Supplementary Information

Study design requirements for RNA sequencing-based breast cancer diagnostics

Arvind Singh Mer, Daniel Klevebring, Henrik Gronberg and Mattias Rantalainen,

Department of Medical Epidemiology and Biostatistics,
Karolinska Institute,
Nobels Väg 12A, SE-17177
Stockholm, Sweden

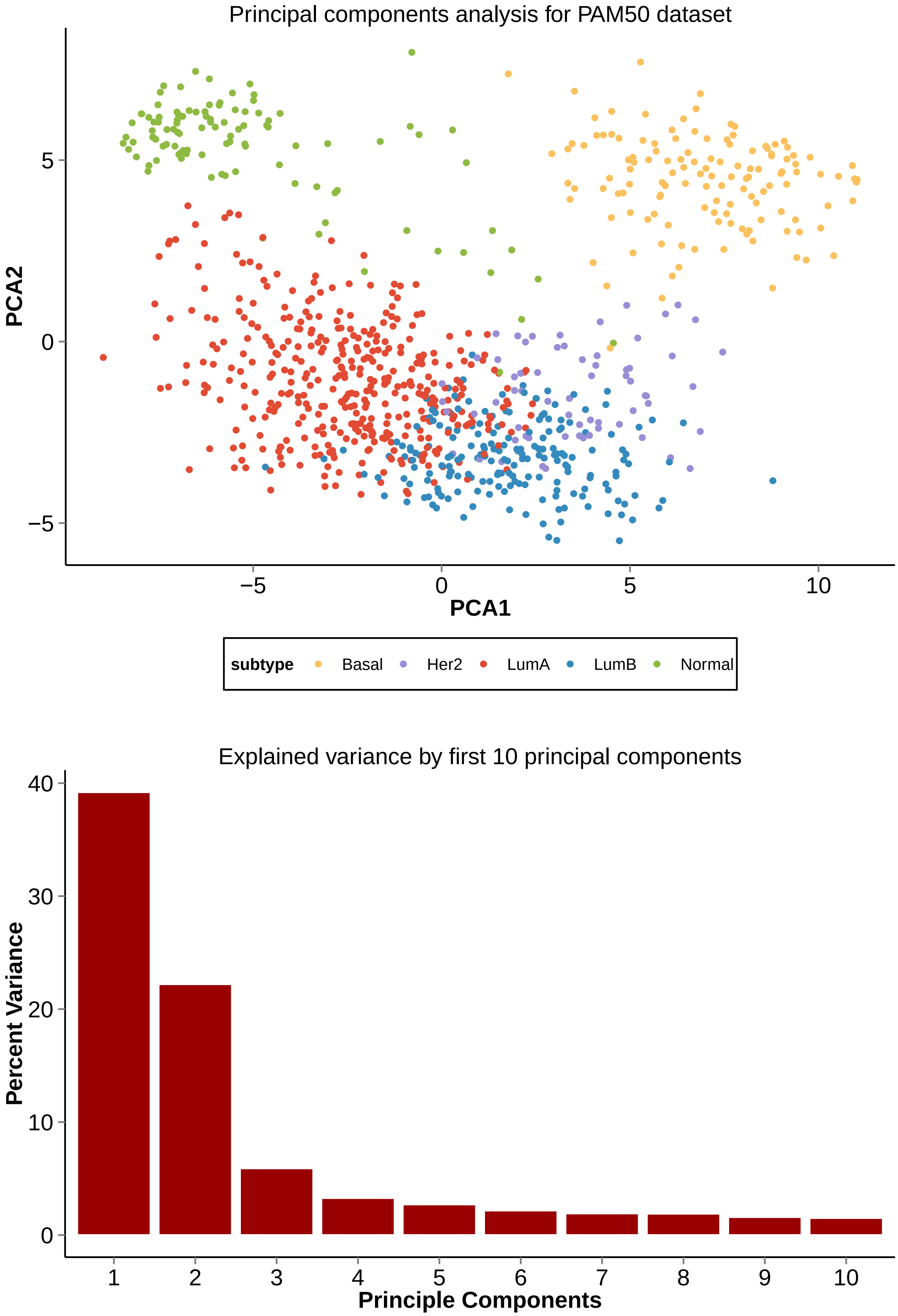
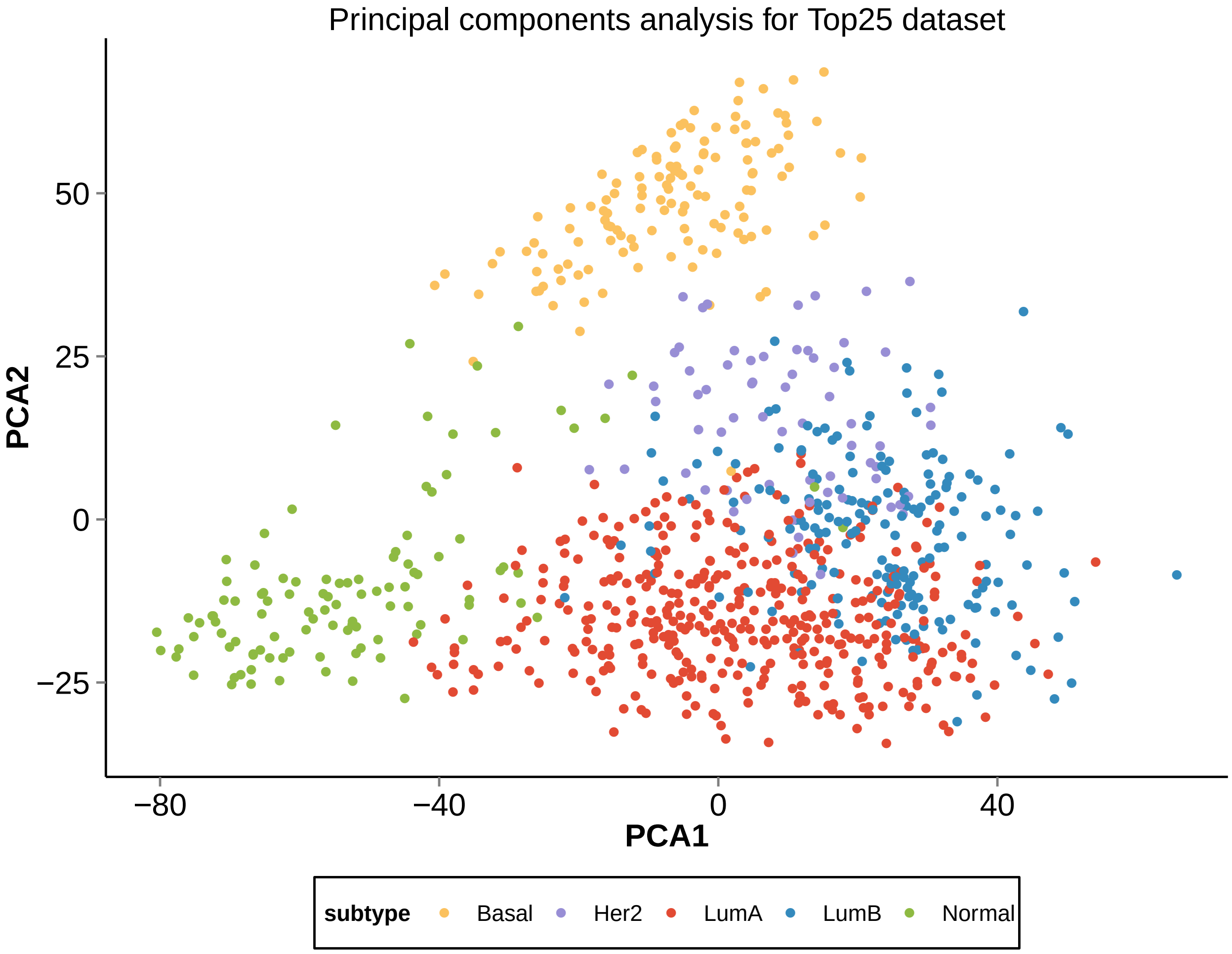


Figure S1: Principal component analysis of PAM50 dataset.



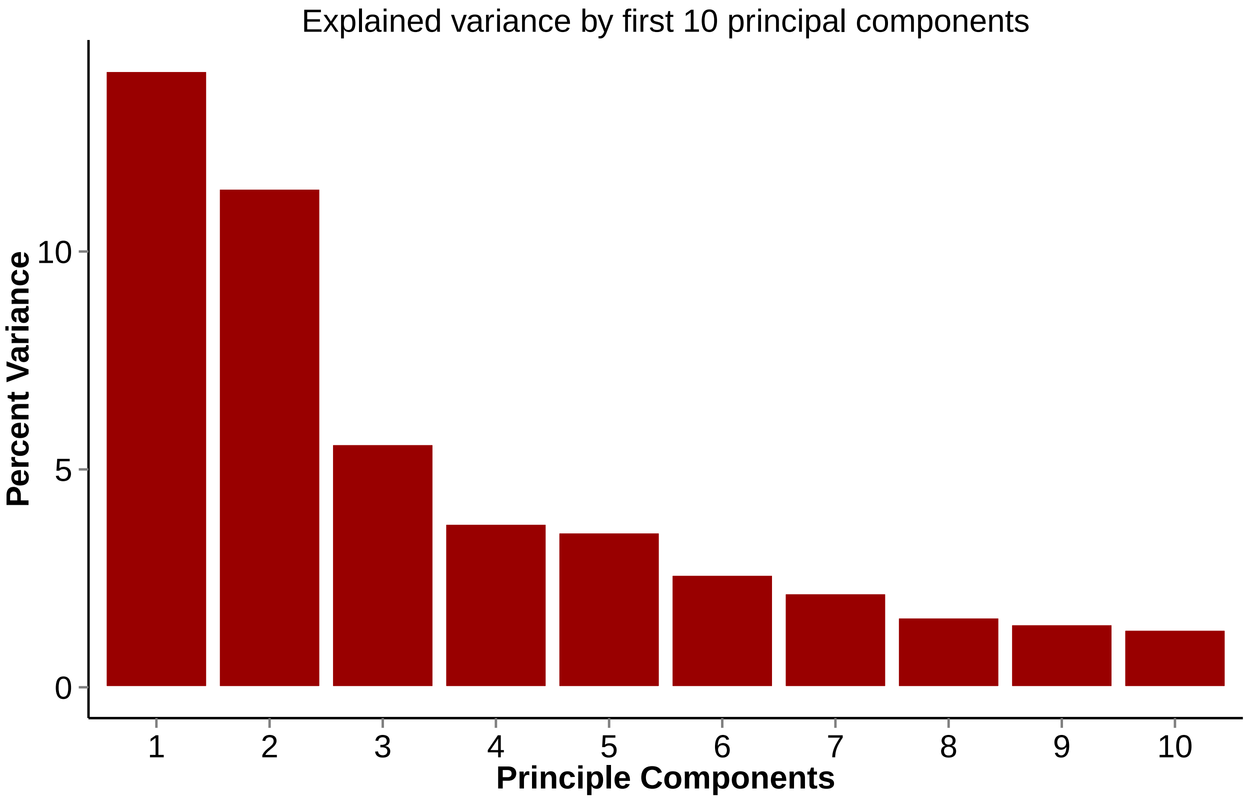


Figure S2: Principal component analysis of Top25 dataset.

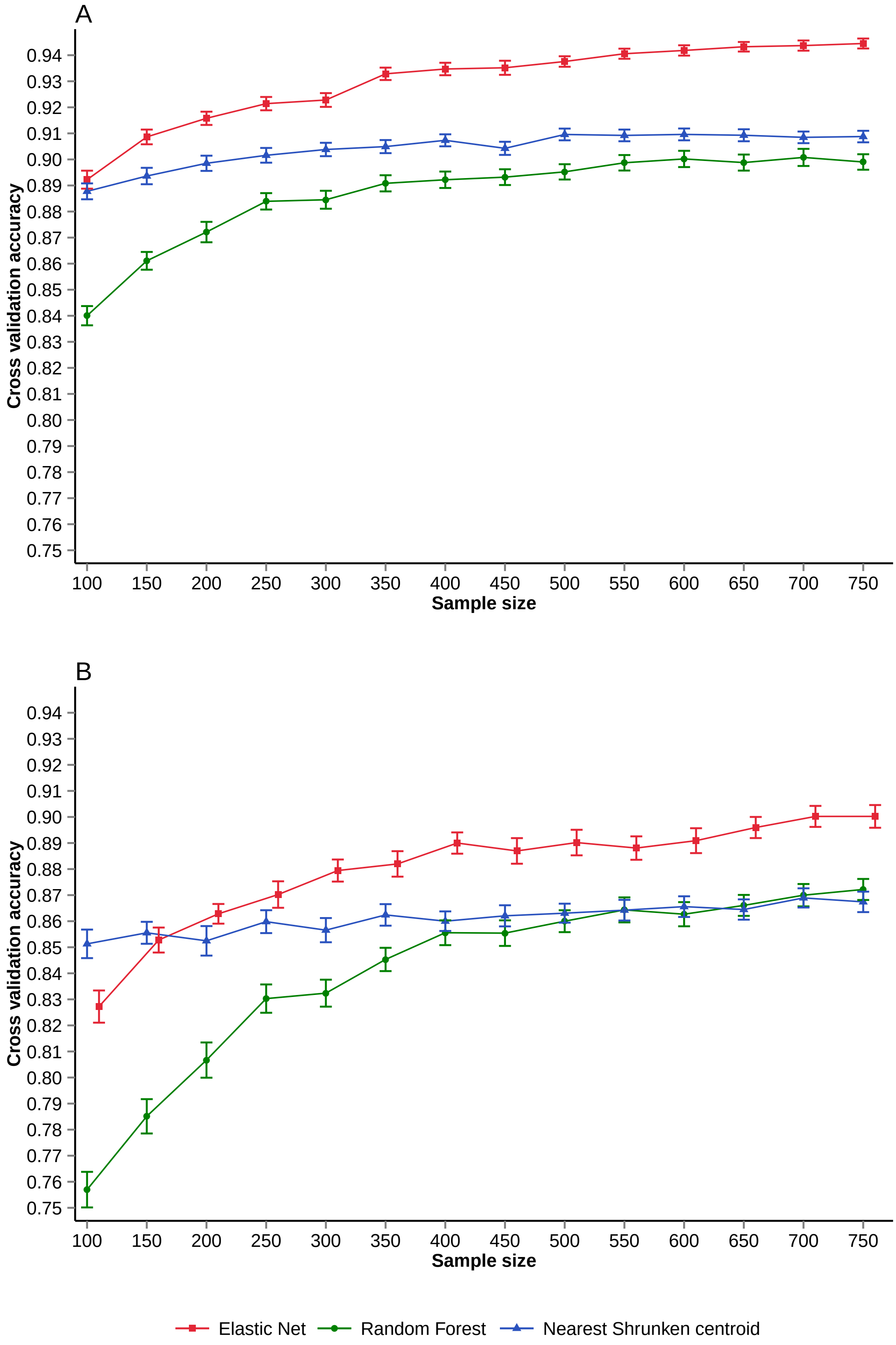


Figure S3: Balance accuracy for (A) PAM50 and (B) Top25 dataset.

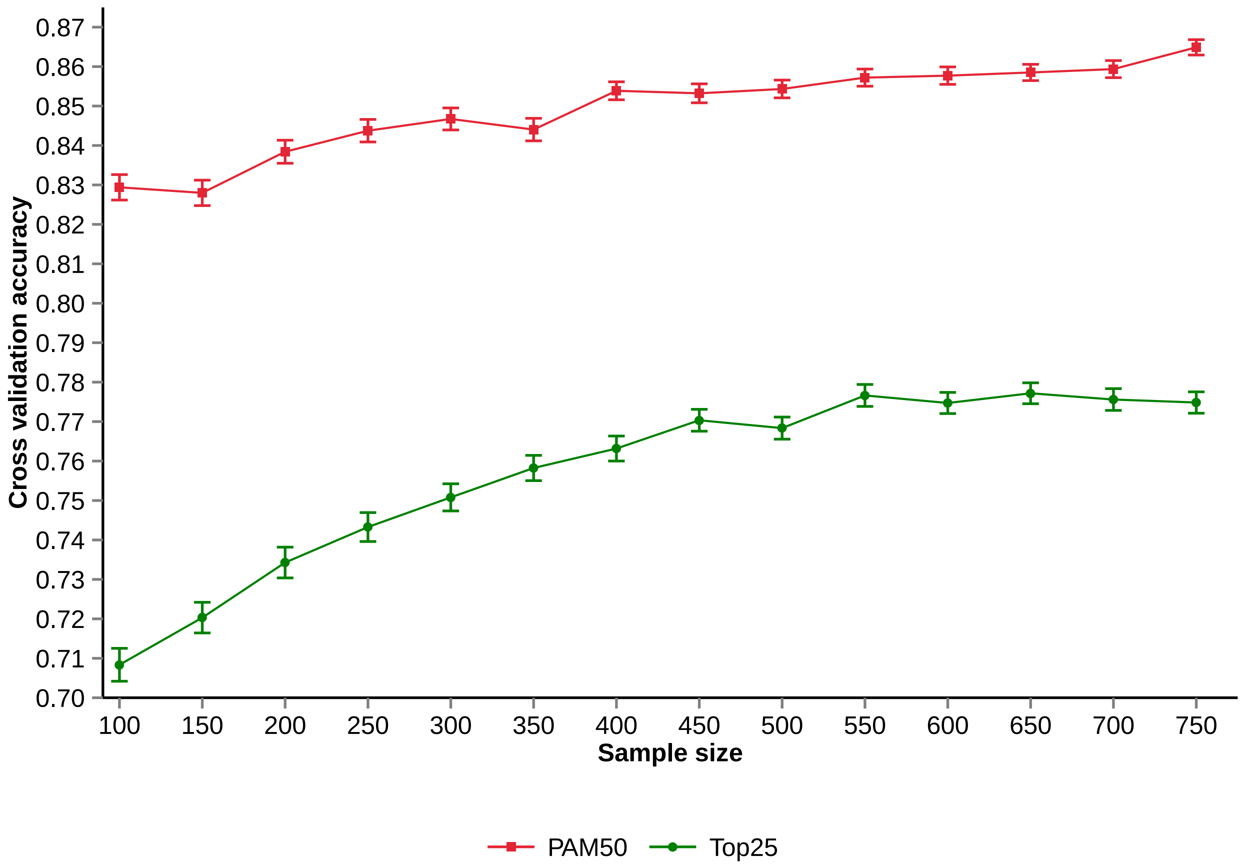
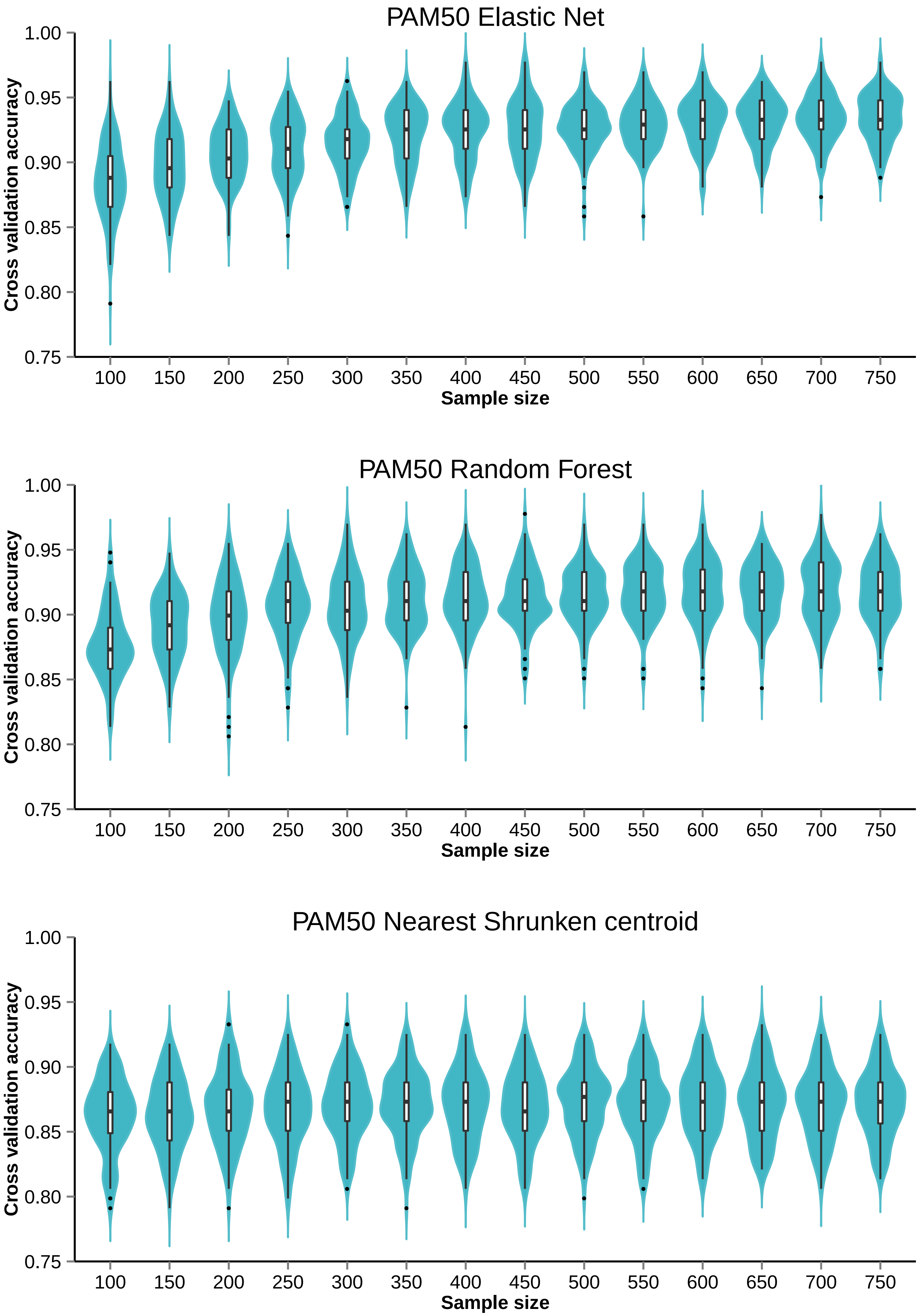


Figure S4: Balance accuracy for unsupervised classification.

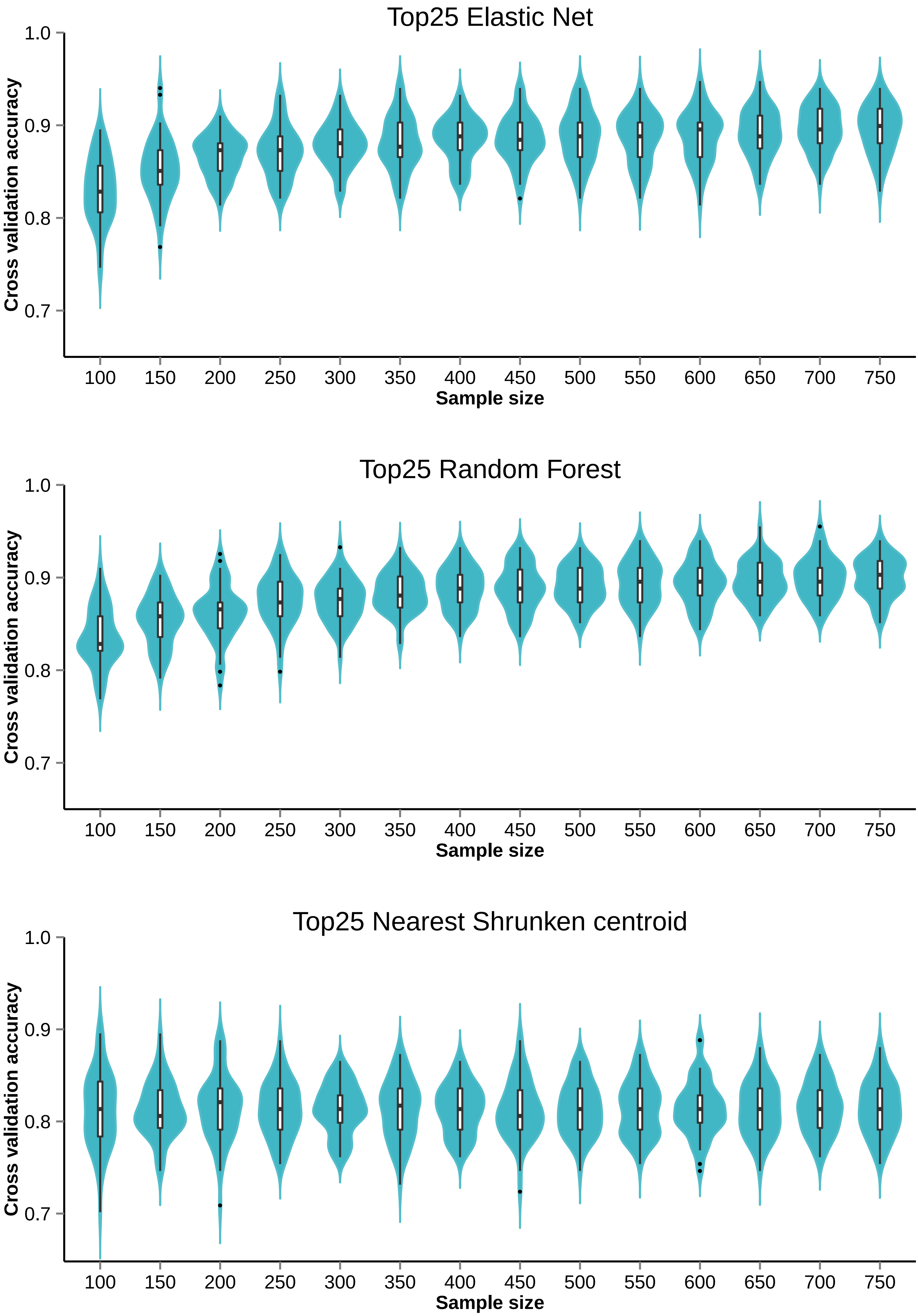


PAM50 Elastic Net

PAM50 Random Forest

PAM50 Nearest Shrunken centroid

Figure S5: Violin plot visualisation of prediction accuracy over cross-validation rounds for PAM50 dataset at different training sample size



Top25 Elastic Net

Top25 Random Forest

Top25 Nearest Shrunken centroid

Figure S6: Violin plot visualisation of prediction accuracy over cross-validation rounds for Top25 dataset at different training sample size

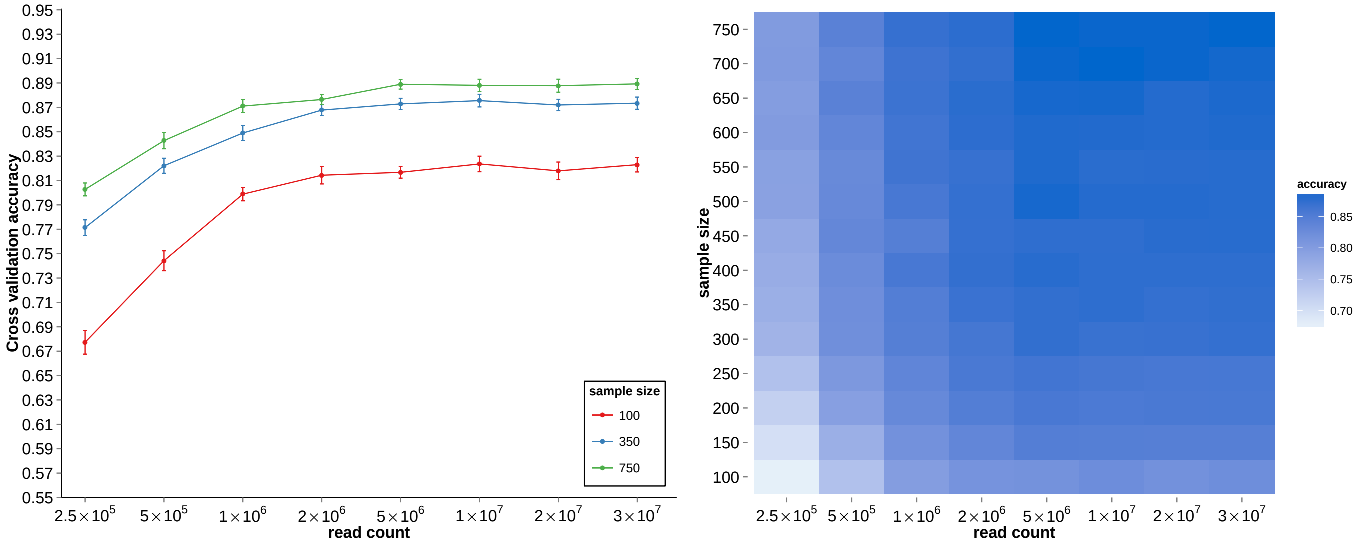


Figure S7: Effect of read count per sample on subtype prediction in Top25 dataset. (A) Classification accuracy at training sample size of 100, 350 and 750 (Error bars represent standard error of the cross-validation mean). (B) Heatmap representation of subtype classification accuracy and read count per sample

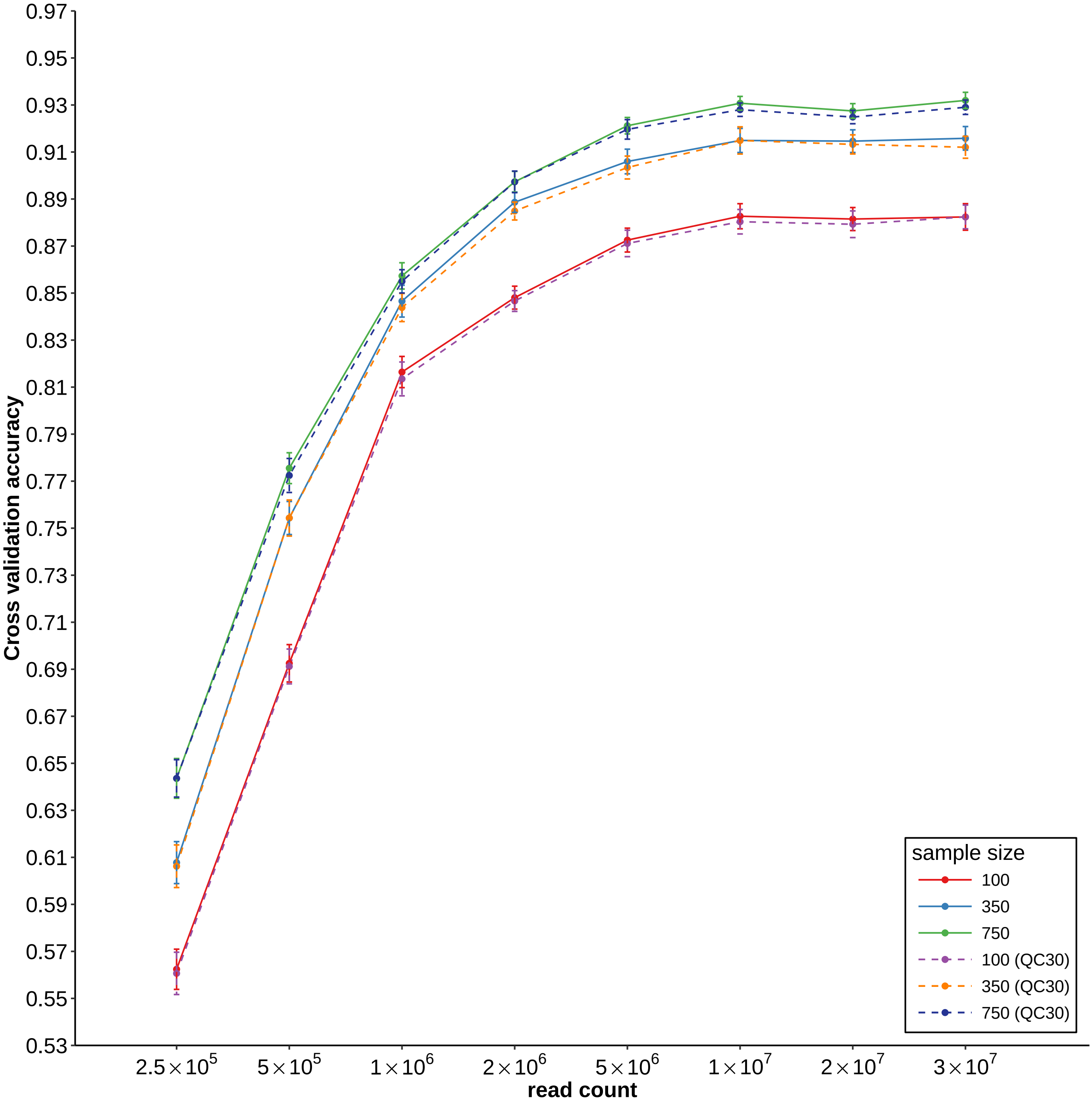
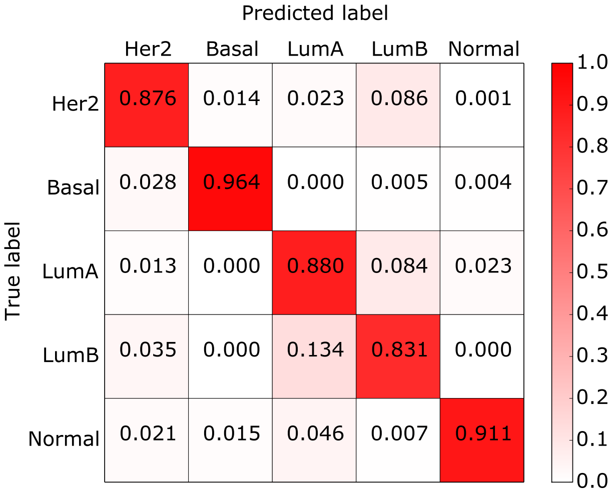


Figure S8: Effect of read count per sample on subtype prediction accuracy in PAM50 dataset, using htseq-count QC threshold 10 (solid lines) and QC threshold 30 (dashed lines). Classification accuracy at training sample size of 100, 350 and 750 (Error bars represent standard error of the cross-validation mean).

Table S1: Confusion matrix for PAM50 dataset and Elastic net classifier

sample size 100

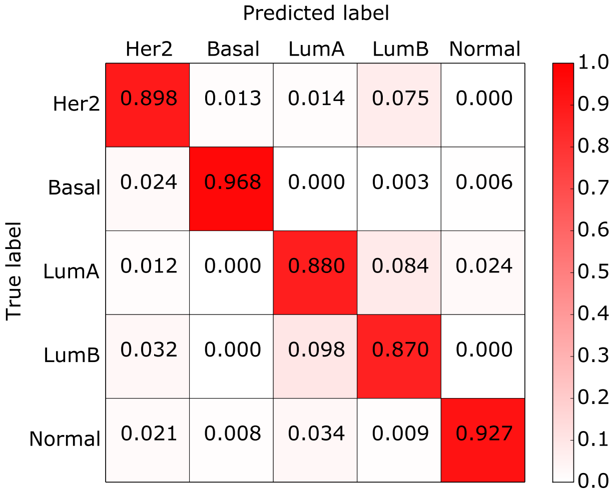


Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.876	0.014	0.023	0.086	0.001
Basal	0.028	0.964	0.000	0.005	0.004
LumA	0.013	0.000	0.880	0.084	0.023
LumB	0.035	0.000	0.134	0.831	0.000
Normal	0.021	0.015	0.046	0.007	0.911

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0

sample size 150

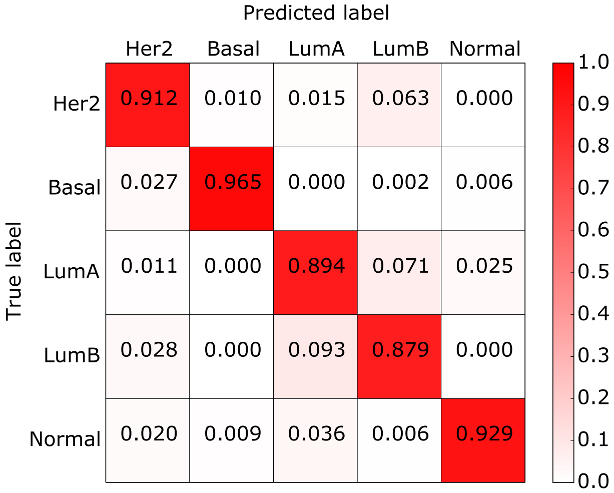


Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.898	0.013	0.014	0.075	0.000
Basal	0.024	0.968	0.000	0.003	0.006
LumA	0.012	0.000	0.880	0.084	0.024
LumB	0.032	0.000	0.098	0.870	0.000
Normal	0.021	0.008	0.034	0.009	0.927

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0

sample size 200



Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.912	0.010	0.015	0.063	0.000
Basal	0.027	0.965	0.000	0.002	0.006
LumA	0.011	0.000	0.894	0.071	0.025
LumB	0.028	0.000	0.093	0.879	0.000
Normal	0.020	0.009	0.036	0.006	0.929

1.0
0.9
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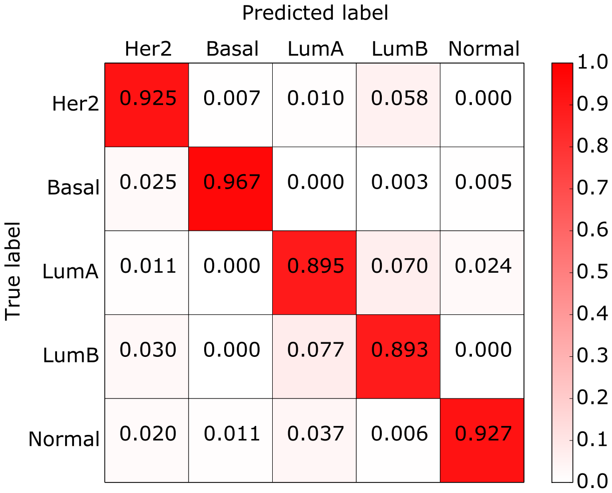
0.2
0.1
0.0

sample size 250



Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.925	0.007	0.010	0.058	0.000
Basal	0.025	0.967	0.000	0.003	0.005
LumA	0.011	0.000	0.895	0.070	0.024
LumB	0.030	0.000	0.077	0.893	0.000
Normal	0.020	0.011	0.037	0.006	0.927

1.0
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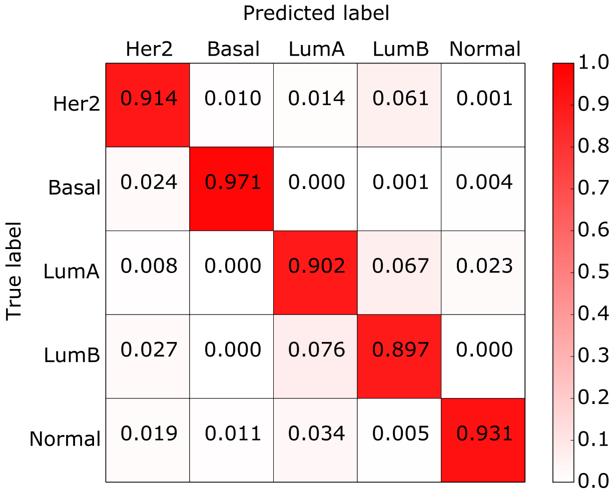
0.2
0.1
0.0

sample size 300



Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.914	0.010	0.014	0.061	0.001
Basal	0.024	0.971	0.000	0.001	0.004
LumA	0.008	0.000	0.902	0.067	0.023
LumB	0.027	0.000	0.076	0.897	0.000
Normal	0.019	0.011	0.034	0.005	0.931

1.0
0.9
0.8
0.7
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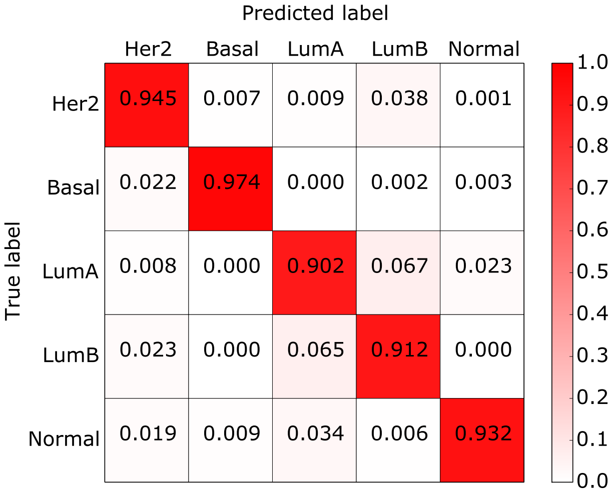
0.5
0.4
0.3
0.2
0.1
0.0

sample size 350



Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.945	0.007	0.009	0.038	0.001
Basal	0.022	0.974	0.000	0.002	0.003
LumA	0.008	0.000	0.902	0.067	0.023
LumB	0.023	0.000	0.065	0.912	0.000
Normal	0.019	0.009	0.034	0.006	0.932

1.0
0.9
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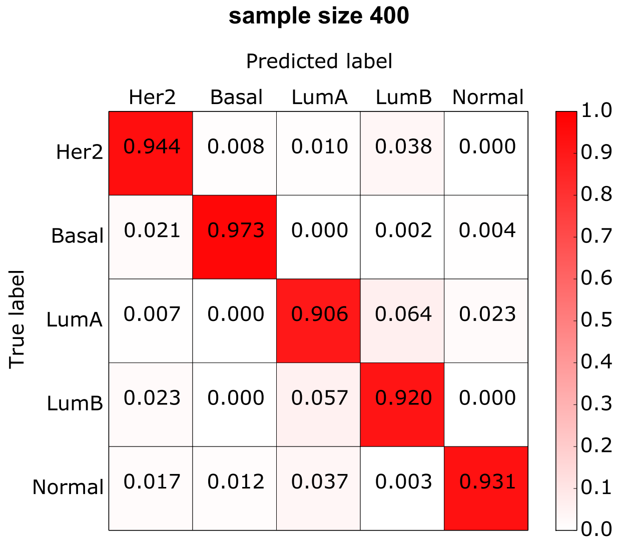
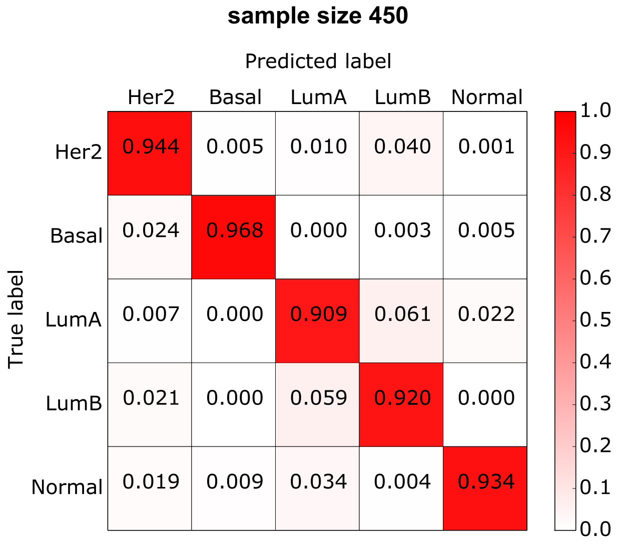


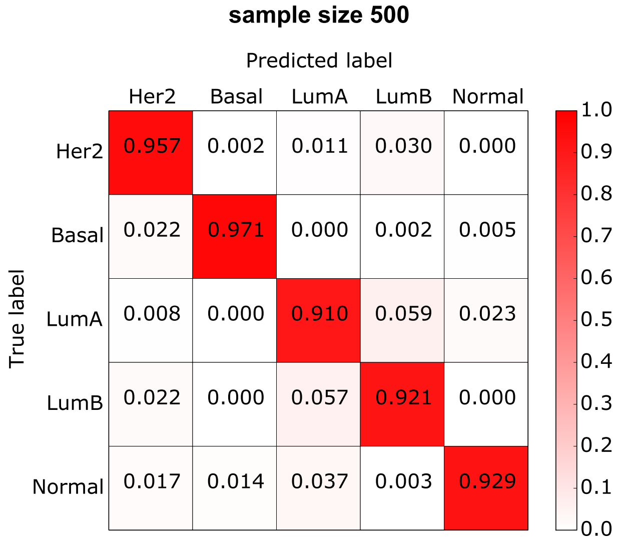
sample size 400

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.944	0.008	0.010	0.038	0.000
Basal	0.021	0.973	0.000	0.002	0.004
LumA	0.007	0.000	0.906	0.064	0.023
LumB	0.023	0.000	0.057	0.920	0.000
Normal	0.017	0.012	0.037	0.003	0.931



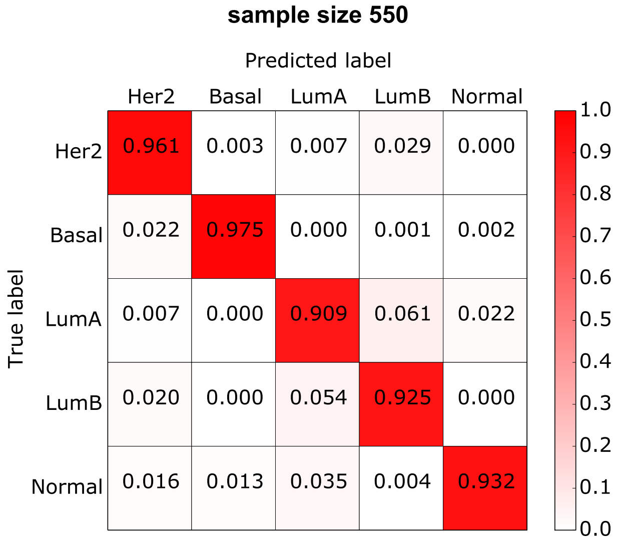
sample size 450

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.944	0.005	0.010	0.040	0.001
Basal	0.024	0.968	0.000	0.003	0.005
LumA	0.007	0.000	0.909	0.061	0.022
LumB	0.021	0.000	0.059	0.920	0.000
Normal	0.019	0.009	0.034	0.004	0.934



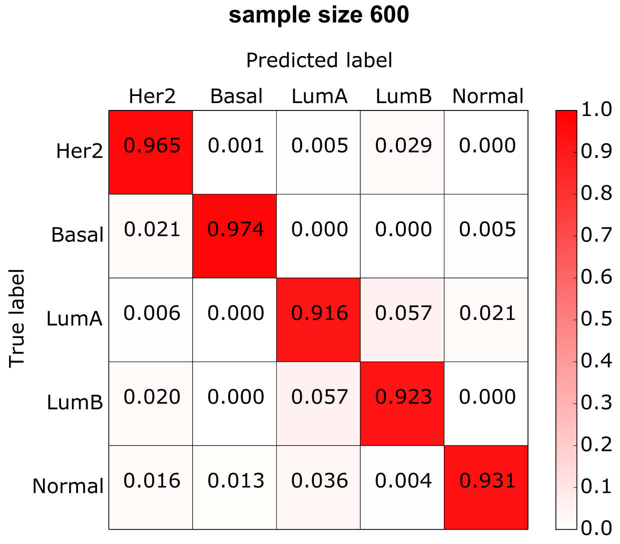
sample size 500

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.957	0.002	0.011	0.030	0.000
Basal	0.022	0.971	0.000	0.002	0.005
LumA	0.008	0.000	0.910	0.059	0.023
LumB	0.022	0.000	0.057	0.921	0.000
Normal	0.017	0.014	0.037	0.003	0.929



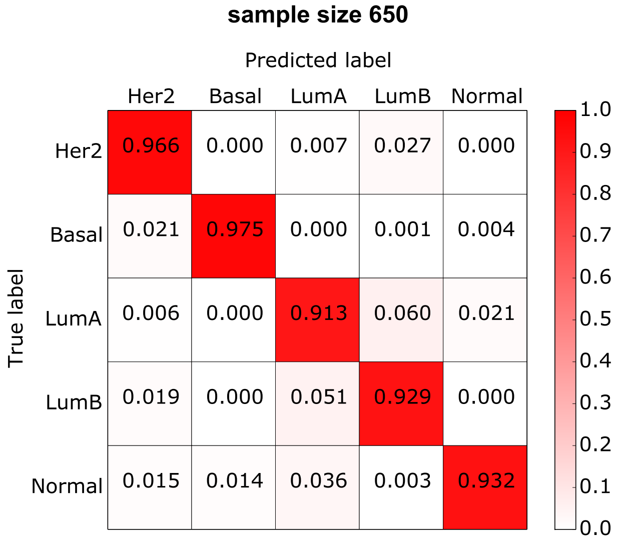
sample size 550

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.961	0.003	0.007	0.029	0.000
Basal	0.022	0.975	0.000	0.001	0.002
LumA	0.007	0.000	0.909	0.061	0.022
LumB	0.020	0.000	0.054	0.925	0.000
Normal	0.016	0.013	0.035	0.004	0.932



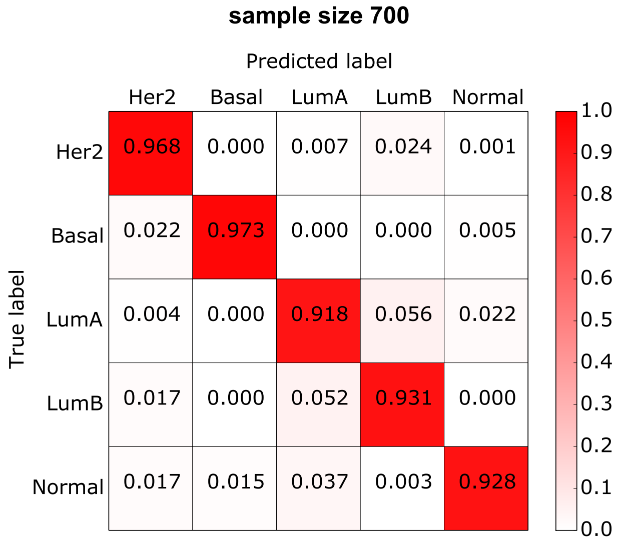
sample size 600

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.965	0.001	0.005	0.029	0.000
Basal	0.021	0.974	0.000	0.000	0.005
LumA	0.006	0.000	0.916	0.057	0.021
LumB	0.020	0.000	0.057	0.923	0.000
Normal	0.016	0.013	0.036	0.004	0.931



sample size 650

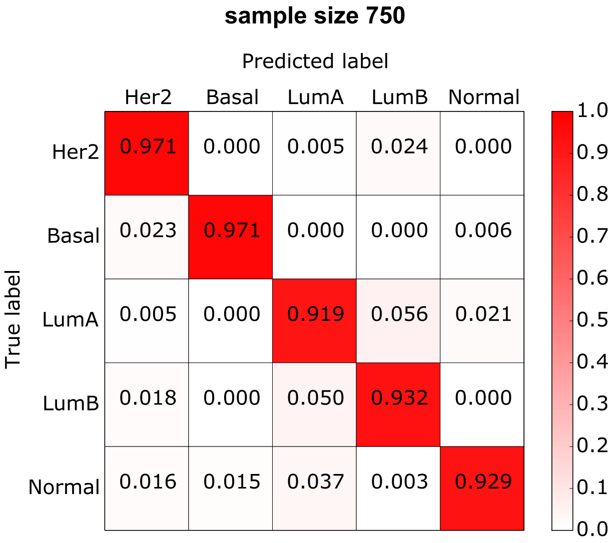
True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.966	0.000	0.007	0.027	0.000
Basal	0.021	0.975	0.000	0.001	0.004
LumA	0.006	0.000	0.913	0.060	0.021
LumB	0.019	0.000	0.051	0.929	0.000
Normal	0.015	0.014	0.036	0.003	0.932



sample size 700

Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.968	0.000	0.007	0.024	0.001
Basal	0.022	0.973	0.000	0.000	0.005
LumA	0.004	0.000	0.918	0.056	0.022
LumB	0.017	0.000	0.052	0.931	0.000
Normal	0.017	0.015	0.037	0.003	0.928

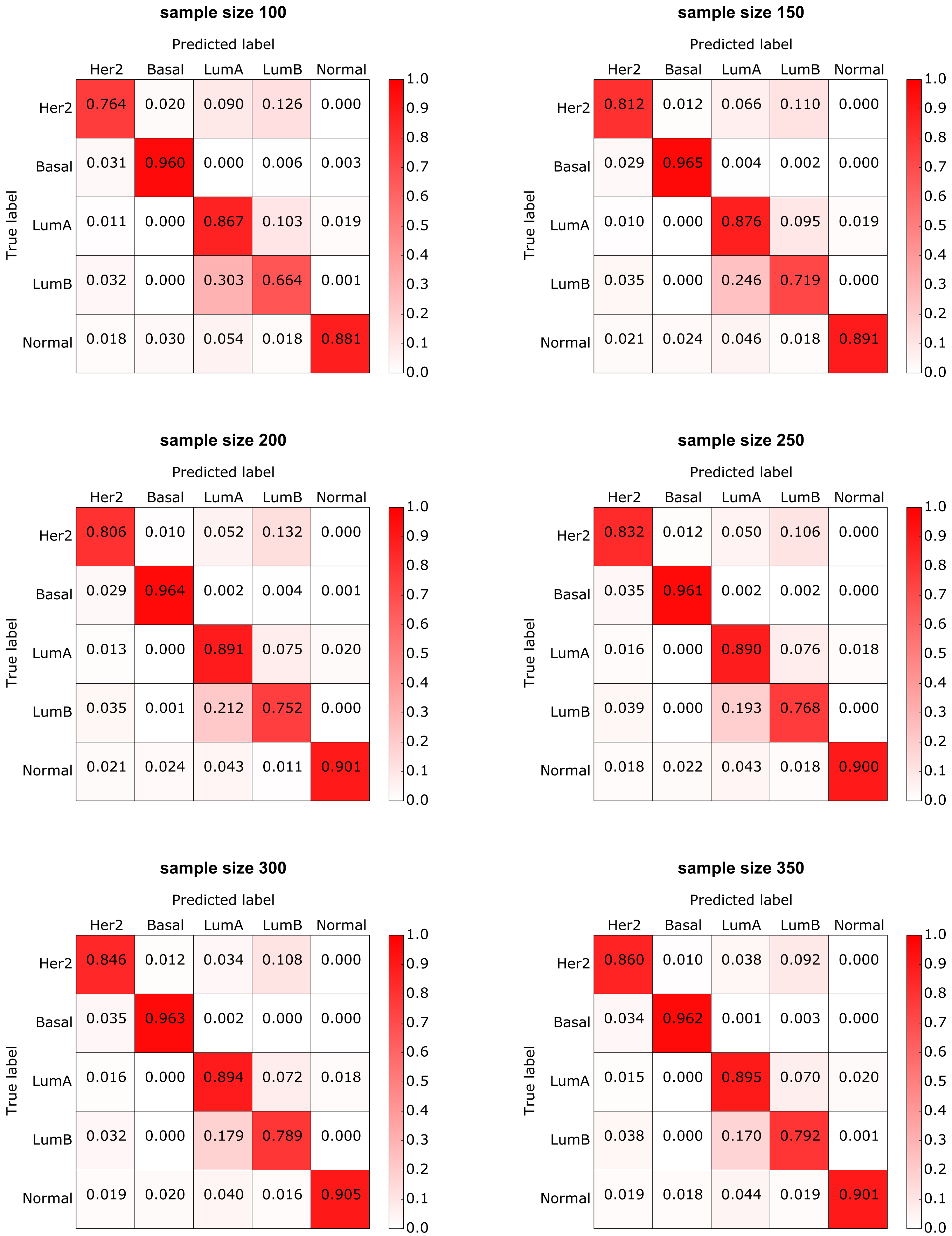


sample size 750

Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.971	0.000	0.005	0.024	0.000
Basal	0.023	0.971	0.000	0.000	0.006
LumA	0.005	0.000	0.919	0.056	0.021
LumB	0.018	0.000	0.050	0.932	0.000
Normal	0.016	0.015	0.037	0.003	0.929

Table S2: Confusion matrix for Top25 dataset and Elastic net classifier



sample size 100

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.764	0.020	0.090	0.126	0.000
Basal	0.031	0.960	0.000	0.006	0.003
LumA	0.011	0.000	0.867	0.103	0.019
LumB	0.032	0.000	0.303	0.664	0.001
Normal	0.018	0.030	0.054	0.018	0.881

sample size 150

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.812	0.012	0.066	0.110	0.000
Basal	0.029	0.965	0.004	0.002	0.000
LumA	0.010	0.000	0.876	0.095	0.019
LumB	0.035	0.000	0.246	0.719	0.000
Normal	0.021	0.024	0.046	0.018	0.891

sample size 200

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.806	0.010	0.052	0.132	0.000
Basal	0.029	0.964	0.002	0.004	0.001
LumA	0.013	0.000	0.891	0.075	0.020
LumB	0.035	0.001	0.212	0.752	0.000
Normal	0.021	0.024	0.043	0.011	0.901

sample size 250

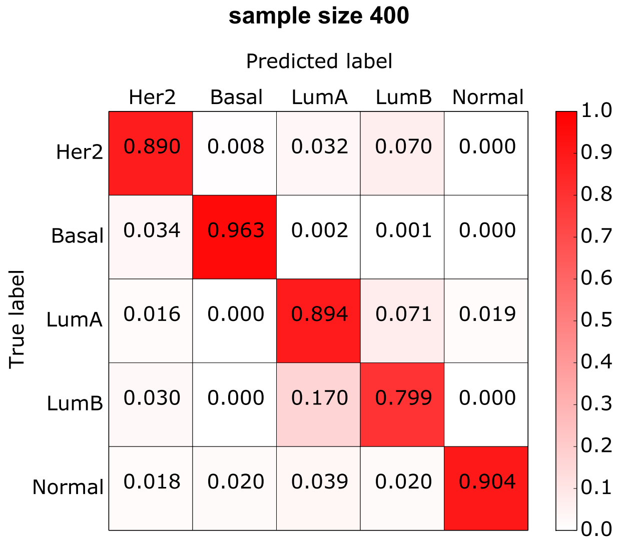
True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.832	0.012	0.050	0.106	0.000
Basal	0.035	0.961	0.002	0.002	0.000
LumA	0.016	0.000	0.890	0.076	0.018
LumB	0.039	0.000	0.193	0.768	0.000
Normal	0.018	0.022	0.043	0.018	0.900

sample size 300

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.846	0.012	0.034	0.108	0.000
Basal	0.035	0.963	0.002	0.000	0.000
LumA	0.016	0.000	0.894	0.072	0.018
LumB	0.032	0.000	0.179	0.789	0.000
Normal	0.019	0.020	0.040	0.016	0.905

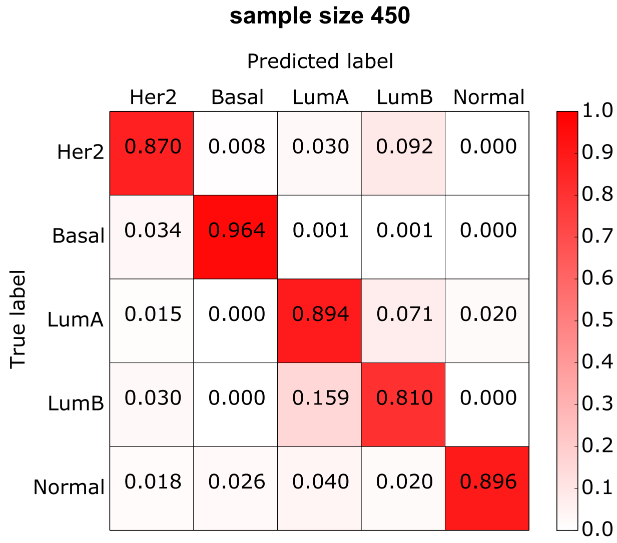
sample size 350

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.860	0.010	0.038	0.092	0.000
Basal	0.034	0.962	0.001	0.003	0.000
LumA	0.015	0.000	0.895	0.070	0.020
LumB	0.038	0.000	0.170	0.792	0.001
Normal	0.019	0.018	0.044	0.019	0.901



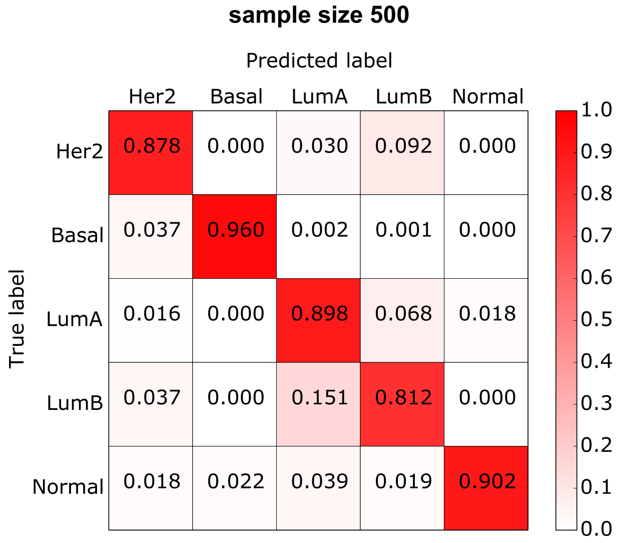
sample size 400

	Her2	Basal	LumA	LumB	Normal
Her2	0.890	0.008	0.032	0.070	0.000
Basal	0.034	0.963	0.002	0.001	0.000
LumA	0.016	0.000	0.894	0.071	0.019
LumB	0.030	0.000	0.170	0.799	0.000
Normal	0.018	0.020	0.039	0.020	0.904



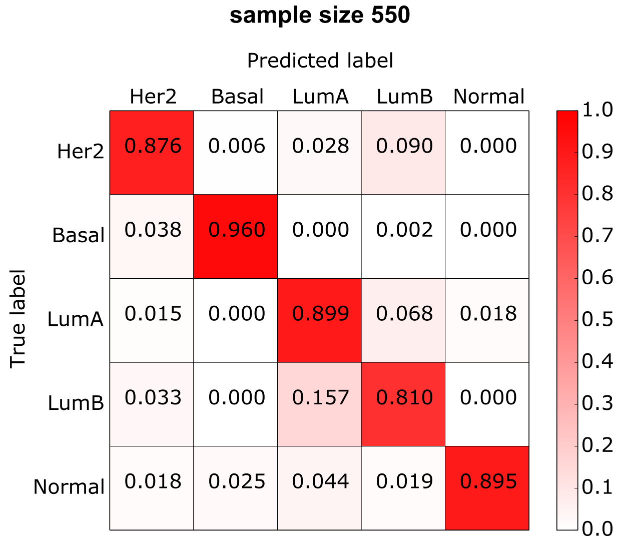
sample size 450

	Her2	Basal	LumA	LumB	Normal
Her2	0.870	0.008	0.030	0.092	0.000
Basal	0.034	0.964	0.001	0.001	0.000
LumA	0.015	0.000	0.894	0.071	0.020
LumB	0.030	0.000	0.159	0.810	0.000
Normal	0.018	0.026	0.040	0.020	0.896



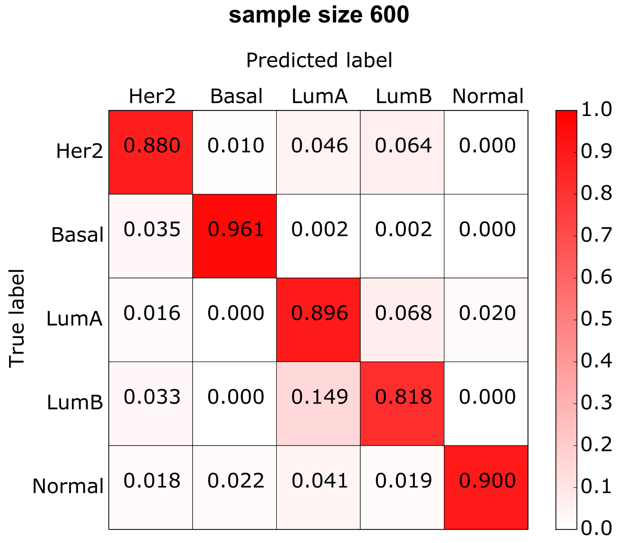
sample size 500

	Her2	Basal	LumA	LumB	Normal
Her2	0.878	0.000	0.030	0.092	0.000
Basal	0.037	0.960	0.002	0.001	0.000
LumA	0.016	0.000	0.898	0.068	0.018
LumB	0.037	0.000	0.151	0.812	0.000
Normal	0.018	0.022	0.039	0.019	0.902



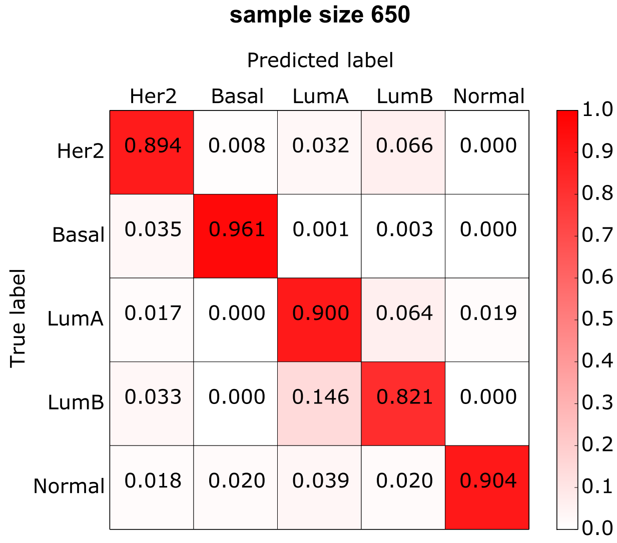
sample size 550

	Her2	Basal	LumA	LumB	Normal
Her2	0.876	0.006	0.028	0.090	0.000
Basal	0.038	0.960	0.000	0.002	0.000
LumA	0.015	0.000	0.899	0.068	0.018
LumB	0.033	0.000	0.157	0.810	0.000
Normal	0.018	0.025	0.044	0.019	0.895



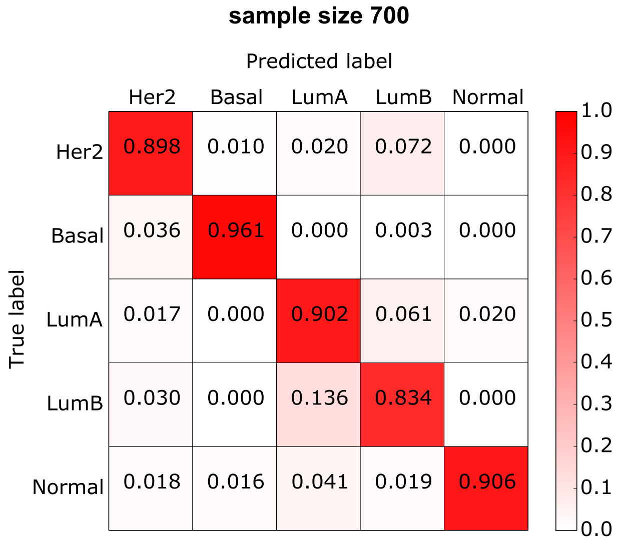
sample size 600

	Her2	Basal	LumA	LumB	Normal
Her2	0.880	0.010	0.046	0.064	0.000
Basal	0.035	0.961	0.002	0.002	0.000
LumA	0.016	0.000	0.896	0.068	0.020
LumB	0.033	0.000	0.149	0.818	0.000
Normal	0.018	0.022	0.041	0.019	0.900



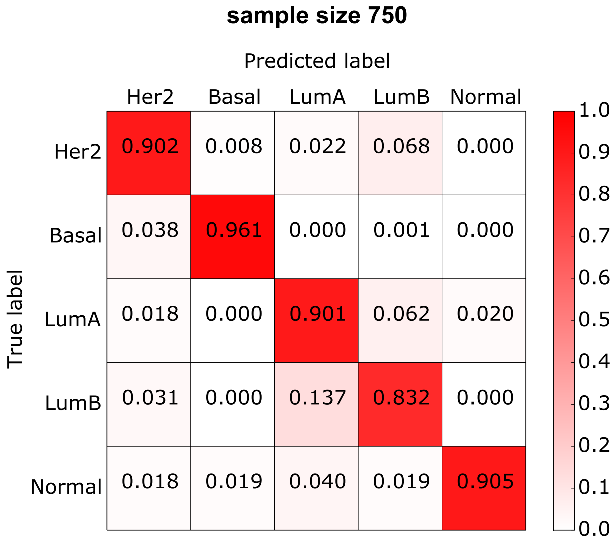
sample size 650

	Her2	Basal	LumA	LumB	Normal
Her2	0.894	0.008	0.032	0.066	0.000
Basal	0.035	0.961	0.001	0.003	0.000
LumA	0.017	0.000	0.900	0.064	0.019
LumB	0.033	0.000	0.146	0.821	0.000
Normal	0.018	0.020	0.039	0.020	0.904



sample size 700

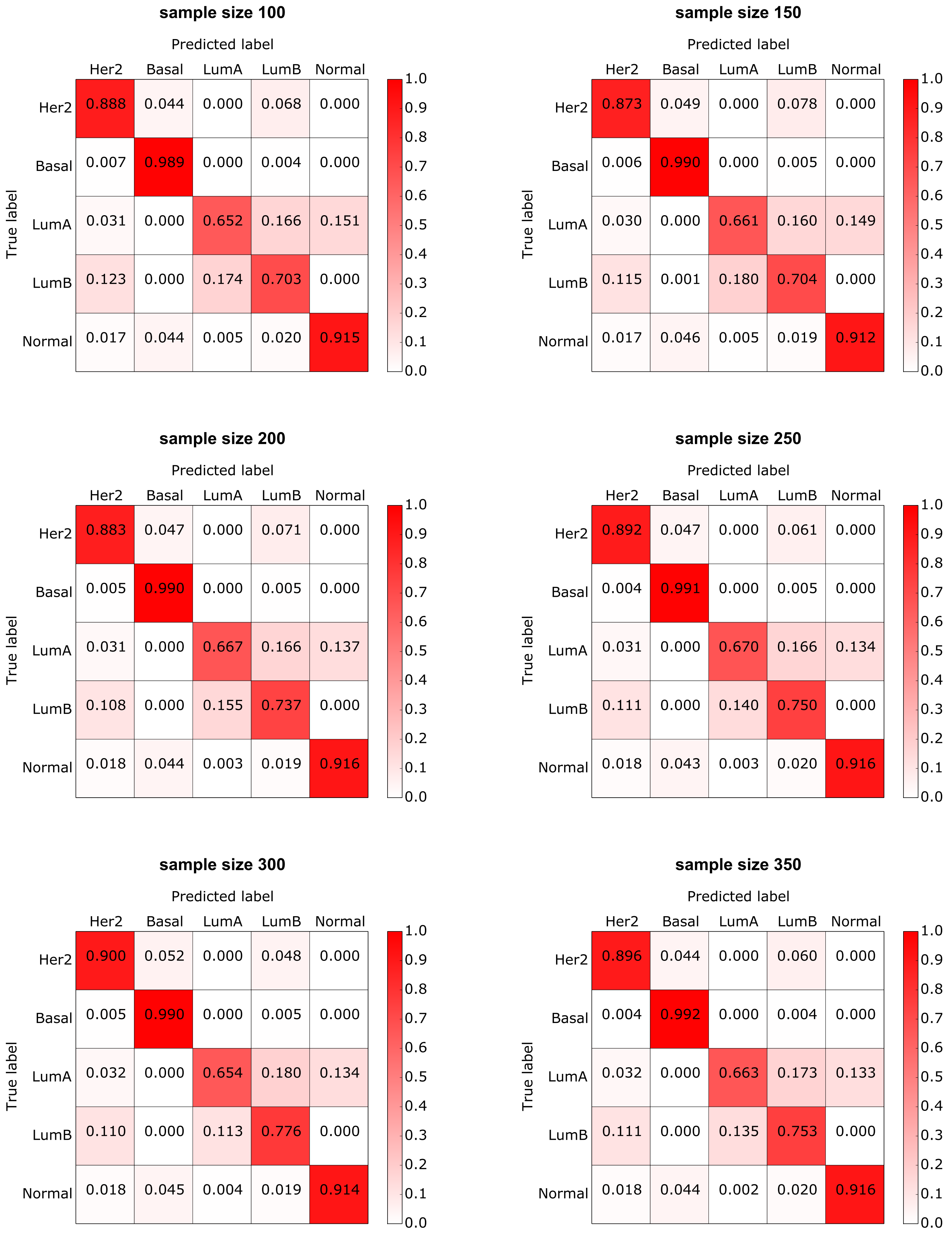
True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.898	0.010	0.020	0.072	0.000
Basal	0.036	0.961	0.000	0.003	0.000
LumA	0.017	0.000	0.902	0.061	0.020
LumB	0.030	0.000	0.136	0.834	0.000
Normal	0.018	0.016	0.041	0.019	0.906



sample size 750

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.902	0.008	0.022	0.068	0.000
Basal	0.038	0.961	0.000	0.001	0.000
LumA	0.018	0.000	0.901	0.062	0.020
LumB	0.031	0.000	0.137	0.832	0.000
Normal	0.018	0.019	0.040	0.019	0.905

Table S3: Confusion matrix for PAM50 dataset and unsupervised learning



sample size 100

Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.888	0.044	0.000	0.068	0.000
Basal	0.007	0.989	0.000	0.004	0.000
LumA	0.031	0.000	0.652	0.166	0.151
LumB	0.123	0.000	0.174	0.703	0.000
Normal	0.017	0.044	0.005	0.020	0.915

sample size 150

Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.873	0.049	0.000	0.078	0.000
Basal	0.006	0.990	0.000	0.005	0.000
LumA	0.030	0.000	0.661	0.160	0.149
LumB	0.115	0.001	0.180	0.704	0.000
Normal	0.017	0.046	0.005	0.019	0.912

sample size 200

Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.883	0.047	0.000	0.071	0.000
Basal	0.005	0.990	0.000	0.005	0.000
LumA	0.031	0.000	0.667	0.166	0.137
LumB	0.108	0.000	0.155	0.737	0.000
Normal	0.018	0.044	0.003	0.019	0.916

sample size 250

Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.892	0.047	0.000	0.061	0.000
Basal	0.004	0.991	0.000	0.005	0.000
LumA	0.031	0.000	0.670	0.166	0.134
LumB	0.111	0.000	0.140	0.750	0.000
Normal	0.018	0.043	0.003	0.020	0.916

sample size 300

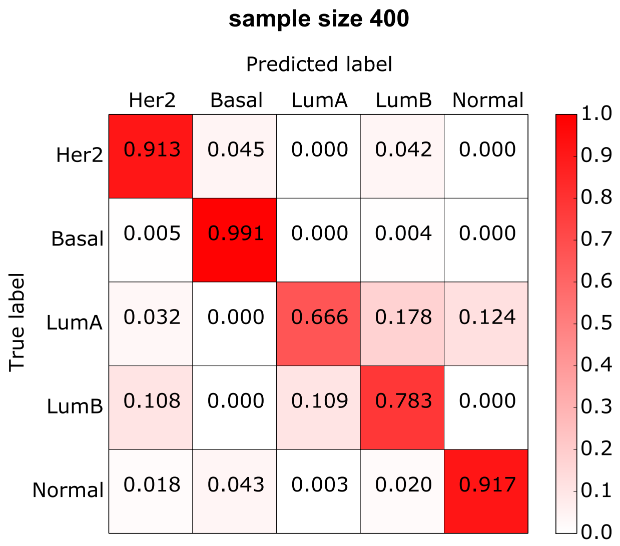
Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.900	0.052	0.000	0.048	0.000
Basal	0.005	0.990	0.000	0.005	0.000
LumA	0.032	0.000	0.654	0.180	0.134
LumB	0.110	0.000	0.113	0.776	0.000
Normal	0.018	0.045	0.004	0.019	0.914

sample size 350

Predicted label

True label	Her2	Basal	LumA	LumB	Normal
Her2	0.896	0.044	0.000	0.060	0.000
Basal	0.004	0.992	0.000	0.004	0.000
LumA	0.032	0.000	0.663	0.173	0.133
LumB	0.111	0.000	0.135	0.753	0.000
Normal	0.018	0.044	0.002	0.020	0.916

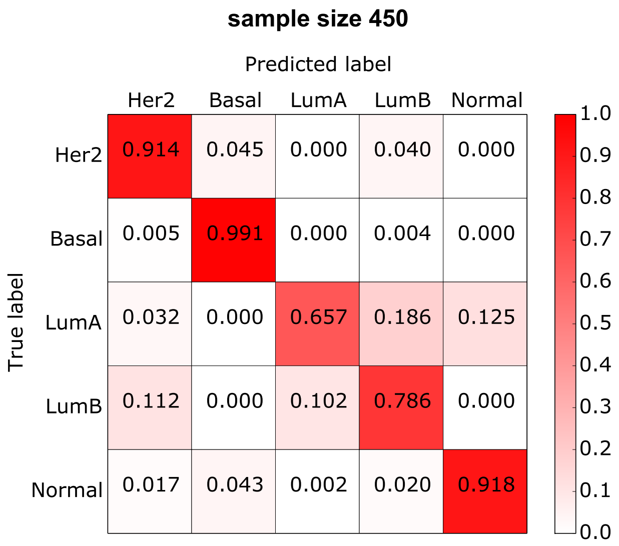


sample size 400

Predicted label

True label

	Her2	Basal	LumA	LumB	Normal
Her2	0.913	0.045	0.000	0.042	0.000
Basal	0.005	0.991	0.000	0.004	0.000
LumA	0.032	0.000	0.666	0.178	0.124
LumB	0.108	0.000	0.109	0.783	0.000
Normal	0.018	0.043	0.003	0.020	0.917

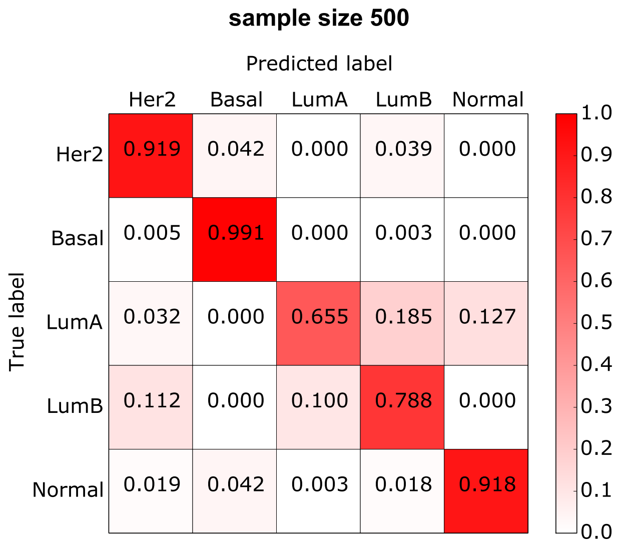


sample size 450

Predicted label

True label

	Her2	Basal	LumA	LumB	Normal
Her2	0.914	0.045	0.000	0.040	0.000
Basal	0.005	0.991	0.000	0.004	0.000
LumA	0.032	0.000	0.657	0.186	0.125
LumB	0.112	0.000	0.102	0.786	0.000
Normal	0.017	0.043	0.002	0.020	0.918

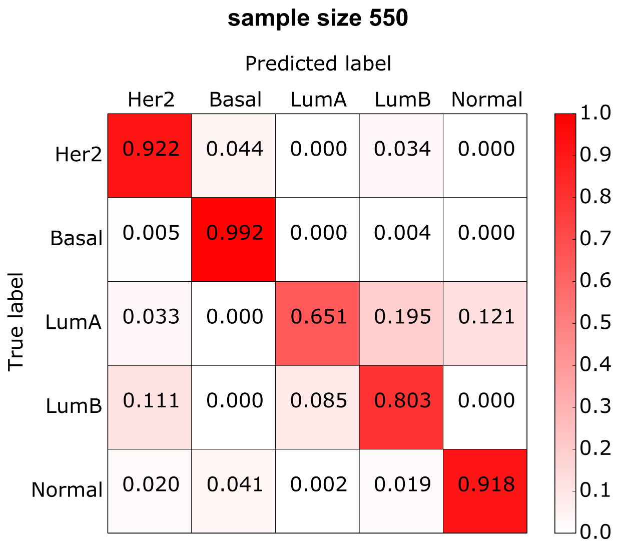


sample size 500

Predicted label

True label

	Her2	Basal	LumA	LumB	Normal
Her2	0.919	0.042	0.000	0.039	0.000
Basal	0.005	0.991	0.000	0.003	0.000
LumA	0.032	0.000	0.655	0.185	0.127
LumB	0.112	0.000	0.100	0.788	0.000
Normal	0.019	0.042	0.003	0.018	0.918

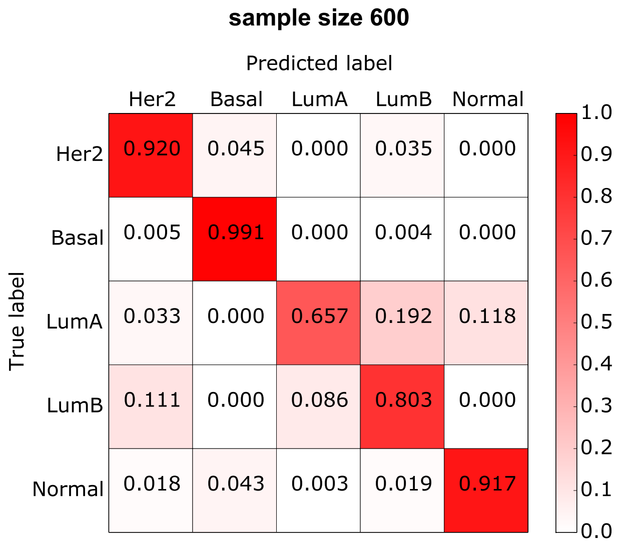


sample size 550

Predicted label

True label

	Her2	Basal	LumA	LumB	Normal
Her2	0.922	0.044	0.000	0.034	0.000
Basal	0.005	0.992	0.000	0.004	0.000
LumA	0.033	0.000	0.651	0.195	0.121
LumB	0.111	0.000	0.085	0.803	0.000
Normal	0.020	0.041	0.002	0.019	0.918

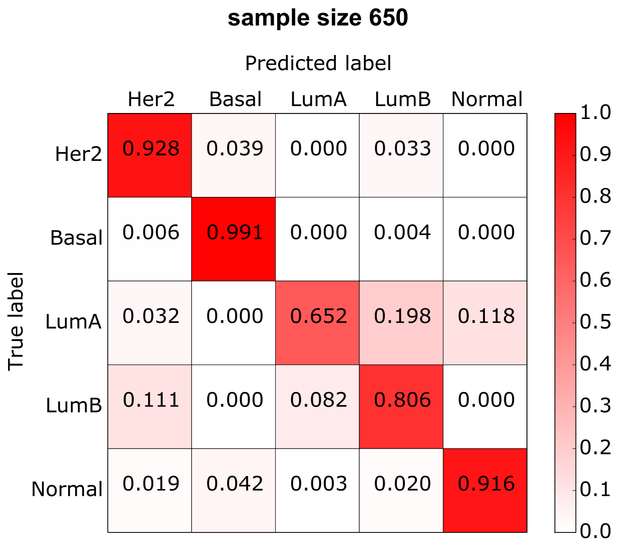


sample size 600

Predicted label

True label

	Her2	Basal	LumA	LumB	Normal
Her2	0.920	0.045	0.000	0.035	0.000
Basal	0.005	0.991	0.000	0.004	0.000
LumA	0.033	0.000	0.657	0.192	0.118
LumB	0.111	0.000	0.086	0.803	0.000
Normal	0.018	0.043	0.003	0.019	0.917

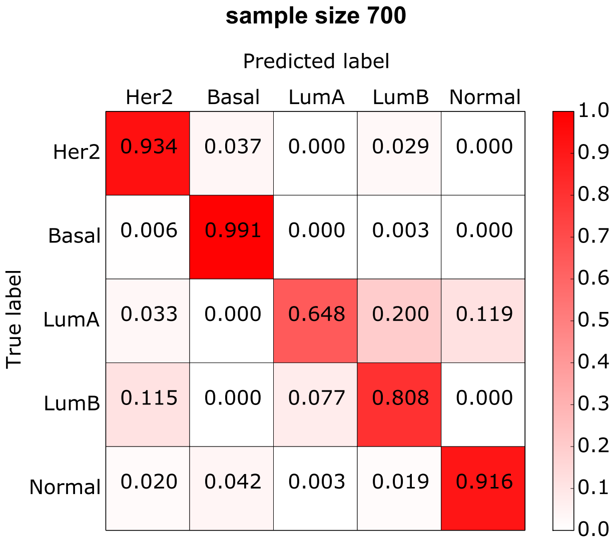


sample size 650

Predicted label

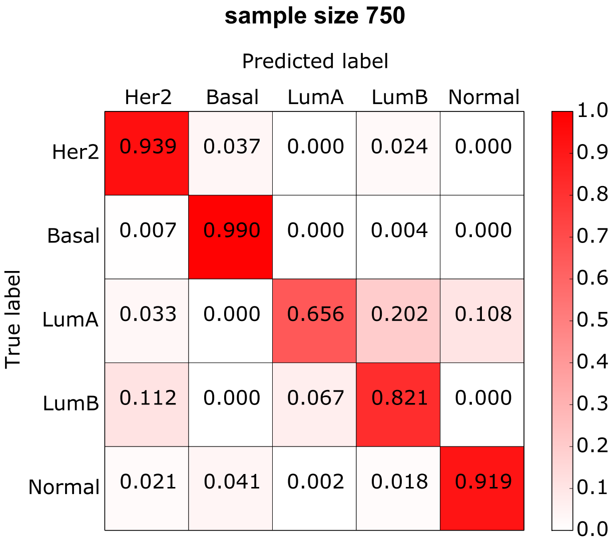
True label

	Her2	Basal	LumA	LumB	Normal
Her2	0.928	0.039	0.000	0.033	0.000
Basal	0.006	0.991	0.000	0.004	0.000
LumA	0.032	0.000	0.652	0.198	0.118
LumB	0.111	0.000	0.082	0.806	0.000
Normal	0.019	0.042	0.003	0.020	0.916



sample size 700

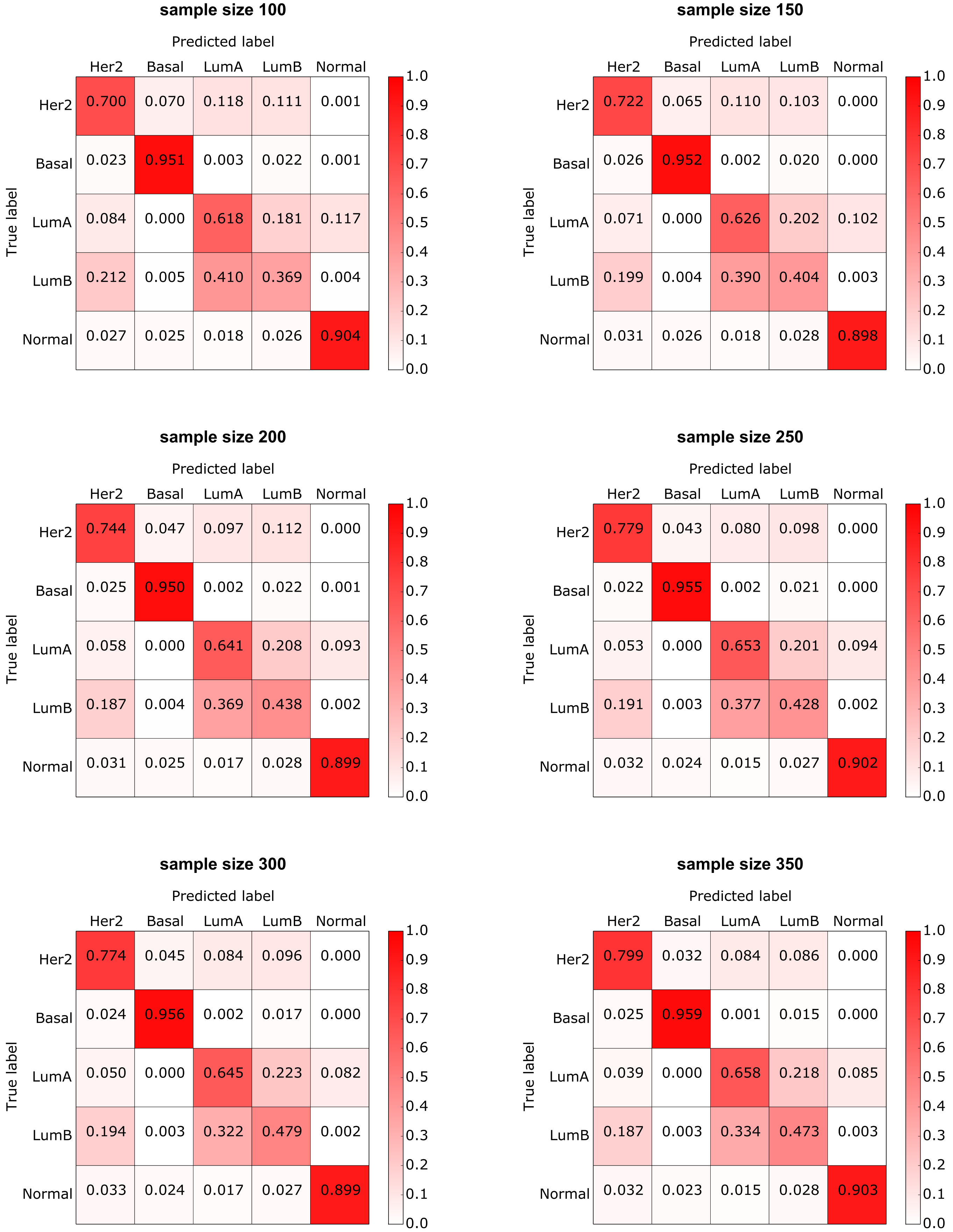
True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.934	0.037	0.000	0.029	0.000
Basal	0.006	0.991	0.000	0.003	0.000
LumA	0.033	0.000	0.648	0.200	0.119
LumB	0.115	0.000	0.077	0.808	0.000
Normal	0.020	0.042	0.003	0.019	0.916



sample size 750

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.939	0.037	0.000	0.024	0.000
Basal	0.007	0.990	0.000	0.004	0.000
LumA	0.033	0.000	0.656	0.202	0.108
LumB	0.112	0.000	0.067	0.821	0.000
Normal	0.021	0.041	0.002	0.018	0.919

Table S4: Confusion matrix for Top25 dataset and unsupervised learning



sample size 100

	Her2	Basal	LumA	LumB	Normal
Her2	0.700	0.070	0.118	0.111	0.001
Basal	0.023	0.951	0.003	0.022	0.001
LumA	0.084	0.000	0.618	0.181	0.117
LumB	0.212	0.005	0.410	0.369	0.004
Normal	0.027	0.025	0.018	0.026	0.904

sample size 150

	Her2	Basal	LumA	LumB	Normal
Her2	0.722	0.065	0.110	0.103	0.000
Basal	0.026	0.952	0.002	0.020	0.000
LumA	0.071	0.000	0.626	0.202	0.102
LumB	0.199	0.004	0.390	0.404	0.003
Normal	0.031	0.026	0.018	0.028	0.898

sample size 200

	Her2	Basal	LumA	LumB	Normal
Her2	0.744	0.047	0.097	0.112	0.000
Basal	0.025	0.950	0.002	0.022	0.001
LumA	0.058	0.000	0.641	0.208	0.093
LumB	0.187	0.004	0.369	0.438	0.002
Normal	0.031	0.025	0.017	0.028	0.899

sample size 250

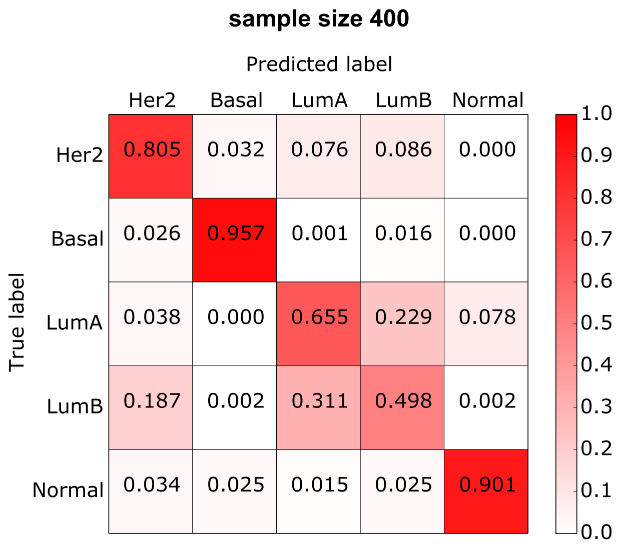
	Her2	Basal	LumA	LumB	Normal
Her2	0.779	0.043	0.080	0.098	0.000
Basal	0.022	0.955	0.002	0.021	0.000
LumA	0.053	0.000	0.653	0.201	0.094
LumB	0.191	0.003	0.377	0.428	0.002
Normal	0.032	0.024	0.015	0.027	0.902

sample size 300

	Her2	Basal	LumA	LumB	Normal
Her2	0.774	0.045	0.084	0.096	0.000
Basal	0.024	0.956	0.002	0.017	0.000
LumA	0.050	0.000	0.645	0.223	0.082
LumB	0.194	0.003	0.322	0.479	0.002
Normal	0.033	0.024	0.017	0.027	0.899

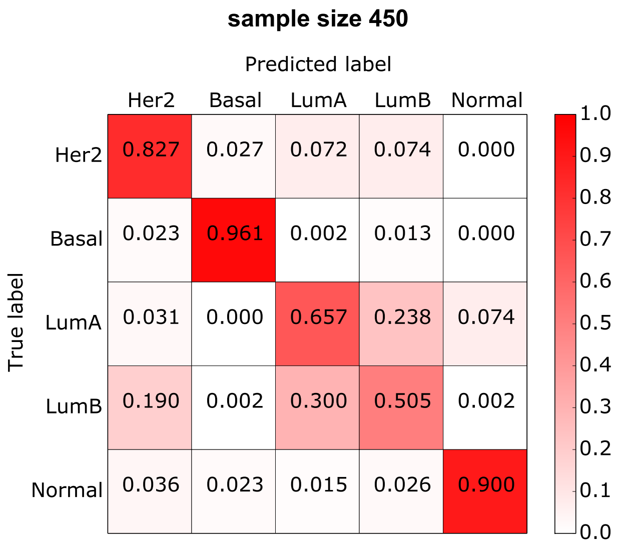
sample size 350

	Her2	Basal	LumA	LumB	Normal
Her2	0.799	0.032	0.084	0.086	0.000
Basal	0.025	0.959	0.001	0.015	0.000
LumA	0.039	0.000	0.658	0.218	0.085
LumB	0.187	0.003	0.334	0.473	0.003
Normal	0.032	0.023	0.015	0.028	0.903



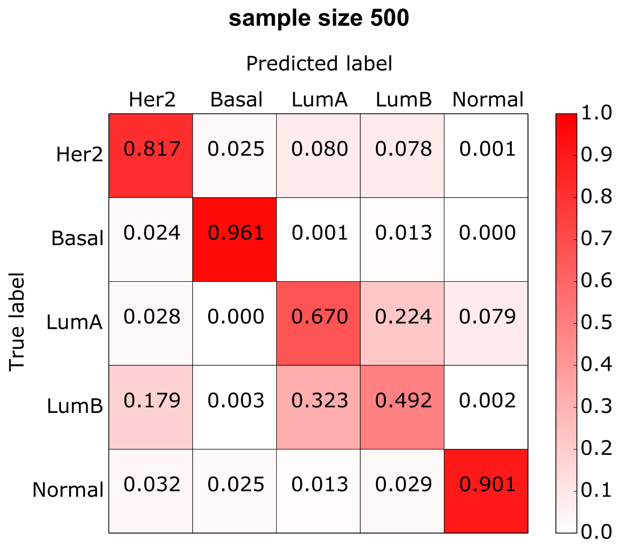
sample size 400

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.805	0.032	0.076	0.086	0.000
Basal	0.026	0.957	0.001	0.016	0.000
LumA	0.038	0.000	0.655	0.229	0.078
LumB	0.187	0.002	0.311	0.498	0.002
Normal	0.034	0.025	0.015	0.025	0.901



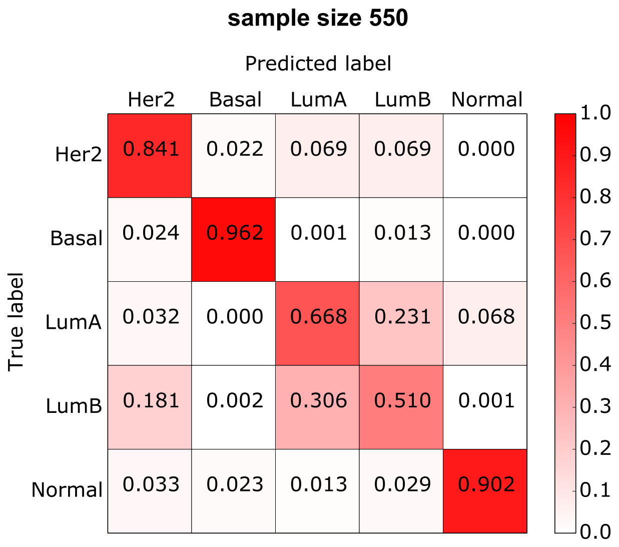
sample size 450

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.827	0.027	0.072	0.074	0.000
Basal	0.023	0.961	0.002	0.013	0.000
LumA	0.031	0.000	0.657	0.238	0.074
LumB	0.190	0.002	0.300	0.505	0.002
Normal	0.036	0.023	0.015	0.026	0.900



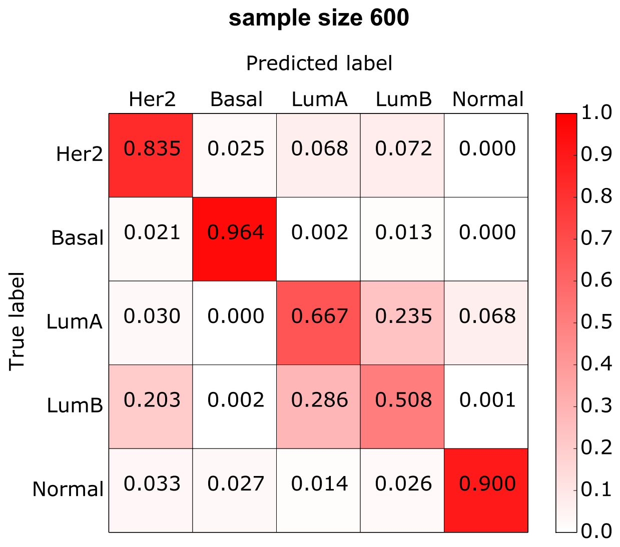
sample size 500

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.817	0.025	0.080	0.078	0.001
Basal	0.024	0.961	0.001	0.013	0.000
LumA	0.028	0.000	0.670	0.224	0.079
LumB	0.179	0.003	0.323	0.492	0.002
Normal	0.032	0.025	0.013	0.029	0.901



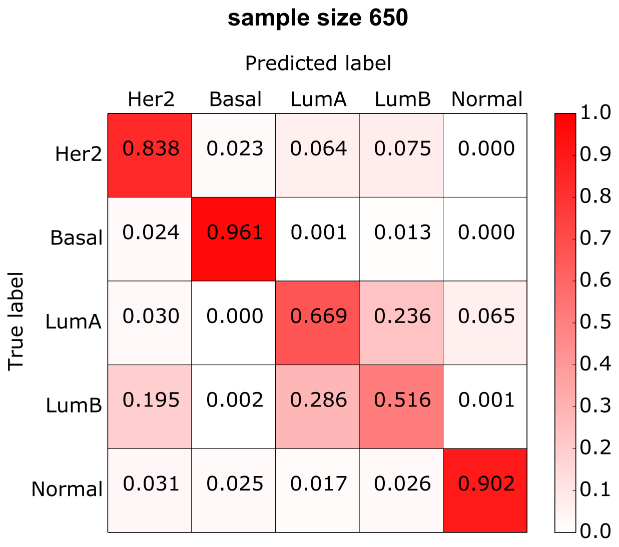
sample size 550

True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.841	0.022	0.069	0.069	0.000
Basal	0.024	0.962	0.001	0.013	0.000
LumA	0.032	0.000	0.668	0.231	0.068
LumB	0.181	0.002	0.306	0.510	0.001
Normal	0.033	0.023	0.013	0.029	0.902



sample size 600

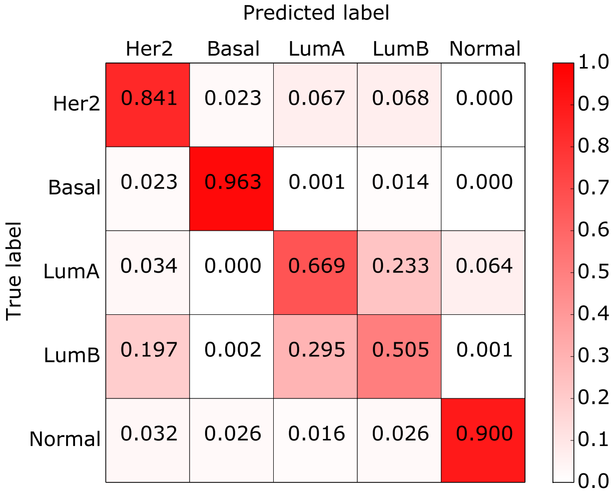
True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.835	0.025	0.068	0.072	0.000
Basal	0.021	0.964	0.002	0.013	0.000
LumA	0.030	0.000	0.667	0.235	0.068
LumB	0.203	0.002	0.286	0.508	0.001
Normal	0.033	0.027	0.014	0.026	0.900



sample size 650

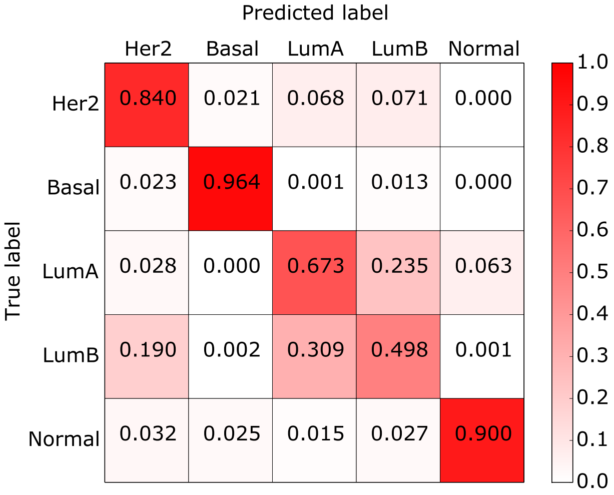
True label \ Predicted label	Her2	Basal	LumA	LumB	Normal
Her2	0.838	0.023	0.064	0.075	0.000
Basal	0.024	0.961	0.001	0.013	0.000
LumA	0.030	0.000	0.669	0.236	0.065
LumB	0.195	0.002	0.286	0.516	0.001
Normal	0.031	0.025	0.017	0.026	0.902

sample size 700



	Her2	Basal	LumA	LumB	Normal
Her2	0.841	0.023	0.067	0.068	0.000
Basal	0.023	0.963	0.001	0.014	0.000
LumA	0.034	0.000	0.669	0.233	0.064
LumB	0.197	0.002	0.295	0.505	0.001
Normal	0.032	0.026	0.016	0.026	0.900

sample size 750



	Her2	Basal	LumA	LumB	Normal
Her2	0.840	0.021	0.068	0.071	0.000
Basal	0.023	0.964	0.001	0.013	0.000
LumA	0.028	0.000	0.673	0.235	0.063
LumB	0.190	0.002	0.309	0.498	0.001
Normal	0.032	0.025	0.015	0.027	0.900