*	2.6 (1.2-7.2)
miR	-1.51	0.004*	0.3 (0.1-0.7)
miR	-1.20	0.017*	0.3 (0.1-0.8)

Conclusions

We identified 38 miRNAs differentially expressed between CIN3+ and \leq CIN1 with good diagnostic performance to identify CIN3+ in hrHPV positive women, five of them with action over *VEGF*.

Six of these miRNAs have good diagnostic performance to differentiate CIN3+ from \leq CIN1 in cervical exfoliated cells samples. In addition, the predictive model shows 9 of the miRNAs have an AUC= 0.89 95% IC (0.83-0.97).

Further validation on a larger cohort of cervical exfoliated cells samples is needed to confirm the potential role of these miRNAs to triage hrHPV+ women.

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ASCUS-COL Trial Group:

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References

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